BMJ Open

The prevalence, incidence and longevity of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium: a prospective cohort study with 12 months of follow-up

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-065897
Article Type:	Original research
Date Submitted by the Author:	22-Jun-2022
Complete List of Authors:	Adriaenssens, Niels; University of Antwerp, General Practice; Universiteit Antwerpen Scholtes, Beatrice; Liege University, General Practice Department - Primary Care and Health Research Unit Bruyndonckx, Robin; Hasselt University, Interuniversity Institute for Biostatistics and statistical Bioinformatics (I-BIOSTAT) Van Ngoc, Pauline; Liege University, Department of General Medicine Verbakel, Jan; University of Oxford, Nuffield Department of Primary Care Health Sciences; KU Leuven, Department of Public Health and Primary Care De Sutter, An; Ghent University, Department of Public Health and Primary Care Heytens, Stefan; University of Ghent, Department of Public Health and Primary Care Van Den Bruel, Ann; KU Leuven Desombere, Isabelle; Sciensano, Department of Infectious Diseases in Humans Vandamme, Pierre; University of Antwerp, Centre for the Evaluation of Vaccination Goossens, Herman; University of Antwerp, of Medical Microbiology, Vaccine & Infectious Diseases Institute (VAXINFECTIO) Buret, Laetitia; Liege University, General Practice Department - Primary Care and Health Research Unit Duysburgh, Els; Sciensano, Department of Epidemiology and Public Health Coenen, Samuel; University of Antwerp, Department of Family Medicine & Population Health (FAMPOP); University of Antwerp, Vaccine & Infectious Disease Institute (VAXINFECTIO)e & Infectious Disease Institute (VAXINFECTIO)
Keywords:	PRIMARY CARE, COVID-19, GENERAL MEDICINE (see Internal Medicine), Epidemiology < INFECTIOUS DISEASES

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

The prevalence, incidence and longevity of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium: a prospective cohort study with 12 months of follow-up

Niels Adriaenssens[1†], Beatrice Scholtes[2†], Robin Bruyndonckx[3,4,5], Pauline Van Ngoc[2], Jan Y Verbakel[6,7], An De Sutter[8], Stefan Heytens[8], Ann Van den Bruel[6], Isabelle Desombere[9], Pierre Van Damme[10], Herman Goossens[4], Laëtitia Buret[2], Els Duysburgh[11‡], Samuel Coenen[1,4‡]

1. Centre for General Practice, Department of Family Medicine & Population Health (FAMPOP), University of Antwerp, Antwerp, Belgium
2. Research unit of Primary Care and Health, Department of General Medicine, , Department of Clinical Sciences, University of Liège, Liège, Belgium
3. Interuniversity Institute for Biostatistics and statistical Bioinformatics (I-BioStat), Data Science Institute, Hasselt University, Diepenbeek, Belgium
4. Laboratory of Medical Microbiology, Vaccine & Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Antwerp, Belgium
5. P95 Epidemiology & Pharmacovigilance, Leuven, Belgium
6. EPI-Centre, Department of Public Health and Primary Care, KU Leuven, Leuven, Belgium
7. NIHR Community Healthcare Medtech and IVD cooperative, Nuffield Department of Primary Care Health Sciences, University of Oxford, Oxford, United Kingdom
8. Department of Public Health and Primary Care, Ghent University, Ghent, Belgium
9. Service Immune response, Department of Infectious Diseases in humans, Sciensano, Brussels, Belgium
10. Centre for the Evaluation of Vaccination, Vaccine & Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Antwerp, Belgium
11. Department of Epidemiology and Public Health, Sciensano, Brussels, Belgium

Corresponding author:
Samuel Coenen
Gouverneur Kinsbergencentrum
Doornstraat 331
BE-2610 Wilrijk
Belgium
samuel.coenen@uantwerpen.be

Word count: 3953

[†]These authors contributed equally to this work as first author

[‡]These authors contributed equally to this work as last author

Abstract

Objectives: To estimate the prevalence, incidence, and longevity of antibodies against SARS-CoV-2 among primary healthcare providers (PHCPs).

Design: Prospective cohort study with 12 months of follow-up.

Setting: Primary care in Belgium

Participants: Any general practitioner (GP) working in primary care in Belgium and any other PHCP from the same GP practice who physically manages (examines, tests, treats) patients were eligible. A convenience sample of 3,648 eligible PHCPs from 2,001 GP practices registered for this study (3,044 and 604 to start in December 2020 and January 2021, respectively). 3,390 PHCPs (92,9%) participated in their first testing timepoint (2,820 and 565, respectively) and 2,557 PHCPs (70,1%) in the last testing timepoint (December 2021).

Interventions: Participants were asked to perform a rapid serological test (RST) targeting IgM and IgG against the receptor binding domain (RBD) of SARS-CoV-2 and to complete an online questionnaire at each of maximum 8 testing timepoints.

Primary and secondary outcome measures: The prevalence, incidence, and longevity of antibodies against SARS-CoV-2 both after natural infection and after vaccination.

Results: Among all participants, 67% were women and 77% GPs. Median age was 43 years. The seroprevalence in December 2020 (before vaccination availability) was 15.1% (95% CI: 13.5% to 16.6%), increased to 84.2% (95% CI: 82.9% to 85.5%) in March 2021 (after vaccination availability) and reached 93.9% (95% CI: 92.9% to 94.9%) in December 2021 (during booster vaccination availability and fourth (delta variant dominant) covid wave). Among not (yet) vaccinated participants the first monthly incidence of antibodies against SARS-CoV-2 was estimated to be 2.91% (95% CI: 1.80% to 4.01%). The longevity of antibodies is higher in PHCPs with self-reported COVID-19 infection.

Conclusions: This study confirms that occupational health measures provided sufficient protection when managing patients. High uptake of vaccination resulted in high seroprevalence of SARS-CoV-2 antibodies in PHCPs in Belgium. Longevity of antibodies was supported by booster vaccination and virus circulation.

Registration: Trial registration number: NCT04779424

Key words: cohort study; primary care; SARS-CoV-2; COVID-19; prevalence; incidence; antibodies; seroprevalence

Strengths and limitations of this study

- This large cohort study with 12 months follow-up could provide precise estimates of the prevalence and incidence of antibodies against SARS-CoV-2 among primary health care providers (PHCPs) at national and regional level in Belgium.
- The rapid serological test (RST) used targets IgM and IgG against the receptor binding domain of SARS-CoV-2 and could therefore also assess the antibody response after vaccination, and longevity of antibodies against SARS-CoV-2 both after natural infection and after vaccination, but cannot distinguish between both.
- The results in PHCPs could be compared to that of the general population and other population groups, e.g. health care workers in hospitals and nursing homes.
- The use of a convenience sample, missing data points and reduced RST accuracy when performed and interpreted by many different participants could limit the validity of the study results.

Introduction

As of 8th June 2022, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused over 530 million infections worldwide (4,164,698 in Belgium) and caused over 6.3 million deaths from coronavirus disease (COVID-19) worldwide (over 31,000 in Belgium).[1] COVID-19 can be a lethal respiratory tract infection (RTI), but often presents with mild symptoms or remains asymptomatic.

Since the start of the COVID-19 pandemic, SARS-CoV-2 seroprevalence estimates have provided essential information about population exposure to infection and helped predict the early course of the epidemic.[2,3] When setting up this study, seroprevalence studies in Iceland[4] and Spain[5] showed different levels of population antibody positivity, lasting up to at least 4 months in Iceland. In addition, early cohort studies have suggested waning of antibody levels in individuals is associated with, for example, illness severity, age and co-morbidities.[6-8] Meanwhile, other seroprevalence studies showed antibody positivity lasting up to 9 months.[9,10] Additionally, after vaccination, longevity of antibody positivity could differ depending on the type of vaccination and vaccination regime.[11,12] For Belgium, Sciensano (the Belgian national institute of public health, www.sciensano.be) performs national seroprevalence studies of SARS-CoV-2 antibodies in the general population[13] and several relevant populations including school-aged children and school staff,[14] hospital staff,[15] nursing homes residents and their staff.[16,17] These results are publicly available and regularly updated on an online dashboard.[18]

This article focuses on the seroprevalence among primary healthcare providers (PHCPs).[19] PHCPs manage the vast majority of patient contacts, including COVID-19 patients and therefore play an essential role in the efficient organisation of healthcare.[20,21] Among the PHCPs, general practitioners (GPs) in particular, act as gatekeepers to the next levels of care. Therefore, preserving the capacity of GPs, together with that of their co-workers, throughout the COVID-19 epidemic is essential.[22] In Belgium, this is particularly concerning given that the GP workforce consists of mainly older adults and is therefore at higher risk for COVID-19-related morbidity and mortality.[23] In Italy, GPs represented up to 38% of the physicians who died from COVID-19 early in the epidemic.[24]

Before the start of this study (December 2020) data on how many PHCPs in Belgium had been infected by SARS-CoV-2 was not readily available,[25] and effective vaccines for PHCPs were not anticipated to be available in the near future.

During the COVID-19 crisis rapid serological tests (RSTs) have been developed to identify the presence of antibodies to SARS-CoV-2. Compared to laboratory tests, a valid easy-to-use RST could speed up the availability of the test results for both the participants and the national health authorities.[25] Furthermore, by using RSTs in this study, PHCPs got the opportunity to become more familiar with this type of technology.

Sciensano has validated five RSTs using finger prick blood, identifying one test with appropriate sensitivity (92.9%) and specificity (96.3%) for use in seroprevalence studies.[26] We used this RST for the present study. It targets IgM and IgG against the receptor binding domain (RBD) of SARS-CoV-2 and could therefore also provide valuable information in a vaccinated population.

Given the availability of vaccines for PHCPs soon after the start of this study, we now report on the prevalence of antibodies against SARS-CoV-2 among a cohort of PHCPs in Belgium followed-up for 12-months, and on the incidence and longevity of those antibodies both after natural infection and after vaccination.

Methods

This study was a prospective cohort study. Data collection was performed according to the publicly available protocol, providing more details on the study methods.[19]

Study population

Any GP working in Belgium (including those in professional training) working in primary care and any PHCP from the same GP practice in a clinical role (clinical examination, testing or treating patients) were eligible if they were able to comply with the study protocol and provided informed consent to participate in the study. Staff hired on a temporary (interim) basis were excluded as follow-up over time would be compromised. Administrative staff or technical staff without any prolonged (longer than 15 minutes) face-to-face contact with patients and PHCPs who were not professionally active during the inclusion period were not eligible either.

PHCPs were recruited between 15 November 2020 and 15 January 2021. GPs working in clinical practice in Belgium were invited to register online for participation in this national epidemiological study and were asked to invite the other PHCPs in their practice to do the same. We emphasized that PHCPs who had already been diagnosed with COVID-19 were also eligible. Information about the study was disseminated to GPs and PHCPs via professional organisations (Domus Medica and Collège de Médecine Générale), university networks across the country and through professional media channels. We checked our convenience sample for representativeness in terms of geographic and demographic characteristics.[23]
To assess the geographical representativeness of our sample, we compared the distribution by region and by province of active GPs in Belgium in 2020 (source www.ima-aim.be) with the distribution of participating GPs.

Data Collection

Upon inclusion in the study, participants were assigned a unique study code by the researchers and received testing material at their workplace through regular mail. At their first testing timepoint they received an invitation by email inviting them to auto-collect a capillary blood sample and analyse it using the RST (OrientGene®) and to complete a baseline questionnaire available in Dutch, French and English via a personalised link through a secured online platform hosted by Sciensano (Limesurvey). The invitation email included links to both written and video instructions to perform the RST on yourself and on someone else.

The baseline questionnaire at the first testing timepoint asked for informed consent and for information about the result of the RST, basic socio-demographic data (age, gender, composition of household – e.g. presence of school-aged children in the house), professional data (practice patient size), health status (pre-existing health conditions, regular medication use, presence of symptoms since the start of the epidemic, previous positive test results for COVID-19), professional exposure (contact with confirmed cases, use of infection prevention and control measures and the availability of personal protective equipment) and practice organisational aspects (delayed care for non-urgent conditions).[19] A follow-up questionnaire was sent for each of the subsequent testing timepoints. In addition to the RST result, it collected information on the health status, including the presence of symptoms, COVID-19 testing and results, vaccination status (date of vaccination, type of vaccine, number of doses, presence of side-effects) and professional exposure (contact with confirmed cases, use of infection prevention and control measures).[19]

Follow-up

The study lasted 12 months, from December 2020 to December 2021, and included 8 testing timepoints. Compared to the study protocol, the testing timepoint at the fifth month was skipped because of limited additional epidemiological value based on progressive insights

from studies with similar protocols conducted by Sciensano that longer interval than four weeks between testing time point are suitable.[13-17]

Sample size

This study aimed to include 5,000 PHCPs with a ratio of 4 GPs to 1 other PHCP. The sample size considerations regarding the different objectives of the proposed study are described in more detail in the study protocol.[19] For the objectives reported here, even half the sample size aimed for would allow for precise estimates of the prevalence, incidence and longevity of antibodies against SARS-CoV-2.

Data analysis

In the analysis, we included all PHCPs who provided informed consent and reported RST results at the testing timepoints. If in the questionnaire the entry for the date the RST was performed was missing or implausible, the date of completing the questionnaire was used instead. All analyses were conducted using R version 4.1.0 (www.R-project.org).

Prevalence

To assess the prevalence of antibodies against SARS-CoV-2, we calculated among the valid RST the proportion (95% CI) of positive RST for IgG and/or IgM, and for IgG and IgM separately (crude seroprevalences). In addition, we calculated the proportion (95% CI) of PHCPs that self-reported testing positive for SARS-CoV-2 (no test specified, so this includes both virus or antibody detection) since the outbreak of the COVID-19 pandemic (February 2020), and the proportion (95% CI) of PHCPs with any positive test, either a positive study RST or testing positive since the outbreak at their first testing timepoint. For any subsequent testing timepoints we asked the participants to specify if self-reported testing positive for SARS-CoV-2 since the previous testing timepoint concerned virus or antibody detection.

We also estimated the prevalence of antibodies against SARS-CoV-2 (IgG and/or IgM) taking into account clustering of PHCPs within their practice as well as the distribution of PHCPs across the districts in Belgium (adjusted seroprevalences). Weights were calculated based on the differences between the actual distribution of GPs across districts and the distribution of participating GPs with RST results across districts. These weights were then extrapolated to all other PHCPs. The estimates are based on Generalised Estimating Equations (GEE) assuming a binomial distribution for the RST result, an identity link function and an independent working correlation matrix.[27] In a similar way we also estimated the adjusted prevalence of self-reported positive testing for SARS-CoV-2 since the start of the COVID-19 pandemic and the adjusted prevalence of these two tests results combined, either a positive study RST or testing positive since the outbreak for the first two testing timepoints.

Incidence

To assess the incidence of antibodies against SARS-CoV-2 (IgG and/or IgM) among participants not (yet) vaccinated, first we produced a Kaplan-Meier plot including participants providing a valid negative RST result at their first testing timepoint and not testing positive before, considering a positive RST during follow-up as event and censoring upon vaccination or loss to follow-up. Second, we assessed the monthly incidence of antibodies against SARS-CoV-2 due to natural infection in those not yet vaccinated, by analysing the data collected during the testing timepoints after the first testing timepoint. We included participants providing valid RST results both at the testing timepoint assessed and the preceding testing timepoint. We excluded participants reporting a positive RST at the preceding timepoint or already vaccinated with a first dose. In addition, we corrected the estimates for clustering of participants in general practices.

To assess the incidence of antibodies against SARS-CoV-2 (IgG and/or IgM) due to vaccination in those vaccinated, we calculated the proportion of participants with antibodies against SARS-CoV-2 less than seven days and seven days or more after the first, the second

and the third dose of a COVID-19 vaccine, respectively, and stratified by self-reported history of COVID-19 infection.

Longevity

To assess the longevity of antibodies against SARS-CoV-2 (IgG and/or IgM) among participants not (yet) vaccinated, first we produced a Kaplan-Meier plot including participants without a self-reported history of COVID-19 infection before their first testing timepoint that provided a valid positive RST results before receiving their first dose of a COVID-19 vaccine, considering a negative RST result during follow-up as event (= negative RST result followed by another negative RST result or missing data) and censoring upon vaccination or loss to follow-up (midpoint and interval censoring). Second, we included participants not yet vaccinated, that provided a valid RST result at the testing timepoint assessed and a positive RST result at the previous testing timepoint. We estimated the proportion with a negative test result at the testing timepoint assessed.

To assess the longevity of antibodies against SARS-CoV-2 (IgG and/or IgM) after COVID-19 vaccination, we produced Kaplan-Meier plots by self-reported history of COVID-19 infection, including participants that provided a valid positive RST results at least seven days after receiving their second dose of a COVID-19 vaccine, considering a negative RST result during follow-up as event (= negative RST result followed by another negative RST result or missing data) and censoring upon booster vaccination (date of third dose) or loss to follow-up (midpoint and interval censoring).

Vaccination

The start of the vaccination of PHCPs during the study follow-up provided the opportunity to monitor its progress.

Ethics and dissemination

Ethical approval granted at 16 November 2020 (reference number: 20/46/605) by the Ethics Committee of the University Hospital Antwerp/University of Antwerp (Belgian registration number: 3002020000237).

Patient and Public Involvement

Neither patients (or PHCPs in this specific study) nor the public were involved in the design of the study. During the study the information shown in Figure 1 was shared with the participants and the general population through the publicly available website of the Belgian health authorities (Sciensano) shortly after each testing-timepoint both for Belgium and its three regions, Brussels, Flanders and Wallonia.[18]

Results

Description of the study cohort

In total, 3,648 eligible PHCPs from 2,001 practices registered and were asked to provide informed consent of whom 3,044 and 604 PHCPs were sent personal study materials to be able to collect data for their first testing timepoint starting on 24 December 2020 and 25 January 2021, respectively. 3,390 PHCPs participated in their first testing timepoint by completing the baseline questionnaire, among which 2,597 GPs, 386 GPs in training and 407 other PHCPs (Table 1).

Our sampling procedure resulted in the participation of a reasonably geographically representative sample of GPs at the level of the provinces (Table S1, online supplementary data). At the level of the regions, there is about 8% overrepresentation of GPs in Flanders and corresponding underrepresentation of GPs in Wallonia.

Participant characteristics

Table 1 presents the characteristics of the 3,390 PHCPs who participated in their first (baseline) testing timepoint. These PHCPs, mainly GPs, were relatively young, more often female and working more often in (large) group practices than in solo or duo practices. Table 2 shows in how many testing timepoints primary healthcare providers (PHCPs) participated. 3,415 (93.6%) PHCPs participated in at least one testing timepoint, 2,909 (79,7%) participated in six and 2,141 (58.7%) participated in all eight testing timepoints. The number of PHCPs participating per testing timepoint is presented in Table S2 (online supplementary data). While the response rate gradually decreased, still 2,557 (77.2% of invited PHCPs) participated in the last testing timepoint.

Vaccination status

Overall, 3,227 participants received a full primary vaccination. 2,783 participants received two doses of an m-RNA vaccine (2,639 (81.8%) BNT162b2, 144 (4.5%) mRNA-1273 and 2 (0.1%) mRNA-1273 followed by BNT162b2). 437 participants (13.5%) received two doses of ChAdOx1-S and 5 (0.2%) participants one dose of Ad26.COV2.S.

At the final testing timepoint, 2,211 of the participants had received a booster vaccination. 1,879 (85.0%) participants received a booster with BNT162b2 and 267 (12.1%) with mRNA-1273. 1 participant received ChAdOx1-S and another participant Ad26.COV2.S as third dose.

Table 1. Characteristics of primary healthcare providers (PHCPs), including general practitioners (GPs), GPs in training and other PHCPs who participated in their first testing timepoints[1]

	PHCPs n=3,390		GPs n=2,597		GPs in training n=386		Other PHCPs n=407	
Age[2], median (IQR)	40	(31-54)	44	(34-57)	27	(26-28)	38	(31-47)
Gender[3], n (%)								
- Male	1,119	(33.0)	943	(36.3)	112	(29.0)	64	(15.7)
- Female	2,296	(66.9)	1,652	(63.6)	274	(71.0)	343	(84.3)
- Not reported	2	(0.1)	2	(0.1)	0	(0)	0	(0)
Practice size, n (%)[3]								
- Solo	618	(33.5)	580	(34.7)	54	(16.1)	29	(11.8)
- Duo	361	(19.6)	328	(19.6)	74	(22.1)	32	(13.1)
- Group (<8 employees)	382	(20.7)	351	(21.0)	51	(15.2)	21	(8.6)
- Large group (>7 employees)	444	(24.1)	386	(23.1)	156	(46.6)	150	(61.2)

[1] The first testing timepoint was December 2020 for 2,820 and January 2021 for 570 PHCPs, respectively; [2]Ages < 21 were considered unrealistic and recoded as missing; IQR=interquartile range; [3] if numbers do not add up to the column total, this is due to missing data; numbers of practices for PHCPs=1,845, GPs=1,672, GPs in training=335 and other PHCPs=245.

Table 2. The number of testing timepoints that primary healthcare providers (PHCPs) participated in

Number of testing timepoints participated in	Number of PHCPs (%) N=3,648		Cumulative percentage
8[1]	2,141	(58.7%)	58.7%
7	490	(13.4%)	72.1%
6	278	(7.6%)	79.7%
5	153	(4.2%)	83.9%
4	129	(3.5%)	87.5%
3	91	(2.5%)	90.0%
2	87	(2.4%)	92.4%
1	46	(1.3%)	93.6%
0	233	(6.4%)	100.0%

[1] The eight testing timepoints have the following start and end dates: T1: 24/12/2021-8/1/2021, T2: 25-31/1/2021, T3: 22-28/2/2021, T4: 22-31/3/2021, T5: 19-28/4/2021, T6: 14-27/6/2021, T7, 13-26/9/2021, T8: 13-26/12/2021.

Prevalence

The prevalence of antibodies against SARS-CoV-2 among PHCPs in Belgium from December 2020 to December 2021 is shown in Figure 1 and Table S3. Table S3 also gives the number of eligible PHCPs, i.e. those testing between the start and end date of the respective testing timepoint, as well as the regional differences. At the first testing timepoint (T1), among 2680 eligible PHCPs, 2629 provided valid test results, of which 366 (15.1%) were positive. Afterwards, the prevalence increased substantially up to 84.2% at T4, mainly due to vaccination (see Table S4). Six months later (T7) the prevalence was substantially lower (70.2%), while during the fourth covid wave (delta variant dominant) and after booster vaccination became available it increased again to 93.9% (T8).

Incidence

Among not (yet) vaccinated participants

The incidence of antibodies against SARS-CoV-2 among PHCPs in Belgium among participants that provided a valid negative RST result at their first testing timepoint, did not self-report a COVID-19 infection before and were not (yet) vaccinated is shown in figure 2.

For the second testing timepoint (T2) the monthly incidence of antibodies against SARS-CoV-2 was estimated to be 2.91% (95%CI: 1.80-4.01; n=895), i.e. the proportion of PHCPs not yet vaccinated at T2 and testing negative at T1, that tested positive at T2. For T3 and T4 it was estimated to be 3.93% (95%CI: 2.04-5.82; n=407) and 4.04% (95%CI: 0.16 - 7.92; n=99), respectively. As of T4, the sample size of eligible participants was too small for precise estimates.

Among vaccinated participants

The incidence of antibodies against SARS-CoV-2 among vaccinated PHCPs in Belgium according to their self-reported history of COVID-19 infection is shown in figure 3. The incidence of antibodies is higher in PHCPs with self-reported COVID-19 infection compared to PHCPs with no self-reported COVID-19 infection both less than seven days and seven days or more after the first and the second dose, less than seven days after the third dose, but not seven days or more after the third dose.

Longevity

Among not (yet) vaccinated participants

The longevity of antibodies against SARS-CoV-2 among not (yet) vaccinated PHPCs in Belgium is shown in figure 4.

For T2 the positivity of antibodies against SARS-CoV-2 was estimated to be 18.54% (95%CI: 12.84-24.24; n=178)) lower compared to T1, i.e. the proportion of participants not yet vaccinated at T1 and testing positive at T1 for SARS-CoV-2 antibodies that tested negative for SARS-CoV-2 antibodies at T2. For T3 and T4 it was estimated to be 19.42% (95%CI: 11.76-27.07; n=103) and 12.50% (95%CI: 0.99 - 24.01; n=32), respectively. As of T4, the sample size of eligible participants was too small for precise estimates.

Among participants after full primary vaccination

The longevity of antibodies against SARS-CoV-2 among PHCPs in Belgium who have received their full primary vaccination, but not yet a booster vaccination, according to their self-reported history of COVID-19 infection is shown in figure 5. The longevity of antibodies is higher in PHCPs with self-reported COVID-19 infection compared to PHCPs without self-reported COVID-19 infection after full primary vaccination.

Discussion

The prevalence of antibodies against SARS-CoV-2 among PHCPs in Belgium was 15.1% in December 2020, i.e. before vaccination had started and right after the second Belgian COVID-19 wave that peaked beginning November 2020, and reached 93.9% in December 2021, i.e. after booster vaccination had started and after the fourth Belgian COVID-19 wave in which the Delta variant was dominant and that peaked beginning December 2021. The incidence of antibodies against SARS-CoV-2 within two weeks after COVID-19 vaccination with a first dose was higher in PHCPs with a self-reported history of COVID-19 infection compared to those with no self-reported history of infection. The longevity of antibodies was more pronounced in the former group of PHCPs than in those with no self-reported history of infection.

The seroprevalence in PHCPs before vaccination (15.1%) appeared to be lower than that among the general population (18.7%) and that among hospital health care workers (19.7%) in Belgium, in December 2020, when the Belgian healthcare system was approaching the end of the second COVID-19 wave.[15,18] It should however be noted that the accuracy of the RST might be lower when used by many different PHCPs instead of a few trained and experienced staff (for validation) and lower than analysis of a serum sample in the lab (for seroprevalence in the general population and in hospital health care workers) using conventional lab-tests. This is suggested by the lower seroprevalence in this study for PHCPs in Flanders compared to that in an earlier prospective cohort study using dried blood spots analysed in the lab.[25] Not finding a higher seroprevalence among PHCPs, generally concerned about being at high risk of COVID-19 infections, compared to the general population might be explained by the availability and proper usage of personal protective equipment (PPE).[25]
Most PHCPs in our study (94.49%) received a first vaccine dose in the period January – March explaining the increase in seroprevalence to 84.1% in April 2021. The monthly incidence of antibodies due to natural infection in those not yet vaccinated in the same time period was estimated to be around 4% in this study. Natural course of infection could therefore not have caused a similar rise in seroprevalence.
A gradual decrease in the prevalence of anti-SARS-CoV-2 antibodies among PHCP was observed in the following months leading to a seroprevalence of 70.2% in September 2021. In December 2021 most PHCPs (86.5% of participants in testing timepoint 8) already received a booster dose of a COVID-19 vaccine resulting in a seroprevalence of 93.1% at the end of the study. Although, also the circulation of Delta variant corona virus might have impacted this increase in seroprevalence. For example, the seroprevalence in mainly unvaccinated schoolchildren in Belgium almost doubled during the fourth covid wave (26.6% at 8 October 2021 versus 50.9% at 15 December 2021).[18, 28] Natural infection before vaccination did seem to limit waning of antibodies after vaccination. These findings strengthen the accruing evidence base for reduced protection from infection in vaccinated, but previously uninfected participants.[29]
The clinical significance is however still to be determined. A reduction in vaccine effectiveness against infection could increase transmission to and the risk of infection among high-risk persons who consult PHCPs, some of whom may have progression to severe disease. In addition, recent studies have shown that vaccination confers more durable protection against severe outcomes of hospitalization and death than against mild symptomatic and asymptomatic infection.[30-32]
At this point studies suggest that a third or booster dose provides additional protection on top of simply reversing previous waning, but that the greatest protection from the worst clinical outcomes still remains heavily concentrated in the first two doses.[32-36]
Although studies suggest prolonged protection, it remains unclear to what extent the presence of antibodies (against the RBD) is associated with protection against new variants of the coronavirus.[36,37] Neutralising antibody titers measured in the laboratory remain the strongest correlate of protection against symptomatic and severe illness across multiple variants.[38, 39]

This large cohort study with 12 months follow-up provided precise estimates of the prevalence and incidence of antibodies against SARS-CoV-2 among PHCPs at national and regional level. Another strength of this study is the use of RSTs. This substantially improved the timeliness of

the test result availability and allowed the PHCPs to immediately check their results, which was not the case in our previous work that used dried blood spots (DBS) to assess the prevalence and incidence of antibodies against SARS-CoV-2 among PHCPs in Flanders.[23] Consequently, the results in PHCPs in Belgium could be compared much faster to that of the general population and other population groups, e.g., health care workers in hospitals and nursing homes.

In addition, the RST used in this study allowed us to estimate the incidence and longevity of antibodies against SARS-CoV-2 both after natural infection and after vaccination. This, on the other hand, also limits seroprevalence studies like ours and others,[16] using an RST not able to distinguish antibodies after natural infection (with new variants) from antibodies after vaccination, to assess virus circulation once the target population is highly vaccinated.

Loss to follow-up or missing data, reduced accuracy of the RST in primary care and the use of a convenience sample could also have limited the validity of the study results. However, overall retention and response of PHCPs in the study was good to excellent, we used the best available RST to avoid under- and overestimation of the presence of SARS-CoV-2 among PHCPs due to imperfect testing methods (imperfect sensitivity and specificity), and the estimates were corrected for clustering and potential geographical misrepresentation of the PHCPs.

Selection bias is possible, because the study started at the end of the second COVID-19 wave: if all the most vulnerable PHCPs had already been infected at the time of the start of this study, then the incidence among the remaining PHCPs may be lower (because better immune system, more adherent to personal protection guidelines etc.). Therefore, we explicitly asked for participation regardless of previous SARS-CoV-2 testing and test results.

In conclusion, this national study confirms results from an earlier study at regional level (Flanders only) that for the PHCPs seroprevalence and incidence during the second COVID-19 wave was similar to that of the general population suggesting that the occupational health measures implemented provided sufficient protection when managing patients. A vaccination programme including one booster increased the seroprevalence of antibodies against SARS-CoV-2 leading to a seroprevalence of 93.9% in December 2021. Between primary and booster vaccination longevity of antibodies was more pronounced in PHCPs with a history of self-reported COVID-19 infection. Therefore, continued monitoring of the seroprevalence in PHCPs after booster vaccination, with longer time intervals, could be relevant, provided that the presence of antibodies is associated with protection.

Authors' contributions: The study concept and design was initiated by SC, NA, BS and ED and finalized with contributions from JYV, ADS, SH, AVdB, ID, PVD, HG. SC, NA,BS and PVN conducted registration and data collection. Analysis was performed by RB. NA prepared the first draft of the manuscript. All authors (NA, BS, RB, PVN, JYV, ADS, SH, AVdB, ID, PVD, HG, LB, ED and SC) provided edits and critiqued the manuscript for intellectual content, approved the submitted version, were involved in the interpretation of data, and agree to be accountable for all aspects of the work.

NA and BS contributed equally to this work as first author. ED and SC contributed equally to this work as last author.

Funding statement: 'This work was supported by Sciensano, grant number [OZ8478]' JYV was further supported by the National Institute for Health and Care Research (NIHR) Community Healthcare MedTech and In Vitro Diagnostics Co-operative at Oxford Health NHS Foundation Trust. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care**.**

Competing interests statement: None declared.

Ethics approval: Ethical approval granted at 16 November 2020 (reference number: 20/46/605) by the Ethics Committee of the University Hospital Antwerp/University of Antwerp (Belgian registration number: 3002020000237).

Data availability statement: Data are available on reasonable request. The relevant anonymised patient level data as well as statistical code that support the findings of this study are available from the corresponding author on reasonable request.

References

1. WHO Coronavirus (COVID-19) Dashboard. https://covid19.who.int. (accessed 9 June 2022).
2. Alter G, Seder R. The Power of Antibody-Based Surveillance. *N Engl J Med* 2020;383:1782-84.
3. Koopmans M, Haagmans B. Assessing the extent of SARS-CoV-2 circulation through serological studies. *Nat Med* 2020;26:1171-72.
4. Gudbjartsson DF, Norddahl GL, Melsted P, *et al.* Humoral Immune Response to SARS-CoV-2 in Iceland. *N Engl J Med* 2020;383:1724-34.
5. Pollán M, Pérez-Gómez B, Pastor-Barriuso R, *et al*. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. *Lancet* 2020;396:535-44.
6. Patel MM, Thornburg NJ, Stubblefield WB, *et al*. Change in Antibodies to SARS-CoV-2 Over 60 Days Among Health Care Personnel in Nashville, Tennessee. *JAMA* 2020;324:1781-82.
7. Long QX, Tang XJ, Shi QL, *et al*. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med* 2020;26:1200-04.
8. Ibarrondo FJ, Fulcher JA, Goodman-Meza D, *et al.* Rapid Decay of Anti–SARS-CoV-2 Antibodies in Persons with Mild Covid-19. *N Engl J Med* 2020;383:1085-87.
9. Yao L, Wang G-L, Shen Y, *et al*. Persistence of Antibody and Cellular Immune Responses in COVID-19 patients over Nine Months after Infection. *J Infect Dis* 2021 https://doi.org/10.1093/infdis/jiab255
10. Duysburgh E, Barbezange C, Dierick K, *et al*. Persistence of IgG response to SARS-CoV-2. *Lancet Infect Dis* 2021;21:163-64.
11. Barouch D, Stephenson K, Sadoff J *et al*. Durable Humoral and Cellular Immune Responses 8 Months after Ad26.COV2.S Vaccination. *N Engl J Med* 2021; 385:951-53
12. Favresse J, Bayart JL, Mullier F, *et al*. Antibody titres decline 3-month post-vaccination with BNT162b2. *Emerg Microbes Infect*. 2021;10:1495-98.
13. Leclercq V, Van den Houte N, Gisle L, *et al*. Prevalence of Anti-SARS-CoV-2 Antibodies and Potential Determinants among the Belgian Adult Population: Baseline Results of a Prospective Cohort Study. *Viruses*. 2022;14:920.
14. Merckx J, Vermeulen M, Vandermeulen C, *et al*. Prevalence and incidence of antibodies against SARS-CoV-2 in children and school staff measured for one year in Belgium: a sero-epidemiological prospective cohort study. https://www.sciensano.be/nl/biblio/prevalence-and-incidence-antibodies-against-sars-cov-2-children-and-school-staff-measured-one-year. (accessed 9 June 2022)
15. Mortgat L, Verdonck K, Hutse V, *et al*. Prevalence and incidence of anti-SARS-CoV-2 antibodies among healthcare workers in Belgian hospitals before vaccination: a prospective cohort study. *BMJ open* 2021;11:e050824.
16. De Sutter A, Heytens S, Duysburgh E, *et al.* SARS-CoV-2 seroprevalence among nursing home staff and residents in Belgium: Protocol https://www.sciensano.be/nl/biblio/sars-cov-2-seroprevalence-among-nursing-home-staff-and-residents-belgium-protocol. (accessed 9 June 2022)
17. Pannus P, Neven K, De Craeye S *et al.* Poor antibody response to BioNTech/Pfizer COVID-19 vaccination in SARS-CoV-2 naïve residents of nursing homes. *Clin Infect Dis*. 2021; https://doi.org/10.1093/cid/ciab998
18. Sciensano. Belgium COVID-19 Epidemiological Situation https://datastudio.google.com/embed/u/0/reporting/7e11980c-3350-4ee3-8291-3065cc4e90c2/page/ZwmOB (accessed 9 June 2022)
19. Adriaenssens N, Scholtes B, Bruyndonckx R, *et al.* Prevalence and incidence of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium during 1 year of the COVID-19 epidemic: prospective cohort study protocol *BMJ Open* 2022;12**:**e054688.
20. Starfield B. Is primary care essential? *Lancet* 1994;344:1129-33.

21. Starfield B. Primary care and health. A cross-national comparison. *JAMA* 1991;266:2268-71.
22. Phadke I, McKee A, Conway J, *et al*. Analysing how changes in the health status of healthcare workers affects epidemic outcomes. *Epidemiol Infect* 2021;149:E42.
23. Federale Overheidsdienst Volksgezondheid Veiligheid van de Voedselketen en Leefmilieu. Jaarstatistieken met betrekking tot de beoefenaars van gezondheidszorgberoepen in België. 2020. https://overlegorganen.gezondheid.belgie.be/sites/default/files/documents/statan_2019_-_nl.pdf (accessed 17 May 2021)
24. Federazione Nazionale degli Ordini dei Medici Chirurghi e degli Odontoiatri. Elenco dei Medici caduti nel corso dell'epidemia di Covid-19. https://portale.fnomceo.it/elenco-dei-medici-caduti-nel-corso-dellepidemia-di-covid-19/.(accessed 9 June 2022)
25. Mariën, Joachim, Ann Ceulemans, Diana Bakokimi, *et al*. Prospective SARS-CoV-2 Cohort Study among Primary Health Care Providers during the Second COVID-19 Wave in Flanders, Belgium. *Fam. Pract.* 2021,39:92-8.
26. Triest D, Geebelen L, De Pauw R, *et al.* Performance of five rapid serological tests in mild-diseased subjects using finger prick blood for exposure assessment to SARS-CoV-2. *J Clin Virol*, 2021;142:104897.
27. Liang K, Zeger SL. Longitudinal data analysis using generalized linear models. *Biometrika* 1986;73:13-22.
28. Merckx J, Roelants M, Callies M, *et al.* Prevalence and incidence of antibodies against SARS-CoV-2 in children and school staff measured between December 2020 and December 2021: Findings of the fifth testing period – brief summary https://overlegorganen.gezondheid.belgie.be/sites/default/files/documents/statan_2019_-_nl.pdf (accessed 16 May 2022)
29. Hall V, Foulkes S, Insalata F *et al.* Protection against SARS-CoV-2 after Covid-19 Vaccination and Previous Infection. *N Engl J Med* 2022;386:1207-1220.
30. Andrews N, Tessier E, Stowe J, *et al.* Vaccine effectiveness and duration of protection of Comirnaty, Vaxzevria and Spikevax against mild and severe COVID-19 in the UK. *N Engl J Med* 2022;386:340-350.
31. Tenforde MW, Self WH, Naioti EA, *et al.* Sustained effectiveness of Pfizer-BioNTech and Moderna vaccines against COVID-19 associated hospitalizations among adults — United States, March– July 2021. *MMWR Morb Mortal Wkly Rep* 2021;70:1156-62.
32. Bager P, Wohlfahrt J, Bhatt S, *et al.* Risk of hospitalisation associated with infection with SARS-CoV-2 omicron variant versus delta variant in Denmark: an observational cohort study, *Lancet Infect Dis* 2022; DOI: https://doi.org/10.1016/S1473-3099(22)00154-2.
33. Cevik M, Grubaugh ND, Iwasaki A, *et al.* COVID-19 vaccines: Keeping pace with SARS-CoV-2 variants. *Cell*. 2021;20:5077-81.
34. Patel MK. Booster Doses and Prioritizing Lives Saved. *N Engl J Med* 2021;385:2476-7.
35. Arbel R, Hammerman A, Sergienko R, *et al.* BNT162b2 vaccine booster and mortality due to Covid-19. *N Engl J Med* 2021;385:2413-2420.
36. Bar-On YM, Goldberg Y, Mandel M, *et al.* Protection against Covid-19 by BNT162b2 booster across age groups. *N Engl J Med* 2021;385:2421-2430.
37. Wu M, Wall EC, Car EJ, *et al*. Three-dose vaccination elicits neutralising antibodies against omicron. *Lancet* 2022;399:715-17.
38. Cromer D, Steain M, Reynaldi A, *et al.* Neutralising antibody titres as predictors of protection against SARS-CoV-2 variants and the impact of boosting: a meta-analysis. *Lancet Microbe* 2021;3:e52–61.
39. Khoury DS, Cromer D, Reynaldi A, *et al.* Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nature Med* 2021;27:1205–11.

Figures

Uploaded separately

Figure 1. Prevalence of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium from December 2020 to December 2021.[1]
[1] The eight testing timepoints have the following start and end dates: T1: 24/12/2021-8/1/2021, T2: 25-31/1/2021, T3: 22-28/2/2021, T4: 22-31/3/2021, T5: 19-28/4/2021, T6: 14-27/6/2021, T7, 13-26/9/2021, T8: 13-26/12/2021. For the proportion of primary health care providers vaccinated at each testing timepoint see Table S4.

The green line marks the prevalence of antibodies against SARS-CoV-2 (seroprevalence).
The grey line mark the 95% confidence interval.
The blue lines mark the start of primary and booster vaccination campaign for PHCPs.

The grey boxes mark the third (15/2/2021-27/6/2021) and fourth COVID-19 (4/10/2021-27/12/2021).

Figure 2. Kaplan-Meier plot[1] of incidence of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium not yet vaccinated after self-reported COVID-19 infection.
[1] Interval censoring is taken into account by assuming that the actual event occurred somewhere between the testing timepoint of the event and the testing timepoint before

Figure 3. Incidence of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium after vaccination according to self-reported history of COVID-19 infection.

Figure 4: Kaplan-Meier plot[1] of longevity of antibodies against SARS-CoV-2 among PHCPs in Belgium after self-reported history of COVID-19 infection
[1] Interval censoring is taken into account by assuming that the actual event occurred somewhere between the testing timepoint of the event and the testing timepoint before.

Figure 5: Kaplan-Meier plots of longevity of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium after full primary vaccination according to self-reported history of COVID-19 infection accounting for censoring as of the booster vaccination.
[1] Assuming that the actual event occurred somewhere between the testing timepoint of the event and the testing timepoint before; [2] Assuming that the actual event occurred exactly between the testing timepoint of the event and the testing timepoint before.

Supplementary materials

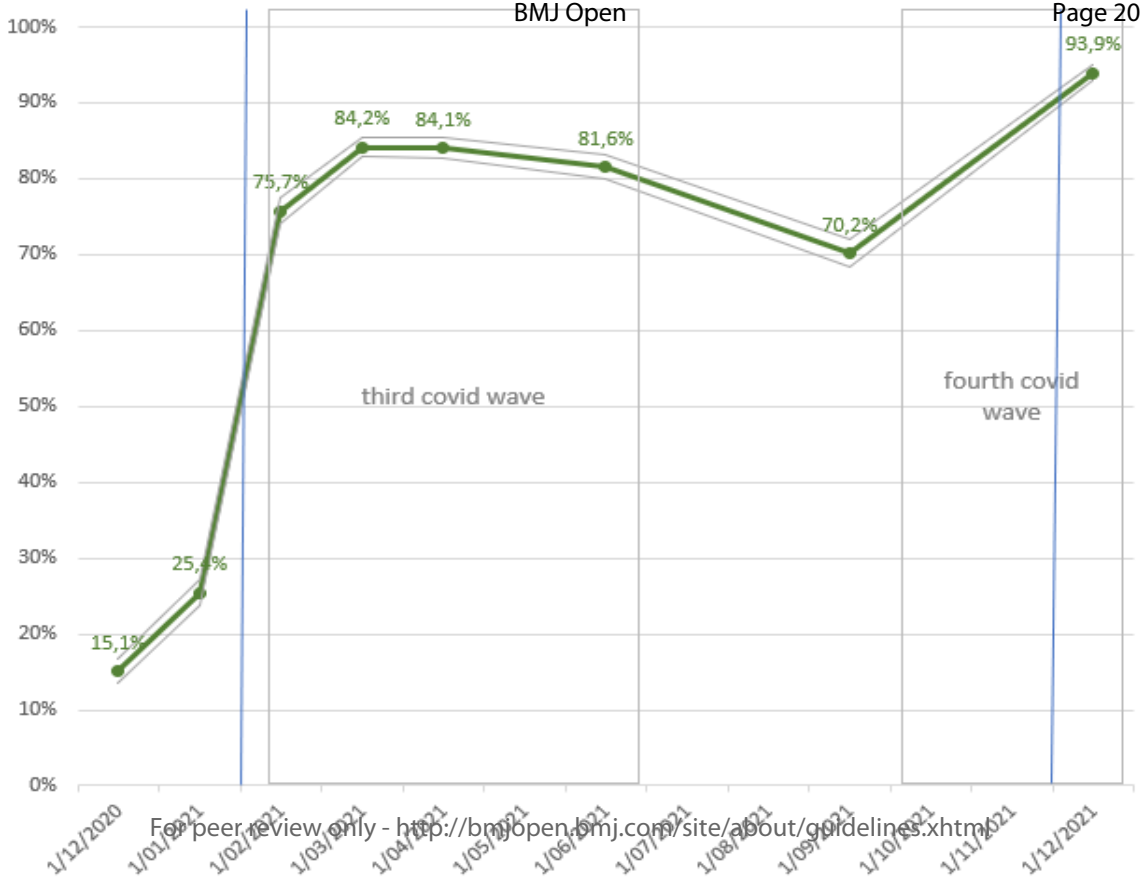
Uploaded separately

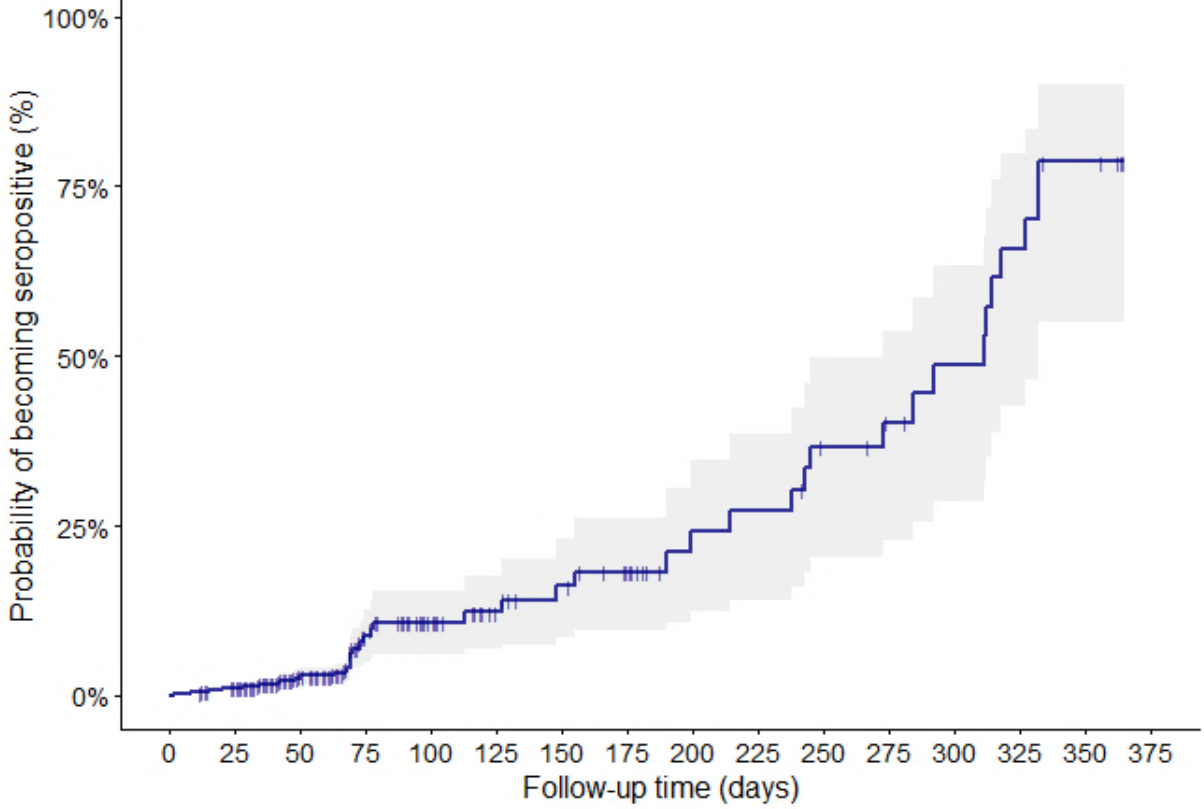
Table S1. Distribution by province of active general practitioners (GPs) in Belgium in 2020 and of GPs who participated in CHARMING in their testing timepoint[1]
[1] The first testing timepoint was December 2020 for 2224 and January 2021 for 373 GPs. PHCPs, respectively.

Table S2. The number of primary healthcare providers (PHCPs) participating per testing timepoint

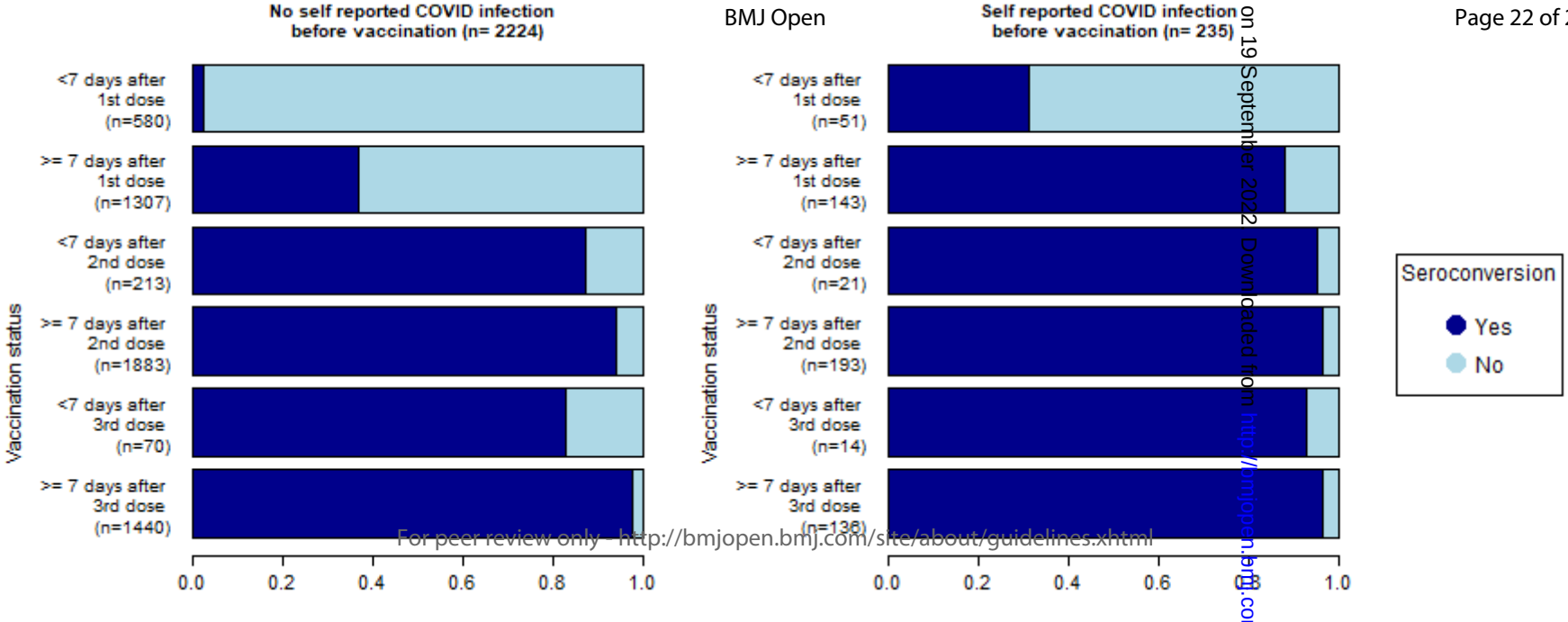
Table S3. Prevalence of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium at eight testing timepoints from December 2020 to December 2021[1]
[1] See Table S4 for the proportions of PHCPs partially and fully vaccinated; [2] RST: Rapid Serological Test; [3] IgG and/or IgM positive among the valid RST; [4] Estimates are based on Generalised Estimating Equations taking into account clustering of PHCPs within their practice and distribution of GPs across districts in Belgium; T1: 24/12/2021-8/1/2021, T2: 25-31/1/2021, T3: 22-28/2/2021, T4: 22-31/3/2021, T5: 19-28/4/2021, T6: 14-27/6/2021, T7, 13-26/9/2021, T8: 13-26/12/2021.

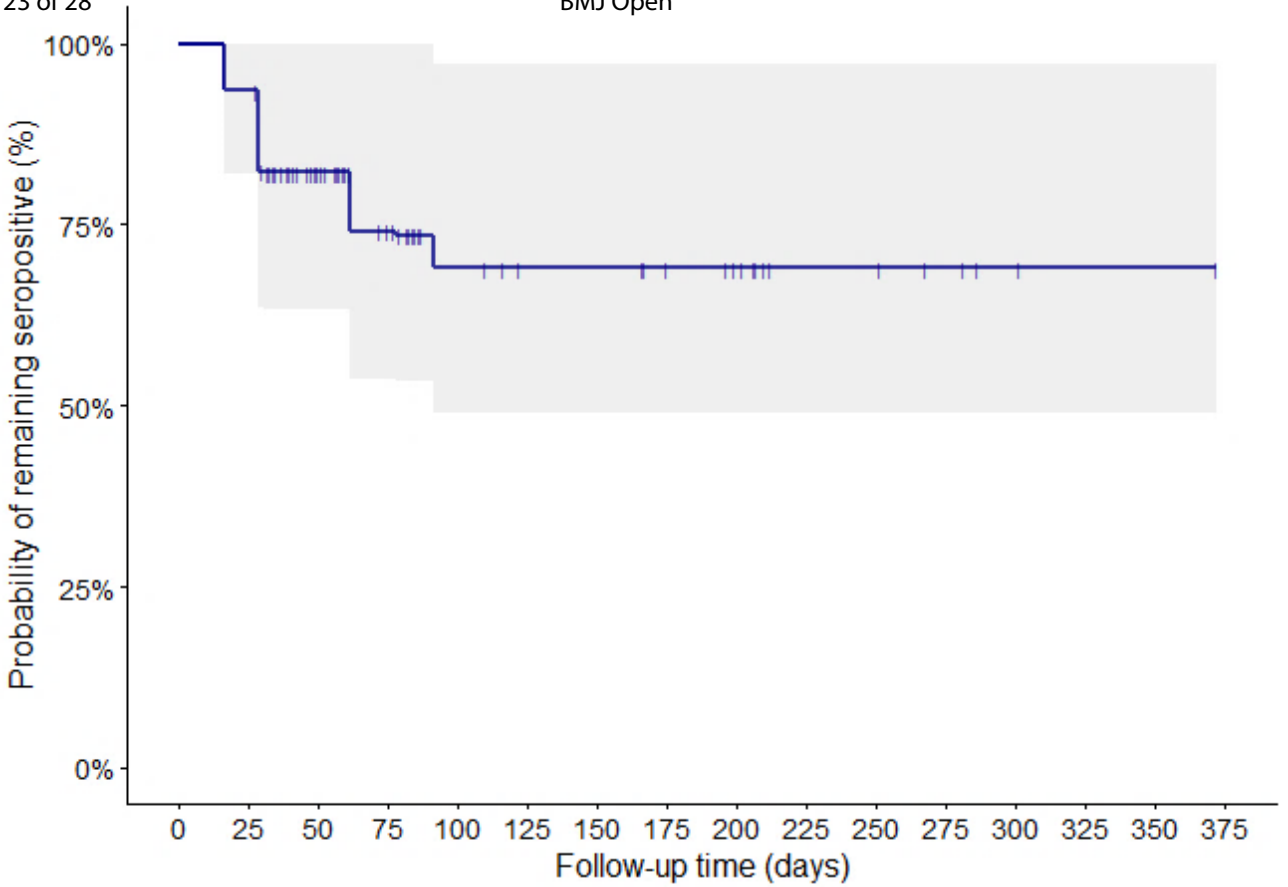
Table S4. Proportions of primary healthcare providers in Belgium with valid rapid serological test results[1] vaccinated at eight testing timepoints from December 2020 to December 2021
[1] See Table S3 for the number of primary healthcare providers with valid rapid serological test results; [2] Received one out of two doses; [3] Received two doses; [4] Received a third dose. T1: 24/12/2021-8/1/2021, T2: 25-31/1/2021, T3: 22-28/2/2021, T4: 22-31/3/2021, T5: 19-28/4/2021, T6: 14-27/6/2021, T7, 13-26/9/2021, T8: 13-26/12/2021.



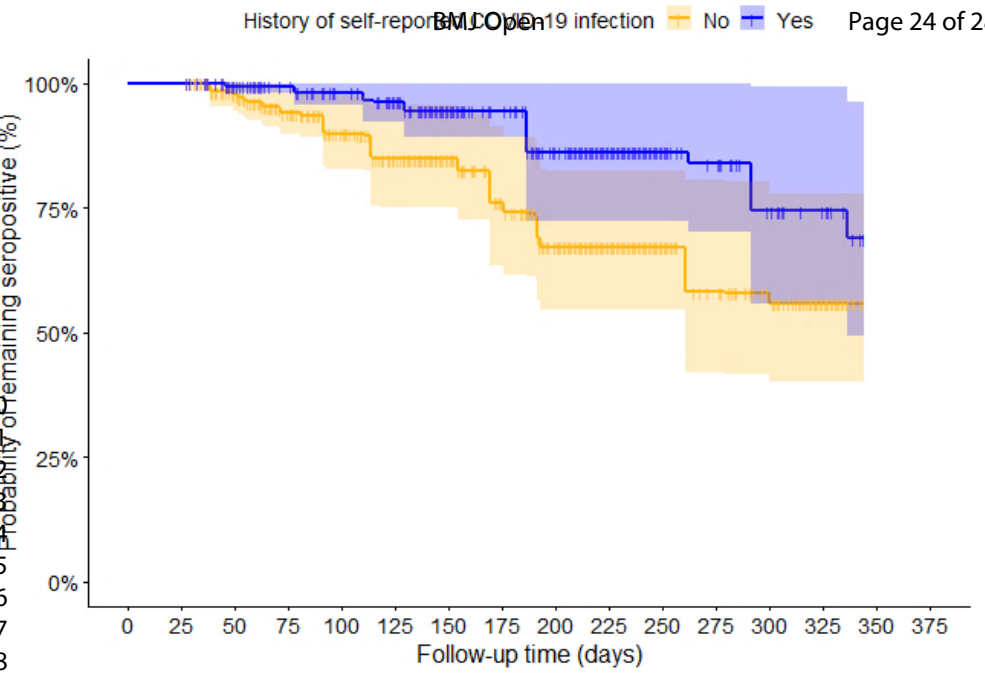


	0	50	100	150	200	250	300
N° at risk	2355	306	65	41	25	19	12

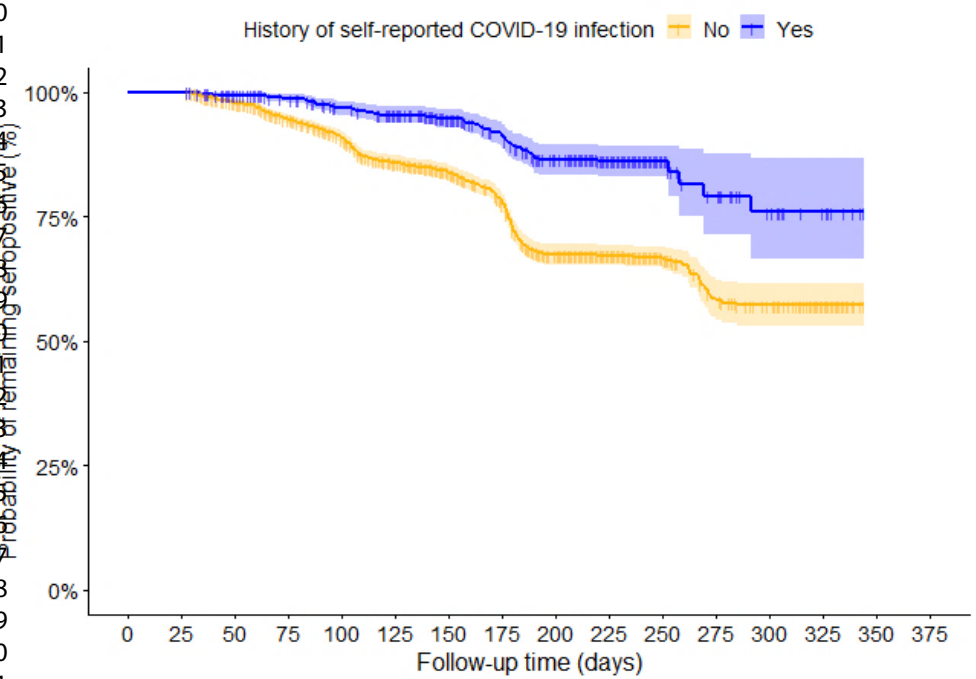




	0	50	100	150	200	250	300
N° at risk	158	64	21	17	12	6	2



A: Interval censoring[1]



B: Midpoint censoring[2]

N°at risk	0	50	100	150	200	250	300
No	2294	2187	1927	1634	1214	200	108
Yes	640	616	565	465	385	48	22

Table S1. Distribution by province of active general practitioners (GPs) in Belgium in 2020 and of GPs who participated in CHARMING in their testing timepoint[1]

Region/Province	Active GPs n (%)		Participating GPs n (%)	
Brussels	1,178	(10.01)	239	(9.2)
Flanders	6,805	(57.83)	1,725	(66.4)
Wallonia	3,784	(32.16)	633	(24.4)
Antwerpen-Anvers	1,806	(15.35)	454	(17.5)
Brussel-Hoofdstad-Bruxelles Capitale	1,178	(10.01)	239	(9.2)
Henegouwen-Hainaut	1,293	(10.99)	175	(6.7)
Limburg-Limbourg	943	(8.01)	235	(9.0)
Luik-Liège	1,125	(9.56)	200	(7.7)
Luxemburg-Luxembourg	301	(2.56)	78	(3.0)
Namen-Namur	594	(5.05)	104	(4.0)
Oost-Vlaanderen-Flandre Orientale	1,556	(13.22)	431	(16.6)
Vlaams-Brabant-Brabant-Flamand	1,241	(10.55)	317	(12.2)
Waals-Brabant-Brabant Wallon	471	(4.00)	76	(2.9)
West-Vlaanderen-Flandre Occidentale	1,259	(10.70)	288	(11.1)
Total	11,767		2,597	(22.1)

[1] The first testing timepoint was December 2020 for 2224 and January 2021 for 373 GPs. PHCPs, respectively.

Table S2. The number of primary healthcare providers (PHCPs) participating per testing timepoint

Number of PHCPs (%) Testing timepoints	Invited (%) N=3,648		Responding (%)		Responding within the testing timeframe[1] (%)	
1	3,044	2,820	(92.6%)	2680	(88.0%)	
2	3,648	3,289	(90.2%)	3060	(83.9%)	
3	3,648	3,162	(86.7%)	3018	(82.7%)	
4	3,409	3,043	(89.3%)	3021	(88.6%)	
5	3,409	2,989	(87.7%)	2891	(84.8%)	
6	3,409	2,802	(82.2%)	2750	(80.7%)	
7	3,313	2,819	(85.1%)	2756	(83.2%)	
8	3,313	2,557	(77.2%)	2516	(75.9%)	

[1]The eight testing timepoints have the following start and end dates: T1: 24/12/2021-8/1/2021, T2: 25-31/1/2021, T3: 22-28/2/2021, T4: 22-31/3/2021, T5: 19-28/4/2021, T6: 14-27/6/2021, T7, 13-26/9/2021, T8: 13-26/12/2021.

Table S3. Prevalence of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium at eight testing timepoints from December 2020 to December 2021[1]

Testing timepoint	Region	PHCPs n	Valid RST[2] n	Positive RST[3] n	Adjusted prevalence[4] % (95% CI)	
T1	Belgium	2,680	2,629	366	15.08	(13.54-16.62)
	Brussels	234	233	43	18.45	(13.47-23.44)
	Flanders	1841	1800	203	11.28	(9.77-12.79)
	Wallonia	605	596	120	20.37	(16.91-23.84)
T2	Belgium	3,060	2,995	716	25.42	(23.75-27.08)
	Brussels	270	263	55	20.91	(15.98-25.84)
	Flanders	2024	1980	389	20.10	(18.29-21.92)
	Wallonia	766	752	272	36.03	(32.46-39.60)
T3	Belgium	3,018	2,967	2,278	75.70	(74.03-77.37)
	Brussels	274	273	168	61.54	(55.72-67.36)
	Flanders	2014	1971	1615	82.35	(80.63-84.06)
	Wallonia	730	723	495	68.80	(65.26-72.34)
T4	Belgium	3,021	2,980	2,509	84.17	(82.86-85.48)
	Brussels	279	274	209	76.28	(71.24-81.31)
	Flanders	1,987	1,963	1,706	86.91	(85.42-88.40)
	Wallonia	755	743	594	79.95	(77.07-82.83)
T5	Belgium	2,891	2,859	2,410	84.07	(82.65-85.48)
	Brussels	274	268	206	76.87	(71.82-81.91)
	Flanders	1,898	1,877	1,622	86.67	(85.09-88.25)
	Wallonia	719	714	582	81.86	(78.97-84.75)
T6	Belgium	2,750	2,725	2,230	81.57	(80.02-83.12)
	Brussels	252	244	197	80.74	(75.81-85.67)
	Flanders	1,839	1,826	1,514	82.76	(80.97-84.55)
	Wallonia	659	655	519	79.78	(76.59-82.98)
T7	Belgium	2,756	2,730	1,917	70.17	(68.36-71.97)
	Brussels	238	237	178	75.11	(69.62-80.59)
	Flanders	1,844	1,823	1,271	69.38	(67.20-71.56)
	Wallonia	674	670	468	70.04	(66.46-73.62)
T8	Belgium	2,516	2,498	2,356	93.91	(92.89-94.93)
	Brussels	222	221	201	90.95	(87.17-94.73)
	Flanders	1,696	1,681	1,607	95.42	(94.36-96.47)
	Wallonia	598	596	548	92.22	(90.02-94.43)

[1] See Table S4 for the proportions of PHCPs partially and fully vaccinated; [2] RST: Rapid Serological Test; [3] IgG and/or IgM positive among the valid RST; [4] Estimates are based on Generalised Estimating Equations taking into account clustering of PHCPs within their practice and distribution of GPs across districts in Belgium; T1: 24/12/2021-8/1/2021, T2: 25-31/1/2021, T3: 22-28/2/2021, T4: 22-31/3/2021, T5: 19-28/4/2021, T6: 14-27/6/2021, T7, 13-26/9/2021, T8: 13-26/12/2021.

Table S4. Proportions of primary healthcare providers in Belgium with valid rapid serological test results[1] vaccinated at eight testing timepoints from December 2020 to December 2021

Testing timepoint	Region	Partially vaccinated[2] % (95%CI)		Fully vaccinated[3] % (95%CI)		Booster vaccinated[4] % (95%CI)	
T1	Belgium	NA		NA		NA	
	Brussels	NA		NA		NA	
	Flanders	NA		NA		NA	
	Wallonia	NA		NA		NA	
T2	Belgium	57.16	(55.39-58.93)	0.87	(0.54-1.20)	NA	
	Brussels	17.49	(12.90-22.08)	1.14	(0.00-2.42)	NA	
	Flanders	67.27	(65.21-69.34)	0.30	(0.06-0.55)	NA	
	Wallonia	44.41	(40.86-47.97)	2.26	(1.20-3.32)	NA	
T3	Belgium	16.92	(15.57-18.27)	66.23	(64.53-67.93)	NA	
	Brussels	50.18	(44.25-56.11)	21.98	(17.07-26.89)	NA	
	Flanders	11.72	(10.30-13.14)	76.15	(74.27-78.04)	NA	
	Wallonia	18.53	(15.70-21.37)	55.88	(52.26-59.50)	NA	
T4	Belgium	16.88	(15.53-18.22)	78.46	(76.98-79.93)	NA	
	Brussels	30.29	(24.85-35.73)	60.22	(54.42-66.01)	NA	
	Flanders	13.40	(11.89-14.90)	84.06	(82.44-85.67)	NA	
	Wallonia	21.13	(18.20-24.07)	70.39	(67.11-73.67)	NA	
T5	Belgium	15.49	(14.17-16.82)	81.11	(79.68-82.55)	NA	
	Brussels	26.46	(22.21-31.78)	66.04	(60.38-71.71)	NA	
	Flanders	13.16	(11.63-14.69)	85.35	(83.75-86.95)	NA	
	Wallonia	17.51	(14.72-20.29)	80.48	(77.13-83.83)	NA	
T6	Belgium	1.54	(1.08-3.00)	95.93	(95.18-96.67)	NA	
	Brussels	2.05	(0.27-3.83)	92.21	(88.85-95.58)	NA	
	Flanders	0.82	(0.41-1.24)	98.68	(97.39-98.67)	NA	
	Wallonia	3.36	(1.42-5.35)	92.90	(90.63-95.17)	NA	
T7	Belgium	0.51	(0.24-0.78)	97.91	(97.38-98.45)	0.73	(0.41-1.05)
	Brussels	2.53	(0.53-4.53)	94.51	(91.62-97.41)	0.42	(0.00-1.25)
	Flanders	0.11	(0.00-0.26)	99.23	(98.88-99.63)	0.93	(0.49-1.37)
	Wallonia	0.90	(0.18-1.61)	95.52	(93.96-97.09)	0.30	(0.00-0.71)
T8	Belgium	0.20	(0.02-0.38)	98.72	(98.28-99.16)	84.78	(83.37-86.18)
	Brussels	0.00	(0.00-0.00)	98.64	(97.12-100.00)	72.07	(66.17-77.97)
	Flanders	0.06	(0.00-0.18)	99.46	(99.12-99.81)	89.74	(88.30-91.18)
	Wallonia	0.67	(0.02-1.33)	96.64	(95.20-98.09)	75.42	(71.97-78.87)

[1] See Table S3 for the number of primary healthcare providers with valid rapid serological test results; [2] Received one out of two doses; [3] Received two doses; [4] Received a third dose. T1: 24/12/2021-8/1/2021, T2: 25-31/1/2021, T3: 22-28/2/2021, T4: 22-31/3/2021, T5: 19-28/4/2021, T6: 14-27/6/2021, T7, 13-26/9/2021, T8: 13-26/12/2021.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1&2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5&6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6&7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6&7
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6&7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6&7
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9&10
		(b) Indicate number of participants with missing data for each variable of interest	9&suppl
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	3 & 11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11&12
Generalisability	21	Discuss the generalisability (external validity) of the study results	11&12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

BMJ Open

The prevalence, incidence and longevity of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium: a prospective cohort study with 12 months of follow-up

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2022-065897.R1
Article Type:	Original research
Date Submitted by the Author:	16-Aug-2022
Complete List of Authors:	Adriaenssens, Niels; University of Antwerp Faculty of Medicine and Health Sciences, Family Medicine & Population Health, Centre for General Practice Scholtes, Beatrice; Liege University, General Practice Department, Primary Care and Health Research Unit Bruyndonckx, Robin; Hasselt University, Interuniversity Institute for Biostatistics and statistical Bioinformatics (I-BIOSTAT); P95, Epidemiology & Pharmacovigilance Van Ngoc, Pauline; Liege University, Department of General Medicine Verbakel, Jan; University of Oxford, Nuffield Department of Primary Care Health Sciences; KU Leuven, Department of Public Health and Primary Care De Sutter, An; Ghent University, Department of Public Health and Primary Care Heytens, Stefan; University of Ghent, Department of Public Health and Primary Care Van Den Bruel, Ann; KU Leuven, Department of Public Health and Primary Care Desombere, Isabelle; Sciensano, Department of Infectious Diseases in Humans Van Damme, Pierre; University of Antwerp Faculty of Medicine and Health Sciences, Vaccine & Infectious Disease Institute, Centre for the Evaluation of Vaccination Goossens, Herman; University of Antwerp Faculty of Medicine and Health Sciences, Vaccine & Infectious Diseases Institute, Laboratory of Medical Microbiology Buret, Laetitia; Liege University, General Practice Department, Primary Care and Health Research Unit Duysburgh, Els; Sciensano, Department of Epidemiology and Public Health Coenen, Samuel; University of Antwerp Faculty of Medicine and Health Sciences, Family Medicine & Population Health, Centre for General Practice; University of Antwerp, Vaccine & Infectious Disease Institute, Laboratory of Medical Microbiology
Primary Subject Heading:	Infectious diseases

Secondary Subject Heading:	Epidemiology, General practice / Family practice
Keywords:	PRIMARY CARE, COVID-19, GENERAL MEDICINE (see Internal Medicine), Epidemiology < INFECTIOUS DISEASES

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

The prevalence, incidence and longevity of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium: a prospective cohort study with 12 months of follow-up

Niels Adriaenssens[1†], Beatrice Scholtes[2†], Robin Bruyndonckx[3,4,5], Pauline Van Ngoc[2], Jan Y Verbakel[6,7], An De Sutter[8], Stefan Heytens[8], Ann Van den Bruel[6], Isabelle Desombere[9], Pierre Van Damme[10], Herman Goossens[4], Laëtitia Buret[2], Els Duysburgh[11‡], Samuel Coenen[1,4‡]

1. Centre for General Practice, Department of Family Medicine & Population Health (FAMPOP), University of Antwerp, Antwerp, Belgium
2. Research unit of Primary Care and Health, Department of General Medicine, , Department of Clinical Sciences, University of Liège, Liège, Belgium
3. Interuniversity Institute for Biostatistics and statistical Bioinformatics (I-BioStat), Data Science Institute, Hasselt University, Diepenbeek, Belgium
4. Laboratory of Medical Microbiology, Vaccine & Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Antwerp, Belgium
5. P95 Epidemiology & Pharmacovigilance, Leuven, Belgium
6. EPI-Centre, Department of Public Health and Primary Care, KU Leuven, Leuven, Belgium
7. NIHR Community Healthcare Medtech and IVD cooperative, Nuffield Department of Primary Care Health Sciences, University of Oxford, Oxford, United Kingdom
8. Department of Public Health and Primary Care, Ghent University, Ghent, Belgium
9. Service Immune response, Department of Infectious Diseases in humans, Sciensano, Brussels, Belgium
10. Centre for the Evaluation of Vaccination, Vaccine & Infectious Disease Institute (VAXINFECTIO), University of Antwerp, Antwerp, Belgium
11. Department of Epidemiology and Public Health, Sciensano, Brussels, Belgium

Corresponding author:
Samuel Coenen
Gouverneur Kinsbergencentrum
Doornstraat 331
BE-2610 Wilrijk
Belgium
samuel.coenen@uantwerpen.be

Word count: 4003

†These authors contributed equally to this work as first author

‡These authors contributed equally to this work as last author

Abstract

Objectives: To estimate the prevalence, incidence, and longevity of antibodies against SARS-CoV-2 among primary healthcare providers (PHCPs).

Design: Prospective cohort study with 12 months of follow-up.

Setting: Primary care in Belgium

Participants: Any general practitioner (GP) working in primary care in Belgium and any other PHCP from the same GP practice who physically manages (examines, tests, treats) patients were eligible. A convenience sample of 3,648 eligible PHCPs from 2,001 GP practices registered for this study (3,044 and 604 to start in December 2020 and January 2021, respectively). 3,390 PHCPs (92,9%) participated in their first testing timepoint (2,820 and 565, respectively) and 2,557 PHCPs (70,1%) in the last testing timepoint (December 2021).

Interventions: Participants were asked to perform a rapid serological test (RST) targeting IgM and IgG against the receptor binding domain (RBD) of SARS-CoV-2 and to complete an online questionnaire at each of maximum 8 testing timepoints.

Primary and secondary outcome measures: The prevalence, incidence, and longevity of antibodies against SARS-CoV-2 both after natural infection and after vaccination.

Results: Among all participants, 67% were women and 77% GPs. Median age was 43 years. The seroprevalence in December 2020 (before vaccination availability) was 15.1% (95% CI: 13.5% to 16.6%), increased to 84.2% (95% CI: 82.9% to 85.5%) in March 2021 (after vaccination availability) and reached 93.9% (95% CI: 92.9% to 94.9%) in December 2021 (during booster vaccination availability and fourth (delta variant dominant) covid wave). Among not (yet) vaccinated participants the first monthly incidence of antibodies against SARS-CoV-2 was estimated to be 2.91% (95% CI: 1.80% to 4.01%). The longevity of antibodies is higher in PHCPs with self-reported COVID-19 infection.

Conclusions: This study confirms that occupational health measures provided sufficient protection when managing patients. High uptake of vaccination resulted in high seroprevalence of SARS-CoV-2 antibodies in PHCPs in Belgium. Longevity of antibodies was supported by booster vaccination and virus circulation.

Registration: Trial registration number: NCT04779424

Key words: cohort study; primary care; SARS-CoV-2; COVID-19; prevalence; incidence; antibodies; seroprevalence

Strengths and limitations of this study

- Prospective cohort study with good response during 12 months of follow-up.
- Rapid serological test (RST) measuring the presence of antibodies against SARS-CoV-2 after infection and vaccination, without distinction.
- Timely and comparable estimates of the prevalence of antibodies against SARS-CoV-2 among primary health care providers.
- Large sample size permitting precise estimates at national and regional level.
- Convenience sample, missing data points and potentially lower actual RST accuracy limiting the study validity.

Introduction

As of 8th June 2022, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused over 530 million infections worldwide (4,164,698 in Belgium) and caused over 6.3 million deaths from coronavirus disease (COVID-19) worldwide (over 31,000 in Belgium).[1] COVID-19 can be a lethal respiratory tract infection (RTI), but often presents with mild symptoms or remains asymptomatic.

Since the start of the COVID-19 pandemic, SARS-CoV-2 seroprevalence estimates have provided essential information about population exposure to infection and helped predict the early course of the epidemic.[2,3] When setting up this study, seroprevalence studies in Iceland[4] and Spain[5] showed different levels of population antibody positivity, lasting up to at least 4 months in Iceland. In addition, early cohort studies have suggested waning of antibody levels in individuals is associated with, for example, illness severity, age and co-morbidities.[6-8] Meanwhile, other seroprevalence studies showed antibody positivity lasting up to 9 months.[9,10] Additionally, after vaccination, longevity of antibody positivity could differ depending on the type of vaccination and vaccination regime.[11,12] For Belgium, Sciensano (the Belgian national institute of public health, www.sciensano.be) performs national seroprevalence studies of SARS-CoV-2 antibodies in the general population[13] and several relevant populations including school-aged children and school staff,[14] hospital staff,[15] nursing homes residents and their staff.[16,17] These results are publicly available and regularly updated on an online dashboard.[18]

This article focuses on the seroprevalence among primary healthcare providers (PHCPs).[19] PHCPs manage the vast majority of patient contacts, including COVID-19 patients and therefore play an essential role in the efficient organisation of healthcare.[20,21] Among the PHCPs, general practitioners (GPs) in particular, act as gatekeepers to the next levels of care. Therefore, preserving the capacity of GPs, together with that of their co-workers, throughout the COVID-19 epidemic is essential.[22] In Belgium, this is particularly concerning given that the GP workforce consists of mainly older adults and is therefore at higher risk for COVID-19-related morbidity and mortality.[23] In Italy, GPs represented up to 38% of the physicians who died from COVID-19 early in the epidemic.[24]

Before the start of this study (December 2020) data on how many PHCPs in Belgium had been infected by SARS-CoV-2 was not readily available,[25] and effective vaccines for PHCPs were not anticipated to be available in the near future.

During the COVID-19 crisis rapid serological tests (RSTs) have been developed to identify the presence of antibodies to SARS-CoV-2. Compared to laboratory tests, a valid easy-to-use RST could speed up the availability of the test results for both the participants and the national health authorities.[25] Furthermore, by using RSTs in this study, PHCPs got the opportunity to become more familiar with this type of technology.

Sciensano has validated five RSTs using finger prick blood, identifying one test with appropriate sensitivity (92.9%) and specificity (96.3%) for use in seroprevalence studies.[26] We used this RST for the present study. It targets IgM and IgG against the receptor binding domain (RBD) of SARS-CoV-2 and could therefore also provide valuable information in a vaccinated population.

Given the availability of vaccines for PHCPs soon after the start of this study, we now report on the prevalence of antibodies against SARS-CoV-2 among a cohort of PHCPs in Belgium followed-up for 12-months, and on the incidence and longevity of those antibodies both after natural infection and after vaccination.

Methods

This study was a prospective cohort study. Data collection was performed according to the publicly available protocol, providing more details on the study methods.[19]

Study population

Any GP working in Belgium (including those in professional training) working in primary care and any PHCP from the same GP practice in a clinical role (clinical examination, testing or treating patients) were eligible if they were able to comply with the study protocol and provided informed consent to participate in the study. Staff hired on a temporary (interim) basis were excluded as follow-up over time would be compromised. Administrative staff or technical staff without any prolonged (longer than 15 minutes) face-to-face contact with patients and PHCPs who were not professionally active during the inclusion period were not eligible either.

PHCPs were recruited between 15 November 2020 and 15 January 2021. GPs working in clinical practice in Belgium were invited to register online for participation in this national epidemiological study and were asked to invite the other PHCPs in their practice to do the same. We emphasized that PHCPs who had already been diagnosed with COVID-19 were also eligible. Information about the study was disseminated to GPs and PHCPs via professional organisations (Domus Medica and Collège de Médecine Générale), university networks across the country and through professional media channels. We checked our convenience sample for representativeness in terms of geographic and demographic characteristics.[23]
To assess the geographical representativeness of our sample, we compared the distribution by region and by province of active GPs in Belgium in 2020 (source www.ima-aim.be) with the distribution of participating GPs.

Data Collection

Upon inclusion in the study, participants were assigned a unique study code by the researchers and received testing material at their workplace through regular mail. At their first testing timepoint they received an invitation by email inviting them to auto-collect a capillary blood sample and analyse it using the RST (OrientGene®) and to complete a baseline questionnaire available in Dutch, French and English via a personalised link through a secured online platform hosted by Sciensano (Limesurvey). The invitation email included links to both written and video instructions to perform the RST on yourself and on someone else.

The baseline questionnaire at the first testing timepoint asked for written informed consent and for information about the result of the RST, basic socio-demographic data (age, gender, composition of household – e.g. presence of school-aged children in the house), professional data (practice patient size), health status (pre-existing health conditions, regular medication use, presence of symptoms since the start of the epidemic, previous positive test results for COVID-19), professional exposure (contact with confirmed cases, use of infection prevention and control measures and the availability of personal protective equipment) and practice organisational aspects (delayed care for non-urgent conditions) (see supplementary file 1).[19]
A follow-up questionnaire was sent for each of the subsequent testing timepoints. In addition to the RST result, it collected information on the health status, including the presence of symptoms, COVID-19 testing and results, vaccination status (date of vaccination, type of vaccine, number of doses, presence of side-effects) and professional exposure (contact with confirmed cases, use of infection prevention and control measures) (see supplementary file 2).[19]

Follow-up

The study lasted 12 months, from December 2020 to December 2021, and included 8 testing timepoints. Compared to the study protocol, the testing timepoint at the fifth month was

skipped because of limited additional epidemiological value based on progressive insights from studies with similar protocols conducted by Sciensano that longer interval than four weeks between testing time point are suitable.[13-17]

Sample size

This study aimed to include 5,000 PHCPs with a ratio of 4 GPs to 1 other PHCP. The sample size considerations regarding the different objectives of the proposed study are described in more detail in the study protocol.[19] For the objectives reported here, even half the sample size aimed for would allow for precise estimates of the prevalence, incidence and longevity of antibodies against SARS-CoV-2.

Data analysis

In the analysis, we included all PHCPs who provided informed consent and reported RST results at the testing timepoints. If in the questionnaire the entry for the date the RST was performed was missing or implausible, the date of completing the questionnaire was used instead. All analyses were conducted using R version 4.1.0 (www.R-project.org).

Prevalence

To assess the prevalence of antibodies against SARS-CoV-2, we calculated among the valid RST the proportion (95% CI) of positive RST for IgG and/or IgM, and for IgG and IgM separately (crude seroprevalences). In addition, we calculated the proportion (95% CI) of PHCPs that self-reported testing positive for SARS-CoV-2 (no test specified, so this includes both virus or antibody detection) since the outbreak of the COVID-19 pandemic (February 2020), and the proportion (95% CI) of PHCPs with any positive test, either a positive study RST or testing positive since the outbreak at their first testing timepoint. For any subsequent testing timepoints we asked the participants to specify if self-reported testing positive for SARS-CoV-2 since the previous testing timepoint concerned virus or antibody detection.

We also estimated the prevalence of antibodies against SARS-CoV-2 (IgG and/or IgM) taking into account clustering of PHCPs within their practice as well as the distribution of PHCPs across the districts in Belgium (adjusted seroprevalences). Weights were calculated based on the differences between the actual distribution of GPs across districts and the distribution of participating GPs with RST results across districts. These weights were then extrapolated to all other PHCPs. The estimates are based on Generalised Estimating Equations (GEE) assuming a binomial distribution for the RST result, an identity link function and an independent working correlation matrix.[27] In a similar way we also estimated the adjusted prevalence of self-reported positive testing for SARS-CoV-2 since the start of the COVID-19 pandemic and the adjusted prevalence of these two tests results combined, either a positive study RST or testing positive since the outbreak for the first two testing timepoints.

Incidence

To assess the incidence of antibodies against SARS-CoV-2 (IgG and/or IgM) among participants not (yet) vaccinated, first we produced a Kaplan-Meier plot including participants providing a valid negative RST result at their first testing timepoint and not testing positive before, considering a positive RST during follow-up as event and censoring upon vaccination or loss to follow-up. Second, we assessed the monthly incidence of antibodies against SARS-CoV-2 due to natural infection in those not yet vaccinated, by analysing the data collected during the testing timepoints after the first testing timepoint. We included participants providing valid RST results both at the testing timepoint assessed and the preceding testing timepoint. We excluded participants reporting a positive RST at the preceding timepoint or already vaccinated with a first dose. In addition, we corrected the estimates for clustering of participants in general practices.

To assess the incidence of antibodies against SARS-CoV-2 (IgG and/or IgM) due to vaccination in those vaccinated, we calculated the proportion of participants with antibodies

against SARS-CoV-2 less than seven days and seven days or more after the first, the second and the third dose of a COVID-19 vaccine, respectively, and stratified by self-reported history of COVID-19 infection.

Longevity

To assess the longevity of antibodies against SARS-CoV-2 (IgG and/or IgM) among participants not (yet) vaccinated, first we produced a Kaplan-Meier plot including participants without a self-reported history of COVID-19 infection before their first testing timepoint that provided a valid positive RST results before receiving their first dose of a COVID-19 vaccine, considering a negative RST result during follow-up as event (= negative RST result followed by another negative RST result or missing data) and censoring upon vaccination or loss to follow-up (midpoint and interval censoring). Second, we included participants not yet vaccinated, that provided a valid RST result at the testing timepoint assessed and a positive RST result at the previous testing timepoint. We estimated the proportion with a negative test result at the testing timepoint assessed.

To assess the longevity of antibodies against SARS-CoV-2 (IgG and/or IgM) after COVID-19 vaccination, we produced Kaplan-Meier plots by self-reported history of COVID-19 infection, including participants that provided a valid positive RST results at least seven days after receiving their second dose of a COVID-19 vaccine, considering a negative RST result during follow-up as event (= negative RST result followed by another negative RST result or missing data) and censoring upon booster vaccination (date of third dose) or loss to follow-up (midpoint and interval censoring).

Vaccination

The start of the vaccination of PHCPs during the study follow-up provided the opportunity to monitor its progress.

Ethics approval

Ethical approval granted at 16 November 2020 (reference number: 20/46/605) by the Ethics Committee of the University Hospital Antwerp/University of Antwerp (Belgian registration number: 3002020000237).

Patient and Public Involvement

Neither patients (or PHCPs in this specific study) nor the public were involved in the design of the study. During the study the information shown in Figure 1 was shared with the participants and the general population through the publicly available website of the Belgian health authorities (Sciensano) shortly after each testing-timepoint both for Belgium and its three regions, Brussels, Flanders and Wallonia.[18]

Results

Description of the study cohort

In total, 3,648 eligible PHCPs from 2,001 practices registered and were asked to provide informed consent of whom 3,044 and 604 PHCPs were sent personal study materials to be able to collect data for their first testing timepoint starting on 24 December 2020 and 25 January 2021, respectively. 3,390 PHCPs participated in their first testing timepoint by completing the baseline questionnaire, among which 2,597 GPs, 386 GPs in training and 407 other PHCPs (Table 1).

Our sampling procedure resulted in the participation of a reasonably geographically representative sample of GPs at the level of the provinces (Table S1, online supplementary data). At the level of the regions, there is about 8% overrepresentation of GPs in Flanders and corresponding underrepresentation of GPs in Wallonia.

Participant characteristics

Table 1 presents the characteristics of the 3,390 PHCPs who participated in their first (baseline) testing timepoint. These PHCPs, mainly GPs, were relatively young, more often female and working more often in (large) group practices than in solo or duo practices. Table 2 shows in how many testing timepoints primary healthcare providers (PHCPs) participated. 3,415 (93.6%) PHCPs participated in at least one testing timepoint, 2,909 (79,7%) participated in six and 2,141 (58.7%) participated in all eight testing timepoints. The number of PHCPs participating per testing timepoint is presented in Table S2 (online supplementary data). While the response rate gradually decreased, still 2,557 (77.2% of invited PHCPs) participated in the last testing timepoint.

Vaccination status

Overall, 3,227 participants received a full primary vaccination. 2,783 participants received two doses of an m-RNA vaccine (2,639 (81.8%) BNT162b2, 144 (4.5%) mRNA-1273 and 2 (0.1%) mRNA-1273 followed by BNT162b2). 437 participants (13.5%) received two doses of ChAdOx1-S and 5 (0.2%) participants one dose of Ad26.COV2.S.

At the final testing timepoint, 2,211 of the participants had received a booster vaccination. 1,879 (85.0%) participants received a booster with BNT162b2 and 267 (12.1%) with mRNA-1273. 1 participant received ChAdOx1-S and another participant Ad26.COV2.S as third dose.

Table 1. Characteristics of primary healthcare providers (PHCPs), including general practitioners (GPs), GPs in training and other PHCPs who participated in their first testing timepoints[1]

	PHCPs n=3,390		GPs n=2,597		GPs in training n=386		Other PHCPs n=407	
Age[2], median (IQR)	40	(31-54)	44	(34-57)	27	(26-28)	38	(31-47)
Gender[3], n (%)								
- Male	1,119	(33.0)	943	(36.3)	112	(29.0)	64	(15.7)
- Female	2,296	(66.9)	1,652	(63.6)	274	(71.0)	343	(84.3)
- Not reported	2	(0.1)	2	(0.1)	0	(0)	0	(0)
Practice size, n (%)[3]								
- Solo	618	(33.5)	580	(34.7)	54	(16.1)	29	(11.8)
- Duo	361	(19.6)	328	(19.6)	74	(22.1)	32	(13.1)
- Group (<8 employees)	382	(20.7)	351	(21.0)	51	(15.2)	21	(8.6)
- Large group (>7 employees)	444	(24.1)	386	(23.1)	156	(46.6)	150	(61.2)

[1] The first testing timepoint was December 2020 for 2,820 and January 2021 for 570 PHCPs, respectively; [2]Ages < 21 were considered unrealistic and recoded as missing; IQR=interquartile range; [3] if numbers do not add up to the column total, this is due to missing data; numbers of practices for PHCPs=1,845, GPs=1,672, GPs in training=335 and other PHCPs=245.

Table 2. The number of testing timepoints that primary healthcare providers (PHCPs) participated in

Number of testing timepoints participated in	Number of PHCPs (%) N=3,648		Cumulative percentage
8[1]	2,141	(58.7%)	58.7%
7	490	(13.4%)	72.1%
6	278	(7.6%)	79.7%
5	153	(4.2%)	83.9%
4	129	(3.5%)	87.5%
3	91	(2.5%)	90.0%
2	87	(2.4%)	92.4%
1	46	(1.3%)	93.6%
0	233	(6.4%)	100.0%

[1] The eight testing timepoints have the following start and end dates: T1: 24/12/2021-8/1/2021, T2: 25-31/1/2021, T3: 22-28/2/2021, T4: 22-31/3/2021, T5: 19-28/4/2021, T6: 14-27/6/2021, T7, 13-26/9/2021, T8: 13-26/12/2021.

Prevalence

The prevalence of antibodies against SARS-CoV-2 among PHCPs in Belgium from December 2020 to December 2021 is shown in Figure 1 and Table S3. Table S3 also gives the number of eligible PHCPs, i.e. those testing between the start and end date of the respective testing timepoint, as well as the regional differences. At the first testing timepoint (T1), among 2680 eligible PHCPs, 2629 provided valid test results, of which 366 (15.1%) were positive. Afterwards,

the prevalence increased substantially up to 84.2% at T4, mainly due to vaccination (see Table S4). Six months later (T7) the prevalence was substantially lower (70.2%), while during the fourth covid wave (delta variant dominant) and after booster vaccination became available it increased again to 93.9% (T8).

Incidence

Among not (yet) vaccinated participants

The incidence of antibodies against SARS-CoV-2 among PHCPs in Belgium among participants that provided a valid negative RST result at their first testing timepoint, did not self-report a COVID-19 infection before and were not (yet) vaccinated is shown in figure 2.

For the second testing timepoint (T2) the monthly incidence of antibodies against SARS-CoV-2 was estimated to be 2.91% (95%CI: 1.80-4.01; n=895), i.e. the proportion of PHCPs not yet vaccinated at T2 and testing negative at T1, that tested positive at T2. For T3 and T4 it was estimated to be 3.93% (95%CI: 2.04-5.82; n=407) and 4.04% (95%CI: 0.16 - 7.92; n=99), respectively. As of T4, the sample size of eligible participants was too small for precise estimates.

Among vaccinated participants

The incidence of antibodies against SARS-CoV-2 among vaccinated PHCPs in Belgium according to their self-reported history of COVID-19 infection is shown in figure 3. The incidence of antibodies is higher in PHCPs with self-reported COVID-19 infection compared to PHCPs with no self-reported COVID-19 infection both less than seven days and seven days or more after the first and the second dose, less than seven days after the third dose, but not seven days or more after the third dose.

Longevity

Among not (yet) vaccinated participants

The longevity of antibodies against SARS-CoV-2 among not (yet) vaccinated PHPCs in Belgium is shown in figure 4.

For T2 the positivity of antibodies against SARS-CoV-2 was estimated to be 18.54% (95%CI: 12.84-24.24; n=178)) lower compared to T1, i.e. the proportion of participants not yet vaccinated at T1 and testing positive at T1 for SARS-CoV-2 antibodies that tested negative for SARS-CoV-2 antibodies at T2. For T3 and T4 it was estimated to be 19.42% (95%CI: 11.76-27.07; n=103) and 12.50% (95%CI: 0.99 - 24.01; n=32), respectively. As of T4, the sample size of eligible participants was too small for precise estimates.

Among participants after full primary vaccination

The longevity of antibodies against SARS-CoV-2 among PHCPs in Belgium who have received their full primary vaccination, but not yet a booster vaccination, according to their self-reported history of COVID-19 infection is shown in figure 5. The longevity of antibodies is higher in PHCPs with self-reported COVID-19 infection compared to PHCPs without self-reported COVID-19 infection after full primary vaccination.

Discussion

The prevalence of antibodies against SARS-CoV-2 among PHCPs in Belgium was 15.1% in December 2020, i.e. before vaccination had started and right after the second Belgian COVID-19 wave that peaked beginning November 2020, and reached 93.9% in December 2021, i.e. after booster vaccination had started and after the fourth Belgian COVID-19 wave in which the Delta variant was dominant and that peaked beginning December 2021. The incidence of antibodies against SARS-CoV-2 within two weeks after COVID-19 vaccination with a first dose was higher in PHCPs with a self-reported history of COVID-19 infection compared to those with no self-reported history of infection. The longevity of antibodies was more pronounced in the former group of PHCPs than in those with no self-reported history of infection.

The seroprevalence in PHCPs before vaccination (15.1%) appeared to be lower than that among the general population (18.7%) and that among hospital health care workers (19.7%) in Belgium, in December 2020, when the Belgian healthcare system was approaching the end of the second COVID-19 wave.[15,18] It should however be noted that the accuracy of the RST might be lower when used by many different PHCPs instead of a few trained and experienced staff (for validation) and lower than analysis of a serum sample in the lab (for seroprevalence in the general population and in hospital health care workers) using conventional lab-tests. This is suggested by the lower seroprevalence in this study for PHCPs in Flanders compared to that in an earlier prospective cohort study using dried blood spots analysed in the lab.[25] Not finding a higher seroprevalence among PHCPs, generally concerned about being at high risk of COVID-19 infections, compared to the general population might be explained by the availability and proper usage of personal protective equipment (PPE).[25]
Most PHCPs in our study (94.49%) received a first vaccine dose in the period January – March explaining the increase in seroprevalence to 84.1% in April 2021. The monthly incidence of antibodies due to natural infection in those not yet vaccinated in the same time period was estimated to be around 4% in this study. Natural course of infection could therefore not have caused a similar rise in seroprevalence.
A gradual decrease in the prevalence of anti-SARS-CoV-2 antibodies among PHCP was observed in the following months leading to a seroprevalence of 70.2% in September 2021. In December 2021 most PHCPs (86.5% of participants in testing timepoint 8) already received a booster dose of a COVID-19 vaccine resulting in a seroprevalence of 93.1% at the end of the study. Although, also the circulation of Delta variant corona virus might have impacted this increase in seroprevalence. For example, the seroprevalence in mainly unvaccinated schoolchildren in Belgium almost doubled during the fourth covid wave (26.6% at 8 October 2021 versus 50.9% at 15 December 2021).[18,28] Natural infection before vaccination did seem to limit waning of antibodies after vaccination. These findings strengthen the accruing evidence base for reduced protection from infection in vaccinated, but previously uninfected participants.[29] The clinical significance is however still to be determined. A reduction in vaccine effectiveness against infection could increase transmission to and the risk of infection among high-risk persons who consult PHCPs, some of whom may have progression to severe disease. In addition, recent studies have shown that vaccination confers more durable protection against severe outcomes of hospitalization and death than against mild symptomatic and asymptomatic infection.[30-32]
At this point studies suggest that a third or booster dose provides additional protection on top of simply reversing previous waning, but that the greatest protection from the worst clinical outcomes still remains heavily concentrated in the first two doses.[32-36]
Although studies suggest prolonged protection, it remains unclear to what extent the presence of antibodies (against the RBD) is associated with protection against new variants of the coronavirus.[36,37] Neutralising antibody titers measured in the laboratory remain the strongest correlate of protection against symptomatic and severe illness across multiple variants.[38,39]

This large cohort study with 12 months follow-up provided precise estimates of the prevalence and incidence of antibodies against SARS-CoV-2 among PHCPs at national and regional level.

Another strength of this study is the use of RSTs. This substantially improved the timeliness of the test result availability and allowed the PHCPs to immediately check their results, which was not the case in our previous work that used dried blood spots (DBS) to assess the prevalence and incidence of antibodies against SARS-CoV-2 among PHCPs in Flanders.[23] Consequently, the results in PHCPs in Belgium could be compared much faster to that of the general population and other population groups, e.g., health care workers in hospitals and nursing homes.

In addition, the RST used in this study allowed us to estimate the incidence and longevity of antibodies against SARS-CoV-2 both after natural infection and after vaccination. This, on the other hand, also limits seroprevalence studies like ours and others,[16] using an RST not able to distinguish antibodies after natural infection (with new variants) from antibodies after vaccination, to assess virus circulation once the target population is highly vaccinated.

Loss to follow-up or missing data, reduced accuracy of the RST in primary care and the use of a convenience sample could also have limited the validity of the study results. However, overall retention and response of PHCPs in the study was good to excellent, we used the best available RST to avoid under- and overestimation of the presence of SARS-CoV-2 among PHCPs due to imperfect testing methods (imperfect sensitivity and specificity), and the estimates were corrected for clustering and potential geographical misrepresentation of the PHCPs. Still, the RST used is less accurate than the enzyme-linked immunosorbent assay (ELISA) and missing this reference test's quantitative aspect.

Selection bias is possible, because the study started at the end of the second COVID-19 wave: if all the most vulnerable PHCPs had already been infected at the time of the start of this study, then the incidence among the remaining PHCPs may be lower (because better immune system, more adherent to personal protection guidelines etc.). Therefore, we explicitly asked for participation regardless of previous SARS-CoV-2 testing and test results.

In conclusion, this national study confirms results from an earlier study at regional level (Flanders only) that for the PHCPs seroprevalence and incidence during the second COVID-19 wave was similar to that of the general population suggesting that the occupational health measures implemented provided sufficient protection when managing patients. A vaccination programme including one booster increased the seroprevalence of antibodies against SARS-CoV-2 leading to a seroprevalence of 93.9% in December 2021. Between primary and booster vaccination longevity of antibodies was more pronounced in PHCPs with a history of self-reported COVID-19 infection. Therefore, continued monitoring of the seroprevalence in PHCPs after booster vaccination, with longer time intervals, could be relevant, provided that the presence of antibodies is associated with protection.

Authors' contributions: The study concept and design was initiated by SC, NA, BS and ED and finalized with contributions from JYV, ADS, SH, AVdB, ID, PVD, HG. SC, NA,BS and PVN conducted registration and data collection. Analysis was performed by RB. NA prepared the first draft of the manuscript. All authors (NA, BS, RB, PVN, JYV, ADS, SH, AVdB, ID, PVD, HG, LB, ED and SC) provided edits and critiqued the manuscript for intellectual content, approved the submitted version, were involved in the interpretation of data, and agree to be accountable for all aspects of the work.

NA and BS contributed equally to this work as first author. ED and SC contributed equally to this work as last author.

Funding statement: 'This work was supported by Sciensano, grant number [OZ8478]' JYV was further supported by the National Institute for Health and Care Research (NIHR) Community Healthcare MedTech and In Vitro Diagnostics Co-operative at Oxford Health NHS Foundation Trust. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care**.**

Competing interests statement: None declared.

Ethics approval: Ethical approval granted at 16 November 2020 (reference number: 20/46/605) by the Ethics Committee of the University Hospital Antwerp/University of Antwerp (Belgian registration number: 3002020000237).

Data availability statement: Data are available on reasonable request. The relevant anonymised patient level data as well as statistical code that support the findings of this study are available from the corresponding author on reasonable request.

References

1. WHO Coronavirus (COVID-19) Dashboard. https://covid19.who.int. (accessed 9 June 2022).
2. Alter G, Seder R. The Power of Antibody-Based Surveillance. *N Engl J Med* 2020;383:1782-84.
3. Koopmans M, Haagmans B. Assessing the extent of SARS-CoV-2 circulation through serological studies. *Nat Med* 2020;26:1171-72.
4. Gudbjartsson DF, Norddahl GL, Melsted P, *et al.* Humoral Immune Response to SARS-CoV-2 in Iceland. *N Engl J Med* 2020;383:1724-34.
5. Pollán M, Pérez-Gómez B, Pastor-Barriuso R, *et al*. Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based seroepidemiological study. *Lancet* 2020;396:535-44.
6. Patel MM, Thornburg NJ, Stubblefield WB, *et al*. Change in Antibodies to SARS-CoV-2 Over 60 Days Among Health Care Personnel in Nashville, Tennessee. *JAMA* 2020;324:1781-82.
7. Long QX, Tang XJ, Shi QL, *et al*. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med* 2020;26:1200-04.
8. Ibarrondo FJ, Fulcher JA, Goodman-Meza D, *et al.* Rapid Decay of Anti–SARS-CoV-2 Antibodies in Persons with Mild Covid-19. *N Engl J Med* 2020;383:1085-87.
9. Yao L, Wang G-L, Shen Y, *et al.* Persistence of Antibody and Cellular Immune Responses in COVID-19 patients over Nine Months after Infection. *J Infect Dis* 2021 https://doi.org/10.1093/infdis/jiab255
10. Duysburgh E, Barbezange C, Dierick K, *et al*. Persistence of IgG response to SARS-CoV-2. *Lancet Infect Dis* 2021;21:163-64.
11. Barouch D, Stephenson K, Sadoff J *et al*. Durable Humoral and Cellular Immune Responses 8 Months after Ad26.COV2.S Vaccination. *N Engl J Med* 2021; 385:951-53
12. Favresse J, Bayart JL, Mullier F, *et al*. Antibody titres decline 3-month post-vaccination with BNT162b2. *Emerg Microbes Infect*. 2021;10:1495-98.
13. Leclercq V, Van den Houte N, Gisle L, *et al*. Prevalence of Anti-SARS-CoV-2 Antibodies and Potential Determinants among the Belgian Adult Population: Baseline Results of a Prospective Cohort Study. *Viruses*. 2022;14:920.
14. Merckx J, Vermeulen M, Vandermeulen C, *et al*. Prevalence and incidence of antibodies against SARS-CoV-2 in children and school staff measured for one year in Belgium: a sero-epidemiological prospective cohort study. https://www.sciensano.be/nl/biblio/prevalence-and-incidence-antibodies-against-sars-cov-2-children-and-school-staff-measured-one-year. (accessed 9 June 2022)
15. Mortgat L, Verdonck K, Hutse V, *et al*. Prevalence and incidence of anti-SARS-CoV-2 antibodies among healthcare workers in Belgian hospitals before vaccination: a prospective cohort study. *BMJ open* 2021;11:e050824.
16. De Sutter A, Heytens S, Duysburgh E, *et al.* SARS-CoV-2 seroprevalence among nursing home staff and residents in Belgium: Protocol https://www.sciensano.be/nl/biblio/sars-cov-2-seroprevalence-among-nursing-home-staff-and-residents-belgium-protocol. (accessed 9 June 2022)
17. Pannus P, Neven K, De Craeye S *et al.* Poor antibody response to BioNTech/Pfizer COVID-19 vaccination in SARS-CoV-2 naïve residents of nursing homes. *Clin Infect Dis*. 2021; https://doi.org/10.1093/cid/ciab998
18. Sciensano. Belgium COVID-19 Epidemiological Situation https://datastudio.google.com/embed/u/0/reporting/7e11980c-3350-4ee3-8291-3065cc4e90c2/page/ZwmOB (accessed 9 June 2022)
19. Adriaenssens N, Scholtes B, Bruyndonckx R, *et al.* Prevalence and incidence of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium during 1 year of the COVID-19 epidemic: prospective cohort study protocol *BMJ Open* 2022;12**:**e054688.
20. Starfield B. Is primary care essential? *Lancet* 1994;344:1129-33.

21. Starfield B. Primary care and health. A cross-national comparison. *JAMA* 1991;266:2268-71.
22. Phadke I, McKee A, Conway J, *et al*. Analysing how changes in the health status of healthcare workers affects epidemic outcomes. *Epidemiol Infect* 2021;149:E42.
23. Federale Overheidsdienst Volksgezondheid Veiligheid van de Voedselketen en Leefmilieu. Jaarstatistieken met betrekking tot de beoefenaars van gezondheidszorgberoepen in België. 2020. https://overlegorganen.gezondheid.belgie.be/sites/default/files/documents/statan_2019_-_nl.pdf (accessed 17 May 2021)
24. Federazione Nazionale degli Ordini dei Medici Chirurghi e degli Odontoiatri. Elenco dei Medici caduti nel corso dell'epidemia di Covid-19. https://portale.fnomceo.it/elenco-dei-medici-caduti-nel-corso-dellepidemia-di-covid-19/.(accessed 9 June 2022)
25. Mariën, Joachim, Ann Ceulemans, Diana Bakokimi, *et al*. Prospective SARS-CoV-2 Cohort Study among Primary Health Care Providers during the Second COVID-19 Wave in Flanders, Belgium. *Fam. Pract.* 2021,39:92-8.
26. Triest D, Geebelen L, De Pauw R, *et al.* Performance of five rapid serological tests in mild-diseased subjects using finger prick blood for exposure assessment to SARS-CoV-2. *J Clin Virol*, 2021;142:104897.
27. Liang K, Zeger SL. Longitudinal data analysis using generalized linear models. *Biometrika* 1986;73:13-22.
28. Merckx J, Roelants M, Callies M, *et al.* Prevalence and incidence of antibodies against SARS-CoV-2 in children and school staff measured between December 2020 and December 2021: Findings of the fifth testing period – brief summary https://overlegorganen.gezondheid.belgie.be/sites/default/files/documents/statan_2019_-_nl.pdf (accessed 16 May 2022)
29. Hall V, Foulkes S, Insalata F *et al.* Protection against SARS-CoV-2 after Covid-19 Vaccination and Previous Infection. *N Engl J Med* 2022;386:1207-1220.
30. Andrews N, Tessier E, Stowe J, *et al.* Vaccine effectiveness and duration of protection of Comirnaty, Vaxzevria and Spikevax against mild and severe COVID-19 in the UK. *N Engl J Med* 2022;386:340-350.
31. Tenforde MW, Self WH, Naioti EA, *et al.* Sustained effectiveness of Pfizer-BioNTech and Moderna vaccines against COVID-19 associated hospitalizations among adults — United States, March– July 2021. *MMWR Morb Mortal Wkly Rep* 2021;70:1156-62.
32. Bager P, Wohlfahrt J, Bhatt S, *et al.* Risk of hospitalisation associated with infection with SARS-CoV-2 omicron variant versus delta variant in Denmark: an observational cohort study, *Lancet Infect Dis* 2022; DOI: https://doi.org/10.1016/S1473-3099(22)00154-2.
33. Cevik M, Grubaugh ND, Iwasaki A, *et al.* COVID-19 vaccines: Keeping pace with SARS-CoV-2 variants. *Cell*. 2021;20:5077-81.
34. Patel MK. Booster Doses and Prioritizing Lives Saved. *N Engl J Med* 2021;385:2476-7.
35. Arbel R, Hammerman A, Sergienko R, *et al.* BNT162b2 vaccine booster and mortality due to Covid-19. *N Engl J Med* 2021;385:2413-2420.
36. Bar-On YM, Goldberg Y, Mandel M, *et al.* Protection against Covid-19 by BNT162b2 booster across age groups. *N Engl J Med* 2021;385:2421-2430.
37. Wu M, Wall EC, Car EJ, *et al*. Three-dose vaccination elicits neutralising antibodies against omicron. *Lancet* 2022;399:715-17.
38. Cromer D, Steain M, Reynaldi A, *et al.* Neutralising antibody titres as predictors of protection against SARS-CoV-2 variants and the impact of boosting: a meta-analysis. *Lancet Microbe* 2021;3:e52–61.
39. Khoury DS, Cromer D, Reynaldi A, *et al.* Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nature Med* 2021;27:1205–11.

Figures

Uploaded separately

Figure 1. Prevalence of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium from December 2020 to December 2021.[1]
[1] The eight testing timepoints have the following start and end dates: T1: 24/12/2021-8/1/2021, T2: 25-31/1/2021, T3: 22-28/2/2021, T4: 22-31/3/2021, T5: 19-28/4/2021, T6: 14-27/6/2021, T7, 13-26/9/2021, T8: 13-26/12/2021. For the proportion of primary health care providers vaccinated at each testing timepoint see Table S4.

The green line marks the prevalence of antibodies against SARS-CoV-2 (seroprevalence).
The grey line mark the 95% confidence interval.
The blue lines mark the start of primary and booster vaccination campaign for PHCPs.

The grey boxes mark the third (15/2/2021-27/6/2021) and fourth COVID-19 (4/10/2021-27/12/2021).

Figure 2. Kaplan-Meier plot[1] of incidence of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium not yet vaccinated after self-reported COVID-19 infection.
[1] Interval censoring is taken into account by assuming that the actual event occurred somewhere between the testing timepoint of the event and the testing timepoint before.

Figure 3. Incidence of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium after vaccination according to self-reported history of COVID-19 infection.

Figure 4: Kaplan-Meier plot[1] of longevity of antibodies against SARS-CoV-2 among PHCPs in Belgium after self-reported history of COVID-19 infection.
[1] Interval censoring is taken into account by assuming that the actual event occurred somewhere between the testing timepoint of the event and the testing timepoint before.

Figure 5: Kaplan-Meier plots of longevity of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium after full primary vaccination according to self-reported history of COVID-19 infection accounting for censoring as of the booster vaccination.
[1] Assuming that the actual event occurred somewhere between the testing timepoint of the event and the testing timepoint before; [2] Assuming that the actual event occurred exactly between the testing timepoint of the event and the testing timepoint before.

Supplementary materials

Uploaded separately

Supplementary file 1. Prevalence and incidence of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium – Consent & baseline questionnaire

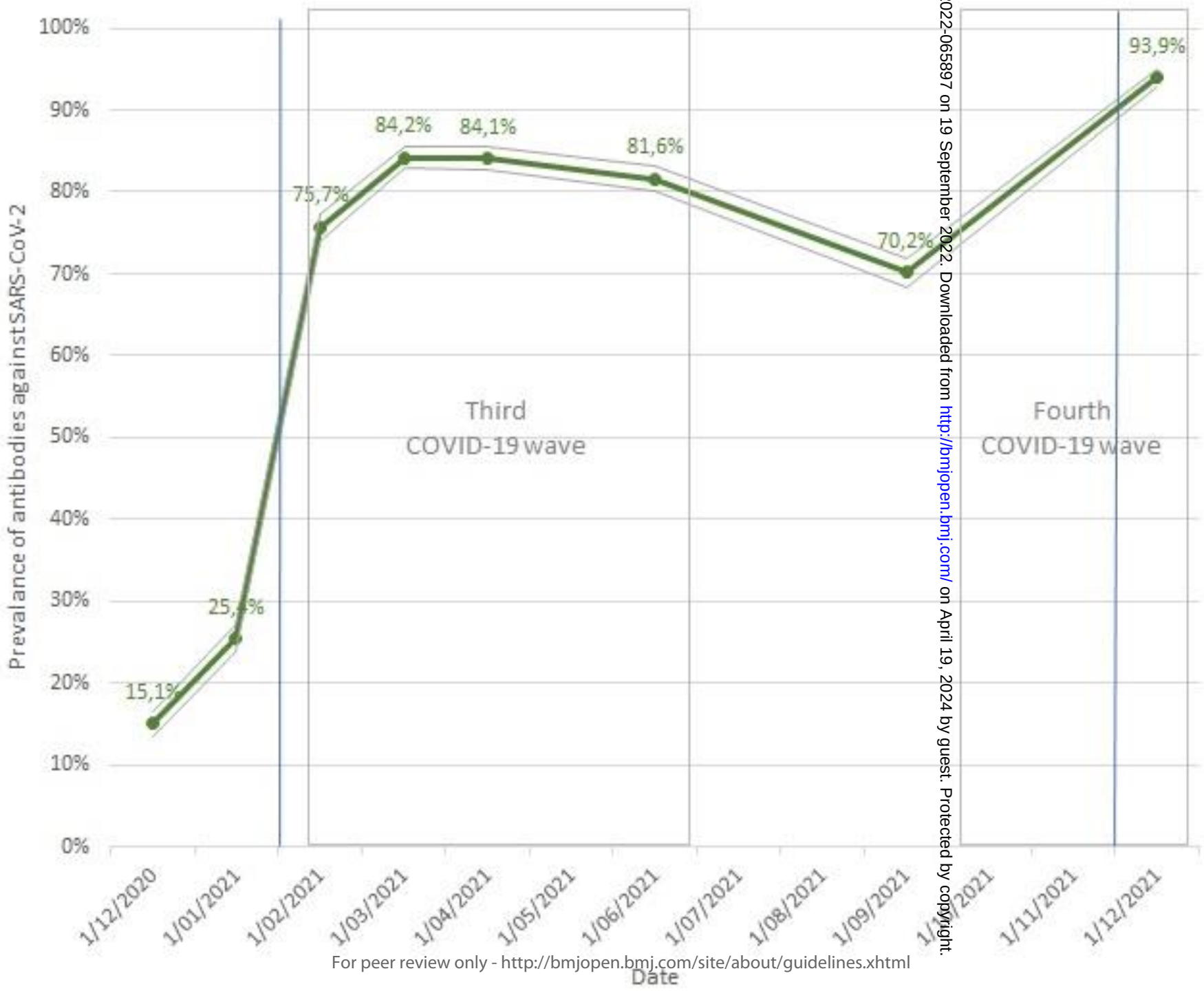
Supplementary file 2. Prevalence and incidence of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium – Follow-up questionnaire

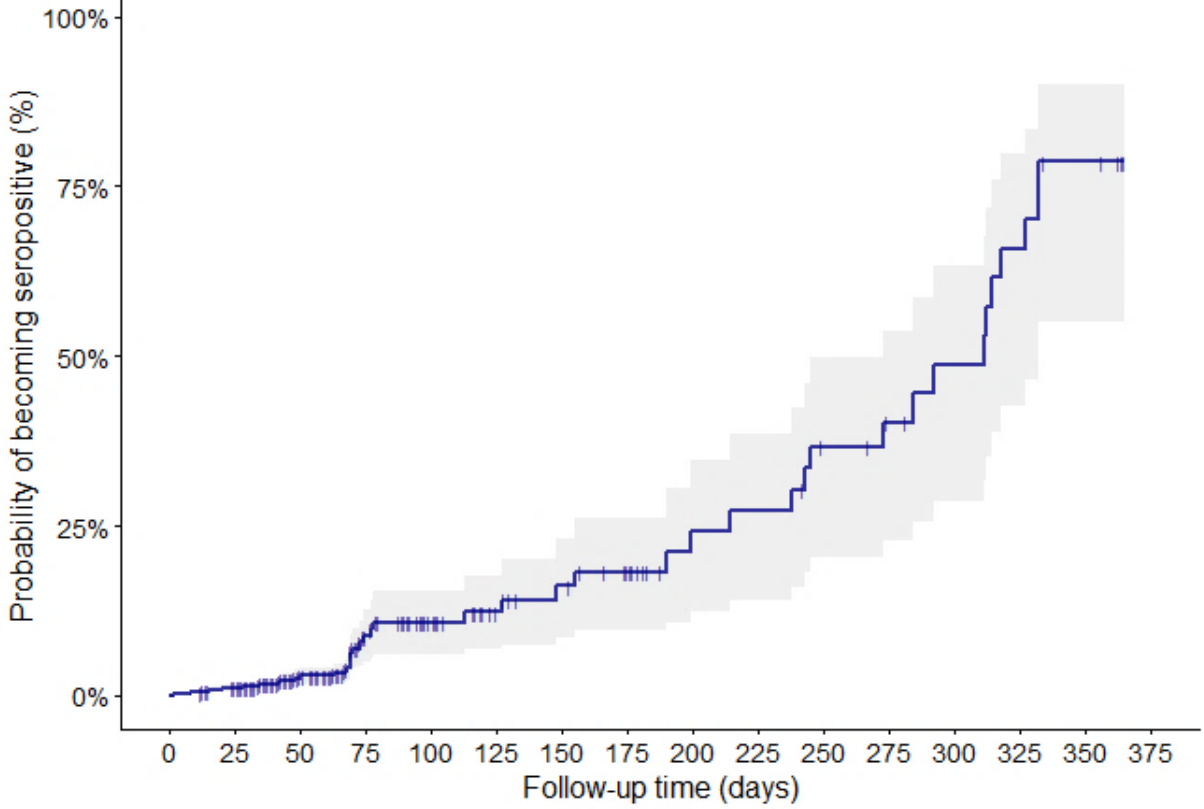
Table S1. Distribution by province of active general practitioners (GPs) in Belgium in 2020 and of GPs who participated in their testing timepoint[1]
[1] The first testing timepoint was December 2020 for 2224 and January 2021 for 373 GPs. PHCPs, respectively.

Table S2. The number of primary healthcare providers (PHCPs) participating per testing timepoint

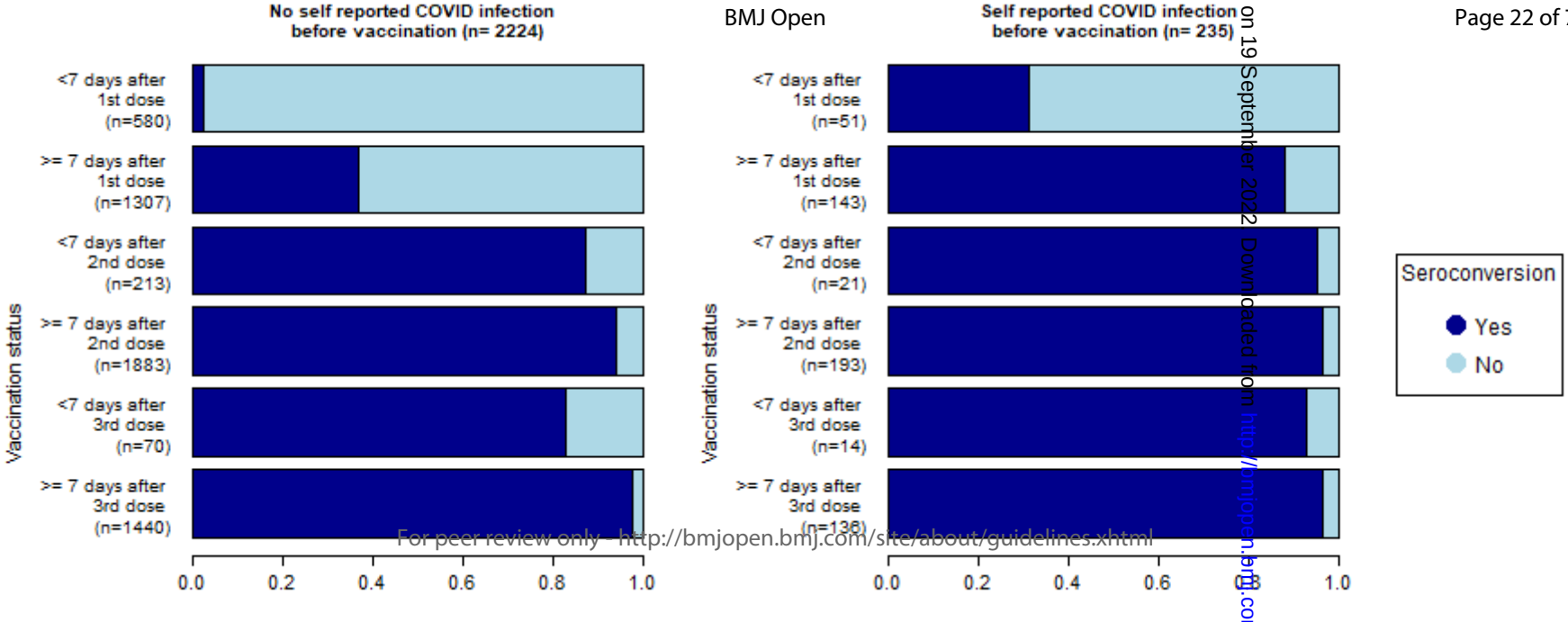
Table S3. Prevalence of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium at eight testing timepoints from December 2020 to December 2021[1]
[1] See Table S4 for the proportions of PHCPs partially and fully vaccinated; [2] RST: Rapid Serological Test; [3] IgG and/or IgM positive among the valid RST; [4] Estimates are based on Generalised Estimating Equations taking into account clustering of PHCPs within their practice and distribution of GPs across districts in Belgium; T1: 24/12/2021-8/1/2021, T2: 25-31/1/2021, T3: 22-28/2/2021, T4: 22-31/3/2021, T5: 19-28/4/2021, T6: 14-27/6/2021, T7, 13-26/9/2021, T8: 13-26/12/2021.

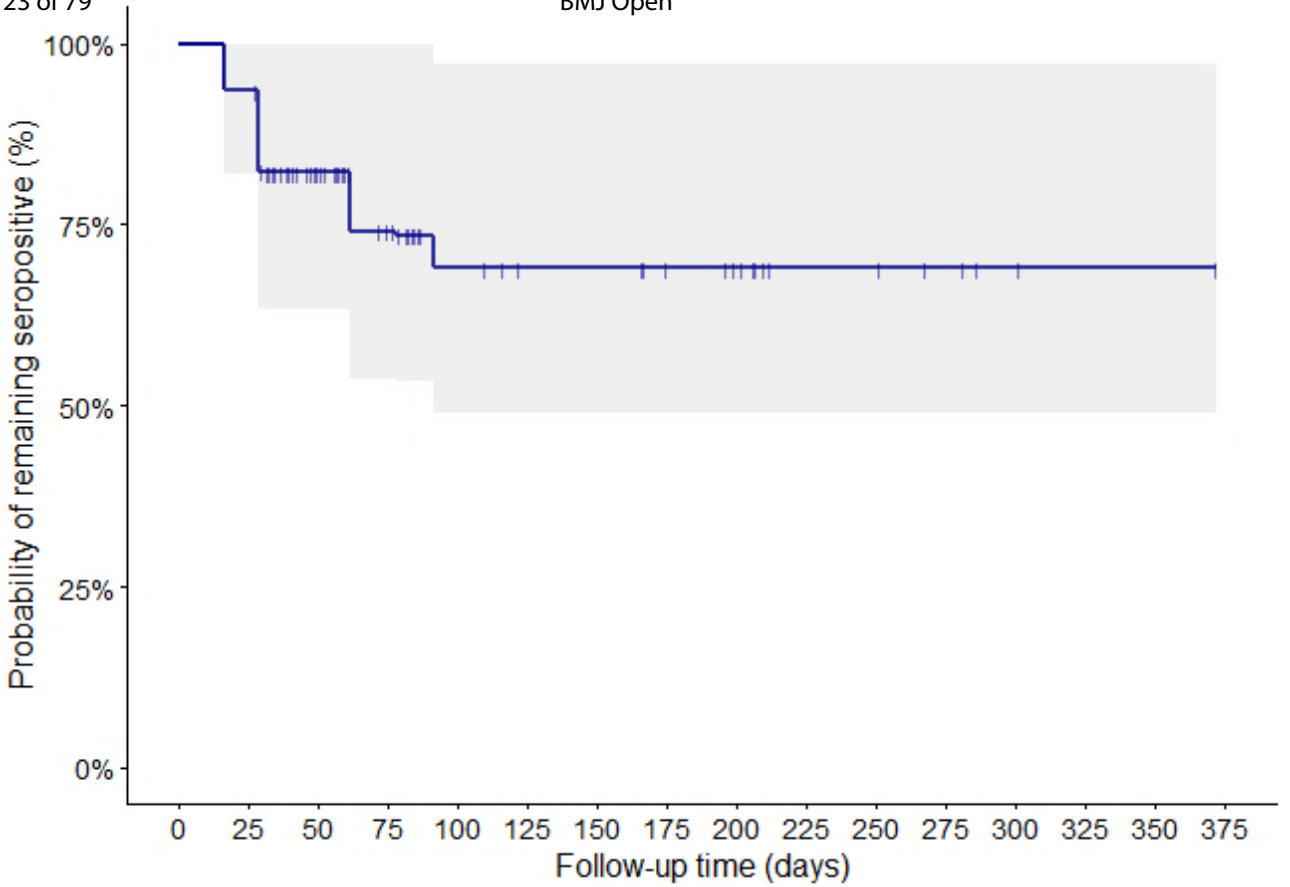
Table S4. Proportions of primary healthcare providers in Belgium with valid rapid serological test results[1] vaccinated at eight testing timepoints from December 2020 to December 2021
[1] See Table S3 for the number of primary healthcare providers with valid rapid serological test results; [2] Received one out of two doses; [3] Received two doses; [4] Received a third dose. T1: 24/12/2021-8/1/2021, T2: 25-31/1/2021, T3: 22-28/2/2021, T4: 22-31/3/2021, T5: 19-28/4/2021, T6: 14-27/6/2021, T7, 13-26/9/2021, T8: 13-26/12/2021.



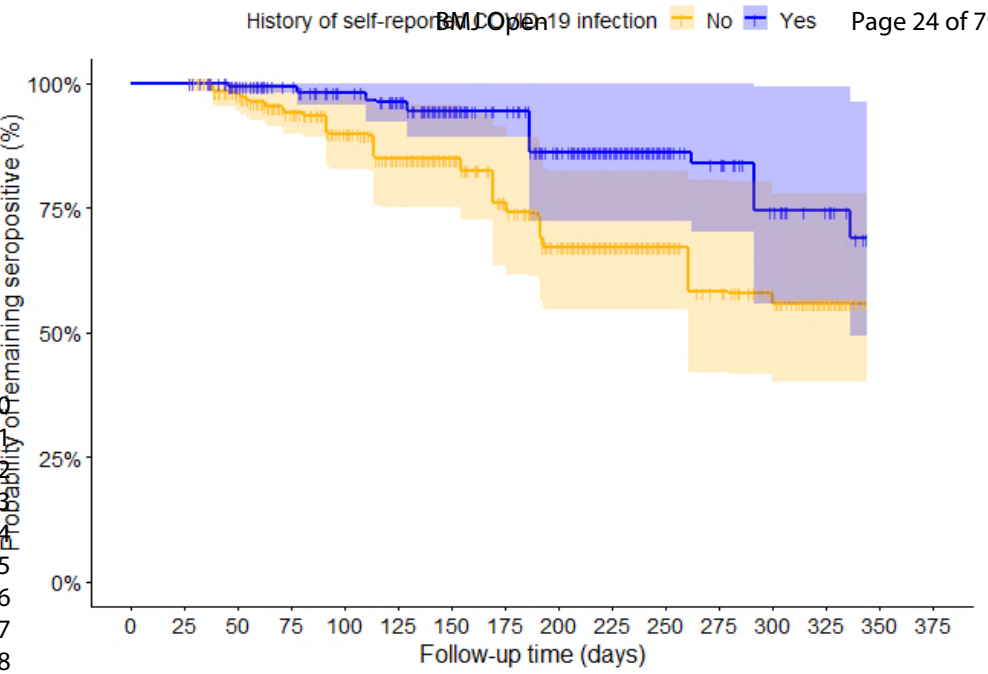


	0	50	100	150	200	250	300
N° at risk	2355	306	65	41	25	19	12

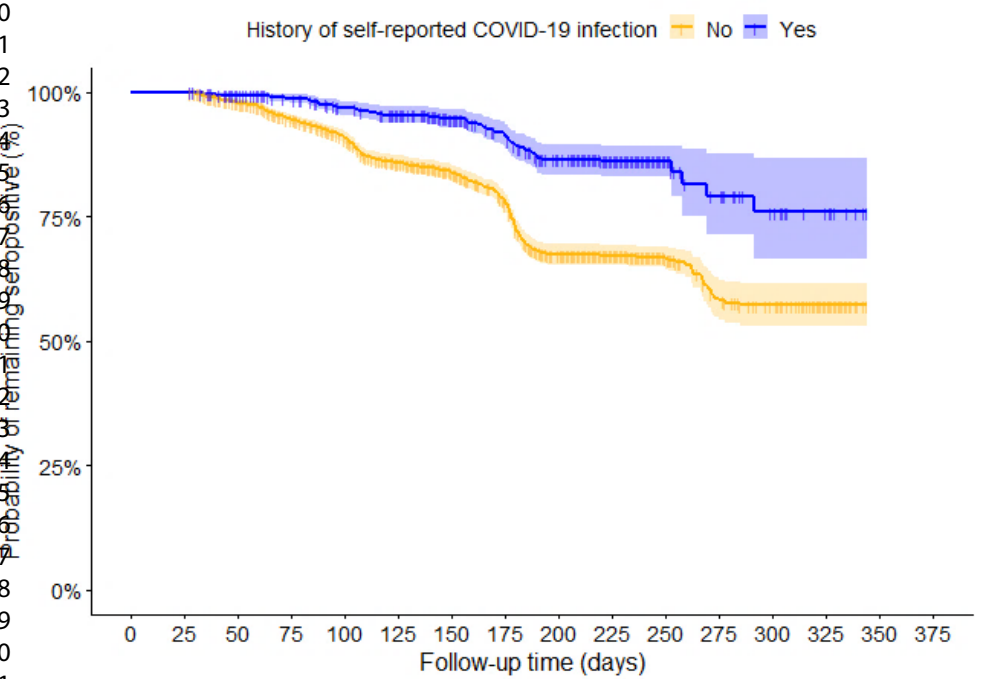




	0	50	100	150	200	250	300
N° at risk	158	64	21	17	12	6	2



A: Interval censoring[1]



B: Midpoint censoring[2]

N°at risk	0	50	100	150	200	250	300
No	2294	2187	1927	1634	1214	200	108
Yes	640	616	565	465	385	48	22

Prevalence and incidence of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium – consent and baseline questionnaire

Dear Participant,

Thank you for your registration for the CHARMING study. We have provided you with your personal study materials for the first three testing time points.

Here we first ask for your formal consent to the study. All questions in the consent section need to be answered before you can proceed. Next we ask for your results on the rapid test, and questions about your health, household, practice and views on the SARS-COV2 pandemic.

If you have questions about CHARMING, please email us at covid-dmg@uliege.be (mailto:covid-dmg@uliege.be).

Many thanks in advance for carefully completing this questionnaire. We hope this will go smoothly for you.

The CHARMING study team

CHARMING
Coronavirus HuisARtsenpraktijk - MédecINe Générale

There are 74 questions in this survey.

E - Consent

Before giving your consent it is important that you have reviewed the information document about this study available in French **here (https://dox.uliege.be/index.php/s/n64T153cp07BOkG)** and in Dutch **here (https://dox.uliege.be/index.php/s/OYp4cIlx8oxERBt).**

1 Your study code (C followed by 4 numbers; see our email of 25.01.2021 with your link to this questionnaire): *

Please write your answer here:

2
I have received an information sheet (version 2.2, 26-11-2020). All my questions concerning this study have been answered satisfactorily. I was given sufficient time to reflect before agreeing to participate in this study.

*

Please choose **only one** of the following:

◯ Yes

3
My participation is voluntary. I have the right to withdraw my consent at any time without giving a reason.

*

Please choose **only one** of the following:

◯ Yes

4
In order to meet the needs of this study, I consent to the collection and use of my data (including the result of the rapid test).
*

Please choose **only one** of the following:

◯ Yes

5
I authorise the consultation of my data to the persons collaborating in this research (these persons are listed in the information form).
*

Please choose **only one** of the following:

◯ Yes

6
I agree that the data recorded in this study will be kept for 20 years and may be processed for future research on respiratory infections and coronaviruses.
*

Please choose **only one** of the following:

◯ Yes

7
I agree to provide a blood sample to validate the rapid test.
*

Please choose **only one** of the following:

◯ Yes

◯ No

8
I agree to provide a blood sample to examine the T-cell response.
*

Please choose **only one** of the following:

◯ Yes

◯ No

9
I agree that the blood samples taken in this study will be stored for 20 years and can be processed at a later date.
*

Please choose **only one** of the following:

◯ Yes

◯ No

10
After this study, I agree to be approached for further research.
*

Please choose **only one** of the following:

◯ Yes
◯ No

11
I wish to participate in this survey.
*

Please choose **only one** of the following:

◯ Yes

Results of the rapid test

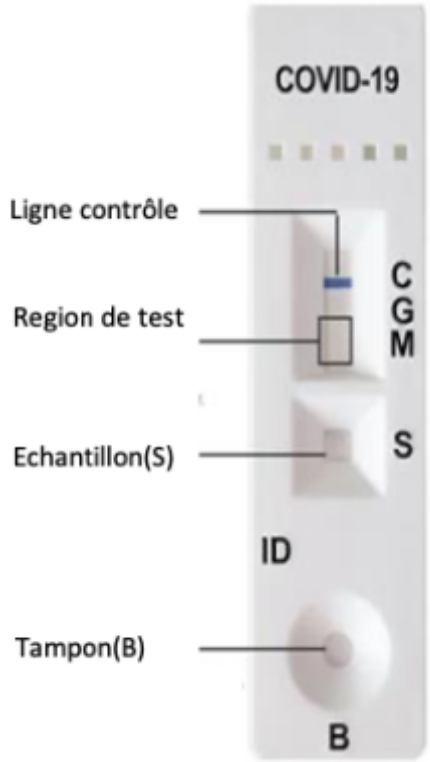
12 *Date on which you carried out the rapid test (dd.mm.yyyy)?* *

Please enter a date:

13

Did the control line "C" change from blue to red?

If not, the test is invalid.



*

Please choose **only one** of the following:

◯ Yes

◯ No

14
Result of your quick test for IgG?

A red line visible next to G = positive (see figure).

*

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '13 [Q00013]' (Did the control line "C" change from blue to red? If not, the test is invalid.)

Please choose **only one** of the following:

◯ Positive
◯ Negative
◯ Unclear

15
Result of your quick test for IgM?

A red line visible next to M = positive (see figure).

*

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '13 [Q00013]' (Did the control line "C" change from blue to red? If not, the test is invalid.)

Please choose **only one** of the following:

◯ Positive

◯ Negative

◯ Unclear

16 Date on which you completed this questionnaire (dd.mm.yyyy)? *

Please enter a date:

17 How many sealed tests do you have left after this testing time point? *

Choose one of the following answers
Please choose **only one** of the following:

- ◯ 0 sealed tests
- ◯ 1 sealed test
- ◯ 2 sealed tests
- ◯ 3 sealed tests
- ◯ 4 sealed tests
- ◯ 5 sealed tests

Your health

18

Do you smoke?
*

If you choose 'not for_____ years' please also specify your choice in the accompanying text field.
Only numbers may be entered in 'not for_____ years' accompanying text field.
Please choose **only one** of the following:

- ◯ Yes
- ◯ I have stopped smoking
- ◯ I have never smoked

19 How many years ago did you stop smoking?

Only answer this question if the following conditions are met:
Answer was 'I have stopped smoking' at question '18 [Q00018]' (Do you smoke?)

Please write your answer here:

years

20

How many alcoholic drinks do you consume per week?
*

Please choose **only one** of the following:

- ◯ 0
- ◯ 1 - 5
- ◯ 6 - 10
- ◯ 11 - 15
- ◯ 16 - 20
- ◯ > 20

21 Have you been vaccinated against pneumococcus? *

Please choose **only one** of the following:

- ◯ Yes
- ◯ No
- ◯ I don't know

22 Have you been vaccinated against influenza for this winter season (2020-2021)? *

Please choose **only one** of the following:

- ◯ Yes
- ◯ No
- ◯ I don't know yet

23 Have you been vaccinated against COVID-19? *

Please choose **only one** of the following:

- ◯ Yes
- ◯ No

24 Which vaccine did you receive? *

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '23 [Q00023]' (Have you been vaccinated against COVID-19?)

Please choose **only one** of the following:

- ◯ Pfizer/BioNTech
- ◯ Moderna
- ◯ Oxford/AstraZeneca
- ◯ Other [ ]

25 How many doses have you received? *

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '23 [Q00023]' (Have you been vaccinated against COVID-19?)

Please choose **only one** of the following:

◯ 1 dose
◯ 2 doses

26 When did you receive the first dose of the vaccine (dd.mm.yyyy)?

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '23 [Q00023]' (Have you been vaccinated against COVID-19?)

Please enter a date:

27

Do you have one or more chronic diseases?
*

Please choose **only one** of the following:

◯ Yes
◯ No

28 What chronic disease(s) do you have? (multiple answers possible) *

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '27 [Q00027]' (Do you have one or more chronic diseases?)

Please choose **all** that apply:

- ☐ Hypertension
- ☐ Diabetes
- ☐ Obesity
- ☐ Other

29 Please list other chronic diseases

Only answer this question if the following conditions are met:
Answer was at question '28 [Q00028]' (What chronic disease(s) do you have? (multiple answers possible))

Please write your answer here:

30 Do you take medicines for chronic diseases? *

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '27 [Q00027]' (Do you have one or more chronic diseases?)

Please choose **only one** of the following:

- ◯ Yes
- ◯ No

31 If yes which ones? *

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '30 [Q00030]' (Do you take medicines for chronic diseases?)

Please choose **all** that apply:

- [ ] ACE inhibitors
- [ ] Immunosuppressants
- [ ] Corticosteroids (also inhalation)
- [ ] NSAID
- [ ] Other

32 Other medicines for chronic disease

Only answer this question if the following conditions are met:
Answer was at question '31 [Q00031]' (If yes which ones?)

Please write your answer here:

33 Other medicines in the last six months

Only answer this question if the following conditions are met:
Answer was 'Yes' at question ' [Q00034]' (Have you taken medicines other than those for chronic diseases in the last six months?)

Please write your answer here:

34 Have you taken medicines other than those for chronic diseases in the last six months? *

Please choose **only one** of the following:

- ◯ Yes
- ◯ No

Your general practice

35 I work in general practice as... *

Please choose **only one** of the following:

- ◯ General practitioner
- ◯ General practitioner in training
- ◯ Other healthcare providers, e.g. nurse, dietician, ...

36 Which year of your training are you in?

Only answer this question if the following conditions are met:
Answer was 'General practitioner in training' at question '35 [Q00035]' (I work in general practice as...)

Please choose **only one** of the following:

- ◯ Year 1
- ◯ Year 2
- ◯ Year 3

37 Please select your profession *

Only answer this question if the following conditions are met:
Answer was 'Other healthcare providers, e.g. nurse, dietician, ...' at question '35 [Q00035]' (I work in general practice as...)

Please choose **only one** of the following:

- ◯ Nurse
- ◯ Psychologist
- ◯ Dietician
- ◯ Speech therapist
- ◯ Other

38 I have been doing this job for… *

Please choose **only one** of the following:

- ◯ Less than 2 years
- ◯ 2 to 5 years
- ◯ 6 to 10 years
- ◯ More than 10 years

39 I also work at... *

Please choose **all** that apply:

- [ ] As CRA (coordinating and advising doctor)
- [ ] In a hospital
- [ ] In an institution (e.g. psychiatry, care for the disabled, ...)
- [ ] I don't have any other activity
- [ ] Other:

40 Which other healthcare professionals work in your practice? (multiple answers possible) *

Please choose **all** that apply:

- [ ] General practitioner
- [ ] Dietician
- [ ] Psychologist
- [ ] Nurse
- [ ] Practice assistant
- [ ] None of the above
- [ ] Other:

41 What is the (estimated) number of patients assigned to your practice? *

Please write your answer here:

42 What is the (estimated) proportion of patients younger than 15 years of age (%) ? *

Your answer must be between 0 and 100

Please write your answer here:

%

43 What is the (estimated) proportion of patients over 65 years of age (%)? *

Your answer must be between 0 and 100

Please write your answer here:

%

44 What is the estimated proportion of patients with increased benefits (%) ? *

Your answer must be between 0 and 100

Please write your answer here:

%

45 What is the (estimated) proportion of patients with a migration background (%) ? *

Please write your answer here:

%

46 What is the (estimated) proportion of patients who do not speak Dutch, French or German (%) ? *

Please write your answer here:

%

Your household

47 What is the composition of your household? *

Each answer must be at least 0

Please write your answer(s) here:

How many family members does your household include, including yourself?

How many children attend a crèche (less than 2.5 years) ?

How many children attend pre-school (2,5 to 6 years)?

How many children attend primary school (typically 6 to 12 years)?

How many children attend secondary school (typically 12 - 18 years?

How many household members are university/college students (typically aged over 18 years) AND sleeping in the family home more than 3 nights per week?

How many household members (typically over 18 years) in employment AND sleeping in the family home more than 3 nights per week?

48 Is your partner employed in healthcare with patient contact? *

Please choose **only one** of the following:

- ◯ Yes
- ◯ No
- ◯ Not applicable

49 How many household members had complaints this year that are compatible with COVID-19, including yourself? *

Please write your answer here:

50 If you had complaints, what were they? (multiple answers possible) *

Only answer this question if the following conditions are met:
Answer was greater than or equal to '1' at question '49 [Q00049]' (How many household members had complaints this year that are compatible with COVID-19, including yourself?)

Please choose **all** that apply:

- [ ] I didn't have any complaints
- [ ] Cough
- [ ] Headache
- [ ] Sore throat
- [ ] Fever
- [ ] Shortness of breath
- [ ] Runny nose
- [ ] Muscle pain
- [ ] Loss of sense of smell
- [ ] Loss of taste
- [ ] General weakness/ fatigue
- [ ] Nausea/ vomiting
- [ ] Diarrhoea
- [ ] Other:

51 How many members of your household, including yourself, have been tested for COVID-19 (excluding tests for research purposes)? *

Please write your answer here:

52 How often have you been tested (except for the research purposes)? *

Please write your answer here:

times

53 How many days have you spent in quarantine? *

Please choose **only one** of the following:

- ◯ 0 days
- ◯ up to 5 days
- ◯ up to 7 days
- ◯ up to 10 days
- ◯ up to 14 days
- ◯ up to 20 days
- ◯ more than 20 days

54 Have you ever tested positive for COVID-19? *

Please choose **only one** of the following:

- ◯ Yes
- ◯ No

55 If you tested positive, when was the positive sample taken? *

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '54 [Q00054]' (Have you ever tested positive for COVID-19?)

Please choose **only one** of the following:

- ◯ February
- ◯ March
- ◯ April
- ◯ May
- ◯ June
- ◯ July
- ◯ August
- ◯ September
- ◯ October
- ◯ November
- ◯ December
- ◯ January 2021

56 if you know the exact date of the positive sample enter it here:

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '54 [Q00054]' (Have you ever tested positive for COVID-19?)

Please enter a date:

57
For the positive test result which test(s) was/were used? (multiple answers possible) *

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '54 [Q00054]' (Have you ever tested positive for COVID-19?)

Please choose **all** that apply:

- ☐ PCR (for virus detection)
- ☐ Rapid test (for virus detection)
- ☐ Blood sample (for antibody detection)
- ☐ Rapid test (for antibody detection)
- ☐ Other: _____

58
If you tested positive, who was the suspected source of the infection?

*

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '54 [Q00054]' (Have you ever tested positive for COVID-19?)

Please choose **all** that apply:

- ☐ Patient
- ☐ Co-worker
- ☐ Family member
- ☐ Other: _____

59 If you were treated for COVID-19, what treatment did you have? *

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '54 [Q00054]' (Have you ever tested positive for COVID-19?)

Please choose **all** that apply:

- ☐ Symptomatic treatment of pain, fever and other complaints
- ☐ Hydroxychloroquine
- ☐ Antibiotics
- ☐ No treatment
- ☐ Other: _____

60 If you were admitted for COVID-19, how many days did you spend in hospital?

(if you were not admitted to hospital put '0')

*

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '54 [Q00054]' (Have you ever tested positive for COVID-19?)

Please write your answer here:

_____

days

61
If you were admitted for COVID-19, how many days did you stay in intensive care? (if you were not admitted to intensive care put '0')
*

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '54 [Q00054]' (Have you ever tested positive for COVID-19?)

Please write your answer here:

days

62 How many household members have tested positive for COVID-19, **not** including yourself? *

Please write your answer here:

63 How many household members have been admitted to hospital for (suspected) COVID-19, **not** including yourself? *

Please write your answer here:

64 How many household members have been treated for (suspected) COVID-19, **not** including yourself? *

Please write your answer here:

Risk factors for COVID-19

65 Have you continued to work since the outbreak? *

Please choose **only one** of the following:

- ◯ Yes
- ◯ No

66 Have you been in physical contact with patients with confirmed COVID-19 since the outbreak? *

Please choose **only one** of the following:

- ◯ Yes
- ◯ No

67 If so, how many? *

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '66 [Q00066]' (Have you been in physical contact with patients with confirmed COVID-19 since the outbreak?)

Please choose **only one** of the following:

- ◯ 1 - 5 patients
- ◯ 6 - 10 patients
- ◯ 11 - 15 patients
- ◯ 16 - 20 patients
- ◯ > 20 patients

68 Have you lacked protective equipment since the outbreak? *

Please choose **only one** of the following:

- ◯ Yes
- ◯ No

69 If so which equipment? (multiple answers possible) *

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '68 [Q00068]' (Have you lacked protective equipment since the outbreak?)

Please choose **all** that apply:

- ☐ Gloves
- ☐ Surgical mouth mask
- ☐ Other mouth mask (FFP2 or FFP3)
- ☐ Safety goggles
- ☐ Apron / body protection
- ☐ Other: _____

70 If available, which protective material do you use in patients with (suspected) COVID-19)? (multiple answers possible) *

Please choose **all** that apply:

- ☐ Gloves
- ☐ Surgical mouth mask
- ☐ Other mouth mask (FFP2 or FFP3)
- ☐ Safety goggles
- ☐ Apron/body protection
- ☐ Other: _____

71 If available, what protective material do you use with your other patients? (multiple answers possible) *

Please choose **all** that apply:

- ☐ Gloves
- ☐ Surgical mouth mask
- ☐ Other mouth mask (FFP2 or FFP3)
- ☐ safety goggles
- ☐ Apron/body protection
- ☐ Other: _____

72 Have you participated in the COVID patient triage? *

Please choose **only one** of the following:

- ◯ Yes
- ◯ No

73 If so, how many patients did you physically examine who subsequently turned out to be COVID-19 positive? *

Only answer this question if the following conditions are met:
Answer was 'Yes' at question '72 [Q00072]' (Have you participated in the COVID patient triage?)

Please choose **only one** of the following:

- ◯ 0 patients
- ◯ 1 - 5 patients
- ◯ 6 - 10 patients
- ◯ 11 - 15 patients
- ◯ 16 - 20 patients
- ◯ > 20 patients

74
Indicate to what extent you agree with the following statements

(1= totally disagree; 5= totally agree): *

Please choose the appropriate response for each item:

	1	2	3	4	5
The personal protection equipment that I use, protects me sufficiently against more contagious variants of SARS-CoV-2.	◯	◯	◯	◯	◯
A temporary ban on non-essential international travel is needed.	◯	◯	◯	◯	◯
I am sure I am already infected with COVID-19.	◯	◯	◯	◯	◯
I will certainly be infected with COVID-19 during this epidemic.	◯	◯	◯	◯	◯
I am afraid I am contaminating my relatives.	◯	◯	◯	◯	◯
The guidelines for primary care are clearly communicated.	◯	◯	◯	◯	◯
The guidelines for primary care are scientifically based.	◯	◯	◯	◯	◯
The Belgian healthcare system is strong enough to cope with this epidemic.	◯	◯	◯	◯	◯
The testing capacity in Belgium is sufficient.	◯	◯	◯	◯	◯
Rapid diagnostic tests are relevant for general practice.	◯	◯	◯	◯	◯
Rapid diagnostic tests for SARS-CoV-2 viral detection are manageable for general practice.	◯	◯	◯	◯	◯

	1	2	3	4	5
The measures imposed by the government are sufficient.	◯	◯	◯	◯	◯
Everyone should wear a mask if they go outdoors.	◯	◯	◯	◯	◯
I have every confidence in the scientific COVID-19 expert committee.	◯	◯	◯	◯	◯
Most of my patients follow the rules of 'social distancing'.	◯	◯	◯	◯	◯
Most of my patients adhere to hygiene rules.	◯	◯	◯	◯	◯
La plupart de mes patients symptomatiques respectent les règles de quarantaine.	◯	◯	◯	◯	◯
This period is more stressful than during a busy flu period.	◯	◯	◯	◯	◯
I want to get the COVID-19 vaccination as soon as it is available.	◯	◯	◯	◯	◯

Thank you very much for completing this questionnaire.

You will shortly receive an email that will explain what your test result means. We will send you an overview of your consent to participate in the study in the coming weeks.

The CHARMING study team

CHARMING
Coronavirus HuisARtsenpraktijk - MédecINe Générale

09.12.2021 – 16:19

Submit your survey.
Thank you for completing this survey.

Prevalence and incidence of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium - Follow-up questionnaire February 2021

Dear Participant,

Thank you for your participation in CHARMING. This follow-up questionnaire refers to the period since the last testing period.

Many thanks in advance for carefully completing this questionnaire. We hope this will go smoothly for you.

The CHARMING study team

PS If you have questions about CHARMING, please email us at covid-dmg@uliege.be (mailto:covid-dmg@uliege.be).

CHARMING
Coronavirus HuisARtsenpraktijk - MédecINe Générale

There are 46 questions in this survey.

Part 1

1 Your personal study code (C followed by 4 numbers; see our email of 26.02.2021 with your link to this questionnaire): *

Please write your answer here:

Part 2

Instructions on how to perform the rapid test can be found in French **here** (https://dox.uliege.be/index.php/s/1duglah08HN8YIr) and in Dutch **here** (https://dox.uliege.be/index.php/s/hqqiswSGBxKw3yf). Short instruction videos are available here:

- French test on yourself : https://vimeo.com/492411023/7b2bedb700 (https://vimeo.com/492411023/7b2bedb700)
- French test on someone else: https://vimeo.com/492427669/b42bb624b6 (https://vimeo.com/492427669/b42bb624b6)
- Dutch test on yourself: https://vimeo.com/492430777/92626224d1 (https://vimeo.com/492430777/92626224d1)
- Dutch test on someone else : https://vimeo.com/492428827/d565f20bc2 (https://vimeo.com/492428827/d565f20bc2)

2 *Date on which you carried out the rapid test (dd.mm.yyyy)?* *

Please enter a date:

3
Did the control line "C" change from blue to red?
If not, the test is invalid.

*

Please choose **only one** of the following:

◯ Yes
◯ No

4
Result of your quick test for IgG?

A red line visible next to G = positive (see figure).
*

Please choose **only one** of the following:

- ◯ Positive
- ◯ Negative
- ◯ Unclear

5
Result of your quick test for IgM?

A red line visible next to M = positive (see figure).
*

Please choose **only one** of the following:

- ◯ Positive
- ◯ Negative
- ◯ Unclear

6 Date on which you completed this questionnaire (dd.mm.yyyy)? *

Please enter a date:

7 How many sealed tests do you have left after this testing time point? *

Please choose **only one** of the following:

- ◯ 0 sealed tests
- ◯ 1 sealed test
- ◯ 2 sealed tests
- ◯ 3 sealed tests
- ◯ 4 sealed tests
- ◯ 5 sealed tests

Part 3

8 Since **your first** testing period (end December 2020 or end January 2021), how many days have you spent in quarantine? *

Please choose **only one** of the following:

- ◯ 0 days
- ◯ up to 5 days
- ◯ up to 7 days
- ◯ up to 10 days
- ◯ up to 14 days
- ◯ up to 20 days
- ◯ more than 20 days

9 Since **your first** testing period (end December 2020 or end January 2021), how often have you been tested for COVID-19 (except for research purposes)? *

Please write your answer here:

times

10 I work in general practice as... *

Please choose **only one** of the following:

- ◯ General practitioner
- ◯ General practitioner in training
- ◯ Other healthcare providers, e.g. nurse, dietician, ...

11 Please select your profession *

Please choose **only one** of the following:

- ◯ Nurse
- ◯ Psychologist
- ◯ Dietician
- ◯ Speech therapist
- ◯ Other

12 Since the **last** testing period I have also worked... *

Please choose **all** that apply:

- [ ] As CRA (coordinating and advising doctor)
- [ ] In a hospital
- [ ] In an institution (e.g. psychiatry, care for the disabled, ...)
- [ ] I don't have any other activity
- [ ] Other:

Part 4

13 Since the last testing phase of CHARMING how many family members had complaints that are compatible with COVID-19, including yourself? *

Please write your answer here:

14 If you had complaints, since the last testing period, what were they? (multiple answers possible) *

Please choose **all** that apply:

- ☐ I didn't have any complaints
- ☐ Cough
- ☐ Headache
- ☐ Sore throat
- ☐ Fever
- ☐ Shortness of breath
- ☐ Runny nose
- ☐ Muscle pain
- ☐ Loss of sense of smell
- ☐ Loss of taste
- ☐ General weakness/ fatigue
- ☐ Nausea/ vomiting
- ☐ Diarrhoea
- ☐ Other: _____

15 Since the last testing period how many family members, including yourself, have been tested for COVID-19 (excluding tests for research purposes)? *

Please write your answer here:

_____

16 Have you tested positive for COVID-19 since the last testing period? (multiple answers possible) *

Please choose **only one** of the following:

◯ Yes

◯ No

17
For the positive test result which test(s) was/were used? (multiple answers possible) *

Please choose **all** that apply:

☐ PCR (for virus detection)

☐ Rapid test (for virus detection)

☐ Blood sample (for antibody detection)

☐ Rapid test (for antibody detection)

☐ Other:

18 If you tested positive when was the positive sample taken (dd.mm.yyyy)?

Please enter a date:

19 If you were treated for COVID-19, what treatment did you have? *

Please choose **all** that apply:

- ☐ Symptomatic treatment of pain, fever and other complaints
- ☐ Hydroxychloroquine
- ☐ Antibiotics
- ☐ No treatment
- ☐ Other:

20
If you tested positive, who was the suspected source of the infection? *

Please choose **all** that apply:

- ☐ Patient
- ☐ Co-worker
- ☐ Family member
- ☐ Other:

21
If you were admitted for COVID-19, how many days did you spend in hospital?

(if you were not admitted to hospital put '0') *

Please write your answer here:

days

22
If you were admitted for COVID-19, how many days did you stay in intensive care? (if you were not admitted to intensive care put '0') *

Please write your answer here:

days

23 Since the last testing period how many family members have tested positive for COVID-19, **not** including yourself? *

Please write your answer here:

24 Since the last testing period how many family members have been admitted to hospital for (suspected) COVID-19, **not** including yourself? *

Please write your answer here:

25 Since the last testing period how many family members have been treated for (suspected) COVID-19, **not** including yourself? *

Please write your answer here:

Part 5

26 Have you continued to work in primary care since the **last** testing period? *

Please choose **only one** of the following:

- ◯ Yes
- ◯ No

27 Have you been in physical contact with patients with confirmed COVID-19 since the last testing period? *

Please choose **only one** of the following:

- ◯ Yes
- ◯ No

28 If so, how many? *

Please choose **only one** of the following:

- ◯ 1 - 5 patients
- ◯ 6 - 10 patients
- ◯ 11 - 15 patients
- ◯ 16 - 20 patients
- ◯ > 20 patients

29 Have you lacked protective equipment since the **last** testing period? *

Please choose **only one** of the following:

◯ Yes

◯ No

30 If so which equipment? (multiple answers possible) *

Please choose **all** that apply:

☐ Gloves

☐ Surgical mouth mask

☐ Other mouth mask (FFP2 or FFP3)

☐ Safety goggles

☐ Apron / body protection

☐ Other: _____

31 If available, which protective material have you used since the last testing period in patients with (suspected) COVID-19)? (multiple answers possible) *

Please choose **all** that apply:

☐ Gloves

☐ Surgical mouth mask

☐ Other mouth mask (FFP2 or FFP3)

☐ Safety goggles

☐ Apron/body protection

☐ Other: _____

32
If available, what protective material have you used with your other patients? (multiple answers possible) *

Please choose **all** that apply:

- [ ] Gloves
- [ ] Surgical mouth mask
- [ ] Other mouth mask (FFP2 or FFP3)
- [ ] safety goggles
- [ ] Apron/body protection
- [ ] Other:

33 Have you participated in the COVID patient triage since the last testing period? *

Please choose **only one** of the following:

- ◯ Yes
- ◯ No

34 If so, how many patients did you physically examine who subsequently turned out to be COVID-19 positive? *

Please choose **only one** of the following:

- ◯ 0 patients
- ◯ 1 - 5 patients
- ◯ 6 - 10 patients
- ◯ 11 - 15 patients
- ◯ 16 - 20 patients
- ◯ > 20 patients

35 Have you been vaccinated against COVID-19? *

Please choose **only one** of the following:

- ◯ Yes
- ◯ No

36 Which vaccine did you receive? *

Please choose **only one** of the following:

- ◯ Pfizer/BioNTech
- ◯ Moderna
- ◯ Oxford/AstraZeneca
- ◯ Other

37 How many doses have you received? *

Please choose **only one** of the following:

- ◯ 1 dose
- ◯ 2 doses

38 When did you receive the **first** dose of the vaccine (dd.mm.yyyy)? *

Please enter a date:

39 Did you experience side-effects after receiving the **first** dose? *

Please choose **only one** of the following:

- ◯ No side-effects
- ◯ Negligible side-effects
- ◯ Mild side-effects
- ◯ Moderate side-effects
- ◯ Severe side-effects

40 For how many days did you experience the following side-effects after the **first** dose (if you did not experience the side-effect put '0'): *

41 What other moderate or severe side-effects did you experience after receiving the **first** dose?

Please write your answer here:

42 When did you receive the **second** dose of the vaccine (dd.mm.yyyy)? *

Please enter a date:

43 Did you experience side-effects after receiving the **second** dose? *

Please choose **only one** of the following:

- ◯ No side-effects
- ◯ Negligible side-effects
- ◯ Mild side-effects
- ◯ Moderate side-effects
- ◯ Severe side-effects

44 For how many days after receiving the **second** dose of the vaccine did you experience the following side-effects (if you did not experience the side-effect put '0')? *

45 What other moderate or severe side-effects did you experience after receiving the **second** dose?

Please write your answer here:

46

Indicate to what extent you agree with the following statements

(1= totally disagree; 5= totally agree): *

Please choose the appropriate response for each item:

	1	2	3	4	5
The personal protection equipment that I use, protects me sufficiently against more contagious variants of SARS-CoV-2.	◯	◯	◯	◯	◯
A temporary ban on non-essential international travel is still needed.	◯	◯	◯	◯	◯
The Belgian healthcare system is strong enough to cope with this epidemic.	◯	◯	◯	◯	◯
The testing capacity in Belgium is sufficient.	◯	◯	◯	◯	◯
Rapid diagnostic tests are relevant for general practice.	◯	◯	◯	◯	◯
Rapid diagnostic tests for SARS-CoV-2 viral detection are manageable for general practice.	◯	◯	◯	◯	◯
The measures imposed by the government are sufficient.	◯	◯	◯	◯	◯
Everyone should wear a mask when they work inside with other people.	◯	◯	◯	◯	◯
I have every confidence in the scientific COVID-19 expert committee.	◯	◯	◯	◯	◯
Most of my patients follow the rules of 'social distancing'.	◯	◯	◯	◯	◯
Most of my patients adhere to hygiene rules.	◯	◯	◯	◯	◯

	1	2	3	4	5
Most of my symptomatic patients respect the quarantine rules.	◯	◯	◯	◯	◯
This period is more stressful than during a busy flu period.	◯	◯	◯	◯	◯
For health care personnel the COVID-19 vaccination should be obligatory.	◯	◯	◯	◯	◯

Thank you very much for completing this questionnaire.

You will shortly receive an email that will explain what your test result means.

If you experience side-effects after receiving the vaccination you can report them officially here:

In Dutch: https://www.fagg.be/nl/bijwerking (https://www.fagg.be/nl/bijwerking)
In French: https://www.afmps.be/fr/effet_indesirable (https://www.afmps.be/fr/effet_indesirable)

The CHARMING study team

CHARMING
Coronavirus HuisARtsenpraktijk - MédecINe Générale

21.03.2021 – 20:58

Submit your survey.
Thank you for completing this survey.

Table S1. Distribution by province of active general practitioners (GPs) in Belgium in 2020 and of GPs who participated in CHARMING in their testing timepoint[1]

Region/Province	Active GPs n	Active GPs (%)	Participating GPs n	Participating GPs (%)
Brussels	1,178	(10.01)	239	(9.2)
Flanders	6,805	(57.83)	1,725	(66.4)
Wallonia	3,784	(32.16)	633	(24.4)
Antwerpen-Anvers	1,806	(15.35)	454	(17.5)
Brussel-Hoofdstad-Bruxelles Capitale	1,178	(10.01)	239	(9.2)
Henegouwen-Hainaut	1,293	(10.99)	175	(6.7)
Limburg-Limbourg	943	(8.01)	235	(9.0)
Luik-Liège	1,125	(9.56)	200	(7.7)
Luxemburg-Luxembourg	301	(2.56)	78	(3.0)
Namen-Namur	594	(5.05)	104	(4.0)
Oost-Vlaanderen-Flandre Orientale	1,556	(13.22)	431	(16.6)
Vlaams-Brabant-Brabant-Flamand	1,241	(10.55)	317	(12.2)
Waals-Brabant-Brabant Wallon	471	(4.00)	76	(2.9)
West-Vlaanderen-Flandre Occidentale	1,259	(10.70)	288	(11.1)
Total	11,767		2,597	(22.1)

[1] The first testing timepoint was December 2020 for 2224 and January 2021 for 373 GPs. PHCPs, respectively.

Table S2. The number of primary healthcare providers (PHCPs) participating per testing timepoint

Number of PHCPs (%) Testing timepoints	Invited (%) N=3,648		Responding (%)		Responding within the testing timeframe[1] (%)	
1	3,044	2,820	(92.6%)	2680	(88.0%)	
2	3,648	3,289	(90.2%)	3060	(83.9%)	
3	3,648	3,162	(86.7%)	3018	(82.7%)	
4	3,409	3,043	(89.3%)	3021	(88.6%)	
5	3,409	2,989	(87.7%)	2891	(84.8%)	
6	3,409	2,802	(82.2%)	2750	(80.7%)	
7	3,313	2,819	(85.1%)	2756	(83.2%)	
8	3,313	2,557	(77.2%)	2516	(75.9%)	

[1]The eight testing timepoints have the following start and end dates: T1: 24/12/2021-8/1/2021, T2: 25-31/1/2021, T3: 22-28/2/2021, T4: 22-31/3/2021, T5: 19-28/4/2021, T6: 14-27/6/2021, T7, 13-26/9/2021, T8: 13-26/12/2021.

Table S3. Prevalence of antibodies against SARS-CoV-2 among primary healthcare providers in Belgium at eight testing timepoints from December 2020 to December 2021[1]

Testing timepoint	Region	PHCPs n	Valid RST[2] n	Positive RST[3] n	Adjusted prevalence[4] % (95% CI)	
T1	Belgium	2,680	2,629	366	15.08	(13.54-16.62)
	Brussels	234	233	43	18.45	(13.47-23.44)
	Flanders	1841	1800	203	11.28	(9.77-12.79)
	Wallonia	605	596	120	20.37	(16.91-23.84)
T2	Belgium	3,060	2,995	716	25.42	(23.75-27.08)
	Brussels	270	263	55	20.91	(15.98-25.84)
	Flanders	2024	1980	389	20.10	(18.29-21.92)
	Wallonia	766	752	272	36.03	(32.46-39.60)
T3	Belgium	3,018	2,967	2,278	75.70	(74.03-77.37)
	Brussels	274	273	168	61.54	(55.72-67.36)
	Flanders	2014	1971	1615	82.35	(80.63-84.06)
	Wallonia	730	723	495	68.80	(65.26-72.34)
T4	Belgium	3,021	2,980	2,509	84.17	(82.86-85.48)
	Brussels	279	274	209	76.28	(71.24-81.31)
	Flanders	1,987	1,963	1,706	86.91	(85.42-88.40)
	Wallonia	755	743	594	79.95	(77.07-82.83)
T5	Belgium	2,891	2,859	2,410	84.07	(82.65-85.48)
	Brussels	274	268	206	76.87	(71.82-81.91)
	Flanders	1,898	1,877	1,622	86.67	(85.09-88.25)
	Wallonia	719	714	582	81.86	(78.97-84.75)
T6	Belgium	2,750	2,725	2,230	81.57	(80.02-83.12)
	Brussels	252	244	197	80.74	(75.81-85.67)
	Flanders	1,839	1,826	1,514	82.76	(80.97-84.55)
	Wallonia	659	655	519	79.78	(76.59-82.98)
T7	Belgium	2,756	2,730	1,917	70.17	(68.36-71.97)
	Brussels	238	237	178	75.11	(69.62-80.59)
	Flanders	1,844	1,823	1,271	69.38	(67.20-71.56)
	Wallonia	674	670	468	70.04	(66.46-73.62)
T8	Belgium	2,516	2,498	2,356	93.91	(92.89-94.93)
	Brussels	222	221	201	90.95	(87.17-94.73)
	Flanders	1,696	1,681	1,607	95.42	(94.36-96.47)
	Wallonia	598	596	548	92.22	(90.02-94.43)

[1] See Table S4 for the proportions of PHCPs partially and fully vaccinated; [2] RST: Rapid Serological Test; [3] IgG and/or IgM positive among the valid RST; [4] Estimates are based on Generalised Estimating Equations taking into account clustering of PHCPs within their practice and distribution of GPs across districts in Belgium; T1: 24/12/2021-8/1/2021, T2: 25-31/1/2021, T3: 22-28/2/2021, T4: 22-31/3/2021, T5: 19-28/4/2021, T6: 14-27/6/2021, T7, 13-26/9/2021, T8: 13-26/12/2021.

Table S4. Proportions of primary healthcare providers in Belgium with valid rapid serological test results[1] vaccinated at eight testing timepoints from December 2020 to December 2021

Testing timepoint	Region	Partially vaccinated[2] % (95%CI)		Fully vaccinated[3] % (95%CI)		Booster vaccinated[4] % (95%CI)	
T1	Belgium	NA		NA		NA	
	Brussels	NA		NA		NA	
	Flanders	NA		NA		NA	
	Wallonia	NA		NA		NA	
T2	Belgium	57.16	(55.39-58.93)	0.87	(0.54-1.20)	NA	
	Brussels	17.49	(12.90-22.08)	1.14	(0.00-2.42)	NA	
	Flanders	67.27	(65.21-69.34)	0.30	(0.06-0.55)	NA	
	Wallonia	44.41	(40.86-47.97)	2.26	(1.20-3.32)	NA	
T3	Belgium	16.92	(15.57-18.27)	66.23	(64.53-67.93)	NA	
	Brussels	50.18	(44.25-56.11)	21.98	(17.07-26.89)	NA	
	Flanders	11.72	(10.30-13.14)	76.15	(74.27-78.04)	NA	
	Wallonia	18.53	(15.70-21.37)	55.88	(52.26-59.50)	NA	
T4	Belgium	16.88	(15.53-18.22)	78.46	(76.98-79.93)	NA	
	Brussels	30.29	(24.85-35.73)	60.22	(54.42-66.01)	NA	
	Flanders	13.40	(11.89-14.90)	84.06	(82.44-85.67)	NA	
	Wallonia	21.13	(18.20-24.07)	70.39	(67.11-73.67)	NA	
T5	Belgium	15.49	(14.17-16.82)	81.11	(79.68-82.55)	NA	
	Brussels	26.46	(22.21-31.78)	66.04	(60.38-71.71)	NA	
	Flanders	13.16	(11.63-14.69)	85.35	(83.75-86.95)	NA	
	Wallonia	17.51	(14.72-20.29)	80.48	(77.13-83.83)	NA	
T6	Belgium	1.54	(1.08-3.00)	95.93	(95.18-96.67)	NA	
	Brussels	2.05	(0.27-3.83)	92.21	(88.85-95.58)	NA	
	Flanders	0.82	(0.41-1.24)	98.68	(97.39-98.67)	NA	
	Wallonia	3.36	(1.42-5.35)	92.90	(90.63-95.17)	NA	
T7	Belgium	0.51	(0.24-0.78)	97.91	(97.38-98.45)	0.73	(0.41-1.05)
	Brussels	2.53	(0.53-4.53)	94.51	(91.62-97.41)	0.42	(0.00-1.25)
	Flanders	0.11	(0.00-0.26)	99.23	(98.88-99.63)	0.93	(0.49-1.37)
	Wallonia	0.90	(0.18-1.61)	95.52	(93.96-97.09)	0.30	(0.00-0.71)
T8	Belgium	0.20	(0.02-0.38)	98.72	(98.28-99.16)	84.78	(83.37-86.18)
	Brussels	0.00	(0.00-0.00)	98.64	(97.12-100.00)	72.07	(66.17-77.97)
	Flanders	0.06	(0.00-0.18)	99.46	(99.12-99.81)	89.74	(88.30-91.18)
	Wallonia	0.67	(0.02-1.33)	96.64	(95.20-98.09)	75.42	(71.97-78.87)

[1] See Table S3 for the number of primary healthcare providers with valid rapid serological test results; [2] Received one out of two doses; [3] Received two doses; [4] Received a third dose. T1: 24/12/2021-8/1/2021, T2: 25-31/1/2021, T3: 22-28/2/2021, T4: 22-31/3/2021, T5: 19-28/4/2021, T6: 14-27/6/2021, T7, 13-26/9/2021, T8: 13-26/12/2021.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1&2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5&6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6&7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6&7
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6&7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6&7
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9&10
		(b) Indicate number of participants with missing data for each variable of interest	9&suppl
		(c) Summarise follow-up time (eg, average and total amount)	9
Outcome data	15*	Report numbers of outcome events or summary measures over time	

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	10
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	3 & 11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11&12
Generalisability	21	Discuss the generalisability (external validity) of the study results	11&12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.