



## Note

## Patients with B-cell malignancies experience reduced antibody responses with class switching defect following BNT162b2 SARS-CoV-2 vaccination

Yu Nakagama<sup>a,b</sup>, Sung-Gi Chi<sup>c</sup>, Yosuke Minami<sup>c</sup>, Reiko Watanabe<sup>d</sup>, Michiteru Yamagishi<sup>e</sup>, Uno Atsuko<sup>e</sup>, Yasutoshi Kido<sup>a,b,\*</sup>

<sup>a</sup> Department of Parasitology, Graduate School of Medicine, Osaka Metropolitan University, 1-4-3 Asahi-machi, Abeno-ku, Osaka, 545-8585, Japan

<sup>b</sup> Research Center for Infectious Disease Sciences, Graduate School of Medicine, Osaka Metropolitan University, 1-4-3 Asahi-machi, Abeno-ku, Osaka, 545-8585, Japan

<sup>c</sup> Department of Hematology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba, 277-8577, Japan

<sup>d</sup> Department of Pathology and Clinical Laboratories, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba, 277-8577, Japan

<sup>e</sup> Clinical Laboratory Department, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba, 277-8577, Japan



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## ABSTRACT

Vaccines having aided in escaping the majority of the population from immunological naïvety, our strategies are now shifting towards an increased focus on identifying and protecting the extremely vulnerable. We here describe the results of testing 12 patients, those with lymphoid malignancies having been targeted their B-cells for therapy with rituximab-containing regimens or a Bruton tyrosine kinase inhibitor, for anti-SARS-CoV-2 spike antibodies after receiving the BNT162b2 mRNA vaccine doses. The interval from last dosing of B-cell depletion therapy to SARS-CoV-2 vaccination was at median 5.3 (range 3.1–6.6) months. Using the ‘seroprotection’ threshold of 775 [BAU/mL] for the anti-spike antibody titer, our finding points out the crucial unresponsiveness of the targeted population with 0/12 (0%) achieving ‘seroprotection’. Although IgG seroconversion was observed in 4/12 (33%), supporting the overall benefit of vaccination, the figures still point out a potential need for optimization of practice. IgA was further less responsive (unsuccessful ‘seroconversion’ in 11/12 (92%)), implicating an underlying class switch defect. Those with depletion on B-cells are caught at a dilemma between, being too early and too late on receiving SARS-CoV-2 vaccines. They wish to get over their immunological naïvety at the earliest, while, in order to assure quality immune memory, are also required to hold the patience for their B-cells to repopulate. Although it remains an issue whether intensified vaccine schedules and/or regimens will lead to stronger immunogenicity or more effective boosters for non-responders, we shall take advantage of every increasing evidence in order to optimize current options.

SARS-CoV-2 vaccines having aided in escaping the majority of the population from immunological naïvety, our strategies against the pandemic are now shifting towards an increased focus on identifying and protecting the persistently vulnerable. The increased risk of morbimortality against COVID-19 in patients with hematological malignancies, having been observed ever since the beginning of the pandemic [1], seemingly extends beyond the completion of the scheduled vaccine doses [2]. The population has been given highest priority in the Japanese national SARS-CoV-2 vaccination campaign, while their immunological response to the initial vaccine doses has been less intensely studied. We thus planned to prospectively monitor the vaccine’s immunogenicity in patients with lymphoid malignancies with specific

focus on those receiving B-cell-directed therapy for treatment.

The study was approved by the Osaka Metropolitan University Institutional Ethics Committee (#2020–101) and performed upon written consent from the participants. Participants provided sera for anti-SARS-CoV-2 serological testing at median 2.0 (range 1.1–4.7) months after the second scheduled Pfizer-BioNTech BNT162b2 mRNA vaccine dose. Clinical variables, regarding the diagnosis of underlying malignancy, treatment regimen, its timing, disease status, absolute lymphocyte count before the first dose of vaccination, and the dates of the two scheduled vaccine doses were recorded. Anti-spike IgG and IgA antibody titers were measured with the Abbott Architect SARS-CoV-2 IgG II Quant (Chicago, IL, USA) and EUROIMMUN anti-SARS-CoV-2

\* Corresponding author. Department of Parasitology, Graduate School of Medicine, Osaka Metropolitan University, 1-4-3 Asahi-machi, Abeno-ku, Osaka, 545-8585, Japan.

E-mail address: [kidoyasu@omu.ac.jp](mailto:kidoyasu@omu.ac.jp) (Y. Kido).

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ELISA IgA (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany) immunoassays, respectively. Rates of achieving ‘seroconversion’ and ‘seroprotection’ were assessed. Successful ‘seroconversion’ was defined as a titer  $\geq 50$  [AU/mL] for anti-spike IgG, and a titer  $\geq 1.1$  [S/C ratio] for anti-spike IgA according to the manufacturers’ definitions. Extrapolating from a previous clinical trial estimating the 90%-efficacy antibody titer in preventing symptomatic COVID-19 [3], the ‘seroprotection’ threshold was defined as a post-vaccine IgG antibody titer of 775 [BAU/mL] in the WHO standard unit, equivalent to 5458 [AU/mL] (derived from division with the assay-specific conversion factor of 0.142). Comparisons of titer used the Mann-Whitney *U* test and two-sided *p*-values  $< 0.05$  were considered significant.

Twelve patients (8/12 were male), aged at median 71.5 (range 41–88) years old and having received B-cell-directed therapy for their treatment of lymphoma/leukemia (11 with rituximab-containing regimens (RTX); 1 with Bruton tyrosine kinase inhibitor (BTKi)) at the National Cancer Center Hospital East, Kashiwa, Japan, composed the ‘RTX/BTKi’ group. 11/11 (100%) and 0/1 (0%) of those having been treated with RTX and BTKi, respectively, were in remission. All participants were seronegative against SARS-CoV-2 upon entry and were monitored of their humoral immune response to the scheduled two doses of the BNT162b2 mRNA vaccine. Patients of the RTX/BTKi group demonstrated significantly attenuated anti-spike antibody responses compared with vaccine recipients with other lymphoid malignancies (‘Other’ group,  $n = 5$ ; one each with multiple myeloma, myeloproliferative neoplasm, myeloid sarcoma, anaplastic large cell lymphoma, and peripheral T-cell lymphoma) visiting the department (3/5 were male; aged at median 50 years old, range 29–59) (Fig. 1). Between the RTX/BTKi and Other groups, there was a surprising 280-fold difference in their anti-spike IgG geometric mean titer [95% confidence interval] (10.3 [1.1–93.2] vs. 2889 [790–10570] AU/mL,  $p = 0.0013$ ). Successful IgG seroconversion was achieved in 4/12 (33%) of the patients with B-cell malignancies on RTX/BTKi, supporting the overall benefit of vaccination. However, their rate of seroconversion was relatively low compared with 5/5 (100%) in the Other group. More importantly, seroprotection was achieved in 0/12 (0%) participants from the RTX/BTKi group, while 1/5 (20%) from the Other group achieved seroprotection. In a recent study from Japan targeting the healthy population, 67% were seroprotected after the second dose [4]. A similar study targeting patients with

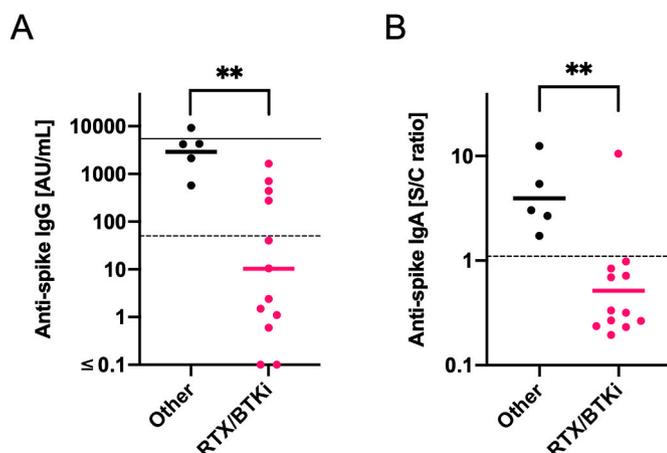
lung cancer showed an overall seroprotection rate of 25% [5]. Altogether, our observation adds to the evidence that patients with hematological malignancies are among the most compromised of the post-vaccine immune response. Anti-spike IgA response was significantly reduced ( $p = 0.0039$ ) in the RTX/BTKi group when compared with that of the Other group. Interestingly, the rate of successful IgA seroconversion decreased to as low as 1/12 (8%) (Figure B). The finding may indicate a further class switching defect from IgG to IgA in the RTX- or BTKi-exposed participants. The interval from last RTX or BTKi dosing to SARS-CoV-2 vaccination in our cohort was at median 5.3 (range 3.1–6.6) months, reaching the proposed ‘vaccine-responsive’ threshold of 9 months in none of our non-seroconverted individuals [6]. Low lymphocyte count has also been reported to predict reduced response to SARS-CoV-2 vaccines, likewise that to the influenza vaccines [7]. Excluding the one participant with active, non-remitting disease, the proposed threshold lymphocyte count of 900/ $\mu$ L at the timing of vaccination was achieved in only 5/8 (63%) of the non-seroconverted RTX/BTKi group participants, in contrast to 3/4 (75%) of the seroconverted individuals.

The present study indicates a reduced vaccine response in patients with B-cell-malignancies recently exposed to RTX or BTKi. The finding adds to the literature on real-world immunogenicity data from vaccine recipients carrying lymphoid malignancies with implications to a further defect on the class switching machinery [8–12]. For the utmost SARS-CoV-2 vulnerable populations, several nations have adopted aggressive vaccination programs, where individuals with an insufficient serological response following the initial dose(s) have been allotted an extra booster dose [13]. In fact, a third dose has shown positive impact on neutralizing antibody titer for responders of the initial dosing, while those who failed to respond to the initial vaccination remained seronegative even after the third dose [14]. Here again, the interval between last RTX administration and date of boosting was a predictor of positive IgG response. Another recent study has shown that a booster dose, administered at least 3 months after and up to 6 months following the second dose, potentiated the neutralizing antibody response in a significant portion of RTX/BTKi recipients and more so in those not on active RTX/BTKi therapy [15]. Very importantly, the only two participants from our RTX/BTKi cohort, having received the third booster dose, both remained non-responsive: one with a 10-months interval since the last RTX dose and one on active BTKi therapy. The incremental protective effect of booster vaccinations is a subject requiring further appraisal in our RTX/BTKi cohort.

Patients with hematological malignancies who fail to respond to a full vaccination can develop severe and often fatal COVID-19 disease. Japanese guidelines have recommended a delay of, at minimum, 6 months following the last dosing of B-cell-directed therapy for the administration of SARS-CoV-2 vaccines to patients with B-cell malignancies, based on the wealth of data on their reduced humoral immune responses to preceding vaccines. To optimize the seroconversion rate, however, the interval following the last dosing of B-cell-directed therapy may better be set longer than what is currently pursued in practice. Another option worth consideration for enhancing vaccine efficacy in poor responders may be intensifying of booster dosing regimens. Needless to say, adherence to non-pharmacological interventions and household vaccination remain to be important measures in mitigating the pandemic.

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**Figure 1.** Post-vaccine anti-spike antibody titer in patients with B-cell malignancies on rituximab-containing regimens (RTX) or a Bruton tyrosine kinase inhibitor (BTKi), in comparison to those with other lymphoid malignancies. Presented are anti-spike (A) IgG, and (B) IgA titers, measured using the Abbott Architect SARS-CoV-2 IgG II Quant and the Euroimmun Anti-SARS-CoV-2 IgA immunoassays, respectively. Bars indicate the geometric mean titers. Dotted and solid horizontal lines indicate the seroconversion and seroprotection thresholds, respectively. Level of statistical significance is indicated as follows: \*\* $p < 0.01$ .

## Authorship statement

YN, SGC, YM, and YK designed the study; SGC, YM, RW, MY and UA enrolled the participants; RW, MY and UA were responsible for the specimen/data collection and processing; YN and YK performed the serological analyses; YN and SGC analyzed the data; YN drafted the manuscript; SGC, YM, RW, MY, UA and YK critically reviewed and revised the manuscript; and all authors approved of the final version of the manuscript.

## Conflict of interest disclosures

None.

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