Dana Wefer, Esq.
Law Offices of Dana Wefer, LLC
375 Sylvan Ave, Suite 32
Englewood Cliffs, NJ 07632

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF NEW JERSEY

ERICH SMITH, FRANK E. GARWOOD, JR., MARIBEL LORENZO, and Dr. DANIEL DONOFRIO Plaintiffs, vs. PRESIDENT JOSEPH R. BIDEN, JR. (in his official capacity and any successor to the Office of the President) Defendants.	The Honorable Judge Christine P. O'Hearn and The Honorable Judge Sharon A. King CIVIL ACTION NO: 1:21-cv-19457-CPO-SAK **DECLARATION OF COUNSEL IN SUPPORT OF PLAINTIFFS' MOTION FOR A TEMPORARY RESTRAINING ORDER AND/OR PRELIMINARY INJUNCTION**

DECLARATION OF COUNSEL DANA WEFER

I, Dana Wefer, being of full age and sound mind declare:

1. I am an attorney with the Law Offices of Dana Wefer, counsel for Plaintiffs in the above captioned matter.
2. I make this declaration to place certain documents before the Court in support of Plaintiffs' motion for a temporary restraining order and/or preliminary injunction.
3. Attached hereto as Exhibit 1 is a true and accurate copy of the article *CDC Data Suggests Vaccinated Don't Carry, Can't Spread Virus, New York Magazine*, by Paola Rosa-Aquino, The Intelligencer (April 1, 2021) available at https://nymag.com/intelligencer/2021/04/cdc-data-

suggests-vaccinated-dont-carry-cant-spread-virus.html (last accessed October 28, 2021).

4. Attached hereto as Exhibit 2 is a true and accurate copy of: CDC, *Statement from CDC Director Rochelle P. Walensky, MD, MPH on Today's MMWR (Media Statement)* (July 30, 2021) available at https://www.cdc.gov/media/releases/2021/s0730-mmwr-covid-19.html (last accessed October 28, 2021).
5. Attached hereto as Exhibit 3 is a true and accurate copy of: Oriana Gonzalez, *CDC director: U.S. may change definition of "fully vaccinated" as boosters roll out*, *Axios* (October 22, 2021) available at https://www.axios.com/cdc-fully-covid-vaccinated-definition-update-5c2312d9-64f4-4bb7-a289-04c00889a573.html (last accessed October 29, 2021).
6. Attached hereto as Exhibit 4 is a true and accurate copy of: Berkeley Lovelace, Jr., *CDC safety group says there's a likely link between rare heart inflammation in young people after Covid shot*, cnbc.com (updated October 28, 2021) available at https://www.cnbc.com/2021/06/23/cdc-reports-more-than-1200-cases-of-rare-heart-inflammation-after-covid-vaccine-shots.html (last accessed November 3, 2021).
7. Attached hereto as Exhibit 5 is: National Institute of

Health, *COVID-19 Vaccines and the Menstrual Cycle: NIH encourages researchers to investigate reported changes in menstruation after COVID-19 vaccination* (last updated August 2, 2021) available at https://covid19.nih.gov/news-and-stories/covid-19-vaccines-and-menstrual-cycle (last accessed September 7, 2021).

8. Attached hereto as Exhibit 6 is a true and accurate copy of: Reuters Staff, *US jury's Neurontin ruling to cost Pfizer $141 mln*, March 25, 2010 available at https://www.reuters.com/article/pfizer-neurontin-idUSN259778920100325 (last accessed November 3, 2021).

9. Attached hereto as Exhibit 7 is a true and accurate copy of: Ranking Member's News | Newsroom | The United States Senate Committee on Financehttps://www.finance.senate.gov/ranking-members-news/-senator-grassleys-testimony-to-house-oversight-hearing-on-the-adequacy-of-fda-efforts-to-assure-the-safety-of-the-drug-supply (last accessed October 28, 2021).

10. Attached hereto as Exhibit 8 is a true and accurate copy of Edmond J. Safra Center for Ethics, About Donald Light.

11. Attached hereto as Exhibit 9 is a true and accurate copy of Donald Light, "*Risky Drugs: Why The FDA Cannot Be Trusted*," Harvard Edmund J. Safra Center for Ethics,

available at https://ethics.harvard.edu/blog/risky-drugs-why-fda-cannot-be-trusted (last accessed October 29, 2021).

12. Attached hereto as Exhibit 10 is a true and accurate copy of NIH, *Lasting immunity found after recovery from COVID-19,* (January 26, 2021) available at https://www.nih.gov/news-events/nih-research-matters/lasting-immunity-found-after-recovery-covid-19 (last accessed September 7, 2021). (last accessed October 28, 2021).

13. Attached hereto as Exhibit 11 is a true and accurate copy of: Turner, J.S., Kim, W., Kalaidina, E. *et al. SARS-CoV-2 infection induces long-lived bone marrow plasma cells in humans.* Nature 595, 421–425 (2021). ("Overall, our results indicate that mild infection with SARS-CoV-2 induces robust antigen-specific, long-lived humoral immune memory in humans") available at https://doi.org/10.1038/s41586-021-03647-4 May 24 (last accessed September 7, 2021)

14. Attached hereto as Exhibit 12 is a true and accurate copy of: John P A Ioannidis, *Infection fatality rate of COVID-19 inferred from seroprevalence data*, Bull World Health Organ 2021;99:19–33F (October 14, 2020).

I declare under penalty of perjury under the laws of the

United States of America that the foregoing is true and correct.

Executed on November 3, 2021 ____/s/ Dana Wefer_____

Dana Wefer, Esq.

EXHIBIT 1

gencer

COVID-19 | UPDATED APR. 1, 2021

CDC Data Suggests Vaccinated Don't Carry, Can't Spread Virus

By Paola Rosa-Aquino

The good news keeps coming. Photo: Grant Hindsley/AFP via Getty Images

After warning for months that vaccinated people should still be cautious in order to not infect others, the Centers for Disease Control and Prevention suggests they may not be at much risk of transmitting the coronavirus.

"Vaccinated people do not carry the virus — they don't get sick," Dr. Rochelle Walensky, director of the CDC, told MSNBC's Rachel Maddow on Tuesday. That's "not just in the clinical trials, but it's also in real-world data."

Walensky was referring to a new CDC study that suggests those fully inoculated with the vaccines produced by Moderna and Pfizer don't transmit the virus. Researchers looked at how the shots protected nearly 4,000 health-care workers, first responders, and other essential workers toiling in eight U.S. locations against the virus and more-contagious variants. Following a single dose of either vaccine, the participants' risk of infection was reduced by 80 percent, and that figure jumped to 90 percent after the second dose. Without infection, people are unable to spread the virus. The results are similar to what scientists saw in clinical trials for the vaccines, which found that two doses of either two-dose vaccine had an efficacy rate of around 95 percent.

The study is the agency's first to analyze how well the vaccines worked among working-age front-line adults, who are at a higher risk of being exposed to the virus and spreading it. "These findings should offer hope to the millions of Americans receiving COVID-19 vaccines each day and to those who will have the opportunity to roll up their sleeves and get vaccinated in the weeks ahead," Dr. Rochelle Walensky, director of the CDC, said in a statement. "The authorized vaccines are the key tool that will help bring an end to this devastating pandemic." Still, the CDC has not issued new guidance on how the vaccinated should behave; its current guidance is that they continue to take precautions such as masking.

Though the study is an impressive piece of evidence of the effectiveness of the Moderna and Pfizer vaccines, some public-health experts pushed back on Walensky's pandemic-changing takeaway. "There cannot be any daylight between what the research shows — really impressive but incomplete protection — and how it is described," Dr. Peter Bach, director of the Center for Health Policy and Outcomes at Memorial Sloan Kettering Cancer Center, told the New York *Times* on Thursday. "This opens the door to the skeptics who think the government is sugarcoating the science," Bach added, "and completely undermines any remaining argument why people should keep wearing masks after being vaccinated."

Even the Centers for Disease Control hedged on Walensky's claims. "Dr. Walensky spoke broadly during this interview," a CDC spokesperson told the *Times*. "It's possible that some people who are fully vaccinated could get Covid-19. The evidence isn't clear whether they can spread the virus to others. We are continuing to evaluate the evidence."

More than 142 million doses of the Moderna and Pfizer vaccines have been administered in the U.S. as of March 30, according to the CDC. The third vaccine currently on the American market is a single-dose shot made by Johnson & Johnson, which was shown to be 66 percent effective in thwarting moderate to severe COVID-19-related illness.

This post has been updated to reflect a statement from the CDC provided to the New York Times.

TAGS: COVID-19 COVID-19 VACCINES CDC

44 COMMENTS

Mixing up the most famous *Star Trek* characters is a very de Blasio thing to do — or maybe his "Captain Kirk" costume had a deeper meaning.

5:57 P.M. POLITICS

SEC Investigating Richard Burr for Possible Insider Trading

By BENJAMIN HART

The North Carolina senator's conveniently timed transactions at the beginning of the pandemic are under scrutiny again.

MOST POPULAR

1. **'The Problem Is Him'**
   By JAMES D. WALSH
2. **Bidenomics Is Working**
   By ERIC LEVITZ
3. **Polling in America Is Still Broken. So Who Is Really Winning in Virginia?**
   By GABRIEL DEBENEDETTI
4. **Lina Khan Isn't Worried About Going Too Far**
   By NANCY SCOLA
5. **Zuckerberg Pivots to Creators and Renames Facebook Meta**
   By CHOIRE SICHA

5:44 P.M. BUILD BACK BETTER

The Build Back Better Framework: The Good, the Bad, the Ugly

By ERIC LEVITZ

The diet-version of Joe Biden's signature legislation has some heartening strengths and dismaying weaknesses.

4:59 P.M. THE CITY POLITIC

Who Will Be Eric Adams's Partner in Power?

By ERROL LOUIS

City Council Speaker is an extremely powerful position that can help shape a political era in NYC. But it's not on the ballot.

3:45 P.M. SATIRE

6 Wildly Popular Proposals Senator Manchin Killed

By BENJAMIN HART

No to free chocolate chip cookies? Thanks a lot, Joe.

3:05 P.M. EXPLAINER

Who Is Ron Watkins, the QAnon Celebrity Running for Congress?

By MATT STIEB

Everything you need to know about why people think Watkins is Q, his campaign to unseat an Arizona congressman, and whether he actually has a shot.

3:00 P.M. THE LAW

You Can Still Say 'Woman.' But You Shouldn't Stop There

By IRIN CARMON

What does trans-inclusive abortion advocacy look like?

2:29 P.M. FACEBOOK

Zuckerberg Pivots to Creators and Renames Facebook Meta

By CHOIRE SICHA

Were your predictions correct?

1:31 P.M. BIG TECH

Inside Jedi Blue, Facebook's Shady Deal With Google

By JACOB SILVERMAN

Behind a geeky code name lies a sinister plot.

10:52 A.M. THE NATIONAL INTEREST

Biden's Build Back Better Plan Can Be Great, But Congress Needs to Fix It

By JONATHAN CHAIT

Lock in the revenue and climate, pare down the social spending.

10:21 A.M. THE ECONOMY

The Delta Variant Pumped the Brakes on the Economy

By BENJAMIN HART

New data show that surging cases and supply-chain issues slowed growth over the past three months.

10:02 A.M. 2022 MIDTERMS

David Perdue May Primary Trump Foe Brian Kemp in Georgia

By ED KILGORE

It seemed Governor Kemp had outfoxed Trump, but now the former president may have reeled in a serious challenger.

8:05 A.M. TOMORROW

Weathering the Weather

By BRIDGET READ

Mental-health professionals are trying to figure out how to talk about the climate.

8:00 A.M. RODENTS

Eric Adams Won't Stop Talking About His Rat Bucket

By WILLY BLACKMORE

As mayor, he wants to take his favorite trapping device citywide.

6:00 A.M. THE INSIDE GAME

Polling in America Is Still Broken. So Who Is Really Winning in Virginia?

By GABRIEL DEBENEDETTI

The polls were disastrously wrong in 2020. That has political insiders extra nervous about a pivotal state election.

10/27/2021 ABORTION

Gun Guy Mark McCloskey Wants to Ban Abortions for Victims of Incestuous Rape

By ED KILGORE

The GOP Senate hopeful who pointed guns at protesters wants to run wild with abortion policy in Missouri, the home of Todd "Legitimate Rape" Akin.

10/27/2021 RANSOMWARE

Russian Cybercriminals Claim to Have Hacked the NRA

By MATT STIEB

More trouble for the troubled guns-rights group.

10/27/2021 THE FUTURE

Lessons in Tending Your Metaverse

By CHOIRE SICHA

What Facebook could learn from Reddit.

10/27/2021 VIRGINIA GOVERNOR'S RACE

If McAuliffe Loses to Youngkin, Don't Blame Princess Blanding

By ED KILGORE

Major-party supporters have a bad habit of blaming close losses on "spoilers" like Blanding, who represent distinct and legitimate points of view.

10/27/2021 FACEBOOK

What Is Being Leaked in the Facebook Papers?

By CHAS DANNER

A guide to the newly reported revelations from a trove of internal company documents shared by a company whistleblower.

10/27/2021 THE FACEBOOK PAPERS

The Facebook Leaks Have Caught the FTC's Attention

By BENJAMIN HART

The agency is reportedly investigating whether leaked files prove Mark Zuckerberg & Co. misled consumers.

10/27/2021 COVID-19

Where Are All the At-Home COVID Tests?

By MARGARET HARTMANN

Here's why rapid antigen tests are still scarce weeks after Biden promised to boost at-home testing, and when they'll finally be widely available.

10/27/2021 THE ECONOMY

Bidenomics Is Working

By ERIC LEVITZ

Even though voters think otherwise.

10/27/2021 ENCOUNTER

Lina Khan Isn't Worried About Going Too Far

By NANCY SCOLA

The FTC's very young new boss thinks corporations such as Facebook are abusing their power. To fight them, she's consolidating some clout of her own.

READ MORE ON THE Intelligencer HOMEPAGE

EXHIBIT 2

Centers for Disease Control and Prevention

Statement from CDC Director Rochelle P. Walensky, MD, MPH on Today's MMWR

Media Statement

For Immediate Release: Friday, July 30, 2021
Contact: Media Relations
(404) 639-3286

On July 27th, CDC updated its guidance for fully vaccinated people, recommending that everyone wear a mask in indoor public settings in areas of substantial and high transmission, regardless of vaccination status. This decision was made with the data and science available to CDC at the time, including a valuable public health partnership resulting in rapid receipt and review of unpublished data.

Today, some of those data were published in CDC's *Morbidity and Mortality Weekly Report (MMWR)*, demonstrating that Delta infection resulted in similarly high SARS-CoV-2 viral loads in vaccinated and unvaccinated people. High viral loads suggest an increased risk of transmission and raised concern that, unlike with other variants, vaccinated people infected with Delta can transmit the virus. This finding is concerning and was a pivotal discovery leading to CDC's updated mask recommendation. The masking recommendation was updated to ensure the vaccinated public would not unknowingly transmit virus to others, including their unvaccinated or immunocompromised loved ones.

This outbreak investigation and the published report were a collaboration between the Commonwealth of Massachusetts Department of Public Health and CDC. I am grateful to the commonwealth for their collaboration and rigorous investigation. I would also like to humbly thank the residents of Barnstable County who leaned in to assist with the investigation through their swift participation in interviews by contact tracers, willingness to provide samples for testing, and adherence to safety protocols following notification of exposure.

This outbreak investigation is one of many CDC has been involved in across the country and data from those investigations will be rapidly shared with the public when available. The agency works every day to use the best available science and data to quickly and transparently inform the American public about threats to health.

###

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

CDC works 24/7 protecting America's health, safety and security. Whether disease start at home or abroad, are curable or preventable, chronic or acute, or from human activity or deliberate attack, CDC responds to America's most pressing health threats. CDC is headquartered in Atlanta and has experts located throughout the United States and the world.

Page last reviewed: July 30, 2021

EXHIBIT 3

AXIOS

Oct 22, 2021 - Health

CDC director: U.S. may change definition of "fully vaccinated" as boosters roll out

Oriana Gonzalez

CDC Director Rochelle Walensky. Photo: Greg Nash-Pool/Getty Images

Rochelle Walensky, director of the Centers for Disease Control and Prevention, said Friday the U.S. "may need

The big picture: The CDC and the FDA have officially approved boosters with every authorized vaccine in the U.S. for people who meet specific requirements. Walensky explained that since not everyone is eligible for a booster, the definition has not been changed "yet."

- Currently, the CDC's definition is the following: "Fully vaccinated persons are those who are ≥14 days post-completion of the primary series of an FDA-authorized COVID-19 vaccine."

What they're saying: "We have not yet changed the definition of 'fully vaccinated.' We will continue to look at this. We may need to update our definition of 'fully vaccinated' in the future," Walensky said during a press briefing.

- She also encouraged those eligible to get boosters: "If you're eligible for a booster, go ahead and get your booster," she said.

Go deeper

Yacob Reyes
Updated 22 hours ago - Politics & Policy

Florida Gov. Ron DeSantis. Photo: Paul Hennessy/SOPA Images/LightRocket via Getty Images

Florida Gov. Ron DeSantis on Thursday announced a lawsuit against the Biden administration's order requiring federal contractors to be vaccinated against the coronavirus no later than Dec. 8.

Why it matters: This is the Republican governor's latest attempt to undermine federal vaccine requirements, with the lawsuit alleging that such measures are a "radical intrusion on the personal autonomy" of U.S. workers.

Go deeper (1 min. read) →

Chelsea Cirruzzo
Oct 28, 2021 - Axios Washington D.C.

Montgomery Co. drops its mask mandate

Illustration: Brendan Lynch/Axios

Montgomery County, Maryland dropped its indoor mask mandate Thursday after community spread levels dropped to moderate transmission for 7 consecutive days, per the CDC's criteria.

Montgomery County is now one of the only counties in the DMV region to meet the CDC's definition of moderate transmission levels, an achievement that county officials on Wednesday linked to public health measures.

Driving the news: While the indoor mask mandate is lifted, businesses are still allowed to implement their own directives, County Executive Marc Elrich said Wednesday.

Go deeper (2 min. read) ⟶

Shawna Chen, Rebecca Falconer
Updated Oct 28, 2021 - Health

NYC firefighters union urges members to defy mayor's vaccine mandate

AXIOS

New York City Mayor Bill de Blasio. Photo: Michael M. Santiago/Getty Images

The president of New York City's firefighters' union told reporters Wednesday that he's advised unvaccinated members to ignore Mayor Bill de Blasio's COVID-19 vaccine mandate for city workers, per Reuters.

Why it matters: Under De Blasio's order that's due to take effect Friday, unvaccinated city employees would be placed on unpaid leave. But Uniformed Firefighters Association head Andrew Ansbro said he told members that "if they choose to remain unvaccinated, they must still report for duty," according to Reuters.

Go deeper (1 min. read) ⟶

AXIOS

EXHIBIT 4

HEALTH AND SCIENCE

CDC safety group says there's a likely link between rare heart inflammation in young people after Covid shot

PUBLISHED WED, JUN 23 2021•11:13 AM EDT UPDATED THU, OCT 28 2021•1:44 PM EDT

Berkeley Lovelace Jr.
@BERKELEYJR

KEY POINTS

There have been more than 1,200 reported cases of a myocarditis or pericarditis mostly in people 30 and under who received Pfizer's or Moderna's Covid-19 vaccine, according to CDC data.

Roughly 300 million of the shots had been administered as of June 11, the agency said.

For both vaccines combined, there were 12.6 heart inflammation cases per million doses.

VIDEO 01:49

CDC panel set to discuss post-vaccine heart issues and booster shots

A CDC safety group said there's a "likely association" between a rare heart inflammatory condition in adolescents and young adults mostly after they've received their second Covid-19 vaccine shot, citing the most recent data available.

There have been more than 1,200 reported cases of a myocarditis or pericarditis mostly in people 30 and under who received Pfizer's or Moderna's Covid vaccine, according to a series of slide presentations published Wednesday for a meeting of the Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices.

Myocarditis is the inflammation of the heart muscle, while pericarditis is the inflammation of the membrane surrounding the heart.

"Clinical presentation of myocarditis cases following vaccination has been distinct, occurring most often within one week after dose two, with chest pain as the most common presentation," said Dr. Grace Lee, who chairs the committee's safety group. CDC officials are gathering more data to fully understand the potential risks, how to manage it and whether there are any long-term issues, she said.

CDC

The agency said there have been 267 cases of myocarditis or pericarditis reported after receiving one dose of the mRNA vaccines and 827 reported cases after two doses through June 11. There are 132 additional cases where the number of doses received is unknown, the CDC said.

Roughly 300 million of the shots had been administered as of June 11, the agency said.

"This is still a rare event," Dr. Tom Shimabukuro said at the meeting. For both vaccines combined, there were 12.6 heart inflammation cases per million doses. The cases were more frequent among Moderna's vaccine recipients at 19.8 cases per million versus eight cases per million for Pfizer's, he said.

CNBC Health & Science

Read CNBC's latest global coverage of the Covid pandemic:

Florida sues Biden over contractor Covid vaccine mandate

Global Covid cases and deaths rise for the first time in two months, WHO says

Some 5% of unvaccinated adults quit their jobs over Covid vaccine mandates, survey shows

1.5 million Americans got a Moderna or J&J Covid booster shot in the first 5 days, White House says

Men under 30 make up the bulk of the cases, the CDC said, and most cases appear to be mild. Of the 295 people who have developed the condition and have been discharged, 79% of them have fully recovered, according to the presentation. Nine people were hospitalized, with two in intensive care as of June 11, according to the agency.

VIDEO 04:52

Dr. Anthony Fauci: Delta variant is the greatest threat to eliminating Covid-19

CDC officials said the benefits of getting the Covid vaccine still outweigh the risks.

Cases among younger people are on the rise as older people get vaccinated at higher rates. The U.S. has vaccinated 177.6 million people with at least one dose, roughly 53% of the population, according to the CDC. Just 13.6% of 18- to-24-year-olds have had at least one vaccine dose in the U.S., compared with 26% of people ages 50 to 64, the data shows.

While older age groups have seen hospitalization rates fall, they've barely budged among adolescents and young adults, said the CDC's Dr. Megan Wallace.

"Adolescents and young adults make up a greater proportion of total cases; 33% of cases reported in May were in persons aged 12 to 29 years, compared with 28% last December," she said. Since the beginning of the pandemic, 2,767 people ages 12 to 29 years old have died from Covid, she said, noting that 316 of those fatalities have happened since April 1.

After Wednesday's meeting, the Department of Health and Human Services released a statement co-signed by the CDC and several medical professional groups that stressed the heart condition is extremely rare.

"Only an exceedingly small number of people will experience it after vaccination," HHS

myocarditis and pericarditis are much more common if you get COVID-19, and the risks to the heart from COVID-19 infection can be more severe."

The CDC is coordinating its investigation with the Food and Drug Administration, which last month authorized the Pfizer-BioNTech vaccine for adolescents ages 12 to 15. Symptoms, which include chest pain and shortness of breath, typically develop within a week of receiving the shot with most developing within four days, the agency said.

-- CNBC's Rich Mendez contributed to this article.

Correction: The majority of cases of people who experienced myocarditis occurred in people 30 years old and under. An earlier version misstated the age. The number of cases per million doses administered was 12.6. An earlier version misstated the figure.

EXHIBIT 5

COVID-19 Vaccines and the Menstrual Cycle

Update: October 5, 2021

NICHD recently awarded five institutions one-year supplemental grants totaling $1.67 million to explore potential links between COVID-19 vaccination and menstrual changes. Researchers at Boston University, Harvard Medical School, Johns Hopkins University, Michigan State University, and Oregon Health and Science University will investigate whether such changes may be linked to the COVID-19 vaccine itself or if they are coincidental, the mechanism underlying any vaccine-related changes, and how long any changes last.

Several of these studies will use blood, tissue, and saliva samples collected before and after vaccination to analyze any immune or hormone changes. Other studies will use established resources — such as large cohort studies and menstrual cycle tracking apps — to collect and analyze data from racially, ethnically, and geographically diverse populations. Two studies will focus on specific populations, including adolescents and people with endometriosis.

People have reported menstrual cycle changes after COVID-19 vaccines, but more research is needed to understand if they are related, which women may be affected, and the exact mechanisms for why.

What you need to know

Increased stress, changes in weight and exercise, and other major lifestyle changes can affect menstrual cycles — and all of those changes are common during the COVID-19 pandemic. Additionally, studies have shown that some women who had COVID-19 experienced changes in the duration and flow of their menstrual cycles.

Some people have reported changes in their menstruation after receiving the COVID-19 vaccine, including changes in duration, flow, and accompanying symptoms such as pain.

What will researchers be doing?

To learn whether there is a connection between vaccination and changes in menstruation, the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) recently released a notice of special interest for researchers to compare the menstruation experiences of vaccinated and unvaccinated people. NICHD will support research focused on menstruation before and after vaccination and how vaccination as well as other factors, such as stress, might influence menstrual changes.

Why is this research important?

As more people are vaccinated for COVID-19, it is possible to gain better understanding of short- and long-term effects of the vaccines. Scientific evidence could also help unvaccinated people understand what, if any, menstruation-related side effects to expect from a COVID-19 vaccine.

Where can I go to learn more?

Notice of Special Interest (NOSI) to Encourage Administrative Supplement Applications to Investigate COVID-19 Vaccination and Menstruation

- NICHD calls on researchers to study the possible effects of the COVID-19 vaccine on menstruation.

Top

Menstruation and Menstrual Problems

- NICHD shares information about menstruation and menstrual cycle irregularities.

Sources

Li, K., Chen, G., Hou, H., Liao, Q., Chen, J., Bai, H., Lee, S., Wang, C., Li, H., Cheng, L., & Ai, J. (2021). Analysis of sex hormones and menstruation in COVID-19 women of child-bearing age. *Reproductive Biomedicine Online, 42*(1), 260–267. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7522626/

This article has been updated and edited for clarity.

News and Stories

Read stories about the efforts underway to prevent, detect, and treat COVID-19 and its effects on our health.

NIH COVID-19 Resources by Topic

COVID-19 research information and resources by topic from NIH institutes and centers

Select a Topic GO

Page last updated: October 5, 2021

For NIH Staff

NIH Strategic Response to COVID-19

Research Initiatives

Funding Information

Open Funding Opportunities

Researcher Resources

Testing

Treatments and Vaccines

Treatments

Vaccines

Clinical Trials

News and Stories

Subscribe for Updates

Email Address Submit

Follow NIH

Coronavirus.gov | CDC.gov/coronavirus

EXHIBIT 6

PHARMACEUTICALS - DIVERSIFIED

MARCH 25, 2010 / 5:07 PM / UPDATED 12 YEARS AGO

US jury's Neurontin ruling to cost Pfizer $141 mln

By Reuters Staff

* Pfizer ordered to pay $47 million in Neurontin case

* Penalty triples under RICO law

* Pfizer to appeal decision

NEW YORK, March 25 (Reuters) - Pfizer Inc PFE.N violated federal racketeering law by improperly promoting the epilepsy drug Neurontin, a Boston jury found on Thursday, and the world's largest drugmaker was ordered to pay $47 million in damages.

Under federal RICO law (Racketeer Influenced and Corrupt Organizations act) the penalty is automatically tripled, so the finding will cost Pfizer $141 million.

Pfizer said it would appeal the decision.

The jury agreed with the plaintiffs, Kaiser Foundation Hospitals and Kaiser Foundation Health Plan, that Pfizer had illegally promoted the drug for unapproved uses, such as for migraine headaches, pain and bipolar disorder, for which plaintiffs attorneys argued the drug does not work.

promote them for uses approved by the U.S. Food and Drug Administration.

Kaiser was seeking about $100 million in damages and was awarded just under half of that, Pfizer said.

"We are disappointed with the verdict and will pursue post-trial motions and an appeal," Pfizer spokesman Chris Loder said in a statement. "The verdict and the judge's rulings are not consistent with the facts and the law."

In 2004, Pfizer agreed to pay $430 million to federal and state governments and pleaded guilty to criminal charges of illegally marketing Neurontin, a drug the company obtained with its 2000 acquisition of Warner Lambert Corp.

Pfizer contends that the judge improperly allowed details of that case and settlement to be considered by the Boston jury.

The drugmaker also said Kaiser doctors continue to prescribe Neurontin for the so-called off-label uses despite Kaiser attorney contentions that the medicine does not work for those unapproved indications.

"Kaiser itself continues to recommend Neurontin for the same uses they sought recovery for in this case. Kaiser's own physicians and several of their expert witnesses prescribed Neurontin for their patients based on their sound medical judgment," Loder said. (Reporting by Bill Berkrot)

Our Standards: The Thomson Reuters Trust Principles.

The Federal Reserve says it will begin trimming monthly bond purchases in Novembe...

EXHIBIT 7

United States Senate
Committee on Finance

Sen. Chuck Grassley · Iowa
Ranking Member

http://finance.senate.gov
Press_Office@finance-rep.senate.gov

Statement of U.S. Senator Chuck Grassley of Iowa
The Adequacy of FDA Efforts to Assure the Safety of the Drug Supply
Subcommittee on Oversight and Investigations
House of Representatives Committee on Energy and Commerce
Tuesday, February 13, 2007

Chairman Dingell, Chairman Stupak, Ranking Members Barton and Whitfield and distinguished colleagues, thank you for holding this important hearing on drug safety and the Food and Drug Administration. Thank you also for inviting me to speak today on this important subject.

During the last three years, I conducted extensive oversight of the Food and Drug Administration while I was Chairman of the Senate Finance Committee, which is responsible for Medicare and Medicaid. I view my role as working to ensure the safety and well-being of the more than 80 million Americans who are beneficiaries of these programs. The Medicare and Medicaid programs spend a lot of money on prescription drugs and medical devices, and that money should be spent on drugs and devices that are safe and effective.

In the course of my oversight of the federal bureaucracy, I have developed many good relationships with whistleblowers. And it was FDA whistleblowers and concerned FDA scientists who first drew my attention to problems at the Food and Drug Administration.

It started in early 2004 with an FDA psychiatrist named Dr. Andrew Mosholder, who realized through his work that there was a serious suicide risk for teenagers taking certain antidepressants. He wanted to make a presentation about his findings to an FDA advisory committee. But for some reason, FDA supervisors didn't want this information to get out. They canceled Dr. Mosholder's presentation and instructed him to write a script approved by his supervisors that he would use if anybody asked him why he was no longer presenting.

That fall, I held a hearing on drug safety in the aftermath of Vioxx - the blockbuster pain medication - being pulled from the market by its manufacturer, rather than the Food and Drug Administration. The testimony at my hearing turned a bright spotlight on problems with the FDA's postmarket surveillance effort. The FDA works tirelessly, as it should, to approve new life-saving and life-enhancing drugs. But it could do a lot better job of keeping track of

developments with these drugs after they're on the market. Reviewing what happened inside the FDA with Vioxx, and in working with a number of whistleblowers who bravely stuck their necks out and came to me after that landmark hearing, I've identified problems at the FDA that consistently fit into a few themes.

First, scientific dissent is discouraged, quashed, and sometimes muzzled inside the Food and Drug Administration. Second, the FDA's relationship with drug makers is too cozy. The FDA worries about smoothing things over with industry much more than it should with its regulatory responsibilities. Third, inside the FDA there's widespread fear of retaliation for speaking up about problems. And fourth, the public safety would be better served if the agency was more transparent and forthcoming about drug safety and drug risks.

These problems involve the culture of the Food and Drug Administration. They're not isolated but systemic. And they can be partly attributed to the organizational structure of the FDA.

My concerns are not isolated either. During the last year, they've been validated by the highly regarded Institute of Medicine, as well as the independent Government Accountability Office and respected medical journals. What's at stake is public safety and public confidence in our nation's world-renowned Food and Drug Administration.

My investigations of FDA issues have also revealed a deeply troubling disregard for Congress' responsibility to conduct oversight of the executive branch of government. The FDA and the Department of Health and Human Services have put up so much resistance to my effort to find out what happened inside the FDA with a relatively new antibiotic called Ketek that I can only wonder what there is to cover up.

Every excuse under the sun has been used to create roadblocks, even in the face of Congressional subpoenas requesting information and access to FDA employees.

In denying access to documents responsive to the subpoenas, the Department and FDA have claimed "prosecutorial deliberative process," "confidential communications," and "agency prerogative to determine who will be interviewed or testify before a jurisdictional committee." Yet, during my years in the Senate, my investigators have obtained access to every single one of these categories of so-called confidential information from HHS as well as other executive branch agencies.

Furthermore, I asked the Congressional Research Service to look into the Department's policies regarding this matter and CRS told me that there is "no legal basis" for the Department's executive branch assertions.

Nevertheless, the Department and FDA not only withheld documents that do not appear to be privileged, but they also won't say what has been withheld and why. The subpoenas compel a privilege log, but the Department and FDA will not provide one.

The Department and FDA say that they have been responsive to the Finance Committee's Ketek investigation because they made available millions of pages of documents to the Committee. But what they provided is quantity, not quality.

They delivered hundreds of pages simply marked, for example, "57 pages removed," or "43 pages removed." (see attachments 1-5) Other documents have whole pages, paragraphs or sentences redacted with no explanation for what has been withheld or redacted and why. In fact, the FDA redacted some of the same documents differently and even redacted one of my own letters to them on a different matter (see attachment 6)

When I point out the absurdities in the Department's responses to my requests for documents and interviews related to Ketek, the Department argues it could not provide access to information and individuals related to open criminal investigations. But I didn't ask for access to open criminal investigations; I don't want to jeopardize a criminal matter. The Department and the FDA know that, yet they keep using that excuse anyway.

Even so, what I've learned about what happened with Ketek troubles me. I've learned that:

- FDA gave its advisory committee questionable data on Ketek and did not tell them about problems with that data. I sent a letter to the FDA in December regarding my findings on this matter and am awaiting a response from the agency.
- FDA approved Ketek without much safety data from the U.S.; the agency relied almost exclusively on foreign, post-marketing safety data; and
- Ketek's sponsor in all likelihood was aware of the fact that it submitted some questionable data to the FDA regarding its large safety study; the sponsor was informed of problems with one of the study sites prior to data submission to the FDA. However, according to FDA reviewers, the sponsor never raised these problems to the FDA. FDA learned about them after its own investigators inspected the site.

I plan to continue my investigation of Ketek and issue more reports. But I am heartened to hear that FDA came to a decision yesterday that mirrors the recommendations of its internal scientists as well as its advisory committees.

During the last three years, I've also tried to work in a productive way with the Commissioners and Acting Commissioners of the FDA. It will take bold leadership to get on top of the FDA's troubles and turn the agency around. So far, the lip service has been fine. The reality a lot less so.

Last month, Senator Chris Dodd and I reintroduced two reform bills that we first proposed in 2005 to get at the safety shortcomings of the FDA. Our first bill would elevate and empower the office with the FDA that is responsible for monitoring FDA-approved drugs after they're on the market. It would make the "postmarket drug safety" function independent within the FDA, instead of under the thumb of the office and center that puts the drugs on the market in the first place, the way it is today.

Chairman Dingell, the Wall Street Journal has reported that you're intrigued by the idea of a drug safety center within the FDA. I appreciate that view. It doesn't make any sense that the FDA officials who are supposed to monitor the safety of a drug on the market serve only as consultants to the FDA officials who approved the drug in the first place. The officials who approved the drug would obviously be conflicted in making a judgment that approval is no longer appropriate or was a mistake in the first place. A separate center for drug safety within the FDA is a vital lynchpin when it comes to meaningful reform and improvement of the agency's postmarket surveillance work.

The second bill that Senator Dodd and I introduced would expand an existing public database by mandating the registry of all clinical trials and the results of those trials. This reform is key to establishing greater transparency regarding clinical trials, the good ones and the bad ones, and to holding drug makers and drug regulators accountable.

Both of these legislative initiatives would make drug information used by doctors and patients more complete and more accessible. American consumers should not have to second guess the safety of the pills in their medicine cabinets.

I appreciate the attention all of you are giving to this important national issue with this hearing. You will hear from some of the heroic whistleblowers who have helped my work, without whom my work wouldn't have been possible. Two of the whistleblowers have left the FDA. It's a tremendous loss for our country when an agency like the Food and Drug Administration gets so dysfunctional that specialists like these whistleblowers are forced to leave the agency to avoid retaliation. I want to work closely with you to make sure FDA whistleblowers can communicate to Congress without fear.

In addition, the existing agreement between the Inspector General for the Department of Health and Human Services and the Food and Drug Administration gives too much power to the FDA when it comes to how allegations of criminal misconduct by FDA employees are investigated. That agreement should be revisited by reform minded leaders in Congress. (see attachment 7)

I look forward to reform opportunities in the year ahead. There's no doubt that the FDA needs additional tools and resources to do its work. The FDA also needs an overhaul to make the agency more transparent, more forthcoming, and more independent-minded.

I look forward to working with this Committee and in particular with you, Chairmen Dingell and Stupak and Ranking Members Barton and Whitfield, as well as my colleagues in the Senate to enact reforms at the FDA. Thank you.

EXHIBIT 8

EDMOND J. SAFRA
Center for Ethics

Donald Light

Light received a BA in history from Stanford, an MA in sociology from the University of Chicago, and a PhD in sociology from Brandeis. His research at the Center concerned the historical roots of institutional corruption in the development of prescription drugs and its consequences.

Websites

Related Content on this Site

Network Fellow

2013-2014

Lab Fellow

2012-2013

Fellows & Scholars by Year ›

EXHIBIT 9

EDMOND J. SAFRA Center for Ethics

Risky Drugs: Why The FDA Cannot Be Trusted

July 17, 2013

*by **Donald W. Light***

A **forthcoming article** for the special issue of the *Journal of Law, Medicine and Ethics* (JLME), edited by Marc Rodwin and supported by the Edmond J. Safra Center for Ethics, presents evidence that about 90 percent of all new drugs approved by the FDA over the past 30 years are little or no more effective *for patients* than existing drugs.

All of them may be better than indirect measures or placebos, but most are no better for patients than previous drugs approved as better against these measures. The few superior drugs make important contributions to the growing medicine chest of effective drugs.

The bar for "safe" is equally low, and over the past 30 years, approved drugs have caused an epidemic of harmful side effects, even when properly prescribed. Every week, about 53,000 excess hospitalizations and about 2400 excess deaths occur in the United States among people taking properly prescribed drugs to be healthier. One in every five drugs approved ends up causing serious harm,[1] while one in ten provide substantial benefit compared to existing, established drugs. This is the opposite of what people want or expect from the FDA. Prescription drugs are the 4th leading cause of death. Deaths and hospitalizations from over-dosing, errors, or recreational drug use would increase this total. American patients also suffer from about 80 million mild side effects a year, such as aches and pains, digestive discomforts, sleepiness or mild dizziness.

The forthcoming article in JLME also presents systematic, quantitative evidence that since the industry started making large contributions to the FDA for reviewing its drugs, as it makes large contributions to Congressmen who have promoted this substitution for publicly funded regulation, the FDA has sped up the review process with the result that drugs approved are significantly more likely to cause serious harm, hospitalizations, and deaths. New FDA policies are likely to increase the epidemic of harms. This will increase costs for insurers but increase revenues for providers.

This evidence indicates why we can no longer trust the FDA to carry out its historic mission to protect the public from harmful and ineffective drugs. Strong public demand that government "do something" about periodic drug disasters has played a central role in developing the FDA.[2] Yet close, constant contact by companies with FDA staff and officials has contributed to vague, minimal criteria of what "safe" and "effective" mean. The FDA routinely approves scores of new minor variations each year, with minimal evidence about

risks of harm. Then very effective mass marketing takes over, and the FDA devotes only a small percent of its budget to protect physicians or patients from receiving biased or untruthful information.[3][4] The further corruption of medical knowledge through company-funded teams that craft the published literature to overstate benefits and understate harms, unmonitored by the FDA, leaves good physicians with corrupted knowledge.[5] [6] Patients are the innocent victims.

Although it now embraces the industry rhetoric about “breakthrough” and “life-saving” innovation, the FDA in effect serves as the re-generator of patent-protected high prices for minor drugs in each disease group, as their therapeutic equivalents lose patent protection. The billions spent on promoting them results in the **Inverse Benefit Law**: the more widely most drugs are marketed, the more diluted become their benefits but more widespread become their risks of harm.

The FDA also legitimates industry efforts to lower and widen criteria prescribing drugs, known by critics as “**the selling of sickness.**” Regulations conveniently prohibit the FDA from comparing the effectiveness of new drugs or from assessing their cost-effectiveness. Only the United States allows companies to charge what they like and raise prices annually on last year’s drugs, without regard to their added value.[7]

A New Era?

Now the FDA is going even further. The New England Journal of Medicine has published, without comment, proposals by two senior figures from the FDA to loosen criteria drugs that allege to prevent Alzheimer’s disease by treating it at an early stage.[8] The authors seem unaware of how their views about Alzheimer’s and the role of the FDA incorporate the language and rationale of marketing executives for the industry. First, they use the word “disease” to refer to a hypothetical “early-stage Alzheimer’s disease” that supposedly exists “before the earliest symptoms of Alzheimer’s disease are apparent.” Notice that phrasing assumes that the earliest symptoms will become apparent, when in fact it’s only a hypothetical model for claiming that cognitive lapses like not remembering where you put something or what you were going to say are signs of incipient Altzheimer’s disease. The proposed looser criteria would legitimate drugs as “safe and effective” that have little or no evidence of being effective and expose millions to risks of harmful side effects.

No proven biomarkers or clinical symptoms exist, the FDA officials note, but nevertheless they advocate accelerated approval to allow “drugs that address an unmet medical need.” What “unmet need"? None exists. This market-making language by officials who are charged with protecting the public from unsafe drugs moves us towards the 19-century hucksterism of peddling cures of questionable benefits and hidden risks of harm, only now fully certified by the modern FDA.[9]

The main reason for advocating approvals of drugs for an unproven need with unproven benefits, these FDA officials explain, is that companies cannot find effective drugs for overt Alzheimer’s. Their drug-candidates have failed again and again in trials. The core rationale of the proposed loosening of criteria is that “the focus of drug development has sifted to earlier stages of Alzheimer’s disease…and the regulatory framework under which such therapies are evaluated should evolve accordingly.” Yet they admit there are no “therapies” in this much larger market where (with the help of the industry-funded FDA) companies will not have to

prove their drugs are effective. In fact, these FDA officers propose to approve the drugs without ever knowing if they are therapeutic or not. Their commercialized language presumes the outcome before starting. The job of the FDA, it seems, is to help drug companies open up new markets to increase profits for the FDA's corporate paymasters.

These two FDA officials maintain that "the range of focus must extend to healthy people who are merely at risk for the disease but could benefit from preventive therapies." Yet they admit we do not know who is "at risk," nor whether there is a "disease," nor whether anyone "could benefit," nor whether the drugs constitute "preventive therapies." Similar FDA-encouraged shifts have been made for drugs treating pre-diabetes, pre-psychosis, and pre-bone density loss, with few or no benefits to offset risks of harm. This week, based on policy research at the Edmond J. Safra Center for Ethics, **a letter of concern** was published in the New England Journal of Medicine. The authors write that approval for drugs to treat "early stage Altzheimer's disease" must meet "a much higher bar – evidence of slowed disease progression." But without clinical manifestations or biomarkers for an alleged disease, how will such progression be measured?

Advice to readers: Experienced, independent physicians recommend not to take a new drug approved by the FDA until it is out for 7 years, unless you have to, so that evidence can accumulate about its real harms and benefits.[10]

Disclaimer: The assessment and views expressed here are solely the author's and do not necessarily reflect those of persons or institutions to which he is associated. The comments and suggestions of Gordon Schiff, an expert in prescribing at Brigham and Women's Hospital, and Robert Whitaker are gratefully acknowledged.

References

1. Lexchin J. New drugs and safety: what happened to new active substances approved in Canada between 1995 and 2010? Archives of Internal Medicine 2012 (Nov 26);172:1680-81.

2. Hilts PJ. Protecting America's Health: The FDA, Business and One Hundred Years of Regulation. New York: Alfred A. Knopf; 2003.

3. Rodwin M. Conflicts of interest, institutional corruption, and Pharma: an agenda for reform. Journal of Law, Medicine & Ethics 2012;40:511-22.

4. Rodwin M. Reforming pharmaceutical industry-physician financial relationships: lessons from the United States, France, and Japan. Journal of Law, Medicine & Ethics 2011(Winter):2-10.

5. Sismondo S. Ghost management. PLoS Medicine 2007;4:1429-33.

6. Sismondo S, Doucet M. Publication ethics and the ghost management of medical publication. Bioethics 2010;24:273-83.

7. Schondelmeyer S, Purvis L. Rx Price Watch Report. Washington DC: American Association of Retired Persons 2012.

8. Kozauer N, Katz R. Regulatory innovation and drug development for early-stage Alzheimer's disease. New England Journal of Medicine 2013 (Mar 13);DOI: 10.1056/NEJMp1302513

9. Young JH. The Toadstool Millionaires: a social history of patent medicines in America before federal regulation. Princeton, NJ: Princeton University Press; 1961.

10. Schiff G, Galanter W, Duhig J, et al. Principles of conservative prescribing. Archives of Internal Medicine 2011;171:1433-30.

See also: **Public Health**, **Donald Light**

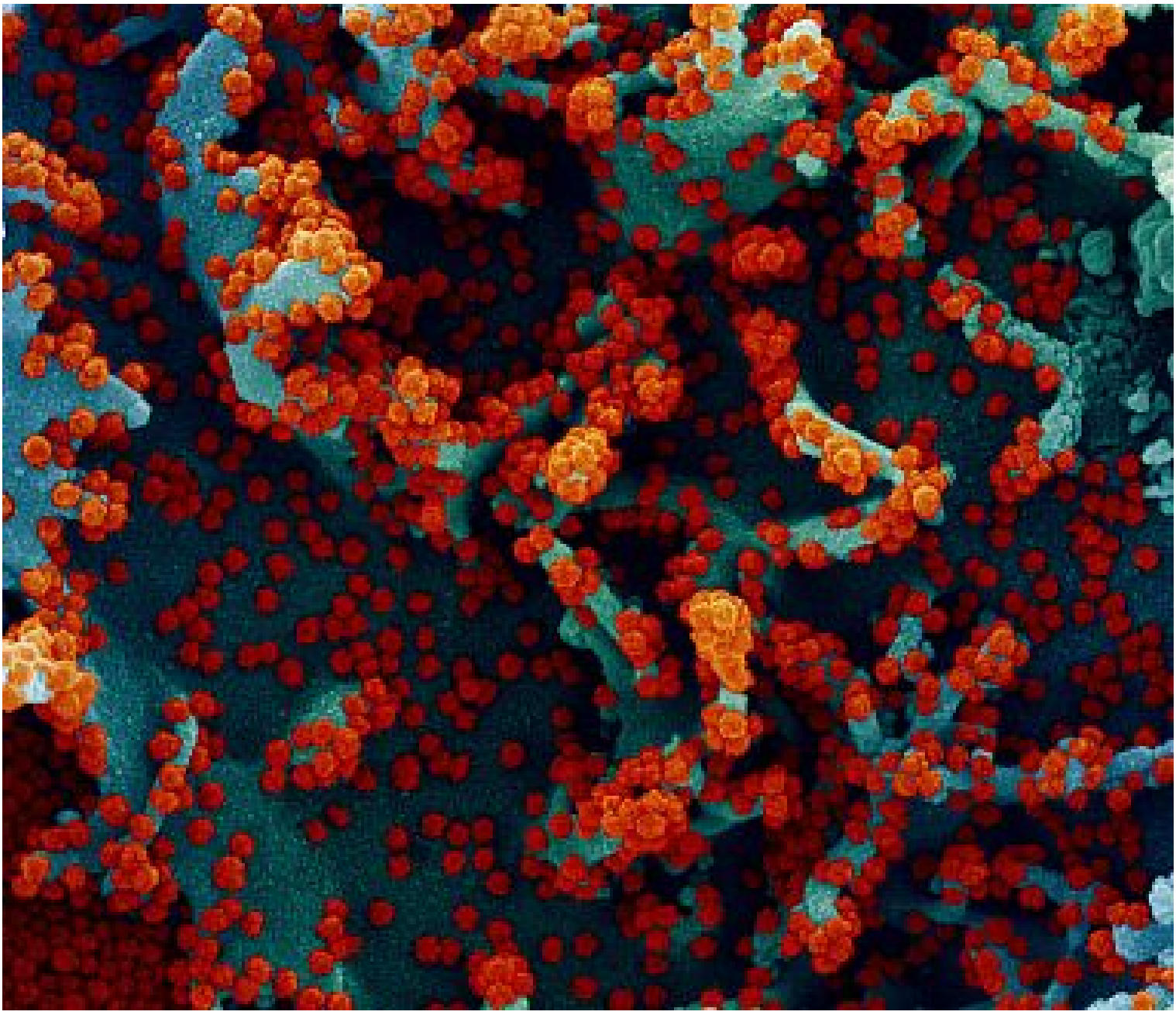
EXHIBIT 10

January 26, 2021

Lasting immunity found after recovery from COVID-19

At a Glance

- The immune systems of more than 95% of people who recovered from COVID-19 had durable memories of the virus up to eight months after infection.
- The results provide hope that people receiving SARS-CoV-2 vaccines will develop similar lasting immune memories after vaccination.



Colorized scanning electron micrograph of a cell, isolated from a patient sample, that is heavily infected with SARS-CoV-2 virus particles (red). *NIAID Integrated Research Facility, Fort Detrick, Maryland*

After people recover from infection with a virus, the immune system retains a memory of it. Immune cells and proteins that circulate in the body can recognize and kill the pathogen if it's encountered again, protecting against disease and reducing illness severity.

This long-term immune protection involves several components. Antibodies—proteins that circulate in the blood—recognize foreign substances like viruses and neutralize them. Different types of T cells help recognize and kill pathogens. B cells make new antibodies when the body needs them.

All of these immune-system components have been found in people who recover from SARS-CoV-2, the virus that causes COVID-19. But the details of this immune response and how long it lasts after infection have been unclear. Scattered reports of reinfection with SARS-CoV-2 have raised concerns that the immune response to the virus might not be durable.

To better understand immune memory of SARS-CoV-2, researchers led by Drs. Daniela Weiskopf, Alessandro Sette, and Shane Crotty from the La Jolla Institute for Immunology analyzed immune cells and antibodies from almost 200 people who had been exposed to SARS-CoV-2

and recovered.

Time since infection ranged from six days after symptom onset to eight months later. More than 40 participants had been recovered for more than six months before the study began. About 50 people provided blood samples at more than one time after infection.

The research was funded in part by NIH's National Institute of Allergy and Infectious Diseases (NIAID) and National Cancer Institute (NCI). Results were published on January 6, 2021, in *Science*.

The researchers found durable immune responses in the majority of people studied. Antibodies against the spike protein of SARS-CoV-2, which the virus uses to get inside cells, were found in 98% of participants one month after symptom onset. As seen in previous studies, the number of antibodies ranged widely between individuals. But, promisingly, their levels remained fairly stable over time, declining only modestly at 6 to 8 months after infection.

Virus-specific B cells increased over time. People had more memory B cells six months after symptom onset than at one month afterwards. Although the number of these cells appeared to reach a plateau after a few months, levels didn't decline over the period studied.

Levels of T cells for the virus also remained high after infection. Six months after symptom onset, 92% of participants had CD4+ T cells that recognized the virus. These cells help coordinate the immune response. About half the participants had CD8+ T cells, which kill cells that are infected by the virus.

As with antibodies, the numbers of different immune cell types varied substantially between individuals. Neither gender nor differences in disease severity could account for this variability. However, 95% of the people had at least 3 out of 5 immune-system components that could recognize SARS-CoV-2 up to 8 months after infection.

"Several months ago, our studies showed that natural infection induced a strong response, and this study now shows that the responses last," Weiskopf says. "We are hopeful that a similar pattern of responses lasting over time will also emerge for the vaccine-induced responses."

—by Sharon Reynolds

Related Links

- Experimental Coronavirus Vaccine Highly Effective (https://www.nih.gov/news-events/nih-research-matters/experimental-coronavirus-vaccine-highly-effective)
- Antibodies and T Cells Protect Against SARS-CoV-2 (https://www.nih.gov/news-events/nih-research-matters/antibodies-t-cells-protect-against-sars-cov-2)
- Immune Cells for Common Cold May Recognize SARS-CoV-2 (https://www.nih.gov/news-events/nih-research-matters/immune-cells-common-cold-may-recognize-sars-cov-2)
- Potent Neutralizing Antibodies Target New Regions of Coronavirus Spike (https://www.nih.gov/news-events/nih-research-matters/potent-neutralizing-antibodies-target-new-regions-coronavirus-spike)
- Potent Antibodies Found in People Recovered from COVID-19 (https://www.nih.gov/news-events/nih-research-matters/potent-antibodies-found-people-recovered-covid-19)
- Novel Coronavirus Structure Reveals Targets for Vaccines and Treatments (https://www.nih.gov/news-events/nih-research-matters/novel-coronavirus-structure-reveals-targets-vaccines-treatments)
- Coronavirus (COVID-19) (https://covid19.nih.gov/)
- Coronavirus Prevention Network (https://www.coronaviruspreventionnetwork.org/)

- Coronavirus (COVID-19) (https://www.coronavirus.gov/)

References: Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. Dan JM, Mateus J, Kato Y, Hastie KM, Yu ED, Faliti CE, Grifoni A, Ramirez SI, Haupt S, Frazier A, Nakao C, Rayaprolu V, Rawlings SA, Peters B, Krammer F, Simon V, Saphire EO, Smith DM, Weiskopf D, Sette A, Crotty S. *Science*. 2021 Jan 6:eabf4063. doi: 10.1126/science.abf4063. Online ahead of print. PMID: 33408181.

Funding: NIH's National Institute of Allergy and Infectious Diseases (NIAID) and National Cancer Institute (NCI); La Jolla Institute for Immunology; John and Mary Tu Foundation; Bill and Melinda Gates Foundation; Mastercard; Wellcome; Emergent Ventures; Collaborative Influenza Vaccine Innovation Centers; JPB Foundation; Cohen Foundation; Open Philanthropy Project.

In this Edition

Search NIH Research Matters

Connect with Us

Subscribe to get NIH Research Matters by email

RSS Feed

Facebook

Email us

Mailing Address:
NIH Research Matters
Bldg. 31, Rm. 5B52, MSC 2094
Bethesda, MD 20892-2094

Popular Stories

Moderna COVID-19 vaccine generates long-lasting immune memory

Autoimmune response found in many with COVID-19

Most COVID-19 hospitalizations due to four conditions

Misdirected antibodies linked to severe COVID-19

Understanding SARS-CoV-2 antibody binding

About NIH Research Matters

Editor: Harrison Wein, Ph.D. **Assistant Editor:** Erin Bryant

NIH Research Matters is a weekly update of NIH research highlights reviewed by NIH's experts. It's published by the Office of Communications and Public Liaison in the NIH Office of the Director.

ISSN 2375-9593

NIH...Turning Discovery Into Health®

National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20892

U.S. Department of Health and Human Services

EXHIBIT 11

Article

SARS-CoV-2 infection induces long-lived bone marrow plasma cells in humans

https://doi.org/10.1038/s41586-021-03647-4

Received: 20 December 2020

Accepted: 14 May 2021

Published online: 24 May 2021

Check for updates

Jackson S. Turner[1], Wooseob Kim[1], Elizaveta Kalaidina[2], Charles W. Goss[3], Adriana M. Rauseo[4], Aaron J. Schmitz[1], Lena Hansen[1,5], Alem Haile[6], Michael K. Klebert[6], Iskra Pusic[7], Jane A. O'Halloran[4], Rachel M. Presti[4,8] & Ali H. Ellebedy[1,8,9] ✉

Long-lived bone marrow plasma cells (BMPCs) are a persistent and essential source of protective antibodies[1–7]. Individuals who have recovered from COVID-19 have a substantially lower risk of reinfection with SARS-CoV-2[8–10]. Nonetheless, it has been reported that levels of anti-SARS-CoV-2 serum antibodies decrease rapidly in the first few months after infection, raising concerns that long-lived BMPCs may not be generated and humoral immunity against SARS-CoV-2 may be short-lived[11–13]. Here we show that in convalescent individuals who had experienced mild SARS-CoV-2 infections ($n = 77$), levels of serum anti-SARS-CoV-2 spike protein (S) antibodies declined rapidly in the first 4 months after infection and then more gradually over the following 7 months, remaining detectable at least 11 months after infection. Anti-S antibody titres correlated with the frequency of S-specific plasma cells in bone marrow aspirates from 18 individuals who had recovered from COVID-19 at 7 to 8 months after infection. S-specific BMPCs were not detected in aspirates from 11 healthy individuals with no history of SARS-CoV-2 infection. We show that S-binding BMPCs are quiescent, which suggests that they are part of a stable compartment. Consistently, circulating resting memory B cells directed against SARS-CoV-2 S were detected in the convalescent individuals. Overall, our results indicate that mild infection with SARS-CoV-2 induces robust antigen-specific, long-lived humoral immune memory in humans.

Reinfections by seasonal coronaviruses occur 6 to 12 months after the previous infection, indicating that protective immunity against these viruses may be short-lived[14,15]. Early reports documenting rapidly declining antibody titres in the first few months after infection in individuals who had recovered from COVID-19 suggested that protective immunity against SARS-CoV-2 might be similarly transient[11–13]. It was also suggested that infection with SARS-CoV-2 could fail to elicit a functional germinal centre response, which would interfere with the generation of long-lived plasma cells[3–5,7,16]. More recent reports analysing samples that were collected approximately 4 to 6 months after infection indicate that SARS-CoV-2 antibody titres decline more slowly than in the initial months after infection[8,17–21]. Durable serum antibody titres are maintained by long-lived plasma cells—non-replicating, antigen-specific plasma cells that are detected in the bone marrow long after the clearance of the antigen[1–7]. We sought to determine whether they were detectable in convalescent individuals approximately 7 months after SARS-CoV-2 infection.

Biphasic decay of anti-S antibody titres

Blood samples were collected approximately 1 month after the onset of symptoms from 77 individuals who were convalescing from COVID-19 (49% female, 51% male, median age 49 years), the majority of whom had experienced mild illness (7.8% hospitalized, Extended Data Tables 1, 2). Follow-up blood samples were collected three times at approximately three-month intervals. Twelve convalescent participants received either the BNT162b2 (Pfizer) or the mRNA-1273 (Moderna) SARS-CoV-2 vaccine between the last two time points; these post-vaccination samples were not included in our analyses. In addition, bone marrow aspirates were collected from 18 of the convalescent individuals at 7 to 8 months after infection and from 11 healthy volunteers with no history of SARS-CoV-2 infection or vaccination. Follow-up bone marrow aspirates were collected from 5 of the 18 convalescent individuals and from 1 additional convalescent donor approximately 11 months after infection (Fig. 1a, Extended Data Tables 3, 4). We first performed a longitudinal analysis of circulating anti-SARS-CoV-2 serum antibodies. Whereas anti-SARS-CoV-2 spike protein (S) IgG antibodies were undetectable in blood from control individuals, 74 out of the 77 convalescent individuals had detectable serum titres approximately 1 month after the onset of symptoms. Between 1 and 4 months after symptom onset, overall anti-S IgG titres decreased from a mean $\log_e$-transformed half-maximal dilution of 6.3 to 5.7 (mean difference 0.59 ± 0.06, $P < 0.001$). However, in the interval between 4 and 11 months after symptom onset, the rate

[1]Department of Pathology and Immunology, Washington University School of Medicine, St Louis, MO, USA. [2]Division of Allergy and Immunology, Department of Internal Medicine, Washington University School of Medicine, St Louis, MO, USA. [3]Division of Biostatistics, Washington University School of Medicine, St Louis, MO, USA. [4]Division of Infectious Diseases, Department of Internal Medicine, Washington University School of Medicine, St Louis, MO, USA. [5]Influenza Centre, Department of Clinical Science, University of Bergen, Bergen, Norway. [6]Clinical Trials Unit, Washington University School of Medicine, St Louis, MO, USA. [7]Division of Oncology, Department of Internal Medicine, Washington University School of Medicine, St Louis, MO, USA. [8]Center for Vaccines and Immunity to Microbial Pathogens, Washington University School of Medicine, St Louis, MO, USA. [9]The Andrew M. and Jane M. Bursky Center for Human Immunology & Immunotherapy Programs, Washington University School of Medicine, St Louis, MO, USA. ✉e-mail: ellebedy@wustl.edu

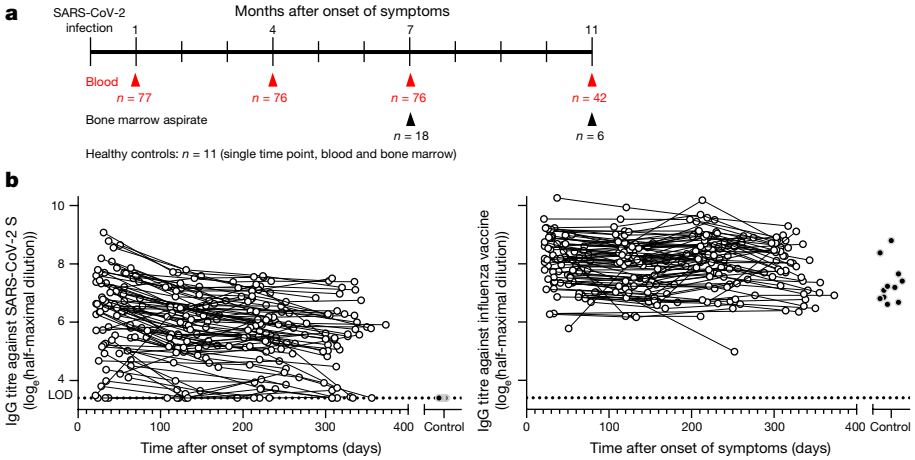


Fig. 1 | SARS-CoV-2 infection elicits durable serum anti-S antibody titres. **a**, Study design. Seventy-seven convalescent individuals who had experienced mild SARS-CoV-2 infections (aged 21–69 years) were enrolled and blood was collected approximately 1 month, 4 months, 7 months and 11 months after the onset of symptoms. Bone marrow aspirates were collected from 18 of the convalescent individuals 7 to 8 months after infection and from 11 healthy volunteers (aged 23–60 years) with no history of SARS-CoV-2 infection. Follow-up bone marrow aspirates were collected from 5 of the 18 convalescent donors and 1 additional convalescent donor approximately 11 months after infection. **b**, Blood IgG titres against SARS-CoV-2 S (left) and influenza virus vaccine (right) measured by enzyme-linked immunosorbent assay (ELISA) in convalescent individuals (white circles) at the indicated time after onset of symptoms, and in control individuals (black circles). The dotted lines indicate the limit of detection (LOD). Mean titres and pairwise differences at each time point were estimated using a linear mixed model analysis.

of decline slowed, and mean titres decreased from 5.7 to 5.3 (mean difference 0.44 ± 0.10, $P < 0.001$; Fig. 1a). In contrast to the anti-S antibody titres, IgG titres against the 2019–2020 inactivated seasonal influenza virus vaccine were detected in all control individuals and individuals who were convalescing from COVID-19, and declined much more gradually, if at all over the course of the study, with mean titres decreasing from 8.0 to 7.9 (mean difference 0.16 ± 0.06, $P = 0.042$) and 7.9 to 7.8 (mean difference 0.02 ± 0.08, $P = 0.997$) across the 1-to-4-month and 4-to-11-month intervals after symptom onset, respectively (Fig. 1b).

Induction of S-binding long-lived BMPCs

The relatively rapid early decline in the levels of anti-S IgG, followed by a slower decrease, is consistent with a transition from serum antibodies being secreted by short-lived plasmablasts to secretion by a smaller but more persistent population of long-lived plasma cells generated later in the immune response. The majority of this latter population resides in the bone marrow[1–6]. To investigate whether individuals who had recovered from COVID-19 developed a virus-specific long-lived BMPC compartment, we examined bone marrow aspirates obtained approximately 7 and 11 months after infection for anti-SARS-CoV-2 S-specific BMPCs. We magnetically enriched BMPCs from the aspirates and then quantified the frequencies of those secreting IgG and IgA directed against the 2019–2020 influenza virus vaccine, the tetanus–diphtheria vaccine and SARS-CoV-2 S by enzyme-linked immunosorbent spot assay (ELISpot) (Fig. 2a). Frequencies of influenza- and tetanus–diphtheria-vaccine-specific BMPCs were comparable between control individuals and convalescent individuals. IgG- and IgA-secreting S-specific BMPCs were detected in 15 and 9 of the 19 convalescent individuals, respectively, but not in any of the 11 control individuals (Fig. 2b). Notably, none of the control individuals or convalescent individuals had detectable S-specific antibody-secreting cells in the blood at the time of bone marrow sampling, indicating that the detected BMPCs represent bone-marrow-resident cells and not contamination from circulating plasmablasts. Frequencies of anti-S IgG BMPCs were stable among the 5 convalescent individuals who were sampled a second time approximately 4 months later, and frequencies of anti-S IgA BMPCs were stable in 4 of these 5 individuals but had decreased to below the limit of detection in one individual (Fig. 2c). Consistent with their stable BMPC frequencies, anti-S IgG titres in the 5 convalescent individuals remained consistent between 7 and 11 months after symptom onset. IgG titres measured against the receptor-binding domain (RBD) of the S protein—a primary target of neutralizing antibodies—were detected in 4 of the 5 convalescent individuals and were also stable between 7 and 11 months after symptom onset (Fig. 2d). Frequencies of anti-S IgG BMPCs showed a modest but significant correlation with circulating anti-S IgG titres at 7–8 months after the onset of symptoms in convalescent individuals, consistent with the long-term maintenance of antibody levels by these cells ($r = 0.48$, $P = 0.046$). In accordance with previous reports[22–24], frequencies of influenza-vaccine-specific IgG BMPCs and antibody titres exhibited a strong and significant correlation ($r = 0.67$, $P < 0.001$; Fig. 2e). Nine of the aspirates from control individuals and 12 of the 18 aspirates that were collected 7 months after symptom onset from convalescent individuals yielded a sufficient number of BMPCs for additional analysis by flow cytometry. We stained these samples intracellularly with fluorescently labelled S and influenza virus haemagglutinin (HA) probes to identify and characterize antigen-specific BMPCs. As controls, we also intracellularly stained peripheral blood mononuclear cells (PBMCs) from healthy volunteers one week after vaccination against SARS-CoV-2 or seasonal influenza virus (Fig. 3a, Extended Data Fig. 1a–c). Consistent with the ELISpot data, low frequencies of S-binding BMPCs were detected in 10 of the 12 samples from convalescent individuals, but not in any of the 9 control samples (Fig. 3b). Although both recently generated circulating plasmablasts and S- and HA-binding BMPCs expressed BLIMP-1, the BMPCs were differentiated by their lack of expression of Ki-67—indicating a quiescent state—as well as by higher levels of CD38 (Fig. 3c).

Robust S-binding memory B cell response

Memory B cells form the second arm of humoral immune memory. After re-exposure to an antigen, memory B cells rapidly expand and differentiate into antibody-secreting plasmablasts. We examined the frequency of SARS-CoV-2-specific circulating memory B cells in individuals who were convalescing from COVID-19 and in healthy control individuals. We stained PBMCs with fluorescently labelled S probes and determined the frequency of S-binding memory B cells among isotype-switched $IgD^{lo}CD20^{+}$ memory B cells by flow cytometry. For comparison, we

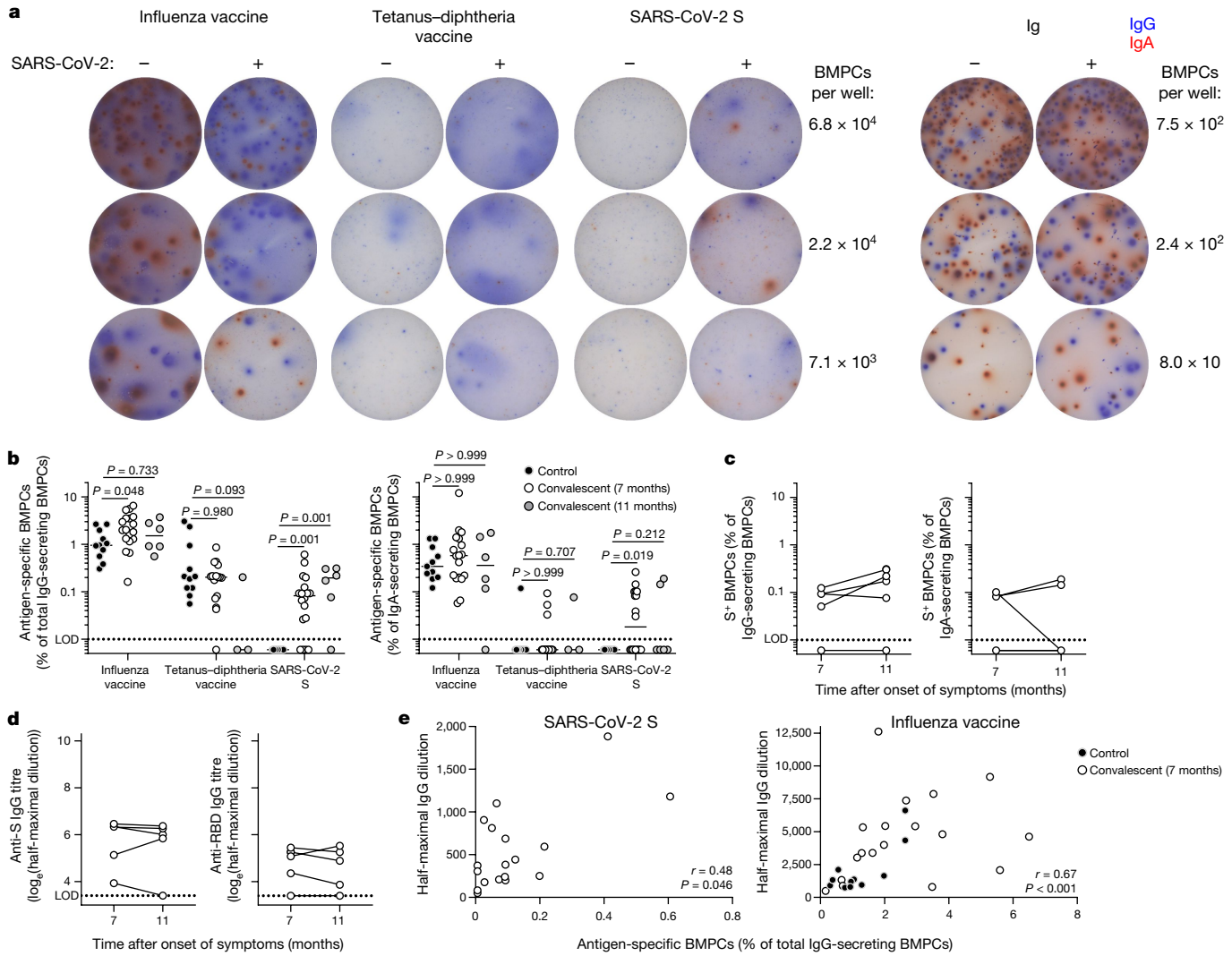


Fig. 2 | SARS-CoV-2 infection elicits S-binding long-lived BMPCs. **a**, Representative images of ELISpot wells coated with the indicated antigens or anti-immunoglobulin (Ig) and developed in blue and red for IgG and IgA, respectively, after incubation of magnetically enriched BMPCs from control individuals and convalescent individuals. **b**, Frequencies of BMPCs secreting IgG (left) or IgA (right) antibodies specific for the indicated antigens, indicated as percentages of total IgG- or IgA-secreting BMPCs in control individuals (black circles) or convalescent individuals 7 months (white circles) or 11 months (grey circles) after symptom onset. Horizontal lines indicate the median. *P* values from two-sided Kruskal–Wallis tests with Dunn's correction for multiple comparisons between control individuals and convalescent individuals. Each symbol represents one sample ($n = 18$ convalescent, $n = 11$ control). **c**, Paired frequencies of S-binding BMPCs among IgG-secreting (left) and IgA-secreting (right) BMPCs from convalescent individuals 7 months and 11 months after symptom onset. **d**, Paired anti-S (left) and anti-RBD (right) IgG serum antibody titres from convalescent individuals 7 months and 11 months after symptom onset. Data in **c** and **d** (left) are also shown in **b** and Fig. 1b, respectively. Each symbol represents one sample ($n = 5$). Dotted lines indicate the limit of detection. **e**, Frequencies of BMPCs secreting IgG antibodies specific for SARS-CoV-2 S (left) and influenza virus vaccine (right) plotted against respective IgG titres in paired blood samples from control individuals (black circles) or convalescent individuals 7 months after symptom onset (white circles). *P* and *r* values from two-sided Spearman's correlations. Each symbol represents one sample ($n = 18$ convalescent, $n = 11$ control).

co-stained the cells with fluorescently labelled influenza virus HA probes (Fig. 4a, Extended Data Fig. 1d). S-binding memory B cells were identified in convalescent individuals in the first sample that was collected approximately one month after the onset of symptoms, with comparable frequencies to influenza HA-binding memory B cells (Fig. 4b). S-binding memory B cells were maintained for at least 7 months after symptom onset and were present at significantly higher frequencies relative to healthy controls—comparable to the frequencies of influenza HA-binding memory B cells that were identified in both groups (Fig. 4c).

Discussion

This study sought to determine whether infection with SARS-CoV-2 induces antigen-specific long-lived BMPCs in humans. We detected SARS-CoV-2 S-specific BMPCs in bone marrow aspirates from 15 out of 19 convalescent individuals, and in none from the 11 control participants. The frequencies of anti-S IgG BMPCs modestly correlated with serum IgG titres at 7–8 months after infection. Phenotypic analysis by flow cytometry showed that S-binding BMPCs were quiescent, and their frequencies were largely consistent in 5 paired aspirates collected at 7 and 11 months after symptom onset. Notably, we detected no S-binding cells among plasmablasts in blood samples collected at the same time as the bone marrow aspirates by ELISpot or flow cytometry in any of the convalescent or control samples. Together, these data indicate that mild SARS-CoV-2 infection induces a long-lived BMPC response. In addition, we showed that S-binding memory B cells in the blood of individuals who had recovered from COVID-19 were present at similar frequencies to those directed against influenza virus HA. Overall, our

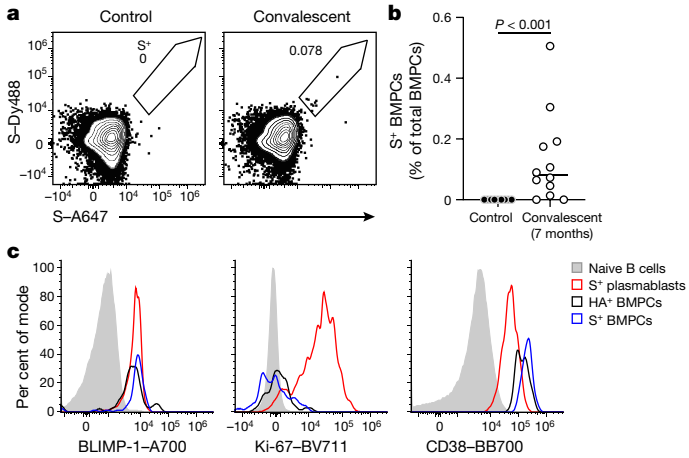


Fig. 3 | SARS-CoV-2 S-binding BMPCs are quiescent and distinct from circulating plasmablasts. a, Representative plots of intracellular S staining in $CD20^{lo}CD38^{+}IgD^{lo}CD19^{+/lo}CD3^{-}$ live singlet BMPCs (gating in Extended Data Fig. 1a) from magnetically enriched BMPCs from control individuals (left) or convalescent individuals 7 months after symptom onset (right). **b**, Frequencies of S-binding BMPCs in total BMPCs from control individuals (black circles) or convalescent individuals 7 months after symptom onset (white circles). Horizontal lines indicate the median. *P* value from two-sided Mann–Whitney *U* test. Each symbol represents one sample ($n = 12$ convalescent, $n = 9$ control). **c**, Histograms of BLIMP-1 (left), Ki-67 (centre), and CD38 (right) staining in S^{+} (blue) and HA^{+} (black) BMPCs from magnetically enriched BMPCs 7 months after symptom onset, and in S^{+} plasmablasts (red) and naive B cells (grey) from healthy donor PBMCs 1 week after SARS-CoV-2 S immunization.

results are consistent with SARS-CoV-2 infection eliciting a canonical T-cell-dependent B cell response, in which an early transient burst of extrafollicular plasmablasts generates a wave of serum antibodies that decline relatively quickly. This is followed by more stably maintained levels of serum antibodies that are supported by long-lived BMPCs.

Although this overall trend captures the serum antibody dynamics of the majority of participants, we observed that in three participants, anti-S serum antibody titres increased between 4 and 7 months after the onset of symptoms, after having initially declined between 1 and 4 months. This could be stochastic noise, could represent increased net binding affinity as early plasmablast-derived antibodies are replaced by those from affinity-matured BMPCs, or could represent increases in antibody concentration from re-encounter with the virus (although none of the participants in our cohort tested positive a second time). Although anti-S IgG titres in the convalescent cohort were relatively stable in the interval between 4 and 11 months after symptom onset, they did measurably decrease, in contrast to anti-influenza virus vaccine titres. It is possible that this decline reflects a final waning of early plasmablast-derived antibodies. It is also possible that the lack of decline in influenza titres was due to boosting through exposure to influenza antigens. Our data suggest that SARS-CoV-2 infection induces a germinal centre response in humans because long-lived BMPCs are thought to be predominantly germinal-centre-derived[7]. This is consistent with a recent study that reported increased levels of somatic hypermutation in memory B cells that target the RBD of SARS-CoV-2 S in convalescent individuals at 6 months compared to 1 month after infection[20].

To our knowledge, the current study provides the first direct evidence for the induction of antigen-specific BMPCs after a viral infection in humans. However, we do acknowledge several limitations. Although we detected anti-S IgG antibodies in serum at least 7 months after infection in all 19 of the convalescent donors from whom we obtained bone marrow aspirates, we failed to detect S-specific BMPCs in 4 donors. Serum anti-S antibody titres in those four donors were low, suggesting that S-specific BMPCs may potentially be present at very low frequencies that are below the limit of detection of the assay. Another limitation is that we do not know the fraction of the S-binding BMPCs detected in our study that encodes neutralizing antibodies. SARS-CoV-2 S protein is the main target of neutralizing antibodies[17,25–30] and a correlation between serum anti-S IgG binding and neutralization titres has been documented[17,31]. Further studies will be required to determine the

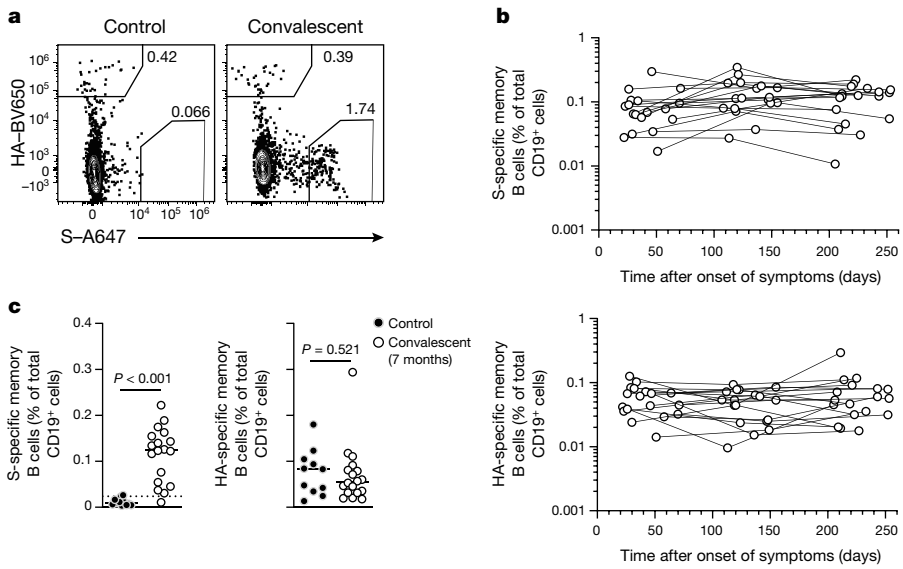


Fig. 4 | SARS-CoV-2 infection elicits a robust memory B cell response. a, Representative plots of surface influenza virus HA and S staining in $CD20^{+}CD38^{lo/int}IgD^{lo}CD19^{+}CD3^{-}$ live singlet memory B cells (gating in Extended Data Fig. 1d) from PBMCs from control individuals (left) and convalescent individuals 7 months after symptom onset (right). **b**, Kinetics of S- (top) and HA- (bottom) binding memory B cells in PBMCs from convalescent individuals, collected at the indicated days after symptom onset. Data from the 7-month time point are also shown in **c**. **c**, Frequencies of S- (left) and HA- (right) binding memory B cells in PBMCs from control individuals (black circles) and convalescent individuals 7 months after symptom onset (white circles). The dotted line in the left plot indicates the limit of sensitivity, which was defined as the median + 2× s.d. of the controls. Each symbol represents one sample ($n = 18$ convalescent, $n = 11$ control). Horizontal lines indicate the median. *P* values from two-sided Mann–Whitney *U* tests.

epitopes that are targeted by BMPCs and memory B cells, as well as their clonal relatedness. Finally, although our data document a robust induction of long-lived BMPCs after infection with SARS-CoV-2, it is critical to note that our convalescent individuals mostly experienced mild infections. Our data are consistent with a report showing that individuals who recovered rapidly from symptomatic SARS-CoV-2 infection generated a robust humoral immune response[32]. It is possible that more-severe SARS-CoV-2 infections could lead to a different outcome with respect to long-lived BMPC frequencies, owing to dysregulated humoral immune responses. This, however, has not been the case in survivors of the 2014 Ebola virus outbreak in West Africa, in whom severe viral infection induced long-lasting antigen-specific serum IgG antibodies[33].

Long-lived BMPCs provide the host with a persistent source of preformed protective antibodies and are therefore needed to maintain durable immune protection. However, the longevity of serum anti-S IgG antibodies is not the only determinant of how durable immune-mediated protection will be. Isotype-switched memory B cells can rapidly differentiate into antibody-secreting cells after re-exposure to a pathogen, offering a second line of defence[34]. Encouragingly, the frequency of S-binding circulating memory B cells at 7 months after infection was similar to that of B cells directed against contemporary influenza HA antigens. Overall, our data provide strong evidence that SARS-CoV-2 infection in humans robustly establishes the two arms of humoral immune memory: long-lived BMPCs and memory B cells. These findings provide an immunogenicity benchmark for SARS-CoV-2 vaccines and a foundation for assessing the durability of primary humoral immune responses that are induced in humans after viral infections.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-021-03647-4.

1. Benner, R., Meima, F., van der Meulen, G. M. & van Muiswinkel, W. B. Antibody formation in mouse bone marrow. I. Evidence for the development of plaque-forming cells in situ. *Immunology* **26**, 247–255 (1974).
2. Manz, R. A., Thiel, A. & Radbruch, A. Lifetime of plasma cells in the bone marrow. *Nature* **388**, 133–134 (1997).
3. Slifka, M. K., Antia, R., Whitmire, J. K. & Ahmed, R. Humoral immunity due to long-lived plasma cells. *Immunity* **8**, 363–372 (1998).
4. Hammarlund, E. et al. Duration of antiviral immunity after smallpox vaccination. *Nat. Med.* **9**, 1131–1137 (2003).
5. Halliley, J. L. et al. Long-lived plasma cells are contained within the $CD19^{-}CD38^{hi}CD138^{+}$ subset in human bone marrow. *Immunity* **43**, 132–145 (2015).
6. Mei, H. E. et al. A unique population of IgG-expressing plasma cells lacking CD19 is enriched in human bone marrow. *Blood* **125**, 1739–1748 (2015).
7. Nutt, S. L., Hodgkin, P. D., Tarlinton, D. M. & Corcoran, L. M. The generation of antibody-secreting plasma cells. *Nat. Rev. Immunol.* **15**, 160–171 (2015).
8. Hall, V. J. et al. SARS-CoV-2 infection rates of antibody-positive compared with antibody-negative health-care workers in England: a large, multicentre, prospective cohort study (SIREN). *Lancet* **397**, 1459–1469 (2021).
9. Houlihan, C. F. et al. Pandemic peak SARS-CoV-2 infection and seroconversion rates in London frontline health-care workers. *Lancet* **396**, e6–e7 (2020).
10. Lumley, S. F. et al. Antibodies to SARS-CoV-2 are associated with protection against reinfection. Preprint at https://doi.org/10.1101/2020.11.18.20234369 (2020).
11. Long, Q.-X. et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat. Med.* **26**, 1200–1204 (2020).
12. Ibarrondo, F. J. et al. Rapid decay of anti-SARS-CoV-2 antibodies in persons with mild Covid-19. *N. Engl. J. Med.* **383**, 1085–1087 (2020).
13. Seow, J. et al. Longitudinal observation and decline of neutralizing antibody responses in the three months following SARS-CoV-2 infection in humans. *Nat. Microbiol.* **5**, 1598–1607 (2020).
14. Edridge, A. W. D. et al. Seasonal coronavirus protective immunity is short-lasting. *Nat. Med.* **26**, 1691–1693 (2020).
15. Callow, K. A., Parry, H. F., Sergeant, M. & Tyrrell, D. A. The time course of the immune response to experimental coronavirus infection of man. *Epidemiol. Infect.* **105**, 435–446 (1990).
16. Kaneko, N. et al. Loss of Bcl-6-expressing T follicular helper cells and germinal centers in COVID-19. *Cell* **183**, 143–157 (2020).
17. Wajnberg, A. et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. *Science* **370**, 1227–1230 (2020).
18. Isho, B. et al. Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. *Sci. Immunol.* **5**, eabe5511 (2020).
19. Dan, J. M. et al. Immunological memory to SARS-CoV-2 assessed for up to 8 months after infection. *Science* **371**, eabf4063 (2021).
20. Gaebler, C. et al. Evolution of antibody immunity to SARS-CoV-2. *Nature* **591**, 639–644 (2021).
21. Rodda, L. B. et al. Functional SARS-CoV-2-specific immune memory persists after mild COVID-19. *Cell* **184**, 169–183 (2021).
22. Davis, C. W. et al. Influenza vaccine-induced human bone marrow plasma cells decline within a year after vaccination. *Science* **370**, 237–241 (2020).
23. Turesson, I. Distribution of immunoglobulin-containing cells in human bone marrow and lymphoid tissues. *Acta Med. Scand.* **199**, 293–304 (1976).
24. Pritz, T. et al. Plasma cell numbers decrease in bone marrow of old patients. *Eur. J. Immunol.* **45**, 738–746 (2015).
25. Shi, R. et al. A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2. *Nature* **584**, 120–124 (2020).
26. Cao, Y. et al. Potent neutralizing antibodies against SARS-CoV-2 identified by high-throughput single-cell sequencing of convalescent patients' B cells. *Cell* **182**, 73–84 (2020).
27. Robbiani, D. F. et al. Convergent antibody responses to SARS-CoV-2 in convalescent individuals. *Nature* **584**, 437–442 (2020).
28. Kreer, C. et al. Longitudinal isolation of potent near-germline SARS-CoV-2-neutralizing antibodies from COVID-19 patients. *Cell* **182**, 843–854 (2020).
29. Alsoussi, W. B. et al. A potently neutralizing antibody protects mice against SARS-CoV-2 infection. *J. Immunol.* **205**, 915–922 (2020).
30. Wang, C. et al. A human monoclonal antibody blocking SARS-CoV-2 infection. *Nat. Commun.* **11**, 2251 (2020).
31. Wang, K. et al. Longitudinal dynamics of the neutralizing antibody response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Infection. *Clin. Infect. Dis.* **2020**, ciaa1143 (2020).
32. Chen, Y. et al. Quick COVID-19 healers sustain anti-SARS-CoV-2 antibody production. *Cell* **183**, 1496–1507 (2020).
33. Davis, C. W. et al. Longitudinal analysis of the human B Cell response to ebola virus infection. *Cell* **177**, 1566–1582 (2019).
34. Ellebedy, A. H. et al. Defining antigen-specific plasmablast and memory B cell subsets in human blood after viral infection or vaccination. *Nat. Immunol.* **17**, 1226–1234 (2016).

Methods

Data reporting

No statistical methods were used to predetermine sample size. The experiments were not randomized and the investigators were not blinded during outcome assessment.

Sample collection, preparation and storage

All studies were approved by the Institutional Review Board of Washington University in St Louis. Written consent was obtained from all participants. Seventy-seven participants who had recovered from SARS-CoV-2 infection and eleven control individuals without a history of SARS-CoV-2 infection were enrolled (Extended Data Tables 1, 4). Blood samples were collected in EDTA tubes and PBMCs were enriched by density gradient centrifugation over Ficoll 1077 (GE) or Lymphopure (BioLegend). The remaining red blood cells were lysed with ammonium chloride lysis buffer, and cells were immediately used or cryopreserved in 10% dimethyl sulfoxide in fetal bovine serum (FBS). Bone marrow aspirates of approximately 30 ml were collected in EDTA tubes from the iliac crest of 18 individuals who had recovered from COVID-19 and the control individuals. Bone marrow mononuclear cells were enriched by density gradient centrifugation over Ficoll 1077, and the remaining red blood cells were lysed with ammonium chloride buffer (Lonza) and washed with phosphate-buffered saline (PBS) supplemented with 2% FBS and 2 mM EDTA. Bone marrow plasma cells were enriched from bone marrow mononuclear cells using the CD138 Positive Selection Kit II (Stemcell) and immediately used for ELISpot or cryopreserved in 10% dimethyl sulfoxide in FBS.

Antigens

Recombinant soluble spike protein (S) and its receptor-binding domain (RBD) derived from SARS-CoV-2 were expressed as previously described[35]. In brief, mammalian cell codon-optimized nucleotide sequences coding for the soluble version of S (GenBank: MN908947.3, amino acids (aa) 1–1,213) including a C-terminal thrombin cleavage site, T4 foldon trimerization domain and hexahistidine tag cloned into the mammalian expression vector pCAGGS. The S protein sequence was modified to remove the polybasic cleavage site (RRAR to A) and two stabilizing mutations were introduced (K986P and V987P, wild-type numbering). The RBD, along with the signal peptide (aa 1–14) plus a hexahistidine tag were cloned into the mammalian expression vector pCAGGS. Recombinant proteins were produced in Expi293F cells (Thermo Fisher Scientific) by transfection with purified DNA using the ExpiFectamine 293 Transfection Kit (Thermo Fisher Scientific). Supernatants from transfected cells were collected 3 (for S) or 4 (for RBD) days after transfection, and recombinant proteins were purified using Ni-NTA agarose (Thermo Fisher Scientific), then buffer-exchanged into PBS and concentrated using Amicon Ultracel centrifugal filters (EMD Millipore). For flow cytometry staining, recombinant S was labelled with Alexa Fluor 647- or DyLight 488-NHS ester (Thermo Fisher Scientific); excess Alexa Fluor 647 and DyLight 488 were removed using 7-kDa and 40-kDa Zeba desalting columns, respectively (Pierce). Recombinant HA from A/Michigan/45/2015 (aa 18–529, Immune Technology) was labelled with DyLight 405-NHS ester (Thermo Fisher Scientific); excess DyLight 405 was removed using 7-kDa Zeba desalting columns. Recombinant HA from A/Brisbane/02/2018 (aa 18–529) and B/Colorado/06/2017 (aa 18–546) (both Immune Technology) were biotinylated using the EZ-Link Micro NHS-PEG4-Biotinylation Kit (Thermo Fisher Scientific); excess biotin was removed using 7-kDa Zeba desalting columns.

ELISpot

Plates were coated with Flucelvax Quadrivalent 2019/2020 seasonal influenza virus vaccine (Sequiris), tetanus–diphtheria vaccine (Grifols), recombinant S or anti-human Ig. Direct ex vivo ELISpot was performed to determine the number of total, vaccine-binding or recombinant S-binding IgG- and IgA-secreting cells present in BMPC and PBMC samples using IgG/IgA double-colour ELISpot Kits (Cellular Technology) according to the manufacturer's instructions. ELISpot plates were analysed using an ELISpot counter (Cellular Technology).

ELISA

Assays were performed in 96-well plates (MaxiSorp, Thermo Fisher Scientific) coated with 100 µl of Flucelvax 2019/2020 or recombinant S in PBS, and plates were incubated at 4 °C overnight. Plates were then blocked with 10% FBS and 0.05% Tween-20 in PBS. Serum or plasma were serially diluted in blocking buffer and added to the plates. Plates were incubated for 90 min at room temperature and then washed 3 times with 0.05% Tween-20 in PBS. Goat anti-human IgG–HRP (Jackson ImmunoResearch, 1:2,500) was diluted in blocking buffer before adding to wells and incubating for 60 min at room temperature. Plates were washed 3 times with 0.05% Tween-20 in PBS, and then washed 3 times with PBS before the addition of *o*-phenylenediamine dihydrochloride peroxidase substrate (Sigma-Aldrich). Reactions were stopped by the addition of 1 M HCl. Optical density measurements were taken at 490 nm. The half-maximal binding dilution for each serum or plasma sample was calculated using nonlinear regression (GraphPad Prism v.8). The limit of detection was defined as 1:30.

Statistics

Spearman's correlation coefficients were estimated to assess the relationship between 7-month anti-S and anti-influenza virus vaccine IgG titres and the frequencies of BMPCs secreting IgG specific for S and for influenza virus vaccine, respectively. Means and pairwise differences of antibody titres at each time point were estimated using a linear mixed model analysis with a first-order autoregressive covariance structure. Time since symptom onset was treated as a categorical fixed effect for the 4 different sample time points spaced approximately 3 months apart. *P* values were adjusted for multiple comparisons using Tukey's method. All analyses were conducted using SAS v.9.4 (SAS Institute) and Prism v.8.4 (GraphPad), and *P* values of less than 0.05 were considered significant.

Flow cytometry

Staining for flow cytometry analysis was performed using cryo-preserved magnetically enriched BMPCs and cryo-preserved PBMCs. For BMPC staining, cells were stained for 30 min on ice with CD45-A532 (HI30, Thermo Fisher Scientific, 1:50), CD38-BB700 (HIT2, BD Horizon, 1:500), CD19-PE (HIB19, 1:200), CXCR5-PE-Dazzle 594 (J252D4, 1:50), CD71-PE-Cy7 (CY1G4, 1:400), CD20-APC-Fire750 (2H7, 1:400), CD3-APC-Fire810 (SK7, 1:50) and Zombie Aqua (all BioLegend) diluted in Brilliant Stain buffer (BD Horizon). Cells were washed twice with 2% FBS and 2 mM EDTA in PBS (P2), fixed for 1 h using the True Nuclear permeabilization kit (BioLegend), washed twice with perm/wash buffer, stained for 1h with DyLight 405-conjugated recombinant HA from A/Michigan/45/2015, DyLight 488- and Alexa 647-conjugated S, Ki-67-BV711 (Ki-67, 1:200, BioLegend) and BLIMP-1-A700 (646702, 1:50, R&D), washed twice with perm/wash buffer, and resuspended in P2. For memory B cell staining, PBMCs were stained for 30 min on ice with biotinylated recombinant HAs diluted in P2, washed twice, then stained for 30 min on ice with Alexa 647-conjugated S, IgA-FITC (M24A, Millipore, 1:500), IgG-BV480 (goat polyclonal, Jackson ImmunoResearch, 1:100), IgD-SB702 (IA6-2, Thermo Fisher Scientific, 1:50), CD38-BB700 (HIT2, BD Horizon, 1:500), CD20-Pacific Blue (2H7, 1:400), CD4-BV570 (OKT4, 1:50), CD24-BV605 (ML5, 1:100), streptavidin-BV650, CD19-BV750 (HIB19, 1:100), CD71-PE (CY1G4, 1:400), CXCR5-PE-Dazzle 594 (J252D4, 1:50), CD27-PE-Cy7 (O323, 1:200), IgM-APC-Fire750 (MHM-88, 1:100), CD3-APC-Fire810 (SK7, 1:50) and Zombie NIR (all BioLegend) diluted in Brilliant Stain buffer (BD Horizon), and washed twice with P2. Cells were acquired on an Aurora using SpectroFlo v.2.2 (Cytek). Flow cytometry data were analysed using FlowJo v.10 (Treestar). In each experiment,

PBMCs were included from convalescent individuals and control individuals.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Relevant data are available from the corresponding author upon reasonable request.

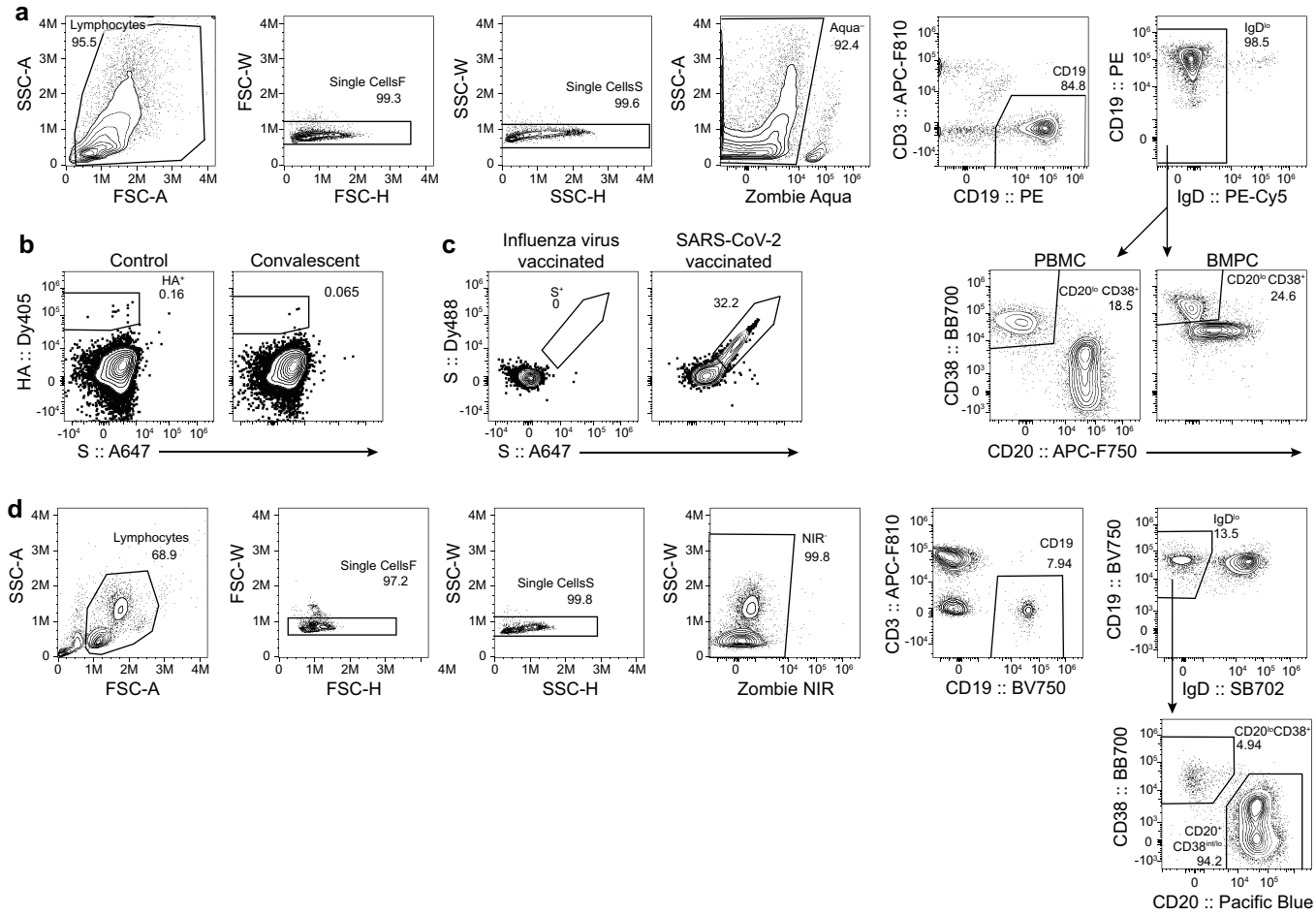
35. Stadlbauer, D. et al. SARS-CoV-2 seroconversion in humans: a detailed protocol for a serological assay, antigen production, and test setup. *Curr. Protoc. Microbiol*. **57**, e100 (2020).

Acknowledgements We thank the donors for providing specimens; T. Lei for assistance with preparing specimens; and L. Kessels, A. J. Winingham, the staff of the Infectious Diseases Clinical Research Unit at Washington University School of Medicine and the nursing team of the bone marrow biopsy suite at Washington University School of Medicine and Barnes Jewish Hospital for sample collection and providing care for donors. The SARS-CoV-2 S and RBD protein expression plasmids were provided by F. Krammer. The Ellebedy laboratory was supported by National Institute of Allergy and Infectious Diseases (NIAID) grants U01AI141990 and 1U01AI150747, NIAID Centers of Excellence for Influenza Research and Surveillance contracts HHSN272201400006C and HHSN272201400008C and NIAID Collaborative Influenza Vaccine Innovation Centers contract 75N93019C00051. J.S.T. was supported by NIAID 5T32CA009547. L.H. was supported by Norwegian Research Council grant 271160 and National Graduate School in Infection Biology and Antimicrobials grant 249062. This study used samples obtained from the Washington University School of Medicine's COVID-19 biorepository, which is supported by the NIH–National Center for Advancing Translational Sciences grant UL1 TR002345. The content is solely the responsibility of the authors and does not necessarily represent the view of the NIH. The WU353, WU367 and WU368 studies were reviewed and approved by the Washington University Institutional Review Board (approval nos. 202003186, 202009100 and 202012081, respectively).

Author contributions A.H.E. conceived and designed the study. J.S.T. and A.H.E. designed experiments and composed the manuscript. A.H., M.K.K., I.P., J.A.O. and R.M.P. wrote and maintained the Institutional Review Board protocol, recruited and phlebotomized participants and coordinated sample collection. J.S.T., W.K., E.K., A.J.S. and L.H. processed specimens. A.J.S. expressed S and RBD proteins. J.S.T., W.K. and E.K. performed ELISA and ELISpot. J.S.T. performed flow cytometry. J.S.T., A.M.R., C.W.G. and A.H.E. analysed data. All authors reviewed the manuscript.

Competing interests The Ellebedy laboratory received funding under sponsored research agreements that are unrelated to the data presented in the current study from Emergent BioSolutions and from AbbVie. J.S.T., A.J.S. and A.H.E. are recipients of a licensing agreement with Abbvie that is unrelated to the data presented in the current study. A.H.E. is a consultant for Mubadala Investment Company and the founder of ImmuneBio Consulting. All other authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41586-021-03647-4.
Correspondence and requests for materials should be addressed to A.H.E.
Peer review information *Nature* thanks Stanley Perlman, Andreas Radbruch and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.



Extended Data Fig. 1 | Flow cytometry identification of SARS-CoV-2-elicited plasma cells and memory B cells. a, **d**, Flow cytometry gating strategies for BMPCs in magnetically enriched BMPCs and plasmablasts in PBMCs (**a**) and isotype-switched memory B cells and plasmablasts in PBMCs (**d**). **b**, Representative plots of intracellular SARS-CoV-2 S and influenza virus HA staining in BMPCs from samples from control individuals (left) and individuals who were convalescing from COVID-19 (right) 7 months after symptom onset. **c**, Representative plots of intracellular S staining in plasmablasts in PBMCs one week after vaccination against seasonal influenza virus or SARS-CoV-2.

Extended Data Table 1 | Demographics of patients with COVID-19

	Total N=77 N (%)	Bone marrow biopsy N=19 N (%)
Age (median [range])	49 (21-69)	52 (30-69)
Sex		
Female	38 (49.4)	7 (36.8)
Male	39 (50.6)	12 (63.2)
Race		
White	70 (90.9)	18 (94.7)
Black	1 (1.3)	0 (0)
Asian	4 (5.2)	0 (0)
Other	2 (2.6)	1 (5.3)
Comorbidities		
Asthma	13 (16.9)	3 (15.8)
Lung disease	0 (0)	0 (0)
Heart disease	3 (3.9)	0 (0)
Hypertension	13 (16.9)	6 (31.6)
Diabetes mellitus	3 (3.9)	3 (15.8)
Cancer	10 (13)	3 (15.8)
Autoimmune disease	4 (5.2)	2 (10.5)
Hyperlipidemia	8 (10.4)	2 (10.5)
Hypothyroidism	5 (6.5)	3 (15.8)
Gastroesophageal reflux disease	5 (6.5)	2 (10.5)
Other	26 (33.8)	10 (52.6)
Solid organ transplant	1 (1.3)	1 (5.3)
Obesity	1 (1.3)	0 (0)

Extended Data Table 2 | Symptoms of patients with COVID-19

	Total N=77 N (%)	Bone marrow biopsy N=19 N (%)
First symptom		
Cough	12 (15.6)	3 (15.8)
Diarrhea	1 (1.3)	0 (0)
Dyspnea	2 (2.6)	1 (5.3)
Fatigue	7 (9.1)	0 (0)
Fever	22 (28.6)	9 (47.4)
Headache	8 (10.4)	2 (10.5)
Loss of taste	3 (3.9)	2 (10.5)
Malaise	4 (5.2)	1 (5.3)
Myalgias	9 (11.7)	0 (0)
Nasal congestion	2 (2.6)	0 (0)
Nausea	1 (1.3)	0 (0)
Night sweats	1 (1.3)	0 (0)
Sore throat	5 (6.5)	1 (5.3)
Symptom present during disease		
Fever	65 (84.4)	17 (89.5)
Cough	54 (70.1)	14 (73.7)
Dyspnea	31 (40.3)	11 (57.9)
Nausea	19 (24.7)	4 (21.1)
Vomiting	9 (11.7)	3 (15.8)
Diarrhea	39 (50.6)	10 (52.6)
Headaches	47 (61)	12 (63.2)
Loss of taste	42 (54.5)	11 (57.9)
Loss of smell	42 (54.5)	10 (52.6)
Fatigue	38 (49.4)	7 (36.8)
Malaise	6 (7.8)	1 (5.3)
Myalgias or body aches	34 (44.2)	8 (42.1)
Sore throat	12 (15.6)	1 (5.3)
Chills	25 (32.5)	6 (31.6)
Nasal congestion	6 (7.8)	0 (0)
Other	32 (41.6)	7 (36.8)
Duration of symptoms in days (median [range])	14 (1-43)	13 (6-30)
Days from symptom onset to positive SARS-CoV-2 PCR test (median [range])	6 (0-36)	6 (1-31)
Days from symptom onset to 1-month blood sample collection (median [range])	41 (21-84)	34 (22-71)
Hospitalization	6 (7.8)	1 (5.3)
COVID medications		
Hydroxychloroquine	2 (2.6)	0 (0)
Chloroquine	1 (1.3)	0 (0)
Azithromycin	14 (18.2)	6 (31.6)
Lopinavir/ritonavir	0 (0)	0 (0)
Remdesivir	0 (0)	0 (0)
Convalescent plasma	0 (0)	0 (0)
None	61 (79.2)	12 (63.2)
Other	2 (2.6)	1 (5.3)

Extended Data Table 3 | Symptoms and follow up samples (months 4–11) of convalescent individuals

	Month 4		Month 7		Month 11	
	Total N= 76 N (%)	**Bone marrow biopsy N=19 N (%)**	**Total N= 76 N (%)**	**Bone marrow biopsy N=18 N (%)**	**Total N= 42 N (%)**	**Bone marrow biopsy N=12 N (%)**
Days from positive SARS-CoV-2 PCR test to follow up visit (median [range])	125 (102-192)	117 (105-150)	222 (191-275)	213 (200-247)	308 (283-369)	303 (283-325)
Days from symptom onset to blood sample collection (median [range])	131 (106-193)	124 (108-155)	227 (194-277)	222 (205-253)	314 (288-373)	309 (297-343)
Any symptom present at follow up visit	25 (32.9)	8 (42.1)	33 (43)	10 (55.6)	20 (47.6)	6 (50)
Fever	0 (0)	0 (0)	2 (2.6)	0 (0)	1 (2.4)	0 (0)
Cough	1 (1.3)	1 (5.3)	0 (0)	0 (0)	1 (2.4)	0 (0)
Dyspnea	7 (9.2)	2 (10.5)	6 (7.9)	3 (16.7)	6 (14.3)	3 (25)
Nausea	1 (1.3)	0 (0)	1 (1.3)	0 (0)	0 (0)	0 (0)
Vomiting	1 (1.3)	1 (5.3)	0 (0)	0 (0)	0 (0)	0 (0)
Diarrhea	2 (2.6)	1 (5.3)	1 (1.3)	0 (0)	0 (0)	0 (0)
Headaches	1 (1.3)	0 (0)	3 (3.9)	0 (0)	2 (4.8)	0 (0)
Loss or altered taste	8 (10.5)	0 (0)	9 (11.8)	1 (5.6)	5 (11.9)	1 (8.3)
Loss or altered smell	13 (17.1)	2 (10.5)	12 (15.8)	2 (11.1)	8 (19)	2 (16.7)
Fatigue	9 (11.8)	4 (21.1)	13 (17.1)	5 (27.8)	8 (19)	3 (25)
Forgetfulness/brain fog	8 (10.5)	6 (31.6)	12 (15.8)	6 (33.3)	10 (23.8)	4 (33.3)
Hair loss	5 (6.6)	1 (5.3)	3 (3.9)	1 (5.6)	2 (4.8)	0 (0)
Other	7 (9.2)	3 (15.8)	12 (15.8)	1 (5.6)	10 (23.8)	1 (8.3)
Joint pain	3 (3.9)	1 (5.3)	7 (9.2)	1 (5.3)	3 (7.1)	0 (0)

Extended Data Table 4 | Healthy control demographics

Variable	Total N= 11 N (%)
Age (median [range])	38 (23-53)
Sex	
Female	3 (27.3)
Male	8 (72.7)
Race	
White	9 (71.8)
Black	1 (9.1)
Asian	1 (9.1)

nature research

Corresponding author(s): Ali H. Ellebedy

Last updated by author(s): May 26, 2021

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Flow cytometry data were acquired using SpectroFlo software v2.2.
Data analysis	Flow cytometry data were analyzed using FlowJo v10 and Prism v8 ELISA and ELISpot data were analyzed using Prism v8 and SAS 9.4

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All relevant data are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to determine sample size. 77 convalescent patients and 11 control participants were enrolled based on recruitment; these numbers provided sufficient power to determine differences in SARS-CoV-2 responses between the groups.
Data exclusions	No data were excluded
Replication	Samples were collected from 77 convalescent patients and 11 control participants. ELISA for each participant at each timepoint was performed once with two technical replicates. ELISpot and flow cytometry experiments were performed once for each sample at each timepoint.
Randomization	Different experimental groups were not assigned.
Blinding	No blinding was done in this study; subjective measurements were not made.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	IgG-HRP (goat polyclonal, Jackson ImmuoResearch 109-035-088), IgG-BV480 (goat polyclonal, Jackson Immunoresearch 109-685-098), IgD-SB702 (IA6-2, Thermo 67-9868-42), IgA-FITC (M24A, Millipore CBL114F), CD45-A532 (HI30, Thermo 58-0459-42), CD38-BB700 (HIT2, BD Horizon 566445), Blimp1-A700 (646702, R&D IC36081N), CD20-Pacific Blue (2H7, 302320), CD4-BV570 (OKT4, 317445), CD24-BV605 (ML5, 311124), streptavidin-BV650 (405232), Ki-67-BV711 (Ki-67, 350516), CD19-BV750 (HIB19, 302262), CD19-PE (HIB19, 302254), CD71-PE (CY1G4, 334106), CXCR5-PE-Dazzle 594 (J252D4, 356928), CD27-PE-Cy7 (O323, 302838), CD71-PE-Cy7 (CY1G4, 334112), CD20-APC-Fire750 (2H7, 302358), IgM-APC-Fire750 (MHM-88, 314546), CD3-APC-Fire810 (SK7, 344858); all Biolegend.
Validation	Commercial antibodies were validated by their respective manufacturers per their associated data sheets and titrated in the lab for their respective assay (ELISA or flow cytometry) by serial dilution

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Expi293F (Thermo)
Authentication	The cell line was not authenticated
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination. Growth rates were consistent with manufacturer's published data.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used

Human research participants

Policy information about studies involving human research participants

Population characteristics	77 SARS-CoV-2 convalescent study participants were recruited, ages 21-69, 49.4% female, 50.6% male 11 healthy control participants with no history of SARS-CoV-2 infection were recruited, ages 23-53, 27.3% female, 72.7% male
Recruitment	Study participants were recruited from the St. Louis metropolitan area by the Washington University Clinical Trials Unit. Potential self-selection and recruiting biases are unlikely to affect the parameters we measured.
Ethics oversight	The study was approved by the Washington University IRB

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Peripheral blood and bone marrow mononuclear cells were isolated from EDTA anticoagulated blood and bone marrow aspirates, respectively using density gradient centrifugation, and remaining RBCs were lysed with ammonium chloride lysis buffer. Bone marrow plasma cells were magnetically enriched from bone marrow mononuclear cells and immediately used for ELISpot or cryopreserved in 10% dimethylsufoxide in FBS for flow cytometric analysis. PBMCs were immediately used or cryopreserved in 10% DMSO in FBS.
Instrument	Cytek Aurora
Software	Flow cytometry data were acquired using Cytek SpectroFlo software, and analyzed using FlowJo (Treestar) v10.
Cell population abundance	Cells were not sorted
Gating strategy	Gating strategies are shown in extended data figure

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

EXHIBIT 12

Infection fatality rate of COVID-19 inferred from seroprevalence data

John P A Ioannidis[a]

Objective To estimate the infection fatality rate of coronavirus disease 2019 (COVID-19) from seroprevalence data.
Methods I searched PubMed and preprint servers for COVID-19 seroprevalence studies with a sample size ≥ 500 as of 9 September 2020. I also retrieved additional results of national studies from preliminary press releases and reports. I assessed the studies for design features and seroprevalence estimates. I estimated the infection fatality rate for each study by dividing the cumulative number of COVID-19 deaths by the number of people estimated to be infected in each region. I corrected for the number of immunoglobin (Ig) types tested (IgG, IgM, IgA).
Findings I included 61 studies (74 estimates) and eight preliminary national estimates. Seroprevalence estimates ranged from 0.02% to 53.40%. Infection fatality rates ranged from 0.00% to 1.63%, corrected values from 0.00% to 1.54%. Across 51 locations, the median COVID-19 infection fatality rate was 0.27% (corrected 0.23%): the rate was 0.09% in locations with COVID-19 population mortality rates less than the global average (< 118 deaths/million), 0.20% in locations with 118–500 COVID-19 deaths/million people and 0.57% in locations with > 500 COVID-19 deaths/million people. In people younger than 70 years, infection fatality rates ranged from 0.00% to 0.31% with crude and corrected medians of 0.05%.
Conclusion The infection fatality rate of COVID-19 can vary substantially across different locations and this may reflect differences in population age structure and case-mix of infected and deceased patients and other factors. The inferred infection fatality rates tended to be much lower than estimates made earlier in the pandemic.

Abstracts in عربي, 中文, Français, Русский and Español at the end of each article.

Introduction

The infection fatality rate, the probability of dying for a person who is infected, is one of the most important features of the coronavirus disease 2019 (COVID-19) pandemic. The expected total mortality burden of COVID-19 is directly related to the infection fatality rate. Moreover, justification for various non-pharmacological public health interventions depends on the infection fatality rate. Some stringent interventions that potentially also result in more noticeable collateral harms[1] may be considered appropriate, if the infection fatality rate is high. Conversely, the same measures may fall short of acceptable risk–benefit thresholds, if the infection fatality rate is low.

Early data from China suggested a 3.4% case fatality rate[2] and that asymptomatic infections were uncommon,[3] thus the case fatality rate and infection fatality rate would be about the same. Mathematical models have suggested that 40–81% of the world population could be infected,[4,5] and have lowered the infection fatality rate to 1.0% or 0.9%.[5,6] Since March 2020, many studies have estimated the spread of the virus causing COVID-19 – severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) – in various locations by evaluating seroprevalence. I used the prevalence data from these studies to infer estimates of the COVID-19 infection fatality rate.

Methods

Seroprevalence studies

The input data for calculations of infection fatality rate were studies on the seroprevalence of COVID-19 done in the general population, or in samples that might approximately represent the general population (e.g. with proper reweighting), that had been published in peer-reviewed journals or as preprints (irrespective of language) as of 9 September 2020. I considered only studies with at least 500 assessed samples because smaller data sets would result in large uncertainty for any calculations based on these data. I included studies that made seroprevalence assessments at different time intervals if at least one time interval assessment had a sample size of at least 500 participants. If there were different eligible time intervals, I selected the one with the highest seroprevalence, since seroprevalence may decrease over time as antibody titres decrease. I excluded studies with data collected for more than a month that could not be broken into at least one eligible time interval less than one month duration because it would not be possible to estimate a point seroprevalence reliably. Studies were eligible regardless of the exact age range of participants included, but I excluded studies with only children.

I also examined results from national studies from preliminary press releases and reports whenever a country had no other data presented in published papers or preprints. This inclusion allowed these countries to be represented, but information was less complete than information in published papers or preprints and thus requires caution.

I included studies on blood donors, although they may underestimate seroprevalence and overestimate infection fatality rate because of the healthy volunteer effect. I excluded studies on health-care workers, since this group is at a potentially high exposure risk, which may result in seroprevalence estimates much higher than the general population and thus an improbably low infection fatality rate. Similarly, I also excluded studies on communities (e.g. shelters or religious or other shared-living communities). Studies were eligible regardless of whether they aimed to evaluate seroprevalence in large or small regions, provided that the population of reference in the region was at least 5000 people.

I searched PubMed® (LitCOVID), and medRxiv, bioRxiv and Research Square using the terms "seroprevalence" OR "antibodies" with continuous updates. I made the first search in early May and did monthly updates, with the last update

[a] Meta-Research Innovation Center at Stanford (METRICS), Stanford University, 1265 Welch Road, Stanford, California 94305, United States of America.
Correspondence to John P A Ioannidis (email: jioannid@stanford.edu).
(*Submitted: 13 May 2020 – Revised version received: 13 September 2020 – Accepted: 15 September 2020 – Published online: 14 October 2020*)

on 9 September 2020. I contacted field experts to retrieve any important studies that may have been missed.

From each study, I extracted information on location, recruitment and sampling strategy, dates of sample collection, sample size, types of antibody measured (immunoglobulin G (IgG), IgM and IgA), the estimated crude seroprevalence (positive samples divided by all samples tested), adjusted seroprevalence and the factors that the authors considered for adjustment.

Inferred infection fatality rate

If a study did not cover an entire country, I collected information on the population of the relevant location from the paper or recent census data so as to approximate as much as possible the relevant catchment area (e.g. region(s) or county(ies)). Some studies targeted specific age groups (e.g. excluding elderly people and/or excluding children) and some estimated numbers of people infected in the population based on specific age groups. For consistency, I used the entire population (all ages) and, separately, the population 0–70 years to estimate numbers of infected people. I assumed that the seroprevalence would be similar in different age groups, but I also recorded any significant differences in seroprevalence across age strata so as to examine the validity of this assumption.

I calculated the number of infected people by multiplying the relevant population size and the adjusted estimate of seroprevalence. If a study did not give an adjusted seroprevalence estimate, I used the unadjusted seroprevalence instead. When seroprevalence estimates with different adjustments were available, I selected the analysis with largest adjustment. The factors adjusted for included COVID-19 test performance, sampling design, and other factors such as age, sex, clustering effects or socioeconomic factors. I did not adjust for specificity in test performance when positive antibody results were already validated by a different method.

For the number of COVID-19 deaths, I chose the number of deaths accumulated until the date 1 week after the midpoint of the study period (or the date closest to this that had available data) – unless the authors of the study had strong arguments to choose some other time point or approach. The 1-week lag accounts for different delays in developing antibodies versus dying from infection. The number of deaths is an approximation because it is not known when exactly each patient who died was infected. The 1-week cut-off after the study midpoint may underestimate deaths in places where patients are in hospital for a long time before death, and may overestimate deaths in places where patients die soon because of poor or even inappropriate care. Whether or not the health system became overloaded may also affect the number of deaths. Moreover, because of imperfect diagnostic documentation, COVID-19 deaths may have been both overcounted and undercounted in different locations and at different time points.

I calculated the inferred infection fatality rate by dividing the number of deaths by the number of infected people for the entire population, and separately for people younger than 70 years. I took the proportion of COVID-19 deaths that occurred in people younger than 70 years from situational reports for the respective locations that I retrieved at the time I identified the seroprevalence studies. I also calculated a corrected infection fatality rate to try and account for the fact that only one or two types of antibodies (among IgG, IgM, IgA) might have been used. I corrected seroprevalence upwards (and inferred infection fatality rate downwards) by one tenth of its value if a study did not measure IgM and similarly if IgA was not measured. This correction is reasonable based on some early evidence,[7] although there is uncertainty about the exact correction factor.

Data synthesis

The estimates of the infection fatality rate across all locations showed great heterogeneity with I^2 exceeding 99.9%; thus, a meta-analysis would be inappropriate to report across all locations. Quantitative synthesis with meta-analysis across all locations would also be misleading since locations with high COVID-19 seroprevalence would tend to carry more weight than locations with low seroprevalence. Furthermore, locations with more studies (typically those that have attracted more attention because of high death tolls and thus high infection fatality rates) would be represented multiple times in the calculations. In addition, poorly conducted studies with fewer adjustments would get more weight because of spuriously narrower confidence intervals than more rigorous studies with more careful adjustments which allow for more uncertainty. Finally, with a highly skewed distribution of the infection fatality rate and with large between-study heterogeneity, typical random effects models would produce an incorrectly high summary infection fatality rate that approximates the mean of the study-specific estimates (also strongly influenced by high-mortality locations where more studies have been done); for such a skewed distribution, the median is more appropriate.

Therefore, in a first step, I grouped estimates of the infection fatality rate from studies in the same country (or for the United States of America, the same state) together and calculated a single infection fatality rate for that location, weighting the study-specific infection fatality rates by the sample size of each study. This approach avoided inappropriately giving more weight to studies with higher seroprevalence estimates and those with seemingly narrower confidence intervals because of poor or no adjustments, while still giving more weight to larger studies. Then, I used the single summary estimate for each location to calculate the median of the distribution of location-specific infection fatality rate estimates. Finally, I explored whether the location-specific infection fatality rates were associated with the COVID-19 mortality rate in the population (COVID-19 deaths per million people) in each location as of 12 September 2020; this analysis allowed me to assess whether estimates of the infection fatality rate tended to be higher in locations with a higher burden of death from COVID-19.

Results

Seroprevalence studies

I retrieved 61 studies with 74 eligible estimates published either in the peer-reviewed literature or as preprints as of 9 September 2020.[8–68] Furthermore, I considered another eight preliminary national estimates.[69–76] This search yielded a total of 82 eligible estimates (Fig. 1).

The studies varied substantially in sampling and recruitment designs (Table 1; available at: http://www.who.int/bulletin/volumes/99/1/20-265892). Of the 61 studies, 24 studies[8,10,16,17,20,22,25,33,34,36,37,42,46–49,52–54,57,61,63,65,68]

explicitly aimed for random sampling from the general population. In principle, random sampling is a stronger design. However, even then, people who cannot be reached (e.g. by email or telephone or even by visiting them at a house location) will not be recruited, and these vulnerable populations are likely to be missed. Moreover, several such studies[8,10,16,37,42] focused on geographical locations with high numbers of deaths, higher than other locations in the same city or country, and this emphasis would tend to select eventually for a higher infection fatality rate on average.

Eleven studies assessed blood donors,[12,15,18,24,28,31,41,44,45,55,60] which might underestimate COVID-19 seroprevalence in the general population. For example, 200 blood donors in Oise, France showed 3.00% seroprevalence, while the seroprevalence was 25.87% (171/661) in pupils, siblings, parents, teachers and staff at a high school with a cluster of cases in the same area; the true population seroprevalence may be between these two values.[13]

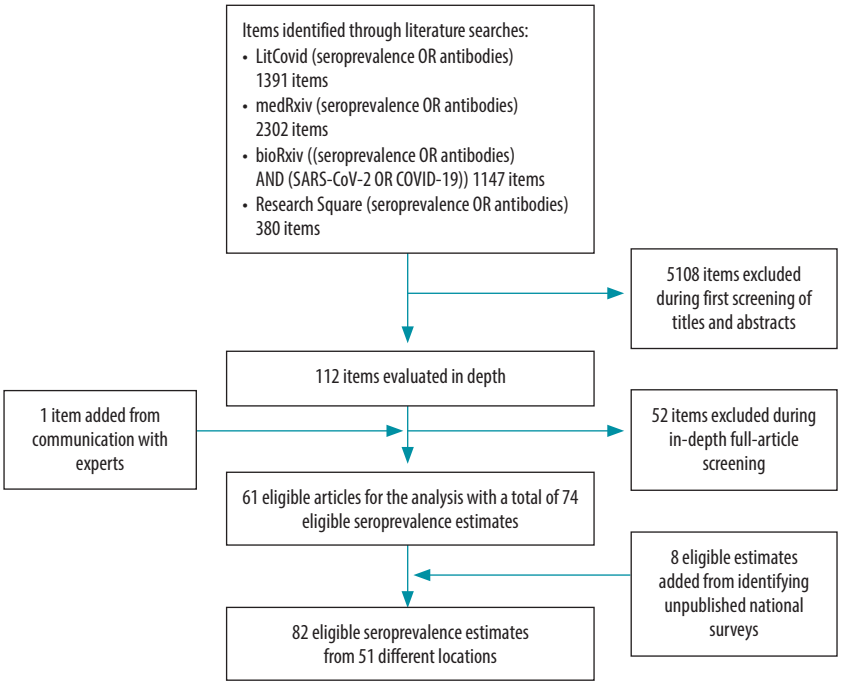
For other studies, healthy volunteer bias[19] may underestimate seroprevalence, attracting people with symptoms[26] may overestimate seroprevalence, and studies of employees,[14,21,25,32,66] grocery store clients[23] or patient cohorts[11,14,27–30,36,38,40,50,51,56,59,62,64,67] risk sampling bias in an unpredictable direction.

All the studies tested for IgG antibodies but only about half also assessed IgM and few assessed IgA. Only seven studies assessed all three types of antibodies and/or used pan-Ig antibodies. The ratio of people sampled versus the total population of the region was more than 1:1000 in 20 studies (Table 2; available at: http://www.who.int/bulletin/volumes/99/1/20-265892).

Seroprevalence estimates

Seroprevalence for the infection ranged from 0.02% to 53.40% (58.40% in the slum sub-population in Mumbai; Table 3). Studies varied considerably depending on whether or not they tried to adjust their estimates for test performance, sampling (to get closer to a more representative sample), clustering (e.g. when including household members) and other factors. The adjusted seroprevalence occasionally differed substantially from the unadjusted value. In studies that used samples from multiple locations, between-location heterogeneity was seen (e.g. 0.00–25.00% across 133 Brazilian cities).[25]

Fig. 1. **Flowchart for selection of seroprevalence studies on severe acute respiratory syndrome coronavirus 2, 2020**



COVID-19: coronavirus disease 2019; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

Inferred infection fatality rate

Inferred infection fatality rate estimates varied from 0.00% to 1.63% (Table 4). Corrected values also varied considerably (0.00–1.54%).

For 15 locations, more than one estimate of the infection fatality rate was available and thus I could compare the infection fatality rate from different studies evaluating the same location. The estimates of infection fatality rate tended to be more homogeneous within each location, while they differed markedly across locations (Fig. 2). Within the same location, infection fatality rate estimates tend to have only small differences, even though it is possible that different areas within the same location may also have real differences in infection fatality rate. France is one exception where differences are large, but both estimates come from population studies of outbreaks from schools and thus may not provide good estimates of population seroprevalence and may lead to an underestimated infection fatality rate.

I used summary estimates weighted for sample size to generate a single estimate for each location. Data were available for 51 different locations (including the inferred infection fatality rates from the eight preliminary additional national estimates in Table 5).

The median infection fatality rate across all 51 locations was 0.27% (corrected 0.23%). Most data came from locations with high death tolls from COVID-19 and 32 of the locations had a population mortality rate (COVID-19 deaths per million population) higher than the global average (118 deaths from COVID-19 per million as of 12 September 2020;[79] Fig. 3). Uncorrected estimates of the infection fatality rate of COVID-19 ranged from 0.01% to 0.67% (median 0.10%) across the 19 locations with a population mortality rate for COVID-19 lower than the global average, from 0.07% to 0.73% (median 0.20%) across 17 locations with population mortality rate higher than the global average but lower than 500 COVID-19 deaths per million, and from 0.20% to 1.63% (median 0.71%) across 15 locations with more than 500 COVID-19 deaths per million. The corrected estimates of the median infection fatality rate were

Table 3. **Estimated prevalence of COVID-19 and estimated number of people infected, 2020**

Country (location)	Seroprevalence, %			Estimated no. of people infected
	Crude	Adjusted		
		Value	Adjustments	
Argentina (Barrio Padre Mugica)[47]	ND	53.4	Age, sex, household, non-response	26 691
Belgium[38]	5.7	6.0	Sampling, age, sex, province	695 377
Brazil (133 cities)[25]	1.39	1.62 overall (0–25.0 across the 133 cities)	Test, design	1 209 435[a]
Brazil (Espirito Santo)[34]	2.1	ND	NA	84 391
Brazil (Maranhao)[68]	37	40.4	Clustering, stratification, non-response	2 877 454
Brazil (Rio de Janeiro), blood donors[41]	6	4.7	Age, sex, test	811 452
Brazil (Rio Grande do Sul)[17]	0.222	0.222[b]	Sampling	25 283
Brazil (Sao Paulo)[42]	5.2	4.7	Sampling design	14 017
Canada (British Columbia)[50]	0.45	0.55	Age	27 890
Chile (Vitacura)[43]	11.2	ND	NA	9 500
China, blood donors[55]				
Wuhan	3.87	ND	NA	433 827
Shenzhen	0.06	ND	NA	7 818
Shijiazhuang	0.02	ND	NA	2 206
China (Wuhan)[14]	10	ND	NA	1 108 000
China (Wuhan)[32]	8.36	ND	NA	926 288
Entire period	3.53	2.80	Age, sex, test	–
China (Guangzhou), blood donors[60]	0.09	ND	NA	104 783
China (several regions)[40]				
Hubei (not Wuhan)	3.6	ND	NA	1 718 110
Chongqing	3.8	ND	NA	11 956 109
Sichuan	0.6	ND	NA	487 847
Guangdong	2.2	ND	NA	2 522 010
Croatia[26]	1.27[c]	ND	NA	51 765
Denmark, blood donors[12]	2	1.9	Test	109 665
Denmark (Faroe Islands)[52]	0.6	0.7	Test	365
France (Crepy-en-Valois)[39]	10.4	ND	NA	620 105
France (Oise)[13]	25.9	ND	NA	1 548 000
Germany (Gangelt)[16]	15	20.0	Test, cluster, symptoms	2 519
Germany (Frankfurt)[21]	0.6	ND	NA	16 086
Greece[62]	0.42 (April)	0.49[d]	Age, sex, region	51 023
Hungary[57]	0.67	0.68	Design, age, sex, district	65 671
Iceland[58]	2.3 (quarantined), 0.3 (unknown exposure)	0.9	Including those positive by RT-PCR	3 177
India (Mumbai)[61]				534 750
Slum areas	54.1	58.4	Test, age, sex	–
Non-slum areas	16.1	17.3	Test, age, sex	–
India (Srinagar)[67]	3.8	3.6	Age, sex	54 000
Islamic Republic of Iran (Guilan)[8]	22	33.0	Test, sampling	770 000
Italy (Apulia), blood donors[31]	0.99	ND	NA	39 887
Japan (Kobe)[11]	3.3	2.7	Age, sex	40 999
Japan (Tokyo)[29]	3.83	ND	NA	532 450
Japan (Utsunomiya City)[48]	0.4	1.23	Age, sex, distance to clinic, district, cohabitants	6 378
Kenya, blood donors[44]	5.6	5.2	Age, sex, region, test	2 783 453
Luxembourg[20]	1.9	2.1	Age, sex, district	12 684
Netherlands, blood donors[15]	2.7	ND	NA	461 622
Netherlands (Rotterdam)[64]	3	ND	NA	512 910
Pakistan (Karachi)[49]	16.3	11.9	Age, sex	1 987 300
East	20.0	15.1	Age, sex	–
Malir	12.7	8.7	Age, sex	–
Pakistan (urban)[66]	17.5	ND	NA	13 825 000
Qatar[51]	30.4	ND	NA	851 200

(*continues. . .*)

(. . .*continued*)

Country (location)	Seroprevalence, %			Estimated no. of people infected
	Crude	Adjusted		
		Value	Adjustments	
Entire period	24.0	ND	NA	–
Republic of Korea[59]	0.07	ND	NA	1 867
Spain[36]	ND	5.0[e]	Sampling, age, sex, income	2 347 000
Spain (Barcelona)[30]	14.3	ND	NA	1 081 938
Switzerland (Geneva)[10]	10.6	10.9	Test, age, sex	54 500
Switzerland [28]				
Zurich[f]	Unclear	1.3	Multivariate Gaussian conditioning	19 773
Zurich and Lucerne[g]	Unclear	1.6	Multivariate Gaussian conditioning	30 888
United Kingdom (England)[65]	5.6	6.0	Test, sampling	3 360 000
United Kingdom (Scotland) blood donors[18]	1.2	ND	NA	64 800
USA (10 states)[35]				
Washington, Puget Sound	1.3	1.1	Age, sex, test	48 291
Utah	2.4	2.2	Age, sex, test	71 550
New York, New York City	5.7	6.9	Age, sex, test	641 778
Missouri	2.9	2.7	Age, sex, test	161 936
Florida, south	2.2	1.9	Age, sex, test	117 389
Connecticut	4.9	4.9	Age, sex, test	176 012
Louisiana	ND	5.8	Age, sex, test	267 033
California, San Francisco Bay	ND	1	Age, sex, test	64 626
Pennsylvania, Philadelphia	ND	3.2	Age, sex, test	156 633
Minnesota, Minneapolis	ND	2.4	Age, sex, test	90 651
USA (California, Bay Area) blood donors[24]	0.4	0.1	Test and confirmation	7 753
USA (California, Los Angeles)[22]	4.06	4.65	Test, sex, race and ethnicity, income	367 000
USA (California, San Francisco), in census tract 022 901[33]	4.3	6.1	Age, sex, race and ethnicity, test	316
USA (California, Santa Clara)[19]	1.5	2.6	Test, sampling, cluster	51 000
USA (Idaho, Boise)[9]	1.79	ND	NA	8620
USA (Georgia, DeKalb and Fulton counties)[53]	2.7	2.5	Age, sex, race and ethnicity	45 167
USA (Idaho, Blaine County)[46]	22.4	23.4	Test, age, sex, household	5 403
USA (Indiana)[54]	2.3 (IgG and RT-PCR)[h]	2.8	Age, race, Hispanic ethnicity	187 802
USA (Louisiana, Baton Rouge)[63]	6	6.6	Census, race, parish, including RT-PCR positives	46 147
USA (Louisiana, Orleans and Jefferson Parish)[37]	6.9 (IgG and RT-PCR)[h]	6.9 for IgG	Census weighting, demographics	56 578
USA (New York)[23]	12.5	14.0	Test, sex, age race and ethnicity, region	2 723 000
USA, New York[66]				
Columbia University Medical Center, New York City	5	ND	NA	463 044
CareMount central laboratory, five New York state counties	1.8	ND	NA	183 404
USA (New York, Brooklyn)[27]	47	ND	NA	1 203 154
USA (Rhode Island), blood donors[45]	3.9	ND	NA	41 384

COVID-19: coronavirus disease 2019; NA: not applicable; ND: no data available; RT-PCR: real-time polymerase chain reaction; test: test performance.

[a] The authors calculated 760 000 to be infected in the 90 cities that had 200–250 samples tested, but many of the other 43 cities with < 200 samples may be equally or even better represented since they tended to be smaller than the 90 cities (mean population 356 213 versus 659 326).

[b] An estimate is also provided adjusting for test performance, but the assumed specificity of 99.0% seems inappropriately low, since as part of the validation process the authors found that several of the test-positive individuals had household members who were also infected, thus the estimated specificity was deemed by the authors to be at least 99.95%.

[c] 1.20% in workers in Split without mobility restrictions, 3.37% in workers in Knin without mobility restrictions, 1.57% for all workers without mobility restrictions; Split and Knin tended to have somewhat higher death rates than nationwide Croatia, but residence of workers is not given, so the entire population of the country is used in the calculations.

[d] An estimate is also provided adjusting for test performance resulting in adjusted seroprevalence of 0.23%, but this seems inappropriately low, since the authors report that all positive results were further validated by ELISA (enzyme-linked immunosorbent assay).

[e] 5.0% with point of care test, 4.6% with immunoassay, 3.7% with both tests positive, 6.2% with at least one test positive.

[f] Patients during 1–15 April.

[g] Blood donors in May.

[h] The study counts in prevalence also those who were currently/recently infected as determined by a positive RT-PCR.

Notes: Of the studies where seroprevalence was evaluated at multiple consecutive time points, the seroprevalence estimate was the highest in the most recent time interval with few exceptions, for example: in the Switzerland (Geneva) study,[10] the highest value was seen 2 weeks before the last time interval; in the Switzerland (Zurich) study,[28] the highest value was seen in the period 1–15 April for patients at the university hospital and in May for blood donors; and in the China (Wuhan) study,[32] the highest value was seen about 3 weeks before the last time interval.

Table 4. **Deaths from COVID-19 and inferred infection fatality rates, overall and in people younger than 70 years, by location, 2020**

Location	No. of site-specific cumulative deaths from COVID-19 (to date)[a]	Inferred infection fatality rate, % (corrected)	% of site-specific cumulative deaths from COVID-19 in people < 70 years[a]	Infection fatality rate in people < 70 years, % (corrected)
Argentina (Barrio Padre Mugica)[47]	44 (1 July)	0.16 (0.13)	~70	0.11 (0.09)
Belgium[38]	7594 (30 April)	1.09 (0.87)	10	0.13 (0.10)
Brazil (133 cities)[25]	–[b]	Median 0.30 (0.27)	31 (< 60 years)	0.10 (0.09)
Brazil (Espirito Santo)[34]	363 (21 May)	0.43 (0.39)	31 (Brazil, < 60 years)	0.14 (0.13)
Brazil (Maranhao)[68]	4272 (8 August)	0.15 (0.14)	23	0.04 (0.03)
Brazil (Rio de Janeiro), blood donors[41]	1019 (3 May)	0.12 (0.11)	31 (Brazil, < 60 years)	0.04 (0.04)
Brazil (Rio Grande do Sul)[17]	124 (14 May)	0.49 (0.39)	31 (Brazil, < 60 years)	0.19 (0.15)
Brazil (Sao Paulo)[42]	NA[c] (15 May)	Unknown, but likely > 0.4	31 (Brazil, < 60 years)	Unknown, but likely > 0.1
Canada (British Columbia)[50]	164 (28 May)	0.59 (0.59)	13	0.08 (0.08)
Chile (Vitacura)[43]	NA[c] (18 May)	Unknown, but likely < 0.2	36 (Chile)	Unknown, but likely < 0.1
China, blood donors[55]				
Wuhan	1935 (20 February)	0.45 (0.41)	50	0.24 (0.22)
Shenzhen	1 (5 March)	0.01 (0.01)	About 50 (if similar to Wuhan)	0.01 (0.01)
Shijiazhuang	1 (27 February)	0.05 (0.04)	About 50 (if similar to Wuhan)	0.03 (0.02)
China (Wuhan)[14]	3869 (2 May)	0.35 (0.31)	50	0.19 (0.15)
China (Wuhan)[32]	3869 (13 April)	0.42 (0.38)	50	0.23 (0.21)
China (Guangzhou), blood donors[60]	8 (5 April)	0.00 (0.00)	About 50 (if similar to Wuhan)	0.00 (0.00)
China (several regions)[40]				
Hubei (not Wuhan)	643 (12 April)	0.04 (0.03)	About 50 (if similar to Wuhan)	0.02 (0.02)
Chongqing	6 (12 April)	0.00 (0.00)	About 50 (if similar to Wuhan)	0.00 (0.00)
Guangdong	8 (12 April)	0.00 (0.00)	About 50 (if similar to Wuhan)	0.00 (0.00)
Sichuan	3 (12 April)	0.00 (0.00)	About 50 (if similar to Wuhan)	0.00 (0.00)
Croatia[26]	79 (3 May)	0.15 (0.14)	13	0.02 (0.02)
Denmark, blood donors[12]	370 (21 April)	0.34 (0.27)	12	0.05 (0.04)
Faroe Islands[52]	0 (5 May)	0.00 (0.00)	0	0.00 (0.00)
France (Crepy-en-Valois)[39]	2325 (5 May)[d]	0.37 (0.30)	7 (France, < 65 years)	0.04 (0.03)
France (Oise)[13]	932 (7 April)[d]	0.06 (0.05)	7 (France, < 65 years)	0.01 (0.01)
Germany (Gangelt)[16]	7 (15 April)	0.28 (0.25)	0	0.00 (0.00)
Germany (Frankfurt)[21]	42[e] (17 April)	0.26 (0.21)	14 (Germany)	0.04 (0.03)
Greece[62]	121 (22 April)	0.24 (0.19)	30	0.09 (0.07)
Hungary[57]	442 (15 May)	0.67 (0.54)	No data	No data
Iceland[58]	10 (1 June)	0.30 (0.30)	30	0.10 (0.10)
India (Mumbai)[61]	495 (13–20 July)	0.09 (0.07)	50 (< 60 years, India)	0.04 (0.03)
India (Srinagar)[67]	35 (15 July)[f]	0.06 (0.05)	50 (< 60 years, India)	0.03 (0.03)
Islamic Republic of Iran (Guilan)[8]	617 (23 April)	0.08 (0.07)	No data	No data
Italy (Apulia), blood donors[31]	530 (22 May)	1.33 (1.20)	15 (Italy)	0.24 (0.22)
Japan (Kobe)[11]	10 (mid-April)	0.02 (0.02)	21 (Japan)	0.01 (0.01)
Japan (Tokyo)[29]	189 (11 May)	0.04 (0.03)	21 (Japan)	0.01 (0.01)
Japan (Utsunomiya City)[48]	0 (14 June)	0.00 (0.00)	0	0.00 (0.00)
Kenya, blood donors[44]	64 (31 May)	0.00 (0.00)	58 (< 60 years)	0.00 (0.00)
Luxembourg[20]	92 (2 May)	0.73 (0.58)	9	0.07 (0.06)
Netherlands, blood donors[15]	3134 (15 April)	0.68 (0.68)	11	0.09 (0.09)
Netherlands (Rotterdam)[64]	3134 (15 April)	0.65 (0.52)	11	0.08 (0.06)
Pakistan (Karachi)[49]	~1500 (9 July)[g]	0.08 (0.07)	~70	0.06 (0.05)
Pakistan (urban)[66]	5266 (13 July)[h]	0.04 (0.04)	~70	0.03 (0.03)
Qatar[51]	93 (19 June)	0.01 (0.01)	74	0.01 (0.01)
Republic of Korea[59]	2 (3 June)[i]	0.10 (0.09)	0	0.00 (0.00)
Spain[36]	26 920 (11 May)	1.15 (0.92)	13	0.18 (0.14)
Spain (Barcelona)[30]	5137 (2 May)	0.48 (0.48)	13 (Spain)	0.07 (0.07)
Switzerland (Geneva)[10]	243 (30 April)	0.45 (0.36)	8	0.04 (0.03)

(*continues. . .*)

(. . .*continued*)

Location	No. of site-specific cumulative deaths from COVID-19 (to date)[a]	Inferred infection fatality rate, % (corrected)	% of site-specific cumulative deaths from COVID-19 in people < 70 years[a]	Infection fatality rate in people < 70 years, % (corrected)
Switzerland (Zurich)[28]	107 (15 April, Zurich), 147 (22 May, Zurich and Lucerne)	0.51 (0.41)	8 (Switzerland)	0.05 (0.04)
England[65]	38 854 (9 July)	1.16 (0.93)	20	0.27 (0.22)
Scotland, blood donors[18]	47 (1 April)	0.07 (0.06)	9 (< 65 years)	0.01 (0.01)
USA (10 states)[35]				
Washington, Puget Sound	207 (4 April)	0.43 (0.43)	10 (state, < 60 years)	0.05 (0.05)
Utah	58 (4 May)	0.08 (0.08)	28 (< 65 years)	0.03 (0.03)
New York	4146 (4 April)	0.65 (0.65)	34 (state)	0.25 (0.25)
Missouri	329 (30 April)	0.20 (0.20)	23	0.05 (0.05)
Florida, south	295 (15 April)	0.25 (0.25)	28 (state)	0.08 (0.08)
Connecticut	2718 (6 May)	1.54 (1.54)	18	0.31 (0.31)
Louisiana	806 (11 April)	0.30 (0.30)	32	0.10 (0.10)
California, San Francisco Bay	321 (1 May)	0.50 (0.50)	25	0.14 (0.14)
Pennsylvania, Philadelphia	697 (26 April)	0.45 (0.45)	21 (state)	0.10 (0.10)
Minnesota, Minneapolis	436 (13 May)	0.48 (0.48)	20 (state)	0.10 (0.10)
USA (California, Bay Area)[24]	12 (22 March)	0.15 (0.12)	25	0.04 (0.03)
USA (California, Los Angeles)[22]	724 (19 April)	0.20 (0.18)	24 (< 65 years)	0.06 (0.05)
USA (California, San Francisco)[33]	0 (4 May)	0.00 (0.00)	0	0.00 (0.00)
USA (California, Santa Clara)[19]	94 (22 April)	0.18 (0.17)	35	0.07 (0.06)
USA (Idaho, Boise)[9]	14 (24 April)	0.16 (0.13)	14 (Idaho)	0.02 (0.02)
USA (Georgia)[53]	198 (7 May)	0.44 (0.44)	30	0.15 (0.15)
USA (Idaho, Blaine County)[46]	5 (19 May)	0.10 (0.08)	14 (Idaho)	0.02 (0.01)
USA (Indiana)[54]	1099 (30 April)	0.58 (0.46)	24	0.16 (0.13)
USA (Louisiana, Baton Rouge)[63]	420 (30 July)	0.91 (0.73)	32 (Louisiana)	0.32 (0.25)
USA (Louisiana, Orleans and Jefferson Parish)[37]	925 (16 May)	1.63 (1.31)	32	0.57 (0.46)
USA (New York)[23]	18 610 (30 April)[j]	0.68 (0.54)[j]	34	0.26 (0.23)
USA (New York Columbia University Medical Center, New York City and CareMount central laboratory, five New York state counties)[56]	965 (28 March, New York state)	0.15 (0.14)	34	0.06 (0.05)
USA (New York, Brooklyn)[27]	4894 (19 May)[j]	0.41 (0.33)[j]	34 (New York state)	0.15 (0.14)
USA (Rhode Island), blood donors[45]	430 (11 May)	1.04 (0.83)	17	0.20 (0.16)

COVID-19: coronavirus disease 2019; NA: not available.

[a] Whenever the number or proportion of COVID-19 deaths at age < 70 years was not provided in the paper, I retrieved the proportion of these deaths from situation reports of the relevant location. If I could not find this information for the specific location, I used a larger geographic area. For Brazil, the closest information that I found was from a news report.[77] For Croatia, I retrieved data on age for 45/103 deaths through Wikipedia.[78] Geographical location in parentheses specifies the population

[b] Data are provided by the authors for deaths per 100 000 population in each city along with inferred infection fatality rate in each city, with wide differences across cities; the infection fatality rate shown here is the median across the 36 cities with 200–250 samples and at least one positive sample (the interquartile range for the uncorrected infection fatality rate is 0.20–0.60% and across all cities is 0–2.4%, but with very wide uncertainty in each city). A higher infection fatality rate is alluded to in the preprint, but the preprint also shows a scatter diagram for survey-based seroprevalence versus reported deaths per population with a regression slope that agrees with an infection fatality rate of 0.3%.

[c] Information on deaths was not available for the specific locations. In the Sao Paulo study, the authors selected six districts of Sao Paulo most affected by COVID-19; they do not name the districts and the number of deaths as of mid-May is not available, but using data for death rates across all Sao Paulo would give an infection fatality rate of > 0.4% overall. In the Vitacura study, similarly one can infer from the wider Santiago metropolitan area that the infection fatality rate in the Vitacura area would probably be < 0.2% overall.

[d] For France, government situation reports provide the number of deaths per region only for in-hospital deaths; therefore, I multiplied the number of in-hospital deaths by a factor equal to: total number of deaths/in-hospital deaths for all of France.

[e] Estimated from number of deaths in Hesse province on 17 April × proportion of deaths in the nine districts with key enrolment (enrolment ratio > 1:10 000) in the study among all deaths in Hesse province.

[f] I calculated the approximate number of deaths assuming the same case fatality ratio in the Srinagar district as in the Jammu and Kashmir state where it is located.

[g] For Karachi, it is assumed that about 30% of COVID-19 deaths in Pakistan are in Karachi (since about 30% of the cases are there).

[h] The number of deaths across all Pakistan; I assumed that this number is a good approximation of deaths in urban areas (most deaths occur in urban areas and there is some potential underreporting).

[i] I calculated the approximate number of deaths from the number of cases in the study areas in south-western Seoul, assuming a similar case fatality as in Seoul overall.

[j] Confirmed COVID-19 deaths; inclusion of probable COVID-19 deaths would increase the infection fatality rate estimates by about a quarter.

Note: Cumulative deaths are sourced from the specific study or from situation report on the same location unless otherwise stated.

0.09%, 0.20% and 0.57%, respectively, for the three location groups.

For people younger than 70 years old, the infection fatality rate of COVID-19 across 40 locations with available data ranged from 0.00% to 0.31% (median 0.05%); the corrected values were similar.

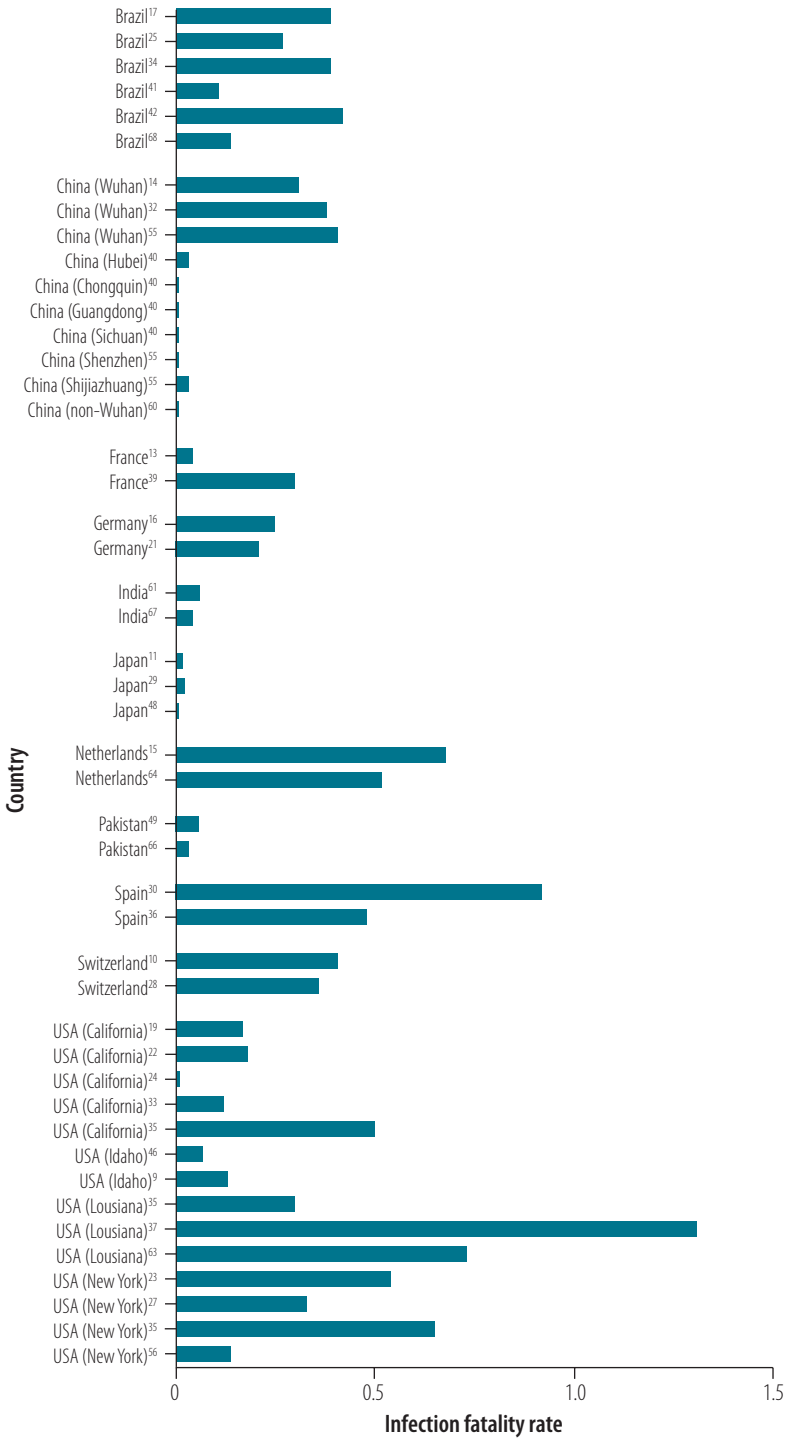
Discussion

The infection fatality rate is not a fixed physical constant and it can vary substantially across locations, depending on the population structure, the case-mix of infected and deceased individuals and other, local factors. The studies analysed here represent 82 different estimates of the infection fatality rate of COVID-19, but they are not fully representative of all countries and locations around the world. Most of the studies are from locations with overall COVID-19 mortality rates that are higher than the global average. The inferred median infection fatality rate in locations with a COVID-19 mortality rate lower than the global average is low (0.09%). If one could sample equally from all locations globally, the median infection fatality rate might even be substantially lower than the 0.23% observed in my analysis.

COVID-19 has a very steep age gradient for risk of death.[80] Moreover, in European countries that have had large numbers of cases and deaths[81], and in the USA[82], many, and in some cases most, deaths occurred in nursing homes. Locations with many nursing home deaths may have high estimates of the infection fatality rate, but the infection fatality rate would still be low among non-elderly, non-debilitated people.

Within China, the much higher infection fatality rate estimates in Wuhan compared with other areas of the country may reflect widespread nosocomial infections,[83] as well as unfamiliarity with how to manage the infection as the first location that had to deal with COVID-19. The very many deaths in nursing homes, nosocomial infections and overwhelmed hospitals may also explain the high number of fatalities in specific locations in Italy[84] and New York and neighbouring states.[23,27,35,56] Poor decisions (e.g. sending COVID-19 patients to nursing homes), poor management (e.g. unnecessary mechanical ventilation and hydroxychloroquine) may also have contributed to worse outcomes.

Fig. 2. **Estimates of infection fatality rates for COVID-19 in locations that had two or more estimates, 2020**



COVID-19: coronavirus disease 2019.
Notes: Locations are defined at the level of countries, except for the United States of America where they are defined at the level of states and China is separated into Wuhan and non-Wuhan areas. Corrected infection fatality rate estimates are shown (correcting for what types of antibodies were assayed).

High levels of congestion (e.g. in busy public transport systems) may also have exposed many people to high infectious loads and, thus, perhaps more severe disease. A more aggressive viral clade has also been speculated.[85] The infection fatality rate may be very high among disadvantaged populations and in settings with a combination of factors predisposing to higher fatalities.[37]

Very low infection fatality rates seem common in Asian coun-

Table 5. **Infection fatality rates for COVID-19 inferred from preliminary nationwide seroprevalence data, 2020**

Country	Sample size	Date	Reported seroprevalence (%)	Population, no.	Deaths, no. (date)	Inferred infection fatality rate (corrected), %
Afghanistan[75]	9 500 (NR)	NR	31.5	39 021 453	1 300 (8 May)	0.01 (0.01)
Czechia[71]	26 549 (IgG)	23 April–1 May	0.4	10 710 000	252 (4 May)	0.59 (0.47)
Finland[69]	674 (IgG)	20–26 April[a]	2.52	5 541 000	211 (30 April)	0.15 (0.12)
Georgia[76]	1 068 (NR)	18–27 May	1	3 988 264	12 (30 May)	0.03 (0.03)[b]
Israel[72]	1 709 (NR)	May	2–3	9 198 000	299 (10 June)	0.13 (0.10)[c]
Russian Federation[74]	650 000 (NR)	NR	14	145 941 776	5 859 (7 June)	0.03 (0.03)
Slovenia[73]	1 368 (NR)	April	3.1	2 079 000	92 (1 May)	0.14 (0.11)
Sweden[70]	1 200 (IgG)	18–24 May	6.3	10 101 000	4 501 (28 May)	0.71 (0.57)

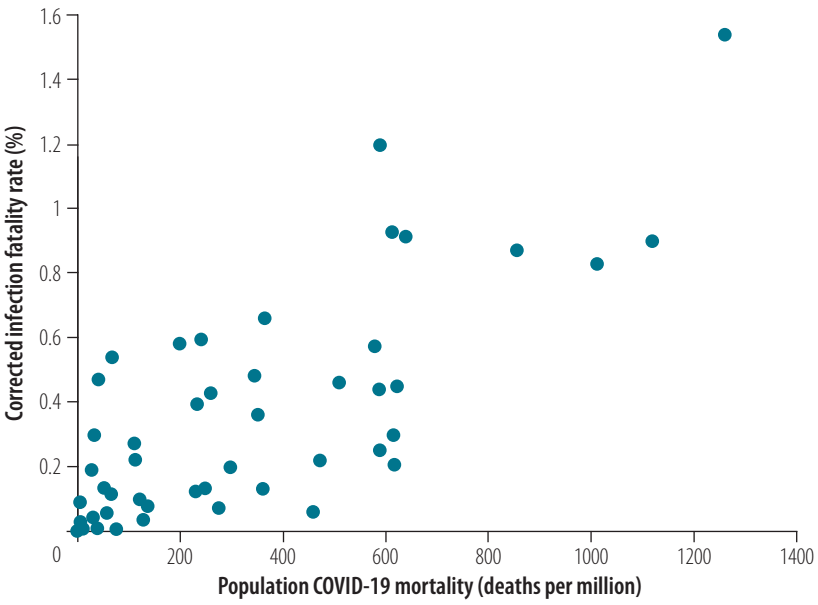
COVID-19: coronavirus disease 2019; Ig: immunoglobin; NR: not reported.
[a] The seroprevalence was slightly lower in subsequent weeks.
[b] The survey was done in Tbilisi, the capital city with a population 1.1 million. I could not retrieve the count of deaths in Tbilisi, but if more deaths happened in Tbilisi, then the infection fatality rate may be higher, but still < 0.1%.
[c] Assuming a seroprevalence of 2.5%.
Notes: These are countries for which no eligible studies were retrieved in the literature search. The results of these studies have been announced to the press and/or in preliminary reports, but are not yet peer reviewed and published.

tries.[8,11,29,48,49,51,59,61,67] A younger population in these countries (excluding Japan), previous immunity from exposure to other coronaviruses, genetic differences, hygiene etiquette, lower infectious load and other unknown factors may explain these low rates. The infection fatality rate is low also in low-income countries in both Asia and Africa,[44,49,66,67] perhaps reflecting the young age structure. However, comorbidities, poverty, frailty (e.g. malnutrition) and congested urban living circumstances may have an adverse effect on risk and thus increase infection fatality rate.

Antibody titres may decline with time[10,28,32,86,87] and this would give falsely low prevalence estimates. I considered the maximum seroprevalence estimate when multiple repeated measurements at different time points were available, but even then some of this decline cannot be fully accounted for. With four exceptions,[10,28,32,51] the maximum seroprevalence value was at the latest time point.

Positive controls for the antibody assays used were typically symptomatic patients with positive polymerase chain reaction tests. Symptomatic patients may be more likely to develop antibodies.[87–91] Since seroprevalence studies specifically try to reveal undiagnosed asymptomatic and mildly symptomatic infections, a lower sensitivity for these mild infections could lead to substantial underestimates of the number of

Fig. 3. **Corrected estimates of COVID-19 infection fatality rate in each location plotted against COVID-19 cumulative deaths per million as of September 12 2020 in that location**



COVID-19: coronavirus disease 2019.
Notes: Locations are defined at the level of countries, except for the United Kingdom of Great Britain and Northern Ireland where they are defined by jurisdiction, United States of America (USA) are defined at the level of states and China is separated into Wuhan and non-Wuhan areas. Included locations are: Afghanistan; Argentina; Belgium; Brazil; Canada; Chile; China (non-Wuhan and Wuhan); Croatia; Czechia; Denmark; Faroe Islands; Finland; France; Georgia; Germany; Greece; Hungary; Iceland; India; Iran (Islamic Republic of); Israel; Italy; Japan; Kenya; Luxembourg; Netherlands; Pakistan; Qatar; Republic of Korea; Russian Federation; Slovenia; Spain; Sweden; Switzerland; United Kingdom (England, Scotland); and USA (California, Connecticut, Florida, Georgia, Idaho, Indiana, Louisiana, Minnesota, Missouri, New York, Pennsylvania, Rhode Island, Utah, Washington). When several infection fatality rate estimates were available from multiple studies for a location, the sample size-weighted mean is used. One outlier location with very high deaths per million population (1702 for New York) is not shown.

infected people and overestimates of the inferred infection fatality rate.

A main issue with seroprevalence studies is whether they offer a representative picture of the population in the assessed region. A generic problem is that vulnerable people at high risk of infection and/or death may be more difficult to recruit in survey-type studies. COVID-19 infection is particularly widespread and/or lethal in nursing homes, in homeless people, in prisons and in disadvantaged minorities.[92] Most of these populations are very difficult, or even impossible, to reach and sample and they are probably under-represented to various degrees (or even entirely missed) in surveys. This sampling obstacle would result in underestimating the seroprevalence and overestimating infection fatality rate.

In principle, adjusted seroprevalence values may be closer to the true estimate, but the adjustments show that each study alone may have unavoidable uncertainty and fluctuation, depending on the type of analysis chosen. Furthermore, my corrected infection fatality rate estimates try to account for undercounting of infected people when not all three antibodies (IgG, IgM and IgA) were assessed. However, the magnitude of the correction is uncertain and may vary in different circumstances. An unknown proportion of people may have responded to the virus using immune mechanisms (mucosal, innate, cellular) without generating any detectable serum antibodies.[93–97]

A limitation of this analysis is that several studies included have not yet been fully peer-reviewed and some are still ongoing. Moreover, despite efforts made by seroprevalence studies to generate estimates applicable to the general population, representativeness is difficult to ensure, even for the most rigorous studies and despite adjustments made. Estimating a single infection fatality rate value for a whole country or state can be misleading, when there is often huge variation in the population mixing patterns and pockets of high or low mortality. Furthermore, many studies have evaluated people within restricted age ranges, and the age groups that are not included may differ in seroprevalence. Statistically significant, modest differences in seroprevalence across some age groups have been observed in several studies.[10,13,15,23,27,36,38] Lower values have been seen in young children and higher values in adolescents and young adults, but these patterns are inconsistent and not strong enough to suggest that major differences are incurred by extrapolating across age groups.

Acknowledging these limitations, based on the currently available data, one may project that over half a billion people have been infected as of 12 September 2020, far more than the approximately 29 million documented laboratory-confirmed cases. Most locations probably have an infection fatality rate less than 0.20% and with appropriate, precise non-pharmacological measures that selectively try to protect high-risk vulnerable populations and settings, the infection fatality rate may be brought even lower. ■

Funding: METRICS has been supported by a grant from the Laura and John Arnold Foundation.

Competing interests: I am a co-author (not principal investigator) of one of the seroprevalence studies.

ملخص

معدل وفيات عدوى كوفيد 19 المستدل عليه من بيانات الانتشار المصلي

الغرض تقدير معدل الوفيات الناجمة عن الإصابة بمرض فيروس كورونا 2019 (كوفيد 19) من بيانات الانتشار المصلي.

الطريقة قمت بالبحث في خوادم PubMed وخوادم ما قبل الطباعة عن دراسات الانتشار المصلي لكوفيد 19، بحجم عينة أكبر من أو تساوي 500 بدءاً من 9 سبتمبر/أيلول 2020. كما أنني استرجعت النتائج الإضافية للدراسات الوطنية من البيانات الصحفية والتقارير الأولية. قمت بتقييم دراسات ميزات التصميم وتقديرات الانتشار المصلي. لقد قمت بتقدير معدل الوفيات الناجمة عن الإصابة لكل دراسة عن طريق قسمة العدد الإجمالي للوفيات الناتجة عن جائحة كوفيد 19، على عدد الأشخاص المقدر إصابتهم في كل منطقة. وقمت بتصحيح عدد أنواع الأجسام المضادة التي تم اختبارها (الغلوبين المناعي، IgA ، IgM ، IgG).

النتائج قمت بتضمين 61 دراسة (74 تقديرًا) وثمانية تقديرات وطنية أولية. تراوحت تقديرات الانتشار المصلي من 0.02% إلى 53.40%. تراوحت معدلات وفيات العدوى من 0.00% إلى 1.63%، وتم تصحيح القيم من 0.00% إلى 1.54%. عبر 51 موقعًا، كان متوسط معدل وفيات عدوى كوفيد 19 هو 0.27% (تصحيح بنسبة 0.23%): كان المعدل 0.09% في المواقع التي تقل فيها معدلات وفيات السكان المصابين بكوفيد 19 عن المتوسط العالمي (أكثر من 118 حالة وفاة/مليون نسمة)، و0.20% في المواقع التي يوجد بها من 118 إلى 500 حالة وفاة/مليون نسمة مصابين بكوفيد 19، و0.57% في مواقع بها أكثر من 500 حالة وفاة/مليون نسمة بسبب كوفيد 19. في الأشخاص الذين تقل أعمارهم عن 70 عامًا، تراوحت معدلات وفيات الإصابة بالعدوى من 0.00% إلى 0.31% بمتوسطات مبدئية ومصححة قدرها 0.05%.

الاستنتاج يمكن أن يختلف معدل وفيات الإصابة بفيروس كوفيد 19 بشكل كبير عبر المواقع المختلفة، وقد يعكس هذا الاختلافات في التركيب العمري للسكان، ومزيج الحالات من المرضى المصابين والمتوفين، وعوامل أخرى. تميل معدلات الوفيات المستدل عنها من العدوى إلى أن تكون أقل بكثير من التقديرات التي تم إجراؤها في وقت سابق في الجائحة.

摘要

根据血清阳性率数据推断新型冠状病毒肺炎的感染死亡率

目的 根据血清阳性率数据估计 2019 年冠状病毒病（新型冠状病毒肺炎）的感染死亡率。

方法 在 PubMed 和预印本服务器上查找截至 2020 年 9 月 9 日新型冠状病毒肺炎相关的血清阳性率研究，样本量为 500 个。另外根据初步新闻稿和报告检索了其他全国性研究结果。并评估了与设计特征和血清阳性率估计值相关的研究。通过将新型冠状病毒肺炎累计死亡人数除以每个地区估计感染人数，估算出了每项研究的感染死亡率。然后校正了测试的抗体类型（免疫球蛋白、免疫球蛋白 G、免疫球蛋白 M、免疫球蛋白 A）的数量。

结果 我汇总了 61 项研究（74 个估计值）和 8 个全国性初步估计值。血清阳性率估计值介于 0.02% 至 53.40% 之间。感染死亡率介于 0.00% 至 1.63% 之间，校正值则介于 0.00% 至 1.54% 之间。在 51 个地区中，新型冠状病毒肺炎感染死亡率的中位数为 0.27%（校正值为 0.23%）：在新型冠状病毒肺炎导致的人口死亡率低于全球平均水平（每一百万人口中死亡病例小于 118 例）的地区中，该比率为 0.09%；在每一百万人口中新型冠状病毒肺炎死亡病例介于 118 – 500 例之间的地区，该比率为 0.20%；而在每一百万人口中新型冠状病毒肺炎死亡病例大于 500 例的地区，该比率则为 0.57%。70 岁以下人群的感染死亡率介于 0.00% 至 0.31% 之间，经粗略校正后该比率的中位数为 0.05%。

结论 不同地区的新型冠状病毒肺炎感染死亡率可能存在很大的差异，据此可反映出在人口年龄结构、感染和死亡病例组合以及其他因素方面存在差异。推断的感染死亡率往往比全球性流行病爆发初期的估计值要低得多。

Résumé

Ratio de létalité réel de la COVID-19 déduit à partir des données de séroprévalence

Objectif Estimer le ratio de létalité réel de la maladie à coronavirus 2019 (COVID-19) à partir des données de séroprévalence.

Méthodes J'ai effectué des recherches sur PubMed et sur les serveurs de prépublication afin de trouver des études consacrées à la séroprévalence de la COVID-19, avec des échantillons ≥ 500, au 9 septembre 2020. J'ai également prélevé des résultats supplémentaires dérivés d'études nationales qui figurent dans les versions préliminaires de divers rapports et communiqués de presse. J'ai analysé les études pour y déceler des caractéristiques de conception et des estimations de séroprévalence. Ensuite, j'ai calculé le ratio de létalité réel pour chaque étude en divisant le nombre cumulé de décès dus à la COVID-19 par le nombre d'individus qui auraient été infectés dans chaque région. Enfin, j'ai apporté des corrections en fonction des types d'anticorps testés (immunoglobulines, IgG, IgM, IgA).

Résultats J'ai pris 61 études en compte (74 estimations) et huit estimations nationales préliminaires. Les estimations en matière de séroprévalence étaient comprises entre 0,02% et 53,40%. Les ratios de létalité réels allaient de 0,00% à 1,63%, les valeurs corrigées de 0,00% à 1,54%. Dans les 51 lieux étudiés, la médiane du ratio de létalité réel pour la COVID-19 s'élevait à 0,27% (0,23% après correction): le ratio était de 0,09% dans les endroits où le taux de mortalité dû à la COVID-19 était inférieur à la moyenne mondiale (< 118 décès/million d'habitants), de 0,20% dans les endroits dénombrant 118–500 décès COVID-19/million d'habitants, et de 0,57% là où la COVID-19 était responsable de > 500 décès/million d'habitants. Chez les personnes de moins de 70 ans, les ratios de létalité réels se situaient entre 0,00% et 0,31% avec des médianes brutes et corrigées de 0,05%.

Conclusion Le ratio de létalité réel de la COVID-19 peut considérablement varier d'un endroit à l'autre, ce qui pourrait correspondre aux différences de structure de pyramide des âges au sein de la population, au case-mix entre patients infectés et décédés, ainsi qu'à d'autres facteurs. Les ratios de létalité réels que j'ai pu déduire avaient tendance à être nettement inférieurs aux estimations formulées précédemment durant la pandémie.

Резюме

Показатель летальности при инфицировании COVID-19, определенный на основании данных о сераспространенности

Цель Оценить показатель летальности при инфицировании коронавирусным заболеванием 2019 г. (COVID-19) на основании данных о сераспространенности.

Методы Автор провел поиск на серверах PubMed и серверах предварительной публикации на предмет исследований сераспространенности COVID-19 с размером выборки ≥500 по состоянию на 9 сентября 2020 года. Были также получены дополнительные результаты национальных исследований из предварительных пресс-релизов и отчетов. Автор оценил исследования по элементам дизайна и оценкам сераспространенности. Автор оценил показатель летальности при инфицировании для каждого исследования, разделив общее количество смертей от COVID-19 на количество людей, предположительно инфицированных в каждом регионе. При этом была сделана поправка на количество протестированных типов антител (иммуноглобины, IgG, IgM, IgA).

Результаты В работу вошло 61 исследование (74 прогноза) и восемь предварительных национальных прогнозов. Прогнозы сераспространенности варьировались в диапазоне от 0,02 до 53,40%. Показатели летальности при инфицировании варьировались в диапазоне от 0,00 до 1,63%, скорректированные значения — от 0,00 до 1,54%. В 51 регионе средний показатель летальности при инфицировании COVID-19 составил 0,27% (скорректированный показатель 0,23%): этот показатель составил 0,09% в регионах с уровнем летальности населения от COVID-19 ниже, чем в среднем по миру (<118 смертей на миллион), 0,20% в регионах, в которых зарегистрировано 118–500 случаев смерти от COVID-19 на миллион человек, и 0,57% в регионах, где зарегистрировано более 500 случаев смерти от COVID-19 на миллион человек. У людей младше 70 лет показатель летальности при инфицировании колебался в пределах от 0,00 до

0,31% с приблизительными и скорректированными медианными значениями 0,05%.

Вывод Показатель летальности при инфицировании COVID-19 может существенно различаться в разных регионах, и это может отражать различия в возрастной структуре населения, структуре случаев инфицирования и смерти пациентов, а также в других факторах. Предполагаемые показатели летальности при инфицировании, как правило, были намного ниже, чем прогнозы, сделанные ранее во время пандемии.

Resumen

Tasa de letalidad por la infección de la COVID-19 calculada a partir de los datos de seroprevalencia

Objetivo Estimar la tasa de letalidad por la infección de la enfermedad por coronavirus de 2019 (COVID-19) a partir de los datos de seroprevalencia.

Métodos Se buscaron los estudios de seroprevalencia de la COVID-19 con un tamaño de muestra mayor o igual a 500 a partir del 9 de septiembre de 2020 en PubMed y en los servidores de preimpresión. Además, se recuperaron los resultados adicionales de los estudios nacionales a partir de los comunicados de prensa y de los informes preliminares. Se evaluaron los estudios para determinar las características de diseño y las estimaciones de seroprevalencia. Para calcular la tasa de letalidad por la infección de cada estudio, se dividió la cantidad acumulada de muertes por la COVID-19 por la cantidad de personas que se estima que están infectadas en cada región. Asimismo, se corrigió la cantidad de tipos de anticuerpos probados (inmunoglobulinas, IgG, IgM, IgA).

Resultados Se incluyeron 61 estudios (74 estimaciones) y 8 estimaciones nacionales preliminares. Las estimaciones de seroprevalencia oscilaban entre el 0,02 % y el 53,40 %. Las tasas de letalidad por la infección oscilaron entre el 0,00 % y el 1,63 %, los valores corregidos entre el 0,00 % y el 1,54 %. En 51 lugares, la mediana de la tasa de letalidad por la infección de la COVID-19 fue del 0,27 % (corregida en un 0,23 %): la tasa fue del 0,09 % en lugares donde las tasas de letalidad de la población con la COVID-19 eran inferiores al promedio mundial (menos de 118 muertes/millón), del 0,20 % en lugares con 118-500 muertes a causa de la COVID-19/millón de personas y del 0,57 % en lugares con más de 500 muertes a causa de la COVID-19/millón de personas. En personas menores de 70 años, las tasas de letalidad por la infección oscilaron entre el 0,00 % y el 0,31 % con medianas brutas y corregidas del 0,05 %.

Conclusión La tasa de letalidad por infección de la COVID-19 puede variar de manera sustancial en diferentes lugares y esto puede reflejar diferencias en la estructura de edad de la población y en la variedad de casos de los pacientes infectados y fallecidos, así como en otros factores. Las tasas de letalidad por infección que se calculan tienden a ser mucho más bajas que las estimaciones realizadas a principios de la pandemia.

References

1. Melnick ER, Ioannidis JPA. Should governments continue lockdown to slow the spread of covid-19? BMJ. 2020 Jun 3;369:m1924. doi: http://dx.doi.org/10.1136/bmj.m1924 PMID: 32493767
2. WHO Director-General's opening remarks at the media briefing on COVID-19 – 3 March 2020. Geneva: World Health Organization; 2020. Available from: https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---3-march-2020 [cited 2020 May 10].
3. Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19) 16–24 February 2020. Geneva: World Health Organization; 2020. Available from: https://www.who.int/publications-detail/report-of-the-who-china-joint-mission-on-coronavirus-disease-2019-(covid-19) [cited 2020 May 10].
4. McGinty JC. How many people might one person with coronavirus infect? Wall Street Journal. February 14, 2020. Available from: https://www.wsj.com/articles/how-many-people-might-one-person-with-coronavirus-infect-11581676200 [cited 2020 Feb 27].
5. Ferguson NM, Laydon D, Nedjati-Gilani G, Imai N, Ainslie K, Baguelin M, et al. Report 9: Impact of non-pharmaceutical interventions (NPIs) to reduce COVID-19 mortality and healthcare demand. London: Imperial College; 2020. Available from: https://www.imperial.ac.uk/media/imperial-college/medicine/sph/ide/gida-fellowships/Imperial-College-COVID19-NPI-modelling-16-03-2020.pdf [cited 2020 May 10].
6. Meyerowitz-Katz G, Merone L. A systematic review and meta-analysis of published research data on COVID-19 infection-fatality rates [online ahead of print]. Int J Infect Dis. 2020 Sep 29;S1201(20):32180–9. PMID: 33007452
7. Garcia-Basteiro AL, Moncunill G, Tortajada M, Vidal M, Guinovart C, Jiménez A, et al. Seroprevalence of antibodies against SARS-CoV-2 among health care workers in a large Spanish reference hospital. Nat Commun. 2020 Jul 8;11(1):3500. doi: http://dx.doi.org/10.1038/s41467-020-17318-x PMID: 32641730
8. Shakiba M, Nazari S, Mehrabian F, Rezvani SM, Ghasempour Z, Heidarzadeh A. Seroprevalence of COVID-19 virus infection in Guilan province, Iran [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.04.26.20079244doi: http://dx.doi.org/10.1101/2020.04.26.20079244
9. Bryan A, Pepper G, Wener MH, Fink SL, Morishima C, Chaudhary A, et al. Performance characteristics of the Abbott Architect SARS-CoV-2 IgG assay and seroprevalence in Boise, Idaho. J Clin Microbiol. 2020 Jul 23;58(8):e00941-20. doi: http://dx.doi.org/10.1128/JCM.00941-20 PMID: 32381641
10. Stringhini S, Wisniak A, Piumatti G, Azman AS, Lauer SA, Baysson H, et al. Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Geneva, Switzerland (SEROCoV-POP): a population-based study. Lancet. 2020 Aug 1;396(10247):313–19. doi: http://dx.doi.org/10.1016/S0140-6736(20)31304-0 PMID: 32534626
11. Doi A, Iwata K, Kuroda H, Hasuike T, Nasu S, Kanda A, et al. Estimation of seroprevalence of novel coronavirus disease (COVID-19) using preserved serum at an outpatient setting in Kobe, Japan: a cross-sectional study [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.04.26.20079822doi: http://dx.doi.org/10.1101/2020.04.26.20079822
12. Erikstrup C, Hother CE, Pedersen OBV, Mølbak K, Skov RL, Holm DK, et al. Estimation of SARS-CoV-2 infection fatality rate by real-time antibody screening of blood donors. Clin Infect Dis. 2020 Jun 25; ciaa849. doi: http://dx.doi.org/10.1093/cid/ciaa849 PMID: 32584966
13. Fontanet A, Tondeur L, Madec Y, Grant R, Besombes C, Jolly N, et al. Cluster of COVID-19 in northern France: a retrospective closed cohort study [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.04.18.20071134doi: http://dx.doi.org/10.1101/2020.04.18.20071134
14. Wu X, Fu B, Chen L, Feng Y. Serological tests facilitate identification of asymptomatic SARS-CoV-2 infection in Wuhan, China. J Med Virol. 2020 Apr 20;92(10):1795–6. doi: http://dx.doi.org/10.1002/jmv.25904 PMID: 32311142
15. Slot E, Hogema BM, Reusken CBEM, Reimerink JH, Molier M, Karregat HM, et al. Herd immunity is not a realistic exit strategy during a COVID-19 outbreak [preprint]. Durham: Research Square; 2020. doi: http://dx.doi.org/https://doi.org/10.21203/rs.3.rs-25862/v1doi: http://dx.doi.org/10.21203/rs.3.rs-25862/v1
16. Streeck H, Schulte B, Kümmerer BM, Richter E, Höller T, Fuhrmann C, et al. Infection fatality rate of SARS-CoV-2 infection in a German community with a super-spreading event [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.05.04.20090076doi: http://dx.doi.org/10.1101/2020.05.04.20090076

17. Silveira MF, Barros AJD, Horta BL, Pellanda LC, Victora GD, Dellagostin OA, et al. Population-based surveys of antibodies against SARS-CoV-2 in Southern Brazil. Nat Med. 2020 Aug;26(8):1196–9. doi: http://dx.doi.org/10.1038/s41591-020-0992-3 PMID: 32641783
18. Thompson C, Grayson N, Paton RS, Lourenco J, Penman BS, Lee L. Neutralising antibodies to SARS coronavirus 2 in Scottish blood donors – a pilot study of the value of serology to determine population exposure [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.04.13.20060467doi: http://dx.doi.org/10.1101/2020.04.13.20060467
19. Bendavid E, Mulaney B, Sood N, Shah S, Ling E, Bromley-Dulfano R, et al. COVID-19 Antibody Seroprevalence in Santa Clara County, California [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.04.14.20062463doi: http://dx.doi.org/10.1101/2020.04.14.20062463
20. Snoeck CJ, Vaillant M, Abdelrahman T, Satagopam VP, Turner JD, Beaumont K, et al. Prevalence of SARS-CoV-2 infection in the Luxembourgish population: the CON-VINCE study [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.05.11.20092916doi: http://dx.doi.org/10.1101/2020.05.11.20092916
21. Kraehling V, Kern M, Halwe S, Mueller H, Rohde C, Savini M, et al. Epidemiological study to detect active SARS-CoV-2 infections and seropositive persons in a selected cohort of employees in the Frankfurt am Main metropolitan area [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.05.20.20107730doi: http://dx.doi.org/10.1101/2020.05.20.20107730
22. Sood N, Simon P, Ebner P, Eichner D, Reynolds J, Bendavid E, et al. Seroprevalence of SARS-CoV-2-specific antibodies among adults in Los Angeles County, California, on April 10–11, 2020. JAMA. 2020 06 16;323(23):2425–7. doi: http://dx.doi.org/10.1001/jama.2020.8279 PMID: 32421144
23. Rosenberg ES, Tesoriero JM, Rosenthal EM, Chung R, Barranco MA, Styer LM, et al. Cumulative incidence and diagnosis of SARS-CoV-2 infection in New York. Ann Epidemiol. 2020 Aug;48:23–29.e4. doi: http://dx.doi.org/10.1016/j.annepidem.2020.06.004 PMID: 32648546
24. Ng D, Goldgof G, Shy B, Levine A, Balcerek J, Bapat SP, et al. SARS-CoV-2 seroprevalence and neutralizing activity in donor and patient blood from the San Francisco Bay Area [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.05.19.20107482doi: http://dx.doi.org/10.1101/2020.05.19.20107482
25. Hallal PC, Hartwig FP, Horta BL, Victora GD, Silveira MF, Struchiner CJ, et al. Remarkable variability in SARS-CoV-2 antibodies across Brazilian regions: nationwide serological household survey in 27 states [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.05.30.20117531doi: http://dx.doi.org/10.1101/2020.05.30.20117531
26. Jerkovic I, Ljubic T, Basic Z, Kruzic I, Kunac N, Bezic J, et al. SARS-CoV-2 antibody seroprevalence in industry workers in Split-Dalmatia and Sibenik-Knin County, Croatia [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.05.11.20095158doi: http://dx.doi.org/10.1101/2020.05.11.20095158
27. Reifer J, Hayum N, Heszkel B, Klagsbald I, Streva VA. SARS-CoV-2 IgG antibody responses in New York City [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.05.23.20111427doi: http://dx.doi.org/10.1101/2020.05.23.20111427
28. Emmenegger M, De Cecco E, Lamparter D, Jacquat RPB, Ebner D, Schneider MM, et al. Early plateau of SARS-CoV-2 seroprevalence identified by tripartite immunoassay in a large population [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.05.31.20118554doi: http://dx.doi.org/10.1101/2020.05.31.20118554
29. Takita M, Matsumura T, Yamamoto K, Yamashita E, Hosoda K, Hamaki T, et al. Geographical profiles of COVID-19 outbreak in Tokyo: an analysis of the primary care clinic-based point-of-care antibody testing. J Prim Care Community Health. 2020 Jan-Dec;11:2150132720942695. doi: http://dx.doi.org/10.1177/2150132720942695 PMID: 32674696
30. Crovetto F, Crispi F, Llurba E, Figueras F, Gomez-Roig MD, Gratacos E. Seroprevalence and clinical spectrum of SARS-CoV-2 infection in the first versus third trimester of pregnancy [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.06.17doi: http://dx.doi.org/10.1101/2020.06.17
31. Fiore JR, Centra M, De Carlo A, Granato T, Rosa A, Sarno M, et al. Results from a survey in healthy blood donors in South Eastern Italy indicate that we are far away from herd immunity to SARS-CoV-2. J Med Virol. 2020 Aug 13;jmv.26425. doi: http://dx.doi.org/10.1002/jmv.26425 PMID: 32790086
32. Ling R, Yu Y, He J, Zhang J, Xu S, Sun R, et al. Seroprevalence and epidemiological characteristics of immunoglobulin M and G antibodies against SARS-CoV-2 in asymptomatic people in Wuhan, China [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.06.16.20132423doi: http://dx.doi.org/10.1101/2020.06.16.20132423
33. Chamie G, Marquez C, Crawford E, Peng J, Petersen M, Schwab D, et al. SARS-CoV-2 community transmission during shelter-in-place in San Francisco [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.06.15.20132233doi: http://dx.doi.org/10.1101/2020.06.15.20132233
34. Gomes CC, Cerutti C, Zandonade E, Maciel EKN, Carvalho de Alencar FE, Almada GL, et al. A population-based study of the prevalence of COVID-19 infection in Espírito Santo, Brazil: methodology and results of the first stage [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.06.13.20130559doi: http://dx.doi.org/10.1101/2020.06.13.20130559
35. Havers FP, Reed C, Lim T, Montgomery JM, Klena JD, Hall AJ, et al. Seroprevalence of antibodies to SARS-CoV-2 in 10 sites in the United States, March 23-May 12, 2020. JAMA Intern Med. 2020 Jul 21. doi: http://dx.doi.org/10.1001/jamainternmed.2020.4130 PMID: 32692365
36. Pollán M, Pérez-Gómez B, Pastor-Barriuso R, Oteo J, Hernán MA, Pérez-Olmeda M, et al.; ENE-COVID Study Group. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. Lancet. 2020 08 22;396(10250):535–44. doi: http://dx.doi.org/10.1016/S0140-6736(20)31483-5 PMID: 32645347
37. Feehan AK, Fort D, Garcia-Diaz J, Price-Haywood E, Velasco C, Sapp E, et al. Seroprevalence of SARS-CoV-2 and infection fatality ratio, Orleans and Jefferson Parishes, Louisiana, USA, May 2020. Emerg Infect Dis. 2020 Jul 30;26(11). doi: http://dx.doi.org/10.3201/eid2611.203029 PMID: 32731911
38. Herzog S, De Bie J, Abrams S, Wouters I, Ekinci E, Patteet L, et al. Seroprevalence of IgG antibodies against SARS coronavirus 2 in Belgium – a prospective cross sectional study of residual samples [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.06.08.20125179doi: http://dx.doi.org/10.1101/2020.06.08.20125179
39. Fontanet A, Grant R, Tondeur L, Madec Y, Grzelak L, Cailleau I, et al. SARS-CoV-2 infection in primary schools in northern France: A retrospective cohort study in an area of high transmission [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.06.25.20140178doi: http://dx.doi.org/10.1101/2020.06.25.20140178
40. Xu X, Sun J, Nie S, Li H, Kong Y, Liang M, et al. Seroprevalence of immunoglobulin M and G antibodies against SARS-CoV-2 in China. Nat Med. 2020 08;26(8):1193–5. doi: http://dx.doi.org/10.1038/s41591-020-0949-6 PMID: 32504052
41. Amorim Filho L, Szwarcwald CL, Mateos SOG, Leon ACMP, Medronho RA, Veloso VG, et al.; Grupo Hemorio de Pesquisa em Covid-19. Seroprevalence of anti-SARS-CoV-2 among blood donors in Rio de Janeiro, Brazil. Rev Saude Publica. 2020;54:69. PMID: 32638883
42. Tess BH, Granato CFH, Alves MC, Pintao MC, Rizzatti E, Nunes MC, et al. SARS-CoV-2 seroprevalence in the municipality of São Paulo, Brazil, ten weeks after the first reported case [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/10.1101/2020.06.29.20142331doi: http://dx.doi.org/10.1101/2020.06.29.20142331
43. Torres JP, Piñera C, De La Maza V, Lagomarcino AJ, Simian D, Torres B, et al. SARS-CoV-2 antibody prevalence in blood in a large school community subject to a Covid-19 outbreak: a cross-sectional study. Clin Infect Dis. 2020 Jul 10;ciaa955. doi: http://dx.doi.org/10.1093/cid/ciaa955 PMID: 32649743
44. Uyoga S, Adetifa IMO, Karanja HK, Nyagwange J, Tuju J, Wankiku P, et al. Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Kenyan blood donors [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.07.27.20162693doi: http://dx.doi.org/10.1101/2020.07.27.20162693
45. Nesbitt DJ, Jin D, Hogan JW, Chan PA, Simon MJ, Vargas M, et al. Low seroprevalence of SARS-CoV-2 in Rhode Island blood donors determined using multiple serological assay formats [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.07.20.20157743doi: http://dx.doi.org/10.1101/2020.07.20.20157743
46. McLaughlin CC, Doll MK, Morrison KT, McLaughlin WL, O'Connor T, Sholukh AM, et al. High community SARS-CoV-2 antibody seroprevalence in a ski resort community, Blaine County, Idaho, US. preliminary results [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.07.19.20157198doi: http://dx.doi.org/10.1101/2020.07.19.20157198

47. Figar S, Pagotto V, Luna L, Salto J, Manslau MW, Mistchenko AS, et al. Community-level SARS-CoV-2 seroprevalence survey in urban slum dwellers of Buenos Aires City, Argentina: a participatory research [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.07.14.20153858doi: http://dx.doi.org/10.1101/2020.07.14.20153858
48. Nawa N, Kuramochi J, Sonoda S, Yamaoka Y, Nukui Y, Miyazaki Y, et al. Seroprevalence of SARS-CoV-2 IgG antibodies in Utsunomiya City, Greater Tokyo, after first pandemic in 2020 (U-CORONA): a household- and population-based study [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.07.20.20155945doi: http://dx.doi.org/10.1101/2020.07.20.20155945
49. Nisar I, Ansari N, Amin M, Khalid F, Hotwani A, Rehman N, et al. Serial population based serosurvey of antibodies to SARS-CoV-2 in a low and high transmission area of Karachi, Pakistan [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.07.28.20163451doi: http://dx.doi.org/10.1101/2020.07.28.20163451
50. Skowronski DM, Sekirov I, Sabaiduc S, Zou M, Morshed M, Lawrence D, et al. Low SARS-CoV-2 sero-prevalence based on anonymized residual sero-survey before and after first wave measures in British Columbia, Canada, March–May 2020 [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.07.13.20153148doi: http://dx.doi.org/10.1101/2020.07.13.20153148
51. Abu Raddad LJ, Chemaitelly H, Ayoub HH, Al Kanaani Z, Al Khal A, Al Kuwari E, et al. Characterizing the Qatar advanced-phase SARS-CoV-2 epidemic [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.07.16.20155317doi: http://dx.doi.org/10.1101/2020.07.16.20155317
52. Petersen MS, Strøm M, Christiansen DH, Fjallsbak JP, Eliasen EH, Johansen M, et al. Seroprevalence of SARS-CoV-2-specific antibodies, Faroe Islands. Emerg Infect Dis. 2020 Jul 29;26(11). doi: http://dx.doi.org/10.3201/eid2611.202736 PMID: 32726200
53. Biggs HM, Harris JB, Breakwell L, Dahlgren FS, Abedi GR, Szablewski CM, et al.; CDC Field Surveyor Team. Estimated community seroprevalence of SARS-CoV-2 antibodies - two Georgia counties, April 28–May 3, 2020. MMWR Morb Mortal Wkly Rep. 2020 Jul 24;69(29):965–70. doi: http://dx.doi.org/10.15585/mmwr.mm6929e2 PMID: 32701941
54. Menachemi N, Yiannoutsos CT, Dixon BE, Duszynski TJ, Fadel WF, Wools-Kaloustian KK, et al. Population point prevalence of SARS-CoV-2 infection based on a statewide random sample – Indiana, April 25–29, 2020. MMWR Morb Mortal Wkly Rep. 2020 Jul 24;69(29):960–4. doi: http://dx.doi.org/10.15585/mmwr.mm6929e1 PMID: 32701938
55. Chang L, Hou W, Zhao L, Zhang Y, Wang Y, Wu L, et al. The prevalence of antibodies to SARS-CoV-2 among blood donors in China [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.07.13.20153106doi: http://dx.doi.org/10.1101/2020.07.13.20153106
56. Meyers K, Liu L, Lin W-H, Luo Y, Yin M, Wu Y, et al. Antibody testing documents the silent spread of SARS-CoV-2 in New York prior to the first reported case [preprint]. Durham: Research Square; 2020. doi: http://dx.doi.org/10.21203/rs.3.rs-39880/v1
57. Merkely B, Szabó AJ, Kosztin A, Berényi E, Sebestyén A, Lengyel C, et al.; HUNgarian COronaVirus-19 Epidemiological Research (H-UNCOVER) investigators. Novel coronavirus epidemic in the Hungarian population, a cross-sectional nationwide survey to support the exit policy in Hungary. Geroscience. 2020 Aug;42(4):1063–74. doi: http://dx.doi.org/10.1007/s11357-020-00226-9 PMID: 32677025
58. Gudbjartsson DF, Norddahl GL, Melsted P, Gunnarsdottir K, Holm H, Eythorsson E, et al. Humoral immune response to SARS-CoV-2 in Iceland. N Engl J Med. 2020 Sep 1.NEJMoa2026116 [online ahead of print]. doi: http://dx.doi.org/10.1056/NEJMoa2026116 PMID: 32871063
59. Noh JY, Seo YB, Yoon JG, Seong H, Hyun H, Lee J, et al. Seroprevalence of anti-SARS-CoV-2 antibodies among outpatients in southwestern Seoul, Korea. J Korean Med Sci. 2020 Aug 24;35(33):e311. doi: http://dx.doi.org/10.3346/jkms.2020.35.e311 PMID: 32830472
60. Xu R, Huang J, Duan C, Liao Q, Shan Z, Wang M, et al. Low prevalence of antibodies against SARS-CoV-2 among voluntary blood donors in Guangzhou, China. J Med Virol. 2020 Aug 19.jmv.26445. [online ahead of print]. doi: http://dx.doi.org/10.1002/jmv.26445 PMID: 32813273
61. Malani A, Shah D, Kang G, Lobo GN, Shastri J, Mohanan M, et al. Seroprevalence of SARS-CoV-2 in slums and non-slums of Mumbai, India, during June 29–July 19, 2020 [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.08.27.20182741doi: http://dx.doi.org/10.1101/2020.08.27.20182741
62. Bogogiannidou Z, Vontas A, Dadouli K, Kyritsi MA, Soteriades S, Nikoulis DJ, et al. Repeated leftover serosurvey of SARS-CoV-2 IgG antibodies, Greece, March and April 2020. Euro Surveill. 2020 Aug;25(31):2001369. doi: http://dx.doi.org/10.2807/1560-7917.ES.2020.25.31.2001369 PMID: 32762796
63. Feehan AK, Velasco C, Fort D, Burton JH, Price-Haywood E, Katzmarzyk PT, et al. Racial and workplace disparities in seroprevalence of SARS-CoV-2 in Baton Rouge, Louisiana, 4 July 15–31, 2020 [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.08.26.20180968doi: http://dx.doi.org/10.1101/2020.08.26.20180968
64. Westerhuis BM, de Bruin E, Chandler FD, Ramakers CRB, Okba NMA, Li W, et al. Homologous and he 1 terologous antibodies to coronavirus 229E, NL63, OC43, HKU1, SARS, MERS and SARS-CoV-2 antigens in an age stratified cross-sectional serosurvey in a large tertiary hospital in The Netherlands [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.08.21.20177857doi: http://dx.doi.org/10.1101/2020.08.21.20177857
65. Ward H, Atchinson C, Whitaker M, Ainslie KED, Elliott J, Okell L, et al. Antibody prevalence for SARS-CoV-2 following the peak of the pandemic in England: REACT2 study in 100,000 adults [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.08.12.20173690doi: http://dx.doi.org/10.1101/2020.08.12.20173690
66. Javed W, Baqar J, Abidi SHB, Farooq W. Sero-prevalence findings from metropoles in Pakistan: implications for assessing COVID-19 prevalence and case-fatality within a dense, urban working population [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/10.1101/2020.08.13.20173914doi: http://dx.doi.org/10.1101/2020.08.13.20173914
67. Khan MS, Qurieshi MA, Haq I, Majid S, Akbar A. Seroprevalence of SARS-CoV-2 specific IgG antibodies in District Srinagar, northern India – a cross-sectional study [preprint].Cold Spring Harbor: bioRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.09.04.282640doi: http://dx.doi.org/10.1101/2020.09.04.282640
68. Da Silva A, Lima-Neto L, de Azevedo C, da Costa L, Braganca M, Filho A, et al. Population-based seroprevalence of SARS-CoV-2 is more than halfway through the herd immunity threshold in the State of Maranhão, Brazil [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/10.1101/2020.08.28.20180463doi: http://dx.doi.org/ doi: http://dx.doi.org/10.1101/2020.08.28.20180463
69. [Weekly report of the population serology survey of the corona epidemic.] Helsinki: Finland Department of Health and Welfare; 2020. [Finnish]. Available from: https://www.thl.fi/roko/cov-vaestoserologia/sero_report_weekly.html [cited 2020 Jul 12].
70. [First results on antibodies after review of covid-19 in blood donors.] Solna: Swedish Public Health Agency; 2020. [Swedish]. Available from: https://www.folkhalsomyndigheten.se/nyheter-och-press/nyhetsarkiv/2020/juni/forsta-resultaten-om-antikroppar-efter-genomgangen-covid-19-hos-blodgivare/ [cited 2020 Jul 12].
71. [Studie SARS-CoV-2-CZ-Preval.] Prague: Institute of Health Information and Statistics of the Czech Republic; 2020. [Czech]. Available from: https://covid-imunita.uzis.cz/res/file/prezentace/20200506-dusek.pdf [cited 2020 Jul 12].
72. Jaffe-Hoffman M. Coronavirus herd immunity? Not in Israel, according to a serological study. Jerusalem Post. 2020 Jun 2. Available from: https://www.jpost.com/israel-news/coronavirus-herd-immunity-not-in-israel-according-to-a-serological-study-630059 [cited 2020 Jul 12].
73. First study carried out on herd immunity of the population in the whole territory of Slovenia. Ljubljana: Republic of Slovenia; 2020. Available from: https://www.gov.si/en/news/2020-05-06-first-study-carried-out-on-herd-immunity-of-the-population-in-the-whole-territory-of-slovenia/ [cited 2020 Jul 12].
74. [Popova declared immunity to coronavirus in 14% of those tested]. Interfax. 2020 Jun 10. [Russian]. Available from: https://www.interfax.ru/russia/712617 [cited 2020 Aug 12].
75. Gul A. Ten million Afghans likely infected and recovered: COVID-19 survey. VOA. 2020 Aug 5. Available from: https://www.voanews.com/south-central-asia/10-million-afghans-likely-infected-and-recovered-covid-19-survey [cited 2020 Aug 12].
76. Preliminary results of seroprevalence study in Tbilisi: 10 out of 1068 residents have coronavirus antibodies. Agenda.ge. 2020 Jun 29. Available from: https://agenda.ge/en/news/2020/2055 [cited 2020 Aug 12].
77. Genot L. In Brazil, COVID-19 hitting young people harder. The Jakarta Post. 2020 May 22. Available from: https://www.thejakartapost.com/news/2020/05/22/in-brazil-covid-19-hitting-young-people-harder.html [cited 2020 Jul 12].
78. COVID-19 pandemic in Croatia [internet]. Wikipedia; 2020. Available from https://en.wikipedia.org/wiki/COVID-19_pandemic_in_Croatia [cited 2020 Jul 12].

79. COVID-19 coronavirus pandemic [internet]. Dover: Worldometer; 2020. Available from: https://www.worldometers.info/coronavirus/ [cited 2020 Sep 12].
80. Ioannidis JPA, Axfors C, Contopoulos-Ioannidis DG. Population-level COVID-19 mortality risk for non-elderly individuals overall and for non-elderly individuals without underlying diseases in pandemic epicenters. Environ Res. 2020 Sep;188:109890. doi: http://dx.doi.org/10.1016/j.envres.2020.109890 PMID: 32846654
81. Booth R. Half of coronavirus deaths happen in care homes, data from EU suggests. The Guardian. 2020 Apr 13. Available from: https://www.theguardian.com/world/2020/apr/13/half-of-coronavirus-deaths-happen-in-care-homes-data-from-eu-suggests [cited 2020 Apr 27].
82. American Geriatrics Society. American Geriatrics Society Policy Brief: COVID-19 and nursing homes. J Am Geriatr Soc. 2020 May;68(5):908–11. doi: http://dx.doi.org/10.1111/jgs.16477 PMID: 32267538
83. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020 Mar 17;323(11):1061–9. doi: http://dx.doi.org/10.1001/jama.2020.1585 PMID: 32031570
84. Boccia S, Ricciardi W, Ioannidis JPA. What other countries can learn from Italy during the COVID-19 pandemic. JAMA Intern Med. 2020 Jul 1;180(7):927–8. doi: http://dx.doi.org/10.1001/jamainternmed.2020.1447 PMID: 32259190
85. Brufsky A. Distinct viral clades of SARS-CoV-2: implications for modeling of viral spread. J Med Virol. 2020 Apr 20;92(9):1386–90. doi: http://dx.doi.org/10.1002/jmv.25902 PMID: 32311094
86. Rosado J, Cockram C, Merkling SH, Demeret C, Meola A, Kerneis S, et al. Serological signatures of SARS-CoV-2 infection: implications for antibody-based diagnostics [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.05.07.20093963doi: http://dx.doi.org/10.1101/2020.05.07.20093963
87. Long QX, Tang XJ, Shi QL, Li Q, Deng HJ, Yuan J, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med. 2020 Aug;26(8):1200–4. doi: http://dx.doi.org/10.1038/s41591-020-0965-6 PMID: 32555424
88. Wu F, Wang A, Liu M, Wang Q, Chen J, Xia S, et al. Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.03.30.20047365doi: http://dx.doi.org/10.1101/2020.03.30.20047365
89. Seow J, Graham C, Merrick B, Acors S, Steel KJA, Hemmings O, et al. Longitudinal evaluation and decline of antibody responses in SARS-CoV-2 infection [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.07.09.20148429doi: http://dx.doi.org/10.1101/2020.07.09.20148429
90. Edouard S, Colson P, Melenotte C, De Pinto F, Thomas L, La Scola B, et al. Evaluating the serological status of COVID-19 patients using an indirect immunofluorescent assay, France [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.05.05.20092064doi: http://dx.doi.org/10.1101/2020.05.05.20092064
91. Solbach W, Schiffner J, Backhaus I, Burger D, Staiger R, Tiemer B, et al. Antibody profiling of COVID-19 patients in an urban low-incidence region in northern Germany [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.05.30.20111393doi: http://dx.doi.org/10.1101/2020.05.30.20111393
92. Ioannidis JPA. Global perspective of COVID-19 epidemiology for a full-cycle pandemic. Eur J Clin Invest. 2020 Oct 7;e13421. doi: http://dx.doi.org/10.1111/eci.13423 PMID: 33026101
93. Kikkert M. Innate immune evasion by human respiratory RNA viruses. J Innate Immun. 2020;12(1):4–20. doi: http://dx.doi.org/10.1159/000503030 PMID: 31610541
94. Krammer F. The human antibody response to influenza A virus infection and vaccination. Nat Rev Immunol. 2019 Jun;19(6):383–97. doi: http://dx.doi.org/10.1038/s41577-019-0143-6 PMID: 30837674
95. Cervia C, Nilsson J, Zurbuchen Y, Valaperti A, Schreiner J, Wolfensberger A, et al. Systemic and mucosal antibody secretion specific to SARS-CoV-2 during mild versus severe COVID-19 [preprint]. Cold Spring Harbor: bioRxiv. 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.05.21.108308doi: http://dx.doi.org/10.1101/2020.05.21.108308
96. Gallais F, Velay A, Wendling M-J, Nazon C, Partisani M, Sibilia J, et al. Intrafamilial exposure to SARS-CoV-2 induces cellular immune response without seroconversion [preprint]. Cold Spring Harbor: medRxiv; 2020. doi: http://dx.doi.org/https://doi.org/10.1101/2020.06.21.20132449doi: http://dx.doi.org/10.1101/2020.06.21.20132449
97. Sekine T, Perez-Potti A, Rivera-Ballesteros O, Strålin K, Gorin J-B, Olsson A, et al.; Karolinska COVID-19 Study Group. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. Cell. 2020 10 1;183(1):158–168.e14. doi: http://dx.doi.org/10.1016/j.cell.2020.08.017 PMID: 32979941

Table 1. **Eligible seroprevalence studies on COVID-19 published or deposited as preprints as of 9 September 2020: dates, sampling and recruitment**

Author	Country (location)	Dates	Sampling and recruitment
Figar et al.[47]	Argentina (Barrio Padre Mugica)	10–26 June	Probabilistic sampling of a slum neighbourhood, sampling from people 14 years or older across households
Herzog et al.[38]	Belgium	30 March–5 April and 20–26 April	Residual sera from 10 private diagnostic laboratories in Belgium, with fixed numbers per age group, region and periodical sampling, and stratified by sex
Hallal et al.[25]	Brazil	15–22 May	Sampling from 133 cities (the main city in each region), selecting 25 census tracts with probability proportionate to size in each sentinel city, and 10 households at random in each tract. Aiming for 250 participants per city
Gomes et al.[34]	Brazil (Espirito Santo)	13–15 May	Cross-section of major municipalities with houses as the sampling units
Da Silva et al.[68]	Brazil (Maranhao)	27 July–8 August	Three-stage cluster sampling stratified by four state regions in the state of Maranhao; the estimates took clustering, stratification and non-response into account
Amorim Filho et al.[41]	Brazil (Rio de Janeiro)	14–27 April (eligible: 24–27 April)	Blood donors without flulike symptoms within 30 days of donation; had close contact with suspected or confirmed COVID-19 cases in the 30 days before donation; or had travelled abroad in the past 30 days
Silveira et al.[17]	Brazil (Rio Grande do Sul)	9–11 May (third round, after 11–13 April, and 25–27 April)	Multistage probability sampling in each of nine cities to select 500 households, from which one member was randomly chosen for testing
Tess et al.[42]	Brazil (Sao Paulo)	4–12 May	Randomly selected adults and their cohabitants sampled from six districts of Sao Paulo City with high numbers of cases
Skowronski et al.[50]	Canada (British Columbia)	15–27 May (after baseline in 5–13 March)	Specimens from patients attending one of about 80 diagnostic service centres of the only outpatient laboratory network in the Lower Mainland
Torres et al.[43]	Chile (Vitacura)	4–19 May	Classroom stratified sample of children and all staff in a community placed on quarantine after school outbreak
Chang et al.[55]	China	January–April weekly: 3–23 February (Wuhan); 24 February–15 March (Shenzhen); 10 February–1 March (Shijiazhuang)	38 144 healthy blood donors in Wuhan, Shenzhen and Shijiazhuang who met the criteria for blood donation during the COVID-19 pandemic in China
Wu et al.[14]	China (Wuhan)	3–15 April	People applying for permission to resume work ($n = 1021$) and hospitalized patients ($n = 381$)
Ling et al.[32]	China (Wuhan)	26 March–28 April	Age 16–64 years, going back to work, with no fever, headache or other symptoms of COVID-19
Xu et al.[60]	China (Guangzhou)	23 March–2 April	Healthy blood donors in Guangzhou
Xu et al.[40]	China (several regions)	30 March–10 April	Voluntary participation by public call for haemodialysis patients ($n = 979$ in Jingzhou, Hubei and $n = 563$ in Guangzhou/Foshan, Guangdong) and outpatients in Chongqing ($n = 993$), and community residents in Chengdu, Sichuan ($n = 9442$), and required testing for factory workers in Guangzhou, Guandong ($n = 442$)
Jerkovic et al.[26]	Croatia	23–28 April	DIV Group factory workers in Split and Sibenik-Knin invited for voluntary testing
Erikstrup et al.[12]	Denmark	6 April–3 May	All Danish blood donors aged 17–69 years giving blood. Blood donors are healthy and must comply with strict eligibility criteria; they must self-defer for two weeks if they develop fever with upper respiratory symptoms
Petersen et al.[52]	Denmark (Faroe Islands)	27 April–1 May	1 500 randomly selected residents invited to participate, samples collected from 1075
Fontanet et al.[39]	France (Crepy-en-Valois)	28–30 April	Pupils, their parents and relatives, and staff of primary schools exposed to SARS-CoV-2 in February and March 2020 in a city north of Paris
Fontanet et al.[13]	France (Oise)	30 March–4 April	Pupils, their parents and siblings, as well as teachers and non-teaching staff of a high-school
Streeck et al.[16]	Germany (Gangelt)	30 March–6 April	600 adults with different surnames in Gangelt were randomly selected; all household members were asked to participate in the study

(*continues. . .*)

(. . .*continued*)

Author	Country (location)	Dates	Sampling and recruitment
Kraehling et al.[21]	Germany (Frankfurt)	6–14 April	Employees of Infraserv Höchst, a large industrial site operator in Frankfurt am Main. No exclusion criteria
Bogogiannidou et al.[62]	Greece	March and April (April data used)	Leftover blood samples collected from a nationwide laboratory network, including both private and public hospital laboratories (27 laboratories in total)
Merkely et al.[57]	Hungary	1–16 May	Representative sample (n = 17 787) of the Hungarian population ≥ 14 years living in private households (8 283 810)
Gudbjartsson et al.[58]	Iceland	Several cohorts between April and June[a]	30 576 people in Iceland, including those documented to be infected, those quarantined and people not known to have been exposed
Malani et al.[61]	India (Mumbai)	29 June–19 July	Geographically-spaced community sampling of households, one individual per household was tested in slum and non-slum communities in three wards, one each from the three main zones of Mumbai
Khan et al.[67]	India (Srinagar)	1–15 July	Adults (> 18 years) who visited selected hospitals across the Srinagar District
Shakiba et al.[8]	Islamic Republic of Iran (Guilan)	April (until 21 April)	Population-based cluster random sampling design through telephone call invitation, household-based
Fiore et al.[31]	Italy (Apulia)	1–31 May	Blood donors 18–65 years old free of recent symptoms possibly related to COVID-19, no close contact with confirmed cases, symptom-free in the preceding 14 days, no contact with suspected cases
Doi et al.[11]	Japan (Kobe)	31 March–7 April	Randomly selected patients who visited outpatient clinics and received blood testing for any reason. Patients who visited the emergency department or the designated fever consultation service were excluded
Takita et al.[29]	Japan (Tokyo)	21 April–20 May	Two community clinics in the main railway stations in Tokyo (Navitas Clinic Shinjuku and Tachikawa)
Nawa et al.[48]	Japan (Utsunomiya City)	14 June–5 July	Invitations enclosed with a questionnaire were sent to 2290 people in 1 000 households randomly selected from Utsunomiya City's basic resident registry; 742 completed the study
Uyoga et al.[44]	Kenya	30 April–16 June (~90% of samples in last 30 days)	Residual blood donor serum samples from donors 16–65 years in four sites (Mombasa, Nairobi, Eldoret and Kisumu)
Snoeck et al.[20]	Luxembourg	16 April–5 May	Representative sample (no details how ensured), 1807 of 2000 contacted provided data, were < 79 years and had serology results
Slot et al.[15]	Netherlands	1–15 April	Blood donors. Donors must be completely healthy, but they may have been ill in the past, provided that they recovered at least 2 weeks before
Westerhuis et al.[64]	Netherlands (Rotterdam)	Early March and early April	Left-over plasma samples from patients of nine age categories in Erasmus Medical Center in Rotterdam: 879 samples in early March and 729 in early April)
Nisar et al.[49]	Pakistan (Karachi)	25 June–11 July (after baseline on 15–25 April)	Cross-sectional household surveys in a low- (district Malir) and high-transmission (district East) area of Karachi with households selected using simple random sampling (Malir) and systematic random sampling (East)
Javed et al.[66]	Pakistan (urban Karachi, Lahore, Multan, Peshawar and Quetta)	Up to 6 July	Adult, working population aged 18–65 years, recruited from dense, urban workplaces including factories, businesses, restaurants, media houses, schools, banks, hospitals (health-care providers), and from families of positive cases in cities in Pakistan
Abu Raddad et al.[51]	Qatar	12 May–12 July (highest seroprevalence on 12–31 May)	Convenience sample of residual blood specimens collected for routine clinical screening or clinical management from 32 970 outpatient and inpatient departments for a variety of health conditions (n = 937 in 12–31 May)
Noh et al.[59]	Republic of Korea	25–29 May	Outpatients who visited two hospitals in south-west Seoul which serve six administrative areas
Pollán et al.[36]	Spain	27 April–11 May	35 883 households selected from municipal rolls using two-stage random sampling stratified by province and municipality size, with all residents invited to participate (75.1% of all contacted individuals participated)

(*continues. . .*)

Bull World Health Organ 2021;99:19–33F | doi: http://dx.doi.org/10.2471/BLT.20.265892

(. . .*continued*)

Author	Country (location)	Dates	Sampling and recruitment
Crovetto et al.[30]	Spain (Barcelona)	14 April–5 May	Consecutive pregnant women for first trimester screening or delivery in two hospitals
Stringhini et al.[10]	Switzerland (Geneva)	6 April–9 May (5 consecutive weeks)	Randomly selected previous participants of the Bus Santé study with an email (or telephone contact, if email unavailable); participants were invited to bring all members of their household aged 5 years and older
Emmenegger et al.[28]	Switzerland (Zurich)	Prepandemic until June (patients) and May (blood donors)	Patients at the University Hospital of Zurich and blood donors in Zurich and Lucerne
Ward et al.[65]	United Kingdom (England)	20 June–13 July	Random population sample of 100 000 adults over 18 years
Thompson et al.[18]	United Kingdom (Scotland)	21–23 March	Blood donors. Donors should not have felt unwell in the past 14 days; some other deferrals also applied regarding travel and COVID-19 symptoms
Havers et al.[35]	USA (10 states)	23 March–1 April (Washington, Puget Sound and New York, New York City), 1–8 April (Louisiana), 5–10 April (Florida, south), 13–25 April (Pennsylvania, Philadelphia, metropolitan area), 20–26 April (Missouri), 23–27 April (California, San Francisco Bay Area), 20 April–3 May (Utah), 26 April–3 May (Connecticut), 30 April–12 May (Minnesota, Minneapolis)	Convenience samples using residual sera obtained for routine clinical testing (screening or management) by two commercial laboratory companies
Ng et al.[24]	USA (California, Bay Area)	March	1000 blood donors in diverse Bay Area locations (excluding those with self-reported symptoms or abnormal vital signs)
Sood[22]	USA (California, Los Angeles)	10–14 April	Proprietary database representative of the county. A random sample of these residents was invited, with quotas for enrolment for subgroups based on age, sex, race and ethnicity distribution
Chamie et al.[33]	USA (California, San Francisco)	25–28 April	United States census tract 022 901 population-dense area (58% Latin American) in San Francisco Mission district, expanded to neighbouring blocks on 28 April
Bendavid et al.[19]	USA (California, Santa Clara)	2–3 April	Facebook advertisement with additional targeting by zip code
Biggs et al.[53]	USA (Georgia, DeKalb and Fulton)	28 April–3 May	Two-stage cluster sampling design used to randomly select 30 census blocks in DeKalb County and 30 census blocks in Fulton County, with a target of seven participating households per census block
McLaughlin et al.[46]	USA (Idaho, Blaine County)	4–19 May	Volunteers who registered via a secure web link, using prestratification weighting to the population distribution by age and sex within each zip code
Bryan et al.[9]	USA (Idaho, Boise)	Late April	People from the Boise, Idaho metropolitan area, part of the Crush the Curve initiative
Menachemi et al.[54]	USA (Indiana)	25–29 April	Stratified random sampling among all persons aged ≥ 12 years using Indiana's 10 public health preparedness districts as sampling strata
Feehan et al.[63]	USA (Louisiana, Baton Rouge)	15–31 July	Representative sample in a method developed by Public Democracy
Feehan et al.[37]	USA (Louisiana, Orleans and Jefferson Parish)	9–15 May	Pool of potential participants reflecting the demographics of the parishes was based on 50 characteristics, then a randomized subset of 150 000 people was selected, and 25 000 were approached with digital apps, and 2640 were recruited

(*continues. . .*)

(. . .*continued*)

Author	Country (location)	Dates	Sampling and recruitment
Rosenberg et al.[23]	USA (New York)	19–28 April	Convenience sample of people ≥ 18 years living in New York State, recruited consecutively on entering 99 grocery stores and through an in-store flyer
Meyers et al.[56]	USA (New York)	2–30 March (Columbia University Medical Center, New York City); 13–28 March (CareMount central laboratory)	Discarded clinical samples in Columbia Medical Center, New York City ($n = 814$ in 24 February–30 March, 742 of those in the period 2–30 March) and samples from CareMount central laboratory (960 samples on 13/14 March, 505 samples on 20/21 March, and 376 samples on 27/28 March) from its network of clinics in five counties north of New York City
Reifer et al.[27]	USA (New York, Brooklyn)	Early May	Patients seen in an urgent care facility in Brooklyn
Nesbitt et al.[45]	USA (Rhode Island)	27 April–11 May	Consecutive blood donors

COVID-19: coronavirus disease 19; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

[a] Sample collection time for some sub-cohorts may have exceeded 1 month, but more than half of the cases were already documented by polymerase chain reaction testing before any antibody testing and the last death occurred on 20 April.

Note: Some studies included additional data sets that did not fulfil the eligibility criteria (e.g. had sample size < 500 or were health-care workers) and they are not presented here.

Bull World Health Organ 2021;99:19–33F | doi: http://dx.doi.org/10.2471/BLT.20.265892

Table 2. **Sample size, types of antibodies assessed and population size in the studies included to assess COVID-19 infection fatality rate, 2020**

Country (location)	Sample size[a], no.	Antibody	Population,[b,c,d] no.	% of population < 70 years[c]
Argentina (Barrio Padre Mugica)[47]	873	IgG	49 983	99
Belgium[38]	3 391 (20–26 April)	IgG	11 589 623	86
Brazil (133 cities)[25]	24 995	IgG and IgM	74 656 499	94 (Brazil)
Brazil (Espirito Santo)[34]	4 608	IgG and IgM	4 018 650	94 (Brazil)
Brazil (Maranhao)[68]	3 156	IgG and IgM	7 114 598	92
Brazil (Rio de Janeiro), blood donors[41]	669 (24–27 April)	IgG and IgM	17 264 943	94 (Brazil)
Brazil (Rio Grande do Sul)[17]	4 500	IgG	11 377 239	91
Brazil (Sao Paulo)[42]	517	IgG and IgM	298 240 (6 districts)	94 (Brazil)
Canada (British Columbia)[50]	885	IgG, IgM and IgA	5 071 000	94
Chile (Vitacura)[43]	1 244	IgG and IgM	85 000	92 (Chile)
China, blood donors[55]				
Wuhan	930 (3–23 February)	IgG and IgM	11 210 000	93 (China)
Shenzhen	3 507 (24 February–15 March)	IgG and IgM	13 030 000	93 (China)
Shijiazhuang	6 455 (10 February–1 March)	IgG and IgM	11 030 000	93 (China)
China (Wuhan)[14]	1 401	IgG and IgM	11 080 000	93 (China)
China (Wuhan)[32]	1 196 (4–8 April)	IgG and IgM	11 080 000	93 (China)
China (Guangzhou), blood donors[60]	2 199	IgG, IgM and IgA	115 210 000 (Guangdong)	93 (China)
China (several regions)[40]				
Hubei (not Wuhan)	979	IgG and IgM	48 058 000	93 (China)
Chongqing	993	IgG and IgM	31 243 200	93 (China)
Sichuan	9 442	IgG and IgM	83 750 000	93 (China)
Guangdong	1 005	IgG and IgM	115 210 000	93 (China)
Croatia[26]	1 494	IgG and IgM	4 076 000	86
Denmark blood donors[12]	20 640	IgG and IgM	5 771 876	86
Denmark (Faroe Islands)[52]	1 075	IgG and IgM	52 428	88
France (Crepy-en-Valois)[39]	1 340	IgG	5 978 000 (Hauts-de-France)	89
France (Oise)[13]	661	IgG	5 978 000 (Hauts-de-France)	89
Germany (Gangelt)[16]	919	IgG and IgA	12 597	86
Germany (Frankfurt)[21]	1 000	IgG	2 681 000[e]	84 (Germany)
Greece[62]	6 586 (4 511 in April)	IgG	10 412 967	84
Hungary[57]	10 504	IgG (also had RT-PCR)	9 657 451	88
Iceland[58]	30 576	Pan-Ig	366 854	90
India (Mumbai)[61]	6 904 (4 202 in slums, 2 702 not in slums)	IgG	1 414 917 (705 523 in slums, 709 394 in non-slums) in the 3 ward areas	98
India (Srinagar)[67]	2 906	IgG	1 500 000	97
Islamic Republic of Iran (Guilan)[8]	551	IgG and IgM	2 354 848	95
Italy (Apulia), blood donors[31]	909	IgG and IgM	4 029 000	84
Japan (Kobe)[11]	1 000	IgG	1 518 870	79 (Japan)
Japan (Tokyo)[29]	1 071	IgG	13 902 077	79 (Japan)
Japan (Utsunomiya City)[48]	742	IgG	518 610	79 (Japan)
Kenya, blood donors[44]	3 098	IgG	47 564 296	99
Luxembourg[20]	1 807	IgG and IgA[f]	615 729	90
Netherlands blood donors[15]	7 361	IgG, IgM and IgA	17 097 123	86
Netherlands (Rotterdam)[64]	729 (early April)	IgG	17 097 123 (Netherlands)	86
Pakistan (Karachi)[49]	1 004	IgG and IgM	16 700 000	98 (Pakistan)
Pakistan (urban)[66]	24 210	IgG and IgM	79 000 000 (urban)	98
Qatar[51]	937	IgG	2 800 000	99
Republic of Korea[59]	1 500	IgG	2 667 341	90 (Republic of Korea)
Spain[36]	61 075	IgG	46 940 000	85
Spain (Barcelona)[30]	874	IgG, IgM and IgA	7 566 000 (Catalonia)	86
Switzerland (Geneva)[10]	577 (20–27 April)	IgG	500 000	88

(*continues. . .*)

(. . .*continued*)

Country (location)	Sample size[a], no.	Antibody	Population,[b,c,d] no.	% of population < 70 years[c]
Switzerland (Zurich)[28]	1 644 patients (1–15 April)	IgG	1 520 968 (Zurich canton)	88
Switzerland (Zurich and Lucerne)[28]	1 640 blood donors (May)	IgG	1 930 525 (Zurich and Lucerne)	88
United Kingdom (England)[65]	109 076	IgG	56 287 000	86
United Kingdom (Scotland), blood donors[18]	500	IgG	5 400 000	88
USA (10 states)[35]				
Washington, Puget Sound	3 264	Pan-Ig	4 273 548	90 (Washington)
Utah	1 132	Pan-Ig	3 282 120	92
New York, New York City	2 482	Pan-Ig	9 260 870	89
Missouri	1 882	Pan-Ig	6 110 800	88
Florida, south	1 742	Pan-Ig	6 345 345	86 (Florida)
Connecticut	1 431	Pan-Ig	3 562 989	88
Louisiana	1 184	Pan-Ig	4 644 049	92
California, San Francisco Bay	1 224	Pan-Ig	2 173 082	90
Pennsylvania, Philadelphia	824	Pan-Ig	4 910 139	90
Minnesota, Minneapolis	860	Pan-Ig	3 857 479	90
USA (California, Bay Area)[24]	1 000	IgG	7 753 000	90
USA (California, Los Angeles)[22]	863	IgG and IgM	7 892 000	92
USA (California, San Francisco)[33]	3 953	IgG and RT-PCR	5 174 (in census tract 022 901)	95
USA (California, Santa Clara)[19]	3 300	IgG and IgM	1 928 000	90
USA (Idaho, Boise)[9]	4 856	IgG	481 587 (Ada County)	92
USA (Georgia, DeKalb and Fulton Counties)[53]	696	Total Ig	1 806 672	88 (Georgia)
USA (Idaho, Blaine County)[46]	917	IgG	23 089	92
USA (Indiana)[54]	3 629	IgG and RT-PCR	6 730 000	89
USA (Louisiana, Baton Rouge)[63]	138	IgG	699 200 (East Baton Rouge, West Baton Rouge, Ascension, Livingston)	92 (Louisiana)
USA (Louisiana, Orleans and Jefferson Parish)[37]	2 640	IgG	825 057	92 (Louisiana)
USA (New York)[23]	15 101	IgG	19 450 000	90
USA, New York[56]				
Columbia University Medical Center, New York City	742 (2–30 March)	IgG and IgM	9 260 870	89
CareMount central laboratory, five New York state counties	1 841	IgG and IgM	10 189 130 (New York state excluding New York City)	89
USA (New York, Brooklyn)[27]	11 092	IgG	2 559 903	91
USA (Rhode Island), blood donors[45]	1 996	IgG and IgM	1 059 000	88

COVID-19: coronavirus disease 19; Ig: immunoglobin; RT-PCR: real-time polymerase chain reaction.

[a] Dates in brackets are the specific dates used when seroprevalence was evaluated at multiple consecutive time points or settings.

[b] Some studies focused on age-restricted populations of the specific location under study, for example: people 17–70 years in the Denmark blood donor study ($n = 3\,800\,000$); people 18–79 years in the Luxembourg study ($n = 483\,000$); people < 70 years in the Netherlands blood donor study ($n = 13\,745\,768$); people ≥ 18 years in the New York state study ($n = 15\,280\,000$); people > 19 years in the Utah population of the 10-state United States of America study ($n = 2\,173\,082$); people ≥ 18 years in Blaine County, Idaho ($n = 17\,611$); people 15–64 years in the Kenya blood donor study ($n = 27\,150\,165$); people > 14 years living in private premises in Hungary ($n = 8{,}283{,}810$); people > 18 years ($n = 551\,185$) in Baton Rouge, Louisiana; people 18–65 years working in urban locations in Pakistan ($n = 22\,100\,000$); and people > 18 years in Srinagar District, India ($n = 1\,020\,000$). In this table and subsequent analyses, the entire population in the location is considered for consistency across studies.

[c] Information in parentheses specifies the population.

[d] When the population of the relevant location was not given in a specific study, information on recent estimates of the pertinent population was obtained by standard online sources such as: populationpyramid.net, worldpopulationreview.com, worldometers.info/coronavirus, and Wikipedia.

[e] Participants were recruited from a large number of districts, but most districts had very few participants; here I included the population of the nine districts with > 1:10 000 sampling ratio (846/1000 participants came from these nine districts).

[f] Considered positive if both IgG and IgA were positive; in the other studies, detection of any antibody was considered positive.