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Early View

Research letter

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Transition to endemic: Two-year SARS-CoV-2 surveillance follow-up of the ELISA cohort

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Take home message:

The two-year SARS-CoV-2 surveillance follow-up on the ELISA cohort shows the successful transition from COVID-19 pandemic to endemic, confirms occupational risk factors in health care, and identifies household risk factors in a high-incidence period.

To the editor:

The COVID-19 pandemic remains a significant challenge to global health despite the application of vaccines to approximately 70% of the world's population [1]. Unresolved questions include the duration of immunity after infection and vaccination, the effectiveness of vaccines against new virus variants, the relationship between antibody levels and immunity, re-infection risk, and long-term consequences of infection, including Long- and Post-COVID-syndrome. Thus, there is an unmet need for long-term, prospective epidemiological studies addressing these issues in the post-vaccination era.

To our knowledge, the Lüb**e**ck **L**ongitudinal **I**nvestigation of **SA**RS-CoV-2 Infection (ELISA) is the only large-scale prospective study continuing to observe its participants since the beginning of the pandemic. In the first part of the study, we comprehensively examined the complications of widespread pharyngeal swabs for PCR testing [2], identified an increased risk for seropositivity in several high-exposure groups, especially nurses, during a low-incidence period, and revealed a high rate of unreported infections during the first, pre-vaccination period of the pandemic [3]. Furthermore, we provided a transferable model for effective surveillance in this and future pandemics [3].

Here, we report the two-year follow-up results of the ELISA cohort. The primary aim was to analyse potential risk factors during a high-incidence period and the effects of large-scale vaccination programs on the seroprevalence of antibodies against the spike (S1) antigen. The secondary aim was to evaluate the relevance of antibodies against the nucleocapsid (NCP) antigen in discriminating immunisation from infection. The methods have been previously described elsewhere [4].

In April 2022, 1,953 participants (64.0% of the initial ELISA cohort) completed the online questionnaire, and 1,990 participants (65.2%) provided a blood sample (overall 2,094 responses). For 1,849 participants, both data were available. The mean age of the participants was 48.4 years (SD 14.7); 57.7% were female (**Figure a**).

In April 2022, 324 participants who had not tested positive for SARS-CoV-2 before (n=1789), stated that they had tested positive by PCR since the last observation in February 2021. A censored time analysis over the entire observation period (since 2020) yields a prevalence of 19.3% in April 2022 compared to 1.4% in February 2021 (**Figure b**, [3]). In the first quarter of 2022 alone, we registered 264 newly positive PCR results (13.5 % of participants with complete questionnaires), most likely due to the more contagious Omicron variants causing a sharp increase in incidence rates in the first months of 2022.

To discriminate between vaccination and infection, questionnaire data were evaluated for reported positive PCR tests and vaccination against SARS-CoV-2. The vaccination rate in our cohort was exceptionally high. In total, 99.0% of our participants were vaccinated at least twice against SARS-CoV-2, which is in keeping with the high vaccination rate in the adult population of Lübeck (>90%) [5]. The vast majority reported three vaccinations (81.1%, n=1583), while an additional 183 participants (9.4%) were vaccinated four times before filling out the questionnaire. Accordingly, the seroprevalence of antibodies against the S1-antigen amounted to 99.5% compared to 3.2% in February 2021 (**Figure b**) but cannot discriminate between vaccination and/or previous infections. Only 16 participants were not vaccinated against SARS-CoV-2 (0.8%). Among those, nine participants were neither anti-S1 nor anti-NCP IgG positive, four were anti-S1 IgG-positive, and three tested positive for anti-NCP IgG antibodies. Notably, three of these unvaccinated participants (0.2%) reported a positive PCR result although testing negative for both anti-S1- and anti-NCP-IgG antibodies. Whether these

patients had had a mild course, were immunosuppressed, or were infected just recently remains elusive.

NCP is critical for viral genome packaging of coronaviruses [6], and antibodies against NCP may be a sensitive early marker for SARS-CoV-2 infection [7]. In our study, 261 participants (13.1%) displayed increased anti-NCP IgG titres, indicating recent contact with SARS-CoV-2 (**Figure b**). Thirty-four participants were positive for anti-NCP IgG antibodies without reporting a positive PCR test. To adjust for underreporting, we calculated a combined prevalence (PCR^+ or positive anti-NCP IgG) considering only first infections, resulting in an overall prevalence of 22.7% in April 2022, compared to 3.4% combined PCR and anti-S1 IgG seroprevalence in February 2021 (**Figure b**). The here-described prevalence closely resembles official numbers reporting 39,624 confirmed, cumulated infections per 183,668 inhabitants aged 18 years and older (21.6%) in our catchment area until April 1st, 2022 (Hansestadt Lübeck) [5]. Notably, a recent large study from the US reported largely similar trends but exceeded the overall prevalence reported here by far with an estimated seroprevalence of 58.2%, likely indicating lower infection rates in our catchment area compared to the US [8].

Notably, of the participants reporting a positive PCR result in the observation period from March 2020 to April 2022 (n=324), only 139 (42.9%) had a positive anti-NCP IgG result (**Figure c**) in April 2022. This observation is in line with the decline of anti-NCP IgG titres starting as early as 8 weeks following COVID-19, depending on the assay [9,10]. Furthermore, the sensitivity of anti-NCP IgG for SARS-CoV-2 is reduced after mRNA vaccination and negatively correlated with low viral loads during the infection [11]. Thus, the analysis of anti-NCP antibodies is a suitable tool to confirm a previous infection but cannot rule out prior, possibly mild infections in the post-vaccination era. Hence, the 34 participants exhibiting increased anti-NCP IgG titres but not reporting a positive PCR test result likely had clinically undetected infections. Under this conservative assumption (there might be anti-NCP IgG^- persons with undetected infections), the proportion of persons with undetected infections was at least 9.5% (34 persons with undetected infections /(324 PCR^+ + 34 persons with undetected infections), which is considerably lower than in February 2021 (29%) [3].

Fifty-five (18.4%) of the participants with a positive PCR result before March 2022 (n=299) stated that they still experienced COVID-19-related symptoms after the acute phase, even though our cohort was characterised by mainly mild cases, with a hospitalisation rate of only 1.5% (n=5, one needing oxygen supplementation, none requiring ventilation).

Adjusted for age, sex, and education, risk groups for SARS-CoV-2 infection encompassed inpatient nursing staff (OR 1.9; 95%CI 1.1-3.1) and physicians in the outpatient sector (OR 1.8; 95%CI 1.2-2.9), confirming the results of the main study [3]. Additionally, children under 18 years in the household (OR 1.4; 95%CI 1.1-1.8), especially children in day-care, were identified as risk factors for reported positive PCR results. Interestingly, an increased proportion of participants with children in day-care also exhibited positive anti-NCP IgG test results compared to households with children in other school forms (16.1% vs. 9.4%). However, contact with a COVID-19-affected person remained the leading risk factor for SARS-CoV-2 infection (OR 4.7; 95%CI 3.6-6.3) (**Figure d**).

In summary, the ELISA study is unique in providing longitudinal population-based cohort data from the beginning of the COVID-19 pandemic, now reporting on the prospective follow-up after two years in the post-vaccination and virus variant era. Our results confirm that large-scale prospective cohorts are a suitable tool to survey the epidemiology of infections during the COVID-19 and future pandemics. With the emergence of the first Omicron variant in late 2021, the prevalence in the ELISA cohort has risen sharply, resulting in a more than six-fold increase in one year. The number of unreported cases remains high, with one in ten infections

going undetected or unreported. We confirmed occupational risk factors for SARS-CoV-2 infection, especially in health care, and identified additional risk factors in the high-incidence period, including having children under the age of 18 in the household. A follow-up on these results in our cohort and additional analytical and epidemiological studies are warranted to investigate a possible link between SARS-CoV-2 antibody dynamics and the resolution of COVID-19-related symptoms over time.

FOOTNOTES:

Conflict of interest:
The authors have no conflict of interest to disclose.

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FIGURE LEGEND:

Figure: Results of the ELISA-study two-year follow-up. a) Invitation and inclusion of participants in the follow-up study protocol. Ultimately, 2094 data sets (68.6% of invited), including an online questionnaire and/or DBS-antibody detection, were included in the further analysis. **b)** Cumulative, positive PCR results for SARS-CoV-2, the seroprevalence of anti-S1 IgG, anti-NCP IgG, and combined PCR and seroprevalences in February 2021 and April 2022, as available. **c)** Anti-NCP IgG antibody status of participants reporting positive PCR test results for SARS-CoV-2 since March 2021. 27 participants reported positive PCR results but didn't provide a DBS sample for antibody analysis **d)**. Odds ratios for SARS-CoV-2 infection (as detected by PCR) for selected parameters of interest. The antibody detection assays were performed as previously described [3] but from dried blood spots. The EUROIMMUN SARS-CoV-2 S1 IgG (#EI 2606-9601 G) and the EUROIMMUN SARS-CoV-2 NCP IgG (#EI 2606-9601-2 G) enzyme-linked immunosorbent assays were performed according to the manufacturer's instructions. DBS = Dried blood spot; ELISA study = The Lüb**e**ck **L**ongitudinal **I**nvestigation of **SA**RS-CoV-2 Infection study; n.d. = not determined; NCP = nucleocapsid; S1 = spike protein S1.

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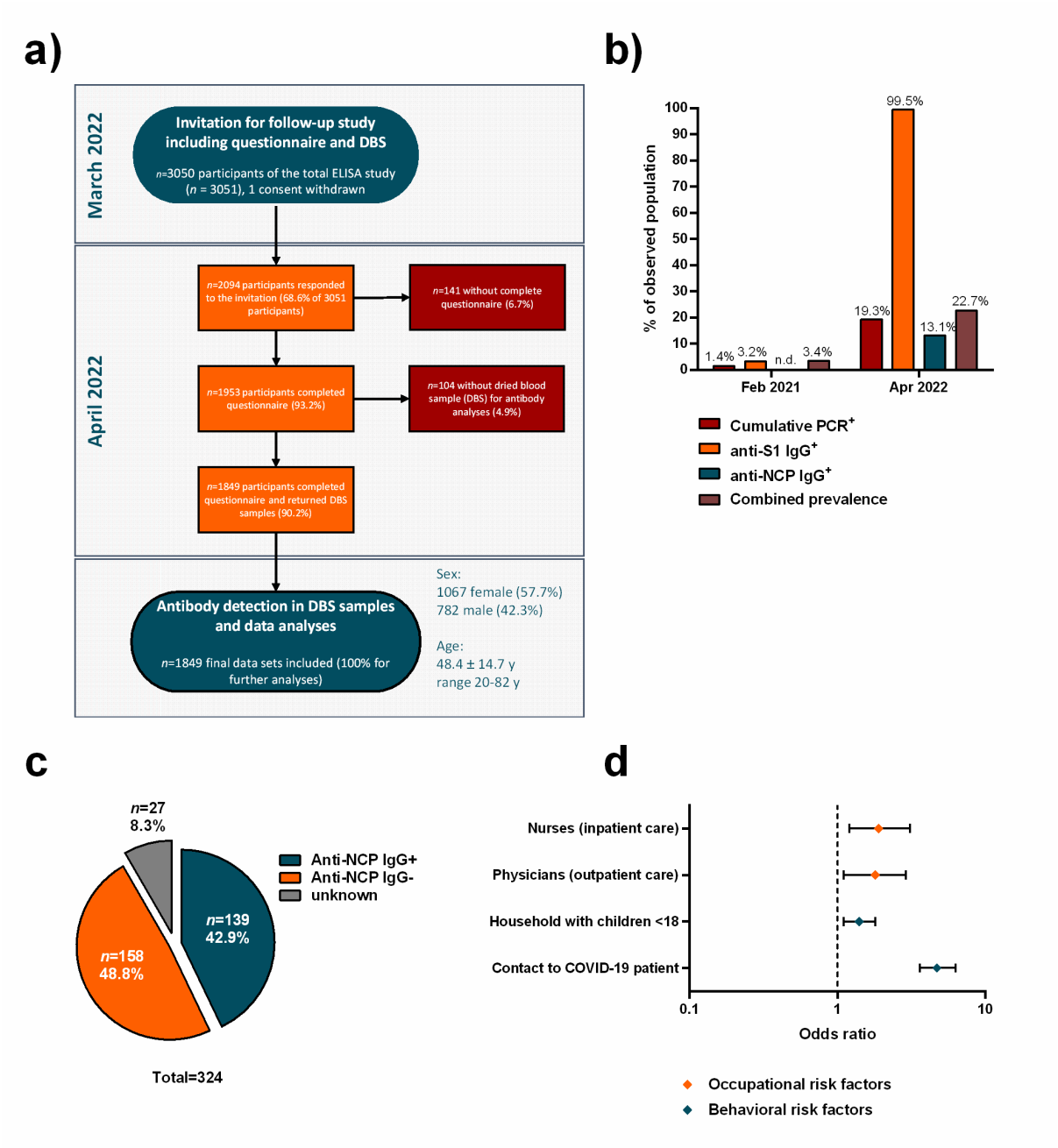
a)
March 2022
Invitation for follow-up study including questionnaire and DBS
n=3050 participants of the total ELISA study (n = 3051), 1 consent withdrawn
April 2022
n=2094 participants responded to the invitation (68.6% of 3051 participants)
n=141 without complete questionnaire (6.7%)
n=1953 participants completed questionnaire (93.2%)
n=104 without dried blood sample (DBS) for antibody analyses (4.9%)
n=1849 participants completed questionnaire and returned DBS samples (90.2%)
Antibody detection in DBS samples and data analyses
n=1849 final data sets included (100% for further analyses)
Sex:
1067 female (57.7%)
782 male (42.3%)
Age:
48.4 ± 14.7 y
range 20-82 y
b)
% of observed population
100
90
80
70
60
50
40
30
20
10
0
1.4%
3.2%
n.d.
3.4%
19.3%
99.5%
13.1%
22.7%
Feb 2021
Apr 2022
Cumulative PCR+
anti-S1 IgG+
anti-NCP IgG+
Combined prevalence
c
n=27
8.3%
n=139
42.9%
n=158
48.8%
Anti-NCP IgG+
Anti-NCP IgG-
unknown
Total=324
d
Nurses (inpatient care)
Physicians (outpatient care)
Household with children <18
Contact to COVID-19 patient
0.1
1
10
Odds ratio
Occupational risk factors
Behavioral risk factors