SARS-CoV-2 seroprevalence and characteristics of post-infection immunity in a general population cohort study in Catalonia, Spain.

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Article

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Abstract

Sparse data exist on the complex natural immunity to SARS-CoV-2 at the population level. We applied a well-validated multiplex serology test in 5000 participants of a general population study in Catalonia in blood samples collected from end June to mid November 2020. We detected a seroprevalence of 18.1% in adults (n=4740), and modeled extrapolation to the general population of Catalonia indicated a 15.3% seroprevalence. Antibodies persisted up to 9 months after infection. Immune profiling of infected individuals revealed that with increasing severity of infection (asymptomatic, 1-3 symptoms, ≥4 symptoms, admitted to hospital/ICU), seroresponses were more robust with a shift towards IgG over IgA and anti-spike over anti-nucleocapsid responses. Seropositive smokers showed lower seroresponses than non-smokers. In adolescents (n=260) seroprevalence was 11.5% and IgG anti-spike responses were dominant. Our study provides an unbiased estimate of SARS-CoV-2 seroprevalence in Catalonia and new evidence on the durability and heterogeneity of post-infection immunity.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) surveillance based on diagnostic testing, incomplete screening of all possible infections and imperfect test sensitivity may lead to a domino-like effect resulting in significant underestimation of the number of coronavirus disease 2019 (COVID-19) cases[1]. Several barriers, such as access to health-care, social stigma and financial worries if tested positive, may increase the magnitude of this underestimation. The high proportion of asymptomatic cases distorts even more the picture of the pandemic[2].

Seroepidemiological studies detect people who have had prior exposure to a pathogen and have developed antibodies and can provide us a better estimate of the proportion of the population infected. The majority of individuals infected with SARS-CoV-2 develop specific antibodies, but there are several challenges for serological surveys[3]. First, the virus has several antigenic epitopes that are the target of antibodies but not everyone responds to the same antigens[4]. Additionally, detection of certain isotype responses depends on the time since infection[5–7]. Within days of symptom onset, specific immunoglobulins M (IgM) are detected and after a lag period strong immunoglobulins G (IgG) responses typically occur. Immunoglobulin A (IgA) reponses are detected almost concurrently to IgM or earlier. With time, attenuation of antibody levels is expected due to decay of immune responses and transition of immunoglobulin production from short to long-lived plasma cell; thus cut-offs for seropositivity should take into account levels of waning immunity [8,9]. Moreover, the magnitude and type of antibody response correlates with disease severity. For example, most studies show that seroresponses are higher in more severe cases[10,11]. Collectively, the diagnostic power of serosurveys will be improved with simultaneous testing for multiple isotype-antigen combinations and systematic errors related to undetected old, recent, asymptomatic or mild infections will be prevented. Nonetheless, most sero-epidemiological studies published up to date have been mainly geared toward studying IgG responses to only one antigen[12].

Limited data exist on the trajectories of immune responses to SARS-CoV-2 over time and the factors that determine their heterogeneity. Notably, most studies consider individuals hospitalized or at least requiring some outpatient treatment[7,13–18]. Describing the characteristics of an effective immune response, as such encountered by asymptomatics or those with mild infections, is valuable. Early data show that some antigen and/or isotype responses dominate among milder infections[6,18,19]. Children are also facing effectively the infection, and studies comparing immune responses between SARS-CoV-2 infected children and adults have already provided some insights[20,21]. Considering members of the same family may resolve further questions related to time of infection, genetics, and other shared environmental exposures.

Taking advantage of multiplex serology to SARS-CoV-2, we describe the sero-epidemiology in a population of 13-93 years old participants of existing cohort studies in Catalonia up to mid-November 2020. Catalonia in northeast Spain, has been among the hardest-hit populations in Europe from COVID-19.

Results

The COVID-19 Cohorts in CATalonia (COVICAT) study

The study design and the distribution of self-reported COVID-19 tests over time are presented in Figure 1. Among the 10,837 adult participants, 10,390 provided information on being previously tested for COVID-19 of which 1,780 (17.1%) have undergone testing. Those tested were more likely to be younger, females, of higher education, working in their usual workplace during confinement, reporting contact with a COVID-19 case, and reporting more symptoms (Supplementary Resource 1). A positive test result was reported by 232 participants (13.0% of those tested) which occured more often among people working in their usual workplace, reporting contact with a COVID-19 case, having more symptoms, non-smokers and overweight/obese people (Supplementary Resource 1). Among the 260 adolescents of the study, only one adolescent received a test, with a negative result.

SARS-CoV-2 serology

Among the 10,837 adult participants of the COVICAT cohort, the 4,740 (44%) who donated a blood sample for serological testing were more likely to report symptoms, not having been tested before, of higher education and less likely to work in their usual workplace and be smokers compared to those who participated only with questionnaire data (Supplementary Resource 2). A blood sample was available for all adolescents.

Table 1 presents the seroprevalence of SARS-CoV-2 based on the serostatus of fifteen isotype-antigen combinations [three isotypes: IgM, IgA and IgG; five viral target antigens: spike full protein (S), S2 fragmnet (S2), receptor binding domain (RBD), nucleocapsid full protein (NFL) and nucleocapsid C-terminal region (NCt)]. Details on the contribution of each isotype-antigen combination in the overall serostatus are available in Supplementary resource 3. The overall seroprevalence of SARS-CoV-2 among adults was 18.1% (IgM 3.7%, IgA 14.6%, IgG 9.0%) while 11.6% had an undetermined status. The highest prevalences were observed for RBD IgG (8.0%), S IgG (7.4%), RBD IgA (7.1%) and S IgA (6.9%). Adult participants of the second sampling period (n=1089, 23%) (median date: 30 October 2020, range 8 September-17 November 2020) were more likely to be seropositive compared to those of the first sampling period (median date: 19 July 2020, range 23 June-31 July 2020) (23.8% versus 16.4%) (Supplementary resource 4). To extrapolate the study results to Catalonia's adult population, we used raked weights to balance the study sample characteristics (age, sex, educational level, health region, smoking) to those of the total population (more details in methods). We estimated a seroprevalence of 15.3% in adults in Catalonia. Among the 260 adolescents (13-15 years old), 11.5% were seropositive and 7.3% had an undetermined status (Table 1).

Antibody levels in time since infection

We examined the association of time since infection with antibody levels using cross-sectional data from all seropositive adults with an estimated time since infection ranging from 23-273 days (Figure 2). For each isotype-antigen combination, levels are plotted irrespective of the serostatus to the specific combination to not affect the heterogeneity of responses expected with time. We observed that RBD, S, and S2 IgM levels decreased significantly over time, with NFL and NCt IgMs being less affected but very few participants were actually seropositive to the specific isotype-antigen combinations. IgA responses to NFL, NCt and RBD sustained in time but those of IgA to S and S2 declined after around 120 days to lower levels. IgG responses seemed to be stable or peaking around 100 days after infection and then started to decline rapidly for NFL IgG, modestly for RBD and S IgG, while NCt and S2 IgG levels were minimally affected. In a subgroup of 99 participants who were previously tested positive (self-reported result), 92% had a positive multiplex serology at a median of 102 days after first diagnosis (range: 13-233 days) (Supplementary resource 5).

SARS-CoV-2 serology by COVID-19 symptoms

We present the distribution of symptoms among adults and adolescents by SARS-CoV-2 serostatus in Table 2. Among adults, all symptoms were more prevalent in SARS-CoV-2 seropositive versus seronegative participants with most remarkable differences seen for loss of odor/taste and fever. Among seropositives, 38.4% were asymptomatic and 28.5% reported ≥4 symptoms. The distribution of symptoms among seropositive adolescents was slightly different compared to adults, with a statistically significant higher proportion reporting chest pain (25% vs. 8% in adolescents and adults respectively), and a lower proportion reporting fever (16.7% vs. 30.6%) and respiratory symptoms (cough & dyspnea 25% vs. 39.1%). Having four or more symptoms, fatigue, chest pain, runny nose, loss of odor/taste and fever were statistically significant more prevalent in seropositive versus seronegative adolescents.

Demographic and clinical characteristics of adult participants according to SARS-CoV-2 serostatus and severity of infection are presented in Supplementary Resource 6. Participants reporting contact with a COVID-19 case and non-smokers were more likely to be seropositive. Among seropositive participants, those admitted to hospital/intensive care unit (ICU) were 40-80 years old. Seropositive women compared to men were more likely to be symptomatic and report more symptoms. The proportion of participants reporting contact with a COVID-19 case, having done a test before, with any chronic disease or being overweight/obese increased with the severity of infection.

Antibody responses by the severity of infection

To determine whether the severity of infection is associated with quantitatively and qualitatively different antibody responses we performed four analyses. Firstly, we compared antibody levels of the fifteen isotype-antigen combinations between asymptomatic individuals (n=322), those reporting 1-3 symptoms (n=276), those reporting ≥4 symptoms (n=216) and those admitted to hospital/ICU (n=24) (Figure 3a). We observed lower levels among asymptomatics and higher levels among those admitted to hospital/ICU (apart from NFL and NCt IgMs). Gradient differences were most evident among IgG to NFL and NCt and all RBD, S and S2 responses. Secondly, we found that the breadth of positive immune responses with all isotype-antigen combinations contributing to the score, increased with severity of infection (Figure 3b). Thirdly, we explored differences in isotype responses. We observed a higher increase in levels of IgG than IgA responses with increasing severity (Figure 3a). In Figure 3c, using IgA/IgG ratios, we observed that IgA levels were closer to IgG levels among asymptomatics and more likely to exceed them compared to those with more severe infection. Also, we found that among asymptomatics, a higher proportion (46%) had more positive IgA than IgG responses compared to the 13% displaying more IgG than IgA responses (Figure 3d). We observed reverse findings among those hospitalized. Finally, we compared responses related to spike protein versus nucleocapsid antigens. Experiencing a more severe infection was associated with a shift towards spike over nucleocapsid antibody responses (Figure 3e). The overall trend is reflected in the last graph of Figure 3e depicting differences in the number of features that had greater spike than nucleocapsid related responses (ratios over one). Because time since infection may impact the associations mentioned above, we repeated all analyses in two strata of seropositive individiuals those sampled before 120 days and after 120 days since infection. Results were materially unchanged (data not shown).

Antibody responses by age, sex, and lifestyle characteristics

We examined differences in antibody levels and breadth of positive immune responses among seropositive adults with respect to age, sex, smoking, and body mass index (BMI) status before confinement (table 3 & supplementary resource 8). Participants 60 years of age or older had lower responses to almost all isotype-antigen combinations and a lower breadth of positive responses but had higher levels of NFL IgA. Females had statistically significant higher NFL, NCt and S2 IgM responses but lower NFL, NCt, and S2 IgA responses. Overweight or obese people had higher levels to almost all IgA and IgG responses and a higher breadth of positive responses. On the other hand, smokers displayed lower levels of almost all antibodies and a lower breadth of positive responses. We additionally adjusted for the severity of infection, considering it as a mediator of the associations. After adjustment, age >60 years old was associated with lower levels of IgM to S2 but higher levels of IgA to NFL. Associations with sex remained, with women showing an overall lower number of positive responses. Associations with overweight/obesity were largely diluted but remained positive. Smoking was consistently associated with lower levels and a lower breadth of positive responses. We repeated all the analyses excluding those seronegative for each isotype-antigen combination and results for smoking and BMI status were similar (supplementary resource 8)

Antibody responses among adolescents

Serological data among 260 parent-child pairs showed a much lower risk for seropositivity among adolescents (n=30) than their parents (n=50) [RR: 0.6, 95% CI: 0.39-0.91)]. Sample collection took place at the same day for parents and their children. Among seropositive, adolescents had higher responses to S, S2 and RBD IgG, whereas parents had higher responses to NFL and NCt IgA (supplementary Resource 9). The dominant IgG responses related to spike protein (S, S2, RBD) observed among adolescents were further confirmed when we compared the ratios of spike versus nucleocapsid responses (figure 4a) and the number of positive IgG compared to IgA or IgM (figure 4b). When we restricted our analysis to the 16 parent-child pairs that were both tested seropositive, we observed similar results although most were no longer statistically significant (data not shown). Among

seropositive adolescents, antibody levels were not different with respect to the severity of infection (0 symptoms, 1-3 symptoms, ≥4 symptoms) (data not shown).

Discussion

The COVICAT study is one of the largest studies examining the complex natural immunity to SARS-CoV-2 at a population level. Based on multiplex serology testing of 5000 participants, we detected a SARS-CoV-2 seroprevalence of 18.1% in adults and much lower, of 11.5%, in adolescents. Additionally, 11.6% of adults and 7.3% of adolescents showed marginal seroresponses (undetermined). Severity of infection, determined the magnitude, breadth and specificity (towards certain antigens and/or isotypes) of immune responses long time after the acute phase of infection. We also identified diverse associations between individuals' characteristics, including age, sex, smoking, and BMI status, with antibody responses.

Up to mid-November, there were 238,596 confirmed COVID-19 cases in Catalonia in people>20 years old which corresponds to 3.9% of the population[22]. This proportion is much lower than our estimation of infected individuals based on serology. We expected differences with the official sources, as surveillance systems are restricted by the emergency and load of testing clinically evident infections and high-risk individuals. Meanwhile, updated data from the fourth phase of a nationwide seroprevalence study in Spain (ENE-COVID) reported a seroprevalence of 9.9% in Spain and 11.6% in Catalonia until the end of November[23]. They based their serology on two tests, a point-of-care rapid test determining IgG against RBD and an immunoassay detecting IgG against nucleocapsid (not yet available for the fourth phase). Although the seroprevalence in ENE-COVID for Catalonia is lower than in our study it is not so different when compared with the seroprevalence for RBD and/or NCt IgG being 8.1% in our study. This observation reinforces the idea that the difference in seroprevalence between the two studies could be partially attributable to the less extensive serological testing in ENE-COVID compared to our multiplex approach. Another argument for this scenario is that seroprevalence in adolescents, whose responses were primarily IgG anti-RBD, was not so different between the two studies (8.6% for 10-14 years of age in ENECOVID and 11.5% for 13-15 years old in our study). An increasing number of seroprevalence surveys now utilize multi-antigen and multi-isotype antibody responses because seroresponses might be skewed to different antigens and isotypes depending on clinical and individuals' characteristics[14,24,25]. Also, the importance of IgA isotype in diagnostic accuracy of SARS-CoV-2 serological tests is emerging[5,15,26].

We found no differences in seroprevalence between females and males or with age among adults. Interestingly, seropositive participants older than 60 years of age had higher NFL IgA levels and women had lower antibody levels and number of seropositive responses (apart from NFL and NCt IgM). Age and sex-specific antibody responses against SARS-CoV-2 have been documented but results are mixed[14,27,28]. Similar to other studies, seroprevalence among young adolescents was lower than among adults[29]. Within one family, adolescents were at lower risk for seropositivity compared to their parents. We cannot make direct conclusions about children's role in transmitting SARS-CoV-2 within the household, but evidence argues for a reduced, marginal or conditional contribution[29,30]. It remains unclear why children are less susceptible to infection but mechanisms related to the number of ACE-2 receptors[31], the naivety of innate immunity[32] and preexisting human coronaviruses-elicited immunity[33] are proposed.

A striking observation was that over 90% of previously tested positive participants had detectable antibodies up to 7 months after their first diagnosis, but most of them were either hospitalized or had experienced ≥4 symptoms. In the overall seropositive population of our study including infections of varying severity, we observed sustained levels for IgA and IgG responses at least 4 months after infection. More stable responses up to 9 months after infection were evident for NCt, RBD IgA and NCt, S2 IgG. We did not have repeated samples but a number of other studies did and showed limited loss of IgG antibodies and some loss of IgA antibodies over time[7,14,16,34,35]. More importantly, two recent studies showed that seropositive participants had a significantly decreased risk of re-infection up to 6 months after first infection[36,37]. It remains to be determined what levels of antoibodies to what specific antigen epitopes protect people from recurrent infections.

Our findings are in agreement with previous reports showing that asymptomatics account for a significant proportion of the infected population (approximately 40%) and that a range of symptoms occurs with COVID-19 infection with the most specific being the loss of odor/taste and fever[38,39]. Importantly, the clinical spectrum of SARS-CoV-2 infection reflects the spectrum of

immune responses. With increasing severity of infection, we observed that hosts mounted more robust and rich responses. A recent study showed that the immune response of severely infected subjects was spread to subdominant viral antigens as well[40]. A novel finding in our study was that asymptomatics were more likely to have greater IgA than IgG responses compared to those experiencing more severe disease nonetheless the magnitude of IgA responses remained in lower levels in asymptomatics compared to those admitted to hospital/ICU. IgA could contribute to virus neutralization early in the infection to a greater extent compared with IgG[6]. Similar to us, most studies have described higher levels of antibodies among those with more severe disease, and some have suggested that a robust IgA response, in particular, may have a pathological role in SARS-CoV-2 infection[7,14,15,21]. Collectively with our data, it seems that IgA at low levels may be able to control the infection, but it could be associated with detrimental effects when boosted to higher levels along with other responses. In our study, we cannot disentangle to which extent the severity of infection drives these immune responses or whether these responses play a role in the pathogenesis of SARS-CoV-2.

Contradictory to two previous smaller studies, we found a shift towards spike over nucleocapsid responses with increasing severity of infection[17,18]. This discrepancy might be related to the fact that each study examined different immune features and we collected samples long after infection. Different rates of decay of anti-spike versus anti-nucleocapsid antibodies might have affected our results[41,42]. Although unclear, it is possible that the severity of infection determines the antibody production in the longterm (e.g. from long-lived plasma cells) in an antigen-specific manner[43]. For example, a recent study demonstrated that subjects with more severe disease mounted a larger memory B cell formation against the spike, but not the nucleocapsid[40]. Serological data from the group of adolescents point to very specific responses mainly of IgG against spike protein, consistent with previous studies[20,21]. We should note that children until late adolescence have a lower capacity of generating IgA[44]. These data suggest that adolescents use IgG alone to control the infection and that lack of anti-nucleocapsid responses might indicate a less widespread infection than adults. Such differences between adult and childhood immune responses should be delineated given the less harmful effects of SARS-CoV-2 infection in children.

Consistent with other studies, we detected higher levels of antibodies among overweight/obese participants. This association largely diluted when we adjusted for severity of infection, suggesting that higher levels were a consequence of the more severe disease experienced by obese people (adjustment by severity of infection not considered in previous studies) [14,45]. In our population, the highest proportion of overweight/obese people was among those experiencing more severe infection. World Obesity Forum recently reported that COVID-19 mortality increased along with the countries' prevalence of obesity, even after adjusting for age and wealth[46]. These data suggest that despite displaying robust serological responses, overweight/obese infected people are more likely to develop severe infection than non-overweight/obese people. Reduced levels of SARS-CoV-2 antibodies and breadth of immune responses were detected among seropositive smokers compared to non-smokers irrespective of the severity of infection. Two studies report similar results concerning levels of antibodies[14,47]. These data suggest that smokers present a weakened immune response to SARS-CoV-2. Thus, the higher COVID-19 morbidity among smokers might be due to impaired immunity as reflected in lower antibody levels[48]. Simultaneously, the paradox of low prevalence of smoking among SARS-CoV-2 infected people might be due low/non-detectable levels of antibodies. Of course, this would not affect results relevant to PCR but it has been suggested, although not investigated, that smoking might decrease nasopharyngeal viral load resulting more often in false-negative results[49]. We need more studies in this perspective.

We acknowledge that this is not a random population-based study as it recruited participants from pre-existing cohorts. On the other hand, existing cohorts allow us to quickly contact a population, achieve a high participation rate and access pre-pandemic information. Moreover, we believe that the selection of our population is not biased because the distribution of positive self-reported COVID-19 tests in time was consistent with the general distribution of COVID-19 cases in Catalonia until the second wave[22]. The study primarily consisted of people 40-70 years old (≈90%), owing to the age distribution in original cohorts but we combined several cohorts in order to include younger and older people. Volunteer bias is always of concern, as we observed a lower participation rate for those who had received a test; this would have lead to underestimation of true seroprevalence. We assumed that all persons infected with SARS-CoV-2 have detectable antibodies at the time of sampling. The 8% of previously tested positive participants with a negative serology in our study might indicate the proportion of the population who lost immunity, were antibody non-responders or false-positive results in the first test. Moreover, we could not verify whether the

symptoms reported were attributable to a SARS-CoV-2 infection given the limited access of the population to diagnostic tests at the initial months of the pandemic.

Conclusion

Collectively, the data presented here argue for a higher number of exposed individuals to SARS-CoV-2 in Catalonia, than what had been described, but still the majority of the population remains unexposed. Although further analysis with repeated samples will allow us to describe the progression of antibody levels in time, we observed that even 4-9 months after infection, responses against SARS-CoV-2 were evident. Individuals presented strikingly different immune responses depending on the severity of infection. Factors such as obesity and smoking that are related to significant COVID-19 morbidity and mortality probably determine immune responses. The key now is to identify the mediators of these immune responses.

Methods

Study design and setting

The COVICAT study includes participants from diferent pre-existing ongoing population-based cohorts in Catalonia and was developed following the COVID-19 pandemic. Eligible participants were from three adult population-based cohorts (GCAT, Genomes for life[50]; MCC-Spain, population controls from Catalunya[51]; and ECRHS, European Community Respiratory Health Survey[52]), two mother-child cohorts [INMA-Sabadell, (INfancia y Medio Ambiente)[53] children born in 2005-2007 and their mothers; BiSC, Barcelona Life Study Cohort pregnant women recruited immediately before and during the pandemic] and two small general population cohorts in special populations (Urban Training, older persons; and LeRAgs, rural population). The eligible adult population consists of 19,424 people and we were able to contact 18,737 (96%) using email and telephone messages or calls. Of them, 10,837 (58%) participated in the study and were invited to provide a blood sample. To overcome several barriers related to providing a blood sample, we offered the option for older people and those living in remote areas of collecting a blood fingerprick sample at their residence, as well as a second opportunity for donating a blood sample during September-November 2020 for all participants not able to provide a sample previously. The majority of COVICAT adult participants (88%) were from GCAT cohort. All adolescent participants (n=260) were from INMA-Sabadell cohort.

All participants gave written informed consent before participation in the study. For individuals younger than 18 years, parents or a legal representative provided consent. The study was approved by the Parc de Salut Mar Drug Research Ethical Committee (IBR number: 2020/9307/I)

Procedures

Just after the strict first confinement period, we invited participants to complete an online questionnaire. In 5.6% of the population who were unfamiliar with the use of online approaches we did shorter telephone interviews. Questions were relevant to COVID-19 compatible symptoms, diagnostics, clinical course of infection including hospitalization and referral to an ICU. Moreover, questions regarding the response to the epidemic, occupational and financial aspects, sociodemographic and lifestyle characteristics, mental health, chronic diseases, and anthropometric data were included. After completing this main questionnaire, participants were asked to donate a blood sample in different facilities of the Blood and Tissue Bank (Banc de Sang i Teixits) or the Barcelona Biomedical Research Park or we planned a visit in their residence to collect a blood fingerprick sample (27% of all samples). INMA participants were invited to the local health center in Sabadell where trained nurses collected a blood fingerprick sample. Blood samples were collected from June 23 to July 31, 2020 and from September 8 to November 17, 2020. Participants of the second sampling period (22% of all) completed an additional questionnaire related to COVID-19 symptoms and testing in order to update the relative information from the main questionnaire. Details on the sources and methods of assessment of all variables included in this analysis are presented in the Supplementary resource 10.

Serology

The levels of IgG, IgM and IgA were assessed by high-throughput multiplex quantitative suspension array technology, including, as SARS-CoV-2 antigens, the S protein (aa 1-1213 expressed in Expi293 and His tag-purified) and the S2 fragment (purchased

from SinoBiologicals), the RBD (donated by the Krammer lab, Mount Sinai, NY), the NFL and the specific NCt (expressed in *E. coli* and His tag-purified). Assay performance was previously established as 100% specificity and 95.78% sensitivity for seropositivity 14 days after symptoms onset [54].

Antigen-coupled microspheres were added to a 384-well µClear® flat bottom plate (Greiner Bio-One, Frickenhausen, Germany) in multiplex (2000 microspheres per analyte per well) in a volume of 90 µL of Luminex Buffer (1% BSA, 0.05% Tween 20, 0.05% sodium azide in PBS) using 384 channels Integra Viaflo semi-automatic device (96/384, 384 channel pipette). Hyperimmune pools were used as positive controls prepared at 2-fold, 8 serial dilutions from 1:12.5. Pre-pandemic samples were used as negative controls to estimate the cut-off of seropositivity. Ten µL of each dilution of the positive control, negative controls and test samples (prediluted 1:50 in 96 round-bottom well plates), were added to a 384-well plate using Assist Plus Integra device with 12 channels Voyager pipette (final test sample dilution of 1:500). To quantify IgM, test samples and controls were pre-treated with anti-Human IgG (Gullsorb) at 1:10 dilution, to avoid IgG interferences. Technical blanks consisting of Luminex Buffer and microspheres without samples were added in 4 wells to control for non-specific signals. Plates were incubated for 1 h at room temperature in agitation (Titramax 1000) at 900 rpm and protected from light. Then, the plates were washed three times with 200 µL/well of PBS-T (0.05% Tween 20 in PBS), using BioTek 405 TS (384-well format). Twenty five µL of goat anti-human IgG-phycoerythrin (PE) (GTIG-001, Moss Bio) diluted 1:400, goat anti-human IgA-PE (GTIA-001, Moss Bio) 1:200, or goat anti-human IgM-PE (GTIM-001, Moss Bio) 1:200 in Luminex buffer were added to each well and incubated for 30 min. Plates were washed and microspheres resuspended with 80 µL of Luminex Buffer, covered with an adhesive film and sonicated 20 seconds on sonicator bath platform, before acquisition on the Flexmap 3D reader. At least 50 microspheres per analyte per well were acquired, and median fluorescence intensity (MFI) was reported for each analyte.

Statistics

Assay positivity cut-offs specific for each isotype and analyte were calculated as 10 to the mean plus 3 standard deviations of $\log_{10}$-transformed MFI of 128 pre-pandemic controls. Results were defined as undetermined when the MFI levels for a given isotype-analyte were between the positivity threshold and an upper limit at 10 to the mean plus 4.5 standard deviations of the $\log_{10}$-transformed MFIs of pre-pandemic samples, and no other isotype-antigen combination was above the positivity cut-off. We calculated the breadth of positive responses referring to the aggregate number of isotype-antigen combinations that participants were seropositive. Similarly we calculated the number of positive IgA or IgM or IgG responses. Antibody levels were $\log_{10}$-transformed to normalise their distribution. In order to compare the isotype responses, we calculated the ratios of IgA/IgG, IgA/IgM and IgM/IgG for each of the five antigens using the $\log_{10}$-transformed values of antibody levels. We also calculated the ratios of anti-spike (S, S2, RBD) over anti-nucleocapsid (NFL, NCt) responses for all possible combinations for each isotype (e.g. IgA S/IgA NFL, IgA S/IgA NCt).

Descriptive analyses of the study population characteristics were conducted. We used raked weights to extrapolate seroprevalence to the total population of Catalonia aged more than 20 years. Briefly, raking calculates weigths so that the weighted sample has the same marginal distribution than the reference population in terms of the variables used to calculate the weights[56]. In particular, we used the joint sex-age (in 10-year groups) distribution, educational level, health region and smoking. Population data were obtained from the National Statistics Institute and from the Catalan Health Survey[55]. The COVICAT study includes lower numbers of younger ages, which leads to overdispersed weights and gaps in the distribution of the weights. For this reason, we restricted the extremes weights by trimming the distribution at 99% of the weights. Generalized additive models were used to explore the shape of the relationship between days since infection and antibody levels to each of the fifteen isotype-antigen combination. Differences in antibody levels and ratios by severity of infection were examined using oneway Anova and pairwise comparisons were performed using Tukey post hoc-test. Differences in immune responses between adolescents and parents were examined with t-tests. Multivariable regression models were applied to examine among seropositive individuals the association between age, sex, smoking and BMI status before confinement (all in the same model adjusted also for days since infection) and $\log_{10}$-transformed antibody levels and the breadth of positive responses. All analyses were conducted using Stata version 16 (StataCorp LP, College Station, Texas).

Abbreviations

SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2); COVID-19 (coronavirus disease 2019); Immunoglobulin M (IgM); Immunoglobulin G (IgG); Immunoglobulin A (IgA); COVID-19 Cohorts in CATalonia (COVICAT); spike full protein (S); S2 fragmnet (S2); receptor binding domain (RBD); nucleocapsid full protein (NFL); nucleocapsid C-terminal region (NCt); intensive care unit (ICU); body mass index (BMI); INMA (INfancia y Medio Ambiente); median fluorescence intensity (MFI).

Declarations

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Author contributions:

M. Kar. contributed to conceptualization, data analysis and writing of the original draft. G. M., C. D. and M. Kog. contributed to funding acquisition, project concept, study design, study implementation and drafted the manuscript. G. M. and C. D. co-ordinated immunology analyses. A. E. and X. B. contributed to statistical analysis and extrapolation calculations. A. J., M. V. developed the serology assays, performed antigen coupling and validation and implementation, and executed immunology assays and data preprocessing. R. S. contributed to laboratory data quality control & quality assurance, data preprocessing, and descriptive statistical analysis of antibody data. D. B. ,L.P. and R. A. contributed to sample reception and registry, processing and aliquoting, overall supervision of lab circuits and coordination with sample collection centers. L. M. and R. R. contributed to preparing pre-dilution plates, data preprocessing, analysis of the last batches. L. I. contributed to antigen design and production for immunoassays. A. C. and B. C. contributed to data collection in GCAT. V. P. managed sample recruitment in GCAT. A. E. and G.C.V. were responsible for data management and harmonization. S. F. was responsible for recruitment of participants from the INMA cohort. I. R. was responsible for recruitment of participants from the BiSC cohort. G.C.V. managed the project. All other authors contributed to data curation, investigation, reviewing and editing the manuscript; all authors approved the final version of the manuscript.

Competing interests:

The authors declare no competing interests

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Tables

Table 1. Overall crude SARS-CoV-2 seroprevalence, by isotype and isotype-antigen combination in adult and adolescent participants of the COVICAT study.

	Adults (n=4740)						Adolescents (n=260)					
	positive		negative		undetermined		positive		negative		undetermined	
	n	%	n	%	n	%	n	%	n	%	n	%
Overall	858	18.1	3331	70.3	551	11.6	30	11.5	211	81.2	19	7.3
By isotype												
IgM	175	3.7	4392	92.7	173	3.6	3	1.2	250	96.2	7	2.7
IgA	694	14.6	3616	76.3	430	9.1	19	7.3	233	89.6	8	3.1
IgG	426	9.0	4134	87.2	180	3.8	24	9.2	225	86.5	11	4.2
By isotype-antigen combination												
IgM NFL	6	0.1	4694	99.0	40	0.8	0	0	260	100,0	0	0.0
IgM NCt	18	0.4	4674	98.6	48	1.0	0	0	258	99.2	2	0.8
IgM RBD	88	1.9	4552	96.0	100	2.1	1	0.4	253	97.3	6	2.3
IgM S	49	1.0	4601	97.1	90	1.9	0	0	259	99.6	1	0.4
IgM S2	20	0.4	4637	97.8	83	1.8	0	0	258	99.2	2	0.8
IgA NFL	140	3.0	4310	90.9	290	6.1	1	0.4	256	98.5	3	1.2
IgA NCt	129	2.7	4479	94.5	132	2.8	0	0	257	98.8	3	1.2
IgA RBD	338	7.1	4210	88.8	192	4.1	17	6.5	240	92.3	3	1.2
IgA S	325	6.9	4259	89.9	156	3.3	13	5.0	245	94.2	2	0.8
IgA S2	209	4.4	4201	88.6	330	7.0	4	1.5	255	98.1	1	0.4
IgG NFL	59	1.2	4473	94.4	208	4.4	2	0.8	246	94.6	12	4.6
IgG NCt	98	2.1	4481	94.5	161	3.4	5	1.9	245	94.2	10	3.8
IgG RBD	380	8.0	4308	90.9	52	1.1	21	8.1	237	91.2	2	0.8
IgG S	351	7.4	4307	90.9	82	1.7	21	8.1	233	89.6	6	2.3
IgG S2	104	2.2	4353	91.8	283	6.0	6	2.3	233	89.6	21	8.1

Table 2. Crude prevalence (%) of symptoms by SARS-CoV-2 serostatus in adult and adolescent participants of the COVICAT study.

	Adults			Adolescents	
	seropositive	seronegative or undetermined		seropositive	seronegative or undetermined
Symptoms			**Symptoms**		
Headache	35.1	23.4	Fatigue	37.5	17.3
Muscle/Joint pain	35.0	17.7	Headache	33.3	26.2
Fever	30.6	9.4	Muscle/joint pain	29.2	14.9
Fatigue	30.5	15.0	Chest pain	25.0	6.0
Cough	27.0	13.9	Runny nose	25.0	10.7
Loss of odor or taste	22.2	1.6	Loss odor/taste	25.0	1.2
Runny nose	17.5	14.6	Nausea/vomiting	20.8	6.5
Diarrhea	17.5	10.2	Diarrhea	16.7	7.7
Dyspnea	12.1	5.7	Fever	16.7	5.4
Rash	8.6	4.2	Cough	16.7	10.7
Chest pain	8.0	5.1	Dyspnea	8.3	5.4
Nausea	5.8	3.0	Rash	0	4.2
Number of symptoms			**Number of symptoms**		
0 symptoms	38.4	54.6	0 symptoms	33.3	54.8
1-3 symptoms	33.1	35.8	1-3 symptoms	29.2	35.7
≥4 symptoms	28.5	9.6	≥4 symptoms	37.5	9.5

Symptoms are sorted in adults and adolescents in decreasing frequency as observed among seropositives. Darker red = higher prevalence

Table 3. Adjusted associations (β) between each characteristic and antibody levels for each of the 15 isotype-antigen combinations and the breadth of positive immune responses for SARS-CoV-2 seropositive participants, the COVICAT study.

Characteristic	IgM NFL	IgM NCt	IgM RBD	IgM S	IgM S2	IgA NFL	IgA NCt	IgA RBD	IgA S	IgA S2	IgG NFL	IgG NCt	IgG RBD	IgG S	IgG S2	breadth
Above 60 years old	-0.02	-0.03	**-0.1**	**-0.08**	**-0.14**	**0.1**	0	**-0.07**	**-0.09**	**-0.15**	-0.04	**-0.11**	**-0.18**	**-0.17**	**-0.15**	**-0.45**
Female sex	**0.15**	**0.12**	0.02	0.02	**0.07**	**-0.07**	**-0.06**	-0.03	-0.04	**-0.11**	0	-0.05	0.01	-0.01	-0.06	-0.18
Obese/overweight	-0.03	0.01	0.04	0.04	0	0.05	0.03	**0.11**	**0.08**	**0.11**	0.07	0.07	**0.15**	**0.14**	**0.11**	**0.71**
Smoker	0.03	0.02	-0.05	**-0.09**	**-0.09**	-0.07	-0.04	**-0.1**	**-0.16**	**-0.16**	**-0.22**	**-0.21**	**-0.35**	**-0.38**	**-0.3**	**-1.13**
After adjustment for severity of infection																
Above 60 years old	-0.03	-0.03	-0.05	-0.03	**-0.1**	**0.11**	0	-0.02	-0.02	-0.06	0.05	-0.01	-0.02	-0.01	-0.03	0.07
Female sex	**0.16**	**0.12**	-0.01	-0.01	0.05	**-0.08**	**-0.06**	**-0.05**	**-0.07**	**-0.15**	-0.04	**-0.09**	-0.06	-0.08	**-0.12**	**-0.41**
Obese/overweight	-0.03	0.01	0.02	0.02	-0.02	0.05	0.03	**0.09**	0.05	0.07	0.03	0.04	0.09	0.08	0.06	**0.51**
Smoker	0.03	0.01	-0.01	-0.06	-0.06	-0.06	-0.04	-0.06	**-0.12**	-0.1	**-0.15**	**-0.14**	**-0.24**	**-0.27**	**-0.22**	**-0.78**

Associations (β coefficients) of each listed characteristic with $\log_{10}$ transformed MFI values of each isotype-antigen combination adjusted for all the listed characteristics and days since infection

Bold indicates statistically significant associations (Online resource 8 presents corresponding p-values and 95% CI)

The red color indicates a positive association while the blue color a negative association (associations with breadth are not comparable with those of antibody levels and thus not colored).

Figures

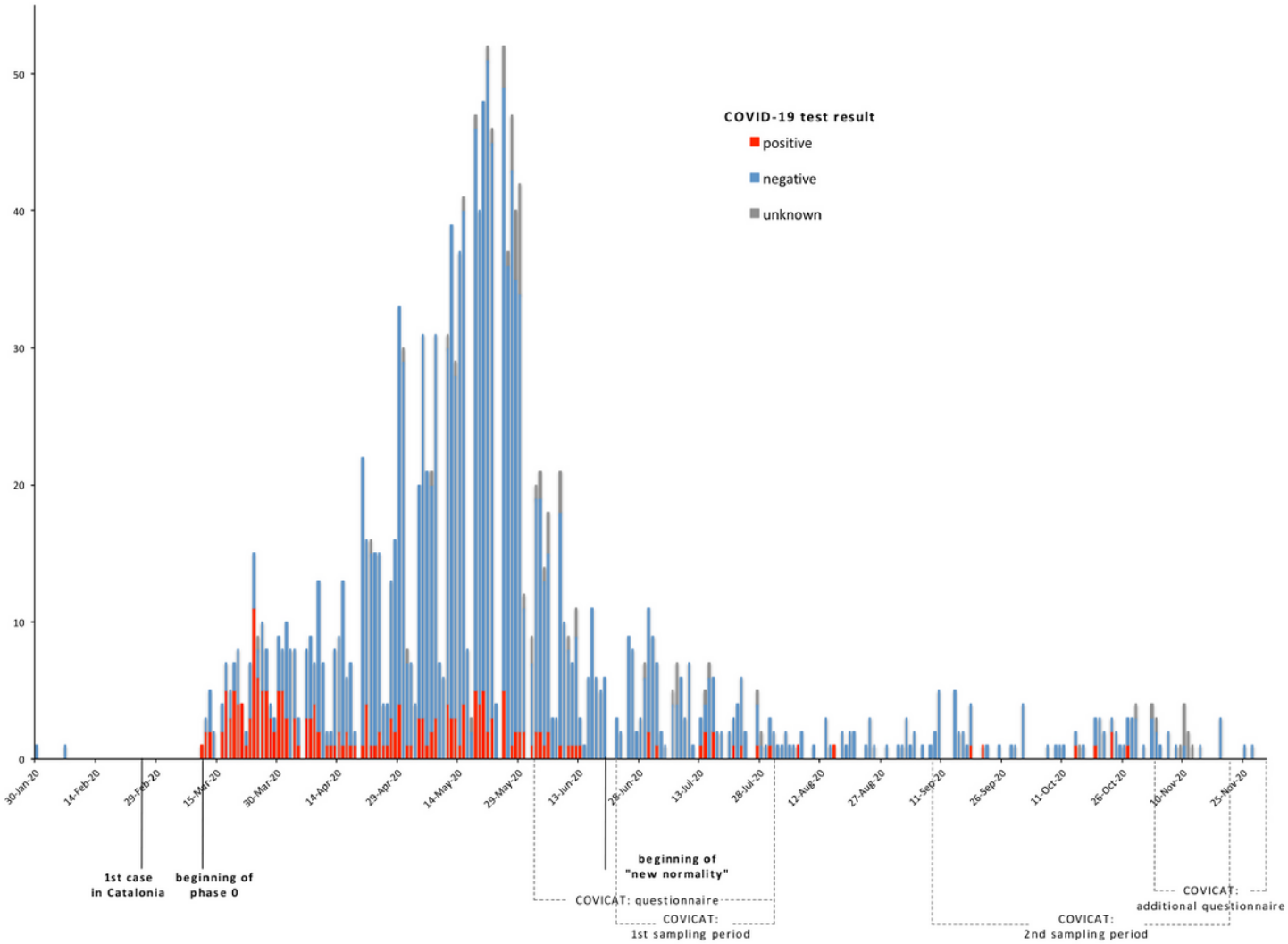

Figure 1

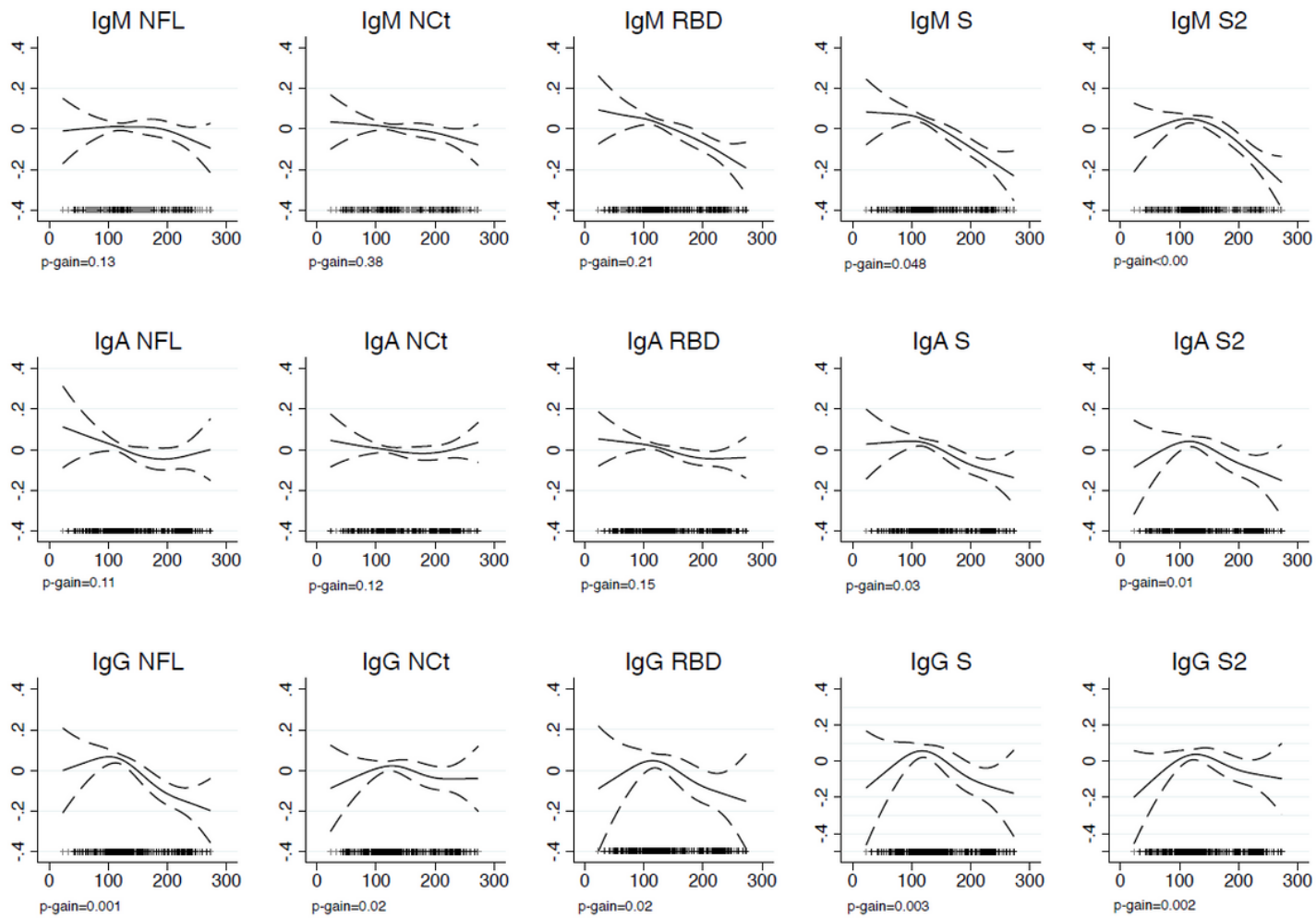

Figure 2

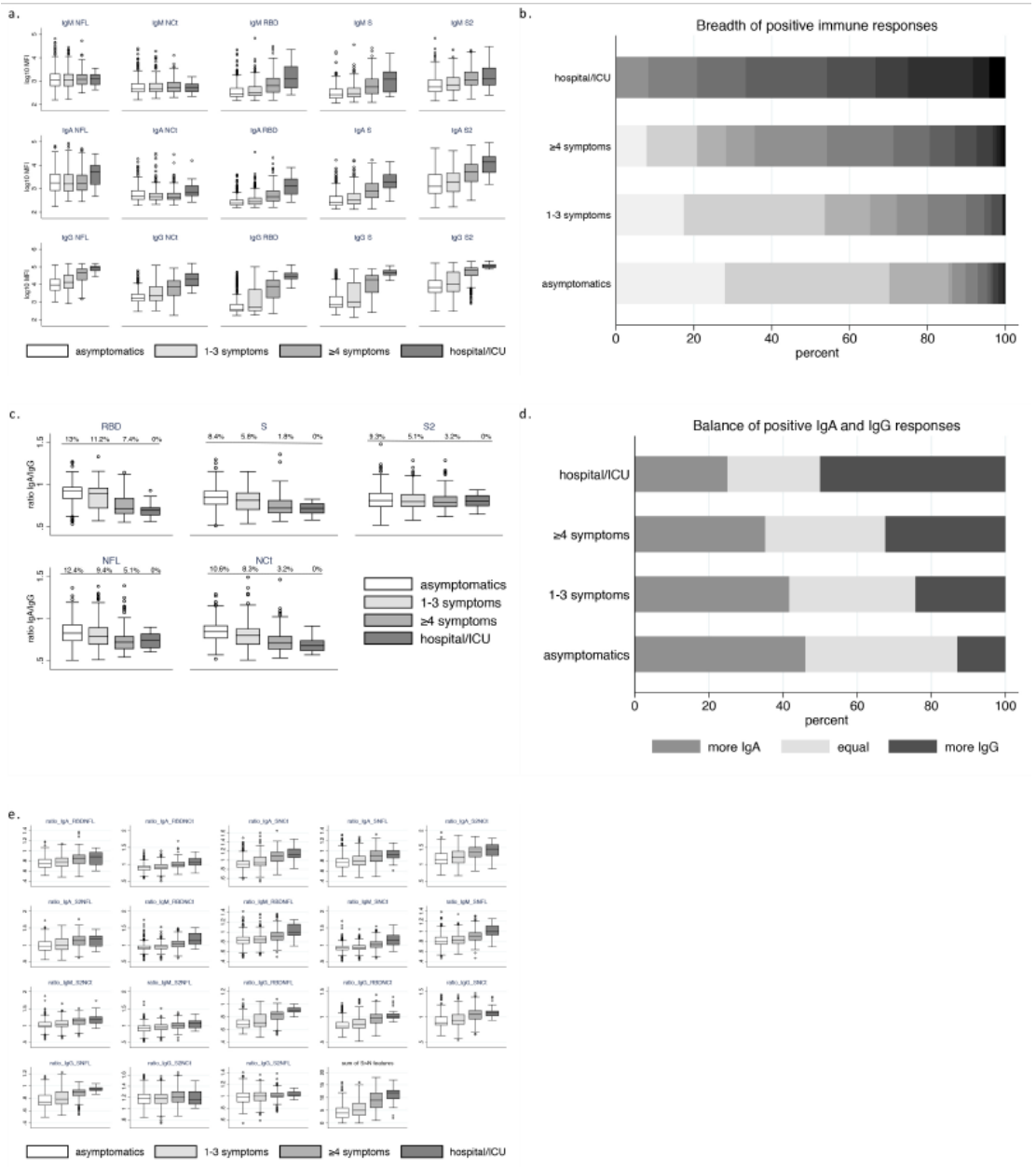
a.
IgM NFL
IgM NCt
IgM RBD
IgM S
IgM S2
IgA NFL
IgA NCt
IgA RBD
IgA S
IgA S2
IgG NFL
IgG NCt
IgG RBD
IgG S
IgG S2
asymptomatics
1-3 symptoms
≥4 symptoms
hospital/ICU
b.
Breadth of positive immune responses
hospital/ICU
≥4 symptoms
1-3 symptoms
asymptomatics
0
20
40
60
80
100
percent
c.
RBD
S
S2
NFL
NCt
ratio IgA/IgG
asymptomatics
1-3 symptoms
≥4 symptoms
hospital/ICU
d.
Balance of positive IgA and IgG responses
hospital/ICU
≥4 symptoms
1-3 symptoms
asymptomatics
0
20
40
60
80
100
percent
more IgA
equal
more IgG
e.
asymptomatics
1-3 symptoms
≥4 symptoms
hospital/ICU

Figure 3

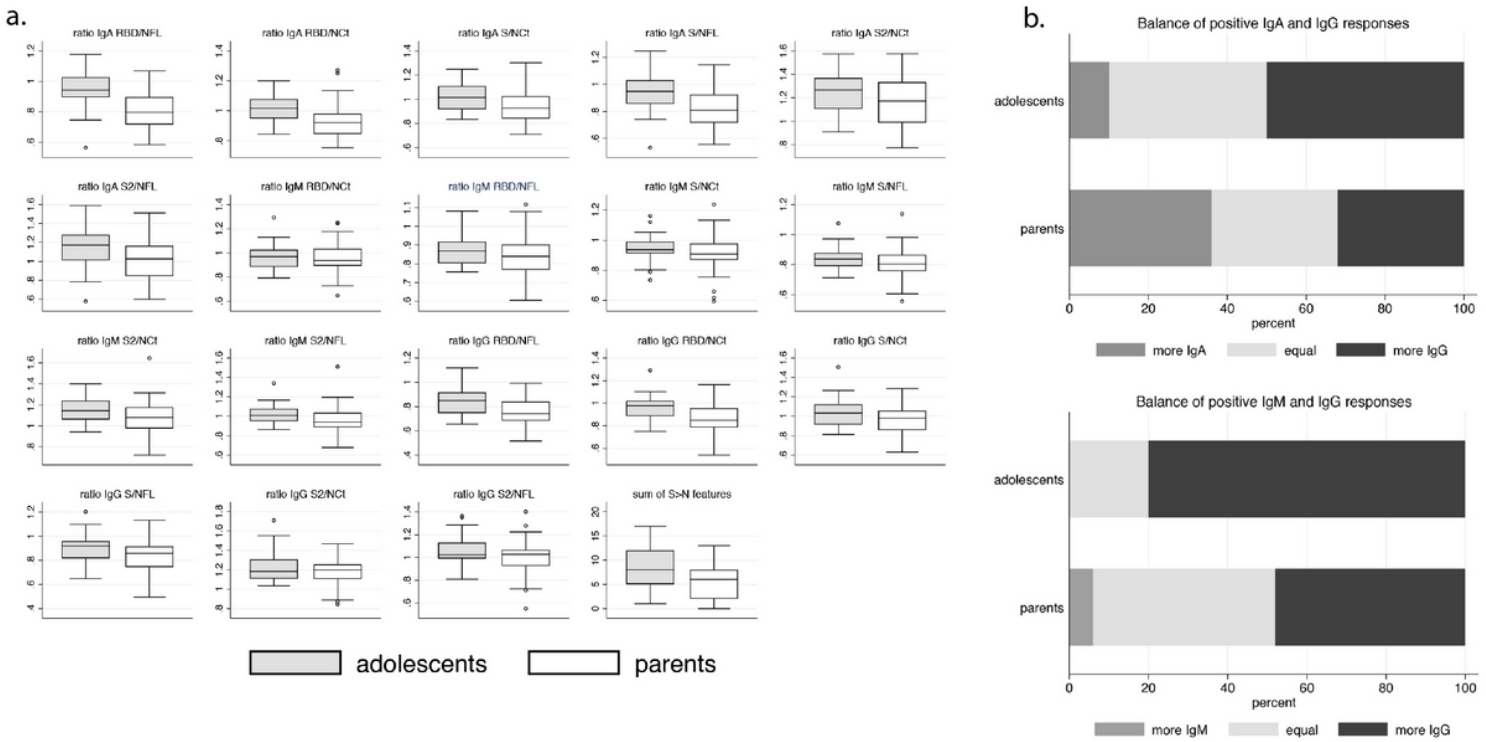

Figure 4

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryResources.pdf