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Two-year follow-up study on the occurrence of antinuclear autoantibodies and clinical manifestations in a population of hospital healthcare workers after anti-SARS-CoV-2 vaccination

Studio di follow-up di due anni sulla comparsa di autoanticorpi antinucleo e manifestazioni cliniche in una popolazione di operatori sanitari ospedalieri dopo vaccinazione anti-SARS-CoV-2

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Abstract

Background: although COVID-19 vaccination has been essential for controlling the pandemic, concerns have been raised about possible autoimmune adverse events. This two-year follow-up study evaluated the occurrence of Antinuclear Antibodies (ANA) and related clinical symptoms in Healthcare Workers (HCWs) receiving BNT162b2 or mRNA-1273 vaccines.
Methods: fifty-two HCWs who had received at least three mRNA vaccine doses were enrolled; 35 completed blood sampling at baseline (T0) and at 3 (T1), 12 (T2), and 24 months (T3) after the first dose. ANA were assessed by indirect immunofluorescence on HEp-2 cells.
Results: seventeen ANA-negative HCWs who remained uninfected with SARS-CoV-2 throughout follow-up were included in the final analysis. Ten developed *de novo* ANA at different time points, and five remained positive at T1, T2, and T3 without developing clinically significant autoimmune symptoms.
Conclusions: ANA may develop after COVID-19 vaccination in previously ANA-negative HCWs, but no associated clinical manifestations were observed.

Background: sebbene la vaccinazione anti-COVID-19 sia stata fondamentale nel controllo della pandemia, sono emerse preoccupazioni circa possibili eventi avversi autoimmuni.
L'obiettivo era di valutare, in un follow-up di due anni, la comparsa di anticorpi antinucleo (ANA) e di sintomi clinici correlati in operatori sanitari vaccinati con BNT162b2 o mRNA-1273.
Materiali e Metodi: sono stati arruolati 52 operatori sanitari che avevano ricevuto almeno tre dosi di vaccino a mRNA; 35 hanno completato i prelievi al basale (T0) e dopo 3 (T1), 12 (T2) e 24 mesi (T3). Gli ANA sono stati valutati mediante immunofluorescenza indiretta su cellule HEp-2.
Risultati: nell'analisi finale sono stati inclusi 17 soggetti ANA-negativi, rimasti non infetti da SARS-CoV-2 durante il follow-up. Dieci hanno sviluppato ANA *de novo* in tempi diversi; cinque sono risultati positivi a T1, T2 e T3, senza sintomi autoimmuni clinicamente significativi.
Conclusioni: gli ANA possono comparire dopo vaccinazione anti-COVID-19, ma senza manifestazioni cliniche associate.

Key words: autoantibodies, COVID-19, SARS-CoV-2 mRNA vaccination.

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Introduction

COVID-19, or coronavirus disease 2019, is a respiratory illness caused by the novel coronavirus SARS-CoV-2. The virus was first identified in Wuhan, China, in late 2019 and led to a global pandemic.[1] COVID-19 can cause a range of symptoms, from mild respiratory issues to severe illness and even fatalities, especially in older adults and individuals with underlying health conditions.

Effective preventive measures such as wearing masks, practicing social distancing, frequent handwashing and vaccination are crucial for curbing the spread of the virus.

Extensive scientific evidence underlines the critical role of COVID-19 vaccination in pandemic management, estimating a significant reduction in mortality due to vaccination adherence.[2] However, vaccines can occasionally lead to adverse events, including transient or permanent autoimmune diseases.[1] The BNT162b2 and mRNA-1273 vaccines were the first mRNA-based vaccines authorized for use. The potential association of mRNA-based anti-SARS-CoV-2 vaccines with autoimmune reactions has been suggested, given the virus propensity to disrupt self-tolerance and trigger autoimmune reactions.[3,4] Ongoing studies continue to explore these effects and their potential to induce autoimmune reactions. The most common adverse events following COVID-19 vaccination include immune thrombocytopenia, myocarditis and Guillain-Barré syndrome and the new onset cases often occurred after the initial dose of vaccine.[5]

This two-year follow-up study aimed to evaluate the development and persistence of autoantibodies, particularly Antinuclear Antibodies (ANA), and assess any associated clinical symptoms in Healthcare Workers (HCWs) who received either the BNT162b2 mRNA or mRNA-1273 vaccines.

Materials and Methods

The study was approved by the ethics committee of the "SS Antonio and Biagio and Cesare Arrigo" University Hospital, Alessandria, Italy, and conducted in accordance with the ethical principles of the Declaration of Helsinki.

All participants were healthcare workers at the "SS Antonio and Biagio and Cesare Arrigo" University Hospital, Alessandria, Italy at the time of the study and provided informed consent. Initially, we enrolled 52 subjects from a previous study[6] who had received three doses of mRNA-based vaccines and were ANA-negative at baseline. Blood samples were collected before vaccine administration (T0), at 3 months (T1), and 12 months (T2) after the first dose, as previously described.[6] Thus, after T1 the subjects received two doses of vaccine and after T2 3 doses. Among these 52 subjects, only 35 agreed to perform blood collection at 24 months after the first dose (T3). Furthermore, subjects who developed COVID-19 after the third vaccine dose during the study (between 12 and 24 months after the first dose) were excluded from the analysis. Thus, our final population was based on 17 naïve subjects.

Blood samples were collected and immediately centrifuged to obtain serum, which was then stored at -20°C until further analysis.

All samples underwent analysis for ANA using indirect immunofluorescence [IIF] on HEp-2 substrate (EUROIMMUN, Luebeck, Germany) considering as dilution: 1:80, 1:160. 1:320 and 1:640. The samples were considered positive with A titre >1:80.

Clinical data were collected and recorded using REDCap software (REDCap version 10.2.3©2020 Vanderbilt University). Excel and GraphPad Prism 9 software were employed for data analysis.

Results

This follow-up study aimed to investigate the potential correlation between SARS-CoV-2 vaccination and autoimmunity in a subgroup of healthcare professional workers inoculated with mRNA vaccines, two years after their initial dose. These subjects were part of the cohort previously analyzed up to the booster dose as reported in our earlier publication.[6]

We initially included 52 subjects (38 females and 14 males, aged 26-67 years, median age 48 years) who completed the vaccination schedule, including the booster dose, and tested negative for ANA at baseline T0 (Figure 1).

Of these, only 35 participants completed sample collection up to the final follow-up at 24 months post-first vaccine dose (T3). However, 18 volunteers contracted SARS-CoV-2 infection between the 12-month (T2) and 24-month (T3) follow-up visits, thus resulting in a final analysis cohort of 17 naïve subjects.

All 17 participants were tested for the presence of ANA at each designated time point. Among them, 2 individuals tested positive at T1 and maintained positivity until T3. At T2, 6 subjects were ANA-positive, leading to a total of 8 ANA-positive subjects at this time point. Among the 6 individuals ANA-positive at T2, 3 remained positive at T3, whereas the other 3 became ANA-negative. At T3, an additional 2 subjects became ANA-positive, resulting in a total of 7 ANA-positive subjects, at this final time point (Figure 2).

Based on our results the number of subjects that developed *de novo* antinuclear antibodies was: 2 at T1, 6 at T2, and 2 at T3. Seven out of the 17 subjects remained ANA-negative throughout the entire study period of two years (Figure 3).

Among the participants, 3 volunteers received a fourth vaccine dose and consistently tested negative for ANA.

Furthermore, of the 7 subjects ANA positive at T3, only 5 subjects remained positive from T1 to T3 for at least two time points and were the ones considered to evaluate the antibody persistence. It is known that a positive ANA result alone does not necessarily indicate

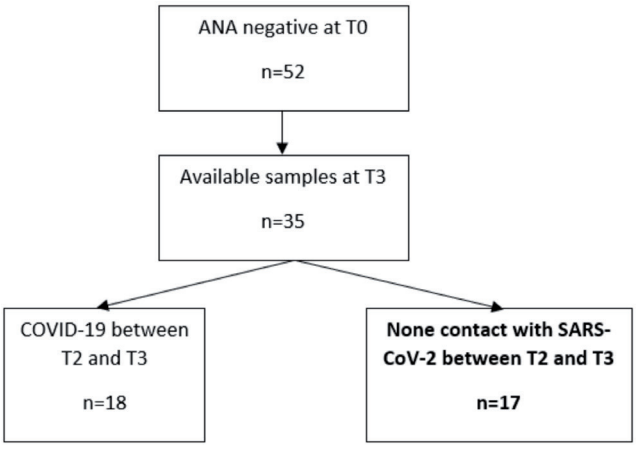

Figure 1. The flow chart shows the population available for follow-up analysis 24 months after the first vaccine dose.

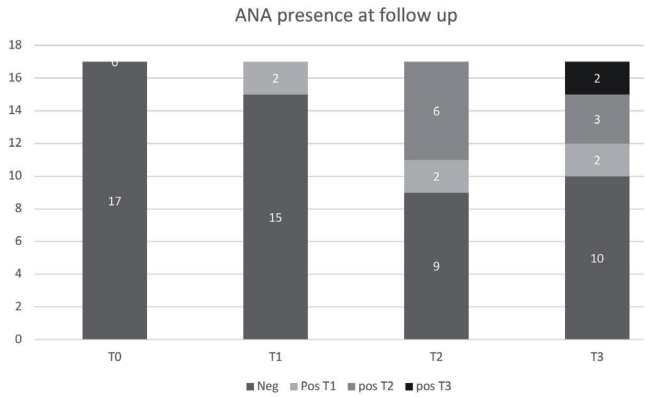

Figure 2. The graph illustrates the distribution of Antinuclear Antibodies (ANA)-positive and ANA-negative subjects across the different time points.

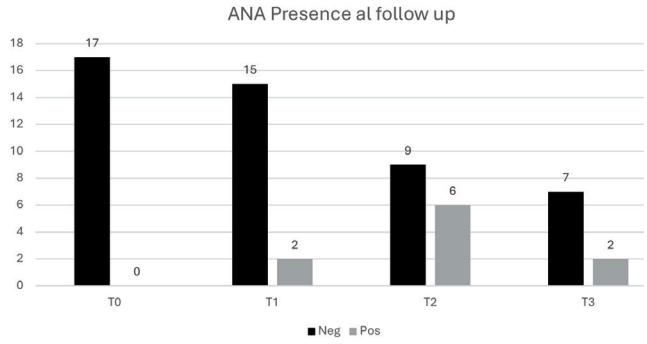

Figure 3. The graph shows data on the presence of Antinuclear Antibodies (ANA) developed *de novo* from T0 to T3.

that a patient will develop an autoimmune disease and, in some cases, might be just a transient phenomenon. Therefore, to assess symptoms consistent with a possible systemic autoimmune disease, these 5 subjects were clinically evaluated by a rheumatologist.

Based on the anamnestic interviews, no clinically significant symptoms related to new-onset autoimmune diseases emerged. Only one case reported a slight worsening of pre-existing joint pain symptoms.

Discussion

Autoimmunity following vaccination is a multifactorial phenomenon related to genetic risk factors, environmental factors, and over-stimulation of the immune response.[8-12] Various immunological mechanisms, including molecular mimicry (antigen-specific) and bystander activation (non-specific) have been proposed as potential triggers for autoimmune diseases or autoinflammatory conditions following viral vaccines.[12] An autoimmune signature has been suggested for the coronavirus disease 2019 (COVID-19) due to molecular mimicry and the deregulated immune response caused by the virus.[7,13,14] If molecular mimicry with SARS-CoV-2, particularly with the Spike protein, is implicated in COVID-19, then the SARS-CoV-2 vaccines could potentially trigger an autoimmune response as well. Autoimmune diseases are known for their long incubation periods, with associated autoantibodies detectable years before clinical manifestations.[15,16] Based on these observations, this two-year follow-up study comprehensively examined the incidence of autoantibody development post-COVID-19 vaccination in HCWs who received either the BNT162b2 mRNA or mRNA-1273 vaccines, with a specific focus on ANA. Among the 17 participants who were initially ANA-negative and never contracted COVID-19, 10 developed ANA seroconversion, with 5 showing persistent positivity at multiple time points. None of these individuals showed clinical signs suggestive of new-onset autoimmune disease. Only one case reported a slight worsening of pre-existing joint pain symptoms. This finding is in accordance with the data shown in another study, that had shorter follow-up period.[10] Our findings are consistent with prior studies showing that ANA seroconversion may occur following mRNA vaccination, yet without a clear correlation with autoimmune symptoms. For instance, Thurm *et al.*[16] and Gazitt *et al.*[17] reported ANA development post-vaccination, but with minimal clinical relevance. Specifically, the study by Thurm *et al.*,[16] conducted on a cohort of healthcare workers comparable to ours and with a shorter follow-up (up to 6 months), also found the onset of specific autoantibodies (such as ANA, aPL, AGA, RF) without disease development. Similarly, Gazitt *et al.*,[17] in a one-year prospective study on patients with Autoimmune Inflammatory Rheumatic Diseases (AIIRD) and the general population, observed autoantibody development post-BNT162b2 vaccination without significant disease flares. Consistent with these findings, Noureldine *et al.*[18] found a low incidence of autoimmune symptoms in seroconverted individuals after BNT162b2 vaccination, although their study had a shorter follow-up. Likewise, Blank *et al.*[19] highlighted a low incidence and transient elevation of autoantibodies post-mRNA COVID-19 vaccination in inflammatory arthritis patients, without significant clinical impact. In contrast, Świerkot *et al.*[20] found no significant increase in autoantibody levels or new autoimmune diseases 7-9 months after mRNA COVID-19 vaccination, supporting its immunological safety in healthcare workers. Unlike our findings, which showed a higher percentage of ANA expression changes, this study reported relevant variation in only two subjects; nonetheless, both studies observed no development of autoimmune diseases, further supporting the vaccine's immunological safety.

Unlike previous studies with larger sample sizes, our investigation is limited by a small cohort and attrition over time, which reduces the statistical power and generalizability of the findings. Additionally, we did not stratify the results according to vaccine type, which might influence immune reactivity. To address these issues, larger and more stratified populations are required for future studies.

Nevertheless, the strength of this study is to add to the existing literature one of the long-term two-year follow-ups on ANA positivity following COVID-19 mRNA vaccination in a healthy population, excluding the possible confounding factor of SARS-CoV-2 infection, which creates an autoimmune response. Our focus has been on clinical immunological monitoring of vaccinated individuals, as it may inform future surveillance strategies. Indeed, despite the persistence of ANA in some individuals, no autoimmune diseases have been diagnosed, strengthening the hypothesis that mRNA vaccines may occasionally trigger transient immunologic responses without clinical consequences.

In conclusion, our findings show that ANA seroconversion can occur after mRNA vaccination. However, it has a minimal clinical impact on healthy individuals, even with extended follow-up. These results reinforce the existing evidence that mRNA vaccines are safe regarding autoimmunity. They also highlight the need for ongoing longitudinal studies.

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Informed consent: all participants were healthcare workers at the “SS. Antonio and Biagio and Cesare Arrigo” University Hospital, Alessandria, Italy at the time of the study and provided informed consent.

Availability of data and materials: the data that support the findings of this study are available from the corresponding author upon reasonable request.