COVID-19 mRNA Vaccines May Cause False Reactivity in Some Serologic Laboratory Tests, Including Rapid Plasma Reagin Tests

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ABSTRACT

Objectives: Acute viral infections and some vaccines have been shown to increase false positivity in serologic assays. We assessed if the messenger RNA coronavirus disease 2019 (COVID-19) vaccines could cause false reactivity in common serologic assays in a pilot longitudinal cohort.

Methods: Thirty-eight participants with sera available prevaccination, 2 weeks after each vaccine dose, and monthly thereafter for up to 5 months were tested for common infectious disease serologies and antiphospholipid syndrome (APS) serology markers on the BioPlex 2200, Sure-Vue rapid plasma reagin (RPR), and Macro-Vue RPR. Twenty-two participants received the Moderna vaccine and 16 received the Pfizer vaccine.

Results: Most assays had no change in reactivity over the course of the sample draws, including APS markers. Epstein-Barr virus immunoglobulin G (IgG), measles IgG, and rubella immunoglobulin M all had possible false reactivity in one to two participants. RPR tests demonstrated false reactivity, with baseline nonreactive participant samples becoming reactive following vaccination. There were more false reactive participants (7/38) in the BioPlex RPR than in the Sure-Vue (2/38) and Macro-Vue (1/38) tests. All falsely reactive RPR tests were in participants who received the Moderna vaccine.

Conclusions: Serologic assays with results that do not fit the clinical picture following COVID-19 vaccination should be repeated. Effects of false reactivity can last more than 5 months in some assays. In particular, RPR is susceptible to false reactivity, and there is variability among assays. Larger longitudinal studies are needed to determine the incidence and window of false reactivity.

KEY POINTS

- False reactivity is noted in some serologic assays following vaccination with severe acute respiratory syndrome coronavirus 2 messenger RNA vaccines in a longitudinal cohort.
- We observed false reactivity in three rapid plasma reagin assays; it was more likely to occur in those receiving the Moderna vaccine.
- On the basis of our findings, we suggest that serologic assays with results not fitting the clinical picture following coronavirus disease 2019 vaccination should be repeated.

KEY WORDS

RPR; COVID-19 vaccine; Serology

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INTRODUCTION

The introduction of vaccines to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been a turning point in the coronavirus disease 2019 (COVID-19) pandemic, with the largest known scale of vaccine creation and distribution in history.[1] Immune responses created to vaccination with messenger RNA (mRNA) SARS-CoV-2 vaccines include B- and T-cell–specific responses to the mRNA aided by lipid nanoparticles functioning as

adjuvants.[2] In addition, work on previous vaccines has demonstrated that transient interference in serologic assays after vaccination may occur.[3] Specifically, following smallpox vaccination, several studies since the 1940s have found biologically false-positive serologic results in nontreponemal tests.[4-7] Acute viral infections such as hepatitis C virus, Epstein-Barr virus (EBV), cytomegalovirus (CMV), hepatitis B virus, and SARS-CoV-2 have been implicated in falsely reactive serologies for a host of infectious disease (ID) and autoimmune markers.[8-10] We sought to determine if SARS-CoV-2 vaccination could cause false reactivity in standard ID assays.

MATERIALS AND METHODS

Cohort

Serum specimens were previously described.[11] This work was carried out under the auspices of the University of Pittsburgh Institutional Review Board (#21060107). Inclusion criteria from the original cohort required a prevaccine baseline sample, samples to 3 or more months following a second vaccine, and sufficient sample volume. Time from vaccination, sex, age, and vaccine type received (Pfizer or Moderna) were available. Participants (n = 38) with six to eight specimens each were included. Specimens were drawn up to 1 week prior to the first vaccination (time point 0), 2 weeks following each dose (time points 1 and 2), and monthly after the second dose (time points 3-7).

Assays

All testing was performed within the College of American Pathologists-accredited University of Pittsburgh Medical Center clinical laboratories by certified technologists, medical directors, and pathology residents. Sure-Vue rapid plasma reagin (RPR) test (Thermo Fisher Scientific) and Macro-Vue RPR test (Becton Dickinson) are RPR card tests. All other tests listed in TABLE 1 were run on the BioPlex 2200 (Bio-Rad). All testing was performed per manufacturer recommendations. Calibrations and quality control were run in compliance with clinical laboratory regulations. Equivocal results were classified as reactive or nonreactive based on the prior time point. If all specimens from a participant were equivocal, they were classified as nonreactive TABLE 1.

RESULTS

To evaluate the possibility of SARS-CoV-2 mRNA vaccination causing transient interference with serologic assays, we tested longitudinal sera collected pre- and postvaccination.[11] We included 38 participants: 27 (71%) were women, aged 19 to 70 years (mean, 50 years); 16 of 38 received the Pfizer vaccine and 22 of 38 received the Moderna vaccine.

Specimens were assessed for common ID serologic markers as well as antiphospholipid syndrome (APS) markers, as we were concerned that immune responses against the lipid carrier could cross-react TABLE 1. We found no change in reactivity for APS markers anticardiolipin and anti–β2-glycoprotein 1 (B2GP1) for immunoglobulin G (IgG), immunoglobulin A, or immunoglobulin M (IgM). One participant showed baseline reactivity for anticardiolipin IgG, and two participants had baseline anticardiolipin and B2GP1 IgM reactivity.

Testing for syphilis involves treponemal and nontreponemal tests, with the RPR being among the most commonly used nontreponemal tests. Treponemal testing for total syphilis antibodies demonstrated no change from baseline (nonreactive for all participants), but RPR demonstrated nonreactive to reactive changes (NR to R) for seven participants in the Bio-Rad RPR assay FIGURE 1A, with two of seven demonstrating continued reactivity at 5 months after second dose. All seven participants received the Moderna vaccine, and the association was statistically significant (Fisher exact test, $P = .0092$). While six of seven participants demonstrated low index values of reactivity FIGURE 1B, one of the NR to R participants and a low-level reactive baseline participant both demonstrated a sharp increase in index value following the second dose of the Moderna vaccine FIGURE 1B. To test whether this trend in RPR reactivity was present in other manual RPR assays, we tested all available samples on Sure-Vue and Macro-Vue RPR card tests. We found Sure-Vue to have two NR to R participants, who were also NR to R for the Bio-Rad assay. Macro-Vue had one NR to R participant, who was NR to R for other RPR assays. Bio-Rad RPR demonstrated reactivity across multiple time points for some participants, while the manual tests had a single time point that was weakly reactive. We did not have sufficient specimen volume to titrate reactive RPR samples.

EBV IgM, herpes simplex virus (HSV) type 1, HSV type 2, Lyme total antibodies, rubella IgG, mumps IgG, varicella zoster IgG, toxoplasma IgG and IgM, CMV IgM, and all human immunodeficiency virus (HIV) markers demonstrated no change in reactivity from baseline for any participant TABLE 1. Three participants had a single assay reactive at baseline, and at least one subsequent sample was nonreactive (R to NR; measles IgG, CMV IgG, Bio-Rad RPR). For these assays, the reactive samples had an index value of 1.3 or less (≥1.0 positive), and all received the Moderna vaccine.

Several assays had one to two participants who were nonreactive at baseline and had at least one subsequently reactive sample (NR to R). EBV early antigen IgG had one NR to R participant who had transient reactivity following the Pfizer vaccine; however, their prevaccine sample could not be tested on this assay due to insufficient volume; the 2-week post–first dose sample was nonreactive, with subsequent samples becoming equivocal, reactive, and declining back to equivocal at 4 months after the second dose. This participant was also reactive for EBV capsid and nuclear IgG. A second NR to R participant was nonreactive until month 2 after the second dose of Moderna, when they became equivocal for 2 months, followed by 2 months of reactivity. This participant was also reactive for EBV capsid and nuclear IgG. The EBV nuclear antigen IgG assay also had one NR to R participant who became equivocal at months 2 to 4 after the second dose of Pfizer and reactive during month 5. This participant was also reactive for EBV capsid IgG. Measles IgG and rubella IgM each had one NR to R participant with reactivity who subsequently declined to either nonreactive or equivocal, respectively,

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TABLE 1 Changes in Serologic Test Reactivity After Coronavirus Disease 2019 Vaccination

Assay	NR→NR, No.	NR→R, No.	R→R, No.	R→NR, No.	Total No. of Participants
B2GP1 IgA	35	0	0	0	35
Cardiolipin IgA	35	0	0	0	35
B2GP1 IgG	35	0	0	0	35
Cardiolipin IgG	34	0	1	0	35
B2GP1 IgM	24	0	2	0	26
Cardiolipin IgM	31	0	2	0	33
EBV capsid Ag IgG	3	0	34	0	37
EBV early Ag IgG	31	2	4	0	37
EBV nuclear Ag IgG	4	1	32	0	37
EBV capsid Ag IgM	35	0	2	0	37
EBV heterophile IgM	37	0	0	0	37
HSV-1 IgG	18	0	20	0	38
HSV-2 IgG	34	0	4	0	38
Lyme total Ab	30	0	0	0	30
Rubella IgG	3[a]	0	35	0	38
Rubella IgM	36	1	0	0	37
Mumps IgG	4	0	33	0	37
Measles IgG	8[b]	1	27	1	37
Varicella zoster IgG	2	0	35	0	37
Toxoplasma IgG	36	0	2	0	38
Toxoplasma IgM	36	0	1	0	37
CMV IgG	19	0	18	1	38
CMV IgM	36	0	1	0	37
HIV Ag	38	0	0	0	38
HIV-1 Ab	38	0	0	0	38
HIV-2 Ab	38	0	0	0	38
Syphilis total Ab	38	0	0	0	38
Bio-Rad RPR	29	7	1	1	38
Sure-Vue RPR	36	2	0	0	38
Macro-Vue RPR	37	1	0	0	38

Ab, antibody; Ag, antigen; B2GP1, β2-glycoprotein 1; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; NR, nonreactive; R, reactive, RPR, rapid plasma reagin.
[a]Contains samples equivocal for all time points (n = 1).
[b]Contains samples equivocal for all time points (n = 3).

following Moderna doses. Of the five NR to R participants, none demonstrated this trend on more than a single assay; two received the Pfizer vaccine.

DISCUSSION

With worldwide vaccination against SARS-CoV-2 under way, it is imperative to consider possible interferences in clinical assays that may result from vaccination. Here we find, as demonstrated for other vaccines,[3,5] that there is a possibility of interference in serologic assays following vaccination for SARS-CoV-2, particularly in RPR assays.

Several trends were observed on postvaccination follow-up, and larger cohorts are needed to determine the effect of these trends on the posttest probabilities of serologic assays. All three participants who demonstrated reactivity on an assay at baseline but were subsequently nonreactive for at least one sample had index values close to the cutoff for positivity, indicating that changes from reactive to nonreactive are within the imprecision of the assay, and repeated testing will show results fluctuating between reactive and nonreactive. This fluctuation was seen in two of three samples, while a steady decline was seen in the CMV IgG participant from 1.3 to 0.8 index value, potentially consistent with a naturally occurring waning of IgG.

Of NR to R participants, two of five not associated with RPR changes appeared to fit the assay imprecision with fluctuations between reactivity and nonreactivity noted (measles IgG and EBV nuclear antigen IgG). The three remaining participants had low index value reactivity, which steadily increased, with two subsequently decreasing in the same stepwise manner over time (EBV early antigen IgG, rubella IgM). This may indicate that some participants have SARS-CoV-2 vaccine-related reactivity in these assays.

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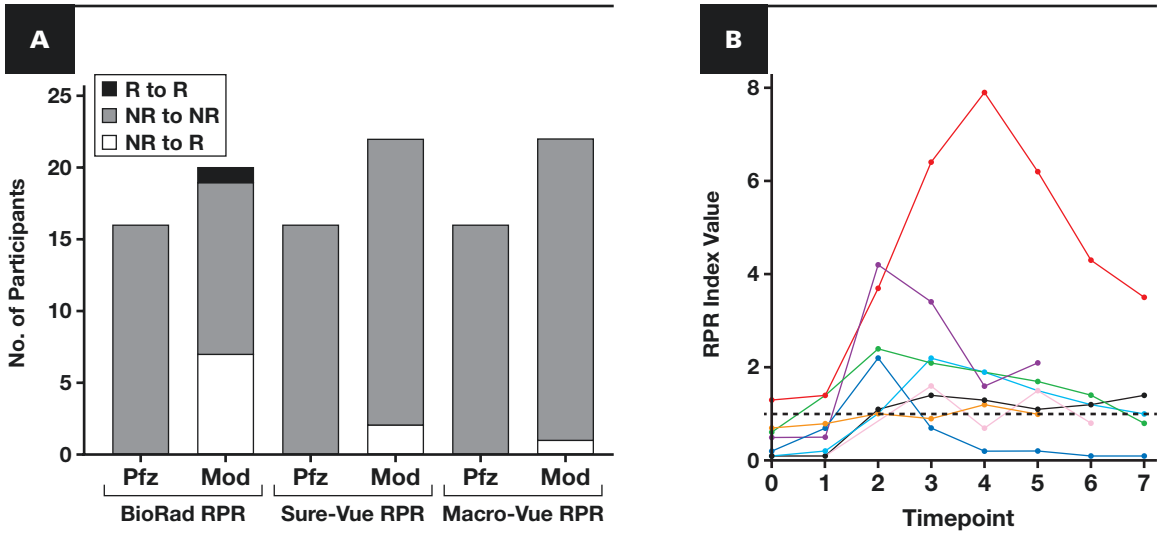

FIGURE 1 Coronavirus disease 2019 messenger RNA vaccines may cause false reactivity in rapid plasma reagin (RPR) tests. **A**, Stacked bar chart demonstrating the number of reactive at baseline to subsequently reactive (R to R), nonreactive at baseline to subsequently nonreactive (NR to NR), and nonreactive at baseline to reactive following vaccination (NR to R) participants in Pfizer (Pfz) and Moderna (Mod) vaccine groups using the Bio-Rad RPR test, Sure-Vue RPR test, and Macro-Vue RPR test. **B**, Evolution of RPR index value over time from baseline (time point 0) to 5 months after the second vaccination (time point 7) for all NR to R participants and the only R to R participant by the Bio-Rad RPR test. Details regarding the exact measurement time points are provided in the Materials and Methods.

It appears there is more false reactivity in IgG assays than in IgM assays, which may be associated with the antibody response to the second vaccine dose being predominantly IgG based.[12]

The number of NR to R participants in the RPR assays is statistically significantly associated with the Moderna vaccine. The lipid nanoparticle (LNP) carriers in each vaccine are composed of four types of lipids: ionizable lipid, phospholipid, cholesterol, and a pegylated lipid. The ionizable lipid and pegylated lipid components are unique to each vaccine.[13,14] It is unclear if the different mRNA dosing correlates to a different LNP dose as well, but prior work has demonstrated that antibodies and other immune responses may be induced against some components of LNPs.[2,15] Differences in LNP composition or dose are a possible explanation for the paucity of NR to R participants in the Pfizer group. It is possible that with a larger cohort some Pfizer vaccinees could demonstrate reactivity. Interestingly, the Bio-Rad RPR assay appears to be more sensitive to potential vaccine-related interference than Sure-Vue and Macro-Vue manual RPR card tests. This may not be surprising given the assay methods, in which the Bio-Rad assay uses fluorescence detection compared with a calculated index value threshold, and the manual card tests use visual flocculation interpreted by technologists.

In our cohort, all participants were treponemal negative, but current recommendations are that high-risk patients who are HIV positive be screened for syphilis quarterly,[16] and many of these patients are treponemal positive. A reactive RPR in this patient population will initiate treatment for reinfection of syphilis, and currently there would be no reliable way to differentiate reinfection from vaccine-induced false reactivity. It appears that manual RPR card tests may be less susceptible to this interference and may be a recommended confirmation for RPR tests with higher incidence of interference. Larger cohorts with a longitudinal study of participants with RPR reactivity due to nonsyphilis conditions and previously syphilis-infected participants are needed to determine the likely incidence and define the testing window for possible false reactivity by commonly used RPR tests.

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