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case Report

Covid-19 Vaccine-Induced Chronic False Positive Rapid Plasma Reagin (Rpr) Tests.

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Abstract

People who receive frequent coronavirus disease 2019 (COVID-19) vaccinations may show persistent reactive plasmin reagin (RPR) responses, as there have been reports of false positive RPR reactivity after a COVID-19 vaccination. Here, we aimed to examine the potential for chronic false RPR reactivity in a longitudinal cohort caused by repeated mRNA COVID-19 vaccinations. Manual RPR card assays were used to screen for RPR reactivity in 119 participants in a longitudinal SARS-CoV-2 cohort study that was approved by the IRB (#20201026). Additional testing was performed on samples that produced reactive results, such as confirmatory fluorescent treponemal antibody (FTA-ABS) testing, anti-nuclear antibody (ANA) testing, and follow-up RPR screening at additional timepoints. Medical histories were gathered.Following booster vaccination, we saw (n = 2) screen-positive RPR results (1.7% [2/119]), with two individuals displaying persistent, vaccine-induced RPR reactivity for up to nine months. Both individuals tested negative for ANA. Clinicians must be aware of the possibility that COVID-19 vaccines may interfere immunologicly with common infectious disease tests, such as RPR testing. Detailed medical histories and clinical contexts, including recent vaccination, should be reviewed prior to proceeding with distressing and invasive workups.

Keywords : *COVID-19; RPR; ID assay.*

INTRODUCTION

Timely detection and diagnosis are essential because syphilis infections brought on by the spirochete bacterium Treponema pallidum can have serious neurological and cardiovascular consequences. When beef-derived cardiolipin-lecithin-cholesterol antigen is present, the reagins—antibodies, usually IgE, produced during infection—agglutinate in the rapid plasma reagin (RPR) test, a straightforward card assay first presented by Portnoy and associates in 1962 [1]. Numerous studies on the RPR card test for syphilis attest to the test's quickness, ease of use, and sufficient sensitivity and specificity [2]. However, despite its well-described utility, some studies have reported that non-syphilis diseases and conditions can induce biological false positives.It is unknown what fundamental mechanism leads to a biological false positive, which is an abnormal antibody response to cardiolipin that is not caused by a T. pallidum infection.

According to one study, a lecithin linked to the globulin fraction of human serum inhibits the globulin that causes biological false positive reactions, which makes it distinct from the syphilis antibody. Therefore, it is hypothesized that auto-immunization by lipids released during exaggerated tissue breakdown is the cause of the false positive antibody response [3]. On the other hand, Rusnak et al. hypothesized that IgM antibodies might be the cause of false positive tests in HIV-positive individuals. Their results, while not statistically significant, showed that biological false positive tests were associated with patients who had higher IgM levels and did not seem to correlate with serum IgG or IgA levels or anticardiolipin antibody levels [4].However, it should be mentioned that the research currently available indicates that the prozone effect may cause people with HIV to have abnormal syphilis serological test results. When investigating the underlying mechanism behind false positive testing for syphilis, the results of Rusnak et al.'s study involving HIV-positive subjects might not be fully generalizable to the general population, despite the fact that this is an intriguing phenomenon that occurs in the context of false positive serological syphilis testing [5]. Among other populations, this one needs more research. Although the occurrence of false RPR positivity has been extensively documented in the literature, little is known about

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the underlying mechanism. For instance, 206 (0.32%) of the 63,765 blood samples tested in a study conducted between May 2008 and February 2013 at Zhongshan Hospital in the Medical College of Xiamen University experienced biological false positive reactions in RPR serological testing. Additionally, this study described the associations between biological false positive tests and any conditions or diseases in these patients. They discovered that these biological false positive reactions were linked to 60 specific diseases as well as 17 categories of diseases (such as genitourinary and respiratory diseases).False labor, megaloblastic anemias, aplastic anemias, redundant prepuce, congenital heart malformations, and salpingitis were among the 60 conditions linked to false positive RPR reactivity [6]. Diseases or conditions like the hepatitis C virus (especially those with elevated eosinophil counts) [7], leprosy (especially the lepromatous form) [2], malaria, respiratory infections, infectious mononucleosis, undulant fever, measles, vaccinia, and pregnancy [3] are additional causes of non-syphilis-induced RPR-positive tests documented throughout the literature. The most recent example of false positive RPR reactivity was found in participant samples that were initially non-reactive but turned reactive after receiving the COVID-19 vaccine [8].According to a recent study by Korentzelos et al., the kind of RPR test employed may affect false positives. Of the 38 participants in their cohort, 7 (18.4%) tested falsely reactive on the BioPlex RPR, whereas 2 (5.3%) and 1 (2.6%) tested falsely reactive on the Sure-Vue and Macro-Vue tests, respectively.

RESOURCES AND PROCEDURES

In order to account for cross-reactivity coinciding with the peak [12] post-vaccination SARS-CoV-2 antibody response, participants (n = 119) in our IRB-approved (#20201026), longitudinal SARS-CoV-2 cohort study were screened for RPR positivity one month after receiving Dose 3 (n = 94) or Dose 4 (n = 25) of a SARS-CoV-2 booster vaccine. Those whose post-vaccine visits took place outside of the intended window [20–50 days post boost] were not included.Following the guidelines provided by the manufacturer (Arlington Scientific, Inc® (ASI), Springville, UT, USA), manual RPR card testing was carried out. Reactive samples were serially diluted to reach endpoint titers of no more than 1:16. The highest dilution at which observable aggregation took place was thought to be endpoint titers. FTA-ABS testing was used to confirm all reactive results (Labcorp, Tampa, FL, USA). Screen-positive participants underwent screening at additional timepoints, including pre- and post-vaccines or boosters, to examine the potential relationships between antibody magnitude and the persistence of RPR reactivity. Pre-Dose 2 timepoints were analyzed if available.

A well-described assay created by the Icahn School of Medicine at Mount Sinai was used for SARS-CoV-2 ELISAs [13,14]. In short, wild-type Wuhan-Hu-1 SARS-CoV-2 spike protein (2 μg/mL) solution was applied to 96-well plates at 4 °C, and the plates were then incubated for the entire night. After blocking the plates with 3% non-fat milk made in PBS with 0.1% Tween 20 (PBST), they were allowed to sit at room temperature for one hour. Following blocking, heat-inactivated serum samples in serial dilutions were added to the plates, and they were then allowed to sit at room temperature for two hours. After three rounds of washing with 0.1% PBST, 50 μL of goat anti-human IgG–horseradish peroxidase (HRP) conjugated secondary antibody was added at a 1:3000 dilution, and the plates were incubated for one hour. After washing the plates, 100 μL of SIGMAFAST OPD (o-phenylenediamine dihydrochloride) solution was added to each well for 10 minutes. Next, 50 μL of 3 M hydrochloric acid was added to each well to halt the reaction. To measure the optical density at 490 nm (OD490), a Synergy 4 plate reader (BioTek [Santa Clara, CA, USA]) was used. Discrete titers were reported in the following values: 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200, 1:6400, 1:12,800, 1:25,600, 1:51,200, 1:102,400, and 1:204,800. The background value was set at an OD490 of 0.15. At 1:100, the detection limit was established.

CASE DESCRIPTION

Two screened persons (1.7% [2/119]) were discovered to be RPR-positive after booster (i.e., third or fourth) doses (Table 1). The first person, Participant 1, was a 72-year-old woman who identified as White and NonHispanic, was heterosexual, and did not work in healthcare. Early in 2021, she received two doses of Moderna, and in July and April of 2022, she received booster doses. Her fourth dose, the first time point at which RPR reactivity was noticed, had been taken thirty-three days prior. The timepoints demonstrated persistent RPR reactivity, even following the second booster dose, though semi-quantitative titers were very weakly reactive throughout at 1:1. Confirmatory testing(FTA-ABS [LabCorp, Tampa, FL, USA]) was non-reactive for Participant 1. FTA-ABS and ANA testing were non-reactive.A 58-year-old heterosexual male healthcare worker who identified as White and Hispanic/Latino was the second participant. Early in 2021, he received two doses of the Pfizer BNT162b2 vaccine, followed by a Pfizer booster dose in October of the same year. RPR titers were 1:8 at each timepoint tested following vaccination, with the exception of his visit occurring 131 days followingthe booster dose, in which the titers decreased to 1:4. His RPR titer after the fourth dose increased to 1:8 once more. Participant 2's FTA-ABS results were reactive at all post-booster time points available, though none were ANA-positive.Curiously, both RPR and FTA-ABS prior to primary vaccine receipt were non-reactive.

Furthermore, neither participant was found to be RPR reactive, and there was no discernible correlation between the SARS-CoV-2 titer magnitude and the RPR titer magnitude (Table 1). The two assays showed a weak, non-significant negative correlation according to Spearman's rank correlation ($r = -0.55$, $p = 0.12$). Surprisingly, SARS-CoV-2 titer magnitude was not predictive of RPR reactivity, as the remaining participants screened at the initial post-booster timepoints (63% [75/119]) had equivalent or higher titers.

DISCUSSION

In this study, we examined the prevalence of RPR test false positives in a longitudinal cohort after receiving mRNA COVID-19 vaccines. Low, chronic RPR reactivity was present in about 2% of our participants for up to nine months after each vaccination dose. This discovery supports persistent reactivity for up to a year and a half, which is uncommon for minor immune challenges like vaccination [15] and builds on earlier research looking at false positive RPR results after COVID-19 vaccination [8].The nature of the reported RPR titers (results < 1:8 may persist over the course of a lifetime even in treated syphilis cases and are considered "low" [16]) and proximity to COVID-19 vaccination are more suggestive of a chronic false positive result, even though we cannot completely rule out the distinct possibility that Participant 2 contracted an active syphilis infection in the time after Dose 2. Additionally, Participant 2 had no history of known infectious diseases or autoimmune disorders, nor any sociodemographic risk factors that might have increased their risk of contracting syphilis. Therefore, we hypothesize that the intriguing results of Participant 2's non-reactive pre-vaccine RPR and FTA-ABS test and subsequent RPR-positive test may be the consequence of COVID-19 vaccineinduced cross-reactivity, even though the chronic false positive result elicited in Participant 2 may have occurred due to a variety of different mechanisms. Since SARS-CoV-2 mRNA vaccines have been shown to induce or enhance PEG-specific antibodies [9], which may work similarly to syphilis-specific antibodies, theoretical explanations for the COVID-19 vaccine include the generation of and cross-reactivity with anti-PEG antibodies.The underlying genetic susceptibility may also be the cause of the responsible mechanisms, which could account for Participant 20's false RPR test positivity through aberrant molecular mimicry [10] (the production of specific autoimmune autoantibodies) or bystander activation [11] (the activation of T-cells without antigen recognition).

Clinicians and the larger scientific community can benefit from the intriguing findings presented in this case report. The authors stress the non-zero chance of chronic RPR false positivity after receiving the SARS-CoV-2 vaccine and boosters outside of this cohort, in addition to the benefits of following a longitudinal cohort (n = 228). This should be taken into account when doing routine RPR screening, especially for people who have known co-morbidities, such as autoimmune diseases. The limitations of this work include the small percentage of false RPR reactivity represented (~2%), though it stands to reason that, if applied to the total population, these serological findings would result in a rather substantial number of affected individuals.In fact, this would translate to about 94,000,000 afflicted individuals globally if applied to the entire population that has received the SARS-CoV-2 vaccine.

In conclusion, the clinical community must be aware of potential vaccine cross-reactivity with RPR assays since vaccination is still the most effective method for reducing the spread of SARS-CoV-2 and the severity of the disease. It might be necessary to repeat or add more diagnostic tools to RPR assays and other serological tests that do not match clinical presentations after SARSCoV-2 vaccination. Patients may have to undergo invasive and time-consuming procedures such as lumbar punctures [12], which calls for thorough screening questions regarding recent immunizations, including any COVID-19 boosters, at the time of RPR screening.To better understand the frequency and duration of false reactive RPR tests, future research using large sample populations is required, especially in light of CDC recommendations regarding COVID-19 vaccination strategies.

CONCLUSIONS

To examine the underlying mechanisms causing false RPR assays, more research is required. Preventing a misdiagnosis of syphilis in COVID-19 vaccination recipients and in people with other illnesses and conditions that have been demonstrated to produce false RPR test results may be made easier by being aware of potential mechanisms, such as autoimmunization after exaggerated breakdown, high levels of IgM antibodies, anti-PEG antibodies, and underlying genetic susceptibility.

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