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MAJOR ARTICLE

Estimation of SARS-CoV-2 Infection Fatality Rate by Real-time Antibody Screening of Blood Donors

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(See the Editorial Commentary by Busch and Stone on pages 254–6.)

Background. The pandemic due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has tremendous consequences for our societies. Knowledge of the seroprevalence of SARS-CoV-2 is needed to accurately monitor the spread of the epidemic and to calculate the infection fatality rate (IFR). These measures may help the authorities make informed decisions and adjust the current societal interventions. The objective was to perform nationwide real-time seroprevalence surveying among blood donors as a tool to estimate previous SARS-CoV-2 infections and the population-based IFR.

Methods. Danish blood donors aged 17–69 years giving blood 6 April to 3 May were tested for SARS-CoV-2 immunoglobulin M and G antibodies using a commercial lateral flow test. Antibody status was compared between geographical areas, and an estimate of the IFR was calculated. Seroprevalence was adjusted for assay sensitivity and specificity taking the uncertainties of the test validation into account when reporting the 95% confidence intervals (CIs).

Results. The first 20 640 blood donors were tested, and a combined adjusted seroprevalence of 1.9% (95% CI, .8–2.3) was calculated. The seroprevalence differed across areas. Using available data on fatalities and population numbers, a combined IFR in patients <70 years is estimated at 89 per 100 000 (95% CI, 72–211) infections.

Conclusions. The IFR was estimated to be slightly lower than previously reported from other countries not using seroprevalence data. The IFR is likely severalfold lower than the current estimate. We have initiated real-time nationwide anti–SARS-CoV-2 seroprevalence surveying of blood donations as a tool in monitoring the epidemic.

Keywords. SARS-CoV-2; COVID-19; emerging infectious disease; seroprevalence; epidemic monitoring.

Humanity is experiencing a pandemic due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The local severity of the epidemic and experiences from other countries are used by the health authorities to calibrate societal interventions. These interventions—for example, the closing of schools, public institutions, prohibition of group gatherings, and even curfews—have tremendous consequences.

The authorities rely on accurate real-time data to make informed decisions. Thus, numbers of patients who tested positive for SARS-CoV-2, admitted to the hospital, needing respiratory assistance, or deceased from coronavirus disease 2019 (COVID-19) are updated on a daily basis. In contrast, little information exists on the percentage of the population with previous mild or asymptomatic COVID-19. The proportion of the population who have overcome the infection can probably be approximated by testing for antibodies against SARS-CoV-2. Antibodies may confer immunity to repeat infection, and a high proportion of immune individuals can attenuate the epidemic.

Measures of anti–SARS-CoV-2 seroprevalence can also be used to estimate the clinical impact of COVID-19. Statistics on COVID-19 morbidity and mortality vary greatly due to varying testing strategies and, for example, the capacity of the healthcare system to treat infected patients [1]. Countries that diagnose mild infections will report lower morbidity and mortality compared with those with a less comprehensive testing strategy. An accurate measure of seroprevalence can be used to estimate the accumulated number of SARS-CoV-2 infections and thus the infection fatality rate (IFR) in the underlying population.

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Blood donors comprise approximately 4.7% of the Danish population in the same age group [2]. Healthy volunteers donate blood in all areas of the country, ensuring wide geographical coverage. We have initiated a prospective screening of all blood donations for SARS-CoV-2 antibodies to establish a real-time nationwide overview of antibody status. The objective of this study was to perform a seroprevalence survey among blood donors as a tool in the monitoring of the SARS-CoV-2 epidemic.

METHODS

In Denmark, approximately 270 000 blood donations are given annually. All Danish blood donation facilities participated in this survey. From 6 April to 3 May 2020, a total of 20 640 blood donations were given by 17–69-year-old donors. Blood donors are healthy and must comply with strict eligibility criteria [3]. Currently, donors must self-defer for 2 weeks if they develop fever with upper respiratory symptoms.

The first patient with COVID-19 in Denmark was diagnosed on 26 February 2020. Subsequently, diagnostic testing for SARS-CoV-2 viral RNA was primarily performed in symptomatic individuals returning from high-risk areas. On 3 March the government recommended home quarantine for 14 days in case of exposure to COVID-19, including traveling to high-risk areas, and on 6 March, events with more than 1000 individuals were recommended to be postponed or cancelled. On 11 March, the government established a partial lockdown of the country: for public employees only, persons with critical functions were allowed to work; schools and childcare facilities were closed except when parents served in critical functions; workers in the private sector were recommended to work from home; and gatherings of more than 100 were prohibited. No restrictions on private freedom of movement were introduced. The viral RNA testing strategy was changed to include individuals with severe symptoms, vulnerable individuals, or individuals with critical functions. On 14 March, a temporary border closure was implemented, and on 17 March, gatherings of more than 10 individuals were prohibited and selected workplaces with increased risk of transmission due to close contact were shut down (sports facilities, shopping centers, hairdressers, night clubs, bars, cafés, etc). A lift of restrictions was initiated on 15 April 2020 allowing childcare facilities and primary schools to reopen [4].

SARS-CoV-2 immunoglobulin (Ig) G and IgM antibodies were tested on EDTA plasma or whole blood by a lateral flow test according to the manufacturer's recommendations (IgM/IgG Antibody to SARS-CoV-2 lateral flow test; Livzon Diagnostics Inc, Zhuhai, Guangdong, China). Briefly, the test comprises 2 cassettes, 1 for IgG and 1 for IgM SARS-CoV-2 anti-body detection. The test uses colloidal gold chromatography to yield a qualitative result based on the visibility or not of a test band. Samples were concluded to be reactive if the IgM, the IgG, or both bands were visible. A validation of the lateral flow test was performed. A total of 651 plasma samples from blood donors giving blood before November 2019 were tested (3 reactive of 651 samples, 1 inconclusive). Specificity was estimated to be 99.54% (98.66–99.90%). Samples from 155 patients with previous SARS-CoV-2 were tested; 128 were reactive. Sensitivity was thus estimated to be 82.58% (75.68–88.20%). Interobserver agreement was assessed by 3 independent raters of 299 samples. The observed Kappa (Fleiss) score was 92% for the combined rating of either IgM and/or IgG positivity. Validation and testing were performed by experienced staff in 5 regional blood establishments.

We retrieved data on population numbers as of 1 January 2020 [5] and the number of infected and deceased individuals due to COVID-19 using daily updated data [6].

Statistical analysis

Statistical analysis was performed in RStudio 1.2 and R 3.6.0 (R Foundation for Statistical Computing, Vienna, Austria). Results were reported as percentages with 95% confidence intervals (CIs). The EpiR package was used to adjust seroprevalence for sensitivity and specificity. We used the Rogan Gladen estimate to calculate the true prevalence. Confidence intervals were derived by 10^8-sample percentile bootstrapping independently sampling sensitivity, specificity, and apparent prevalence using posterior binomial distributions.

Ethics

SARS-CoV-2 antibody testing was performed as a routine screening of all blood donations. Only consenting donors were tested and informed about their result. Anonymized data were used in this study. The Regional Scientific Ethical Committees for the Zealand Region of Denmark approved the investigation as a register project (20–000013).

RESULTS

We included blood donors aged 17 to 69 years, and a total of 20 640 blood donors were informed and all consented to testing (see Table 1 for characteristics). The distribution between seropositivity for IgM and IgG appears in Table 2. The estimated number of infected individuals was calculated per area in the relevant age group (Table 3). The overall unadjusted seroprevalence was 2.0% (95% CI, 1.8–2.2%). After adjusting for assay sensitivity and specificity including their CIs, the overall seroprevalence was 1.9% (95% CI, .8–2.3%).

The seroprevalence in the Capital region was higher than in the other 4 regions combined (3.2% vs 1.2%; difference, 2.0 percentage points; 95% CI, 1.4–2.6). There was a nonsignificant increase in seroprevalence from the first 2 weeks to the last 2 weeks (1.7% vs 2.0%; difference, .28 percentage points; 95% CI, –.15 to .78).

As of 3 May 2020, 484 individuals are reported to have died from SARS-CoV-2 in Denmark; 65 of these were younger than 70. Thus, the combined IFR in patients younger than

Table 1. Age- and Sex-stratified Seroprevalence of Anti–SARS-CoV-2

	Female			Male			Total		
	Nonreactive	Reactive		Nonreactive	Reactive		Nonreactive	Reactive	
Age Strata in Years	n	n	%	n	n	%	n	n	%
17–29	3009	71	2.3	1934	54	2.7	4943	125	2.5
30–39	1908	20	1.0	2075	36	1.7	3983	56	1.4
40–49	2342	51	2.1	2400	48	2.0	4742	99	2.0
50–59	2035	31	1.5	2389	46	1.9	4424	77	1.7
60–69	930	26	2.7	1206	29	2.3	2136	55	2.5
Total	10 224	199	1.9	10 004	213	2.1	20 228	412	2.0

Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

70 is estimated to be 89 per 100 000 infections (95% CI, 72–211). The total ratio between estimated antibody-positive individuals and the number of confirmed cases was 16 (95% CI, 7–20).

DISCUSSION

In this survey of SARS-CoV-2 antibodies in Danish blood donors we found a seroprevalence of 1.9 (95% CI, .8–2.3) adjusted for the assay performance and a low IFR of 89/100 000 (95% CI, 72–211). This IFR of 0.089% is slightly lower than a recently published COVID-19 IFR estimate of 0.145% (95% CI, .088–.317%; individuals <60 years) not including seroprevalence data [8].

The ratio between estimated antibody-positive individuals and confirmed COVID-19 cases is expected given the targeted early Danish SARS-CoV-2 testing strategy. The lack of large seroprevalence surveys prevents a comparison with other areas/countries.

The low IFR is encouraging, but several caveats exist. Although blood donors represent a very broad population base, they are selected healthy and self-defer for 2 weeks after signs of COVID-19. Conversely, blood donor prevalence increases with income [9] and we speculate that this leads to a higher risk of exposure through travel and social activity. We may therefore either under- or overestimate the true population immunity.

We validated the antibody assay primarily in individuals diagnosed with clinical COVID-19. If silent and mild infections lead to weaker antibody responses, we will underestimate the population immunity. Conversely, we found that 42.7% of donors testing positive were IgM-only reactive. It is possible that some of these individuals had asymptomatic infection and we cannot rule out that some were infectious while reporting for donation. Also, screening only for antibodies may underestimate the prevalence of infections, if cellular cytotoxicity is able to eradicate virally infected cells, as for SARS-CoV, before eliciting a humoral response [10]. Finally, this study only addresses the IFR in 17–69-year-old individuals. The IFR in other population strata (eg, among individuals >80 years or with comorbidity) is higher [8, 11].

Currently, the governments in most countries are trying to balance the economic consequences of a societal lockdown against the risk of an uncontrolled epidemic. Our results underpin that social distancing in a healthy population predominantly acts as a means to protect vulnerable individuals.

It would be challenging to perform an unbiased seroprevalence survey in the background population. As blood donation facilities are located nationwide and operate continuously, the screening is suited to monitor regional differences and temporal changes. With greater knowledge of the seroprevalence in other population strata, the continued monitoring may also be used to effectively model the activity of the SARS-CoV-2 epidemic.

Limitations

We undertook a validation study and found a less than perfect sensitivity of 82.6% (75.7–88.2%) when patients with previous polymerase chain reaction–confirmed COVID-19 were tested. The specificity was acceptable at 99.5% (98.7–99.9%), but leads to a low positive-predictive value in low-prevalence areas.

Table 2. Samples Stratified According to Detectable SARS-CoV-2 IgM or IgG Antibody Isotype

		Reactive		
	Nonreactive	IgM Only	IgG Only	IgM + IgG
n	20 228	176	140	96
Percentage of tested	98.0	0.85	0.68	0.47
Percentage of reactives	…	42.7	34.0	23.3

Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table 3. Distribution of Seroprevalence According to Geographical Area

	Area			
	Capital	South DK, Zealand	Central DK, North DK	Total
Nonreactive, n	6325	7756	6147	20 228
Reactive, n	203	130	79	412
Total, n	6528	7886	6226	20 640
Donor seroprevalence, %				
Unadjusted	3.1 (2.7–3.6)	1.6 (1.4–2.0)	1.3 (1.0–1.6)	2.0 (1.8–2.2)
Adjusted	3.2 (2.1–3.9)	1.4 (0.3–2.0)	1 (0.0–1.5)	1.9 (0.8–2.3)
Citizens aged 17–69 years, n	1 268 550	1 349 455	1 279 208	3 897 213
Expected seropositives, n	40 908 (26 354–49 710)	19 505 (4619–26 593)	12 576 (0–19 226)	72 828 (30 769–90 837)
Registered cases				
Confirmed cases, n	2484	1169	962	4615
Ratio of expected seropositives/confirmed cases	16 (11–20)	17 (4–23)	13 (0–20)	16 (7–20)

A test sensitivity of 82.58% (75.68–88.20%) and a specificity of 99.54% (98.66–99.90%) were used in the adjustment of the seroprevalence percentage. Confirmed cases in each geographical area were defined as confirmed viral RNA reactives as of 6 April 2020 to allow for an extra 2-week lag time between detectable virus and antibody development [7].
Abbreviation: DK, Denmark.

We used a conservative method to estimate the CI and thus took not only the sample variation, but also the uncertainty in the sensitivity and specificity into account. This is necessary because we, unlike most diagnostic and screening tests, do not have a gold standard to confirm positive or negative results. The CI for the regions with lowest antibody prevalence thus reached a lower-limit seroprevalence of 0%.

We chose to use the current lateral flow test because of early availability enabling us to produce the first SARS-CoV-2 antibody seroprevalence estimate in Denmark. While the assay performed acceptably, we recommend the use of a well-validated laboratory-based assay, which are now available, for subsequent seroprevalence studies.

The estimates for the IFR should allow for the lag time from infection to death. Based on current literature, the time from infection to death in nonsurvivors is 23–30 days [12, 13]. Similarly, the lag time from infection to the detection of antibodies may be 16 days [12, 14]. Donor self-deferral due to respiratory symptoms will add to the lag time for the detection of antibodies. We used the available total of deceased citizens due to COVID-19 on the last date of the study (3 May 2020). Using earlier values would result in a lower IFR estimate, while waiting for later death tolls would result in a higher IFR. The death toll among all citizens below 70 years was used, even though only 20 of 65 deaths appeared among individuals with no comorbidity. This was chosen because the denominator included all citizens in the age strata, thus also individuals with comorbidity. The IFR including only individuals with no comorbidity is thus likely severalfold lower than the current estimate.

Rapid tests are read by individuals and interobserver variation often exist. Furthermore, there is uncertainty regarding cross-reactivity of SARS-CoV-2 and other coronavirus antibodies.

The results included in this article will be updated and freely accessible at http://www.bloddonor.dk/antisarscov2.

Conclusions

Our results indicate that the IFR among individuals aged 17 to 69 years is 89/100 000 (95% CI, 72–211). This may have implications for risk mitigation. The IFR in older population strata may be considerably higher. Nationwide continuous seroprevalence surveying of blood donations may be a tool in monitoring the SARS-CoV-2 epidemic.

Notes

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