ORIGINAL ARTICLE

Humoral Immune Response to SARS-CoV-2 in Iceland

D.F. Gudbjartsson, G.L. Norddahl, P. Melsted, K. Gunnarsdottir, H. Holm, E. Eythorsson, A.O. Arnthorsson, D. Helgason, K. Bjarnadottir, R.F. Ingvarsson, B. Thorsteinsdottir, S. Kristjansdottir, K. Birgisdottir, A.M. Kristinsdottir, M.I. Sigurdsson, G.A. Arnadottir, E.V. Ivarsdottir, M. Andresdottir, F. Jonsson, A.B. Agustsdottir, J. Berglund, B. Eiriksdottir, R. Fridriksdottir, E.E. Gardarsdottir, M. Gottfredsson, O.S. Gretarsdottir, S. Gudmundsdottir, K.R. Gudmundsson, T.R. Gunnarsdottir, A. Gylfason, A. Helgason, B.O. Jensson, A. Jonasdottir, H. Jonsson, T. Kristjansson, K.G. Kristinsson, D.N. Magnusdottir, O.T. Magnusson, L.B. Olafsdottir, S. Rognvaldsson, L. le Roux, G. Sigmundsdottir, A. Sigurdsson, G. Sveinbjornsson, K.E. Sveinsdottir, M. Sveinsdottir, E.A. Thorarensen, B. Thorbjornsson, M. Thordardottir, J. Saemundsdottir, S.H. Kristjansson, K.S. Josefsdottir, G. Masson, G. Georgsson, M. Kristjansson, A. Moller, R. Palsson, T. Gudnason, U. Thorsteinsdottir, I. Jonsdottir, P. Sulem, and K. Stefansson

ABSTRACT

BACKGROUND

Little is known about the nature and durability of the humoral immune response to infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

METHODS

We measured antibodies in serum samples from 30,576 persons in Iceland, using six assays (including two pan-immunoglobulin [pan-Ig] assays), and we determined that the appropriate measure of seropositivity was a positive result with both pan-Ig assays. We tested 2102 samples collected from 1237 persons up to 4 months after diagnosis by a quantitative polymerase-chain-reaction (qPCR) assay. We measured antibodies in 4222 quarantined persons who had been exposed to SARS-CoV-2 and in 23,452 persons not known to have been exposed.

RESULTS

Of the 1797 persons who had recovered from SARS-CoV-2 infection, 1107 of the 1215 who were tested (91.1%) were seropositive; antiviral antibody titers assayed by two pan-Ig assays increased during 2 months after diagnosis by qPCR and remained on a plateau for the remainder of the study. Of quarantined persons, 2.3% were seropositive; of those with unknown exposure, 0.3% were positive. We estimate that 0.9% of Icelanders were infected with SARS-CoV-2 and that the infection was fatal in 0.3%. We also estimate that 56% of all SARS-CoV-2 infections in Iceland had been diagnosed with qPCR, 14% had occurred in quarantined persons who had not been tested with qPCR (or who had not received a positive result, if tested), and 30% had occurred in persons outside quarantine and not tested with qPCR.

CONCLUSIONS

Our results indicate that antiviral antibodies against SARS-CoV-2 did not decline within 4 months after diagnosis. We estimate that the risk of death from infection was 0.3% and that 44% of persons infected with SARS-CoV-2 in Iceland were not diagnosed by qPCR.

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Stefansson at deCODE Genetics–Amgen, Sturlugata 8, Reykjavik 102, Iceland, or at kstefans@decode.is.

This article was published on September 1, 2020, at NEJM.org.

DOI: 10.1056/NEJMoa2026116

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 2019 (Covid-19), emerged in December 2019.[1] Seroconversion of most patients with Covid-19 occurs between 7 and 14 days after diagnosis.[2,3] A study of 61,000 persons in Spain showed that 5% of the population had formed antibodies against the spike and nucleoproteins and that approximately one third of infected persons were asymptomatic.[4] It was suggested that a substantial fraction of those infected become antibody-negative early in the convalescence period.[5] Several studies have reported a higher prevalence[4] and levels[3,5] of SARS-CoV-2 antibodies in severely ill patients than in those with no or mild symptoms.

The infection fatality risk of SARS-CoV-2 is difficult to estimate because the total number of diagnosed and undiagnosed cases is needed as the denominator. The infection fatality risk was reported as 0.4% in a small German town after carnival festivities,[6] 0.6% on the Diamond Princess cruise ship,[7] and 0.66% in China.[8]

Well-validated serologic assays for SARS-CoV-2 are urgently needed. Several small comparative studies of commercial SARS-CoV-2 antibody assays have been published.[9-12] A highly specific assay is required for screening populations with a low seroprevalence, such as that in Iceland.

The first case of SARS-CoV-2 infection in Iceland was confirmed on February 28, 2020, and by April 30 the epidemic had to a large extent receded.[13] During this period, 1797 cases were diagnosed by quantitative polymerase-chain-reaction (qPCR), in contrast with only 13 new cases diagnosed between April 30 and June 15. Testing by qPCR has been extensive in Iceland: 15% of the population (54,436 persons) had been tested with qPCR by June 15.

The aim of this study was to assess SARS-CoV-2 seroprevalence in the population of Iceland and to assess longitudinal changes in antibody levels within the first 4 months after SARS-CoV-2 infection and how the changes correlate with sex, age, existing phenotypes, and Covid-19 symptoms. We screened for SARS-Cov-2 reactive serum antibodies, using six different assays, in two groups of qPCR-positive persons and six groups of persons who had not been tested with qPCR or who had been tested and received negative results (Fig. 1; and Tables S1 and S2 in Supplementary Appendix 1, available with the full text of this article at NEJM.org).

Methods

Ethical Considerations

The study was approved by the National Bioethics Committee of Iceland. The Health Care sample collection was performed on behalf of Icelandic health authorities in agreement with the Act no. 19/1997 on Health Security and Communicable Diseases. Participants who were part of the other sample collections provided written informed consent.

Antibody Measurements

We measured SARS-CoV-2–specific antibodies in up to 30,576 persons with six established assays, targeting pan-immunoglobulin (pan-Ig: IgM, IgG, and IgA) antibodies against the nucleoprotein (N) (Roche); pan-Ig antibodies against the receptor binding domain (RBD) in the S1 subunit of the spike protein (pan-Ig anti–S1-RBD) (Wantai); IgM and IgG antibodies against N (IgM anti-N and IgG anti-N) (EDI/Eagle); and IgG and IgA against the S1 subunit of the spike protein (IgG anti-S1 and IgA anti-S1) (Euroimmun). Thresholds for positivity were supplied by the assay manufacturers. We used the two pan-Ig antibody assays to evaluate seroprevalence, requiring positive results for both assays for a test result to be considered positive (Fig. S1 in Supplementary Appendix 1). To quantify antibody levels among qPCR-positive persons, we assayed antibodies against SARS-CoV-2 using IgG anti-N, IgM anti-N, IgG anti-S1, and IgA anti-S1.

Sample Collection

We measured antibodies in two groups of qPCR-positive Icelanders and in six groups who had not been qPCR-tested or who had been tested and had received a negative result (Fig. 1). We collected samples from a group of hospitalized qPCR-positive persons and invited all qPCR-positive persons who had recovered from infection to donate samples, both shortly after recovery and again approximately 3 months after recovery (a total of 2102 samples from 1237 persons). We used two groups of samples collected before the pandemic (in 2017 and in early 2020) to evaluate assay specificity and to determine when the pandemic reached Iceland. We

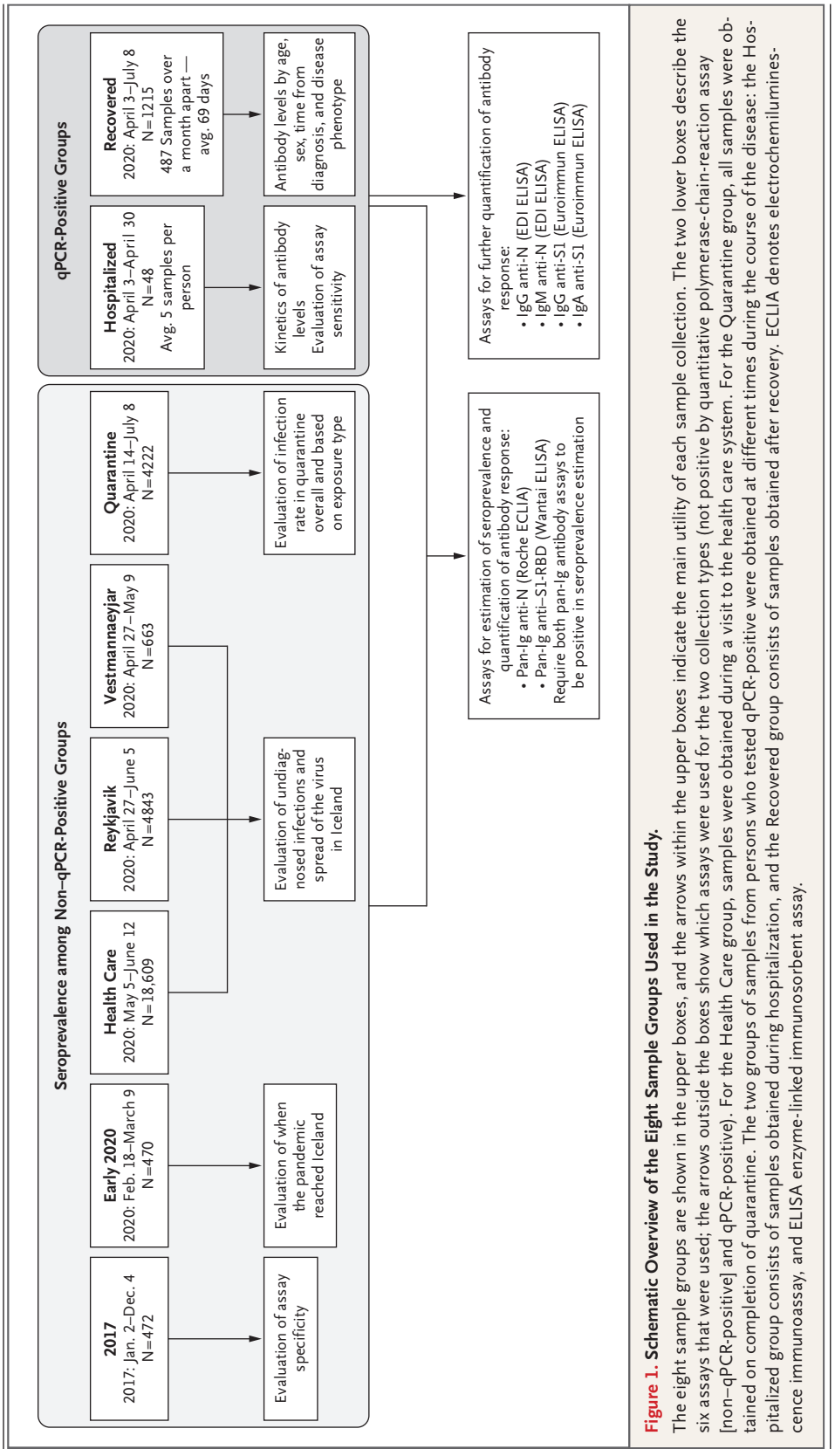

Figure 1. Schematic Overview of the Eight Sample Groups Used in the Study.

The eight sample groups are shown in the upper boxes, and the arrows within the upper boxes indicate the main utility of each sample collection. The two lower boxes describe the six assays that were used; the arrows outside the boxes show which assays were used for the two collection types (not positive by quantitative polymerase-chain-reaction assay [non–qPCR-positive] and qPCR-positive). For the Health Care group, samples were obtained during a visit to the health care system. For the Quarantine group, all samples were obtained on completion of quarantine. The two groups of samples from persons who tested qPCR-positive were obtained at different times during the course of the disease: the Hospitalized group consists of samples obtained during hospitalization, and the Recovered group consists of samples obtained after recovery. ECLIA denotes electrochemiluminescence immunoassay, and ELISA enzyme-linked immunosorbent assay.

collected samples from quarantined persons who had not tested qPCR-positive to evaluate infection during quarantine and the effect of exposure type on the probability of infection. We used three groups of samples collected from persons who had neither tested qPCR-positive nor been quarantined to evaluate seroprevalence outside quarantine and the spread of the virus in Iceland (the Health Care, Reykjavik, and Vestmannaeyjar sample groups, totaling 23,452 persons).

ESTIMATION OF INFECTION RATE

The largest of these six groups, the Health Care group, was enriched for older people. To estimate seroprevalence, we weighted this sample by region, sex, and age in the population (see Supplementary Appendix 1). To estimate the number of infected Icelanders, we added together the number of qPCR-positive persons, the number of quarantined persons times the estimated seroprevalence in this group, and the number of persons outside quarantine times the estimated seroprevalence outside quarantine. We estimated the percentage of Icelanders infected by dividing the number of infected persons by the number of Icelanders. We estimated the infection fatality risk by dividing the number of deaths from Covid-19 by the number of infected persons.

ANTIBODY LEVELS, AGE, SEX, AND CLINICAL CHARACTERISTICS

We tested for associations of age, sex, preexisting conditions (27 phenotypes), and clinical outcome (35 characteristics) with antibody titers (for each of the six assays) in the most recent samples obtained from persons in the Recovered group. We recoded categorical clinical characteristics with their ordinal number in the analysis.

STATISTICAL ANALYSIS

We used a likelihood ratio method to calculate confidence intervals of fractions with the Clopper–Pearson exact method when the estimated fraction was 0 or 1. To test for association between each clinical characteristic and antibody levels, we performed multiple regression analyses with the phenotype as a covariate and quantile normalized antibody levels as a response, adjusting for age, age squared, sex, and time since qPCR diagnosis, excluding the age and sex covariates when testing for association with age and sex, respectively. We quantile-normalized the antibody levels by ranking the levels and transforming them, using the inverse normal transform of the rank divided by one plus the number of observations. Effects estimates were reported in terms of standard deviations of antibody levels. We derived P values and confidence intervals from standard errors estimated by the multiple regression. We used Bonferroni correction to determine significance, with a threshold for significance of $P<[0.05\div 6(2+27+35)]=0.00013$. For effects of the exposure type on the probability of infection among quarantined persons, we used logistic regression to estimate the confidence intervals of odds ratios. We did not adjust confidence intervals for multiple testing.

RESULTS

SPECIFICITY OF SARS-COV-2 ANTIBODY ASSAYS

Both assays measuring pan-Ig antibodies had low numbers of false positives among samples collected in 2017: there were 0 and 1 false positives for the two assays among 472 samples, results that compared favorably with those obtained with the single IgM anti-N and IgG anti-N assays (Table S3). Because of the low prevalence of SARS-CoV-2 infection in Iceland, we required positive results from both pan-Ig antibody assays for a sample to be considered seropositive (see Supplementary Methods in Supplementary Appendix 1). None of the samples collected in early 2020 group were seropositive, which indicates that the virus had not spread widely in Iceland before February 2020.

SARS-COV-2 ANTIBODIES AMONG QPCR-POSITIVE PERSONS

Twenty-five days after diagnosis by qPCR, more than 90% of samples from recovered persons tested positive with both pan-Ig antibody assays, and the percentage of persons testing positive remained stable thereafter (Figs. 2 and S2). Hospitalized persons seroconverted more frequently and quickly after qPCR diagnosis than did nonhospitalized persons (Figs. 2 and S3). Of 1215 persons who had recovered (on the basis of results for the most recently obtained sample from persons for whom we had multiple samples), 1107 were seropositive (91.1%; 95% confidence interval [CI], 89.4 to 92.6) (Tables 1 and S4).

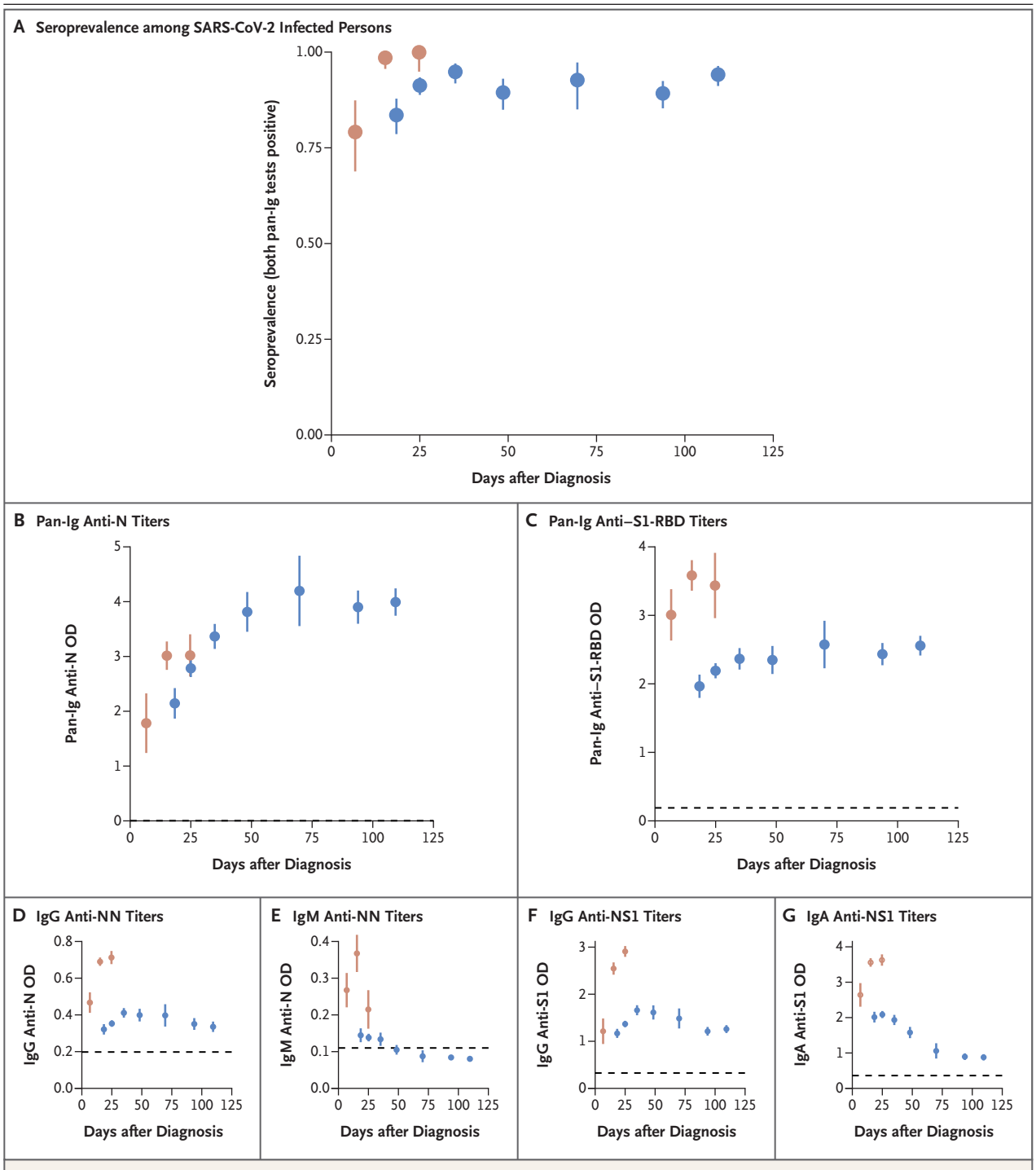

Figure 2. Antibody Prevalence and Titers among qPCR-Positive Cases as a Function of Time since Diagnosis by qPCR.

Shown are the percentages of samples positive for both pan-Ig antibody assays and the antibody titers. Red denotes the count or percentage of samples among persons during their hospitalization (249 samples from 48 persons), and blue denotes the count or percentage of samples among persons after they were declared recovered (1853 samples from 1215 persons). Vertical bars denote 95% confidence intervals. The dashed lines indicated the thresholds for a test to be declared positive. OD denotes optical density, and RBD receptor binding domain.

Table 1. Prevalence of SARS-CoV-2 Antibodies by Sample Collection as Measured by Two Pan-Ig Antibody Assays.*

Sample Collection	No. of Persons Tested	Both Pan-Ig Antibody Assays Positive		Either Pan-Ig Antibody Assay Positive	
		No. of Persons	Frequency	No. of Persons	Frequency
			% (95% CI)		% (95% CI)
2017	472	0	0.0 (0.0–0.4)	1	0.2 (0.0–0.9)
Early 2020	470	0	0.0 (0.0–0.4)	4	0.9 (0.3–2.0)
Health care†	18,609	39	0.2 (0.2–0.3)	119	0.6 (0.5–0.8)
Reykjavik†	4,843	21	0.4 (0.3–0.6)	38	0.8 (0.6–1.1)
Vestmannaeyjar†	663	3	0.5 (0.1–1.2)	7	1.1 (0.5–2.0)
Quarantine	4,222	97	2.3 (1.9–2.8)	131	3.1 (2.6–3.7)
Hospitalized	48	45	93.8 (84.6–98.4)	47	97.9 (91.1–99.9)
Recovered	1,215	1,107	91.1 (89.4–92.6)	1,156	95.1 (93.8–96.3)

* The pan-Ig antibodies are anti-N and anti–S1-RBD. The latest available sample was used.
† Sampling restricted to persons who had not tested qPCR-positive and who had not been quarantined.

Table 2. Results of Repeated Pan-Ig Antibody Tests among Recovered qPCR-Diagnosed Persons.*

First Sample	Second Sample			
	Neither Positive	Single Positive	Both Positive	Total
	number (percent)			
Neither positive	19 (3.9)	1 (0.2)	2 (0.4)	22 (4.5)
Single positive	0	12 (2.5)	10 (2.1)	22 (4.5)
Both positive	1 (0.2)	5 (1.0)	437 (89.7)	443 (91.0)
Total	20 (4.1)	18 (3.7)	449 (92.2)	487 (100.0)

* The two samples were obtained at least 30 days apart. For each sample, a person could be positive for neither test, for a single test, or for both tests. Shown are the number of persons with each result and the percentage of the overall (N=487).

Since some diagnoses may have been made on the basis of false positive qPCR results, we determined that 91.1% represents the lower bound of sensitivity of the combined pan-Ig tests for the detection of SARS-CoV-2 antibodies among recovered persons.

Among the 487 recovered persons with two or more samples, 19 (4%) had different pan-Ig antibody test results at different time points (Table 2 and Fig. S4). It is notable that of the 22 persons with an early sample that tested negative for both pan-Ig antibodies, 19 remained negative at the most recent test date (again, for both antibodies). One person tested positive for both pan-Ig antibodies in the first test and negative for both in the most recent test.

The longitudinal changes in antibody levels among recovered persons were consistent with the cross-sectional results (Fig. S5); antibody levels were higher in the last sample than in the first sample when the antibodies were measured with the two pan-Ig assays, slightly lower than in the first sample when measured with IgG anti-N and IgG anti-S1 assays, and substantially lower than in the first sample when measured with IgM anti-N and IgA anti-S1 assays.

IgG anti-N, IgM anti-N, IgG anti-S1, and IgA anti-S1 antibody levels were correlated among the qPCR-positive persons (Figs. S5 and S6 and Table S5). Antibody levels measured with both pan-Ig antibody assays increased over the first 2 months after qPCR diagnosis and remained at a plateau over the next 2 months of the study. IgM anti-N antibody levels increased rapidly soon after diagnosis and then fell rapidly and were generally not detected after 2 months. IgA anti-S1 antibodies decreased 1 month after diagnosis and remained detectable thereafter. IgG anti-N and anti-S1 antibody levels increased during the first 6 weeks after diagnosis and then decreased slightly.

SARS-CoV-2 Infection in Quarantine

Of the 1797 qPCR-positive Icelanders, 1088 (61%) were in quarantine when SARS-CoV-2 infection was diagnosed by qPCR. We tested for antibodies

Table 3. SARS-CoV-2 Infection among Quarantined Persons According to Exposure Type and Presence of Symptoms.*

Variable	No. of Persons	qPCR			Both Pan-Ig Antibody Assays		
		No. Tested	No. Positive (%)	OR (95% CI)†	No. Tested	No. Positive (%)	OR (95% CI)†
No household exposure	18,877	6839	689 (10.1)		3700	52 (1.4)	
Household exposure	1,889	1092	399 (36.5)	5.2 (4.5–6.1)	503	37 (7.4)	5.2 (3.3–8.0)
No reported symptoms	3,439	1421	142 (10.0)		1007	24 (2.4)	
Reported symptoms	1,639	1397	920 (65.9)	18.2 (14.8–22.4)	237	17 (7.2)	3.2 (1.7–6.2)

* Exposure data were available for 7931 persons who had been tested with qPCR and 4203 tested for antibodies. Symptom data were available for 2818 persons who had been tested with qPCR and 1244 tested for antibodies. The effects of household exposure and symptoms were tested separately among all persons who were tested by qPCR and the collected subset of non qPCR-positive persons tested for antibodies.
† The odds ratios (ORs) comparing exposed with nonexposed and symptomatic with nonsymptomatic were adjusted for sex, age, and age squared.

among 4222 quarantined persons who had not tested qPCR-positive (they had received a negative result by qPCR or had simply not been tested). Of those 4222 quarantined persons, 97 (2.3%; 95% CI, 1.9 to 2.8) were seropositive (Table 1). Those with household exposure were 5.2 (95% CI, 3.3 to 8.0) times more likely to be seropositive than those with other types of exposure (Table 3); similarly, a positive result by qPCR for those with household exposure was 5.2 (95% CI, 4.5 to 6.1) times more likely than for those with other types of exposure. When these two sets of results (qPCR-positive and seropositive) were combined, we calculated that 26.6% of quarantined persons with household exposure and 5.0% of quarantined persons without household exposure were infected. Those who had symptoms during quarantine were 3.2 (95% CI, 1.7 to 6.2) times more likely to be seropositive and 18.2 times (95% CI, 14.8 to 22.4) more likely to test positive with qPCR than those without symptoms.

We also tested persons in two regions of Iceland affected by cluster outbreaks. In a SARS-CoV-2 cluster in Vestfirdir, 1.4% of residents were qPCR-positive and 10% of residents were quarantined. We found that none of the 326 persons outside quarantine who had not been tested by qPCR (or who tested negative) were seropositive. In a cluster in Vestmannaeyjar, 2.3% of residents were qPCR-positive and 13% of residents were quarantined. Of the 447 quarantined persons who had not received a qPCR-positive result, 4 were seropositive (0.9%; 95% CI, 0.3 to 2.1). Of the 663 outside quarantine in Vestmannaeyjar, 3 were seropositive (0.5%; 95% CI, 0.1 to 0.2%).

SARS-COV-2 SEROPREVALENCE IN ICELAND

None of the serum samples collected from 470 healthy Icelanders between February 18 and March 9, 2020, tested positive for both pan-Ig antibodies, although four were positive for the pan-Ig anti-N assay (0.9%), a finding that suggests that the virus had not spread widely in Iceland before March 9.

Of the 18,609 persons tested for SARS-CoV-2 antibodies through contact with the Icelandic health care system for reasons other than Covid-19, 39 were positive for both pan-Ig antibody assays (estimated seroprevalence by weighting the sample on the basis of residence, sex, and 10-year age category, 0.3%; 95% CI, 0.2 to 0.4). There were regional differences in the percentages of qPCR-positive persons across Iceland that were roughly proportional to the percentage of people quarantined (Table S6). However, after exclusion of the qPCR-positive and quarantined persons, the percentage of persons who tested positive for SARS-CoV-2 antibodies did not correlate with the percentage of those who tested positive by qPCR. The estimated seroprevalence in the random sample collection from Reykjavik (0.4%; 95% CI, 0.3 to 0.6) was similar to that in the Health Care group (0.3%; 95% CI, 0.2 to 0.4) (Table S6).

We calculate that 0.5% of the residents of Iceland have tested positive with qPCR. The 2.3% with SARS-CoV-2 seroconversion among persons in quarantine extrapolates to 0.1% of Icelandic residents. On the basis of this finding and the seroprevalence from the Health Care group, we estimate that 0.9% (95% CI, 0.8 to 0.9) of the

population of Iceland has been infected by SARS-CoV-2. Approximately 56% of all SARS-CoV-2 infections were therefore diagnosed by qPCR, 14% occurred in quarantine without having been diagnosed with qPCR, and the remaining 30% of infections occurred outside quarantine and were not detected by qPCR.

DEATHS FROM COVID-19 IN ICELAND

In Iceland, 10 deaths have been attributed to Covid-19, which corresponds to 3 deaths per 100,000 nationwide. Among the qPCR-positive cases, 0.6% (95% CI, 0.3 to 1.0) were fatal. Using the 0.9% prevalence of SARS-CoV-2 infection in Iceland as the denominator, however, we calculate an infection fatality risk of 0.3% (95% CI, 0.2 to 0.6). Stratified by age, the infection fatality risk was substantially lower in those 70 years old or younger (0.1%; 95% CI, 0.0 to 0.3) than in those over 70 years of age (4.4%; 95% CI, 1.9 to 8.4) (Table S7).

AGE, SEX, CLINICAL CHARACTERISTICS, AND ANTIBODY LEVELS

SARS-CoV-2 antibody levels were higher in older people and in those who were hospitalized (Table 4, and Table S8 [described in Supplementary Appendix 1 and available in Supplementary Appendix 2]). Pan-Ig anti–S1-RBD and IgA anti-S1 levels were lower in female persons. Of the preexisting conditions, and after adjustment for multiple testing, we found that body-mass index, smoking status, and use of antiinflammatory medication were associated with SARS-CoV-2 antibody levels. Body-mass index correlated positively with antibody levels; smokers and users of antiinflammatory medication had lower antibody levels. With respect to clinical characteristics, antibody levels were most strongly associated with hospitalization and clinical severity, followed by clinical symptoms such as fever, maximum temperature reading, cough, and loss of appetite. Severity of these individual symptoms, with the exception of loss of energy, was associated with higher antibody levels.

DISCUSSION

We estimate that during the first wave of the SARS-CoV-2 pandemic, the incidence of infection in Iceland was 0.9% (95% CI, 0.8 to 0.9) and the infection fatality risk was 0.3% (95% CI, 0.2 to 0.6). Our estimate of the infection fatality risk is lower than but consistent with estimates described by others.[6-8] We estimate that of the infected persons, 56% had cases previously diagnosed by qPCR, 14% had been in quarantine (but either had not been qPCR-tested or had tested negative), and 30% neither were known to be qPCR-positive nor had been placed in quarantine. We therefore conclude that, despite extensive screening by qPCR, a substantial fraction of infections were not detected, which indicates that many infected persons did not have substantial symptoms.

The case fatality risk is straightforward to estimate but may differ across countries and over time. An accurate calculation of infection fatality risk requires an accurate estimate of the number of infections, both diagnosed and undiagnosed. In Iceland, the high percentage of infections identified through qPCR (56%) as compared with that of other countries (for example, approximately 9% in Spain[4]) renders a commensurately accurate estimate of the total number of infections.

Each of the pan-Ig SARS-CoV-2 antibody assays that we used has high specificity (99.8%, according to the manufacturers' literature), which raises the question of whether using a single pan-Ig assay would have sufficed. One sample obtained in 2017 was positive on only one pan-Ig antibody assay, a finding that supports the use of two separate assays to determine seroprevalence, if the infection rate is below 1%, as in Iceland.

By April 30, a total of 20,766 Icelanders had been placed in quarantine. Of the 1797 Icelanders who tested positive by qPCR, 1088 (61%) were in quarantine when tested. Despite substantial qPCR testing of persons in quarantine, 2.3% of persons in quarantine who did not receive qPCR-positive result (i.e., a diagnosis of infection) developed SARS-CoV-2 antibodies. Household exposure was more likely to lead to infection than other types of exposure, which suggests that people who share a household with an infected person should not have contact during quarantine and that contacts of household members should be quarantined. Seroprevalence in the two regional hot spots (Vestfirdir and Vestmannaeyjar) was absent or low outside quarantine, which indicates that most infections were detected by qPCR screening and that quarantine, social distancing, contact tracing, and limits on

Table 4. Association of Existing Conditions and Covid-19 Severity with SARS-CoV-2 Antibody Levels among Recovered Persons.*

Variable	No. of Persons	Log (Pan-Ig Anti-N) (95% CI)	Pan-Ig Anti–S1-RBD (95% CI)	IgG Anti-N (95% CI)	IgM Anti-N (95% CI)	IgG Anti-S1 (95% CI)	IgA Anti-S1 (95% CI)
Change in levels per 10 yr of life	1215	0.15 (0.11 to 0.18)†	0.10 (0.07 to 0.14)†	0.22 (0.19 to 0.25)†	0.04 (0.01 to 0.08)‡	0.15 (0.09 to 0.20)†	0.15 (–0.05 to 0.34)
Female sex	1215	–0.09 (–0.20 to 0.02)	–0.24 (–0.35 to –0.13)§	–0.11 (–0.22 to 0.00)	–0.04 (–0.15 to 0.07)	–0.09 (–0.15 to –0.03)‡	–0.09 (–0.12 to –0.06)†
Body-mass index¶	542	0.03 (0.02 to 0.05)§	0.02 (0.00 to 0.03)‡	0.02 (0.01 to 0.04)‡	0.02 (0.00 to 0.03)‡	0.03 (0.01 to 0.06)‡	0.03 (0.00 to 0.07)
Smoker	1200	–0.58 (–0.83 to –0.32)§	–0.59 (–0.85 to –0.32)§	–0.62 (–0.87 to –0.36)§	–0.21 (–0.45 to 0.03)	–0.58 (–0.82 to –0.34)§	–0.58 (–0.84 to –0.32)§
Antiinflammation medication¶	538	–0.36 (–0.61 to –0.12)‡	–0.37 (–0.62 to –0.12)‡	–0.35 (–0.59 to –0.11)‡	–0.02 (–0.23 to 0.20)	–0.36 (–0.66 to –0.06)‡	–0.36 (–0.53 to –0.19)§
Hospitalization	1215	–0.09 (–0.20 to 0.02)	–0.24 (–0.35 to –0.13)§	–0.11 (–0.22 to 0.00)	–0.04 (–0.15 to 0.07)	–0.09 (–0.15 to –0.03)‡	–0.09 (–0.12 to –0.06)†
Maximum clinical stage	1215	0.19 (0.11 to 0.27)§	0.23 (0.15 to 0.31)†	0.28 (0.20 to 0.36)†	0.10 (0.02 to 0.17)‡	0.19 (0.14 to 0.24)†	0.19 (0.09 to 0.28)§
Temperature¶	401	0.40 (0.20 to 0.59)§	0.43 (0.23 to 0.63)§	0.48 (0.29 to 0.68)§	0.17 (–0.01 to 0.35)	0.40 (0.24 to 0.55)†	0.40 (0.13 to 0.66)‡
Maximum temperature¶	269	0.37 (0.23 to 0.51)†	0.44 (0.29 to 0.58)†	0.43 (0.29 to 0.57)†	0.21 (0.09 to 0.34)‡	0.37 (0.24 to 0.49)†	0.37 (0.18 to 0.55)‡
Loss of energy¶	545	–0.20 (–0.29 to –0.11)§	–0.17 (–0.26 to –0.08)‡	–0.18 (–0.27 to –0.10)§	–0.02 (–0.10 to 0.06)	–0.20 (–0.36 to –0.05)‡	–0.20 (–0.35 to –0.05)‡
Cough¶	422	0.13 (0.02 to 0.23)‡	0.15 (0.05 to 0.26)‡	0.21 (0.11 to 0.31)§	0.13 (0.04 to 0.22)‡	0.13 (0.06 to 0.20)‡	0.13 (0 to 0.26)
Loss of appetite¶	420	0.14 (0.06 to 0.23)‡	0.14 (0.05 to 0.22)‡	0.20 (0.12 to 0.29)†	0.10 (0.02 to 0.17)‡	0.14 (0.07 to 0.21)§	0.14 (–0.07 to 0.35)

* Shown are significant associations after adjustment for multiple testing (see Table S8 [described in Supplementary Appendix 1 and available in Supplementary Appendix 2] for a complete list of results). All associations except the age and sex associations were adjusted for age, age squared, sex, and time since diagnosis. Information was available on up to 1215 recovered persons with at least one antibody measurement. Of these, 545 persons answered an online questionnaire. The effects are expressed in number of standard deviations of the corresponding antibody assay.

† $P<10^{-6}$.

‡ $P<0.05$.

§ $P<1.3\times10^{-4}$ after adjustment for multiple testing

¶ Data are from an online questionnaire.

public gatherings were effective in limiting spread.

Over 90% of qPCR-positive persons tested positive with both pan-Ig SARS-CoV-2 antibody assays and remained seropositive 120 days after diagnosis, with no decrease of antibody levels as detected by the two pan-Ig assays. We observed some diminution of antibody titer with some of the single-Ig assays. Previous smaller studies reported reduction of IgG antibodies against the N protein and a peptide representing the S protein within 21 to 28 days[5] and against trimeric S protein within 56 days[14] after a positive test by qPCR. These discrepancies may be explained partly by differences in the specificity and sensitivity of the assays used as well as differences in the design and performance of the semiquantitative assays used, including the antigen targeted and the analytic sensitivity and range, as well as differences in the study populations. For example, because of widespread qPCR testing and screening, it is likely that the Icelandic qPCR-positive persons were healthy, as compared with the participants in other studies. Repeated SARS-CoV-2 exposure is unlikely to affect the persistence of antibody levels in Iceland, given the low prevalence of infection. Comparative studies using validated quantitative SARS-CoV-2 antibody assays are needed; those described in the published literature are based on small sample sizes.[9-12]

Of the 22 recovered persons who had a negative result (using the combined pan-Ig antibody tests) for an early sample and who had another sample tested at least a month later, 19 (86%) received a second negative result. Thus, either some persons infected by SARS-CoV-2 produce no antibodies or undetectable levels of antibodies reactive to the S1 and N proteins, even 3 months after infection, or some qPCR delivered false positive results.

Among recovered persons, antibody levels are higher in older persons and in those more severely affected by SARS-CoV-2 infection. Women, who tend to become less sick than men, had lower antibody levels in two spike protein antibody assays. SARS-CoV-2 antibody levels were lower in smokers. Smoking increases the probability of severe Covid-19 illness among young adults,[15] and smoking has been reported to increase the expression of ACE2,[16] the receptor for cellular entry of the SARS-CoV-2 virus.

The humoral immune response is critical for the clearance of cytopathic viruses and is generally important for the prevention of viral reinfection.[17] A relationship between a humoral immune response to SARS-CoV-2 infection and protection against reinfection by this virus has been shown in rhesus macaques[18] but has yet to be established in humans. Regardless of the relationship or lack thereof between seropositivity against SARS-CoV-2 and protection against reinfection, the low SARS-CoV-2 antibody seroprevalence in Iceland indicates that the Icelandic population is vulnerable to a second wave of infection.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

APPENDIX

The authors' full names and academic degrees are as follows: Daniel F. Gudbjartsson, Ph.D., Gudmundur L. Norddahl, Ph.D., Pall Melsted, Ph.D., Kristbjorg Gunnarsdottir, M.Sc., Hilma Holm, M.D., Elias Eythorsson, M.D., Ph.D., Asgeir O. Arnthorsson, M.Sc., Dadi Helgason, M.D., Ph.D., Kristbjorg Bjarnadottir, Ph.D., Ragnar F. Ingvarsson, M.D., Brynja Thorsteinsdottir, B.Sc., Steinunn Kristjansdottir, B.Sc., Kolbrun Birgisdottir, B.Sc., Anna M. Kristinsdottir, M.Sc., Martin I. Sigurdsson, M.D., Ph.D., Gudny A. Arnadottir, M.Sc., Erna V. Ivarsdottir, M.Sc., Margret Andresdottir, M.Sc., Frosti Jonsson, M.Sc., Arna B. Agustsdottir, M.Sc., Jonas Berglund, Ph.D., Berglind Eiriksdottir, Run Fridriksdottir, M.Sc., Elisabet E. Gardarsdottir, Magnus Gottfredsson, M.D., Ph.D., Olafia S. Gretarsdottir, B.Sc., Steinunn Gudmundsdottir, M.Sc., Kjartan R. Gudmundsson, B.Sc., Thora R. Gunnarsdottir, B.Sc., Arnaldur Gylfason, M.Sc., Agnar Helgason, Ph.D., Brynjar O. Jensson, M.Sc., Aslaug Jonasdottir, M.Sc., Hakon Jonsson, Ph.D., Thordur Kristjansson, M.D., Ph.D., Karl G. Kristinsson, M.D., Ph.D., Droplaug N. Magnusdottir, M.Sc., Olafur T. Magnusson, Ph.D., Lovisa B. Olafsdottir, M.D., Solvi Rognvaldsson, B.Sc., Louise le Roux, M.Sc., Gudrun Sigmundsdottir, M.D., Asgeir Sigurdsson, B.Sc., Gardar Sveinbjornsson, M.Sc., Kristin E. Sveinsdottir, B.Sc., Maney Sveinsdottir, B.Sc., Emil A. Thorarensen, B.Sc., Bjarni Thorbjornsson, B.Sc., Marianna Thordardottir, Ph.D., Jona Saemundsdottir, B.Sc., S. Hjortur Kristjansson, M.D., Kamilla S. Josefsdottir, M.D., Gisli Masson, Ph.D., Gudmundur Georgsson, B.Sc., Mar Kristjansson, M.D., Alma Moller, M.D., Ph.D., Runolfur Palsson, M.D., Thorolfur Gudnason, M.D., Unnur Thorsteinsdottir, Ph.D., Ingileif Jonsdottir, Ph.D., Patrick Sulem, M.D., and Kari Stefansson, M.D., Ph.D.

The authors' affiliations are as follows: deCODE Genetics/Amgen (D.F.G., G.L.N., P.M., K.G., H.H., A.O.A., K. Bjarnadottir, B. Thorsteinsdottir, S.K., K. Birgisdottir, A.M.K., G.A.A., E.V.I., M.A., F.J., A.B.A., J.B., B.E., R.F., E.E.G., S.G., K.R.G., A.G., A.H., B.O.J., A.J., H.J., T.K., D.N.M., O.T.M., S.R., L.R., A.S., G. Sveinbjornsson, K.E.S., E.A.T., B. Thorbjornsson, J.S., G.M., G.G., U.T., I.J., P.S., K.S.), the School of Engineering and Natural Sciences (D.F.G., P.M.), the Department of Anthropology (A.H.), the BioMedical Center (K.G.K.), and the Faculty of Medicine, School of Health Sciences (M.I.S., M.G., K.G.K., R.P., U.T., I.J., K.S.), University of Iceland, Internal Medicine and Rehabilitation Services (E.E., D.H., R.F.I., M.G., L.B.O., M.K., R.P.), the Division of Anesthesia and Intensive Care Medicine (M.I.S.), and the Department of Clinical Microbiology (O.S.G., T.R.G., K.G.K., M.S.), Landspitali–the National University Hospital, and the Directorate of Health (G. Sigmundsdottir, M.T., K.S.J., A.M., T.G.), Reykjavik, and the Health Care Institution of South Iceland, Selfoss (S.H.K.) — all in Iceland.

REFERENCES

1. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 2020;395:497-506.
2. Vabret N, Britton GJ, Gruber C, et al. Immunology of COVID-19: current state of the science. Immunity 2020;52:910-41.
3. Zhao J, Yuan Q, Wang H, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. Clin Infect Dis 2020 March 28 (Epub ahead of print).
4. Pollán M, Pérez-Gómez B, Pastor-Barriuso R, et al. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. Lancet 2020;396:535-44.
5. Long Q-X, Tang X-J, Shi Q-L, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med 2020;26:1200-4.
6. Streeck H, Schulte B, Kuemmerer B, et al. Infection fatality rate of SARS-CoV-2 infection in a German community with a super-spreading event (https://www.medrxiv.org/content/10.1101/2020.05.04.20090076v2). preprint.
7. Russell TW, Hellewell J, Jarvis CI, et al. Estimating the infection and case fatality ratio for coronavirus disease (COVID-19) using age-adjusted data from the outbreak on the Diamond Princess cruise ship, February 2020. Euro Surveill 2020; 25(12).
8. Verity R, Okell LC, Dorigatti I, et al. Estimates of the severity of coronavirus disease 2019: a model-based analysis. Lancet Infect Dis 2020;20:669-77.
9. GeurtsvanKessel CH, Okba NMA, Igloi Z, et al. An evaluation of COVID-19 serological assays informs future diagnostics and exposure assessment. Nat Commun 2020;11:3436.
10. Meyer B, Torriani G, Yerly S, et al. Validation of a commercially available SARS-CoV-2 serological immunoassay. Clin Microbiol Infect 2020 June 27 (Epub ahead of print).
11. Beavis KG, Matushek SM, Abeleda APF, et al. Evaluation of the EUROIMMUN anti-SARS-CoV-2 ELISA assay for detection of IgA and IgG antibodies. J Clin Virol 2020;129:104468.
12. Jääskeläinen AJ, Kuivanen S, Kekäläinen E, et al. Performance of six SARS-CoV-2 immunoassays in comparison with microneutralisation. J Clin Virol 2020; 129:104512.
13. Gudbjartsson DF, Helgason A, Jonsson H, et al. Spread of SARS-CoV-2 in the Icelandic population. N Engl J Med 2020; 382:2302-15.
14. Adams ER, Ainsworth M, Anand R, et al. Antibody testing for COVID-19: a report from the National COVID Scientific Advisory Panel (https://www.medrxiv.org/content/10.1101/2020.04.15.20066407v3). preprint.
15. Adams SH, Park MJ, Schaub JP, Brindis CD, Irwin CE Jr. Medical vulnerability of young adults to severe COVID-19 illness — data from the National Health Interview Survey. J Adolesc Health 2020 July 9 (Epub ahead of print).
16. Leung JM, Yang CX, Tam A, et al. ACE-2 expression in the small airway epithelia of smokers and COPD patients: implications for COVID-19. Eur Respir J 2020;55: 2000688.
17. Dörner T, Radbruch A. Antibodies and B cell memory in viral immunity. Immunity 2007;27:384-92.
18. Deng W, Bao L, Liu J, et al. Primary exposure to SARS-CoV-2 protects against reinfection in rhesus macaques. Science 2020;369:818-23.