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Research Note

Long-term evolution of humoral immune response after SARS-CoV-2 infection

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Abstract

Objective

We aimed to characterize the evolution of humoral immune response up to one year after SARS-CoV-2 infection in health care workers (HCWs) during the first wave of COVID-19 in Paris.

Methods

Serum samples from 92 HCWs were tested at month 0 (M0), M6 and M12 after SARS-CoV-2 infection for IgG targeting the nucleocapsid (N), IgG targeting the receptor-binding domain (RBD) of spike (S) protein, IgA targeting S, and anti-RBD neutralizing antibodies. After M6, 46 HCWs received a single-dose of COVID-19 vaccine.

Results

We observed a significant decrease of all SARS-CoV-2 immunologic markers at M6 post-infection: median decreases were 0.26 log binding antibody units (BAU)/mL (M0: 1.9 [interquartile range (IQR) 1.47 – 2.27]; M6: 1.64 [IQR: 1.22 – 1.92]) for anti-RBD IgG, 4.10 (index) (M0: 4.94 [IQR: 2.72 – 6.82]; M6: 0.84 [IQR: 0.25 – 1.55]) for anti-N IgG, 0.64 (index) (M0: 2.50 [IQR: 1.18 – 4.62]; M6: 1.86 [IQR: 0.85 – 3.54]) for anti-S IgA, and 24.4% (M0: 66.4 [IQR: 39.7 – 82.5]; M6: 42.0 [IQR: 16.8 – 68.8]) inhibition activity for the RBD neutralizing antibodies. Between M6 and M12, anti-RBD IgG level, anti-S IgA index, and anti-RBD neutralizing activity, significantly increased among COVID-19 vaccinated HCWs, whereas they remained stable among unvaccinated HCWs. Anti-N IgG index significantly decreased between M6 and M12 among both vaccinated (median: 0.73 [IQR: 0.23 – 1.11] at M6 and 0.52 [IQR: 0.20 – 0.73] at M12) and unvaccinated HCWs (median: 0.79 [IQR: 0.21 – 4.67] at M6 and 0.34 [IQR: 0.24 – 2.78] at M12).

Conclusion

A steady decline in the anti-N IgG response was observed during the first year following SARS-CoV-2 infection among HCWs, whereas the anti-RBD IgG and the anti-S IgA responses remained stable and could be enhanced by COVID-19 vaccination.

Keywords: SARS-CoV-2, IgG, IgA, seroneutralization, vaccines

Introduction

During the beginning of the COVID-19 pandemic, the contamination risk of health care workers (HCWs) by the SARS-CoV-2 was of major concern. The SEROCOV multicenter cohort study conducted among 1062 frontline HCWs from 5 Parisian hospitals reported a rate of SARS-CoV-2 infection of 14.6% at the end of the first COVID-19 wave by detection of anti-nucleocapsid protein (N) IgG in HCW sera [1]. Several studies have shown that anti-SARS-CoV-2 IgG levels decreased after infection throughout time and that COVID-19 vaccination led to a rise of antibodies levels [2,3]. The present retrospective study aimed to characterize the evolution of the humoral immune response among SARS-CoV-2-infected HCWs from the SEROCOV study during the first year post-infection.

Methods

For the SEROCOV study, registered on ClinicalTrials.gov: NCT04304690 first registered on 11/03/2020, and approved by the ethics committee (CPP Sud-Ouest et Outre-Mer I, approval no. 2-20-023 id7257), HCWs from Pitié-Salpêtrière, Bichat, Tenon, Trousseau and Saint-Antoine hospitals were included from March 16, 2020 to

April 24, 2020 for a 3-month follow-up. HCWs with a positive detection of SARS-CoV-2 anti-N IgG in the serum at the end of the initial 3-month period were included in the present study for an additional 9-month follow-up. Humoral immune responses were evaluated at M0 (corresponding to the time of seroconversion), M6 and M12 (5-6 and 11-12 months after seroconversion, respectively). All participant signed an informed consent [1].

Semi-quantification (index) of IgG against N and quantification (log binding antibody units [BAU]/mL) of Ig against receptor-binding domain (RBD) of spike (S) protein were assessed by chemiluminescence assay (ALINITY i System, Abbott). Semi-quantitative (index) ELISA assay was performed for anti-S IgA (ELISA Anti-SARS-CoV-2 IgA kit, Euroimmun). Anti-RBD neutralizing activity of sera was measured with a semi-quantitative ELISA assay (SARS-CoV-2 Surrogate Virus Neutralization Test, GenScript) based on the binding inhibition of labelled RBD to angiotensin converting enzyme 2 (ACE2) by the anti-RBD neutralizing antibodies (results expressed in %).

For statistical analyses, Mann-Whitney U tests and non-parametric Wilcoxon paired tests were performed with the GraphPad Prism version 8.0.2 software. $p<0.05$ was considered statistically significant.

Results

The study included 92 SARS-CoV-2-infected HCWs from the SEROCOV cohort: 22 males, 70 females, median age of 33 years [interquartile range (IQR) 28-41]. A total of 91 and 55 serum samples were available at M6 and M12, respectively. We first evaluated the natural evolution of humoral anti-SARS-CoV-2 immune response between M0 and M6. The anti-RBD IgG median level decreased significantly by 0.26 log BAU/mL between M0 (1.90 log BAU/mL [IQR: 1.47 – 2.27]) and M6 (1.64 log

BAU/mL [IQR: 1.22 – 1.92]) (Fig.1A). The anti-N IgG median index also significantly decreased by 4.10 during this period: 4.94 [IQR: 2.72 – 6.82] at M0 and 0.84 [IQR: 0.25 – 1.55] at M6 (Fig. 1B). We also observed a significant decline by 0.64 of the anti-S IgA median index between M0 (2.50 [IQR: 1.18 – 4.62]) and M6 (1.86 [IQR: 0.85 – 3.54]) (Fig. 1C). Considering the anti-RBD neutralizing activity, a median decay of 24.4% of inhibition was observed: 66.4% [IQR: 39.7 – 82.5] at M0 and 42.0% [IQR: 16.8 – 68.8] at M6 (Fig. 1D).

After M6, 46 (79%) HCWs received a single-dose of COVID-19 vaccine: 35 (76%) Pfizer-BioNTech and 11 (24%) Oxford-AstraZeneca. The anti-SARS-CoV-2 humoral immune response was compared between vaccinated and unvaccinated HCWs. In the unvaccinated group of HCWs, the natural evolution of antibody responses could be analysed. The levels of anti-RBD IgG, anti-S IgA and the anti-RBD neutralizing activity were stable between M6 and M12 (Figs. 2A, 2C and 2D), whereas we observed a significant decrease of the anti-N IgG median index during the same period: 0.79 [IQR: 0.21 – 4.67]) at M6 and 0.34 [IQR: 0.24 – 2.78] at M12 (Fig. 2B). A significant decrease of the anti-N IgG median index was also observed in the vaccinated group of HCWs: 0.73 [IQR: 0.23 – 1.11] at M6 and 0.52 [IQR: 0.20 – 0.73]) at M12 (Fig. 2B). However, the single-dose vaccination induced a strong increase of the anti-RBD IgG level (+1.95 log BAU/mL), the anti-S IgA index (+16.30) and the anti-RBD neutralizing activity (+71.4% of inhibition) (Figs. 2A to 2D).

We also investigated the impact of the COVID-19 vaccine type (Pfizer-BioNTech or Oxford-AstraZeneca) on the humoral immune response of HCWs. No difference was observed between both vaccines at M12 for the anti-N IgG index, the anti-S IgA index and the anti-RBD neutralizing activity between the two vaccines (Fig. 2F to 2H).

Conversely, a significantly higher level of anti-RBD IgG was observed in the Pfizer-BioNTech group of HCWs than among in the Oxford-AstraZeneca group of HCWs (median: 3.56 log BAU/mL [IQR: 3.33 - 3.78] versus 2.94 log BAU/mL [IQR: 2.76 – 3.14] (Fig. 2E).

Discussion

The evolution of the humoral immunity after SARS-CoV-2 infection is an important element to study the dynamic of COVID-19 pandemic. Our study reinforces and brings new evidence to the fact that anti-S antibodies (IgG and IgA) decreased but remained detectable through time, conversely to anti-N antibodies, and can be strongly enhanced after vaccination.

As previously shown [3,4], we observed a continuous decrease of the anti-N IgG over 1 year. The anti-RBD IgG level also decreased until M6 but remained stable above the positive threshold over a year. These data were consistent with those observed in others European HCWs or in symptomatic/asymptomatic patients [2,3,5–7]. We observed the same pattern of evolution for the anti-S IgA antibodies. Previous works have shown that anti-S IgA levels decreased in a less proportion compared to the anti-RDB IgG levels over a time period of 6 to 9 months [8–10]. The present study confirmed this decrease at M6 but showed that, similarly to the anti-RBD IgG, they remained stable over a year. These patterns of antibody evolution is coherent with the kinetics of B-cells and T-cells expansion after SARS-CoV-2 infection [11] and suggest that active and young adult HCWs could exhibit an efficient immune response in case of virus re-exposure after one year.

Moreover, a strong increase of antibodies titers was observed between M6 and M12 after one dose of vaccine. Consistent with previous studies [12,13], only one dose of

vaccine after SARS-CoV-2 infection was enough to increase strongly immune response makers.

One of the limitations of our study it is the low number of available serum samples from SARS-CoV-2-infected HCWs, and particularly for the unvaccinated group and for the Oxford-AstraZeneca group. Indeed, we only observed a lower level of the anti-RBD IgG with the Oxford-AstraZeneca group which is consistent with the literature [13]. No significant differences were observed for the anti-S IgA level and the anti-RDB neutralizing activity. Those results could be explained by the fact that the last two immunologic markers were assessed by semi-quantitative assays, which do not allow a precise quantification.

Conclusion

Anti-RBD IgG and anti-S IgA levels decreased until 6 months and then stabilized until 12 months post-SARS-CoV-2 infection in HCWs. Anti-N IgG levels showed a continuous decline throughout the study period. COVID-19 vaccination (Pfizer-BioNTech and Oxford-AstraZeneca) led to a strong increase of all anti-SARS-CoV-2 immunologic markers, except for the anti-N IgG response.

Authors' contribution

DB, AGM and ET planned the study; ET, KZ, SS, SM, MD, CS and BA conducted the experiments; ET and DB analysed the data; ET, DB and AGM wrote the manuscript. ET, DB, AGM, PH and FT reviewed the manuscript. All authors approved the final version.

Conflict of Interest

Authors declare that they have no conflict of interest.

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Figure legends

Fig. 1. Natural evolution of humoral immune response after SARS-CoV-2 infection among HCWs.

Evolution of antibody response during 6 months for (A) anti-RBD IgG, (B) anti-N IgG, (C) anti-S IgA and (D) anti-RBD neutralizing activity (M0 n=92 and M6 n=91). On each graph, the horizontal dotted line represents the positivity cutoff of the technique: (A) 50 BAU/mL, (B) 0.5 (index), (C) 1.1 (index), (D) 30%. ** p<0.005 **** p<0.0001.

Fig. 2. Evolution of immune response after SARS-CoV-2 infection among COVID-19 vaccinated and unvaccinated HCWs.

Evolution of antibody response between M6 and M12 among vaccinated (dark grey, n=46) and unvaccinated (light grey n=9) HCWs: (A) anti-RDB IgG, (B) anti-N IgG, (C) anti-S IgA and (D) anti-RBD neutralizing activity.

Comparison of antibody response at M12 among HCWs vaccinated with Pfizer-BioNTech vaccine (white, n=35) and with Oxford-AstraZeneca vaccine (grey, n=11): (E) anti-RBD IgG, (F) anti-N IgG, (G) anti-S IgA, and (H) anti-RBD neutralizing activity. On each graph, the horizontal dotted line represents the positivity cutoff of the technique: (A) and (E) 50 BAU/mL, (B) and (F) 0.5 (index), (C) and (G) 1.1 (index), (D) and (H) 30%. *p<0.05 ****p<0.001.

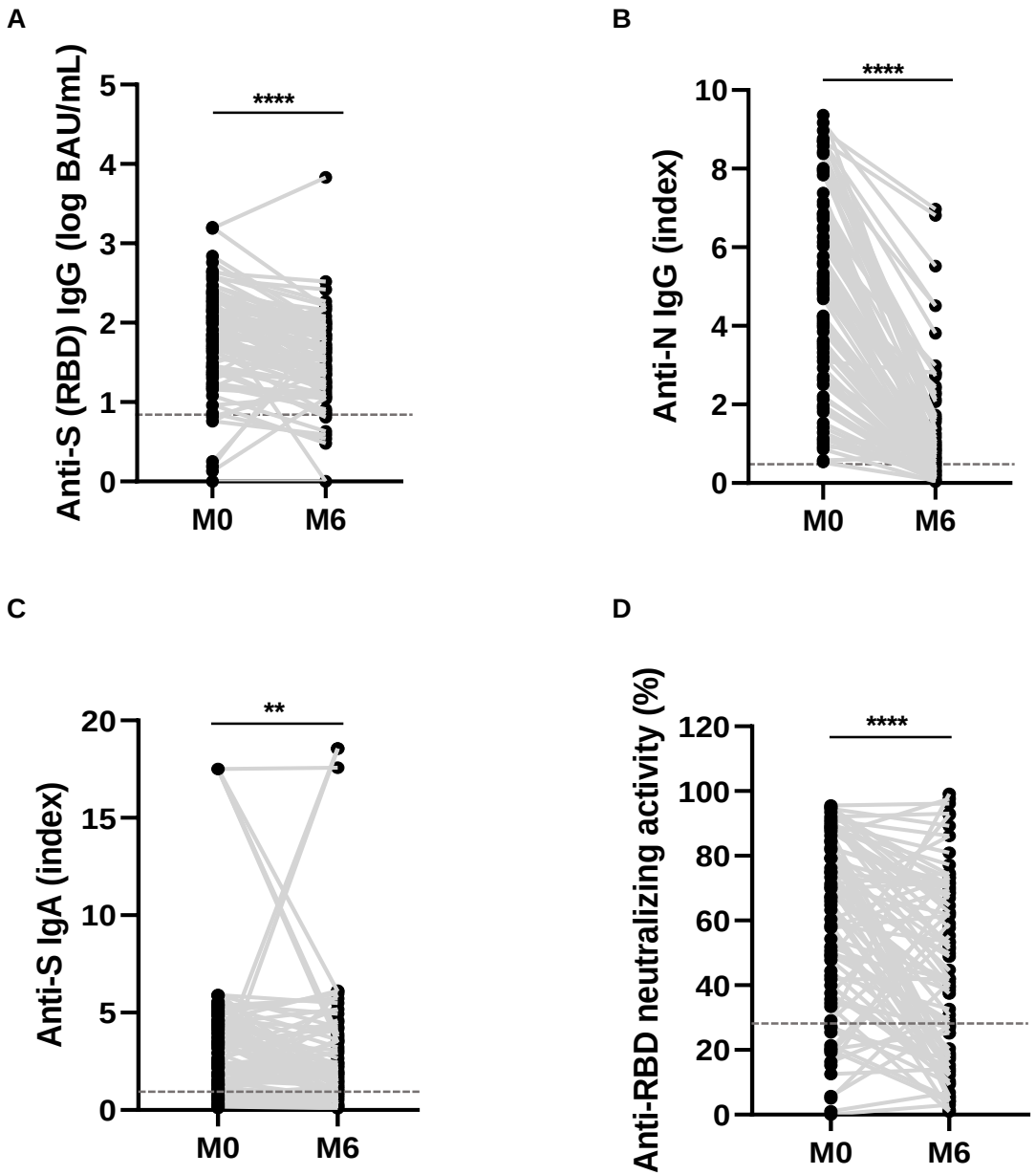
A
Anti-S (RBD) IgG (log BAU/mL)
****
M0
M6
B
Anti-N IgG (index)
****
M0
M6
C
Anti-S IgA (index)
**
M0
M6
D
Anti-RBD neutralizing activity (%)
****
M0
M6

Fig. 1

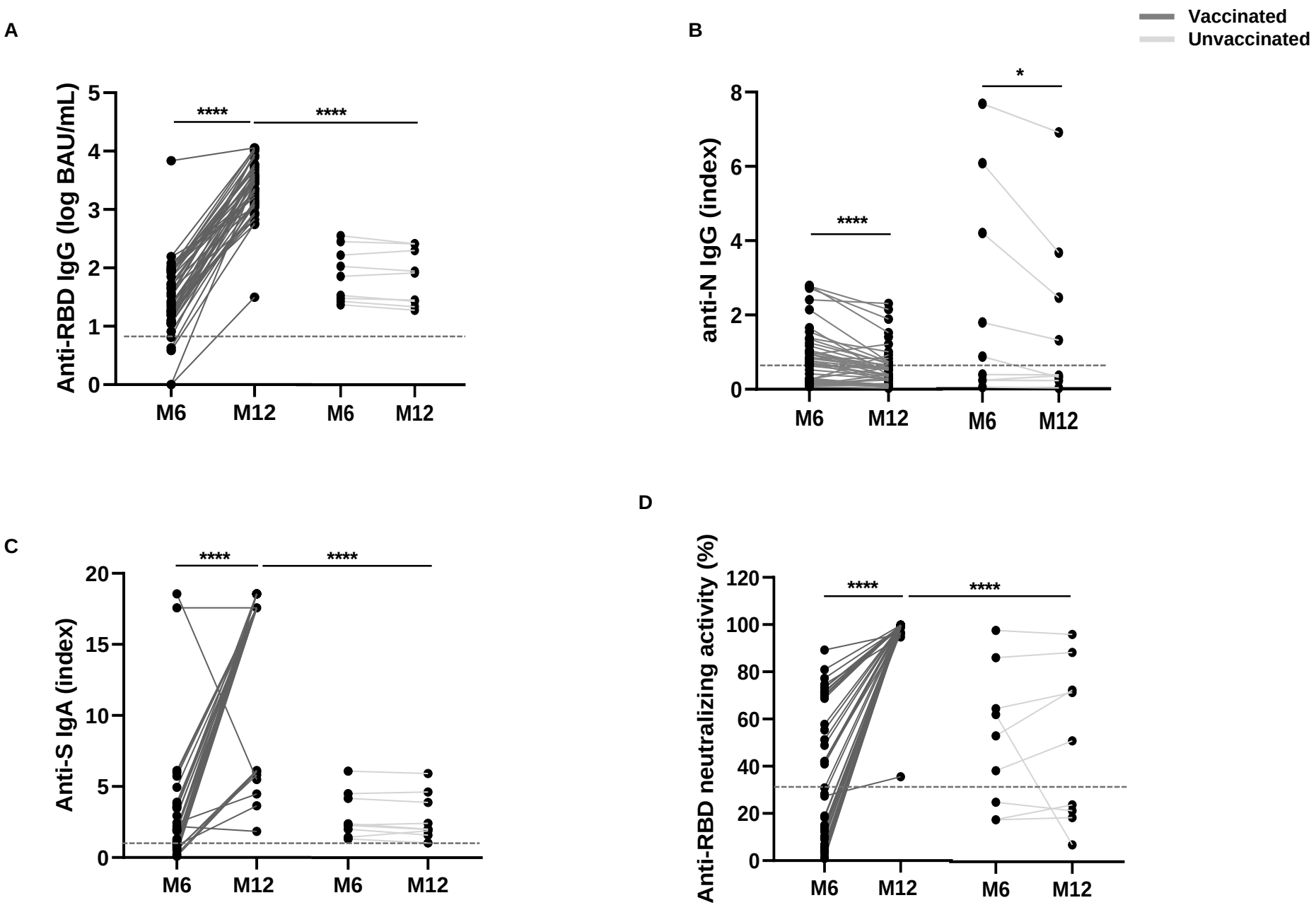
A
B
C
D
Vaccinated
Unvaccinated
Anti-RBD IgG (log BAU/mL)
anti-N IgG (index)
Anti-S IgA (index)
Anti-RBD neutralizing activity (%)
****
*
M6
M12

Fig. 2

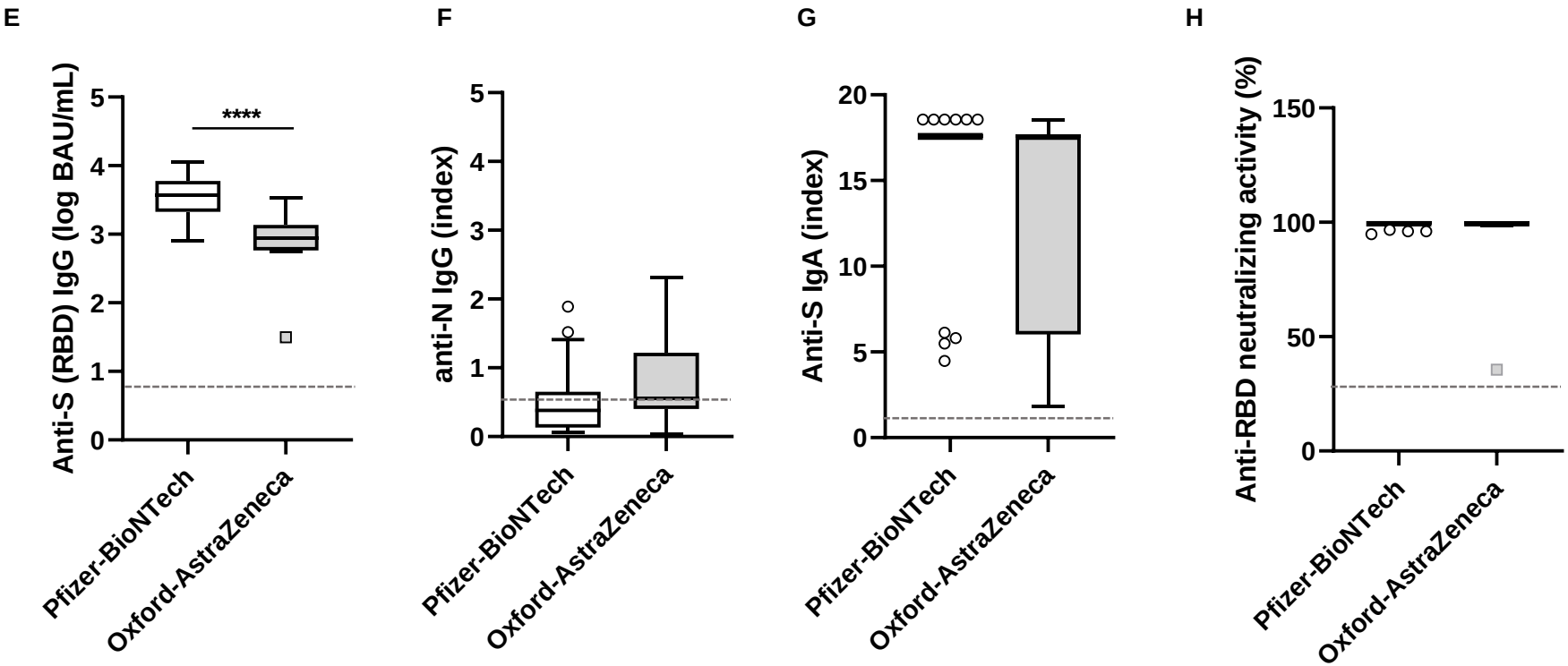
E
Anti-S (RBD) IgG (log BAU/mL)
****
Pfizer-BioNTech
Oxford-AstraZeneca
F
anti-N IgG (index)
Pfizer-BioNTech
Oxford-AstraZeneca
G
Anti-S IgA (index)
Pfizer-BioNTech
Oxford-AstraZeneca
H
Anti-RBD neutralizing activity (%)
Pfizer-BioNTech
Oxford-AstraZeneca

Fig. 2