



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



Original article

Seroprevalence of SARS-CoV-2 antibodies and reduced risk of reinfection through 6 months: a Danish observational cohort study of 44 000 healthcare workers

Kasper Iversen^{1, 2, 3, *}, Jonas Henrik Kristensen^{1, 2, 3}, Rasmus Bo Hasselbalch^{1, 2, 3}, Mia Pries-Heje^{2, 4}, Pernille Brok Nielsen^{1, 2, 3}, Andreas Dehlbæk Knudsen^{2, 4, 5}, Kamille Fogh^{1, 2, 3}, Jakob Boesgaard Norsk^{1, 2, 3}, Ove Andersen^{2, 6}, Thea Køhler Fischer^{7, 8}, Claus Antonio Juul Jensen^{2, 9}, Christian Torp-Pedersen^{7, 8}, Jørgen Rungby^{2, 10}, Sisse Bolm Ditlev^{2, 17}, Ida Hageman^{2, 11}, Rasmus Møgelvang^{2, 4}, Mikkel Gybel-Brask¹⁵, Ram B. Dessau^{19, 20}, Erik Sørensen¹⁵, Lene Harritshøj¹⁵, Fredrik Folke^{1, 2, 12, 13}, Curt Sten^{2, 16}, Maria Elizabeth Engel Møller¹⁴, Thomas Benfield¹⁴, Henrik Ullum¹⁸, Charlotte Sværke Jørgensen¹⁸, Christian Erikstrup²¹, Sisse R. Ostrowski¹⁵, Susanne Dam Nielsen^{2, 5, †}, Henning Bundgaard^{2, 4, †}

¹) Department of Cardiology, Copenhagen University Hospital—Herlev and Gentofte, Borgmester Ib Juuls Vej 1, 2730, Herlev, Denmark

²) Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark

³) Department of Emergency Medicine, Copenhagen University Hospital—Herlev and Gentofte, Borgmester Ib Juuls Vej 1, 2730, Herlev, Denmark

⁴) Department of Cardiology, Copenhagen University Hospital—Rigshospitalet, Blegdamsvej 9, 2100, Copenhagen, Denmark

⁵) Department of Infectious Disease, Copenhagen University Hospital—Rigshospitalet, Blegdamsvej 9, 2100, Copenhagen, Denmark

⁶) Department of Emergency and Department of Clinical Research, Copenhagen University Hospital—Amager og Hvidovre, Kettegård Alle 30, 2650, Hvidovre, Denmark

⁷) Department of Clinical Research, Copenhagen University Hospital—Nordsjællands Hospital, Dyrehavevej 29, 3400, Hillerød, Denmark

⁸) Department of Public Health, University of Copenhagen, Copenhagen, Denmark

⁹) Department of Clinical Biochemistry, Copenhagen University Hospital—Nordsjællands Hospital, Dyrehavevej 29, 3400, Hillerød, Denmark

¹⁰) Department of Endocrinology and Copenhagen Center for Translational Research, Copenhagen University Hospital—Bispebjerg and Frederiksberg, Bispebjerg Bakke 23, 2400, København NV, Denmark

¹¹) Mental Health Services—The Capital Region of Denmark, Kristineberg 3, 2100, København Ø, Denmark

¹²) Copenhagen University Hospital—Copenhagen Emergency Medical Services, Copenhagen, Denmark

¹³) Copenhagen University Hospital—Copenhagen Emergency Medical Services, Telegrafvej 5A, Stairway 2, 2750, Ballerup, Denmark

¹⁴) Department of Infectious Disease, Copenhagen University Hospital—Amager og Hvidovre, Kettegaard Alle 30, 2650 Hvidovre, Denmark

¹⁵) Department of Clinical Immunology, Copenhagen University Hospital—Rigshospitalet, Inge Lehmanns Vej 7, 2100, Copenhagen, Denmark

¹⁶) Diagnostisk Enhed, Copenhagen University Hospital—Bornholm, Ullasvej 8, 3700, Rønne, Denmark

¹⁷) Department of Pulmonary Medicine and Copenhagen Center for Translational Research, Copenhagen University Hospital—Bispebjerg and Frederiksberg, Bispebjerg Bakke 23, 2400, Copenhagen NV, Denmark

¹⁸) Statens Serum Institut, Artillerivej 5, 2300, København S, Copenhagen, Denmark

¹⁹) Department of Clinical Microbiology, Zealand University Hospital—Slagelse, Ingemannsvej 46, 4200, Slagelse, Denmark

²⁰) University of Southern Denmark, Odense, Denmark

²¹) Department of Clinical Immunology, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, 8200, Aarhus, Denmark

ARTICLE INFO

Article history:

Received 27 April 2021

Received in revised form

3 September 2021

ABSTRACT

Objectives: Antibodies to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) are a key factor in protecting against coronavirus disease 2019 (COVID-19). We examined longitudinal changes in seroprevalence in healthcare workers (HCWs) in Copenhagen and the protective effect of antibodies against SARS-CoV-2.

* Corresponding author: Kasper Iversen, Department of Emergency Care and Department of Cardiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen University Hospital—Herlev & Gentofte, Borgmester Ib Juuls Vej 1, 2730, Herlev.

E-mail address: Denmark.Kasper.karmark.iversen@regionh.dk (K. Iversen).

† Susanne Dam Nielsen and Henning Bundgaard share last authorship equally.

Accepted 4 September 2021
Available online 17 September 2021

Editor: R. Chemaly

Keywords:
COVID
Healthcare workers
Immune response
Seroprevalence
SARS-CoV-2

Methods: In this prospective study, screening for antibodies against SARS-CoV-2 (ELISA) was offered to HCWs three times over 6 months. HCW characteristics were obtained by questionnaires. The study was registered at [ClinicalTrials.gov](https://clinicaltrials.gov), NCT04346186.

Results: From April to October 2020 we screened 44 698 HCWs, of whom 2811 were seropositive at least once. The seroprevalence increased from 4.0% (1501/37 452) to 7.4% (2022/27 457) during the period ($p < 0.001$) and was significantly higher than in non-HCWs. Frontline HCWs had a significantly increased risk of seropositivity compared to non-frontline HCWs, with risk ratios (RRs) at the three rounds of 1.49 (95%CI 1.34–1.65, $p < 0.001$), 1.52 (1.39–1.68, $p < 0.001$) and 1.50 (1.38–1.64, $p < 0.001$). The seroprevalence was 1.42- to 2.25-fold higher ($p < 0.001$) in HCWs from dedicated COVID-19 wards than in other frontline HCWs. Seropositive HCWs had an RR of 0.35 (0.15–0.85, $p = 0.012$) of reinfection during the following 6 months, and 2115 out of 2248 (95%) of those who were seropositive during rounds one or two remained seropositive after 4–6 months. The 133 of 2248 participants (5.0%) who seroreverted were slightly older and reported fewer symptoms than other seropositive participants.

Conclusions: HCWs remained at increased risk of infection with SARS-CoV-2 during the 6-month period. Seropositivity against SARS-CoV-2 persisted for at least 6 months in the vast majority of HCWs and was associated with a significantly lower risk of reinfection. **Kasper Iversen, Clin Microbiol Infect 2022;28:710**

© 2021 Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases.

Introduction

The current coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to more than 120 million confirmed cases and almost three million deaths worldwide [1]. Vaccination combined with natural immunity against SARS-CoV-2 is expected to bring the pandemic under control, but whether immunity after vaccination or SARS-CoV-2 infection is lasting remains a key question. This is of importance to the current vaccination strategies deployed worldwide. Currently, recommendations to vaccinate individuals with previously verified SARS-CoV-2 infection differ among countries.

Immunity against SARS-CoV-2 is established by humoral and cell-mediated immune responses, but much is still to be learned [2,3]. In infected individuals, antibodies against SARS-CoV-2 can be detected at an estimated mean of 12–15 days from the onset of symptoms, and virtually all SARS-CoV-2-infected, immunocompetent individuals seroconvert within 19–50 days [4–6]. Antibody development is generally thought to be one of the most important measures to prevent COVID-19 reinfection. However, reinfection has been reported in public media and case reports [7–14]. In these reports reinfected individuals were often asymptomatic during the first course of infection with SARS-CoV-2. Recently, one larger cohort study found reinfection with SARS-CoV-2 to be rare and mild up to 6 months post primary infection, but the rate of seroreversion is still unknown [3].

Seroreversion may be due to a low level of antibodies after mild infection, or immunodeficiency, but as SARS-CoV-2 is a relatively new infectious agent in humans, the percentage of cases that serorevert over time is still being explored [15]. Also, mutations in SARS-CoV-2 may change the properties of the virus, leading to reduced immunity.

We have previously reported that healthcare workers (HCWs) are at higher risk of contracting SARS-CoV-2 than the general population [16]. Extended seroprevalence studies in HCWs may be of importance for safety reasons, and to ensure continued staffing of the healthcare sector, but may also provide knowledge on disease development and immunity before the general population reaches the same seroprevalence levels.

The aim of this study was to examine longitudinal changes in seroprevalence and seroconversion in a highly exposed population of HCWs in the Capital Region of Denmark.

Methods

Study design and participants

In this prospective cohort study, we organized a scheme of repeated, voluntary testing for antibodies against SARS-CoV-2 among HCWs in the Capital Region of Denmark (1.84 million inhabitants) from April to October 2020. Voluntary testing was supported by the administrative and political systems in the region.

All staff at somatic, psychiatric, prehospital and specialized healthcare institutions, as well as administrative and technical staff employed in the healthcare organization in the Capital Region of Denmark, were offered screening. Further, doctors and other staff working in the primary healthcare sector, as well as students working in the healthcare system, were invited to participate. HCWs were stratified by work assignments: those working on dedicated COVID-19 wards, other frontline HCWs, and remaining HCWs. Information on work assignment on dedicated COVID-19 wards were retrieved by questionnaires. Frontline HCWs were defined as all doctors, nurses, assistant nurses, and medical and nursing students who were exposed to patients potentially infected with SARS-CoV-2 but who did not work on COVID-19-dedicated wards. The rest were HCWs not adhering to the first two groups.

Screening for antibodies was offered three times during 2020. The first screening was offered 15th–17th April and 20th–22nd April for hospital staff and 4th–7th May for staff from the primary healthcare sector. The second screening was performed 2nd–4th June and 8th–10th June for all staff. The third and last screening was performed 30th September to 2nd October and 5th–7th October. Participation in the second and third screening was not conditioned by prior participation.

Participants were asked at each round to fill in a survey using a smartphone or computer. The survey was accessed through a link sent to their email at the Danish, governmental, personal, password-protected, email system, e-Boks, or via a QR code at the blood-sampling clinics. Participants filled in information about demographics, exposure to SARS-CoV-2, symptoms and SARS-CoV-2 PCR testing (Supplementary Material Appendix p 2). Study data were collected and managed using Research Electronic Data Capture, a secure, web-based, electronic data capture tool, hosted at the Capital Region's server [17,18]. Participation in the survey was voluntary, and antibody screening was also provided to staff who did not wish to participate in the survey.

Detailed information on dissemination of information regarding the project to HCWs and organization of blood sampling at clinical departments and blood sampling clinics has been published previously [16].

For comparative purposes, SARS-CoV-2 screening data from blood donors were anonymously extracted from the Danish blood bank production system from the same region and for the same time period in which screening in round three was performed for the HCWs [19]. This group was used as a proxy for the general working population. The age range of blood donors was 18–64 years.

SARS-CoV-2 antibody assay

For detection of anti-SARS-CoV-2 (total antibodies of immunoglobulins G, M and A), the enzyme-linked immunosorbent assay (ELISA) from Wantai (Beijing, China) was applied manually on all samples according to the manufacturer's instructions in three different laboratories (Supplementary Material Appendix 20). The cut-off value for a positive result was calculated according to the manufacturer's instruction by adding the negative control value to 0.160. The signal/cut-off ratio (S/CO ratio) ≥ 1.1 was interpreted as a positive result. Borderline S/CO ratios >0.9 and < 1.1 were considered negative for SARS-CoV-2 antibodies. Internal validations with a sensitivity of 96.7% and a specificity of 99.5% were in accordance with the package insert (sensitivity 94.5% and a specificity of 100%).

Approvals and registrations

This study was presented to the scientific ethics committee of the Capital Region. They concluded that the study did not require a scientific ethical approval (J.nr-H-20026288). The study was registered with the Danish Data Protection Authorities (P-2020-361) and the protocol is registered at ClinicalTrials.gov (<https://clinicaltrials.gov/ct2/show/NCT04346186>).

Statistical analyses

Calculations were done using R (version 6.3.1). All results were presented as mean (\pm standard deviation, SD) or median (interquartile range, IQR) according to normality and tested using the χ^2 test, t-test or Wilcoxon rank sum test. Possible associations between exposures and the primary outcome were explored by risk ratios (RRs) presented with 95% confidence intervals (CIs), calculated using the normal approximation (Wald). Significance was examined by Fisher's exact test using the R package *epitools*. A $p < 0.05$ (two-sided) was considered significant. A multivariable logistic regression—including age, sex, asymptomatic versus symptomatic SARS-CoV-2 infection, and being an ever versus never smoker—was used to assess risk factors of seroreversion, with each predictor presented with odds ratios (ORs) and 95% CIs.

Results

Through the three screening rounds a total of 44 698 participants were included. Of these, 37 452 of 44 698 (83.8%) were included in round one, 29 862 of 44 698 (66.8%) in round two, and 27 457 of 44 698 (61.4%) in round three. A total of 18 769 of 44 698 (42.0%) participated in all three rounds (Fig. 1). Baseline characteristics of participants at each round are presented in Table 1. Supplementary Material Tables S1–S4 show baseline characteristics stratified by seroprevalence of all included HCWs, HCWs participating in all rounds, and for each round individually. Flow charts of changes in seroprevalence between rounds are shown in Supplementary Material Figs S1–S4.

The seroprevalence increased from 4.0% (1501/37 452) in round one to 5.8% (1722/29 862) in round two and 7.4% (2022/27 457) in round three ($p < 0.001$). The seropositive participants were younger, more likely to be male, and less likely to smoke (all $p < 0.001$) (Supplementary Material Figs S5 and S6).

Seroprevalence stratified according to proximity to COVID-19 patients

Frontline HCWs had a significantly increased risk of being seropositive throughout the three rounds as compared with non-frontline HCWs, with an RR of 1.49 (95%CI 1.34–1.65, $p < 0.001$), an RR of 1.52 (95%CI 1.39–1.68, $p < 0.001$), and an RR of 1.50 (95%CI 1.38–1.64, $p < 0.001$) for rounds one, two and three, respectively. The risk of seropositivity was even higher in HCWs who worked in dedicated COVID-19 wards compared to frontline HCWs, with an RR of 1.42 (95%CI 1.22–1.66, $p < 0.001$), an RR of 2.48 (95%CI 2.22–2.76, $p < 0.001$), and an RR of 2.25 (95%CI 2.04–2.49, $p < 0.001$) for rounds one, two and three, respectively.

Gradual increases in seropositivity from rounds one to three were seen in the three main categories of HCWs, and were most pronounced in HCWs in COVID-19 wards (Fig. 2). Correspondingly, the incidence of seropositivity was highest in HCWs in COVID-19 wards (6.35% (190/2992), 7.66% (257/3354), and 3.14% (78/2486) in rounds one, two and three, respectively) (Supplementary Material Fig. S7). During the study period the incidence of seropositivity mostly decreased from round one to round three for frontline HCWs (4.5% (759/16 960), 1.98% (244/12 335), and 1.8% (193/10 713) respectively) and the group of remaining HCWs, who were not frontline and not on COVID-19 wards (3.15% (552/17 500), 1.87% (246/13 131), and 2.3% (292/12 727) respectively). At the time of round three the seroprevalence in blood donors was 4.2% (294/6964), i.e., the seroprevalence was significantly higher in all three main categories of HCWs compared with blood donors ($p < 0.001$ for all).

Seroprevalence stratified according to job categories, age and specialty

Fig. 3 shows the seroprevalence among doctors, nurses, and assisting nurses stratified by specialty and round (incidence for each round shown in Supplementary Material Fig. S8). Overall, the differences between the specialties were maintained during the three rounds.

The seroprevalence and incidence for different job categories are shown in Supplementary Material Figs S9 and S10. The highest seroprevalence was observed among medical students where 25.23% (222/880), 28.02% (167/596), and 34.25% (186/543) were seropositive in rounds one, two and three, respectively. The lowest seroprevalence was observed among midwives, where 2.3% (13/555), 1.7% (7/404), and 3.7% (13/356) were seropositive in rounds one, two and three, respectively. Overall, the differences between the job categories were maintained during the three rounds.

In a subgroup analysis of HCWs compared to blood donors stratified by under or over 30 years of age at round three, the difference between blood donors and HCWs persisted in both the young and the old (Supplementary Material Fig. S11). In a subgroup analysis looking only at participants >30 years of age, the incidence of seropositivity was similar to the one seen for all HCWs (Supplementary Material Fig. S12).

Risk of reinfection by seroprevalence

Using a blanking period of 7 days after serological testing, and only including participants with no positive PCR test prior to round

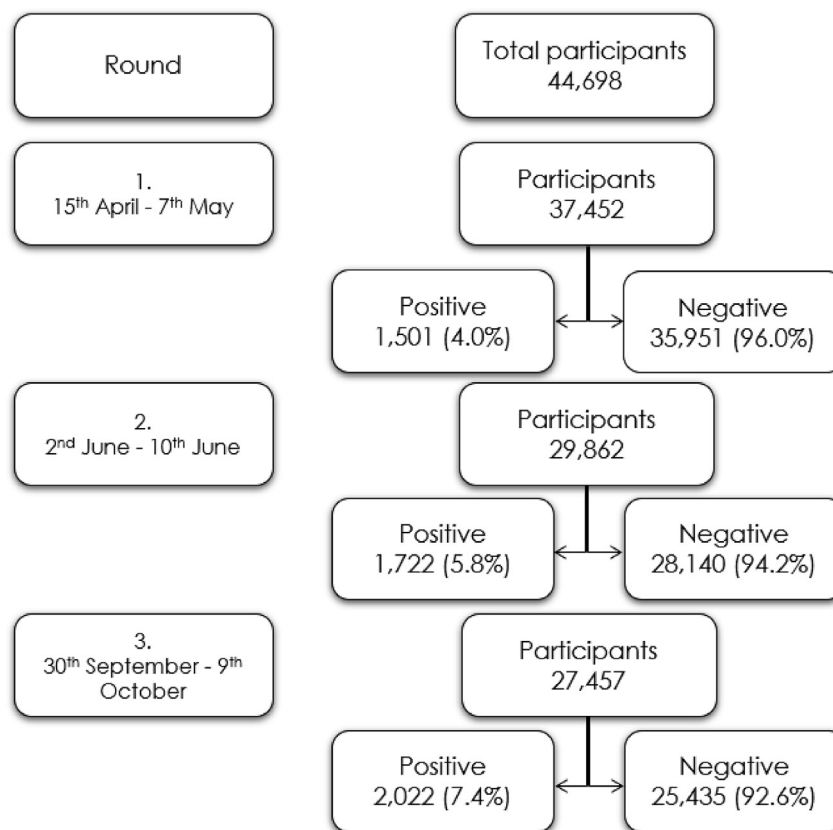


Fig. 1. Flow of participants in the study: healthcare workers (HCWs) participating in the study during the period from April to October 2020.

Table 1

Baseline characteristics of included healthcare workers (HCWs) stratified by round

	Round 1	Round 2	Round 3
N	37 452	29 862	27 457
Age (mean \pm SD)	44.55 \pm 12.72	45.09 \pm 12.57	44.89 \pm 12.81
Body mass index (mean \pm SD)	25.05 \pm 4.69	25.09 \pm 4.75	25.14 \pm 4.79
Female n (%)	28 965 (77.3)	23 887 (80.0)	22 029 (80.2)
Ever smoker n (%)	7026 (21.7)	5574 (21.1)	4546 (19.2)
Seropositive n (%)	1501 (4.0)	1722 (5.8)	2022 (7.4)

one, seven of 801 (0.87%) seropositives and 193 of 25 144 (0.77%) seronegatives reported having had a positive PCR test between rounds one and two (RR 1.14, 95%CI 0.54–2.41, p 0.68). Between rounds one and three, five of 760 seropositives (0.66%) and 389 of 20 894 (1.86%) seronegatives reported having a positive PCR between the rounds, resulting in a significant RR of 0.35 (95%CI 0.15–0.85, p 0.012). Between rounds two and three, three of 796 (0.38%) and 210 of 19 280 (1.09%) seronegatives reported having had a positive PCR test between the rounds (RR 0.35, 95%CI 0.11–1.08, p 0.051).

S/CO ratio for antibodies against SARS-CoV-2

Fig. 4 shows the S/CO ratio for seropositivity for 817 participants who were seropositive in round one and participated in all rounds, as well as the fluctuation of S/CO ratios for seroreverters and HCWs with possible reinfections through rounds one to three. The S/CO ratio of seropositive participants who participated in all rounds increased significantly through rounds one to three (median 11.92, IQR 6.49–17.98; median 16.97, IQR 8.11–23.68; median 19.31, IQR

14.28–23.68 for rounds one, two and three respectively, $p < 0.001$ for all). Looking at the S/CO ratio for these groups in round one, the participants who were seropositive in all three rounds had a significantly higher S/CO ratio (median 11.92, IQR 6.49–17.98) than both participants who seroreverted in round two and did not subsequently seroconvert (median 1.64, IQR 1.38–2.52, $p < 0.001$), the participants who seroreverted between rounds two and three (median 6.22, IQR 1.70–14.91, p 0.01), and the participants with possible reinfections (median 2.76, IQR 1.70–4.76, $p < 0.001$). There was no clear difference in S/CO ratio among the seropositive participants working on the frontline or in dedicated COVID-19 wards (Supplementary Material Fig. S13).

Seroreversion

A total of 2811 participants were found to be seropositive at least one time during the study period, of whom 2248 were seropositive in one of the first two rounds. Of these 2248 participants, 113 (5.0%) had seroreverted at a subsequent round. During the study period of 6 months, 948 of 1003 (94.5%) seropositive participants who participated in both round one and round three stayed seropositive. Table 2 shows a comparison of basic characteristics for seroreverters to participants who stayed seropositive. The participants who seroreverted were significantly older than the other seropositive participants, reported milder illness, and were less likely to think they had been ill because of SARS-CoV-2. In a multivariable logistic regression model including age, sex, asymptomatic versus symptomatic SARS-CoV-2 infection, and ever versus never smoking, only age (OR 1.03, 95%CI 1.01–1.04 $p < 0.001$) and asymptomatic SARS-CoV-2 infection (OR 7.46, 4.8–11.86, $p < 0.001$) were significantly associated with an

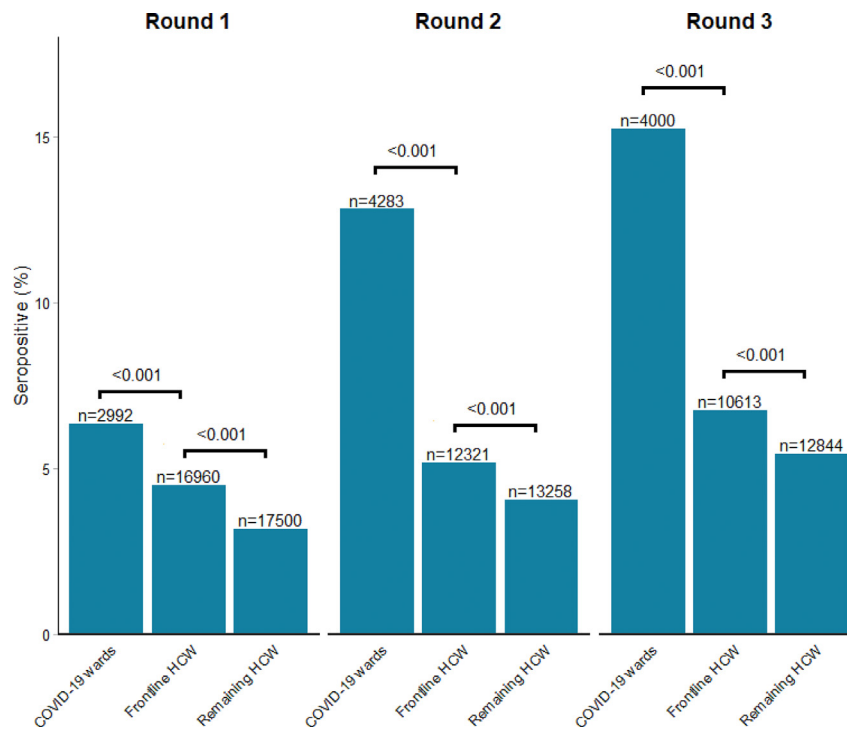


Fig. 2. Risk of seropositivity according to occupational exposure: seroprevalence among healthcare workers (HCWs) at each round stratified by HCWs working in dedicated coronavirus disease 2019 (COVID-19) wards, HCWs not on COVID-19 wards but working frontline, and remaining HCWs.

increased risk of seroreversion. [Supplementary Material Table S6](#) shows the basic characteristics of seroreverters compared to all other participants.

Discussion

We found a gradual and significant increase in seroprevalence in HCWs from April to October 2020. HCWs with antibodies to SARS-CoV-2 had a 65% reduction in risk of reinfection during the following 6 months, and approximately 95% of those who were seropositive during the first round remained seropositive after 6 months. Seroreverters were slightly older and had a milder course of disease.

At all three rounds, HCWs working in COVID-19 wards had the highest seroprevalence, followed by other frontline personnel. The lowest rate was seen in the remaining HCWs. At the end of the study period this group, however, still had a significantly higher seroprevalence than blood donors, which served as a proxy for the general working population. Seroprevalence was lower among females and decreased with age. The highest seroprevalence in all rounds was seen in medical students. HCWs with the medical specialties respiratory medicine, followed by infectious diseases and haematology, had the highest seroprevalence. The differences remained stable during the study period.

Following seroconversion, HCWs reported lower rates of positive PCR tests as compared to seronegative HCWs, indicating protection against reinfection; this is in good agreement with findings in the general population of registry studies [20,21]. This is reassuring, as it indicates that not only seropositive individuals in the general population but also seropositive HCWs seem to be similarly protected despite their increased exposure to SARS-CoV-2.

We have previously reported a higher seroprevalence in HCWs than in blood donors, and that the risk of SARS-CoV-2 infection was related to exposure to infected patients [16]. Despite this knowledge, the observed increase in seroprevalence during the

study period was also highest in those exposed to patients with COVID-19. HCWs in dedicated COVID-19 wards should use adequate protective measures, especially during the second and third screening rounds, since they were directly exposed to patients with COVID-19. Frontline HCWs could, on the other hand, be expected to have had a higher incidence, since compliance with protection measures in treatment of patients without a confirmed diagnosis of COVID-19 may be lower. The reason for our finding of an even greater seropositivity among HCWs in COVID-19 wards is unclear. Provision of healthcare during the COVID-19 pandemic is of utmost importance, and the safety of HCWs is crucial. The present findings strongly support the need for protection of HCWs against transmission and justify the prioritized vaccination of HCWs in most countries.

In a study of British HCWs from the Oxford area [3], a seroprevalence of 9.4% was found in samples collected from March to June 2020. As in our study, the seroprevalence was higher in HCWs than in blood donors in the same area, which was approximately 4% in both March and June 2020 in the UK [22]. No stratification according to specialty or information on whether personnel were working as front-line personnel or in COVID-19 wards was reported in the British study [3].

The observed antibody test characteristics may, in part, explain seroreversion between test rounds. However, the importance of potential (although limited) seroreversion is apparent, but it is reassuring that our data indicate that the antibodies developed in response to infection with SARS-CoV-2 persist in the vast majority for at least 6 months. Similar findings have been reported in a recent study by Wajnberg et al., where stability of IgG antibody titres was found over 148 days in individuals with mild to moderate COVID-19 [23]. While it is unknown to what degree previous infection with SARS-CoV-2 protects individuals from reinfection, a recent study as well as our data suggests that seropositivity is associated with protection against reinfection with SARS-CoV-2 [24].

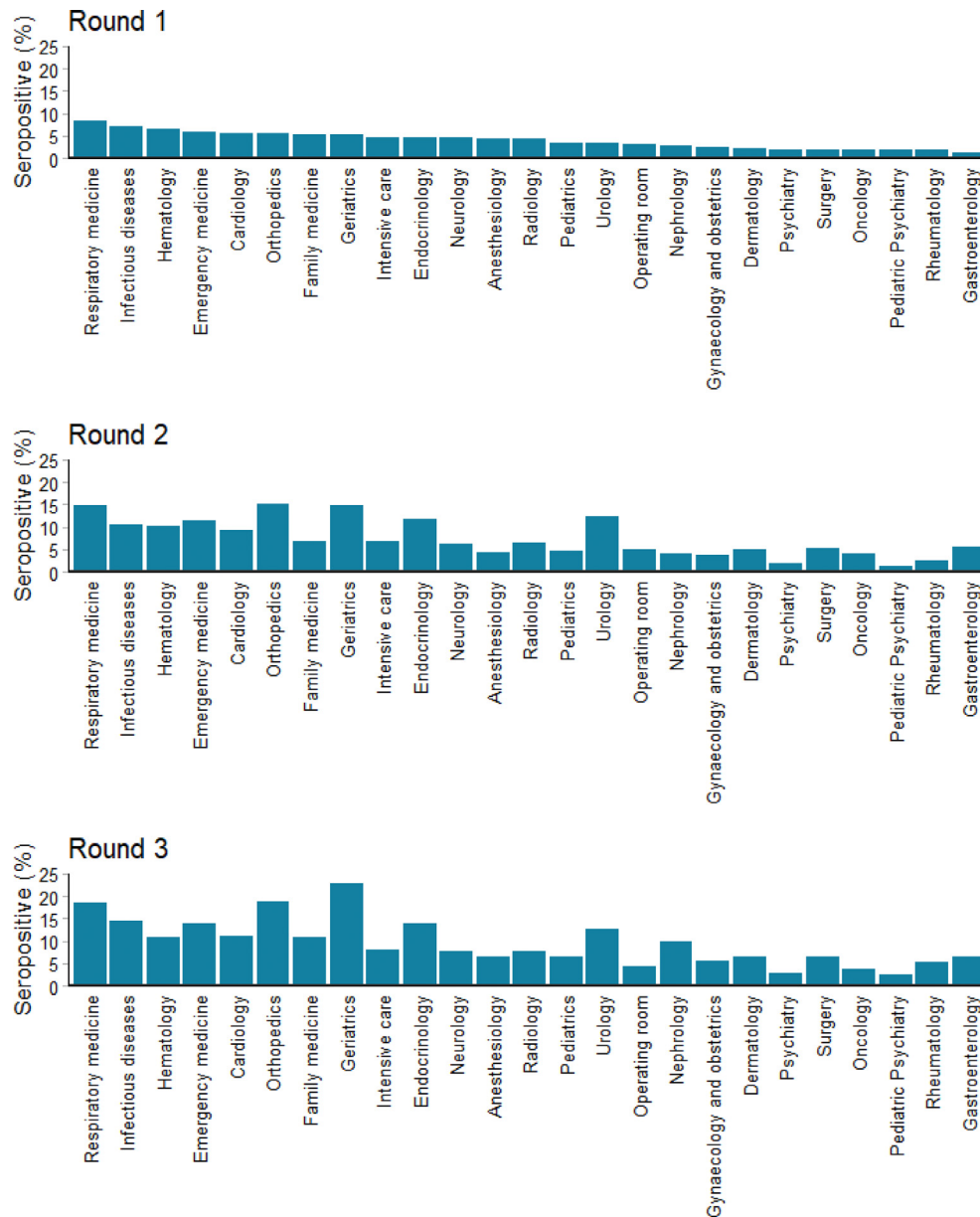


Fig. 3. Seroprevalence stratified by medical specialty for doctors, nurses and assistant nurses. The figure shows seroprevalence among doctors, nurses and assistant nurses stratified by medical specialty. Some specialties are more involved in the treatment of patients with coronavirus disease 2019 (COVID-19) than others, for example a high seroprevalence is noted for respiratory medicine, infectious diseases and emergency medicine. Elderly and/or immunosuppressed patients may shed more virus, which may explain why geriatrics and haematology rank high. Also, in geriatrics, healthcare workers (HCWs) visit patients in their homes where transmission may be higher than in hospitals. Surprisingly, the seroprevalence in intensive care is low compared to other specialties.

The HCWs who seroreverted during the follow-up reported no or mild symptoms of COVID-19. This is in line with previous studies reporting a more rapid decline in antibody levels amongst infected individuals with no or mild symptoms [25]. In a large study of three million patients who were tested for antibodies against SARS-CoV-2, seroreversion was seen in 18.4% of seropositive individuals during a median follow-up of 54 days [24]. However, these data were based on results from several laboratories. Antibody test characteristics and differences in handling of samples may in part also explain the observed high rate of seroreversion compared to our findings.

A high seroprevalence among Copenhagen medical students was also observed in a seroprevalence study of medical students from autumn 2020 [26]. This may be caused by outbreaks at social

events for medical students. Danish students have also been working at SARS-CoV-2 testing facilities during the pandemic, and behavioural patterns may be in play. Also, seroprevalence among medical students contributes to the observed decrease in seroprevalence with increasing age, as medical students are younger than the other participants.

Limitations

Our study has several limitations. Only 42% of participants participated in all study rounds. This may be explained by the introduction of widespread screening options by nasal swab for PCR testing for HCWs during the second and third rounds of our study. In comparison, PCR testing was available only for symptomatic

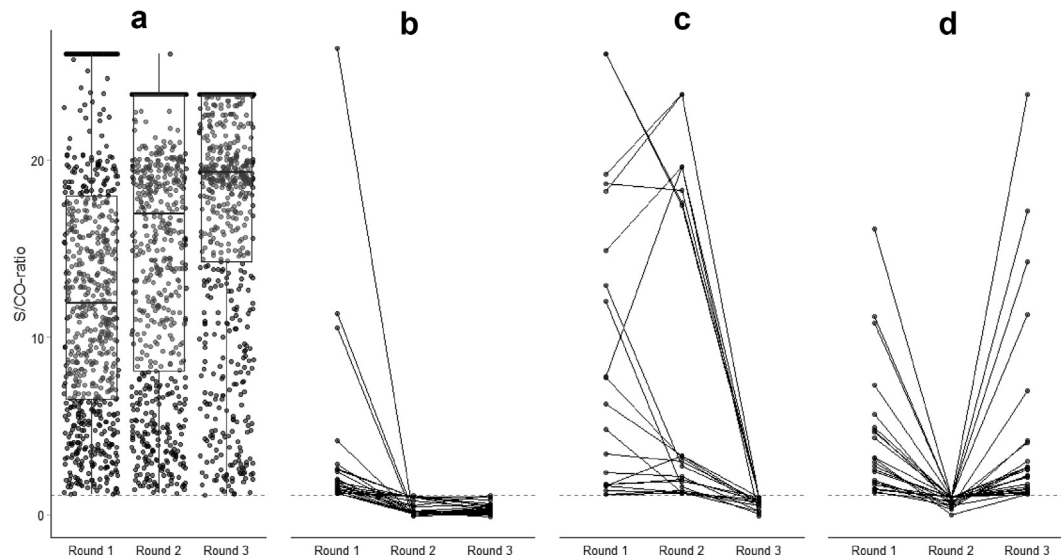


Fig. 4. Signal/cutoff ratio (S/CO ratio) for participants who were seropositive in round one and participated in all three rounds ($n = 817$). (a) S/CO ratio in all three rounds ($n = 745$). (b) Seroreverters in round two ($n = 26$) (positive in round one and negative in rounds two and three). (c) Seroreverters in round three ($n = 21$) (positive in round one and negative in rounds two and three). (d) Possible reinfections ($n = 25$) (positive in round one, negative in round two and positive in round three). The dotted line signifies 1.1, above which was considered positive.

Table 2

Participants who seroreverted compared to all other seropositive participants

	Other seropositive	Seroreverters	p
N	2698	113	
Female n (%)	1967 (72.9)	84 (74.3)	0.820
Age (mean \pm SD)	39.43 \pm 13.27	44.77 \pm 12.80	<0.001
Body mass index (mean \pm SD)	25.02 \pm 4.71	24.48 \pm 4.11	0.244
Ever smoker n (%)	370 (16.4)	23 (21.9)	0.177
Think they have had COVID-19 n (%)	1538 (84.6)	52 (54.7)	<0.001
Severity of symptoms during COVID-19 n (%): ^a			<0.001
I had no symptoms	149 (9.7)	22 (42.3)	
I was at home with few/mild symptoms	405 (26.3)	12 (23.1)	
I was bedridden due to symptoms	949 (61.6)	18 (34.6)	
I was hospitalized due to symptoms	34 (2.2)	0 (0.0)	
I was hospitalized and on a respiratory support machine due to symptoms	4 (0.3)	0 (0.0)	

^a Severity of illness only for participants who reported having been ill with COVID-19.

individuals in March and April, while an antibody test made it possible to tell if an individual was likely to have had SARS-CoV-2. It seems plausible that individuals who did not find it likely that they would have seroconverted since the last screening were less motivated to participate in the consecutive follow-up rounds.

While participation in the questionnaire was possible from home, severely ill persons would not necessarily have been able to participate in follow-up rounds, which may have resulted in a small drop-out among persons who were infected during the study period. Unfortunately, information on ventilator-free days, length of hospital stay, and mortality were not available in the current questionnaire-based study.

Of the 25 participants who were seropositive, then seronegative, and again seropositive in rounds one, two and three, it is unknown whether the seroconversion represents actual reinfection. None of them reported having had a positive PCR test after round one. It is possible that the antibody test results from these individuals are either false positives or false negatives.

In conclusion, this study found that HCWs working frontline and HCWs working in dedicated COVID-19 wards remained at a significantly increased risk of infection with SARS-CoV-2 during the 6-month period of the pandemic. Seropositivity against SARS-CoV-2 was found to last for at least 6 months after infection in nearly all

HCWs and, importantly, reduced the risk of contracting SARS-CoV-2 by 65%. Longitudinal seroprevalence studies with longer follow-up are needed to assess whether immunity is lasting and protects against future SARS-CoV-2 infection.

Author contributions

Conceptualization: KI, JHK, RBH, PBN, KF, JBN, OA, TKF, CAJJ, CTP, JR, SBD, IH, RM, MGB, ES, FF, CS, MEEM, TB, HU, SRO, SDN, HB. Methodology: KI, JHK, RBH, PBN, KF, JBN, OA, TKF, CAJJ, CTP, JR, SBD, IH, RM, MGB, ES, LH, FF, CS, MEEM, TB, HU, SRO, SDN, HB. Validation: MGB, RBD, ES, LH, HU, CSJ, CE, SRO. Formal Analysis: KI, JHK, RBH, HB. Investigation: JHK, RBH, MPH, PBN, ADK, KF, JBN, SBD, IH, MGB, RBD, ES, LH, CSJ, CS, MEEM. Writing—original draft: JHK, RBH, MPH, LH, HB. Writing—review and editing: KI, JHK, RBH, MPH, ADK, KF, JBN, OA, TKF, CAJJ, CTP, JR, SBD, IH, MGB, RBD, ES, LH, FF, MEEM, TB, HU, CSJ, CE, SRO, SDN, HB.

Transparency declaration

Professor Iversen received grants from Lundbeck Foundation to his institution (R349-2020-731); the benefactor had no role in study design, data collection, analysis, interpretation, or writing of the

article. Professor Torp-Pedersen has received grants from Bayer and Novo-Nordisk for studies not related to the current study. Dr Folke has received research grant from the Novo Nordisk Foundation and an unrestricted research grant from the Laerdal Foundation to the Copenhagen EMS. Dr Nielsen has an unrestricted research grant from the Novo Nordic Foundation. Dr Knudsen has received a grant from the Danish Heart Foundation unrelated to this work. Dr Dessau reports personal fees from Roche Diagnostics, outside the submitted work. Professor Benfield received grants for his institution from the Novo Nordisk Foundation, Simonsen Foundation, Lundbeck Foundation, Kai Foundation, and Erik and Susanna Olesen's Charitable Fund. Also, he received an unrestricted grant for his institution and advisory board from GSK; from Pfizer an unrestricted grant for his institution, as principal investigator/clinical trial, and advisory board; from Boehringer Ingelheim as principal investigator for a clinical trial; from Gilead Sciences an unrestricted grant for his institution, as principal investigator for a clinical trial and advisory board; from MSD an unrestricted grant for his institution, as principal investigator and advisory board; from Pentabase as board member; from Roche, Novartis and Kancera AB as principal investigator for clinical trials. Professor Benfield received payment or honoraria from GSK, Pfizer, Gilead Sciences, Boehringer Ingelheim, and Abbvie for lectures. Professor Benfield received a donation of trial medication from Eli Lilly.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2021.09.005>.

References

- [1] WHO Coronavirus (COVID-19) Dashboard. WHO Coronavirus Disease (COVID-19) Dashboard [Internet]. 2021 [cited 24th Mar] Available from: <https://covid19.who.int/>.
- [2] Jeyanathan M, Afkhami S, Smaill F, Miller MS, Lichty BD, Xing Z. Immunological considerations for COVID-19 vaccine strategies. *Nat Rev Immunol* 2020;20:615–32.
- [3] Lumley SF, O'Donnell D, Stoesser NE, Matthews PC, Howarth A, Hatch SB, et al. Antibody status and incidence of SARS-CoV-2 infection in health care workers. *N Engl J Med* 2021;384:533–40.
- [4] Long Q-X, Liu B-Z, Deng H-J, Wu G-C, Deng K, Chen Y-K, et al. Antibody responses to SARS-CoV-2 in patients with COVID-19. *Nat Med* 2020;26:845–8.
- [5] Wajnberg A, Mansour M, Leven E, Bouvier NM, Patel G, Firpo-Betancourt A, et al. Humoral response and PCR positivity in patients with COVID-19 in the New York City region, USA: an observational study. *Lancet Microbe* 2020;1:e283–9.
- [6] Post N, Eddy D, Huntley C, van Schalkwyk MCI, Shrotri M, Leeman D, et al. Antibody response to SARS-CoV-2 infection in humans: a systematic review. *PLoS One* 2020;15:e0244126.
- [7] Tillett RL, Sevinsky JR, Hartley PD, Kerwin H, Crawford N, Gorzalski A, et al. Genomic evidence for reinfection with SARS-CoV-2: a case study. *Lancet Infect Dis* 2021;21:52–8.
- [8] Van Elslande J, Vermeersch P, Vandervoort K, Wawina-Bokalanga T, Vanmechelen B, Wollants E, et al. Symptomatic SARS-CoV-2 reinfection by a phylogenetically distinct strain. *Clin Infect Dis* 2021;73:354–6.
- [9] Prado-Vivar B, Becerra-Wong M, Guadalupe JJ, Marquez S, Gutierrez B, Rojas-Silva P, et al. COVID-19 re-infection by a phylogenetically distinct SARS-CoV-2 variant, first confirmed event in South America. *SSRN Electron J* [Internet] 2020 [cited 2021 Feb 5], <https://papers.ssrn.com/abstract=3686174>.
- [10] To KK, Hung IF, Ip JD, Chu AW, Chan WM, Tam AR, et al. Coronavirus Disease 2019 (COVID-19) re-infection by a phylogenetically distinct severe acute respiratory syndrome coronavirus 2 Strain confirmed by whole genome sequencing. *Clin Infect Dis* 2021;73:e2946–51.
- [11] Gupta V, Bhojar RC, Jain A, Srivastava S, Upadhyay R, Imran M, et al. Asymptomatic reinfection in 2 healthcare workers from India with genetically distinct severe acute respiratory syndrome coronavirus 2. *Clin Infect Dis* 2021;73:e2823–5.
- [12] Mumoli N, Vitale J, Mazzone A. Clinical immunity in discharged medical patients with COVID-19. *Int J Infect Dis* 2020;99:229–30.
- [13] Gousseff M, Penot P, Gally L, Batisse D, Benech N, Bouiller K, et al. Clinical recurrences of COVID-19 symptoms after recovery: viral relapse, reinfection or inflammatory rebound? *J Infect* 2020;81:816–46.
- [14] Reinfection with SARS-CoV-2: considerations for public health response [Internet]. 2020. Available from: <https://www.ecdc.europa.eu/sites/default/files/documents/Re-infection-and-viral-shedding-threat-assessment-brief.pdf>.
- [15] 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;26:1200–4.
- [16] Iversen K, Bundgaard H, Hasselbalch RB, Kristensen JH, Nielsen PB, Pries-Heje M, et al. Risk of COVID-19 in health-care workers in Denmark: an observational cohort study. *Lancet Infect Dis* 2020;20:1401–8.
- [17] Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, O'Neal L, et al. The REDCap consortium: building an international community of software platform partners. *J Biomed Inform* 2019;95:103208.
- [18] Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)—a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009;42:377–81.
- [19] 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;72:249–53.
- [20] Hansen CH, Michlmayr D, Gubbels SM, Mølbak K, Ethelberg S. Assessment of protection against reinfection with SARS-CoV-2 among 4 million PCR-tested individuals in Denmark in 2020: a population-level observational study. *Lancet* 2021;397:1204–12.
- [21] Harvey RA, Rassen JA, Kabelac CA, Turenne W, Leonard S, Klesh R, et al. Association of SARS-CoV-2 seropositive antibody test with risk of future infection. *JAMA Intern Med* 2021;181:672–9.
- [22] Public Health England. Weekly national Influenza and COVID-19 surveillance report. 2020.
- [23] Wajnberg A, Amanat F, Firpo A, Altman DR, Bailey MJ, Mansour M, et al. Robust neutralizing antibodies to SARS-CoV-2 infection persist for months. *Science* 2020;370:1227–30.
- [24] Harvey RA, Rassen JA, Kabelac CA, Turenne W, Leonard S, Klesh R, et al. Real-world data suggest antibody positivity to SARS-CoV-2 is associated with a decreased risk of future infection. *medRxiv* 2020. 2020.12.18.2024833. Preprint.
- [25] Choe PG, Kang CK, Suh HJ, Jung J, Song KH, Bang JH, et al. Waning antibody responses in asymptomatic and symptomatic SARS-CoV-2 infection. *Emerg Infect Dis* 2021;27:327–9.
- [26] Madsen JR, Nielsen JPS, Fogh K, Hansen CB, Nielsen PB, Lange T, et al. Anti-SARS-CoV-2 seropositivity among medical students in Copenhagen. *Open Forum Infect Dis* 2021;8:ofab273.