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Highlights :

- Omicron (sub)variant neutralizing capacity was reduced compared to WT and Delta.
- Lowest neutralizing response was observed with the FL.1.5.1.
- Results support the need to use vaccine antigens that target circulating variants.

# Neutralizing antibody response to XBB.1.5, BA.2.86, FL.1.5.1 and JN.1 six months after the BNT162b2 bivalent booster

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**Abstract**

**Introduction:** An increase evasion of the SARS-CoV-2 virus towards vaccination strategies and natural immunity has been rapidly described notably due to mutations in the spike receptor binding domain and the N-terminal domain.

**Material and methods:** Participants of the CRO-VAX HCP study who received the bivalent booster were followed at 6 months. A pseudovirus-neutralization test was used to assess the neutralization potency of antibodies against D614G, Delta, BA.1, BA.5, XBB.1.5, BA.2.86, FL.1.5.1, and JN-1.

**Results:** The neutralizing capacity of antibodies against Omicron variant or subvariants was significantly reduced compared to D614G and Delta ($p<0.0001$). The lowest neutralizing response that was observed with JN-1 (GMT=22.1) was also significantly lower compared to XBB.1.5 (GMT=29.5, $p<0.0001$), BA.2.86

(GMT=29.6, p<0.0001), and FL.1.5.1 (GMT=25.2, p<0.0001). Participants that contracted a breakthrough infection due to XBB.1.5 had significantly higher neutralizing antibodies against all variants compared to uninfected participants, especially against Omicron variant and subvariants.

**Conclusion:**

Our results confirm that JN.1 is one of the most immune evading variants to date and that the BA.2.86 subvariant did not show an increased immunity escape compared to XBB.1.5. The stronger response in BKI with Omicron variant and subvariants supports the need to use vaccine antigens that target circulating variants.

## Introduction

An increase evasion of the SARS-CoV-2 virus towards vaccination strategies and natural immunity has been rapidly described notably due to mutations in the spike receptor binding domain (RBD) and the N-terminal domain (NTD)C.

At the end of January 2020, the D614G mutation emerged in UK and rapidly became dominant in the world. In late 2020, the Delta variant was identified, bearing 9 mutations in spike[1] followed in November 2021 by the Omicron variant that presented 32 spike mutations compared to the D614G strain[2]. More recently (August 2023), the BA.2.86 subvariant was identified and characterized by 60 amino acid changes, predominantly in RBD and NTD, compared to the wild-type (WT) strain[3, 4]. The BA.2.86 subvariant has over 30 mutations in spike in comparison with BA.2 and XBB.1.5[5]. The FL.1.5.1, also known as XBB.1.9.1.1.5.1, presents 3 additional mutations in spike compared to XBB.1.5 (i.e., F456L, T478R, and A701V)[5]. In late 2023, the BA.2.86 has evolved in JN.1 and rapidly became

dominant. It is characterized with one additional RBD mutation (L455S) and 3 other non-spike mutations[6, 7].

The presence of these mutations raised the possibility of an increase in neutralizing antibody evasion[5].

The aim of the study was to evaluate the impact of recent circulating SARS-CoV-2 subvariants on the neutralizing antibody response of individuals who had been followed for 6 months after having received the bivalent booster.

## Material and methods

- **Study design**

The CRO-VAX-HCP study is a Belgian multicenter, perspective, and interventional study that was designed to assess the humoral response in a population of healthcare workers (HCW) from 18 to 65 years having received two doses of the BNT162b2 mRNA COVID-19 vaccine followed by a homologous booster (third dose) and after by a bivalent booster (BA.1 or BA.4/5; fourth dose) (ethical approval number: 2020-006149-21) (**Supplemental Table 1**). In the present study, we compared the neutralizing antibody response against D614G, Delta, BA.1, BA.5, XBB.1.5, BA.2.86, FL.1.5.1, and JN.1 6 months after the bivalent booster administration in a population of 30 participants.

- **Seroneutralization**

A pseudovirus-neutralization test was used to assess the neutralization potency of antibodies against 8 variants (D614G, Delta, BA.1, BA.5, XBB.1.5, BA.2.86, FL.1.5.1, and JN.1). The antibody titer is determined as the dilution of serum at which 50% of the infectivity is inhibited ($IC_{50}$) as determined by a nonlinear sigmoid regression model. Method details have been described elsewhere[8].

- **Statistical analysis**

The normality of distribution was assessed by the Anderson-Darling's test following log-transformation. Median and interquartile range (IQR) were used to present demographic data and geometric mean titers (GMT) and 95% confidence intervals (95%CIs) to present the results of the humoral response. A multiple comparison test (two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli) was used to assess the potential difference between the type of variants. A Mann-Withney test was used to compare age between genders and to compare neutralizing antibody titers between participants that developed a breakthrough infection (BKI) or not. Statistical analyses were performed using GraphPad Prism 10.2.0 (GraphPad Software, Massachusetts, USA). $p<0.05$ was considered significant.

## Results

- **Demographics**

Twenty-one were females (median age=55 years; IQR=44–59) and 9 were males (median age =54years; IQR=41–60). Ages were non-significantly different between females and males (p=0.90). Most of the participants (25/30; 83%) had a history of SARS-CoV-2 infection (BA.1) before the administration of the bivalent booster. Twenty-six participants received the BA.1 adapted booster while 4 received the BA.4/5 adapted booster. Six participants development a BKI due to XBB.1.5, 3–6 months after the bivalent booster administration (**Supplemental Table 1**).

- **Neutralizing capacity**

Bivalent-booster sera obtained at 6 months neutralized D614G, Delta, BA.1, BA.5, XBB.1.5, BA.86, FL.1.5.1, and JN.1 with GMT of 319, 162, 71.6, 61.4, 29.5, 29.6, 25.2, and 22.1, respectively (**Figure 1**). The neutralizing capacity of antibodies

against Omicron variant or subvariants was significantly reduced compared to D614G and Delta variants (p<0.0001). The lowest neutralizing response that was observed with JN.1 (GMT=22.1, 95%CI: 16.2–30.2) was also significantly lower compared to XBB.1.5 (GMT=29.5, 95%CI: 21.4–40.6; p=0.0002), BA.2.86 (GMT=29.6, 95%CI: 21.4–41.0; p=0.0003), and FL.1.5.1 (GMT=25.2, 95%CI: 18.1-34.9; p=0.0094) (**Figure 1**).

The level of neutralizing antibodies against Delta, BA.1, BA.5, XBB.1.5, BA.2.86, FL.1.5.1, and JN.1 were 1.97, 4.46, 5.20, 10.81, 10.77, 12.67, and 14.43 lower compared to D614G. Compared to XBB.1.5, BA.2.86, and FL.1.5.1, the neutralizing capacity of antibodies against JN.1 was 1.33, 1.34, and 1.14-fold-decrease, respectively (**Figure 1**).

Participants that contracted a BKI had significantly higher neutralizing antibodies against all variants (i.e., cross-reactivity) compared to uninfected participants. The fold change was more pronounced with BA.1, BA.5, XBB.1.5., BA.2.86, FL.1.5.1, and JN.1 (fold change ranging 4.5–5.0) compared to D614G (fold change=2.5) and Delta (fold change=3.0) (**Figure 2**).

## Discussion

The administration of COVID-19 vaccines allowed the reduction of SARS-CoV-2 infections, complications and death. A gradual decline in vaccine efficacy (VE) against infection was however rapidly observed. This waned efficacy was consistent with the decrease of neutralizing antibodies, supporting their role as a strong correlation of COVID-19 protection from infection. The decrease in VE was further heightened by the emergence of variants, especially the Omicron variant and its

subvariants[2, 5, 8, 9]. For instance, a 23.1-fold decrease of NAbs against BA.5 was observed between one and 6 months after the bilvant booster ($T_{1/2}$=16.1 days)[10]. Recently, some concerns were formulated following the identification of the BA.2.86 subvariant that could more easily escape immunity because of its high number of additional mutations compared to XBB.1.5.[3, 5]. This has not happened[11] and was confirmed in our study and by others by the identification of similar neutralizing antibody titers between BA.2.86 and XBB.1.5[5, 9]. In accordance with our evaluation, Hu *et al.* identified that neutralizing antibody response of FL.1.5.1 was also lower compared to BA.2.86 and XBB.1.5. in a population of 48 individuals 14–32 days after the bivalent booster [12].

Compared to BA.2.86, the JN.1 has become the predominant subvariant[6, 13]. Yang *et al.* found an enhance immune escape of JN.1 compared to BA.2.86 (1.1 to 2.1-fold decrease in the 50% neutralization titer ($NT_{50}$) (n=54)[7]. Kaku *et al.* pointed a 4.5-fold decrease in $NT_{50}$ of JN.1 compared to BA.2.86 3-4 weeks after XBB.1.5 vaccination (n=19)[6]. Planas *et al.* confirmed a 2.0-fold neutralization decreased between BA.2.86 and JN.1 after 3 doses of BNTA62b2 (n=13) and 6 months after the BA.5 bivalent booster (n=8)[14]. A less pronounced decreased of 1.15-fold was also observed by Jeworowski *et al.* compared to BA.2.86[13] and is in line with the present study.

Interestingly, individuals who developed a XBB.1.5 BKI in our study presented a boost in neutralizing antibodies against all variants and subvariants compared to uninfected individuals, but mostly on Omicron variants and subvariants, supporting the need to consider the use of vaccines that are adapted to circulating variants, as recommended by the WHO and the FDA[9, 12]. Similar results have been found in

two studies in individuals who developed an XBB.1.5 BKI or who received the XBB .1.5 monovalent vaccine[11, 14].

The sample size of our study was low, especially considering the subcohort of participants that developed a BKI. This represents a limitation.

**Conclusion**

Our results confirm that JN.1 is one of the most immune evading variants to date and that the BA.2.86 subvariant did not show an increased immunity escape compared to XBB.1.5. The fact that only the reduced neutralizing capacity might be the only mechanism that could explain why JN.1 rapidly became predominant need further evaluations. The boost in neutralizing antibody titers observed in subjects who developed a BKI after the administration of the bivalent booster was mostly superior considering Omicron variants and subvariant. This supports the need to use vaccine antigens that target circulating variants.

Conflict of interest: The authors declare no conflict of interest.

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Ethical statement: The study was in accordance with the declaration of Helsinki (ethical approval number: 2020-006149-21).

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**Figure 1:** Comparison of the neutralizing capacity against the D614G strain (WT), the Delta, the BA.1 Omicron variant, the BA.5 Omicron variant, the XBB.1.5 Omicron subvariant, the BA.2.86 Omicron subvariant, and the FL.1.5.1 Omicron subvariant in a population of 30 healthy volunteers 6 months after having received the bivalent booster. Geometric mean titers (GMT) (±95% CI) and percentage of positive samples are represented. The block dotted line represents the positivity cut-offs for neutralizing antibodies ($IC_{50}$ of 1:20). The grey dotted line represents the limit of detection of the assay ($IC_{50}$ of 10). * = significantly higher compared to all other variants (p<0.0001). ** = significantly higher compared to all other variants (p<0.0001) except for the D614G strain (p<0.0001).

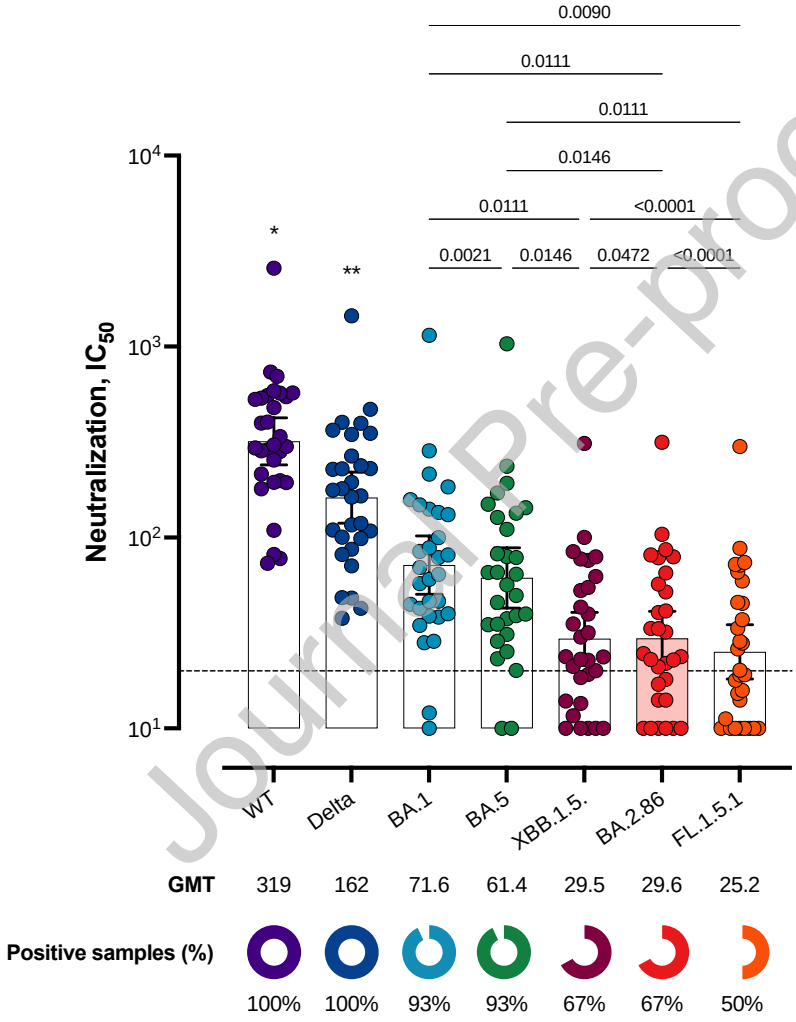
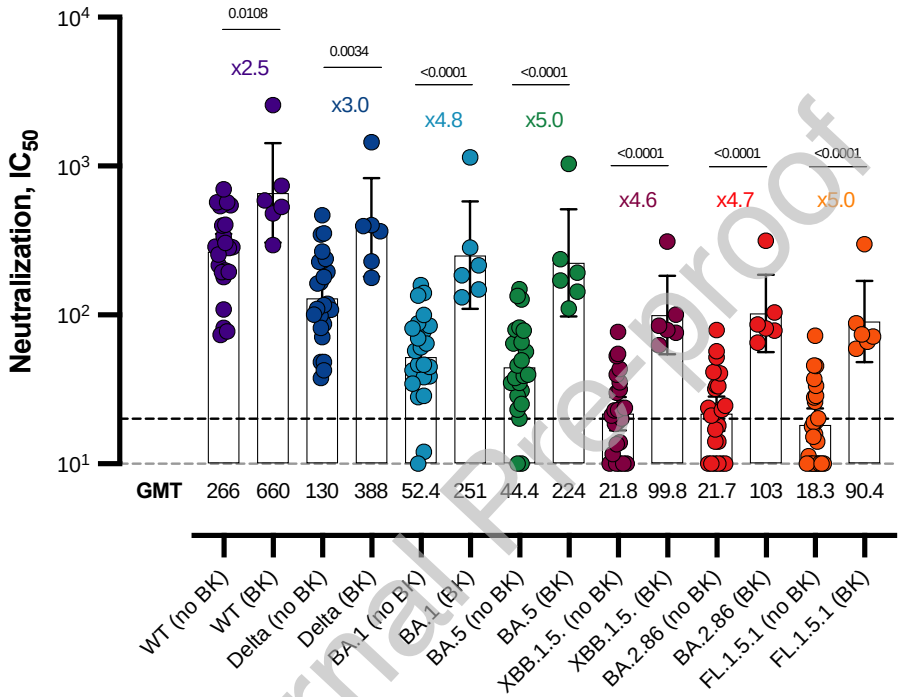


**Figure 2:** Comparison of the neutralizing capacity against the D614G strain (WT), the Delta, the BA.1 Omicron variant, the BA.5 Omicron variant, the XBB.1.5 Omicron subvariant, the BA.2.86 Omicron subvariant, and the FL.1.5.1 Omicron subvariant in individuals who developed a breakthrough infection following administration of the bivalent booster or not. Blood was collected 6 months after having received the bivalent booster. Geometric mean titers (GMT) (±95% CI) are represented as well as the fold-difference between groups. The block dotted line represents the positivity cut-offs for neutralizing antibodies ($IC_{50}$ of 1:20). The grey dotted line represents the limit of detection of the assay ($IC_{50}$ of 10).






**Declaration of interests**

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: