Cancer Cell, Volume *40*

Supplemental information

Increased antibody titers and reduced seronegativity following fourth mRNA COVID-19 vaccination in patients with cancer

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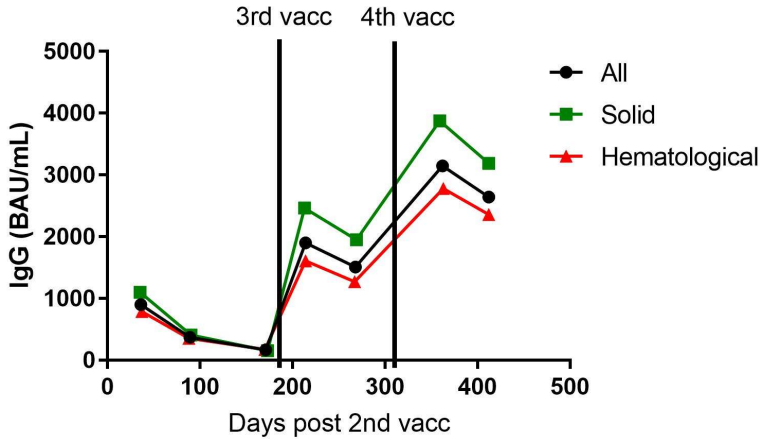
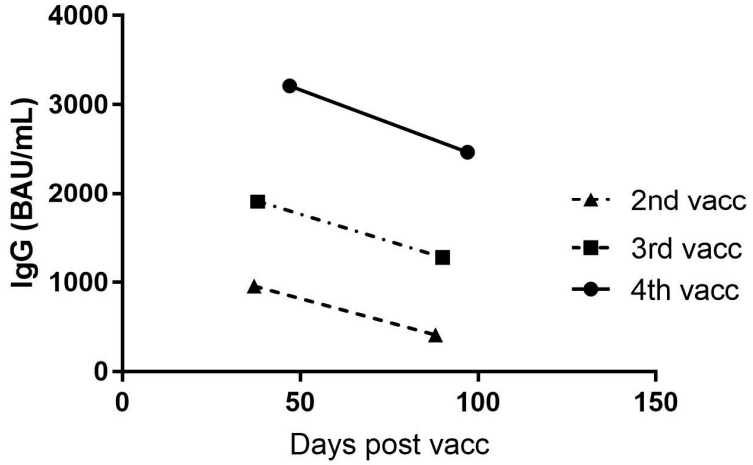
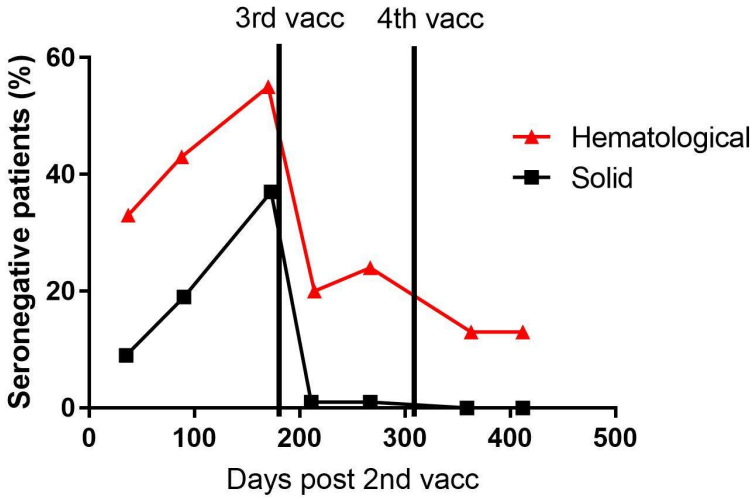
A


3rd vacc
4th vacc
5000
4000
3000
2000
1000
0
IgG (BAU/mL)
0
100
200
300
400
500
Days post 2nd vacc
All
Solid
Hematological

B


4000
3000
2000
1000
0
IgG (BAU/mL)
0
50
100
150
Days post vacc
2nd vacc
3rd vacc
4th vacc

C


3rd vacc
4th vacc
60
40
20
0
Seronegative patients (%)
0
100
200
300
400
500
Days post 2nd vacc
Hematological
Solid

Supplemental figure legend

Figure S1. SARS-CoV-2-specific IgG (anti-S IgG) titers over time in blood samples from patients with cancer after two, three and four doses of mRNA COVID-19 vaccine.

A. Mean anti-S IgG levels in blood samples from patients with hematologic or solid cancers. The graph shows results from blood samples collected 1, 3 and 6 months after the 2nd vaccination, 1 and 3 months after 3rd and 4th vaccinations. The 4th vaccination boosted anti-S IgG levels significantly (Patients received the 4th vaccination, all: n=395, solid cancers: n=139, hematological cancers: n=256).

B. Decreasing mean anti-S IgG titers of the total cohort comparing blood samples collected 1 and 3 months after the 2nd (n=466), 3rd (n=325) and 4th (n=281) vaccinations. Patients with IgG titers that increases between the analyzed blood samples were withdrawn from the analysis.

C. Percentage of seronegative patients (anti-S IgG ≤ 54BAU/mL) with hematological or solid cancers. The number of seronegative patients were calculated for blood samples collected 1, 3 and 6 months after the 2nd vaccination, 1 and 3 months after 3rd and 4th vaccinations. The 4th vaccination reduced the number of seronegative patients with hematological and solid cancers to 13% and 0%, respectively.

Supplemental Table S1. Patient characteristics and association with anti-SARS-CoV-2 immune status.

Supplemental Materials and Methods

Study Design

This is an analytical, observational prospective, single-center cohort study of immune responses after SARS-CoV-2 vaccination among patients with solid and hematologic cancers. Patients eligible for inclusion were adults (age >18 years) diagnosed either with solid tumors (histologically or cytologically verified) and undergoing active systemic cancer treatments, or selected hematologic malignancies either in active systemic cancer treatment or not receiving treatment, during the period of vaccination with two, three and, in most cases, four COVID-19 mRNA vaccinations. The included patients with hematologic cancers were pre-selected based on the expectation of a poor immune response and therefore primarily included patients with lymphoma, chronic lymphocytic leukemia (CLL), or multiple myeloma; patients with acute leukemia, myelodysplastic syndrome or chronic myeloproliferative neoplasms were not included. All included patients were followed and treated at the Departments of Oncology or Hematology at Odense University Hospital, and all were able to provide written and oral informed consent. A total of 2,601 patients were invited to participate in the study. Of those, 715 provided electronic written consent, of which 590 also provided oral consent and thus met the eligibility criteria and were enrolled in the study. Of the included patients, 530 completed the recommended three mRNA vaccinations at time of data interpretation and 395 of those completed the fourth vaccination. Of the included patients, 24 withdrew their consent but allowed us to use the results from the blood samples already taken. Patients were excluded from the study if they did not have a personal digital mailbox or mobile phone, as the communication regarding blood samples was relayed via these means. No patients were lost to follow-up. Twelve patients died of their disease after receiving only two vaccinations, 20 patients died after receiving only three vaccinations, and 6 died after the 4^{th} vaccination (end of study June 10, 2022).

The primary outcomes that were reported in our initial study (Ehmsen et al., 2021a) were the rate of SARS-CoV-2-specific IgG seropositivity and the degree of SARS-CoV-2-specific T cell responses after vaccination. Secondary outcomes included comparisons of IgG titer and interferon (IFN)-γ release and identifying factors associated with vaccine response, including anti-cancer therapies and cancer types associated with seropositivity. The primary outcomes of our previous follow-up study (Ehmsen et al., 2022) were the rate of SARS-CoV-2-specific IgG seropositivity after the 3rd vaccination and the decay of SARS-CoV-2-specific IgG titer over time after the 2nd and 3rd vaccinations. Secondary outcomes included comparisons of IgG titers and identifying factors associated with vaccine response, including anti-cancer therapies and cancer types associated with seropositivity. The primary outcomes of this follow-up study were the rate of SARS-CoV-2-specific IgG seropositivity after the fourth vaccination and the decay of SARS-CoV-2-specific IgG titer over time after the 2nd, 3rd and 4th vaccinations. Secondary outcomes included comparisons of IgG titers and identifying factors associated with vaccine response, including anti-cancer therapies and cancer types associated with seropositivity.

Participants

Clinical information regarding patient medical histories was obtained from the hospital digital medical file system using the patient's Danish Civil Registration numbers (CPR), allowing for follow-up with accurate censoring at emigration or death. All participants received vaccination as part of the national COVID-19 vaccination program. Participants were included from March 7 to November 8, 2021. The first blood sample was drawn 36 days (mean, standard deviations: 10 days) after the 2nd vaccination, while the second and third blood samples were drawn 89 and 171 days (mean, std. dev: 12 days and 12 days) after the 2nd vaccination, respectively. After the 3rd vaccination, the first blood samples were drawn at 39 days (mean, std. dev: 9 days), while the second and third blood samples

were drawn at 93 and 186 days (mean, std. dev: 11 days and 14 days) after 3rd vaccination, respectively. After the 4th vaccination, the first and second blood samples were drawn at 47 days and 97 days (mean, std. dev: 10 days and 10 days) respectively after the 4th vaccination. The last blood sample included in this study was drawn June 10, 2022, 119 days after 4th vaccination.

Baseline

Baseline data included age (at time of 3rd vaccination), sex, cancer type, dates of vaccinations, type of vaccine, other malignancies, and cancer therapy (at time of 4th vaccination). At time of 4th vaccination, most recent cytotoxic drugs were registered during the past three months for cytotoxic drugs and immunotherapy and during the past 12 months for anti-CD20 antibody therapy. Immunoglobulin treatments up to three months and steroid treatment (≥ 50mg) given up to one week prior to 4th vaccine dose were registered.

Cancer types were defined according to the International Classification of Disease (10th revision) diagnostic codes. The registered data was abstracted from medical files by local investigators and entered into the research electronic data capture (REDCap) application and analyzed using Stata, version 17.

Anti-SARS-CoV-2 S RBD and N IgG antibody analysis

For antibody quantification, the samples were separated by centrifugation and IgG antibodies against SARS-CoV-2 spike receptor binding domain (RBD), the main target for virus neutralization, (anti-S IgG) and quantified using a chemiluminescent microparticle immunoassay (SARS-CoV-2 IgG II Quant assay; Abbott Laboratories). The resulting chemiluminescence in relative light units following the addition of anti-human IgG acridinium-labeled conjugate in comparison with the IgG II

calibrator/standard indicates the strength of response, which reflects the quantity of IgG present. This FDA Emergency Use Authorization approved assay has shown excellent correlation with the first WHO International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC code 20/136), enabling the issuing of immunogenicity results in standardized units; binding antibody units (BAU)/mL. The relationship between the Abbott arbitrary units (AU)/mL unit and the WHO BAU/mL unit follows the equation BAU/mL = 0.142 × AU/mL. Cut-off was set at 9 BAU/mL and considered as negative IgG response (IgG negative) and a result of >9 to ≤54 BAU/mL was considered weak IgG antibody response.

In this study, we used the Abbott SARS-CoV-2 IgG (N; Abbott Laboratories) serological assay, which is a two-step chemiluminescent microparticle immunoassay designed to detect IgG antibodies against the SARS-CoV-2 nucleocapsid antigen. A value >1.4 AU was interpreted as positive (manufacturer defined).

Statistical Analysis

Baseline characteristics and immune responses were described proportionately across the pre-defined diagnosis groups. Students t-test was used to investigate the significance of the relationship between SARS-CoV-2 IgG in patients receiving or not receiving the 4^{th} vaccination. A p value < 0.05 was considered statistically significant. Statistical analysis and figures were performed using GraphPad Prism, version 7.

Ethics

This study was approved by the Danish ethics committee (record no. S-20210008C) and data handling approved by Region of Southern Denmark (record no. 21/14199). Data are reported according to STROBE (Strengthening the reporting of observational studies in epidemiology) guidelines.