Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Serum neutralization of SARS-CoV-2 Omicron sublineages BA.1 and BA.2, and cellular immune responses 3 months after booster vaccination

Enagnon Kazali Alidjinou, Julie Demaret, Bénédicte Corroyer-Simovic, Fanny Vuotto, Sophie Miczek, Julien Labreuche, Anne Goffard, Jacques Trauet, Daniela Lupau, Arnaud Dendooven, Dominique Huvent-Grelle, Juliette Podvin, Daniel Dreuil, Karine Faure, Dominique Deplanque, Laurence Bocket, Alain Duhamel, Annie Sobaszek, Didier Hober, Michael Hisbergues, Francois Puisieux, Brigitte Autran, Yazdan Yazdanpanah, Myriam Labalette, Guillaume Lefèvre

PII: S1198-743X(22)00528-6
DOI: https://doi.org/10.1016/j.cmi.2022.10.014
Reference: CMI 3099

To appear in: Clinical Microbiology and Infection

Received Date: 26 June 2022
Revised Date: 7 October 2022
Accepted Date: 9 October 2022

Please cite this article as: Alidjinou EK, Demaret J, Corroyer-Simovic B, Vuotto F, Miczek S, Labreuche J, Goffard A, Trauet J, Lupau D, Dendooven A, Huvent-Grelle D, Podvin J, Dreuil D, Faure K, Deplanque D, Bocket L, Duhamel A, Sobaszek A, Hober D, Hisbergues M, Puisieux F, Autran B, Yazdanpanah Y, Labalette M, Lefèvre G, Serum neutralization of SARS-CoV-2 Omicron sublineages BA.1 and BA.2, and cellular immune responses 3 months after booster vaccination, Clinical Microbiology and Infection, https://doi.org/10.1016/j.cmi.2022.10.014.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.
Research Note

Serum neutralization of SARS-CoV-2 Omicron sublineages BA.1 and BA.2, and cellular immune responses 3 months after booster vaccination

Enagnon Kazali Alidjinou 1, Julie Demaret 2, Bénédicte Corroyer-Simovic 3, Fanny Vuotto 4, Sophie Miczek 5, Julien Labreuche 6, Anne Goffard 7, Jacques Trauet 2, Daniela Lupau 2, Arnaud Dendooven 2, Dominique Huvent-Grelle 3, Juliette Podvin 3, Daniel Dreuil 3, Karine Faure 4, Dominique Deplanque 8, Laurence Bocket 1, Alain Duhamel 9, Annie Sobaszek 10, Didier Hober 1, Michael Hisbergues 11, François Puisieux 3, Brigitte Autran 12, Yazdan Yazdanpanah 13, Myriam Labalette 12, Guillaume Lefèvre 21.

†‡These authors have contributed equally to this work and share authorship

Affiliations:

1) Univ Lille, CHU Lille, Laboratoire de Virologie ULR3610, F-59000, Lille, France.
2) CHU Lille, Institut d’Immunologie, U1286 - INFINITE - Institute for Translational Research in Inflammation Inserm Univ. Lille, Lille, F-59000, Lille, France.
3) CHU Lille, Pôle de Gériatrie, Hôpital gériatrique Les Bateliers, CHU de Lille, Université de Lille, F-59000, Lille, France.
4) CHU Lille, Département de Maladies Infectieuses, F-59000 Lille France.
5) CHU Lille, Médecine et santé-travail, CHU Lille, F-59000 Lille France.
6) CHU Lille, Department of Biostatistics, F59000-Lille, France.
7) Université Lille, CNRS, Inserm, CHU Lille, Institut Pasteur de Lille, U1019 - UMR 8204 - CIIL 2011.
8) U1019 - CIIL-Centre d’Infection et d’Immunité de Lille Centre d’Infection et d’Immunité de Lille, F-59000 Lille, France ; Clinical Microbiology Unit, Institut Pasteur de Lille, F-59000 Lille, France.
9) Université Lille, CHU Lille, EA 2694 - Santé publique : épidémiologie et qualité des soins, Université de Lille, Lille, Hauts-de-France, France.
10) CHU Lille, Médecine et santé-travail, Univ. Lille, CHU Lille, ULR 4483, IMPECS, F-59000 Lille France.
11) CHU Lille, Université Lille, Univ.Lille, Centre de Ressources Biologiques, F-59000 Lille France.
12) Sorbonne-Université, Paris, France; UMR-S Inserm/UPMC 1135, CIMI-Paris (Centre de Recherches Immunité Maladies Infectieuses), Paris, France.
13) INSERM, IAME, Hôpital Bichat - Claude-Bernard, Infectious Diseases Department, France.

Corresponding author:

Guillaume Lefèvre, Institut d’Immunologie, F-59000 Lille France ; Phone: +33 3 62 94 38 55 ; Email: Guillaume.LEFEVRE@chu-lille.fr
Abstract

Objectives
We investigated serum neutralizing activity against BA.1 and BA.2 Omicron sublineages, and T cell response before and 3 months after booster vaccine in healthcare workers (HCWs).

Methods
HCWs aged 18–65 years, vaccinated and boosted with the BNT162b2 vaccine were included. Anti-SARS-CoV-2 IgG levels and cellular response (through IFNγ ELISpot assay) were evaluated in all participants, and neutralizing antibodies against Delta, BA.1 and BA.2 were evaluated in participants with at least one follow-up visit 1 or 3 months after the booster dose.

Results
Among the 118 HCWs who received the booster dose, 102 and 84 participants attended the 1-month and 3-month visits, respectively. Before the booster vaccine dose, a low serum neutralizing activity against Delta, BA.1, and BA.2 was detectable in only n=39/102 (38.2%), n=8/102 (7.8%) and n=12/102 (11.8%) of the participants, respectively. At 3 months, neutralizing antibodies were detected in n=84/84 (100%), n=79/84 (94%) and n=77/84 (92%) against Delta, BA.1 and BA.2, respectively. Geometric mean titers (GMTs) of neutralizing antibodies against BA.1 and BA.2 were 2.2- and 2.8-fold reduced compared to Delta. From 1 to 3 months after the booster dose, participants with a recent history of SARS-CoV-2 infection (n=21/84) had persistent levels of S1 reactive specific T cells and of neutralizing antibodies against Delta and BA.2, and 2.2-fold increase in neutralizing antibodies against BA.1 (p=0.014). Conversely, neutralizing antibody titers declined from 1 to 3 months after
booster dose in individuals without any recent infection, against Delta (2.5-fold decrease, $p<0.0001$), BA.1 (1.5-fold, $p=0.02$) and BA.2 (2-fold, $p<0.0001$).

**Conclusions**

The booster vaccine dose provided significant and similar response against BA.1 and BA.2 Omicron sublineages, but the immune response declines in the absence of recent infection.
Introduction

The Omicron (B.1.1.529) SARS-CoV-2 variant has emerged in late 2021, and has rapidly outcompeted the already highly transmissible Delta variant. The first sublineage of this variant, BA.1, became dominant in Europe between December 2021 and January 2022 and has generated serious concern about the efficacy of vaccines, due to substantial escape from neutralizing antibodies. Indeed, Omicron displayed numerous epitopic modifications of the spike protein, and as available COVID-19 vaccines were prepared with the original lineage, neutralizing activity against Omicron was absent or very low even in vaccinated people [1,2].

The subsequent sublineage BA.2, has rapidly replaced the previous one in Europe around March-April 2022, and rates of new sublineages BA.4 and BA.5 are increasing by June 2022 [3]. BA.2 has shown a selective advantage over BA.1, especially an enhanced transmissibility, and can reinfect previously BA.1-infected individuals [4,5]. Recent data have shown that beyond 6 months after prime vaccination, neutralizing antibodies against Omicron sublineages were undetectable or at very low levels; however, neutralizing antibody titers increased significantly few weeks after booster vaccination (third dose) [6–8].

In this study, we evaluated neutralizing antibody responses against the BA.1 and BA.2 sublineages of the Omicron variant, in parallel with the previous Delta variant in a cohort of healthcare workers (HCWs) who had been vaccinated and boosted with the BNT162b2 mRNA vaccine (Pfizer–BioNTech). In addition, cellular responses to SARS-CoV-2 were investigated, and the occurrence of infection after booster dose analyzed.

Methods
This study is part of the MONITOCOV project in which the protocol amendments concerning the present data, were approved by the Ile-De-France V (ID-CRB 2021-A00119-32) ethics committee.

Health care workers were consecutively included in the study if they had no significant chronic disease and no medication which could influence immune responses (including steroids, immunosuppressive therapies, ..), and were aged 18–65 years. All participants initially received the two-dose BNT162b2 vaccination at a 3-weeks dosing interval. The booster dose was administered to participants in accordance with French national guidelines (at least six months after primary vaccination course). Data from samples collected before and 3 months after primary vaccination were reported previously [9], and data from samples collected before booster, and at 1- and 3-month post-booster are reported in this study.

Anti-SARS-CoV-2 IgG levels, as well as neutralizing antibodies (using a live virus neutralization assay) against Delta variant, and BA.1 and BA.2 Omicron sublineages were evaluated. Cellular responses were evaluated through IFNγ ELISpot assay.

Detailed methods are provided in the supplementary material.

Results

A total of 118 individuals who received the booster dose were included. Samples were collected at 1- month after booster for 102 subjects, and only 84 participants attended the 3-month visit.

The median (interquartile range, IQR) time between the initial vaccination (second dose) and the booster dose was 254 (248-265) days.

Three months after the primary vaccination course (D1/D2), the median level (IQR) of anti-SARS-CoV-2 IgG was 1125 (653;1828) BAU/mL. This value declined to 290
(200;534) BAU/mL before the third dose, and then peaked to more than 2080 BAU/mL, 
1 and 3 months after the booster dose (Figure 1A). The counts of S1 reactive T cells 
also increased 1 and 3 months after the booster dose (Figure 1A).

As shown in Figure 1B, before the booster vaccine dose, serum neutralizing activity 
against Delta, BA.1, and BA.2 was detectable in only n=39/102 (38.2%), n=8/102 
(7.8%) and n=12/102 (11.8%) of the participants, respectively, with low neutralizing 
antibody titers ranging from 20 to 160. Geometric mean titers (GMTs) were below the 
limit of detection of 20 for all variants.

One month after the booster dose, a neutralizing activity against Delta, BA.1 and BA.2 
was observed in n=102/102 (100%), n=99/102 (97%) and n=101/102 (99%) of tested 
sera, respectively. Also, GMTs of neutralizing antibodies (95% confidence interval, 
CI95%) significantly increased to 465 (373;579) for Delta, 105 (85;131) for BA.1 and 
99 (82;121) for BA.2, indicating that the GMTs against Delta were 4.4- and 4.7-fold 
higher compared to BA.1 (p < 0.0001) and BA.2 (p < 0.0001), respectively.

Three months after the booster dose, neutralizing antibodies were detected in 100% 
(n=84/84), 94% (n=79/84) and 92% (n=77/84) against Delta, BA.1 and BA.2, 
respectively, and the GMTs were 260 (203;334), 102 (76;136) and 69 (54;88) 
respectively. GMTs against BA.1 and BA.2 were 2.6- and 3.8-fold reduced compared 
to Delta 3 months after the booster dose (p < 0.0001 in both comparisons).

Within 3 months following the booster dose, a SARS-CoV-2 infection was confirmed 
or strongly suspected in 21 subjects (presumably Omicron infections as this variant 
represented > 95% of cases during this period). The infection was confirmed by a 
positive RT-PCR in 10 individuals, with the infection occurring during the first 4 weeks 
(1 subject) or during the next 8 weeks (9 subjects) following the booster vaccination. 
An asymptomatic infection, not confirmed by RT-PCR for this reason [10], and not
detectable by serological tests using antigens from the Spike protein, was strongly suspected in the remaining 11 individuals by a significant increase of N reactive specific T cells in IFNγ ELISpot assay, as the N protein is not an antigen targeted by BNT162b2 (Figure 1C).

The subgroup analysis showed that 3-month post-booster dose, S1 reactive specific T cells (Figure 1C) and neutralizing antibody levels against all variants were higher in the group with a history of post-booster infection compared to participants without recent infection (Figure 1D, Supplementary Table S1). Indeed, GMT (CI95%) of neutralizing titers declined from 1 to 3 months after booster dose in individuals without any recent infection for Delta (482 (366; 635) vs 190 (143;252), 2.5-fold decrease, p<0.0001), for BA.1 (110 (85;142) vs 74 (85;142), 1.5-fold decrease, p=0.02) and BA.2 (104 (81;133) vs 52 (39;69), 2-fold decrease, p<0.0001). Conversely, participants with a recent history of SARS-CoV-2 infection had persistent levels of S1 reactive specific T cells and of neutralizing antibodies against Delta and BA.2, and neutralizing antibodies against BA.1 increased from 128.5 (72; 229) to 277 (137; 557) (2.2-fold increase, p=0.014). (Figure 1D, Supplementary Table S1).

Discussion

In this work, we have evaluated the vaccine-induced response against Omicron sub-variants. We found that 8.5 months (median) after primary vaccination with two-doses of an mRNA vaccine, HCWs showed minimal neutralization antibody response to Delta variant as well as BA.1 and BA.2 Omicron sublineages. Following the booster dose, the participants exhibited a stronger neutralizing capacity not only against Delta variant but also BA.1 and BA.2. This is consistent with prior reports regarding BA.1 [2,11–15] and BA.2 [6–8]. However, the median neutralizing antibody titers against BA.1 and
BA.2 at 1- and 3-month post-booster were significantly reduced, as compared to the previous Delta variant, indicating a reduced effectiveness of booster vaccination against Omicron variant.

Interestingly, at 1-month post-boost, we observed similar neutralizing antibody levels against BA.1 and BA.2, highlighting that the immune response induced by the BNT162b2 vaccine was similar for both variants. This finding of the early weeks post-booster is in agreement with other reports [6,7]. However, the decline over time seemed to be more pronounced for neutralizing activity against BA.2. This observation which needs to be confirmed could partially explain the surge of BA.2 and the replacement of BA.1 which probably depends on several factors.

As expected, the occurrence of a SARS-CoV-2 infection after the boost leads to a stronger immune response [9]. The maintenance or even increase of neutralizing activity against Omicron variant in patients with SARS-CoV-2 infection after the booster dose, suggests that an additional antigen exposure can enhance a memory response. This observation is likely due to an expanded B cell repertoire and an increased affinity maturation following additional antigen exposure, as previously suggested [16,17].

The main strength of our study is the evaluation of cellular responses along with the humoral response 3 months after the booster dose as well as the positive effect of an early post-boost infection on the persistence of the immune response. The main weakness of our study is that we are not able to compare effectiveness of BNT162b2 to others mRNA vaccines or to COVID-19 vaccine combinations.

In conclusion, vaccine boost, as additional antigen exposure, provided strong response against BA.1 and BA.2 Omicron sublineages. Nevertheless, the response will likely
decline over time, and is one of the factors to consider for the future of the SARS-CoV-2 vaccine strategies. The public health implication of these findings is that additional boost might be needed to maintain consistent immune response in individuals at risk of severe COVID-19 regarding the recent emergence of new sublineages (BA.4 and BA.5).
Transparency declaration
The authors declare no competing interests

Author contributions
Conception and design of the study, data collection, analysis, writing of the manuscript, and revision of the manuscript: EKA, JD, BC-S, ML, and GL. EKA, JD, BC-S, ML, and GL take full responsibility for the integrity of the data and the accuracy of the data analysis. JL, AG, JT, DL, SM, FV, ArD, DH-G, JP, DaD, KF, DoD, LB, AI-D, AS, DH, MH, FP, BA and YY participated in data analysis, and revision of the manuscript. All authors gave final approval for the version to be submitted.

Funding
This study received the Label of COVID-19 National Research Priority, awarded by the National Steering Committee on Therapeutic Trials and Other COVID-19 Research (CAPNET). This work was supported by the French government through the Programme Investissement d’Avenir (I-SITE ULNE/ANR-16-IDEX-0004 ULNE) managed by the Agence Nationale de la Recherche and was also supported by a sponsorship from GMF under the aegis of the ANRS- Maladies Infectieuses Emergentes.

Acknowledgements
We are grateful to Véronique Betrancourt, Virginie Dutriez, Anne Guigo, Coralie Lefebvre, Véronique Lekeux, Marie-Thérèse Meleszka, Catherine Mortka and Anthony Rabat for their technical support and Bertrand Accart for his contribution (Centre de Ressources Biologiques, BRIF number BB 0033-00030). We are also grateful to Séverine Duflos, Marie Broyez, Peggy Bouquet, Clémentine Rolland, Marion...
Lecorche, Abeer Shaikh Al Arab, Isabelle Tonnerre, Floriane Mirgot, Japhete Elenga Koanga, Laurent Schwarb, Emilie Rambaut, and all the nurses implicated in patients sampling and Mathieu Tronchon for data collection.
References

[1] Garcia-Beltran WF, St Denis KJ, Hoelzemer A, Lam EC, Nitido AD, Sheehan ML, et al. mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 Omicron variant. Cell 2022;3;185:457-66.e4. https://doi.org/10.1016/j.cell.2021.12.033.

[2] Planas D, Saunders N, Maes P, Guivel-Benhassine F, Planchais C, Buchrieser J, et al. Considerable escape of SARS-CoV-2 Omicron to antibody neutralization. Nature 2022;602:71-675. https://doi.org/10.1038/s41586-021-04389-z.

[3] SARS-CoV-2 variants of concern as of 25 August 2022. Eur Cent Dis Prev Control n.d. https://www.ecdc.europa.eu/en/covid-19/variants-concern (accessed August 31, 2022).

[4] Lyngse FP, Kirkeby CT, Denwood M, Christiansen LE, Mølbak K, Møller CH, et al. Transmission of SARS-CoV-2 Omicron VOC subvariants BA.1 and BA.2: Evidence from Danish Households. MedRxiv 2022 (published online January 30). https://doi.org/10.1101/2022.01.28.22270044.

[5] Stegger M, Edslev SM, Sieber RN, Ingham AC, Ng KL, Tang M-HE, et al. Occurrence and significance of Omicron BA.1 infection followed by BA.2 reinfection. MedRxiv 2022 (published online February 22) https://doi.org/10.1101/2022.02.19.22271112.

[6] Yu J, Collier AY, Rowe M, Mardas F, Ventura JD, Wan H, et al. Neutralization of the SARS-CoV-2 Omicron BA.1 and BA.2 Variants. N Engl J Med 2022;386:1579–80. https://doi.org/10.1056/NEJMc2201849.

[7] Pedersen RM, Bang LL, Madsen LW, Sydenham TV, Johansen IS, Jensen TG, et al. Serum Neutralization of SARS-CoV-2 Omicron BA.1 and BA.2 after BNT162b2 Booster Vaccination. Emerg Infect Dis 2022;28:1274–5. doi: 10.3201/eid2806.220503.

[8] Evans JP, Zeng C, Qu P, Faraone J, Zheng Y-M, Carlin C, et al. Neutralization of SARS-CoV-2 Omicron sub-lineages BA.1, BA.1.1, and BA.2. Cell Host Microbe 2022;30:1093-1102.e3: https://doi.org/10.1016/j.chom.2022.04.014.

[9] Demaret J, Lefèvre G, Vuotto F, Trauet J, Duhamel A, Labreuche J, et al. Severe SARS-CoV-2 patients develop a higher specific T-cell response. Clin Transl Immunol 2020;9:e1217. https://doi.org/10.1002/cti2.1217.

[10] Vimercati L, Stefanizzi P, De Maria L, Caputli A, Cavone D, Quarato M, et al. Large-scale IgM and IgG SARS-CoV-2 serological screening among healthcare workers with a low infection prevalence based on nasopharyngeal swab tests in an Italian university hospital: Perspectives for public health. Environ Res 2021;195:110793. https://doi.org/10.1016/j.envres.2021.110793.

[11] Jacobsen H, Strengert M, Maab H, Durand MAY, Kessel B, Harries M, et al. Diminished neutralization responses towards SARS-CoV-2 Omicron VoC after mRNA or vector-based COVID-19 vaccinations medRxiv 2021 (published online December) https://doi.org/10.1101/2021.12.21.21267898.

[12] Pérez-Then E, Lucas C, Monteiro VS, Miric M, Brache V, Cochon L, et al. Neutralizing antibodies against the SARS-CoV-2 Delta and Omicron variants following heterologous CoronaVac plus BNT162b2 booster vaccination. Nat Med 2022;28:481–5. https://doi.org/10.1038/s41591-022-01705-6.

[13] Schmidt F, Muecksch F, Weisblum Y, Silva JD, Bednarski E, Cho A, et al. Plasma Neutralization of the SARS-CoV-2 Omicron Variant. N Engl J Med 2022; 10;386:599-601. doi: 10.1056/NEJMc2119641.
[14] Wang X, Zhao X, Song J, Wu J, Zhu Y, Li M, et al. Homologous or heterologous booster of inactivated vaccine reduces SARS-CoV-2 Omicron variant escape from neutralizing antibodies. Emerg Microbes Infect 2022;11:477–81. https://doi.org/10.1080/22221751.2022.2030200.

[15] Xia H, Zou J, Kurhade C, Cai H, Yang Q, Cutler M, et al. Neutralization and durability of 2 or 3 doses of the BNT162b2 vaccine against Omicron SARS-CoV-2. Cell Host Microbe 2022;30:485-488.e3. https://doi.org/10.1016/j.chom.2022.02.015.

[16] Muecksch F, Weisblum Y, Barnes CO, Schmidt F, Schaefer-Babajew D, Wang Z, et al. Affinity maturation of SARS-CoV-2 neutralizing antibodies confers potency, breadth, and resilience to viral escape mutations. Immunity 2021;54:1853-1868.e7. https://doi.org/10.1016/j.immuni.2021.07.008.

[17] Muecksch F, Wang Z, Cho A, Gaebler C, Tanfous TB, DaSilva J, et al. Increased Potency and Breadth of SARS-CoV-2 Neutralizing Antibodies After a Third mRNA Vaccine Dose BioRxiv 2022 (published online February 15). https://doi.org/10.1101/2022.02.14.480394.
Figure legends

Fig 1. Specific antibody and T cell response 1-month and 3-month after booster BNT162b2 mRNA vaccine

(A) Anti-S1 IgG levels (blue, left Y axis) and S1 specific reactive T cells (black, right Y axis), from before initial vaccination (Pre D1/D2) to 3-month post booster vaccine dose. (B) Neutralization of the Delta, BA.1 Omicron and BA.2 Omicron variants before and 1-month and 3-month after booster BNT162b2 mRNA vaccine dose. Positive threshold (titer ≥ 20) is shown with dotted line. Individual values, geometric mean neutralization titers (GMTs) and 95% confidence intervals are shown. Wilcoxon signed rank test was used for within-subject comparisons. **p-values <0.01; ***p-values <0.001; ****p-values <0.0001; ns, not significant. (C) S1 specific reactive T cell counts before, 1-month and 3-month after booster BNT162b2 mRNA vaccine. Individual values and median (interquartile range) are shown. N (left panel) and S1 (right panel) specific reactive T cells in healthcare workers without (circle) or with (square) COVID-19 after the booster dose. Mann-Whitney U test was performed to compare T cell counts between both groups. **p=0.006; ****p-values < 0.0001. (D) Neutralization of the Delta, BA.1 Omicron and BA.2 Omicron variants in healthcare workers without (circle) or with (square) COVID-19 after the booster dose. Positive threshold (titer ≥ 20) is shown with dotted line. Geometric mean neutralization titers (GMTs) and 95% confidence intervals are shown. A mixed model ANOVA was performed to compare both groups. ****p-values <0.0001.
A

B

C

D

- Omicron infection after booster
- No infection

- Omicron infection after booster
- No infection