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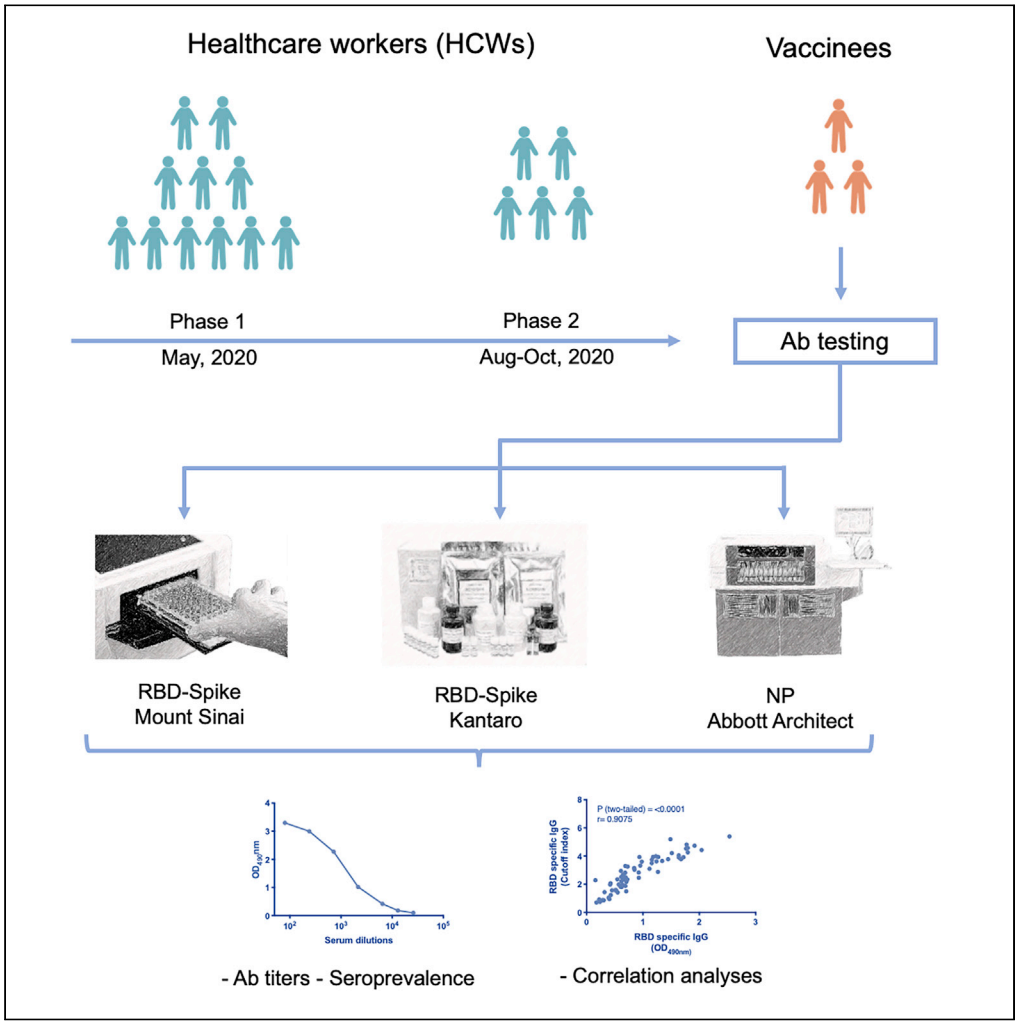

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Highlights

SARS-CoV-2 antibody seroprevalence in HCWs ranged around 28% early during the pandemic

Good correlation was observed between research-grade and commercial RBD-spike ELISAs

NP but not RBD-spike antibody seroprevalence significantly declined

RBD-spike-based assays effectively detected seroconversion in vaccinees

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Longitudinal analysis of severe acute respiratory syndrome coronavirus 2 seroprevalence using multiple serology platforms

Juan Manuel Carreño,[1] Damodara Rao Mendu,[2] Viviana Simon,[1,4,5] Masood A. Shariff,[3] Gagandeep Singh,[1] Vidya Menon,[3,*] and Florian Krammer[1,6,*]

SUMMARY

Current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) serological tests are based on the full-length spike (S), the receptor-binding domain (RBD), or the nucleoprotein (NP) as substrates. Here, we used samples from healthcare workers (HCWs) to perform a longitudinal analysis of the antibody responses using a research-grade RBD and spike-based enzyme-linked immunosorbent assay (ELISA), a commercial RBD and spike-based ELISA, and a commercial NP-based chemiluminescent microparticle immunoassay. Seroprevalence ranged around 28% early during the pandemic and a good correlation was observed between RBD and spike-based ELISAs. Modest correlations were observed between NP and both RBD and spike-based assays. The antibody levels in HCWs declined over time; however, the overall seroprevalence measured by RBD and spike-based assays remained unchanged, while the seroprevalence of NP-reactive antibodies significantly declined. Moreover, RBD and spike-based assays effectively detected seroconversion in vaccinees. Overall, our results consolidate the strength of different serological assays to assess the magnitude and duration of antibodies to SARS-CoV-2.

INTRODUCTION

In the advent of the current pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), methods to detect the prevalence of recent and past infections are key to determine public health and social countermeasures. Nucleic acid amplification tests provide an accurate estimation of acute infections (Chu et al., 2020; Pan et al., 2020), but they fail to inform about past infections. Serological tests that detect antibodies directed against structural targets of the virus, not only are useful to estimate the overall viral seroprevalence and rates of infection in the population (Angulo et al., 2021; Rostami et al., 2020; Stadlbauer et al., 2020b) but also help to assess responses to vaccination (Krammer, 2020), to determine correlates of protection (Brown, 2020; McMahan et al., 2020), and to test and standardize therapeutic approaches such as monoclonal antibody and plasma transfer therapies (Duan et al., 2020). Moreover, estimation of viral seroprevalence and quantification of antibody levels adds to our understanding of the immune response and protection at the individual and population levels (Cohen, 2021).

Currently, serological assays to detect antibodies against SARS-CoV-2 are based on recombinant versions of the spike (S) protein, the receptor-binding domain (RBD) of S, or the nucleoprotein (NP) as substrates (Choi et al., 2020; Krammer and Simon, 2020; Schaffner et al., 2020). A variety of research grade and commercial S-based and NP-based assays are now available, but antibodies to these two targets have different characteristics. Antibodies directed against the viral S are retained for several months after infection (Dan et al., 2021; Isho et al., 2020; Iyer et al., 2020; Seow et al., 2020; Vanshylla et al., 2021; Wajnberg et al., 2020; Wu et al., 2020) and correlate with virus neutralization and protection against reinfection (Chandrashekar et al., 2020; Deng et al., 2020; Hall et al., 2021; Krammer, 2020; Lumley et al., 2020; Vanshylla et al., 2021; Wajnberg et al., 2020). Moreover, vaccination relies uniquely on the viral S, evidencing the importance of detecting antibodies against this target with high levels of sensitivity and specificity (Krammer, 2020).

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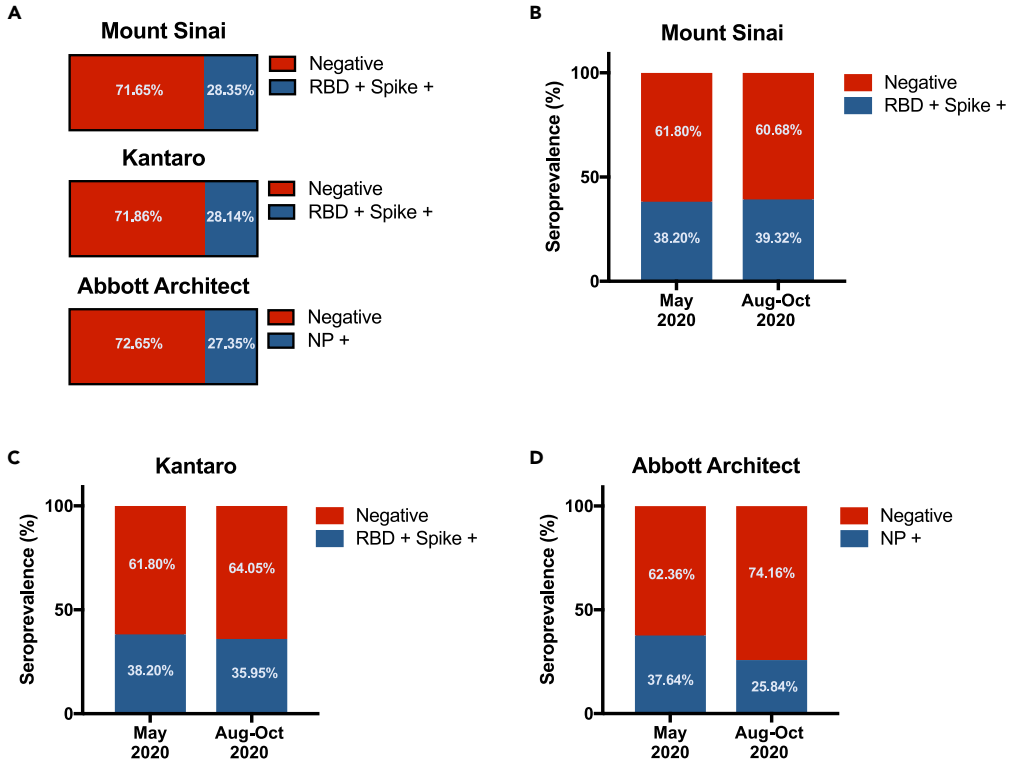

Figure 1. Seroprevalence against SARS-CoV-2 in healthcare workers using different serology platforms

Serum samples from frontline healthcare workers were assessed for antibodies against RBD-spike using a research grade ELISA from Mount Sinai, against RBD-spike using a commercial ELISA from Kantaro Biosciences, or against NP using the Abbott-Architect CMIA. Initially, samples analyzed in the three assays consisted of specimens obtained early during the pandemic on May 2020 (n = 501) (A). Seroprevalence in a subset of subjects (n = 178) who attended a follow up visit between August-October 2020 was determined using the three different serological assays and a comparison of the two time points is shown (B-C).

Several studies evaluated the sensitivity and specificity of individual assays, either S- or NP- based; however, longitudinal side-by-side comparisons of different serological platforms are scarce. Here, we employed samples from a high-risk cohort of healthcare workers (HCWs) using three different serological assays. In addition, SARS-CoV-2 postvaccination samples were included in the analysis. We compared a research-grade RBD and S-based tandem enzyme-linked immunosorbent assay (ELISA) developed at Mount Sinai (MS ELISA, research grade version), the Seroklir commercial RBD-S-based ELISA from Kantaro Biosciences, and the commercial NP-based chemiluminescent microparticle immunoassay (CMIA) for Abbott Architect.

RESULTS

Longitudinal comparison of SARS-CoV-2 seroprevalence using RBD/S- and NP-based assays

Seroprevalence of SARS-CoV-2 across different regions of the world has been described using multiple serological assays based either on the S protein, its RBD, or the NP. Here, we compared side-by-side the research-grade MS ELISA based on RBD and S, an RBD/S-based SeroKlir assay from Kantaro Biosciences and the NP-based Abbott Architect test. We used a set of 501 samples from frontline HCWs collected after the first pandemic wave in the New York City metropolitan area (phase 1, May 2020). Seroprevalence in this set of samples using the research-grade ELISA from Mount Sinai was 28.4% (142/501), 28.1%, using the SeroKlir test from Kantaro Biosciences (141/501), and 27.3% using the Abbott Architect test (137/501) (Figure 1A). A subset of the initial participants (n = 178) provided a second serum sample at a follow-up visit between August–October 2020 (phase 2) allowing assessment of seroprevalence at two different time points. Of note, the seroprevalence in the smaller subset of participants was higher compared to the initial cohort (N = 501). This is likely due to higher compliance of participants that knew their serostatus in the first phase. Overall, the seroprevalence measured by the Mount Sinai and

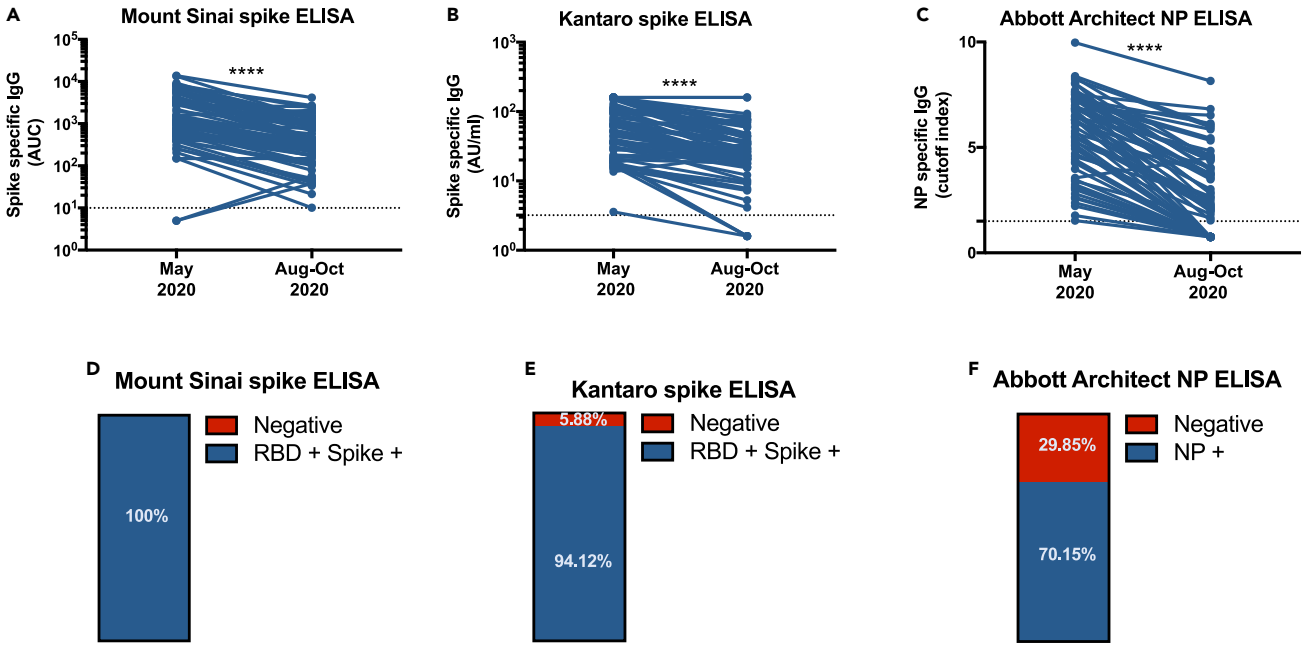

Figure 2. Longitudinal analysis of antibody levels against RBD-spike or NP in seropositive subjects

Serum samples from frontline healthcare workers were obtained on May 2020 or between August-October 2020. Antibodies against RBD-spike were measured using a research grade ELISA from Mount Sinai (A); against RBD-spike using a commercial ELISA from Kantaro Biosciences (B); or against NP using the Abbott-Architect CMIA (C). Antibody levels in specimens obtained early during the pandemic in May 2020 or in a follow up visit between August-October 2020 are shown. Samples with a value above or below the cutoff of the corresponding assay (doted line) are shown. ****$P < 0.0001$. The percentage of seropositive samples that turned negative (red) or that remained positive (blue) as measured in each of the corresponding assays is shown in D-F. See also Figures S1 and S2.

the Kantaro ELISAs did not vary significantly between the two time points (Figures 1B and 1C), but the seroprevalence of NP-reactive antibodies measured by the Abbott Architect test declined (Figure 1D).

We further compared the antibody levels in samples obtained during the first phase (May 2020) and the second phase (August–October, 2020) in the subset of 178 subjects (the distribution of antibody levels is shown in Figure S1 and concordance analyses among the different assays are shown in Figure S2). As expected, antibody levels in the second phase declined in the majority of participants in a manner that was consistent in the three different assays (Figures 2A–2C). A sharper decline of NP-reactive antibody levels as measured by the Abbott Architect test (Figure 2C). Moreover, the percentage of subjects that were seropositive initially and whose antibodies became undetectable in the second phase did not vary significantly in the Mount Sinai and Kantaro ELISAs (Figures 2A and 2B) but approximately 30% of the samples that were positive initially in the Abbott Architect test became negative in the second phase (Figure 2B). 5.88% of the individuals who tested positive for RBD-S-reactive antibodies in phase 1 (May, 2020) using the Kantaro assay showed no reactivity in phase 2 (August–October, 2020) (Figures 2B and 2E), whereas two individuals who tested negative for RBD-S-reactive antibodies in phase 1 (May, 2020) using the MS ELISA seroconverted in phase 2 (Figure 2A). Of note, in these particular cases all the positive/negative values were close to the cutoff of the corresponding assays.

Correlation of antibody levels among the different assays

The antibody response against different antigenic targets of a particular virus exhibits a high degree of complexity. The magnitude and kinetics of the antibody response against RBD/S and the nucleoprotein are not fully understood. To analyze the consistency between the two RBD/S-based assays and to study the relationship of RBD/S-reactive antibodies versus NP-reactive antibodies, we performed correlation analyses among the three different assays. Since a signal against the RBD at one serum dilution is measured in the MS and Kantaro assays followed by a quantification of spike-reactive antibodies we performed the analysis for RBD- and spike-reactive antibodies separately. Using the positive samples from the first

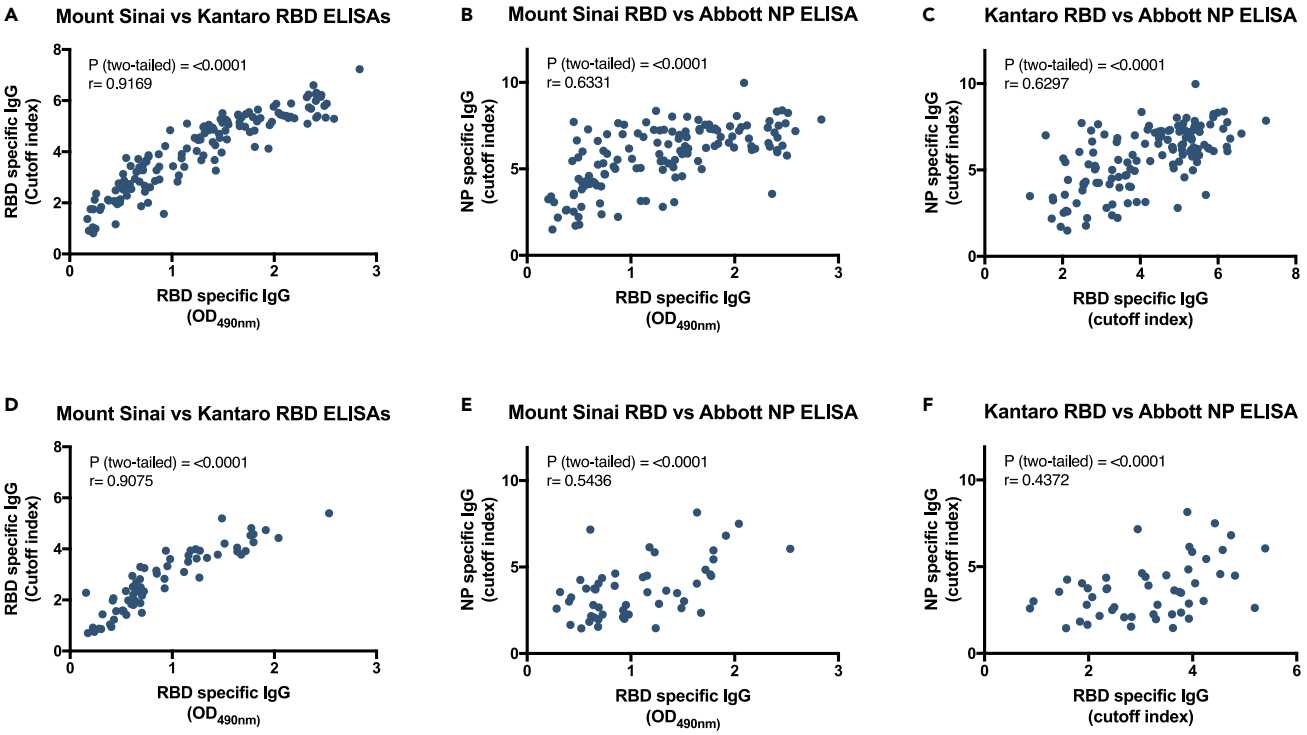

Figure 3. Correlation analysis of antibody levels (RBD-reactive vs. NP-reactive) among the different serology assays

Serum samples were assessed for antibodies against RBD-only using a research grade ELISA from Mount Sinai, against RBD-only using a commercial ELISA from Kantaro Biosciences or against NP using the Abbott-Architect CMIA. Correlation of antibody levels (RBD reactive) among the different assays using serum samples obtained in May 2020 (first time point) is shown (A-C). Correlation of antibody levels (RBD reactive) among the different assays using serum samples obtained between August-October 2020 (second time point) is shown (D-F). Correlation analysis between Mount Sinai RBD and Kantaro RBD ELISAs (A, D); between Mount Sinai RBD and Abbott Architect NP ELISAs (B, E); and between Kantaro RBD ELISAs and Abbott Architect NP CMIAs (C, F) are shown. Pearson correlation was used. Significance and correlation coefficient are shown.

(Figures 3A–3C) and second (Figures 3D–3F) phases, we detected a good correlation of RBD-reactive antibodies (optical density [OD] measured at one dilution) measured by the Mount Sinai ELISA versus the Kantaro ELISA either in phase 1 (r = 0.9169; P two-tailed= <0.0001, Figure 3A) or phase 2 (r = 0.9075; P two-tailed= <0.0001, Figure 3D). However, the correlation of RBD-reactive antibodies measured in the Mount Sinai or Kantaro assays versus the NP-reactive antibodies measured in the Abbott Architect test, either in phase 1 (Figures 3B and 3C) or phase 2 (Figures 3E and 3F) samples, was modest to low.

Next, we performed the same type of analyses but with quantitative S-reactive antibody levels instead of RBD-reactive single-dilution antibody values. Again, we found a good correlation between the Mount Sinai ELISA versus the Kantaro ELISA either in phase 1 (r = 0.6860; P two-tailed= <0.0001, Figure 4A) or phase 2 (r = 0.9135; P two-tailed= <0.0001, Figure 4D) and a weak correlation between S-reactive antibodies measured in the Mount Sinai or Kantaro assays versus the NP-reactive antibodies measured in the Abbott Architect test (Figures 4B and 4C: phase 1; Figures 4E and 4F: phase 2). For both RBD and S, some of the subjects exhibited very high levels of RBD-reactive antibodies and low levels of NP-reactive antibodies and *vice versa*. Overall, as reported by others (Grzelak et al., 2021; Marien et al., 2021), these findings indicate that the magnitude of RBD/S and NP antibody responses differs considerably, highlighting the need for further studies using samples from well-described longitudinal cohorts.

Detection of vaccine induced antibodies in both assays

As an addition to the data available about how *in-house* produced and commercial antibody assays respond to antibodies developed in response to infection (Amanat et al., 2021; Kanji et al., 2021; Stankov et al., 2021; Suhandynata et al., 2021), we determined how the three assays perform against vaccine-induced antibodies. We measured reactivity in serum of individuals who had received two doses of

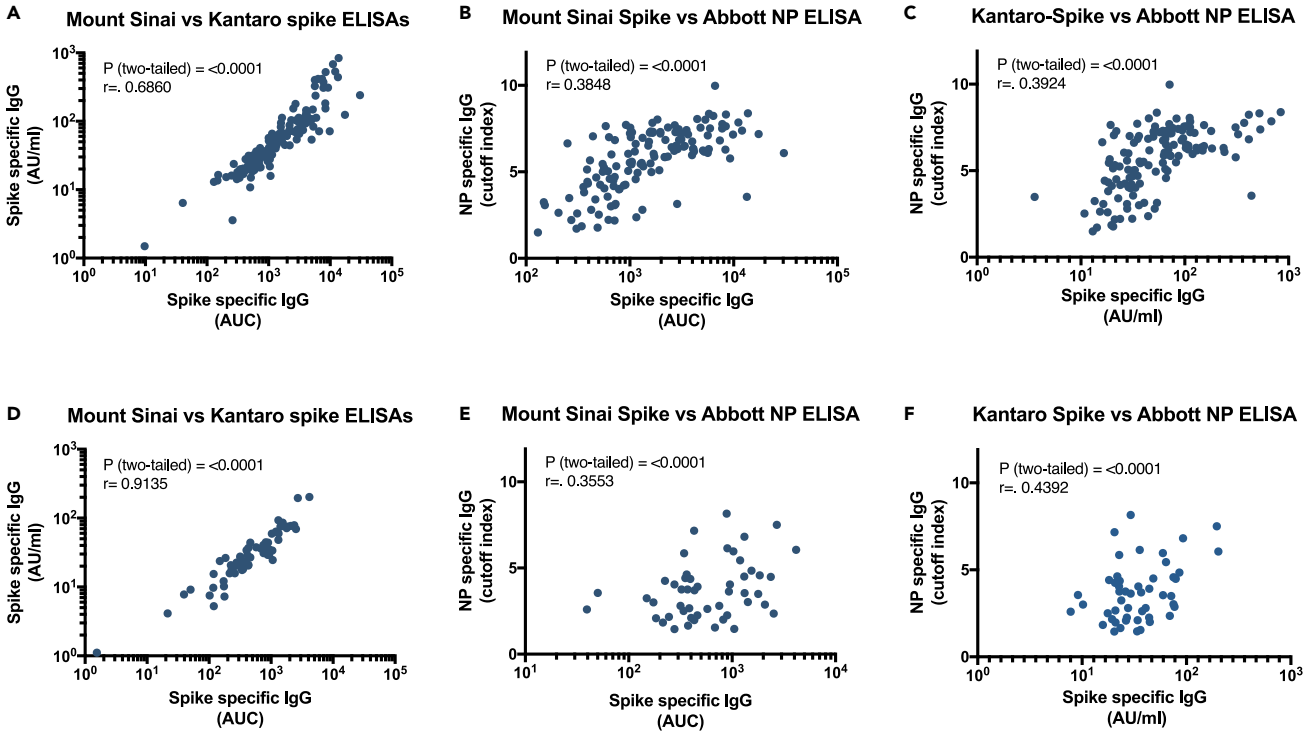

Figure 4. Correlation analysis of antibody levels (spike-reactive vs. NP-reactive) among the different serology assays

Serum samples were assessed for antibodies against spike using a research grade ELISA from Mount Sinai, against spike using a commercial ELISA from Kantaro Biosciences or against NP using the Abbott-Architect CMIA. Correlation of antibody levels (spike reactive) among the different assays using serum samples obtained in May 2020 (first time point) is shown (A-C). Correlation of antibody levels (spike reactive) among the different assays using serum samples obtained between August-October 2020 (second time point) is shown (D-F). Correlation analysis between Mount Sinai spike and Kantaro spike ELISAs (A, D); between Mount Sinai spike and Abbott Architect NP CMIAs (B, E); and between Kantaro spike ELISAs and Abbott Architect NP CMIAs (C, F) are shown. Pearson correlation was used. Significance and correlation coefficient are shown.

SARS-CoV-2 mRNA vaccines. The expectation was that the RBD/S-based assays would detect a signal, while the NP-based assay would not. Indeed, we measured high titers using the S-based assay platforms (the Mount Sinai and Kantaro assays), but the samples produced no signal in the NP-based assay (Figures 5A–5C). Of note, the S titers measured in the Mount Sinai and Kantaro assay correlated very well (Figure 5D).

DISCUSSION

While antibody responses to acute SARS-CoV-2 infection are relatively well understood, less data are available regarding antibody kinetics over longer time frames against different viral antigens. We determined seroprevalence and antibody titers in SARS-CoV-2-infected individuals at two time points (1–2 months (phase 1) and 3–4 months postinfection (phase 2)) using three different assays. One assay, the Mount Sinai ELISA, is a laboratory-developed assay that uses an initial ELISA at a single serum dilution against the RBD followed by a confirmation and titration against the full-length S protein. The second assay tested, the Kantaro SeroKlir assay, is based on the same principle, but commercially available. The third assay, the Abbott Architect, targets the NP and is a CMIA.

There was high concordance among the three assays with respect to seroprevalence during phase 1. However, the titers only correlated well for the two S-based assays. During phase 2, the two S-based assays identified all (Mount Sinai Research grade) or the vast majority (Kantaro) of previously seropositive individuals as seropositive, while the NP-based assay (Abbott) failed to detect a signal above the cutoff in approximately 30% of previously positive individuals. These findings mirror similar results recently published, suggesting that the NP antibody response exhibits a faster decline (Grandjean et al., 2021; Ripperger et al., 2020; Stromer et al., 2020). However, this could also be a reflection of a high cutoff required to ensure

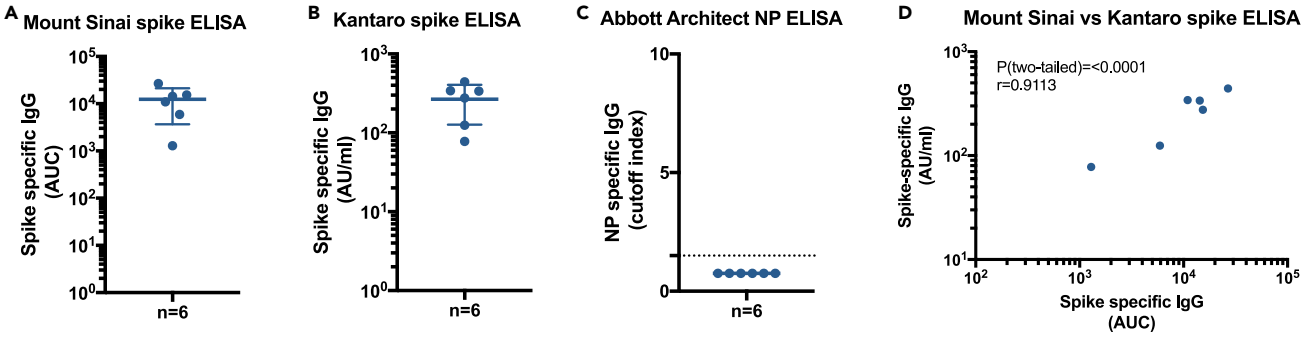

Figure 5. Post SARS-CoV-2 mRNA vaccination serum titers as measured in three different assays

Serum samples of individuals who had received two doses of SARS-CoV-2 mRNA vaccines were assessed for antibodies against RBD-spike using a research grade ELISA from Mount Sinai (A), against RBD-spike using a commercial ELISA from Kantaro Biosciences (B) or against NP using the Abbott-Architect CMIA (C). Correlation of antibody levels (spike reactive) between the Mount Sinai ELISA and the commercial ELISA from Kantaro Biosciences assays using the same serum samples (D). In (A) and (B), mean and standard deviation are indicated. For (D), Pearson correlation was used, significance and correlation coefficient are shown.

high specificity for SARS-CoV-2 in the NP-based assay. Importantly, and as expected, since no NP is included in the FDA-EUA-approved vaccines used in the US, only the S-based assays were able to detect antibodies induced by SARS-CoV-2 mRNA vaccines (Krammer, 2020). Our data highlight the need to understand assay performance before a specific assay is used to study specific aspects of SARS-CoV-2 immunity. All three assays are very valuable to assess seroconversion shortly after infection, but only the two S-based assays were reliable months after recovery. Similarly, only S-based assays are fit for measuring vaccine-induced antibodies, e.g. to determine if vaccination triggered immune responses.

Limitations of the study

We used samples from HCWs to perform a longitudinal analysis of the antibody responses using three distinct serological assays. We detected good correlations between the S-based assays, but modest correlations between the NP-based assay and S-based assays. The seroprevalence as measured by the NP-based assay significantly declined, given that the Abbott test failed to detect a signal above the cutoff in approximately 30% of previously positive individuals. Although these findings mirror similar results already published – which likely indicate a faster decline of NP-specific antibodies – the current work does not exclude the possibility of this effect being a reflection of the high cutoff required to ensure high specificity for SARS-CoV-2 in the NP-based assay. Further studies are needed to explore these findings.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHODS DETAILS
  - Research grade ELISAs
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- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.102937.

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AUTHOR CONTRIBUTIONS

F.K. and V.M. conceptualized study; J.M.C., D.R.M., M.A.S., and G.S. performed experiments; J.M.C. analyzed data; J.M.C., V.M., and F.K. administered the project; F.K., V.M., and V.S. provided resources; J.M.C. and F.K. wrote original draft. All authors reviewed, edited and approved the final version of the manuscript, and have had access to the raw data. J.M.C., V.M., and F.K. can verify the accuracy of the raw data for the study.

DECLARATION OF INTERESTS

The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2 serological assays (the "Serology Assays") and NDV-based SARS-CoV-2 vaccines which list Florian Krammer (the "Serology Assays", vaccines) and Viviana Simon ("Serology Assays") as co-inventors. The foundational "Serology Assay" intellectual property (IP) was licensed by the Icahn School of Medicine at Mount Sinai to commercial entities including Kantaro Biosciences, a company in which Mount Sinai has a financial interest. Kantaro manufactures and markets serologic tests based on the Mount Sinai IP. It is anticipated that the medical school will receive payments related to commercialization of the "Serology Assay" IP and, as faculty inventors, Drs. Krammer and Simon will be entitled to a portion of these payments.

Florian Krammer consulted for Merck, Curevac and Pfizer in the past (before 2020) and is currently consulting for Pfizer, Seqirus and Avimex. The Krammer laboratory is collaborating with Pfizer on animal models of SARS-CoV-2.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-human IgG (Fab-specific) horseradish peroxidase antibody	Sigma-Aldrich	Cat# A0293, RRID:AB_257875
Biological samples		
Human serum samples from healthcare workers	New York City Public Hospital in the South Bronx (NYC Health + Hospitals/Lincoln)	https://www.nychealthandhospitals.org/hospitals/
Human serum samples from subjects receiving the Pfizer mRNA vaccine	Personalized Virology Initiative (PVI) at the Icahn School of Medicine at Mount Sinai	https://labs.icahn.mssm.edu/simonlab/
Chemicals, peptides, and recombinant proteins		
Recombinant receptor binding domain (RBD)	Krammer laboratory at the Icahn School of Medicine at Mount Sinai	https://labs.icahn.mssm.edu/krammerlab/reagents/
Recombinant spike (S)	Krammer laboratory at the Icahn School of Medicine at Mount Sinai	https://labs.icahn.mssm.edu/krammerlab/reagents/
SIGMAFAST™ OPD (o-Phenylenediamine dihydrochloride)	Sigma-Aldrich	Cat# P9187
3-molar hydrochloric acid	Thermo Fisher Scientific	Cat# S25856
Tween-20	Fisher Bioreagents	Cat# BP337-100
Non-Fat Dry Milk Omniblok	American Bio	Cat# AB10109-01000
Critical commercial assays		
Kantaro Quantitative SARS-CoV-2 IgG Antibody Kit. COVID-SeroKlir	Kantaro Biosciences	Cat# COV219
Abbott Architect CMIA	Abbott Laboratories	Cat# 06R86
Software and algorithms		
Prism 7	GraphPad	https://www.graphpad.com

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Florian Krammer (florian.krammer@mssm.edu).

Materials availability

Recombinant proteins generated in this study and plasmids encoding for recombinant proteins can be requested from the Krammer laboratory (https://labs.icahn.mssm.edu/krammerlab/reagents/).

Data and code availability

- All data reported in this paper and codes will be shared by the lead contact upon request
- This paper does not report original code
- Any additional information required to reanalyze the data reported in this paper is available upon request from the lead contact

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The samples used for the longitudinal study, were part of a cross sectional cohort of healthcare workers (HCWs) of the New York City Public Hospital in the South Bronx (NYC Health + Hospitals/Lincoln). This

study was approved by the Institutional Review Board (IRB#20-009). Samples were collected in two phases: Phase 1 samples were obtained in May 2020 and Phase 2 samples were collected from August to October 2020. Informed consent was obtained prior to Phase 1 sample collection. The characteristics of the participants that completed phase 1 and 2 are: average age of 44.6±12.4 years, 65 females (36.5%), 113 males (63.5%), 54 (30.3%) of them had comorbidities (hypertension, diabetes, asthma). 82 of the participants were physicians (46.1%), followed by 58 hospital staff (32.6%) and 29 nurses (16.3%). 104 (58.4%) of the participants were symptomatic with SARS-CoV-2 infection prior to the study period.

Samples from study participants receiving the Pfizer mRNA vaccine were obtained from IRB approved longitudinal observation studies (IRB-16-00791; IRB-20-03374) conducted by the Personalized Virology Initiative (PVI) at the Icahn School of Medicine at Mount Sinai. All participants signed informed consents prior to data and sample collection. All serum samples were coded upon collection and analyzed in a blinded manner in the Krammer laboratory. Number of samples from naturally infected and vaccinated individuals was dependent on the availability of specimens at the time and period of collection.

METHODS DETAILS

Research grade ELISAs

Detection of receptor binding domain (RBD) and full-length spike (S) antibodies in plasma was performed with a research-grade two-step ELISA developed at Mount Sinai closely resembling an assay used in Mount Sinai's CLIA-certified Clinical Pathology Laboratory, which received FDA Emergency Use Authorization in April 2020 (Amanat et al., 2020; Stadlbauer et al., 2020a). The research grade assay has 95% sensitivity and 100% specificity (Stadlbauer et al., 2020b). Before performing the ELISA, samples were heat-inactivated for 1h at 56°C. Briefly, for RBD screening, 96-well plates (Thermo Fisher) were coated with 50ul/well of phosphate-buffered saline (PBS; Gibco) containing 2μg/ml of recombinant RBD protein and incubated overnight at 4°C. Plates were washed three times with PBS containing 0.1% Tween-20 (PBS-T; Fisher Bioreagents) using an automated plate washer (BioTek). For blocking, 200μl/well of PBS-T containing 3% (w/v) of milk powder (American Bio) were added and plates were incubated for 1 h at room temperature. Plasma samples were diluted (1:50) in PBS-T containing 1% milk powder. Blocking solution was removed and dilutions of samples were added. After a 2-hour incubation, plates were washed three times with PBS-T and 50μl/well of anti-human IgG (Fab-specific) horseradish peroxidase antibody (Sigma, A0293) diluted 1:3,000 in PBS-T 1% milk powder were added. Plates were incubated for 1 h at room temperature, followed by three times washing with PBS-T and addition of developing solution (100μl/well, Sigmafast *o*-phenylenediamine dihydrochloride (Sigma)). The reaction was led to proceed for 10 min, and stopped using 50μl/well of 3-molar hydrochloric acid (Thermo Fisher Scientific). Optical density was measured at 490 nm using an automated plate reader (BioTek). Samples with an OD_{490nm} above 0.15 (cut-off value) were considered as presumptive positives and were further tested in the confirmatory ELISA using the full-length recombinant spike protein.

Briefly, to perform the confirmatory ELISAs, plates were coated and blocked as described above, but using full-length spike protein for coating. Presumptive positive plasma samples were serially diluted (1:3) in 1%-milk prepared in PBS-T, starting at an initial dilution of 1:80. Serial dilutions (100μl/well) were added to the plates, followed by a 2-hour incubation at room temperature. The remaining steps were performed as described above. Data was analyzed using GraphPad Prism 7. Samples with an OD_{490nm} above 0.15 (cut-off value) at a 1:80 plasma dilution were considered positive. Samples with an OD_{490nm} above 0.15 at the last dilution were further diluted (1:2160 initially) and re-tested. Only samples positive in both steps of the assay were considered positive.

Kantaro ELISAs

ELISAs to detect antibodies in plasma against the receptor binding domain (RBD) and the full-length spike (S) based on the commercial Kantaro Quantitative SARS-CoV-2 IgG Antibody Kit (COVID-SeroKlir, Kantaro Biosciences) were used. This assay has approximately 99% positive percent agreement and approximately 99% negative percent agreement in PCR+ subjects 15 days post-symptom onset (https://resources.rndsystems.com/pdfs/datasheets/cov219.pdf?v=20210525&_ga=2.12000950.307497989.1621962942-1278575996.1621962942). The assay was performed according to manufacturer's instructions except for additional serum dilution steps in highly reactive individuals. All reagents and microplates were included with the commercial kit. Briefly, for qualitative RBD ELISAs, samples were diluted in sample buffer (1:100) using 96-well microtitre plates, and 100μl/well of pre-diluted samples were transferred to the RBD

pre-coated microplates. Positive and negative controls were added to every plate. Samples were incubated for 2 hours at room temperature, followed by removal of plasma dilutions and washing three times with wash buffer. RBD conjugate was diluted in conjugate buffer and 100µl/well were added to the plates. After 1h incubation, the conjugate was removed and plates were washed three times with wash buffer. Substrate solution was added (100µl/well) and after 20min incubation, 100µl/well of stop solution were added. Samples were read at OD_{450nm} and at OD_{570nm} for wavelength correction. The cutoff index (CI) was calculated by dividing the corrected OD of the clinical sample/corrected OD of RBD positive control. Samples with a CI above 0.7 were considered as presumptive positives and were further tested in the confirmatory quantitative ELISA based on the full-length recombinant spike protein.

For quantitative spike ELISAs, presumptive positive plasma samples were diluted (1:200) in sample buffer. Dilutions were added in duplicate to the pre-coated microplates. Low, medium and high controls, as well as spike calibrators used to generate a standard curve, were added to every microtiter plate. After 2h incubation at room temperature, the remaining steps were performed as described above. Data was analyzed using GraphPad Prism 7. The concentration of spike-reactive antibodies was calculated using a four parameter logistic (4-PL) curve fit. Samples exceeding the range of the standard curve were further diluted (1:5400) and re-tested. Only samples positive in both steps of the assay were considered positive.

Abbott Architect CMIA

The Architect test (Abbott Laboratories) consists of an automated, two-step, qualitative CMIA for qualitatively detecting IgG against the nucleoprotein (N) antigen from SARS-CoV-2. This test has a reported sensitivity of 100% (CI 95.8–100%) and specificity of 99.6 (CI 99–99.9%) 14 days after symptom onset. The assay was performed according to manufacturer's instructions. All reagents were included with the kit. Briefly, sample, SARS-CoV-2 antigen coated paramagnetic microparticles, and assay diluent were combined and incubated. The IgG antibodies to SARS-CoV-2 present in the sample bind to the SARS-CoV-2 antigen coated microparticles. The mixture is washed. Anti-human IgG acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added. The resulting chemiluminescent reaction is measured as relative light units (RLU). There is a direct relationship between the amount of IgG antibodies to SARS-CoV-2 in the sample and the RLU detected by the system optics. This relationship is reflected in the calculated index (S/C). The presence or absence of IgG antibodies to SARS-CoV-2 in the sample is determined by comparing the chemiluminescent RLU in the reaction to the calibrator RLU.

QUANTIFICATION AND STATISTICAL ANALYSIS

Correlations of antibody levels in the different assays were calculated using a standard Pearson's correlation. Correlation coefficients (r) are presented. A paired t-test was used for comparison of phase 1 and phase 2 antibody levels. All adjusted p values of <0.05 were considered statistically significant. Statistical analyses were performed using Prism 7 (GraphPad, USA). Details are described in every figure legend.