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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Hemolytic crisis due to Covid-19 vaccination in a woman with cold agglutinin disease

To the Editor:

A 57-year-old Caucasian female was diagnosed of primary cold agglutinin disease (CAD) in 2016. At that time, she presented with weakness, fatigue, jaundice and distal acrocyanosis when exposed to cold temperatures. Laboratory findings showed a regenerative anemia (hemoglobin of 9.0 g/dl, reticulocyte count of 175 000/μl), red blood cell agglutination on peripheral blood smear, undetectable haptoglobin and elevated bilirubin and lactate dehydrogenase (1.73 mg/dl and 427 U/L respectively). The direct antiglobulin test (DAT) was strongly positive for complement protein C3D and negative for IgG. A cold agglutinin with anti-I specificity was identified, with a titer of 128 and a high thermal amplitude. There was evidence of a serum monoclonal protein IgM lambda (352 mg/dl), a clonal lymphocyte population (0.4% of total leukocytes) and low titer antinuclear antibodies (1:80) at diagnosis. A recent history of infection was excluded. A complete body computed tomography scan ruled out an underlying malignancy.

Her symptoms improved with a short course of corticosteroids and she did not require any other pharmacological therapy or blood transfusion. For the past 5 years, she has presented compensated hemolysis with intermittent mild anemia that occasionally required 5–10 mg of prednisone to control flare-ups. Haptoglobin levels consistently remained undetectable. Although corticosteroid therapy is not an adequate long-term treatment for CAD, rituximab plus/minus bendamustine was not required due to the good response to low dose short-course intermittent prednisone.

During this last pandemic year, she received follow-up every 3 months, and her blood work showed very mild compensated hemolysis, with a hemoglobin greater than 11 g/dl. She did not refer fever or SARS-CoV-2-like symptoms at any time.

As she worked in a nursing home, in January, 2021 she was scheduled to receive a mRNA Covid-19 vaccination. Two days after the inoculation of the first dose, she began with chills, weakness, shortness of breath upon exertion, lumbar pain, jaundice and mild hemoglobinuria. Physical examination showed paleness and mucous jaundice. Laboratory findings revealed a hemoglobin of 8.6 g/dl, increased reticulocyte count, bilirubin (2.9 mg/dl), LDH (462 U/L), and spherocytes on the peripheral blood smear. Inflammation parameters such as ferritin or D-dimer were elevated at 426 and 726 ng/ml respectively. Serologic testing for known viruses and bacteria were negative. Autoimmunity screen was only positive for antinuclear antibodies, as previously detected. Real time PCR detection for SARS-CoV2 was also negative. The patient was treated with prednisone 20 mg daily with improvement of the hemolytic parameters and hemoglobin level to baseline values. Seven days before the second dose of the vaccine, prednisone was reduced to 10 mg daily to avoid dampening of the immune response. Two days after the second inoculation, again, she presented the same signs, symptoms and laboratory findings observed after the first dose, which were consistent with an exacerbation of autoimmune hemolytic anemia (Figure 1).

Five days after receiving the second dose, a serological study was performed, demonstrating the production of >40 000 AU/ml of SARS-CoV-2 IgG by the chemiluminescence immunoassay (CLIA). Prednisone was then increased to 20 mg per day with a subsequent improvement of all clinical and laboratory parameters, which allowed gradual tapering of steroids until discontinuation.

The cryoagglutinin titer increased after the first dose of the vaccine from 256 (4°C), to 512 with a wide range thermal amplitude. The DAT remained positive for CD3. Flow cytometric detection of

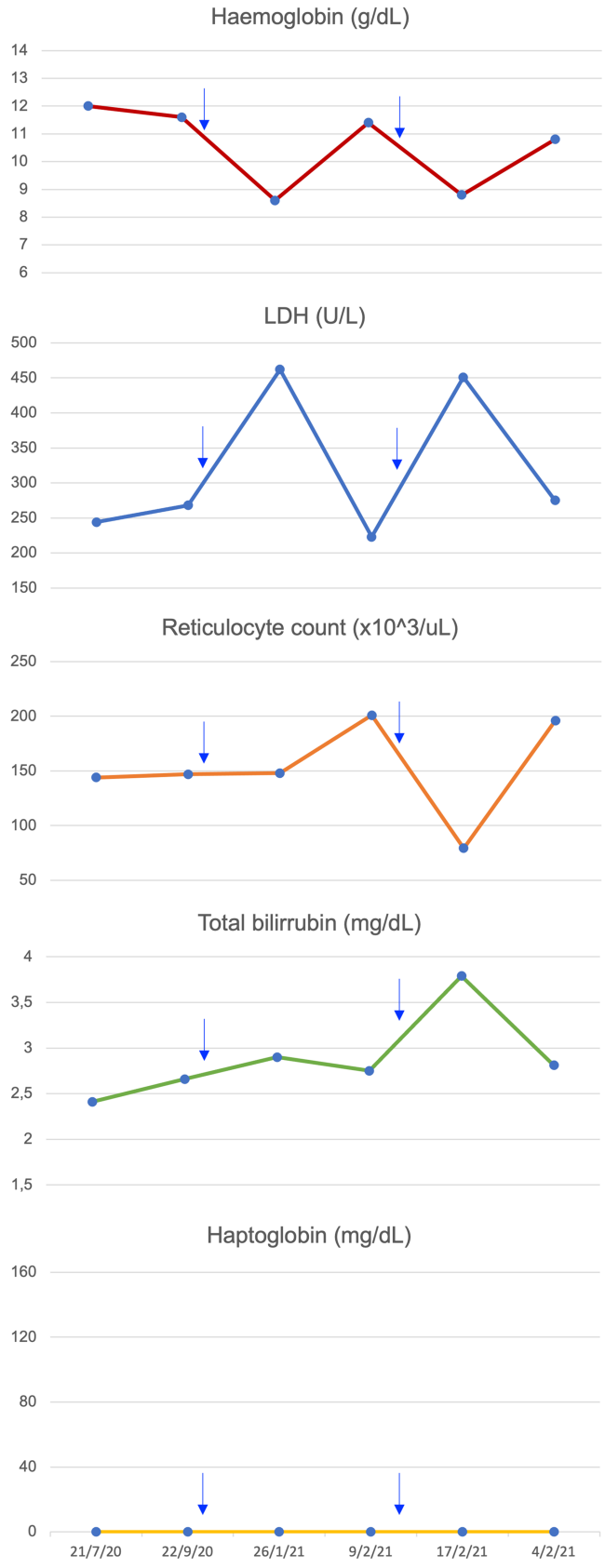

FIGURE 1 Evolution of laboratory findings from before vaccination (baseline values) up to the third week after the second dose of SARS-CoV2 vaccine (times of vaccine marked with blue arrows)

clonal B lymphocytes in peripheral blood was similar to previous determinations, with no other abnormalities.

One year into the SARS-CoV-2 pandemic, there is evidence on the ability of severe SARS-CoV-2 infection to trigger autoimmune disorders in genetically predisposed patients. Autoimmune diseases such as Guillain Barre Syndrome, immune thrombocytopenia (IT), Kawasaki disease, antiphospholipid syndrome and autoimmune hemolytic anemia (AIHA) have been associated with SARS-CoV-2 infection.[1]

The molecular similarity between the pathogenic viruses and human proteins seems to play a crucial role in these autoimmune phenomena. A significant similarity has been described between the SARS-CoV-2 spike (S) glycoprotein, which is one of the major antigens of the virus, and some molecules of the human proteome. A study comparing this similarity describes more than 300 common hexapeptides and more than 25 heptapeptides.[2]

Specifically the ankyrin-1 protein present in the red blood cell membrane was found to share a 100% identity with the SARS-CoV-2 S glycoprotein.[1] These findings could explain the development or worsening of AIHA by a mechanism of molecular mimicry, in which antibodies generated against the virus attack erythrocytes by a mechanism of cross-reactivity.

Similarly, other autoimmune symptoms associated with the virus and the immune system hyperactivation and cytokine storm, could be largely explained by the same mechanism of molecular mimicry, but in most of them a homology between SARS-CoV-2 and a human molecule has not been found yet.

To date the commercialized vaccines in the European Union use mRNA or a non-replicative viral vector to encode the spike glycoprotein as a stimulus to produce neutralizing antibodies against the virus.

Following the previously stated theory and the findings of this similarity, it could be anticipated that the vaccines under development may be capable of producing, similarly to the SARS-CoV-2 virus, significant autoimmune phenomena in genetically predisposed subjects or with an underlying autoimmune disease. At least theoretically, all the vaccines that share this mechanism of action or use the complete attenuated virus, would share the risk of developing these complications.

The SARS-CoV-2 infection-related flare-ups have already been reported in patients previously diagnosed with CAD.[1] However, here we report the first case, to our knowledge, of a Covid-19 vaccine-induced hemolytic crisis in a patient with a previous diagnosis of an autoimmune cytopenia.

In the case described, the close temporal relationship between the hemolytic flare up and vaccination, the lack of previous Covid-19 related symptoms and the negativity of the PCR detection for SARS-CoV2 suggest a central role of vaccination in the hemolytic process. Furthermore, in both hemolytic episodes, other possible triggering causes were reasonably ruled out.

Other vaccines, such as DTP (diphtheria-tetanus-pertussis), are also capable of inducing hemolytic anemia in subjects receiving them, with numerous cases of autoimmune hemolytic anemia due to both warm and cold antibodies described in the literature.[3] The MMR (measles-mumps-rubella) vaccine is a recognized cause of immune thrombocytopenia. This is thought to be due to an autoimmune

mechanism in which cross-reactivity with platelet antigens such as GP Ib/IX, GP Ia/IIa and GP VI occurs.[4] These would be other examples of side effects probably produced by molecular mimicry.

The recently described cases of thrombocytopenia and thrombosis related to ChAdOx1 nCoV-19 vaccine[5] are probably mediated by a different mechanism. The pathogenesis to date remains unknown. It would be reasonable to suspect that they are produced by a mechanism similar to the one produced in heparin-induced thrombocytopenia (HIT), such that a vaccine component (adenoviral sequence, spike protein or other components) binds to platelet factor-4 (PF4), producing a conformational change that leads to the generation of antibodies against this structure. In fact, DNA and RNA have been described as polyanions capable of inducing the conformational change in PF4 required to expose HIT-causing antigens.[6]

Returning to the above, so far, with a low percentage of the world population vaccinated, the cases of vaccine-related autoimmune events described are anecdotal. However, it is worth acknowledging these, since the objective of SARS-CoV-2 vaccination is to have a global reach, and the impact it may have on predisposed patient's population is unknown to date.

In this sense, it would be advisable to seek preventive strategies to avoid, prevent or minimize these phenomena in a susceptible population. In this regard, it seems that immunosuppressive therapy will play a relevant role, taking into account the possible impact on the immune response to the vaccine. In our patient, corticosteroid treatment with prednisone at low doses did not compromise the vaccine's serological response. If other treatments had been required to control the flare up (rituximab, bendamustine, bortezomib) the degree of compromise on the immune response may have been greater.

Another strategy that could be interesting to investigate would be to produce vaccines based on unique immune determinants in pathogens that are absent in the human proteome or to use single hexapeptides instead of hole proteins to avoid these autoimmune complications in susceptible individuals.[2]

With millions of doses of the different vaccines inoculated to date, the SARS-CoV-2 vaccines have demonstrated a very good safety profile. However, as with SARS-CoV-2 viral infection, it appears that they may trigger some autoimmune manifestations in predisposed subjects or those with previously known autoimmune diseases. Given that this vaccine is going to be administered to the entire population in an unprecedented short-term mass immunization strategy, it would be appropriate to evaluate preventive strategies to ameliorate this risk in predisposed patients. It is advisable to warn patients with CAD or other immune cytopenias and monitor the occurrence of these phenomena when they receive the vaccine. It would be useful to explore possible immunosuppressive therapy regimens to control flares, as well as to estimate the impact of these drugs on the vaccine serological response.

CONFLICT OF INTEREST

The authors declare no conflict interest.

AUTHOR CONTRIBUTIONS

Gemma Moreno-Jiménez is the consultant hematologist responsible for the patient, Lucía Pérez-Lamas and Gemma Moreno-Jiménez did the bibliographic research, wrote the clinical case and its discussion. Kyra Velázquez-Kennedy, María C. Tenorio-Núñez, Ana Jiménez-Martín and Ana Vallés-Carboneras provided technical guidance and advice. Carlos Jiménez-Chillón, Beatriz Astibia-Mahillo and Claudia Núñez-Torrónwere involved in critical revision of the report. Javier F. López-Jiménez and Valentín García-Gutiérrez helped with the literature review and provided overall supervision. All authors critically reviewed the final manuscript and approved it in its final version.

DATA AVAILABILITY STATEMENT

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ETHICS STATEMENT

Verbal consent was obtained from the patient for the publication of this report.

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Epidemiology of autoimmune hemolytic anemia: A nationwide population-based study in France

To the Editor:

Autoimmune hemolytic anemia (AIHA) is characterized by the destruction of red blood cells by warm or cold autoantibodies. Its epidemiology is not well known. The incidence rate of AIHA has been estimated at 1.77 per 100 000 person-years between 2008 and 2016 in Denmark.[1] However, these results need to be confirmed and variations by age and sex are not known. The main causes of secondary AIHA among adults are hematological malignancies and especially B-cell lymphomas, systemic autoimmune diseases and some chronic infections.[2] However, their prevalences deserve to be evaluated on a large scale with recent data. Mortality has been estimated in retrospective clinical cohorts between 8 and 21% in adults, with median or mean follow-ups between 1.8 and 3.3 years.[2,3] However, mortality compared to the general population, as well as mortality over time, are not established. Moreover, AIHA has been associated with an increased risk of venous thrombosis.[2,3] The cumulative incidence of arterial thrombosis has not been measured. Infections, including opportunistic infections, are also expected to be more frequent in patients with AIHA.[3] This study aimed to assess the overall incidence rate of AIHA in France, the incidence rate by age, sex and seasons, the prevalence of secondary AIHA causes, mortality and the cumulative incidence of hospitalization for thrombosis and infections compared to the general population.

The data source was the French health insurance database, named *Système National des Données de Santé* (SNDS). This database links individualized and anonymous data covering the entire French population (>66 million inhabitants) including sociodemographics, out-hospital and hospital data.[4] Data from 2010 to 2018 were available. We built the AHEAD cohort (Autoimmune HEmolytic Anemia: a population-baseD study), including all patients with an incident AIHA in France between 2012 and 2017. Patients with AIHA were identified using the international classification of disease, tenth version (ICD-10) code D59.1 as hospital discharge diagnosis or long-term disease. This code yielded a positive predictive value (PPV) of 90.0% in the SNDS.[5] The date of AIHA diagnosis (index date) was defined by the first occurrence of D59.1 code after a prior observation period of at least 2 years. Warm and cold AIHA are not distinguishable in the SNDS. Each patient was matched on year of birth, sex and index date to five comparisons randomly selected from the general French population. Causes of secondary AIHA were searched during the year before the diagnosis of AIHA using specific long-term disease and hospital discharge diagnosis codes (Table S1). Evans syndrome, defined with the ICD-10 code D69.3 of immune thrombocytopenia, was described separately. The included population was subdivided into three subgroups defined at index date: primary AIHA, secondary AIHA associated with hematological malignancies and other secondary AIHAs. Death and first hospitalization for thrombosis and for infection were assessed between the index date and December 31, 2018 (end of follow-up). They were identified in the SNDS by primary hospital discharge diagnoses using appropriate ICD-10 codes (PPVs indicated in Tables S2 and S3). Statistical analyses are detailed in supplementary material.

We identified 9663 patients with incident AIHA between 2012 and 2017. Patients were matched with 47 700 comparisons. Patients' and comparisons' characteristics are described in Table S4. The median age was 69.5 years and 55.6% of patients were women. Evans syndrome represented 5.8% of AIHAs. The Charlson comorbidity index score was ≥3 in 42.4% of patients with AIHA versus 13.6% of comparisons (Table S5).

The overall incidence rate of AIHA was 2.44 per 100 000 person-years (95% confidence interval – 95% CI: 2.39 to 2.48). The incidence rate of AIHA was more than 10 times higher in the elderly (≥75 years) than in people aged <50 years (Figure S1). It was higher in women than in men between 15 and 45 years of age. The same pattern was observed for primary AIHAs (Figure S2). Incidence rates of AIHA by months revealed a trend for a higher incidence rate during winter (Figures S3 and S4).

Note, AIHA was primary in 55.2% of the cases at the time of AIHA diagnosis. In adults, AIHA was associated with hematological malignancy in 31.9%, including lymphoma in 24.1%, B-cell chronic lymphocytic leukemia in 11.3% and myelodysplastic syndrome in 5.4%. Systemic lupus erythematosus accounted for 5.3% of AIHA in adults (Table S6).

During follow-up (27 682 patient-years and 173 370 comparisons-years; median follow-up: 31 months and 43 months, respectively), 3354 patients died, 956 had a hospitalization for thrombosis and 3278 for infection. The categories of thrombosis and infection are shown in Tables S7 and S8, respectively. Infections were opportunistic in 180 patients (5.5%), including 65 *Pneumocystis jirovecii* pneumonias and 32 zosters.

The 1-year mortality was 20.5% (95% CI: 19.7 to 21.3) in AIHA (primary AIHA: 17.9%, 95% CI: 16.8 to 18.9), versus 3.3% (95% CI: 3.1 to 3.5) in comparisons (Figure 1, Table S9 and Figure S5). The hazard ratio (HR) of death adjusted for Charlson comorbidity index score was 3.3 (95% CI: 3.1 to 3.4). It was 2.9 (95% CI: 2.7 to 3.1) in primary AIHA. We observed a higher mortality in all categories of age. The 5-year mortality was 18.2% (95% CI: 16.7 to 19.7) in patients with