Reduced immunogenicity of a third COVID-19 vaccination among recipients of allogeneic haematopoietic stem cell transplantation

by Sigrun Einarsdottir, Ann Martner, Malin Nicklasson, Hanna Grauers Wiktorin, Mohamma Arabpour, Andrea Törnell, Krista Vaht, Jesper Waldenström, Johan Ringlander, Tomas Bergström, Mats Brune, Kristoffer Hellstrand, Per Ljungman, and Martin Lagging

Received: December 9, 2021.
Accepted: February 22, 2022.


Publisher's Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors’ final approval; the final version of the manuscript will then appear in a regular issue of the journal. All legal disclaimers that apply to the journal also pertain to this production process.
Reduced immunogenicity of a third COVID-19 vaccination among recipients of allogeneic haematopoietic stem cell transplantation

Sigrun Einarsdottir¹, Anna Martner², Malin Nicklasson¹, Hanna Grauers Wiktorin², Mohammad Arabpour², Andreas Törnell², Krista Vaht¹, Jesper Waldenström³, Johan Ringlander³, Tomas Bergström³, Mats Brune¹, Kristoffer Hellstrand², Per Ljungman⁵, and Martin Lagging³,⁴*

¹Department of Hematology and Coagulation, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Sweden, ²TIMM Laboratory, Sahlgrenska Center for Cancer Research, Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, ³Region Västra Götaland, Sahlgrenska University Hospital, Department of Clinical Microbiology, Gothenburg, Sweden, ⁴Department of Infectious Diseases, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden, ⁵Department of Cellular Therapy and Allogeneic Stem Cell Transplantation, Karolinska Comprehensive Cancer Center, Karolinska University Hospital, Huddinge and Division of Hematology, Department of Medicine, Huddinge, Karolinska Institutet, Stockholm, Sweden.

*Correspondence to: Martin Lagging Professor, MD, PhD, Dept. of Infectious Diseases/Virology, Guldhedsgatan 10B, SE 413 46 Gothenburg, Sweden Telephone: +46705683759, FAX: +4631411256, E-mail: martin.lagging@medfak.gu.se

Running Title: Response to 3rd dose Covid vaccine in allo-HSCT

Competing Interests: The authors have nothing to disclose.

Clinical trial registration: EudraCT 2021-000349-42
Acknowledgements: This work was supported by the Swedish Medical Research Council (Vetenskapsrådet; Diarienr 2021-04779) and ALF Funds at Sahlgrenska University Hospital (Diarienr ALFGBG-438371).

Author Contributions: SE and ML were responsible for designing and writing the protocol, conducting the study, extracting and analysing data, interpreting results, writing the letter, updating reference lists and creating the table and figure. AM and KH were responsible for designing and writing the protocol, extracting and analysing data, interpreting results, writing the letter, updating reference lists and creating the table and figure. MN participated in interpreting results, writing the letter, and creating the figure. HGW, MA, and AT were responsible for performed the T cell as well as participated in extracting and analysing data and interpreting results. KV, JW, and JR participated in extracting and analysing data and interpreting results. TB, MB, and PL participated in designing and writing the protocol, interpreting results, writing the letter, and updating the reference list.

Data sharing statement: The original data and protocols may be made available to other investigators after contact with the corresponding author.
To the editor

Previous allogeneic haematopoietic stem cell transplantation (allo-HSCT) is a risk factor for severe COVID-19 with mortality rates that may exceed 20%\textsuperscript{1,2}. The efficiency of two doses of mRNA-based COVID-19 vaccines is reportedly lower in allo-transplanted patients than in healthy controls with rates of seronegativity or failure to seroconvert in 15-31%\textsuperscript{3-6}. In a study of allo-transplanted patients with insufficient responses to two doses of the BNT162b2 (Pfizer-BioNTech) mRNA vaccine, only 48% of patients reached a putative threshold (4160 arbitrary units (AU)/ml, corresponding to 590 WHO standard binding antibody units (BAU)/ml) of IgG against the receptor-binding domain of the spike 1 (S1) protein (anti-RBD) following a 3\textsuperscript{rd} vaccine dose\textsuperscript{7}.

Forty recipients of allo-HSCT for haematological malignancies were identified in local transplant registries of the Region Western Götaland (population of approximately 1.6 million) and accepted participation in this sub-study within the DurIRVac study (EudraCT no. 2021-000349-42), at the Sahlgrenska University Hospital. All participants gave written informed consent before enrolment. The DurIRVac study was approved by the Swedish Ethical Review Authority (permit no. 2020-03276, 2021-00374 and 2021-00539) and by the Swedish Medical Products Agency (permit no. 5.1-2021-11118). All patients fulfilled national criteria from the Public Health Agency of Sweden (www.folkhalsomyndigheten.se) for receiving a third dose namely: (i) having undergone transplantation within three years or (ii) having ongoing immunosuppressive treatment for graft-versus-host-disease (GvHD). The European Society for Blood and Marrow transplantation (EBMT) guidelines for COVID-19 vaccination were also followed (www.ebmt.org; Version 6.0, May 31, 2021). Three patients
were excluded based on previously confirmed COVID-19. All enrolled patients \((n=37)\) had received 2 doses of COVID-19 mRNA vaccine \(\geq 8\) weeks prior to screening.

The median time from transplantation to the 3rd vaccination was 23 months (min-max 6-191). Twenty-one (57\%) of participants had chronic GvHD and 25 (68\%) received immunosuppressive treatment at the time of vaccination (Table 1). Patients were given the same vaccine as in their initial 2 doses, \(i.e.,\) either BNT162b2 (Pfizer-BioNTech Comirnaty\(^\circledR\); \(n=24\)) or mRNA-1273 (Moderna Spikevax\(^\circledR\); \(n=13\)) at a median 127 days (min-max 56-174) after the 2nd dose. Peripheral blood was collected immediately before and 4 weeks (median 24 days, range 19-30) after the 3rd vaccination. Patients completed a questionnaire 2 weeks after the 3rd dose to assess side-effects, categorized according to the CTCAE (Common Terminology Criteria for Adverse Events) standards. Severity of GvHD was additionally assessed from medical records.

Chemiluminescent microparticle immunoassays were performed on serum using the automated Alinity system for analysis of IgG antibodies against RBD (SARS-CoV-2 IgG II Quant, Abbott, Illinois, USA) with levels reported in the WHO international standard BAU/ml (quantitative detection range of 14 to 5680 BAU/ml), which correlate well with neutralizing antibody levels \(^8\). To assess T cell responses one ml of peripheral blood, collected in heparinized tubes, was stimulated with peptides spanning the N-terminal spike 1 (S1) domain of the SARS-CoV-2 surface glycoprotein. After two days of incubation at 37\(^\circ\)C plasma was recovered for analysis of interferon-\(\gamma\) (IFN-\(\gamma\)) by ELISA. This assay captures SARS-CoV-2-specific reactivity of CD4\(^+\) and CD8\(^+\) T cells with high specificity and sensitivity \(^9\). S1-induced IFN-\(\gamma\) production is presented with levels in unstimulated samples.
subtracted using a limit of detection of 10 pg/ml. Statistical analyses were performed using SPSS statistical software package (version 24) or GraphPad Prism software (version 9).

The majority (31/37, 84%) of allo-HSCT patients responded to 3rd dose vaccination by increased anti-RBD IgG levels (Figure 1A). A subgroup (12/37, 32%) achieved very high antibody levels (>5680 BAU/ml). However, among the 14 patients seronegative prior to the third dose vaccination, six (42%) remained seronegative four weeks after the third vaccine dose (Figure 1A). All patients who were seropositive before the third dose (23/37, 62%) achieved antibody responses exceeding 100 BAU/ml, a level above which has been proposed to provide protection against COVID-19 10. The characteristics of responders and non-responders to the 3rd vaccine dose are detailed in Table 1. No significant differences in serological responses were noted among patients with or without chronic GvHD or ongoing immunosuppressive therapy.

Regarding T cell immune response, 18/37 (49%) were devoid of measurable responses four weeks after the 3rd vaccination (Figure 1B). T cell responses tended to be lower in patients with chronic GvHD and were significantly diminished in patients receiving immunosuppressive therapy (IST), in particular among those receiving prednisone (Table 1). Seronegativity prior to the third dose predicted poor humoral and cellular responses after vaccination. Treatment with IST was associated with insufficient T cell responses, more so than time from transplantation. Furthermore, 4/5 (80%) of patients on ruxolitinib showed no T cell reactivity. Of note, among the 14 patients who were seronegative for anti-RBD IgG prior to the third dose, 11 (79%) also lacked a T cell response after three vaccine doses, compared with 7/23 (30%) among those seropositive prior to the 3rd dose (P<0.01, Chi-square test). Seronegativity prior to the 3rd vaccination was non-significantly associated with ongoing
GvHD (9/14 vs. 12/23 in seropositive patients) and immunosuppressive therapy (10/14 vs. 15/23). Additionally, a lower fraction of patients mounted SARS-CoV-2 specific T cell responses than developed anti-RBD IgG after 3 vaccinations (P<0.01, Chi-square test). Out of 6 patients who remained seronegative after three vaccine doses, 5 (83%) were also devoid of specific T cells. Vaccine-reported adverse events were observed in 15 (41%) patients after the 3rd dose, with the majority of these categorized as mild local injection-related reactions. No exacerbations of GvHD were noted.

The main findings in this study were that a significant fraction of allo-transplanted patients failed to produce anti-RBD IgG (16%) and that 48% of patients did not mount measurable SARS-CoV-specific T cells despite three vaccinations. Our results confirm and extend a previous report of insufficient anti-RBD responses among allo-transplanted patients 7 to imply that the inherent and treatment-induced T cell deficiency associated with allo-transplantation may translate into lack of COVID-19 mRNA vaccine efficacy. The interval between dose 2 and 3 was longer among patients remaining seronegative following the third dose, implying that a shorter interval between vaccinations may improve responses.

Our results additionally suggest that the SARS-CoV-2-specific T cell response to vaccination is more affected than the humoral response among allo-transplanted patients, based on the finding that a significantly higher fraction of patients showed complete deficiency of T cell responsiveness to SARS-CoV-2-derived peptides compared with those remaining seronegative. Using the same T cell assay, we have previously shown that 13/13 (100%) of healthy donors developed detectable T cell responses four weeks after the 2nd SARS-CoV-2 vaccine dose 11. Notably, 35% of allo-transplanted patients lacked T cell reactivity against S1
peptides despite mounting anti-RBD IgG. The clinical relevance of the observed T cell deficiency remains to be established.

In conclusion, the 3rd dose of COVID-19 mRNA vaccine resulted in elevated antibody titres and measurable SARS-CoV-2-S1 T cell responses in many allo-transplanted patients. However, a substantial proportion of patients did not respond by antibody formation and/or SARS-CoV-2-specific T cells, highlighting the need for additional preventive measures and continued vigilance in this cohort.
References


<table>
<thead>
<tr>
<th></th>
<th>Anti-RBD IgG in serum</th>
<th>T cell reactivity (S1-γ)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (≥14 BAU/mL)</td>
<td>Negative (&lt;14 BAU/mL)</td>
</tr>
<tr>
<td><strong>All patients</strong>$^5$</td>
<td>31 (84%)</td>
<td>6 (16%)</td>
</tr>
<tr>
<td><strong>Vaccine</strong> (Pfizer/Moderna)</td>
<td>21/10</td>
<td>3/3</td>
</tr>
<tr>
<td><strong>Age at vaccination median (range)</strong></td>
<td>60 (19-78)</td>
<td>63 (32-72)</td>
</tr>
<tr>
<td><strong>Median days between dose 2 and 3 (range)</strong></td>
<td>123 (56-157)</td>
<td>139 (127-174)</td>
</tr>
<tr>
<td><strong>Median months from allo-HSCT, (range)</strong></td>
<td>26 (6-188)</td>
<td>19 (13-191)</td>
</tr>
<tr>
<td><strong>Ongoing IST$^6$ (yes/no)</strong></td>
<td>20/11</td>
<td>5/1</td>
</tr>
<tr>
<td><strong>Ongoing prednisone (yes/no)</strong></td>
<td>16/15</td>
<td>4/2</td>
</tr>
</tbody>
</table>

1,2 All comparisons refer to patients responding or not responding to third dose vaccination by anti-RBD IgG1 or SARS-CoV-2-specific T cell reactivity.

3 MannWhitney U-test

4 Chi-square test

5 Patients had received allogenic HCT for acute myeloid leukemia (n=15 patients), acute lymphoblastic leukemia (n=4), myelodysplastic syndrome (n=5), myelofibrosis (n=4), chronic myeloid leukemia (n=4), atypical chronic myeloid leukemia (n=1), myeloma (n=1), chronic lymphocytic leukemia (n=1), Hodgkin’s disease (n=1), and STAT-1 immune deficiency (n=1).
Immunosuppressive therapy comprising prednisone (n=20 patients), photopheresis (n=5), ibrutinib (n=4), ruxolitinib (n=5), photopheresis (n=5), dasatinib (n=2), cyclosporine (n=2), daratumumab (n=1), imatinib (n=1), carfilzomib (n=1), and/or ponatinib (n=1)
**Figure Legend**

**Figure 1.** Serological and virus-specific T cell responses to the S1 region of SARS-CoV-2 before and after 3 doses of COVID-19 vaccines in allo-transplanted patients. A shows serum levels of IgG against the receptor-binding domain (RBD). B shows IFN-γ production in supernatant plasma following stimulation of whole blood with 15 11-mer spike 1 peptides, reflecting reactivity of SARS-CoV-2-specific T cells. The upper dotted line represents the cut-off value of 590 BAU/ml (i.e., corresponding to 4160 Abbott Arbitrary Units (AU) /ml) while the lower dotted line represents the limit of detection (LOD) for respective assay. Statistical comparison by Wilcoxon matched pairs test (n=37). P-values are two-sided and are designated as follows: **P<0.01, ****P<0.0001.