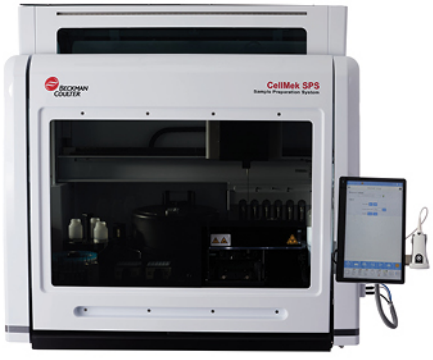

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CORRESPONDENCE

Immunogenicity and clinical efficacy of anti-SARS-CoV-2 vaccination in patients with hematological malignancies: Results of a prospective cohort study of 365 patients

To the Editor:

Since its outbreak, Covid-19 has been responsible for more than 6 000 000 deaths.[1] Cancer is one of the most important risk factors for severe disease and death; hematological malignancies (HMs), specifically, have been associated with an estimated mortality rate of 37%.[2]

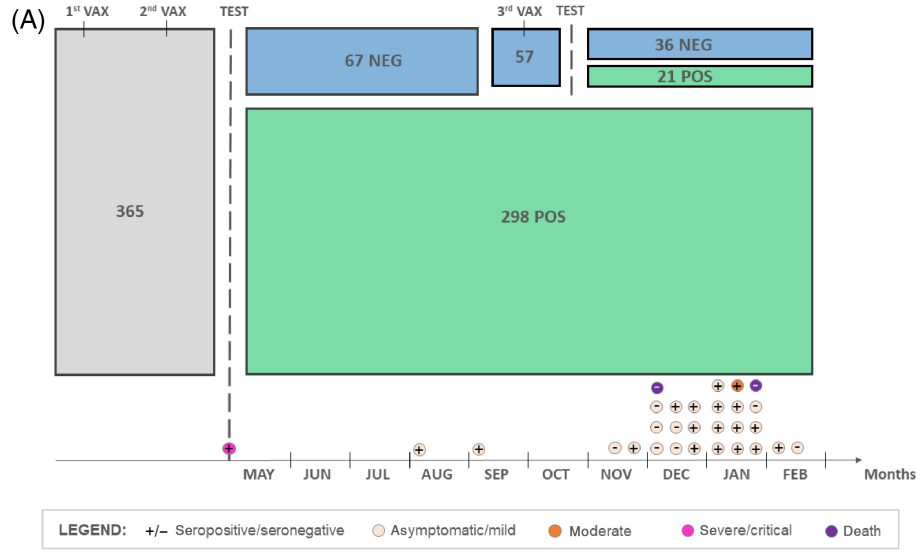
At present, the anti-SARS-CoV-2 vaccination represents the most effective strategy to reduce the incidence and severity of Covid-19.

Here, we present the results of a prospective, cohort study aimed to evaluate the humoral and cell-mediated immune response and the clinical efficacy of anti-SARS-CoV-2 vaccination in adult patients with HMs.

The study included consecutive adult patients with HMs who had completed the first cycle of anti-SARS-CoV-2 vaccination. At enrollment, information was collected regarding patient demographics, HM characteristics, last HM therapy, anti-SARS-CoV-2 vaccination, and previous Covid-19. Active disease was defined as being at the time of diagnosis or having a progressive disease. Active treatment was defined as any ongoing therapy, or any therapy discontinued in the 6 months prior to vaccination, or stem cell transplant (SCT) performed within 3 months from vaccination. Humoral and cellular immunity testing was planned 4 weeks after the completion of the first vaccination cycle. Seronegative patients underwent additional serology testing 4 weeks after the administration of a booster dose of vaccine. During follow-up, all patients were monitored for SARS-CoV-2 breakthrough infections through telephone interviews, starting 7 days after the completion of the first cycle of vaccination. The DiaSorin's LIAISON® SARS-CoV-2 TrimericS IgG assay was used to test humoral immunity. The assay's range was increased from 4.81 - 2080 BAU/ml to 4.81–41 600 BAU/ml for a more accurate determination of Ab titer. Technical details of the assay are summarized in Supplementary Table 1. The anti-spike T-cell-mediated immune response was tested by quantifying spike-specific IFNγ-producing T-cells by enzyme-linked immunosorbent spot (ELISpot) assay and by characterizing different subpopulations of T-cells through multicolor fluorescence-activated cell sorting (FACS). Both analyses were performed ex vivo using whole thawed peripheral blood mononuclear cells (PBMC). To evaluate IFNγ ELISpot response, spot forming cells (SFC) were counted by a stereomicroscope and values were defined as positive if there was at least a two-fold increase from the negative value and above a threshold of 4 SFC per well. Flow cytometry analysis was used to differentiate T-cell subpopulations based on the expression of CD4+, CD8+, CD4 + IFNγ+, and CD8 + IFNγ+. Then, the following subsets of spike-specific CD4+ and CD8+ memory T-cells were characterized: effector memory T-cells (T_{EM}; CD45RA-CCR7−), effector memory T-cells re-expressing CD45RA (T_{EMRA}; CD45RA + CCR7−), central memory T-cells (T_{CM}; CD45RA-CCR7+). Each T-cell subpopulation was also characterized based on the production of either IFNγ or IL-17A. Cells were analyzed by using a BD FACS Fortessa x20 for T-cell subset analysis and for IFNγ and IL-17A production. Flow data were analyzed using the FlowJo v10 software (TreeStar).

All cases of SARS-CoV-2 breakthrough infection in our cohort were registered, with details regarding disease severity, duration, and outcome. We considered December 22, 2021, to be the cutoff date after which the SARS-CoV-2 variant of concern (VOC) Omicron became prevalent in Italy, based on the national epidemiological data.[3, 4] To estimate the vaccination's efficacy in reducing the severity of Covid-19, we compared our results with those collected in a group of patients with HMs, treated at our Institution, who had been affected by Covid-19 during the pre-vaccination period of the pandemic. A detailed description of ELISpot assay, FACS and statistical analysis can be found in Supplemental Material S1. The study was approved by the Institutional Review Board (protocol No. 84–2021) and conducted in accordance with the Helsinki Declaration of 1975 as revised in 2013. All patients signed a written informed consent. The study was registered at ClinicalTrials.gov (identifier, NCT04878822).

Between April 14 and July 26, 2021, we enrolled 414 patients with HMs who had been administered anti-SARS-CoV-2 vaccination. At data cutoff (February 21, 2022), 365 patients vaccinated with the double-dose regimen of mRNA vaccines entered the analysis (Supplementary Figure 1). Patients' characteristics at the time of the first vaccination cycle are reported in Supplementary Table 2. Overall, 298 out of 365 patients (82%) developed a humoral immune response. Lower seroconversion rates were observed in patients receiving anti-CD20-based therapies (4%), BTK inhibitors (42%), JAK2 inhibitors (68%), and daratumumab-based therapies (69%). Detailed results of serology testing are reported in Supplementary Table 3 and Supplementary Figure 2. Multivariate analysis showed that diagnosis of lymphoma (RR 3.01, 95% CI 1.53–5.93; $p = .0014$), immunotherapy (RR 9.42, 95% CI 2.66–33.33; $p = .0005$), treatment with biologics (RR 4.05, 95% CI 1.29–12.71; $p = .0166$), having SCT



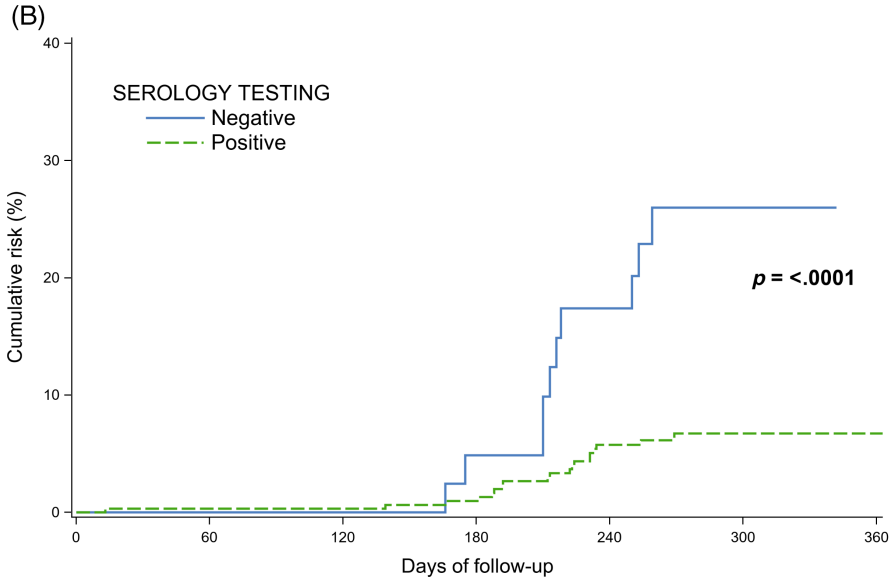


FIGURE 1 Serology test results and SARS-CoV-2 breakthrough infections. Visual description of serology test results and distribution of breakthrough infections during follow-up (A). Cumulative risk of SARS-CoV-2 breakthrough infection according to seroconversion status after vaccination (B). NEG, seronegative; POS, seropositive.

as last treatment (RR 5.59, 95% CI 1.16–26.92; $p = .0319$), and being on active treatment (RR 8.09, 95% CI 2.93–22.31; $p < .0001$) were all significantly associated with negative serology testing (Supplementary Table 4). The overall GM titer of Abs in the subset of seropositive patients was 778 BAU/ml (95% CI 658–920). Supplementary Figure 3 shows that advancing age, active disease, and being on active treatment were significantly associated with a lower GM titer.

Between September 19 and October 15, 2021, 57 out of 67 patients who were seronegative after the first vaccination cycle received a booster dose. All patients were vaccinated with homologous booster vaccines (49 with BNT162b2 and 8 with mRNA-1273). Demographic and clinical characteristics of this subgroup are summarized in Supplementary Table 5.

The administration of a booster dose of vaccine led to seroconversion in 21 (37%).

Notably, in the subset of patients who seroconverted after the booster dose the GM titer of Abs was 258 BAU/ml (95% CI 141–473), significantly lower than the one we found after the first vaccination cycle (778 BAU/ml; $p = .0009$). Results of serology testing after booster vaccination are summarized in Supplementary Figure 4. The ELISpot analysis was performed on 107 patients; among those, 63 were seronegative and 44 were seropositive after double-dose vaccination. Results stratified by demographic and clinical characteristics are reported in Supplementary Table 6. The multivariate analysis showed active disease status to be significantly associated with a lower probability of a positive response to ELISpot assay (RR 0.39, 95% CI 0.19–0.80; $p = .0103$) (Supplementary Table 7). Among the 107 patients who underwent ELISpot analysis, FACS was also performed in a subgroup of 92 to characterize different subpopulations of T-cells. As summarized in Supplementary Figures 5–7, anti-SARS-CoV-2 vaccination stimulates the development of spike-specific memory T-cells

across both seropositive and seronegative patients, although generally lower in the latter.

During a median follow-up of 269 days (min-max, 13–356), 29 patients (8%) developed a SARS-CoV-2 breakthrough infection, with a rate of 2.98 per 10 000 person-days (Figure 1A).

Anti-SARS-CoV-2 vaccination seems to be less effective in preventing infection by the VOC Omicron, as the incidence of breakthrough infections has risen from 1.17 per 10 000 person-days to 9.82 per 10 000 person-days after the spread of Omicron ($p < .0001$). Overall, infection occurred at a median time of 105 days (min-max, 26–257) from the last vaccine dose. This time did not differ significantly before and after the spread of Omicron (149 vs. 103 days; $p = .9627$). The majority of patients (26 out of 29) developed non-severe Covid-19. Seropositive status after vaccination was associated with a lower cumulative risk of breakthrough infection as compared to seronegative status ($p < .0001$) (Figure 1B). This observation was confirmed by a Cox model, which found a lower risk of post-vaccination Covid-19 in seropositive patients (HR 0.11, 95% CI 0.03–0.43; $p = .0017$). Conversely, cellular immunity evaluated by ELISpot did not appear to correlate with the risk of breakthrough infection. By comparing clinical characteristics of Covid-19 diagnosed prior to vaccination versus after vaccination in patients with HMs, we found that the rate of severe or critical disease (10% vs. 33%; $p = .0242$), the rate of hospitalization (17% vs. 50%; $p = .0024$), and the median duration of disease (16 days vs. 22 days; $p = .0094$) were all significantly lower in vaccinated patients. As shown in Supplementary Table 8, the two subgroups of patients affected by Covid-19 (pre-vaccination vs. post-vaccination) are similar in terms of demographic and clinical characteristics.

This study has some limitations, such as the absence of a baseline immune evaluation. However, this was balanced out by a careful anamnestic evaluation at enrollment, which led to the exclusion of 7% of all patients enrolled because of previous SARS-CoV-2 infection. This rate was comparable to the rate of local cumulative Covid-19 cases during the enrollment phase (10%).[5] As previously reported,[6] our study showed that anti-SARS-CoV-2 vaccination is associated with lower immunogenicity in patients with HMs. The seroconversion rate after full vaccination was 82%; the seroconversion rate in 57 seronegative patients who underwent booster vaccination was 37%. Patients undergoing active treatment, especially with anti-CD20 and anti-CD38 monoclonal antibodies, BTK inhibitors, and JAK2 inhibitors are at high risk of seroconversion failure. Cell-mediated immunity showed positive responses across seropositive and seronegative patients. The incidence of Covid-19 was 2.98 per 10 000 person-days, lower in seropositive patients. The rate of severe/critical Covid-19, of hospitalization, and the median duration of disease were all significantly lower in vaccinated patients as compared to non-vaccinated patients.

AUTHOR CONTRIBUTIONS

Francesco Passamonti, Marco Salvini, Paolo A. Grossi, Fabrizio Maggi, Lorenzo Mortara, Antonino Bruno designed research and analyzed data; Fabrizio Maggi and Andreina Baj performed the serological analysis; Lorenzo Mortara and Raffaella Bombelli performed the ELISpot analysis; Antonino Bruno and Matteo Gallazzi performed the FACS analysis; Giacomo Pellegrini and Matteo Franchi did the statistical analysis; Marco Salvini, Camilla Damonte, Francesco Passamonti, Lorenzo Mortara, and Antonino Bruno wrote the paper; Camilla Damonte, Barbara Mora, Marco Brociner, Alessia Ingrassia, Roberta Mattarucchi, Benedetta Bianchi, Davide Sirocchi, Stefania Agnoli, Elisa Rumi, Michele Merli, Alessandro Fossati, Susanna Bassi, and Oscar Borsani collected clinical data.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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SUPPORTING INFORMATION

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