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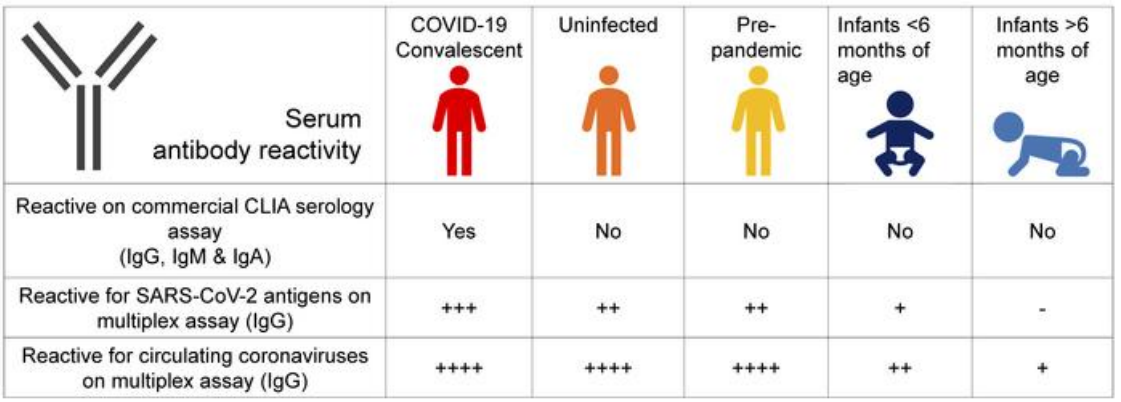
Graphical abstract



A majority of uninfected adults show pre-existing antibody reactivity against SARS-CoV-2

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Abstract

Pre-existing cross-reactivity to SARS-CoV-2 may occur in absence of prior viral exposure. However, this has been difficult to quantify at the population level due to a lack of reliably defined seroreactivity thresholds. Using an orthogonal antibody testing approach, we estimated that ~0.6% of non-triaged adults from the greater Vancouver area, Canada between May 17th and June 19th 2020 showed clear evidence of a prior SARS-CoV-2 infection, after adjusting for false-positive and false-negative test results. Using a highly sensitive multiplex assay and positive/negative thresholds established in infants in whom maternal antibodies have waned, we determine that more than 90% of uninfected adults showed antibody reactivity against the spike, receptor-binding domain (RBD), N-terminal domains (NTD) or the nucleocapsid (N) protein from SARS-CoV-2. This sero-reactivity was evenly distributed across age and sex, correlated with circulating coronaviruses reactivity, and was partially outcompeted by soluble circulating coronaviruses' spike. Using a custom SARS-CoV-2 peptide mapping array, we found that this antibody reactivity broadly mapped to spike, and to conserved non-structural viral proteins. We conclude that most adults display pre-existing antibody cross-reactivity against SARS-CoV-2, which further supports investigation of how this may impact the clinical severity of COVID-19 or SARS-CoV-2 vaccine responses.

Introduction

Coronavirus disease 2019 (COVID-19) was declared a global pandemic on March 11th, 2020 and has resulted in almost 100 million confirmed cases and 2.1 million deaths worldwide as of January 24th, 2020. Almost all individuals infected with SARS-CoV-2 seroconvert within 2-3 weeks, with the spike and nucleocapsid (N) proteins eliciting the strongest responses (1, 2). While much attention has focused on defining immune reactivity in individuals post-infection, other data have indicated that many individuals show pre-existing SARS-CoV-2 cross-reactive T cell and B cells without prior exposure to the virus (3-5). However, the extent of pre-existing SARS-CoV-2 antibody reactivity at the population level has been difficult to estimate, due to a lack of assay sensitivity (6) and clearly definable background thresholds to define meaningful seroreactivity among individuals who have been unexposed to the virus (7).

There are four circulating coronaviruses pre-dating COVID-19 that cause up to 30% of seasonal upper respiratory tract infections (8). The spike proteins of beta-coronaviruses HKU1 and OC43 exhibit ~40% sequence similarity, whereas the alpha-coronaviruses, NL63 and 229E, exhibit ~30% structural similarity with SARS-CoV-2 (9). The common occurrence of circulating coronaviruses year after year and their structural similarity with SARS-CoV-2 raise the possibility that the former may stimulate cross-reactive responses towards SARS-CoV-2, and that this heterotopic immunity may impact clinical susceptibility to COVID-19 and/or modulate responses to the SARS-CoV-2 vaccine (10, 11).

The main objectives of this study were to estimate the extent of the pre-existing seroreactivity against SARS-CoV-2 in the general adult population, and its relationship to circulating coronaviruses. To confirm that SARS-CoV-2 antibody reactivity in uninfected adults was genuinely cross-reactive and not due to widespread unreported, asymptomatic SARS-CoV-2 circulation, we similarly assayed sera collected prior to the emergence of SARS-CoV-2 and from infants before and after maternal antibodies have waned. In addition, we used a SPOT peptide array to map this antibody reactivity on the SARS-CoV-2 proteome.

Results

Study population: In total, 276 healthy adults were recruited for this cohort between May 17th and June 19th 2020. The demographic characteristics and geographical area of residence of participants are shown in **Supplemental Table 1** and **Supplemental Figure 1**, respectively. The majority (n = 196; 71%) were health care workers. Less than half had travelled outside of British Columbia (BC) since January 1st 2020, to the USA, Europe, Iran, the Caribbean, Australia, Mexico and Japan. Two individuals had a history of PCR-confirmed COVID-19.

Prevalence of prior SARS-CoV-2 infection in the study population: To estimate the proportion of individuals who had been previously infected with SARS-CoV-2, we used a multiplex assay to profile antibody reactivity against four viral antigens: the whole SARS-CoV-2 spike protein, N-terminal domain (NTD) and its receptor binding domain (RBD), and N. Clustering analysis based on antibody reactivity for these four antigens identified that three individuals (CW087, CW0150, FH0037) and five control sera from convalescent COVID-19 patients (A, B, C, D and E) clustered together, separately from the rest of the cohort (**Figure 1**). The antibody reactivity profile of these 8 distinct sera showed high reactivity against all four SARS-CoV-2 antigens, whereas all other individuals showed variable antibody reactivity against either spike, RBD or the N protein (**Supplemental Figure 2**).

The three individuals (CW087, CW0150, FH0037) clustered with the five control sera including the two individuals who had a history of PCR-confirmed COVID-19, plus an asymptomatic woman who was not

aware she had COVID-19 initially but who was later identified to have been in contact with a COVID-19 case about 90 days prior to serology testing for this study (**Supplemental Table 2**).

All sera from the cohort who displayed above-the-mean antibody reactivity for at least one of the four SARS-CoV-2 antigens (i.e. for a total of 222 out of 276 individuals) were further tested with a commercial diagnostic CLIA assay, which recognizes the S1 domain of the SARS-CoV-2 spike protein (**Supplemental Figure 3**). With this assay, the same three individuals (CW087, CW0150, FH0037), plus the five control sera mentioned above, tested positive. Therefore, based upon these data, it appeared that 3 out of 276 participants (1.1%) showed clear evidence of a previous infection with SARS-CoV-2. After adjusting for bias by using point estimates of specificity and sensitivity of the CLIA assay, we estimated that the prevalence of a previous SARS-CoV-2 infection was 0.60% [95%CI 0% to 2.71%] in this cohort.

Antibody reactivity to circulating coronaviruses: The multiplex assay also included quantification of antibody reactivity against the spike proteins of circulating coronaviruses (OC43, HKU1, NL63, 229E). All individuals showed high antibody reactivity against these proteins (**Supplemental Figure 2**). To understand the relationship between the antibody reactivity against SARS-CoV-2 and circulating coronaviruses we used correlation analyses. Among the 273 seronegative individuals, we detected significant correlations between antibody reactivity to SARS-CoV-2, and HKU1, NL63, and 229E, but not OC43 (**Supplemental Figure 4**).

Specificity of SARS-CoV-2 antibody reactivity in uninfected individuals: Next, we conducted competition experiments to exclude the possibility that the antibody reactivity against SARS-CoV-2 in uninfected individuals was due to non-specific binding in the multiplex assay and to assess whether this antibody reactivity may represent cross-reactive antibody responses to circulating coronaviruses. To this end, we determined whether the antibody reactivity against antigens in the multiplex assay could be competed using either a cocktail of free SARS-CoV-2 RBD and full-length spike proteins, or of all four other circulating coronavirus (OC43, HKU1, NL63 and 229E) spike proteins pooled (**Figure 2**). Antibody reactivity was measured on serial dilutions from selected COVID-19 convalescent and uninfected sera selected on the basis of a high reactivity to full-length SARS-CoV-2 spike protein, its RBD or low reactivity to both of these antigens. As expected, SARS-CoV-2 spike and RBD reactivity in COVID-19 convalescent sera was efficiently outcompeted by free SARS-CoV-2 spike and RBD proteins, but not by other free circulating coronavirus spike proteins (**Figure 2A**, **B**).

Moreover, antibody reactivity to SARS-CoV-2 spike and RBD was partially outcompeted by circulating coronavirus spikes in uninfected individuals – this latter finding supports the argument that at least some of this SARS-CoV-2 antibody reactivity represented cross-reactivity towards circulating coronaviruses. Conversely, reactivity to circulating coronavirus spike proteins was efficiently outcompeted by spike from circulating coronaviruses, but not by SARS-CoV-2 spike and RBD proteins (**Figure 2C**, **D**). Therefore, this experiment confirms that SARS-CoV-2 spike and RBD antibody reactivity in uninfected individuals is saturatable, essentially excluding non-specific binding in the multiplex assay. Interestingly, competition of SARS-CoV-2 spike antibody reactivity was higher in uninfected individuals with higher detectable SARS-CoV-2 spike or RBD antibody reactivity compared to individuals who showed low reactivity against these two antigens (**Figure 2E**). Curiously, antibody reactivity against SARS-CoV-2 RBD in uninfected individuals was not efficiently competed by a fixed amount of SARS-CoV-2 RBD.

Seroreactivity thresholds defined in sera from immunologically naïve infants: To unequivocally distinguish uninfected individuals who could have SARS-CoV-2 antibody reactivity, we defined the background of antibody reactivity of sera in the multiplex assay. We reasoned that infants would be

immunologically naïve, with the exception of maternal antibodies that are expected to wane gradually after birth, and thus their sera can be used to define antibody reactivity thresholds in uninfected adults in the multiplex assay. Using this assay, we measured antibody reactivity of sera from 45 infants less than 6 months of age and repeated in the same infants ~8 months later, after BC's lockdown period (**Figure 3**), including 21 infants in whom the first sera were obtained before the pandemic (i.e. before January 2020). In infant sera, reactivity to circulating coronaviruses was uniformly detected in the first set of blood samples, albeit at much lower levels than adults. In the second infant sample sets taken after July 1st 2020, antibody recognition of circulating coronaviruses had decreased to ~1000-fold lower levels compared to adults, consistent with a waning of maternal antibodies (**Supplemental Figure 5**). When comparing antibody reactivity of SARS-CoV-2 in the second post-natal infant sera, levels were up to 100-fold higher in uninfected adults compared to infants, for the different SARS-CoV-2 antigens (**Figure 3**).

Thus, these second infant samples allowed us to define effective thresholds for SARS-CoV-2-antibody reactivity in uninfected adults (**Figure 3**). Based on infants' sera, we estimate that between 90% and 99% of adults show positive antibody reactivity for SARS-CoV-2 spike, RBD or the N antigen. Prepandemic sera showed similar antibody reactivity, therefore excluding the possibility that the reactivity in adults after the first pandemic was due to undiagnosed exposures to the virus in the study population. This baseline, pre-existing SARS-CoV-2 cross-reactivity in uninfected adults was evenly distributed according to age, sex, travel history, whether participants were healthcare workers (HCW) or not, and was independent of participants' reporting of "COVID-19-like" symptoms (**Supplemental Figure 6**).

Further characterization of SARS-CoV-2 antibody reactivity in uninfected adults: To map the antibody reactivity on the viral proteome, we used a SPOT array assay where peptides broadly covering the SARS-CoV-2 proteome were directly synthesized on a cellulose membrane (**Supplemental Figure 7**). To enrich for high affinity antibodies, sera from individuals that showed high spike or RBD antibody reactivity where compared to infant samples. As shown in **Figure 4**, we detected high antibody reactivity against non-structural proteins, particularly the non-structural protein 2 (nsp2) and nsp15 encoded in the replicase polypeptides ORF1a and ORF1b. In addition, several structural peptide regions of the S1 and S2 domains of the spike protein. RBD-high samples showed the strongest antibody reactivity encompassing RBD, but also the S1 and the S2 peptides, indicating a diverse anti-SARS-CoV-2 antibody reactivity linked to a high RBD antibody cross-reactivity. This cross-reactivity was also detected in randomly selected prepandemic sera, which demonstrated pre-existing recognition prior to the SARS-CoV-2 pandemic. Importantly, we detected no antibody reactivity against any viral peptides in infants' sera.

Discussion

In this study, we estimated that 0.60% [95%CI 0% to 2.71%] of the study population showed evidence of a prior infection with SARS-CoV-2. The combination of a highly specific commercial CLIA assay and a highly sensitive multiplex assay allowed us to distinguish individuals who have been infected with SARS-CoV-2 from those who have not. This prevalence of SARS-CoV-2 infections was identical to the 0.55% prevalence reported by the BC Centre for Disease Control (BC CDC) on 885 residual sera obtained from an outpatient laboratory network in the Lower Mainland of BC between May 15 and 27, 2020. Data from the BC CDC represented a wider geographical catchment and did not specifically target HCW (12). The current study confirms that COVID-19 transmission in BC after the first wave was low, even among HCW, contrasting with a high seroprevalence reported among HCW in other studies (13-15) which may be attributed to the very low number of total tested cases in BC during the first wave.

The main finding in this study is that at a population level, the vast majority of adults show antibody reactivity against SARS-CoV-2 antigens. BC reported its first COVID-19 case on January 29th, with the first documented case of community transmission on March 5th, 2020. The first pandemic wave peaked between the third week of March and late April (11). As of May 17th, only 2,445 diagnosed COVID-19 cases (~49/100,000 population) had been reported in BC after the first wave, which was the lowest rate in Canada and one of the lowest rates in North America. Because of a relatively low number of COVID-19 cases in BC after the first wave, it is extremely unlikely that this antibody reactivity results from a direct exposure to SARS-CoV-2. Moreover, findings of similar antibody reactivity in prepandemic adult sera and from sera obtained from infants under one year of age confirms that we are detecting genuine cross-reactivity rather than reactivity to SARS-CoV-2 from asymptomatic COVID-19 cases.

Our findings are consistent with another study in which prepandemic sera exhibited cross-reactive IgG antibody reactivity with conserved epitopes in SARS-CoV-2 proteins (S2 and N) (5). The higher prevalence of pre-existing antibody reactivity in uninfected adults in our cohort compared to this previous study may be explained by the high sensitivity of our assay and evidence of positive seroreactivity in those individuals informed by the infant sera. However, whereas these previous studies have quantified cross-reactivity in selected sera, to the best of our knowledge the current study is the first to determine SARS-CoV-2 antibody reactivity at the population level. The fact that we measured antibody reactivity between infected and uninfected individuals in the same population and time period in the current study also eliminates recruitment or sampling biases and is another major strength of this study.

The presence of pre-existing SARS-CoV-2 antibody reactivity in uninfected individuals in the current study is consistent with the detection of T cell reactivity against SARS-CoV-2 in about 40% of uninfected individuals (3, 4). This raises an important question: what is the antigenic source of this antibody reactivity? Competition experiments and correlatives analyses indicate that it may in part be attributable to cross-reactivity against circulating coronaviruses. Most humans become infected with circulating coronaviruses by their second year of age (16). On the one hand, correlations between SARS-CoV-2 and either HKU1, N63L, and 229E, but not OC43 antibody reactivity could reflect seasonal variations in recent exposure to common coronaviruses (10, 17). On the other hand, the high antibody reactivity to SARS-CoV in individuals in this study likely represents cross-reactivity due to the higher (>75%) sequence similarity between SARS-CoV and CoV-2 (18, 19), rather than a previous exposure to SARS-CoV.

The data presented in this study shed light on another important question: what region of the virus does this pre-existing antibody reactivity bind to? We found in our peptide mapping experiments that it is broadly distributed across the viral proteome, including whole spike, and proteins encoding the viral replication complex. The binding to ORF polypeptides could possibly be a sign of infection by circulating coronaviruses that share conserved sequences with SARS-CoV-2. High antibody reactivity against non-structural ORF proteins was reported in another study using a VirScan peptide mapping approach on prepandemic sera (6). However, due to a lower sensitivity of the assay, antibody reactivity against spike was not detected in the latter study. Here, we confirm that this pre-existing antibody reactivity involves structural external elements of the virus in both epitope mapping and competition experiments.

It is unclear whether this antibody reactivity may confer clinical benefits, for instance, modulating the severity of a SARS-CoV-2 infection. Data indicate that a past circulating coronavirus infection may decrease the severity of a subsequent SARS-CoV-2 infection (20). Others have linked pre-existing seroreactivity against circulating coronaviruses to increased SARS-CoV-2 pseudovirus neutralization in vitro (5), although this remains debated. Individuals with high RBD reactivity showed the most

structurally diverse antibody reactivity against spike epitopes, which may enhance viral clearance in addition to the improved neutralizing activity specific to RBD-binding antibodies. Indeed, strong antibody response to RBD have been linked to improved clinical outcomes from COVID-19 (21). However, reactivity against RBD in the multiplex assay was not competed by soluble RBD in uninfected individuals, despite that this reactivity was almost completely abrogated in COVID-19 convalescent sera. The latter finding is consistent with another study that showed that pre-existing antibody reactivity against SARS-CoV-2 in prepandemic sera could be efficiently competed by a soluble S1 (that contains the RBD domain), but not a soluble S2 subunit of the spike protein (5). Notably, we were also unable to detect ACE2 receptor binding inhibition from sera of uninfected individuals (not shown), which could indicate that the pre-existing antibody reactivity against SARS-CoV-2 in uninfected adults represents an excess of low affinity antibodies that have poor overall viral neutralizing potential. This may not be surprising given that viral neutralization improves generally with affinity maturation, an antigen-driven process that requires cognate interaction by B cells, in collaboration with follicular T cells. Similarly, pre-existing, highly variable low avidivity SARS-CoV-2 CD4 memory T cells cross-reactive to circulating coronaviruses appeared less protective in uninfected adults (22). More studies are needed to understand the origin of pre-existing SARS-CoV2 antibodies and their impact on COVID-19 severity.

In conclusion, this study reveals common pre-existing, broadly reactive SARS-CoV-2 antibodies in uninfected adults. These findings warrant larger studies to understand how these antibodies affect the severity of COVID-19, but also the quality and longevity of responses to SARS-CoV-2 vaccines.

Methods

Study design: Prospective cross-sectional study after the first pandemic wave in BC.

Participants: Adults over 18 years of age from the greater Vancouver metropolitan area were included if they did not have active COVID-19, did not require self-isolation as per BC provincial public measures, or had recovered from COVID-19 at least 14 days prior to the study visit and blood collection. Blood was drawn in gold-top serum separator tubes with polymer gel (BD, cat# 367989); after at least 30 minutes of clotting at room temperature, the blood sample was then centrifuged at 1,400 x G to obtain serum aliquots that were frozen at -80°C within four hours of collection. Adult prepandemic sera were all obtained before January 1st 2020. Infants sera were collected before discharge from hospital at birth (1st sample) and after June 11th of 2020 (2nd sample), as part of a study examining antibody responses to respiratory viruses.

Recruitment: Greater Vancouver is the main urban centre in BC and third largest metropolitan area in Canada, with a population of 2.5 million. Study participants were invited by an email sent to clinical departments of the BC Children's & Women's Hospitals (C&W, the largest pediatric referral centre in BC, located in Vancouver, and where no cases of COVID-19 were admitted during the pandemic's first wave) and its affiliated BC Children's Research Institute (BCCHR). The study was also advertised to hospitalists, anesthesiologists and critical care physicians at Surrey Memorial Hospital (SMH, located ~27 km from Vancouver). To minimize recruitment bias, all adults who responded to the invitation email and returned their signed consent form were enrolled sequentially and invited to give a blood sample, without triaging. Blood samples were collected between May 17th and June 19th, 2020.

Study size: Since there was little population seroprevalence data available at the time and none in BC or Canada, no *a priori* sample size calculation was performed. The recruitment period was therefore defined by convenience over a three-week period of enrolment, in order to obtain baseline data.

Multiplex antibody assay: A highly sensitive multiplex (10-plex) assay (Meso Scale Diagnostics, Gaithersburg, USA, cat. #K15369U) where each antigen is 'spotted' into a single well of a 96-well plate (23) was used to measure antibody profiles against four SARS-CoV-2 antigens: the trimeric (whole) S-2P native spike protein, its receptor-binding domain (RBD), its N-terminal domain (NTD) (24) and nucleocapsid (N) protein; the trimeric SARS-CoV spike protein; and spike proteins from circulating beta- (HKU1, OC43) and alpha- (229E, NL63) coronaviruses, plus bovine serum albumin (BSA), a negative control. Briefly, after blocking wells with 5% BSA, sera were added at 4 dilutions (1:100, 1:800, 1:3200 and 1:10,000) and incubated with shaking for two hours. Sulfo-tag-labelled anti-IgG detection antibody were added and the electrochemiluminescence (ECL) signal was read using the MSD Sector 600 instrument (Meso Scale Diagnostics, Rockville, MD). Initial area under the curve of the ECL values for antibody detection were well above the BSA background for all sera for SARS-CoV-2 antigens except for one sera for the RBD and N antigens, and 10 sera for the NTD antigen. Samples were re-screened again in a second set of experiments after MSD provided standards. Results are presented as dilution-corrected interpolated values from a standard curve with assigned Arbitrary Units (AU)/mL. Assignment of AU/mL of serum was performed by Meso Scale Diagnostics and is designed such that values are comparable to an International Standard Serum (ISS), so that bridging to a WHO International Standard will be possible in the future.

Competition experiments: Eight 2-fold dilutions of sera pre-diluted in a ratio of 1:50 assay diluent were added to an equal volume of assay diluent (control), or to assay diluent mixes containing 5 μg/mL SARS-CoV-2 spike and 5 μg/mL RBD proteins (SARS-CoV-2 RBD-spike cocktail), or 5 μg/mL spike proteins from all four circulating coronaviruses (HKU1, OC43, 229E, NL63: cCoVs cocktail) (25), for an on-plate assay dilution of 1:100 through 1:12,800. The dilution series were incubated for 30 minutes at room temperature and then analyzed using the multiplex antibody assay protocol (see above).

Commercial chemiluminescent (CLIA) antibody assay: Total antibody (IgA, IgG and IgM) against recombinant spike (S1) protein was determined using the VITROS 5600 analyser (Ortho-Clinical Diagnostics, Rochester, NY) according to manufacturer instructions. This is a Health Canada and FDA-licensed qualitative assay with reported performance and in-house validation indicating sensitivities >7 days post onset range between 96% and 100% and specificities from 99% to 100% (26, 27).

SPOT peptide array: Forty-one 15-mer peptides selected based on their reactivity on convalescent samples and immunogenicity, that were distributed over the entire SARS-CoV-2 proteome were synthesized on a cellulose trioxatridecanediamine membrane using a MultiPep synthesizer (CEM, NC USA) (28). Additionally, each membrane contained a human-IgG binding peptide as positive control (29). These in-house made membranes were incubated with a 1:400 dilution of sera and incubated for 2 hours at room temperature. A copy of the array was also incubated with Tris buffered saline with Tween 20 only, as a negative control. After washing, membranes were incubated with secondary antibody (HRP conjugated goat anti-human IgA+IgG+IgM polyclonal antibody; Jackson ImmunoResearch Inc.; cat# 109-035-064) at a 1:30,000 dilution for another 2 hours, and detection was carried out using enhanced chemiluminescence (ECL), with 8 images captured over an exposure time of 50 sec. The greyscale of images represented as numeric values between 0 and 10 were used before applying a uniform background correction of 1.

Variables: The following information were collected from participants by questionnaire: age, sex, the first three digits of their postal code, HCW status (and whether they worked at C&W or SMH), history of travel outside BC since January 1st, 2020, and history of COVID-19 symptoms and testing. SARS-CoV-2-

exposed cases were defined by a positive result on the commercial CLIA assay, validated for sensitivity by antibody profiling on the multiplex assay (detailed in Results).

Statistical analyses: The seroprevalence from a SARS-CoV-2 exposure was adjusted for bias due to false positive and false negative tests using the Greenland method (30). Differences in proportions were calculated using a Fisher exact test, with significance threshold at $P<0.05$. Hierarchical clustering of antibody levels (based on the multiplex assay) was performed on log-transformed, z-score normalized serology data, using the complete linkage agglomeration method and Euclidean distance measures. Spearman correlations between antibody levels and metavariables were adjusted for multiple testing using the Benjamini-Hochberg false-discovery rate method (FDR 5%). There were no missing data. Analyses were conducted in R version 4.0.2, R Studio version 3.6.2, and GraphPad Prism version 8.4.

Study approval: Written informed consent was obtained from all participants. The study procedures were approved by the University of British Columbia (UBC) Children's & Women's Research Ethic Board (H20-01205; H18-01724).

Author's contributions: AM, CM and SD coordinated the study sample accrual and blood processing in Vancouver. JG and DM coordinated recruitment at SMH. CC collated the data and helped with data analysis. SEOC performed the multiplex assay, with help from SN and MB. MG provided important input into the study design. AL, IS and ANJ revised the manuscript. MI supervised the statistical analyses. VEB and DMG supervised the commercial CLIA testing of samples. DW and SP performed the SPOT peptide array analysis. PML and ABM supervised the study in Vancouver and at the NAID/NIH, respectively. AM, CM, SD, DCD and PML wrote the manuscript first draft. All authors contributed to the study design, data analysis and reviewing the manuscript, and accept the article submission in its final form. The order of co-first authors was determined based on their earlier involvement at the study design stage.

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Figure legends

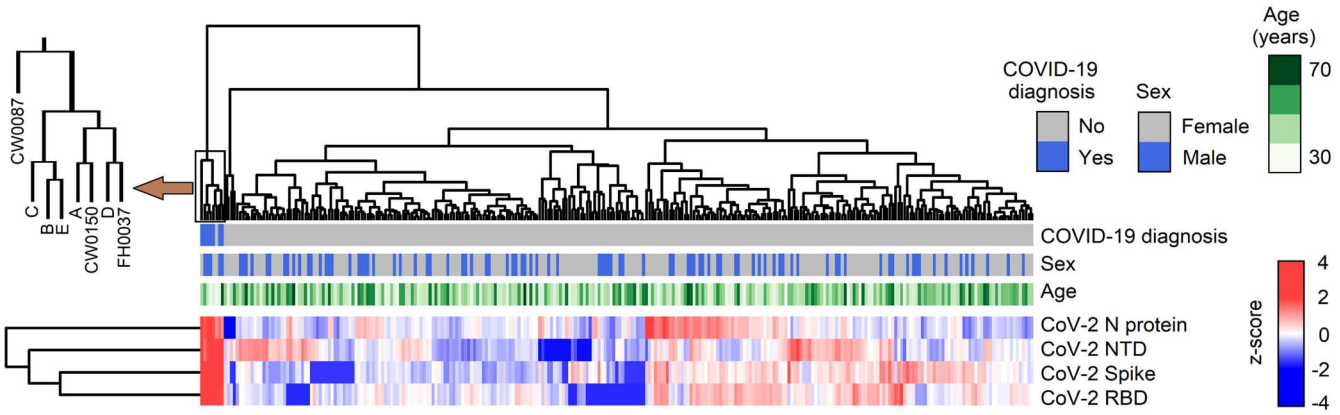

Figure 1. Hierarchical clustering of individual based on serum SARS-CoV-2 antibody reactivity profiles. COVID-19 diagnosis identifies convalescing individuals who had a positive viral test by PCR. This figure combines data from 276 study participants plus the five COVID-19 convalescent control sera. Colour scale represents antibody reactivity as a z-score.

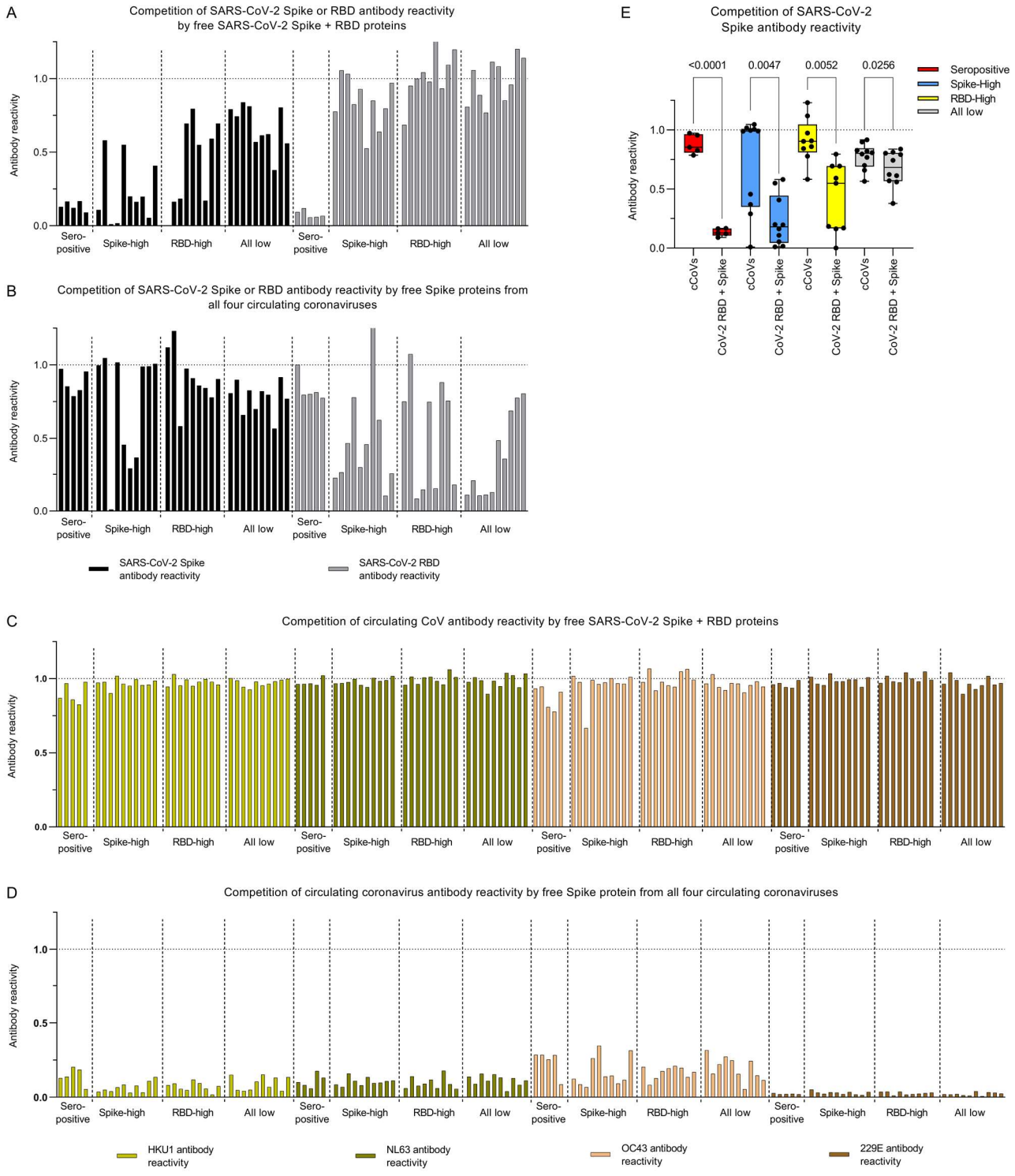

Figure 2: Specificity of SARS-CoV-2 antibody reactivity. Competition of SARS-CoV-2 spike and RBD antibody reactivity by (**A**) SARS-CoV-2 spike or RBD or (**B**) by circulating coronaviruses (cCoVs) spike proteins; Competition of circulating CoVs spike antibody reactivity by (**C**) SARS-CoV-2 spike and RBD proteins or (**D**) circulating CoVs spike proteins; (**E**) Competition of SARS-CoV-2 spike antibody reactivity by SARS-CoV-2 spike and RBD proteins, or cCoVs spike proteins in COVID-19 convalescent sera (seropositive, n=5) or sera from uninfected individuals who showed highest SARS-CoV-2 spike

(n=10), high RBD (n=9) or lowest SARS-CoV-2 spike and RBD antibody reactivity (All low; n=10). All values represent the ratios of antibody reactivity in competed samples over the antibody reactivity measured in absence of competing proteins (dash line). One sample in the RBD-high group failed and these data are not shown. In (E), data is represented as boxes (25th to 75th percentile, line at median) and whiskers (min to max); comparisons were made using paired t-tests.

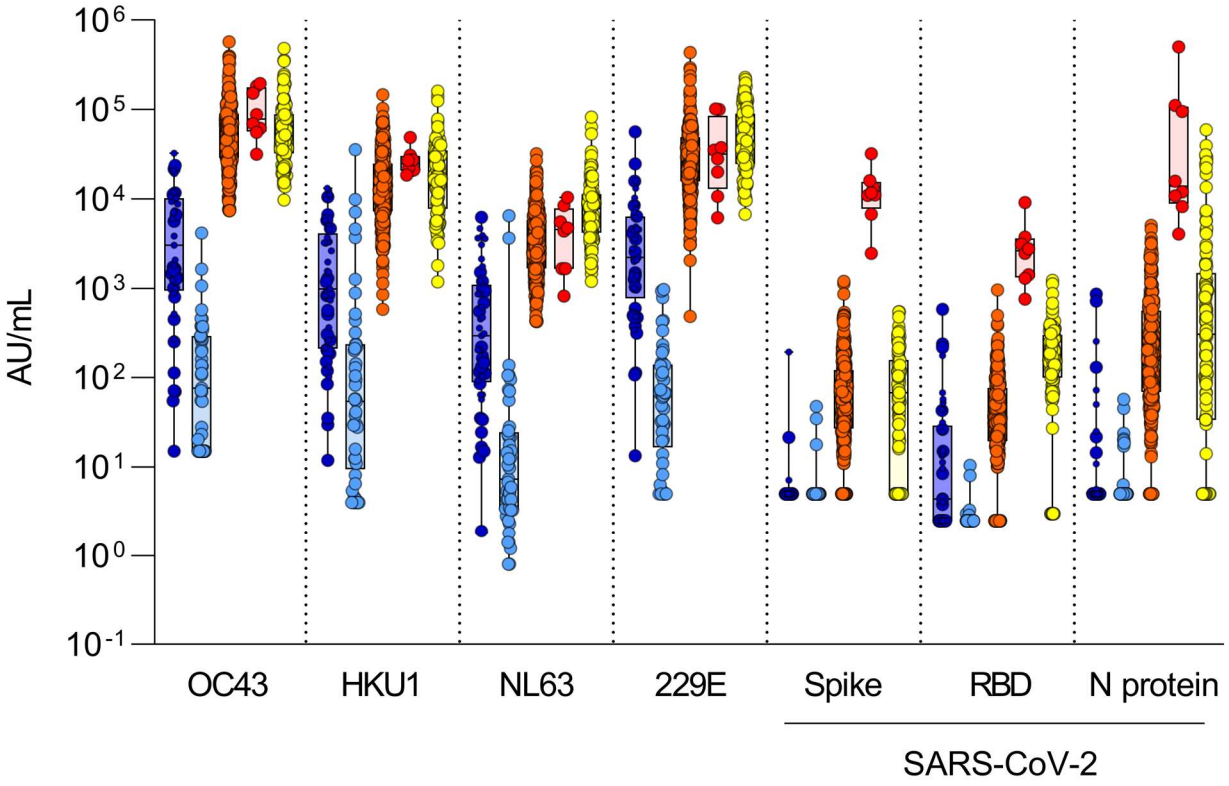

Figure 3. Thresholds of antibody reactivity based on infants' sera. Comparison of antibody reactivity (AU/mL) in infants sampled before 6 months of age (darker blue) and again, ~8 months later (lighter blue; n = 45), SARS-CoV-2-uninfected (orange; n = 273) and SARS-CoV-2-infected (convalescent) adults (red; n = 8) and from prepandemic sera (yellow; n = 99). Infants sampled before the pandemic (Jan 1st, 2020) are represented by the larger circle symbols whereas infants sampled after January 1st 2020 are shown using the small circle symbols. Boxes represent median with 25th and 75th centiles with positive/ negative antibody reactivity thresholds for SARS-CoV-2 spike calculated at the 99th centile for value distribution (10.00 AU/mL), RBD (10.00 AU/mL) and N protein (10.00 AU/mL) as $10^{[\text{mean log(antibody reactivity)} + \text{SD log (antibody reactivity)}*2.33]}$ in infants' sera. Antibody detection for NTD was low and inconsistent between experiments; therefore the data are not presented and reactivity thresholds were not calculated.

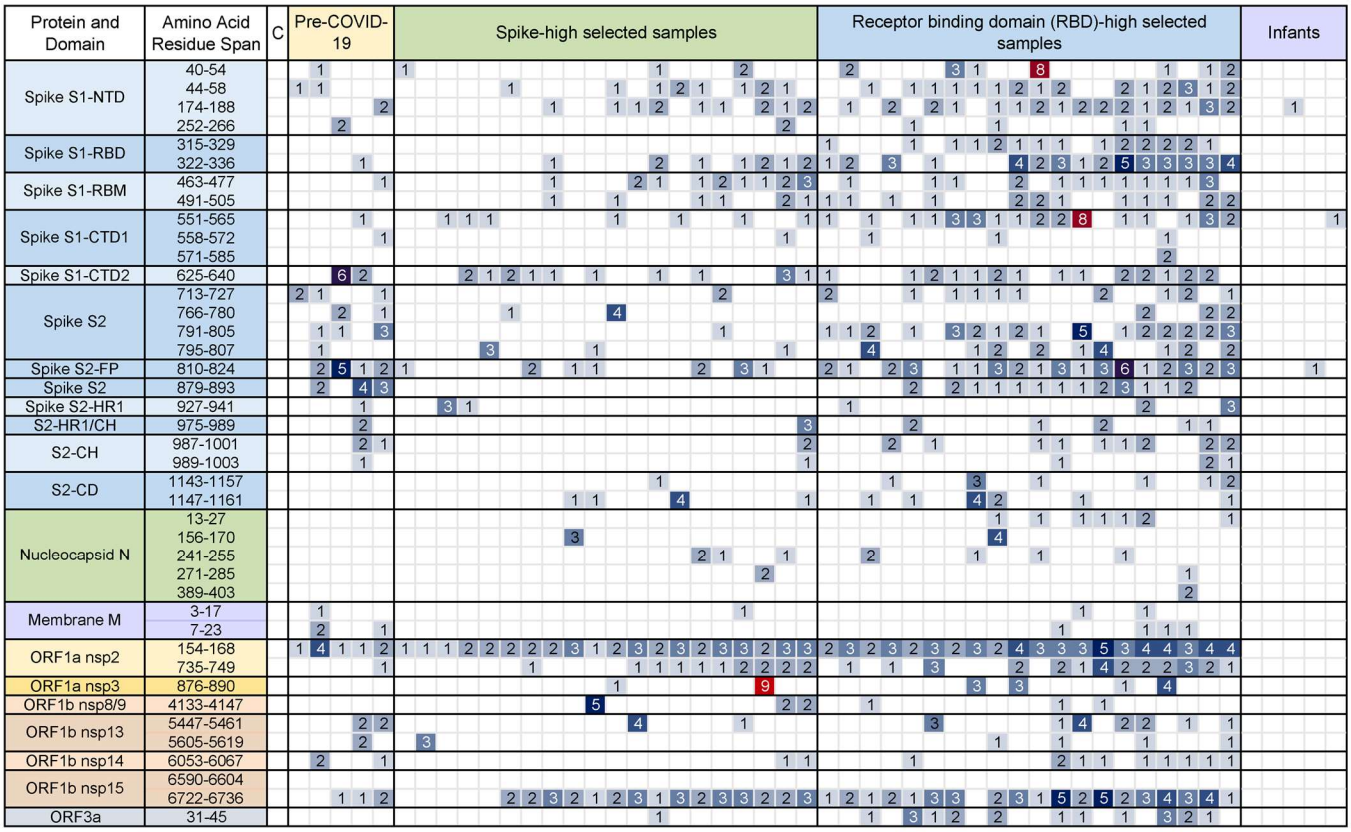

Figure 4. Mapping of SARS-Cov-2 antibody reactivity in sera from uninfected individuals. Serum antibody binding to 15-mer peptides distributed across the SARS-CoV-2 proteome or an IgG-binding peptide (positive control), from 5 randomly selected prepandemic samples, and adults showing high level of spike or RBD reactivity (n = 20 each), or infants (n = 5). Values represent signals on a scale of 0 to 10, after subtracting background. The first column labelled "C" shows the immunoreactivity signal in the absence of sera, but with the addition of anti-human IgA, IgM and IgG horse-radish peroxidase-coupled secondary antibody. NTD: N-terminal and CTD: N- and C-terminal (spike) domains; RBD: receptor-binding domain, HR: hinge region (between spike S1 and S2 domains); HR: heptad repeat; CH: central helix; CD: connector domain; N: nucleocapsid; M: membrane protein; ORF: Open-Reading Frame polypeptide proteins; nsp: non-structural proteins.