SARS-CoV-2 mRNA vaccination of aplastic anemia patients is safe and effective

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CORRESPONDENCE

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To the Editor:
Vaccines are an essential part of the fight against the COVID-19 pandemic. Especially immunocompromised patients at risk for a severe or fatal course of SARS-CoV-2 infection are expected to benefit from vaccination. While studies on SARS-CoV-2 mRNA vaccines have shown that healthy subjects are able to mount both effective humoral and cellular immune responses to these vaccines,[1] the effectiveness and safety of SARS-CoV-2 vaccines for immunocompromised patients remain unclear. Acquired aplastic anemia (AA) is an example of a disease that results in an immunocompromised state. AA patients are immunocompromised either due to the disease itself which is characterized by profound pancytopenia caused by immune-mediated bone marrow failure, or due to the immunosuppressive treatment (IST) consisting of horse-derived antithymocyte globulin (hATG) in combination with cyclosporine A (CsA) that they received.[2] This immunocompromised state of AA patients argues that it is important to vaccinate these patients with a SARS-CoV-2 mRNA vaccine in order to prevent severe COVID-19. However, anecdotal case studies have reported AA relapse after vaccination and, therefore, the international guidelines recommend caution when vaccinating AA patients after IST irrespective of the time between last IST and vaccination.[3] Furthermore, it is not known whether previous IST affects the ability to mount an adequate immune response to a vaccine in these patients. These considerations create a dilemma whether to vaccinate AA patients after IST with SARS-CoV-2 mRNA vaccines.

In this study, we investigated the occurrence of relapse as well as the ability to mount both a humoral and T-cell response to SARS-CoV-2 mRNA vaccination in 18 AA patients treated with IST at a median time of 11.1 years (range 0.3–39) before SARS-CoV-2 vaccination (Table S1). At the time of vaccination, 14 AA patients were transfusion-independent and successfully tapered from IST. Three patients were transfusion-independent but IST-dependent, and one patient was both transfusion- and IST-dependent. All IST-dependent patients ($N = 4$) received CsA at time of vaccination. The AA patients and healthy controls (HCs; $N = 9$) received two SARS-CoV-2 mRNA vaccines (mRNA-1273 (Moderna) or BNT162b2 (Pfizer-BioNtech) vaccines). Whole blood was sampled prior and post-vaccination to measure blood counts, and to isolate serum and peripheral blood mononuclear cells (PBMCs) to measure SARS-CoV-2-specific IgG antibodies and T-cells (see Supplementary material and methods).

To investigate whether AA patients relapsed after SARS-CoV-2 mRNA vaccination, hemoglobin (Hb), thrombocyte, and neutrophil values were determined in peripheral blood. Samples were taken pre-vaccination, post-vaccination (median 27 days after the second vaccination), and at follow-up (median 9.1 months after the first vaccination). The blood values were stable post-vaccination and remained stable without the need for transfusion during the follow-up period in all 17 patients that were transfusion-independent at start of the study (Figure 1A). The transfusion-frequency remained stable in the patient that was transfusion-dependent at the start of the study. These results indicate that no signs of AA relapse are present up to 9 months after first vaccination, which is in accordance with a previous study investigating mRNA vaccination in 16 AA patients.[4] This suggests that the case reports describing AA relapse observed after vaccination may be rare incidents or incidents unrelated to vaccination.

The humoral immune response of AA patients to SARS-CoV-2 mRNA vaccination was measured by determining SARS-CoV-2 anti-Spike IgG levels pre- and post-vaccination. Seventeen of 18 AA patients had an adequate SARS-CoV-2 IgG antibody response (defined as >300 BAU/ml) after vaccination which was similar to HCs (Figure 1B). The patient with antibody levels below threshold had recently received hATG, still received CsA, and was the oldest person (79 years) in the AA patient cohort. An inversed correlation between age and Spike-IgG was found (Table S2), indicating that the amount of Spike-IgG decreased with increasing age. For other factors, such as time between IST (hATG treatment) and vaccination, absolute number of B-cells, absolute number of $CD4^+$ and $CD8^+$ T-cells, no significant correlations were observed (Table S2). In short, the majority of AA patients is able to generate an adequate antibody response and which is accordance with previous literature.[4]

Spike-specific $CD4^+$ and $CD8^+$ T-cell responses were measured by incubating PBMCs with a SARS-CoV-2 spike peptide pool, followed by intracellular cytokine staining for flow cytometry. The frequency of SARS-CoV-2 spike-specific $CD4^+$ and $CD8^+$ T-cells was determined before and after vaccination which showed a significant increase in both AA patients and healthy controls (Figure 1C,D). The SARS-CoV-2 spike-specific $CD4^+$ and $CD8^+$ T-cell frequencies between AA patients and HCs were not significantly different after vaccination, although a trend toward a lower frequency of SARS-CoV-2 specific $CD8^+$ T-cells in AA patients could be observed (Figure 1C,D). As expected, the $CD4^+$ and $CD8^+$ T-cell responses directed against the broad cytomegalovirus, Epstein–barr virus, influenza, and extended peptide pool (CEFX) did not differ pre- and

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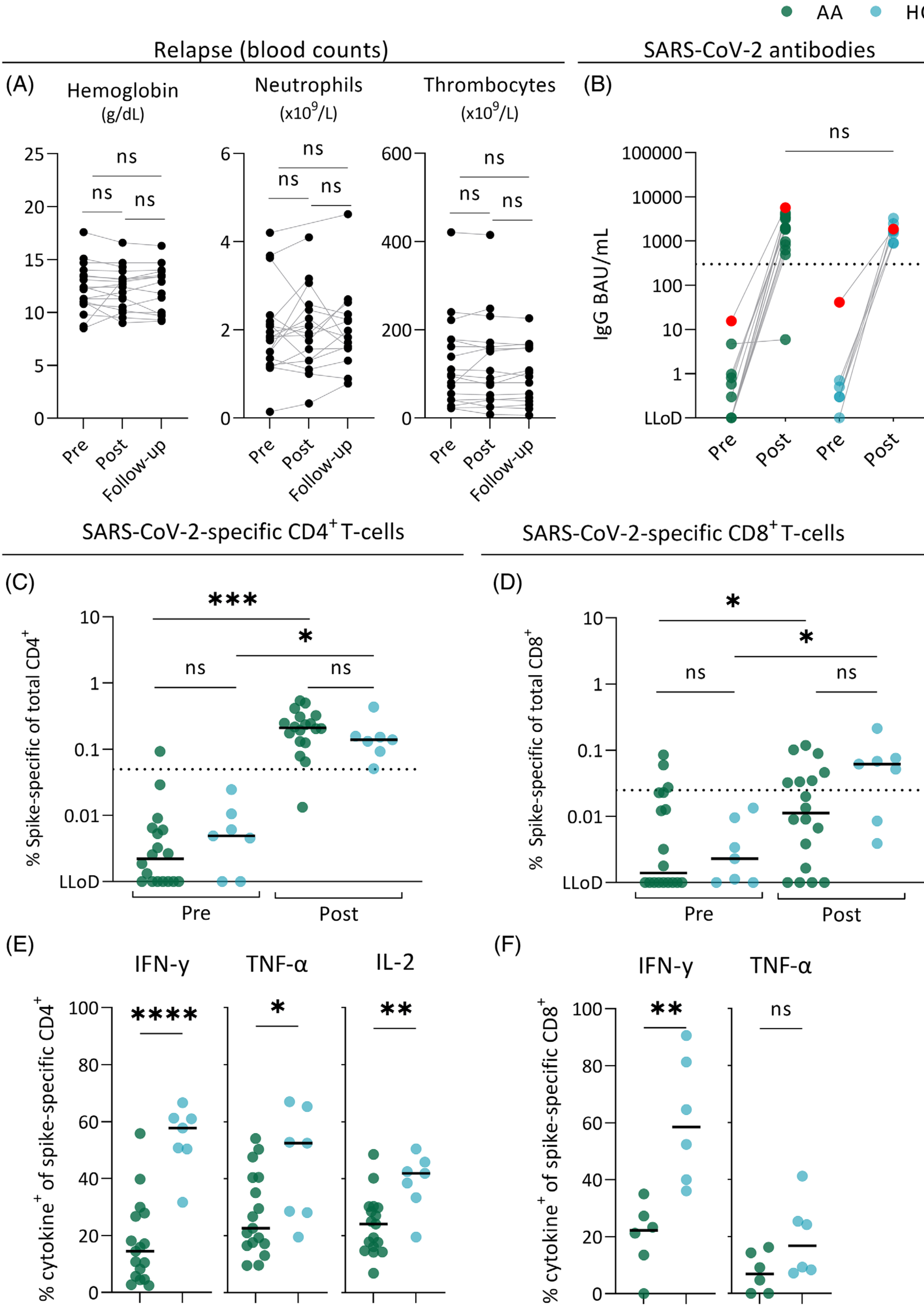

FIGURE 1 Legend on next page.

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post-vaccination in AA patients and HCs, and frequencies of CEFX-specific $CD4^+$ and $CD8^+$ T-cell were comparable for both cohorts (Figure S1). Percentages of SARS-CoV-2 spike-specific $CD4^+$ and $CD8^+$ T-cells that produce interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), or interleukin-2 (IL-2) were significantly lower in the AA patients than in healthy controls (Figure 1 E,F). Interestingly, this trend of reduced cytokine production was also observed for the CEFX-specific $CD4^+$ T-cells in AA patients that produced significantly reduced levels of TNF-α and IL-2 compared to healthy controls (Figure S1). In conclusion, spike-specific $CD4^+$ and $CD8^+$ T-cell frequencies were comparable between AA patients and healthy controls. However, the percentage of spike-specific $CD4^+$ and $CD8^+$ T cells that produced IFN-γ, TNF-α or IL-2 was lower in AA patients compared to healthy controls.

Reduced T-cell cytokine production can be caused by multiple factors. Age, time between IST (hATG treatment) and vaccination, and absolute numbers of the $CD4^+$ and $CD8^+$ T-cell compartment at the time of vaccination were not significantly correlated to the reduced cytokine production seen after IST (Table S2). Since CsA is a known inhibitor of T-cell proliferation and cytokine production, we investigated whether CsA could be responsible for the decreased cytokine production of the SARS-CoV-2 specific T-cells. Although the frequency of $CD4^+$ and $CD8^+$ SARS-CoV-2 spike-specific T-cells was comparable between AA patients who received CsA at time of vaccination and AA patients who did not receive CsA at time of vaccination, we observed that three AA patients who received CsA at time of vaccination tended to have lower percentages of IFN-γ, TNF-α, and IL-2 producing SARS-CoV-2 spike-specific $CD4^+$ T-cells (Figure S2). Interestingly, these AA patients tended to have higher spike-IgG antibody levels (median: 3431 BAU/mL) compared to patients who no longer received CsA (median: 1912 BAU/mL) at the time of vaccination (Figure S2). Due to the low number of patients that received CsA at time of vaccination ($n = 3$) both trends could not be statistically confirmed.

For the AA patients that did not receive CsA during vaccination, we cannot fully explain the lower percentage of cytokine producing SARS-CoV-2 spike-specific T-cells in comparison to HCs. We cannot exclude the possibility that the reduced cytokine production is the result of a lingering effect of the disease or the IST these patients have received. Although no correlation was found between the spike-specific T-cell response and time that patients last received hATG or CsA, hATG or CsA may have had a permanent effect on the repertoire of the T lymphocytes. Based on the analyses of the major T lymphocyte subsets, no obvious difference could be detected (Figure S3). However, it is also possible that the difference is more subtle and could, therefore, not be detected based on the T-cell markers used in this study and the sample size of the study population. Importantly, it is not known whether the reduced cytokine production influences the effectiveness of vaccines in AA patients and whether this might increase by additional vaccination doses.

In summary, no indications of AA relapse was observed up to nine months after the first mRNA vaccination. Additionally, 17 of 18 AA patients were able to mount an adequate humoral response and demonstrated comparable magnitudes of spike-specific $CD4^+$ T-cells and spike-specific $CD8^+$ T-cells. Our study sheds another light on the current view on the risk/benefit discussion for vaccination of AA patients as the results indicate that SARS-CoV-2 mRNA vaccines are more beneficial to AA patients than potentially harmful. The reduced cytokine production by the T-cells further underlines the importance of vaccinating AA patients to protect against a possible severe course of SARS-CoV-2 infection. Larger cohort studies are needed to further study the chance of AA relapse after SARS-CoV-2 mRNA vaccination and vaccine efficacy in AA patients not only after successfully tapered IST but also in AA patients recently treated with hATG who are still using CsA. Furthermore, it has to be determined whether additional vaccination doses result in improved cytokine production by spike-specific T-cells which could affect the vaccination scheme for AA patients.

FIGURE 1 Blood counts, humoral responses, and T-cell responses following SARS-CoV-2 mRNA vaccination in aplastic anemia patients and healthy controls. (A) Hemoglobin, neutrophils, and thrombocytes shown pre-vaccination, post-vaccination (median 27.1 days after second vaccination), and at follow-up (median 9.1 months after start vaccination). Blood value data at follow-up were not available for three patients; therefore, the statistical comparisons of pre/post with follow-up blood values were only performed for the 15 AA patients for whom data were available. (B) SARS-CoV-2 spike IgG response according to WHO standardization of AA patients (green; $n = 18$) and HCs (light blue; $n = 9$). The red dots correspond to individuals that were positive for SARS-CoV-2 IgG before vaccination. Post-vaccination SARS-CoV-2 spike IgG levels were determined in serum of AA patients (median 27.1 days (range 11–49)) and HCs (median 21.4 days (range 18–24)) after second vaccination. Dotted line shows threshold of an adequate IgG response of 300 BAU/mL. (C) Percentage of SARS-CoV-2 spike-specific $CD4^+$ T-cells of total $CD4^+$ T-cells pre- and post-vaccination in AA patients (green) and HC (light blue). Dotted line shows a threshold for a $CD4^+$ T-cell response of 0.05%. (D) Percentage of SARS-CoV-2 spike-specific $CD8^+$ T-cells of total $CD8^+$ T-cells pre- and post-vaccination in AA patients (green) and HC (light blue). The percentage of SARS-CoV-2 spike-specific CD4+ and CD8+ T-cells was corrected for the background signal in the negative control (DMSO). Dotted line shows a threshold for a $CD8^+$ T-cell response of 0.025%. (E) The percentages of IFN-γ, TNF-α, and IL-2 producing spike-specific CD4+ T-cells in AA patients (green) and HC (light blue). (F) The percentages of IFN-γ and TNF-α producing spike-specific $CD8^+$ T-cells in AA patients (green) and HC (light blue). Horizontal bars in figures C-F represent the median. ns: $p > .05$, *: $p \le .05$, **: $p \le .01$, ***: $p \le .001$. AA, aplastic anemia; HC, healthy controls; ns, not significant; LLoD, Lowest limit of detection; BAU, binding antibody units; DMSO, dimethyl sulfoxide; TNF-α, tumor necrosis factor alpha; IFN-γ, interferon gamma; and IL-2, interleukin 2

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AUTHOR CONTRIBUTIONS

Cilia R. Pothast and Kayleigh van Dijk contributed equally to the manuscript. Cilia R. Pothast designed the experimental set-up, analyzed data, interpreted data, and wrote manuscript. Kayleigh van Dijk performed experiments, analyzed data, and wrote the manuscript. Emma S. Pool interpreted experiments and assisted with statistical data analysis. Constantijn J. M. Halkes conceptualized the study and recruited patients. Mirjam H. M. Heemskerk conceptualized the study, designed experimental set-up, interpreted the data, and supervised the project. Jennifer M.-L. Tjon conceptualized the study, recruited patients, interpreted the data, and supervised the project. All authors critically edited and reviewed the manuscript. All authors have access to the raw data used for this study and they all approved the final manuscript.

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CONFLICT OF INTEREST

Authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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