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SUPPORTING INFORMATION

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COVID-19 vaccination with BNT162b2 and ChAdOx1 vaccines has the potential to induce nasal neutralizing antibodies

To the Editor,

To date, several vaccines against severe acute respiratory coronavirus 2 (SARS-CoV-2) have proven to effectively reduce severe illness.[1,2] To promote herd immunity and reduce virus circulation, vaccines need to effectively reduce transmission risk. The nasal cavity is the first entrance point for SARS-CoV-2, and it has been suggested that viral replication is most efficient in the upper airways.[3] Local neutralizing antibodies (NAbs) in the nasal mucosa can play an important role in preventing SARS-CoV-2 infection and transmission by limiting viral replication and shedding. While induction of systemic neutralizing humoral responses has been shown for both natural infection and upon vaccination with BNT162b2 and ChAdOx1, the presence of NAbs in the nasal mucosa upon vaccination remains unclear. A recent not peer-reviewed report described the potential of BNT162b2 to induce NAbs in the nasal cavity, but did not consider prior COVID-19 as a potentiator of this response.[4] Local humoral responses after vaccination with viral vector-based vaccines, another type of frequently used SARS-CoV-2 vaccines, have not been investigated. In the present study, we compared systemic and local immune responses in the serum and nasal secretions of 46 study subjects vaccinated with SARS-CoV-2 mRNA (BNT162b2) or viral vector-based (ChAdOx1) vaccines.

Jozefien Declercq and Els Tobback are shared co-first authorship.

Philippe Gevaert and Vandekerckhove Linos are shared last authorship.

TABLE 1 Subject baseline characteristics

	BNT162b2	ChAdOx1
	No (%)	No (%)
n=	24	22
Sex		
Female	18 (75%)	18 (82%)
Male	6 (25%)	4 (18%)
Age, median (IQR), y	42.5 (38.5–49.0)	36.0 (25.0–42.0)
BMI, median (IQR), kg/m2	25.0 (23.0–27.1)	23.7 (20.6–26.5)
Current smoking	1 (4%)	2 (9%)
Prior COVID-19 infection	12 (50%)	11 (50%)
Confirmed by RT-PCR	9 (75%)	10 (91%)
Confirmed by serology	1 (8%)	1 (9%)
Self-reported	2 (17%)	0 (0%)
Patient-reported allergy to aeroallergens		
Yes	8 (33%)	5 (23%)
No	16 (67%)	17 (77%)

Serum and nasal secretions from subjects visiting the COVID-19 vaccination center at the University Hospital Ghent, Belgium were collected, just prior to the first SARS-CoV-2 vaccination and after the second dose of the same vaccine. Median time between second vaccine dose and sampling was 19 days (IQR: 15–23) for the BNT162b2 group and 18 days (IQ: 15–26) for the ChAdOx1 group. Collection of nasal secretions was performed as described previously.[5] SARS-CoV-2 NAbs in serum and nasal secretions were determined using the Elabsciene SARS-CoV-2 Neutralization Antibody ELISA kit (Gentaur) as per manufacturer's instructions. This surrogate virus neutralization test uses purified receptor-binding domain (RBD) from the S protein and the host cell receptor ACE2 to mimic the virus-host interaction.[6] This RBD-ACE2 interaction is blocked by SARS-CoV-2 specific NAbs in patient samples. Inhibition rates are calculated based on the OD value of the negative control. A cutoff of 20% inhibition is determined as positive for the presence of NAbs by the manufacturer, based on testing 500 negative control sera.

Forty-six subjects, mainly females, were included in the study (Table 1). Twenty-four subjects were vaccinated with BNT162b2 and 22 with ChAdOx1. In both groups, half of the study subjects had a history of prior COVID-19. NAbs were determined in serum and nasal secretions prior and post-vaccination in all study subjects. Prior to vaccination, 16 subjects had NAbs in serum and 4 in nasal secretions. At second sampling, except for one, all subjects showed NAbs in their serum, regardless of the vaccine received (Figure S1). In nasal secretions, NAbs were observed in the majority of subjects (n = 23; 96%) vaccinated with BNT162b2 and in about half of the subjects (n = 13; 59%) vaccinated with ChAdOx1 at second sampling (p = 0.0032; Fisher's exact test) (Figure 1A). Moreover, the ACE2 binding inhibition in nasal secretion was higher in subjects vaccinated with BNT162b2 compared to those vaccinated with ChAdOx1 ($p < 0.0001$; 2-way repeated-measures ANOVA with Sidak's multiple comparisons test) (Figure 1D). Induction of NAbs occurred irrespective of prior SARS-CoV-2 infection or the presence of patient-reported allergy to aeroallergens (pollen, animals and house dust mite) (Figure 1C-F).

Taken together, our study shows that both BNT162b2 and ChAdOx1 vaccines can induce nasal NAbs, albeit variable in the ChAdOx1 arm. Why only some subjects develop local NAbs in the former group is currently unclear. Differences in time between the two vaccine doses or other mechanisms of action of the vaccines might account for the observed differences. Further research is needed to fully understand the underlying immunological mechanisms. Longitudinal follow-up of the described subjects is needed to see whether vaccines can induce long-lasting neutralizing responses in the nasal mucosa. Failure to induce long-lasting NAbs warrants rational booster design or other strategies, such as nasal vaccination. Based on our findings and given that mucosal NAbs might be key to prevent infection and viral shedding, we advocate for the inclusion of nasal mucosa NAbs measurements in vaccine efficacy trials and routine testing procedures.

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CONFLICT OF INTEREST

LV received grants from Gilead Sciences. The other authors declare no conflict of interest.

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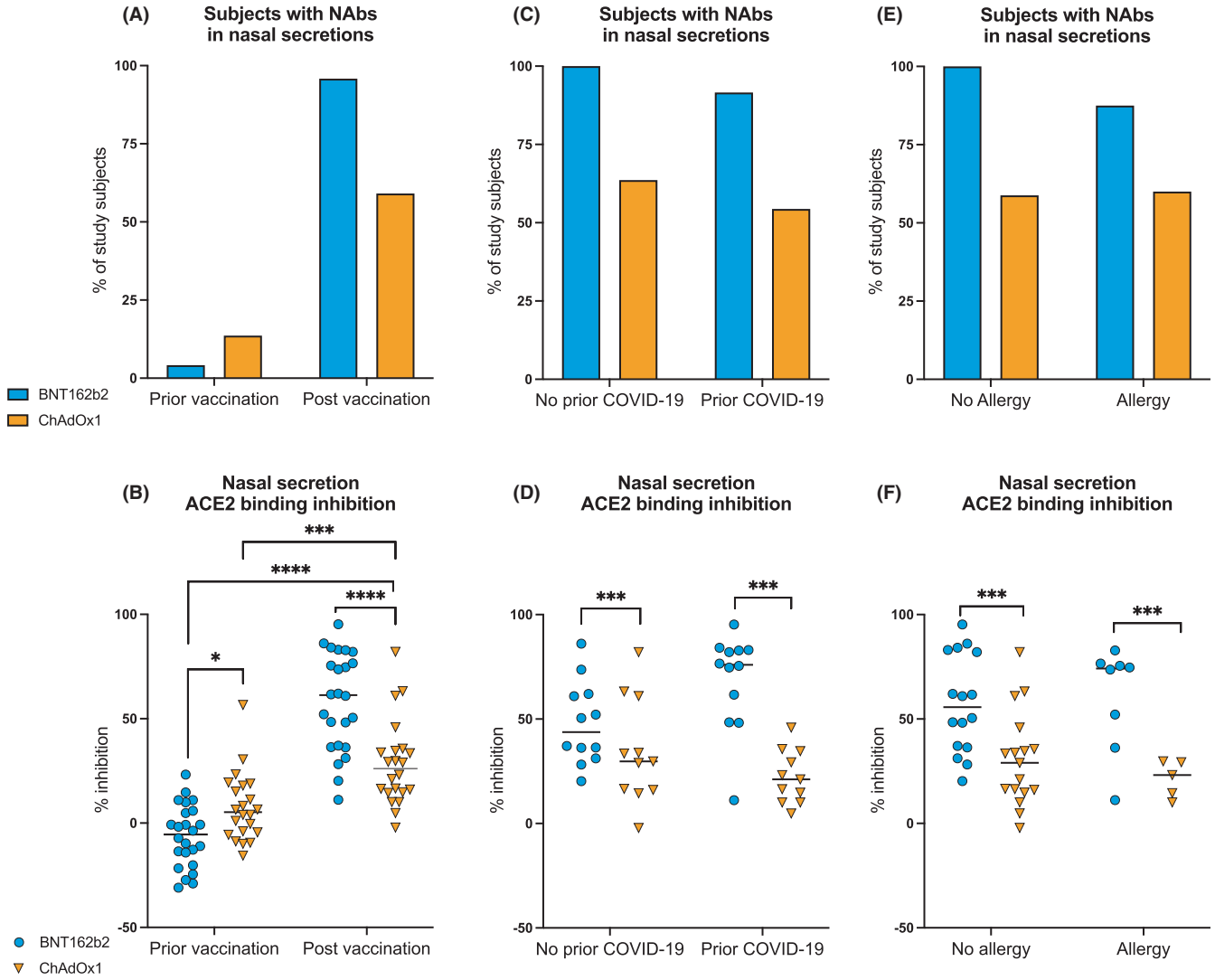

FIGURE 1 Effect of SARS-CoV-2 vaccination on the induction of nasal neutralizing antibodies. A-B, Percentage of patients with SARS-CoV-2 neutralizing antibodies (A) and ACE2 binding inhibition rates (B) in nasal secretions prior and post-vaccination with BNT162b2 and ChAdOx1. (C-E), Percentage of patients with SARS-CoV-2 NAbs (C, E) and ACE2 binding inhibition rates (D, F) post-vaccination in nasal secretions of patients respective to prior COVID-19 (C, D) and to allergy to aeroallergens (E,F). Asterisks indicate statistical significance by two-way repeated-measures ANOVA followed by Sidak's multiple comparisons test for B and by ordinary two-way ANOVA with Tukey's multiple comparisons test for D and F. *$p < 0.05$, **$p < 0.001$, ***$p < 0.0002$, ****$p < 0.0001$

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SUPPORTING INFORMATION

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Nasal epigenetic age and systemic steroid response in pediatric emergency department asthma patients

To the Editor,

Asthma is a leading cause of emergency department visits and hospitalizations in children with health-related costs amounting to over $80 billion annually.[1] Hence, continued efforts to improve the risk stratification and management of pediatric asthma patients remain important. To better predict asthma severity, Zhu et al. performed an epigenome-wide association study in nasal epithelial cells of 55 African American children with asthma.[2] They identified 816 differentially methylated sites and 10 differentially methylated regions. An earlier study of 20 children by this same group similarly identified over 200 sites that were differentially methylated when comparing children who were good versus poor responders to acute systemic steroid treatment.[3] Beyond single methylation sites or regions, a few studies have demonstrated relationships of pediatric asthma with blood and nasal cell epigenetic age acceleration—a novel marker of biological age.[4,5] Despite this evidence, even fewer studies have evaluated relationships of epigenetic age with treatment responses in pediatric asthma. Given the robust associations of epigenetic age with healthspan and lifespan and its potential clinical utility, improving our understanding of its relationship with asthma could have a profound role in informing patient management and risk stratification moving forward.

To further our understanding of epigenetic age and asthma therapeutic relationships, we utilized the publicly available 20 participant systemic steroid response (baseline [T0] and ~24 h after steroid therapy [T1]) nasal DNA methylation data from Zhang et al.[3] Per the Cincinnati Children's Hospital Medical Center evidence-based treatment protocol for in-patient asthma exacerbation, all study participants received 2 mg/kg/day of prednisone during their hospital stay.[3] The Zhang et al. dataset included information on chronological age, sex, race, and hospital length of stay (LOS) [short (≤24 h) and long (>24 h)]. Participants had a median (IQR) chronological age of 10 (6–15) years. Other demographic information has been previously published (Table S3).[3] We calculated the T1-T0 change in epigenetic age acceleration (EAA Horvath) using an online calculator (http://dnamage.genetics.ucla.edu). We found no significant differences in median EAA by LOS, sex, or race (Figure 1A–C). However, females with short LOS were significantly younger than males with long LOS ($p = .049$, Figure 1D). In repeated measures mixed effects models examining relationships of LOS with EAA, we observed no direct associations of LOS, sex, or race with EAA. Nor did we observe any significant effect modification of the LOS and epigenetic age relationship by race. However, we did observe a significant interaction between LOS and sex after adjusting for timepoint and race. Specifically, compared to males with long LOS, females with short LOS were on average 2.32 years younger in nasal EAA (95% CI: −4.06, −0.57, $p = .01$). EAA was not statistically different between males with short LOS ($\beta = .99$ years, 95% CI: −0.38, 2.36, $p = .15$) and males with long LOS. No significant differences were observed in chronological age among the sex and LOS categories (Table 1).

Anti-inflammatory processes are often associated with anti-aging. Conversely, inflammatory diseases and biomarkers are frequently associated with aging processes, including EAA.[6] In the context of pediatric asthma, previous work in children from the Project Viva cohort (mean chronological age of 12.9 years) reported a greater EAA of 0.74 years in children with current asthma compared to their peers.[4] Given this background literature and our study design of nasal methylation samples taken within 24 h of each other, we hypothesized that biologically older individuals may have differences in disease severity and may require different durations of therapeutics like steroids to temporize their disease. When tested directly in our study sample, albeit not statistically significant, the median EAA of individuals with long LOS (a proxy of more severe asthma) was greater than that of individuals with short LOS (a proxy of less severe asthma). Furthermore, statistically significant differences in EAA were observed when we examined the interaction between sex and LOS. Our results of significantly lower EAA in females with short LOS compared to males with long LOS may suggest less severe disease and/or better inflammatory regulation (including effectiveness of systemic steroids in mitigating the inflammatory response) in the former group.