CORESMA - COVID-19-Outbreak Response combining E-health, Serolomics, Modelling, Artificial Intelligence and Implementation Research

WP 2	**Differential serolomics to assess sero-prevalence, cross and pre-existing immunity against coronaviruses**
Deliverable D2.6	**Report**
Title of Deliverable:	**Screening of German national cohort samples**
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Deliverable 2.6 Screening of German national cohort samples

At the beginning of the COVID-19 pandemic, it was hypothesized that a previous exposure to the widely circulating four endemic human "common cold" coronaviruses (hCoVs) HKU1, NL63, OC43 and 229E could offer protection from a SARS-CoV-2 infection based on sequence similarity between the viruses resulting in cross-reactive antibodies or T-cells. While the role of preexisting T-cell driven immunity in preventing SARS-CoV-2 infections has been accounted for[1-4], there is still a lack of scientific consensus on pre-existing humoral SARS-CoV-2 immunity with some authors reporting protection from a SARS-CoV-2 infection while others describe a negative correlation of hCoV antibody levels and COVID-19 severity or strength of the neutralizing SARS-CoV-2 antibody response[5-8]. Our use of MULTICOV-AB in such studies[9-11], has identified equally divergent results with both the presence and absence of a potentially cross-protective effect indicating that participants characteristics or collection time point (i.e. seasonality of hCoVs) might be key in determining whether a correlation will be found or not.

Renk et al[9] (Figure 1a) and Becker et al[10] (Figure 1b) both identified a lack of cross-protection from either of the 4 hCoVs antibody levels, with no significant difference seen between those who were infected with SARS-CoV-2 and those who were not. By contrast, Wratil et al[11] (Figure 1c) found a small significant correlation between nucleocapsid alpha coronavirus antibodies and SARS-CoV-2 infection. Of these three studies, Renk et al[9] represents the most realistic scenario to assess if pre-existing hCoV antibody levels impact on SARS-CoV-2 susceptibility as the study design involved two sample collections approximately 8 months apart from households with adults and children and at least one previous PCR-confirmed SARS-CoV-2 infection within the family. Critically, this manuscript also identified that endemic coronavirus antibody titres itself strongly correlated with increasing age and the associated repeated exposure (Figure 1d).

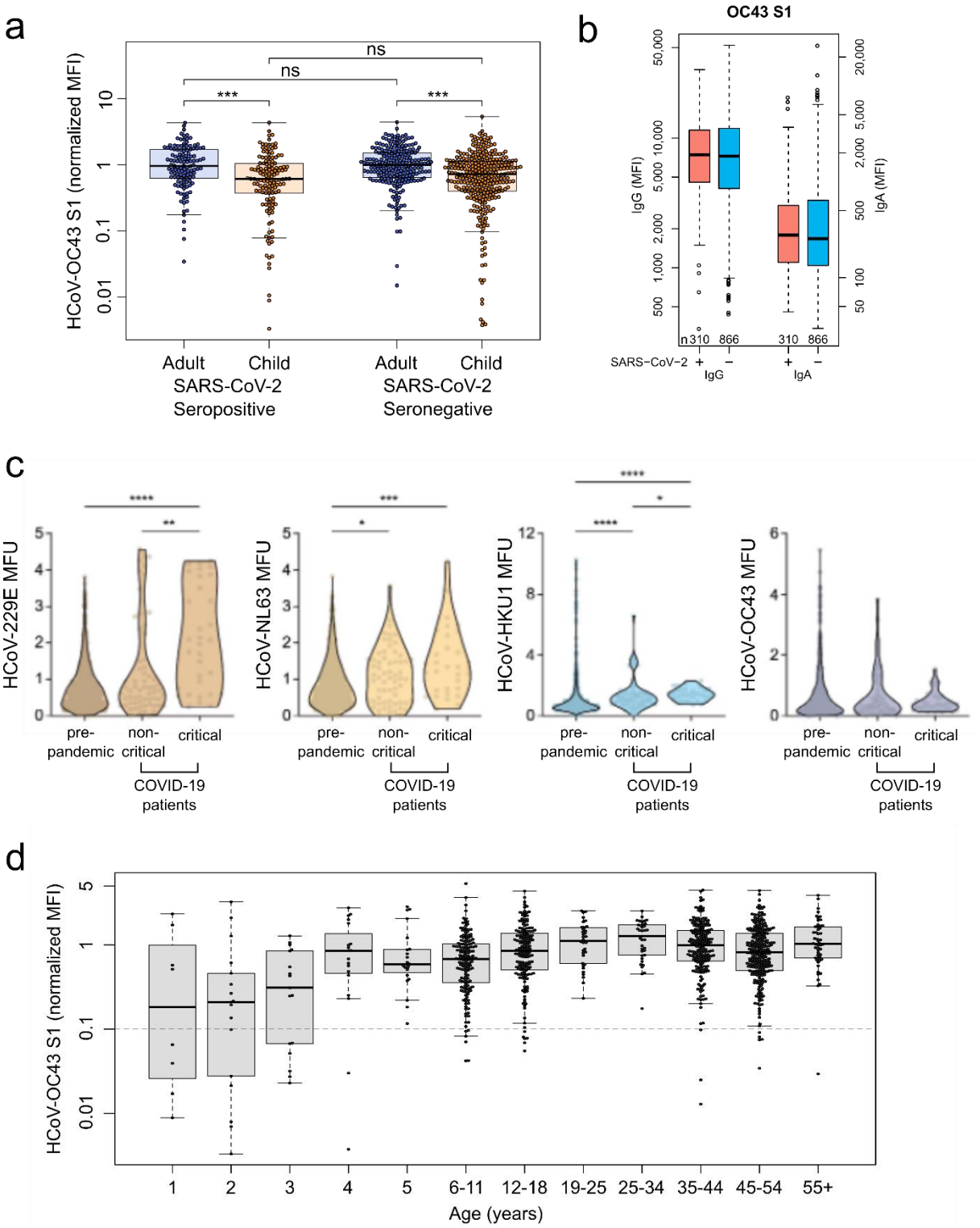

Figure 1: The potential role of hCoVs in SARS-CoV-2 susceptibility. (a and b) No significant difference in hCoV titres in adults and children were identified between those who did and did not seroconvert for SARS-CoV-2. Selection of OC43 S1 antibody levels is exemplary. 229E, NL63 and HKU1 antibody titers were equally comparable. Reproduced from Renk et al[9] and Becker et al[10]. (c) Significant correlations between endemic coronavirus titre and COVID-19 infection/severity. Reproduced from Wratil et al[11]. (d) hCoV titre increases with age due to repeat exposures. Dashed line indicates naïve samples. OC43 S1 antibody levels are again used as an example. Reproduced from Renk et al[9].

By screening approximately 10000 pre-pandemic sera samples collected as part of the German national cohort (GNC), we initially intended to evaluate endemic hCoV antibody levels and determine their implications on SARS-CoV-2 susceptibility. Based on the aforementioned issues such as the seasonal hCoV infection dynamics, we instead analyzed samples from 2992 GNC participants and of 1734 MuSPAD[16,17] participants collected within a tighter time frame between February and July 2022. In contrast to the pre-pandemic sera samples, this second sample cohort does not only allow us to assess actual SARS-CoV-2 population seroprevalence and correlate it to the endemic hCoV antibody levels at the time of sampling, but also to determine with a larger sample size if SARS-CoV-2 vaccination impacts on hCoV antibody levels. To differentiate if SARS-CoV-2-specific antibodies originated from an infection or a vaccination by using the presence of nucleocapsid–specific antibodies in a sample. The capability of MULTICOV-AB to differentiate infected from vaccinated individuals based on their antibody profiles has been shown previously in the analysis of over 5500 samples across various studies[10-15], for which assay sensitivity and specificity always remaining at a minimum of 90% and 100%,respectively. We identified that a total of 4262 samples (90.18%) had SARS-CoV-2 Spike-specific antibodies. Of these, 4106 (96.33%) of samples were classified as vaccinated, while 156 (3.67%) were classified as infected.

To examine the role of endemic coronavirus antibody levels, the corresponding medians and 95% confidence interval for each group are shown in Table 1.

	HCoV-OC43 S1	**HCoV-HKU1 S1**	**HCoV-NL63 S1**	**HCoV-229E S1**
Negative	0.77 (0.69-0.82)	0.95 (0.86-1.01)	1.20 (1.13-1.31)	1.02 (0.97-1.08)
Infected	0.73 (0.57-0.92)	0.94 (0.82-1.06)	1.19 (0.97-1.31)	1.02 (0.90-1.11)
Vaccinated	0.75 (0.73-0.77)	0.90 (0.87-0.93)	1.17 (1.13-1.20)	0.98 (0.96-1.00)

Table 1: Median HCoV normalised MFI (95% CI of median in brackets) sera samples classified as SARS-CoV-2 negative, infected and vaccinated.

In agreement with results of Renk et al. and Becker et al., hCoV antibody titres displayed as normalised MFI values were comparable between the two sample groups defined as SARS-CoV-2 non-infected or infected indicating that there is no protective role for existing endemic coronaviruses antibody levels with regards to SARS-CoV-2 susceptibility. Interestingly, hCoV antibody levels in sera samples from vaccinated individuals did also not differ which implies that SARS-CoV-2 vaccination as an infection only induces SARS-CoV-2 Spike-specific B-cell populations and equally does not confer enhanced levels of protection by creating potentially cross-reactive antibodies. In summary, we have screened approximately 4700 samples from different national population cohorts, identifying no differences in hCoV titers between infected, negative and vaccinated individuals. Should further studies be required to definitively assess the role of humoral pre-existing SARS-CoV-2 immunity after exposure to endemic coronaviruses, hCoV infection seasonality means that those studies would need to be tightly controlled longitudinal studies with frequent sera sampling and molecular testing for SARS-CoV-2 exposure. However, as the majority of the population now already has some form of vaccine-derived protective immunity, it is unclear as to whether such studies would still be relevant.

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