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SARS-CoV-2 Seroprevalence Following a Large-Scale Vaccination Campaign in Portugal: Results of the National Serological Survey, September - November 2021

Seroprevalência de SARS-CoV-2 em Portugal após a Campanha de Vacinação em Larga Escala: Resultados do Inquérito Serológico Nacional, Setembro - Novembro 2021

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ABSTRACT

Introduction: Following a COVID-19 mass vaccination campaign, it is important to evaluate the population level of SARS-CoV-2 antibodies. The aim of this study was to estimate the seroprevalence rate of SARS-CoV-2 specific antibodies acquired due to infection or vaccination in the Portuguese population.

Material and Methods: The National Serological Survey (third wave – ISN3COVID-19) is a cross-sectional nationwide epidemiological study developed on a sample of 4545 Portuguese residents aged one year or older, between the 28th September 2021 and the 19th November 2021. The SARS-CoV-2 anti-nucleoprotein and anti-spike IgG antibody levels were determined in serum samples using Abbott Chemiluminescent Microparticle Immunoassays. Seroprevalence estimates were stratified by age group, sex, administrative region and self-reported chronic conditions. Medians and respective 95% confidence intervals were used to describe the distribution of SARS-CoV-2 specific antibodies in specific population subgroups.

Results: The total seroprevalence rate of SARS-CoV-2 was 86.4% (95% CI: 85.2% to 87.6%). A higher seroprevalence rate was estimated for women (88.3%), 50 to 59 years-old (96.5%) and in those with two or more self-reported chronic conditions (90.8%). A higher IgG (anti-Spike) concentration was observed in individuals vaccinated with the booster dose (median = 1 2601.3 AU/mL; 95% CI: 4127.5 to 19 089.1).

Conclusion: There was a significant increase in SARS-CoV-2 seroprevalence following the mass vaccination campaign in Portugal. It is important to continue to monitor the distribution of specific SARS-COV-2 antibody at the population level to further inform public health policies.

Keywords: COVID-19; COVID-19 Vaccines; Portugal; SARS-COV-2; Seroepidemiologic Studies

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RESUMO

Introdução: Após uma campanha de vacinação em larga escala contra a COVID-19 é importante avaliar o nível de anticorpos anti-SARS-CoV-2 na população. O objetivo deste estudo foi estimar a taxa de seroprevalência dos anticorpos específicos contra SARS-CoV-2 adquiridos após infeção ou vacinação na população portuguesa.

Material e Métodos: O Inquérito Serológico Nacional COVID-19 (terceira fase ISN3COVID-19) é um estudo epidemiológico transversal de âmbito nacional que foi desenvolvido numa amostra de 4545 residentes em Portugal com idade igual ou superior a um ano recrutados entre 28 de setembro e 19 de novembro de 2021. Procedeu-se à deteção de anticorpos IgG contra a proteína da nucleocápside e contra a subunidade 1 da proteína da espícula (anti-S) em amostras de soro usando os ensaios quimioluminiscentes de micropartículas (Abbott). As estimativas de seroprevalência foram estratificadas por grupo etário, sexo, região e presença de doenças crónicas. As medianas e os respetivos intervalos de confiança de 95% foram usados para descrever a distribuição de anticorpos específicos contra SARS-CoV-2 em subgrupos populacionais.

Resultados: A taxa de seroprevalência total de SARS-CoV-2 foi de 86,4% (IC 95%: 85,2% a 87,6%). Uma maior taxa de seroprevalência foi estimada para mulheres (88,3%) entre os 50 e os 59 anos (96,5%) e naquelas que reportaram ter duas ou mais doenças crónicas (90,8%). Uma concentração de IgG (anti-S) mais elevada foi observada em indivíduos vacinados com a dose de reforço (mediana = 12 601,3 UA/mL; IC 95%: 4127,5 a 19 089,1).

Conclusão: Verificou-se um aumento significativo na seroprevalência de SARS-CoV-2 após a campanha de vacinação em Portugal. É importante continuar a monitorizar a distribuição de anticorpos específicos contra SARS-COV-2 ao nível da população para informar futuras políticas de saúde pública.

Palavras-chave: COVID-19; Estudo Seroepidemiológico; Portugal; SARS-COV-2; Vacinas Contra a COVID-19

INTRODUCTION

Since the beginning of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic, Portugal experienced several waves, accounting for 1 117 451 confirmed SARS-CoV-2 infections and 18 300 deaths reported by the National Surveillance System (SINAVE) up to 19 November 2021.[1] The COVID-19 vaccination campaign started in late December 2020, prioritizing those with a higher risk of exposure or complications due to age or medical conditions.[2] In subsequent months, vaccination was extended to the general population and rolled out by age criteria, starting with those aged 80 years or more in February 2021 and reaching the 12 to 15 years-old group in August 2021. Four vaccine brands have been administrated in Portugal: Comirnaty® (Pfizer-BioNTech), Spikevax® (Moderna), Vaxzevria® (Astrazeneca) and JCovden®, previously called COVID-19 vaccine Janssen (Johnson & Johnson) vaccines.[3] By week 46/2021, with 17 216 507 doses administered,[4] Portugal reached the highest primary vaccination coverage among European Union countries.[4] Since September 2021 a booster dose with mRNA vaccines was administered, primarily for high-risk groups and later for those aged 50 and over, which was extended to all the adult population in December 2021. Since the 15th May, 2022 a second booster dose has been administered to all individuals aged 80 years and older and those that are institutionalized.

The systematic monitoring of the distribution of SARS-CoV-2 specific antibodies in the population is an essential tool to understand pandemic dynamics, to evaluate the burden of SARS-CoV-2 infection, to identify susceptible population subgroups and to guide public health decisions to contain the spread of the pandemic. In vaccinated populations, serological surveys remain useful to evaluate the implementation of the vaccination campaigns and monitor population-level immune responses over time.[5] Seroprevalence studies are also indicative of the levels of population protection and are widely used to establish imputed parameters for mathematical modelling of scenarios,[6,7] planning national public health and pandemic response policies.

Seroprevalence rates varied considerably by time and country.[8,9] A recent systematic review covering 97 of 194 World Health Organisation (WHO) Member States, in all six WHO regions, published in July 2021 estimated a worldwide seroprevalence of 45.2%, [95% Confidence Interval (CI); 40.7% to 49.8%].[9] A higher seroprevalence rate was reported for the European region, 72% (95% CI: 55% to 84%), corresponding to a 16-fold increase since the first wave of COVID-19 pandemic.[9]

Based on the WHO guidelines,[10] Portugal has implemented repeated cross-sectional serological surveys aiming at monitoring the seroprevalence of SARS-CoV-2 antibodies in the population during the course of the pandemic. The first survey (ISN COVID-19) showed that, by July 2020, 2.9% (95% CI: 2.0% to 4.2 %) of the population[11] had SARS-CoV-2 specific antibodies. The second survey (ISN-2COVID-19), conducted during the early stages of the vaccination campaign (March 2021), estimated the seroprevalence of SARS-CoV-2 specific antibodies in the Portuguese population to have increased to 15.5% (95% CI: 14.6% to 16.5%).[12]

Following a mass vaccination campaign, this study aimed at estimating the seroprevalence rate of SARS-CoV-2 specific antibodies acquired due to infection or vaccination in the Portuguese population between September and November 2021, and also at characterizing the distribution of the SARS-CoV-2 IgG (anti-Spike) antibodies levels in the population by vaccination status, known previous infection and time since vaccination.

MATERIAL AND METHODS

Study design and settings

The third cross-sectional serological survey (ISN3COVID-19) was conducted between the 28th September 2021 and the 19th November 2021 and targeted Portuguese residents aged one year old or above. Survey participants (n = 4545) were recruited using a two-stage non-probability quota sampling design, described elsewhere.[11,12] The survey included 38 private Clinical Pathology Laboratories and

36 public hospitals and primary health care units of the Portuguese National Health System, comprising a total of 305 data collection sites with nationwide geographical distribution (Fig. 1). Individuals seeking routine blood tests for reasons unrelated to COVID-19 in the above-mentioned sites were invited to participate.

Data collection and processing

All participants filled in a short questionnaire on sociodemographic information, chronic conditions (diabetes, cardiovascular disease, hypertension, hematologic disease, chronic pulmonary disease excluding asthma, autoimmune diseases, obesity, chronic hepatic disease, immunodeficiency or other), previous SARS-CoV-2 exposure, COVID-19 symptoms, and COVID-19 vaccination history (date and brand of each vaccine dose).[13]

Participants were classified as symptomatic, pauci-symptomatic or asymptomatic, based on reported COVID-19 related symptoms using the definition proposed by Pollan *et al*.[14] Individuals were considered vaccinated if they reported to have received at least one dose of any COVID-19 vaccine. In addition, vaccination exposure was classified as partial (one dose of two-dose scheme vaccines), complete vaccination (single dose of JCovden®, previously called COVID-19 vaccine Janssen or two doses of Comirnaty®, Spikevax® or Vaxzevria®) and booster vaccination (uptake of booster dose of Comirnaty® or Spikevax® following primary vaccination).

For all participants, qualitative detection of immunoglobulins (Ig) of type G against the Nucleocapside protein (IgG anti-N; SARS-CoV-2 IgG I, Abbott Diagnostics, IL, USA) and quantitative determination of IgG against the Spike protein (IgG anti-S; SARS-CoV-2 IgG II, Abbott Diagnostics, IL, USA) were performed using Chemiluminescent Microparticle Immunoassays (CMIA) on the ARCHITECT i2000S.[15,16] Based on manufacturer recommendations, a positivity cut-off of 50 AU/mL was used for the quantitative IgG anti-S test, for qualitative IgG anti-N test cut-off of 1.40 Index (S/C) was used. IgG determinations in serum samples were performed by a single laboratory.

Outcomes definitions

Once in contact with SARS-CoV-2, the immune system generates detectable anti-SARS-CoV-2 antibodies. Anti-S antibodies prevent the virus from entering the cell, while anti-N antibodies prevent the proliferation of the virus if it penetrates the cell.

mRNA and viral vector vaccine induce immune response against Spike protein, whereas an infection induces production of both anti-N and anti-S SARS-CoV-2 antibodies. Therefore, detectable anti-N antibody levels imply that the individual was previously infected.

We estimated post-infection and total seroprevalence that combines seropositivity due to infection and vaccination. Any individual who had specific IgG antibodies against SARS-CoV-2 (anti-S and/or anti-N) was considered seropositive for SARS-CoV-2. Any individual who reported not being vaccinated and had specific IgG antibodies against SARS-CoV-2 (anti-S and/or anti-N) and any individual who reported being vaccinated or with unknown vaccination status with positive IgG (anti-N) was considered seropositive for SARS-CoV-2 due to infection.

Statistical analysis

SARS-CoV-2 seroprevalence (total and post-infection) was estimated for the overall sample and stratified by sex, age group (1 – 9, 10 – 19, 20 – 29, 30 – 39, 40 – 49, 50 – 59, 60 – 69, ≥ 70 years old), region (North, Center, Lisbon and Tagus Valley, Alentejo, Algarve, Madeira, Azores), education level (without formal education/elementary school, middle school, secondary school, higher education), any contact with suspected or confirmed case of COVID-19, presence of symptoms compatible with COVID-19 (symptomatic, pauci-symptomatic, asymptomatic), self-reported previous SARS-CoV-2 infection, presence of self-reported chronic conditions (0 – 1, ≥ 2 conditions) and vaccination status. For all point estimates, the respective 95% CI were calculated using the logit method. Seroprevalence estimates were compared among the above-mentioned population subgroups using the design adjusted Rao-Scott chi-square test.[17]

The distributions of SARS-CoV-2 IgG (anti-S) antibody levels by vaccination status and previous infection were described in terms of medians with respective 95% CI determined based on the method proposed by Woodruff.[18] Taking into account the roll-out of the national vaccination campaign (from older to younger age groups and with specific vaccine brands for each age group and sex), we considered several subsamples of the study population. Firstly, we restricted our analysis to those aged 20 to 69 years old with complete vaccination scheme and without previous infection, in order to evaluate the effect of vaccine brand on IgG (anti-S) antibody levels, adjusting for time since vaccination, sex and age group.

Secondly, the distribution of IgG (anti-S) antibodies by time since vaccination was analyzed in a subsample of those aged 20 to 69 years old (to exclude individuals with a booster dose) without known previous infection and fully vaccinated with Comirnaty®. This specific brand was the most administrated in Portugal in our analysis period and, hence, was selected in order to exclude the effect of vaccination brand on the analysis, while maximizing statistical power.

Finally, the effect of age on IgG (anti-S) antibody levels was assessed on a subsample of adults aged 20 or more years old without known previous infection and fully

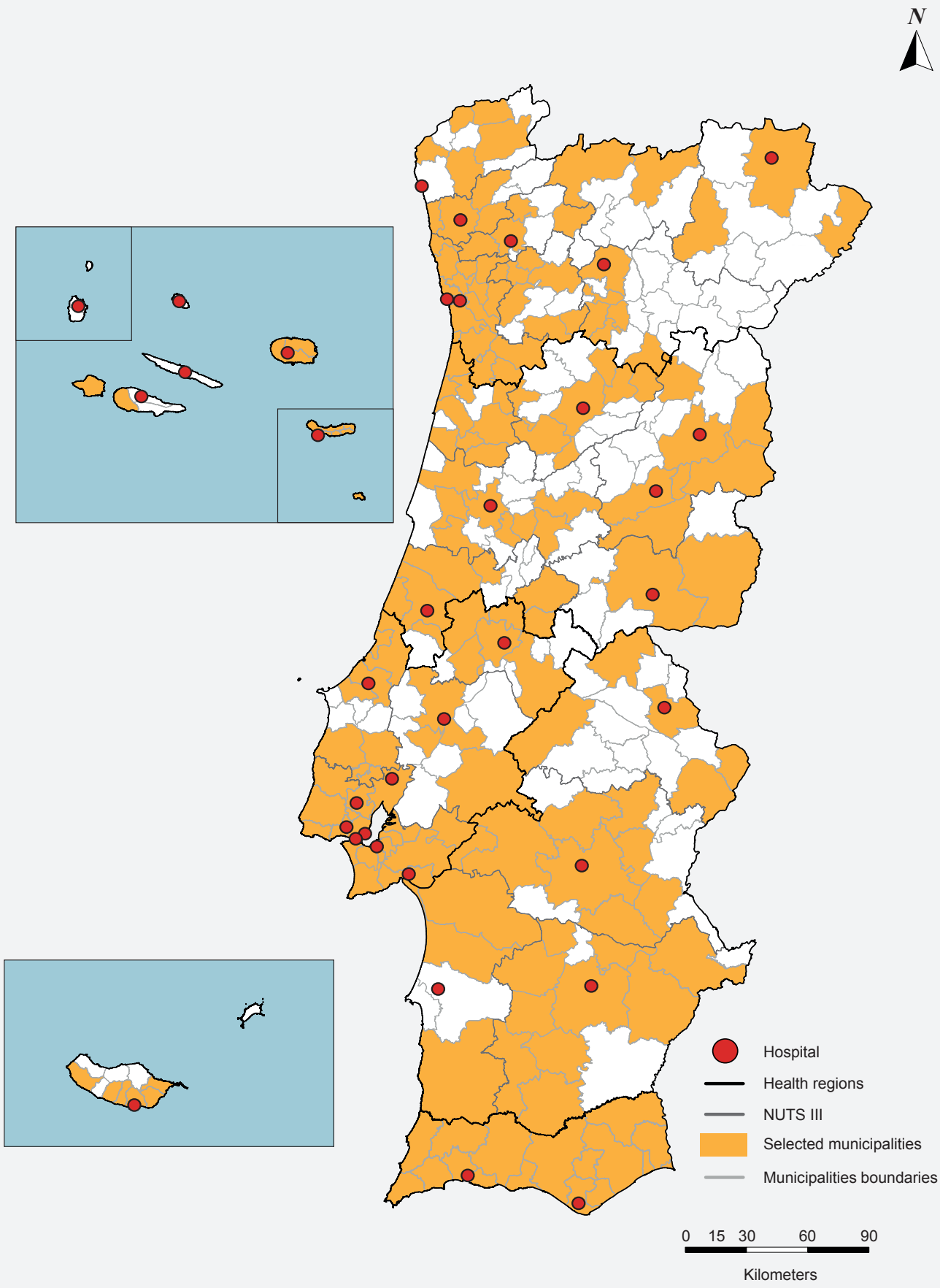

Figure 1 – Distribution of data collection sites ISN3COVID-19

vaccinated with Comirnaty® in July - August 2021. Quantile regression was used to estimate confounder-adjusted difference in medians (MD) between population subgroups with respective 95% CI.

All estimates were weighted for the distribution of the Portuguese resident population by post-stratification by administrative region, sex and age group. Statistical analysis was performed using Stata software, version 15.1 for Windows®.[19] The level of significance was set at 5%.

Ethical procedures

The study protocol had received clearance from the Ethics Committee and the Data Protection Officer of the Portuguese National Institute of Health (INSA). All participants or their legal representatives signed an informed consent form to allow their leftover serum sample, after routine clinical testing, to be used in the study for SARS-CoV-2 antibodies determination and answered a short questionnaire. All data were pseudoanonymized for the study and the participants have the right to receive their serological results according to their will.

RESULTS

Seroprevalence rates

Between September 28th 2021 and November 19th 2021 we collected data and serum samples on 4 545 individuals. The distribution of ISN3COVID-19 participants by sex, age group, region, history of COVID-19 exposure is shown in Table 1.

For the general population, we estimated a total seroprevalence rate of 86.4% (95% CI: 85.2% to 87.6%) and post-infection seroprevalence of 7.5% (95% CI: 6.6% to 8.5%) (Table 2). Total seroprevalence estimates varied significantly by sex, age group, administrative region, education, self-reported chronic condition and previous history of COVID-19 exposure (contact and previous infection). A higher total prevalence rate was observed among women (88.3%; 95% CI: 86.7% to 89.6%), 50 to 59 years-old (96.5%; 95% CI: 94.1% to 98.0%), residents of the North region (88.4%; 95% CI: 86.3% to 90.2%) and among those with two or more chronic conditions (90.8%; 95% CI: 88.2% to 92.9%). Among participants aged 20 or more years old, we estimated a total seroprevalence rate of 70.1% (95% CI: 67.2% to 72.8%) for those with low levels of education, in contrast with the higher education group where the seroprevalence rate was 96.1% (95% CI: 94.3% to 97.3%).

The post-infection seroprevalence rate was higher amongst individuals aged between one and nine years old (17.9%; 95% CI: 14.0% to 22.6%), residents in the North (8.8%; 95% CI: 7.1% to 10.9%) and Algarve regions (8.8%; 95% CI: 6.7% to 11.4%), those without formal education or who completed elementary school (10.3%; 95% CI: 8.6% to 12.4%), as well as among those reporting previous contact

Table 1 – Distribution of ISN3COVID-19 participants by sex, age group, region, history of COVID-19 exposure (population one year of age or older, between 28th September and 19th November 2021 Portugal)

Participants characteristics	n	%
Sex		
Male	2047	47.1
Female	2498	52.9
Age group (years)		
1 – 9	486	7.8
10 – 19	591	10.2
20 – 29	546	10.9
30 – 39	549	11.9
40 – 49	579	15.3
50 – 59	568	14.6
60 – 69	563	12.9
≥ 70	663	16.5
Region of residence		
North	884	34.7
Center	671	16.1
Lisbon and Tagus Valley	815	35.7
Alentejo	585	4.5
Algarve	580	4.2
Madeira	431	2.5
Azores	579	2.4
Education level*		
Without formal education/ Elementary school	828	24.4
Middle school	740	21.6
Secondary school	898	24.7
Higher education	971	29.3
Self-reported chronic condition		
0 – 1	3415	80.5
≥ 2	773	19.5
Vaccination		
Unvaccinated	875	14.6
Vaccinated with at least 1 dose	3642	85.4
Self-reported previous SARS-CoV-2 infection		
No	3864	87.1
Yes	466	12.9
Self-reported symptoms§		
Asymptomatic	1979	46.2
Pauci-symptomatic	1312	28.7
Symptomatic	1116	25.1
Previous contact with COVID-19 case		
No	3089	64.8
Yes	1157	27.2
Unknown	299	8.0

*: only for participants aged 20 years old or above; §: definition proposed by Pöllan *et al*

Table 2 – Seroprevalence rate of specific IgG SARS-CoV-2 antibodies (population one year of age or older, between 28th September and 19th November 2021 Portugal)

	Post-infection % (95% CI)†	Total % (95% CI)†
Overall (n = 4545)	7.5 (6.6 to 8.5)	86.4 (85.2 to 87.6)
Sex (n = 4545)	***p* = 0.900**	***p* = 0.001**
Male	7.5 (6.2 to 9.1)	84.4 (82.4 to 86.2)
Female	7.4 (6.2 to 8.8)	88.3 (86.7 to 89.6)
Age group (years) (n = 4545)	***p* < 0.001**	***p* < 0.001**
1 – 9	17.9 (14.0 to 22.6)	17.9 (14.0 to 22.6)
10 – 19	9.6 (7.0 to 13.1)	76.8 (72.4 to 80.8)
20 – 29	7.1 (4.8 to 10.4)	92.2 (88.8 to 94.6)
30 – 39	7.6 (5.1 to 11)	94.6 (91.9 to 96.4)
40 – 49	6.9 (4.6 to 10.2)	94.3 (91.2 to 96.4)
50 – 59	5.0 (3.2 to 7.7)	96.5 (94.1 to 98.0)
60 – 69	6.1 (4.0 to 9.1)	93.8 (90.9 to 95.8)
≥ 70	5.2 (3.5 to 7.7)	93.0 (90.2 to 95.0)
Region of residence (n = 4545)	***p* = 0.020**	***p* = 0.024**
North	8.8 (7.1 to 10.9)	88.4 (86.3 to 90.2)
Center	6.2 (4.6 to 8.3)	85.8 (83.1 to 88.1)
Lisbon and Tagus Valley	7.4 (5.8 to 9.4)	85.8 (83.3 to 88.0)
Alentejo	4.8 (3.3 to 6.8)	85.8 (82.8 to 88.4)
Algarve	8.8 (6.7 to 11.4)	80.2 (76.9 to 83.2)
Madeira	2.8 (1.5 to 5.1)	86.3 (79.1 to 91.2)
Azores	4.4 (3.0 to 6.5)	84.0 (81.0 to 86.5)
Self-reported chronic conditions (n = 4188)	***p* = 0.498**	***p* < 0.001**
0 – 1	7.7 (6.7 to 9.0)	85.5 (84.1 to 86.8)
≥ 2	6.8 (4.9 to 9.4)	90.8 (88.2 to 92.9)
Education level (n = 3437)*	***p* = 0.745**	***p* < 0.001**
Without formal education/ Elementary school	10.3 (8.6 to 12.4)	70.1 (67.2 to 72.8)
Middle school	6.5 (4.9 to 8.7)	91.4 (89.1 to 93.2)
Secondary school	6.3 (4.6 to 8.6)	93.2 (90.7 to 95.0)
Higher education	6.1 (4.4 to 8.4)	96.1 (94.3 to 97.3)
Previous contact with COVID-19 case (n = 4545)	***p* < 0.001**	***p* < 0.001**
Unknown	4.4 (2.2 to 8.4)	94.5 (91.0 to 96.7)
No	3.9 (3.1 to 4.9)	86.0 (84.5 to 87.4)
Yes	16.7 (14.3 to 19.5)	85.4 (82.9 to 87.6)
Self-reported previous SARS-CoV-2 infection (n = 4330)	***p* < 0.001**	***p* < 0.001**
No	3.2 (2.6 to 3.9)	84.9 (83.5 to 86.2)
Yes	38.9 (33.8 to 44.3)	98.3 (96.6 to 99.2)
Self-reported symptoms (n = 4407)§	***p* < 0.001**	***p* < 0.001**
Asymptomatic	5.8 (4.6 to 7.3)	88.9 (87.1 to 90.4)
Pauci-symptomatic	6.4 (5.0 to 8.3)	84.7 (82.3 to 86.9)
Symptomatic	12.0 (9.8 to 14.6)	84.1 (81.4 to 86.4)
Vaccination status (n = 4517)	***p* < 0.001**	***p* < 0.001**
Unvaccinated	24.7 (21.1 to 28.7)	24.7 (21.1 to 28.7)
Vaccinated with at least one dose	4.5 (3.7 to 5.5)	97.0 (96.2 to 97.6)

†: weighted proportion; *: only for participants aged 20 years old or above; §: definition proposed by Pöllan *et al*

with a COVID-19 case, previous SARS-CoV-2 infection or COVID-19 related symptoms (Table 2). Among individuals vaccinated with at least one dose of a COVID-19 vaccine, the proportion of seropositivity was 97.0% (95% CI: 96.2% to 97.6%), and post-infection seroprevalence was 4.5% (95% CI: 3.7% to 5.5%). No statistically significant differences in the infection-induced seroprevalence estimates were observed by sex and self-report of chronic conditions.

SARS-CoV-2 IgG anti-Spike antibody distribution

Table 3 shows the medians of SARS-CoV-2 IgG (anti-S) antibody concentrations by previous infection and vaccination status among individuals eligible for vaccine uptake. The highest levels (median) of IgG anti-S were observed among those with a booster dose but without previous infection (median = 12 601.3 AU/mL; 95% CI: 4127.5 to 19 089.1).

Restricting the analysis to individuals aged 20 to 69 years old with a complete vaccination scheme and without previous infection or booster dose, we compared medians of IgG anti-S antibody concentrations by vaccine brand, adjusting for time since vaccination, sex and age group (Table 4). The median of IgG anti-S varied from 237.5 AU/ml (95% CI: 177.5 to 297.4) for those who reported complete primary vaccination with JCovden®, previously called COVID-19 vaccine Janssen, to 7012.7 AU/mL (95% CI: 5568.8 to 8456.6) for those with primary vaccination with Spikevax®. Considering complete vaccination with Comirnaty® as a reference group, we estimated statistically significant lower IgG anti-S antibody concentrations for both viral vector vaccines (Vaxzevria® and JCovden®, previously called COVID-19 vaccine Janssen®) and higher for the Spikevax® (MD = 4300.8 AU/mL; 95% CI: 3290.9 to 5310.8).

Regarding the time since vaccination and restricting the analysis to those fully vaccinated with Comirnaty® and without previously known infection or booster dose, we observed statistically significant confounder-adjusted differences in medians, indicating higher IgG concentrations among those vaccinated closer to the date of data collection, compared to those vaccinated between January and February 2021 considered as the reference (Table 4).

To assess the effect of age, we additionally restricted analysis to those aged 20 or more years old without recorded infection and vaccinated with Comirnaty® (complete vaccination scheme). Considering 20 – 29 years old as a reference, we estimated statistically significant lower median of IgG (anti-S) antibodies in the older age groups, with confounder-adjusted differences in medians ranging between -1624.3 AU/mL (95% CI: -2845.3 to -403.4) and -3710.9 AU/mL (95% CI: -4795.6 to -2626.2) for 30 – 39 years old and ≥ 70years-old, respectively (Table 4).

Table 3 – Medians of SARS-CoV-2 IgG anti-S antibodies by vaccination and previous infection status

Characteristics	n	Median (AU/mL) (95% CI)
Unvaccinated, previous infection	192	521.3 (340.8 to 720.8)
Partial, without infection	39	554.9 (0 to 1153.3)
Complete, without infection	3198	1547.2 (1429.4 to 1665.0)
Booster dose, without infection	44	12 601.3 (4127.5 to 19 089.1)
Vaccinated after infection	270	8013.6 (6411.8 to 9326.7)

DISCUSSION

In this nationwide survey of a sample of the Portuguese population aged one year old or above conducted between September and November 2021, following a mass vaccination campaign, we estimated the seroprevalence rate of SARS-CoV-2 antibodies and characterized the population-level distribution of IgG anti-S antibodies.

The estimated total seroprevalence rate (post-infection or vaccination) was 86.4% (95% CI: 85.2% to 87.6%), which is consistent with the vaccine coverage achieved in Portugal during that period.[4] Our results show that the seroprevalence rate varied by age group and administrative region, with lower rates in the Algarve region (80.2%; 95% CI: 76.9% to 83.2%) and in the pediatric population 1 – 9 years (17.9%; 95% CI: 14.0% to 22.6%). Regional differences may be related to the lower vaccine coverage achieved in the Algarve when compared with the other regions, whilst in children there was no vaccine recommendation for those aged below 12 years old at the time of the survey.[2] As expected, the highest seroprevalence rate was observed among individuals aged between 50 – 59 years old (96.5%; 95% CI: 94.1% to 98.0 %), since this age group also had the highest accumulated incidence of SARS-CoV-2 infection, combined with high vaccination coverage.[4] The gender pattern in seroprevalence observed in our study is similar to that previously described in blood donors of the USA population[20] and may be related to higher vaccine coverage among women, as reported in the literature for COVID-19.[21] These results are in line with a study on COVID-19 vaccination adherence conducted in Portugal, which found lower odds of vaccine hesitancy for women, which may, reflect higher engagement of women in preventive behaviors.[22]

A higher seroprevalence rate was also found in those with high education (96.0%; 95% CI: 94.3% to 97.3%), which was also the group with the lowest post-infection seroprevalence (6.1%; 95% CI: 4.4% to 8.4%) rate. A similar result was observed in a population-based serological survey in Switzerland.[23] Considering the differences in the risk of infection according to the socio-economic level de-

Table 4 – Median of SARS-CoV-2 anti-S IgG titers by age group, vaccination brand and time since vaccination

	n	Median AU/mL (95% CI)†	Confounder-adjusted difference in medians (95% CI)
Vaccine brand (n = 2195)§			
Comirnaty®	1385	2126.1 (1898.8 to 2353.3)	ref.
JCovden®	259	237.5 (177.5 to 297.4)	-2045.1 (-2303.5 to -1786.5)
Spikevax®	196	7012.7 (5568.8 to 8456.6)	4300.8 (3290.9 to 5310.8)
Vaxzevria®	355	515.3 (414.9 to 615.7)	-1180.3 (-1417.6 to -943.1)
Month of 2nd dose uptake (n = 1382)§§			
Jan – Feb 2021	108	574.4 (402.3 to 746.4)	
Mar – Apr 2021	65	1212.8 (656.4 to 1769.1)	784.5 (433.9 to 1134.9)
May – Jun 2021	446	1284.9 (1125.6 to 1444.2)	976.6 (685.8 to 1267.5)
Jul – Aug 2021	648	2942.3 (2566.8 to 3317.8)	2509.3 (2130.4 to 2888.2)
Sep – Out 2021	115	8465.8 (6691.9 to 10 239.7)	6654.4 (5222.9 to 8085.8)
Age group (n = 678)§§§			
20 – 29	90	6075.1 (4188.5 to 7961.7)	ref.
30 – 39	153	3976.6 (3008.1 to 4945.1)	-1624.3 (-2845.3 to -403.4)
40 – 49	228	2306.2 (1800.4 to 2812.0)	-2684.7 (-3780.5 to -1588.9)
50 – 59	144	2483.7 (2006.6 to 2960.8)	-2409.6 (-3551.4 to -1267.8)
60 – 69	33	1747.5 (428.8 to 3066.2)	-3581.6 (-4693.2 to -2470.0)
≥ 70	30	1618.2 (817.7 to 2418.7)	-3710.9 (-4795.6 to -2626.2)

†: weighted estimates; §: estimated by quantile regression adjusted for age group, sex, time since vaccination on a sample of individuals aged 20 - 69 years old without previous infection with complete vaccination scheme; §§: estimated by quantile regression adjusted for age group, sex on a sample of individuals aged 20 - 69 years old without previous infection with complete vaccination scheme with Comirnaty® in 2021; §§§: estimated by quantile regression adjusted for sex and month of vaccination on a sample of individuals aged 20 or more years old without previous infection fully vaccinated with Comirnaty® in July - August 2021

scribed in previous research,[24,25] the seroprevalence patterns observed according to educational level possibly indicate a greater adherence to public health measures in subgroups-with a higher level of education. Our results may also reflect lower adherence to vaccination by those with lower health literacy which is correlated with lower levels of education. In a Portuguese study, higher odds of vaccine hesitancy were found within individuals with no education/basic education and secondary education, when compared to those with a university degree, hence corroborating this hypothesis.[22] In addition, during the COVID-19 pandemic, Portugal experienced a significant shift to digital technology in the health sector. A web-based appointment system for vaccination was implemented and the European Union (EU) digital vaccination certificate was introduced. Although vaccines were offered free of charge, digital barriers associated with socioeconomic status may have led to lower adherence to vaccination among those with a lower education level.

At national level, the estimated post-infection seroprevalence rate in this survey (7.5%; 95% CI: 6.6% to 8.5%) was below the estimate from the second survey (ISN2COVID-19) carried out in February - March 2021 (13.5%; 95% CI: 12.6% to 14.4%),[12] while the cumulative percentage of COVID-19 confirmed cases in Portugal increased from 7.9% to 10.9%, between the two surveys.[26] This result may be explained by the shorter half-life of IgG anti-NP antibodies[27,28] that, at the time of the present survey, was already undetectable for infections that occurred in the early stages of the pandemic. In fact, in the present survey, just over a third (38.9%; 95% CI: 33.8% to 44.3%) of participants who reported having had a previous SARS-CoV-2

infection had serological signs of infection. Higher post-infection seroprevalence in the Algarve and Azores, which experienced an intense wave of COVID-19 during the summer of 2021,[26] and lower seropositivity among those who reported infection more than 90 days prior to the sample collection observed in the second survey[12] also corroborate this hypothesis.

Like in previous studies, we observed a more robust IgG antibody response in individuals vaccinated with a booster dose, and in those who reported having been vaccinated with an mRNA vaccine.[29,30] This suggests that the amount of antibodies could be correlated with vaccine protection as mRNA vaccines were also the ones that resulted in higher effectiveness estimates against infection, hospitalization and death.[31-34]

Considering the immune response to specific vaccine brands, the levels of IgG anti-S antibodies were higher among those vaccinated with Spikevax® compared to those vaccinated with Comirnaty®. This result may be explained by the product characteristics (mRNA concentration of 30µg for Comirnaty®, compared to 100 µg for Spikevax®).

Higher IgG anti-S antibody levels were observed in people vaccinated more recently. These results are consistent with previous research on IgG antibodies kinetics following vaccination[35] and corroborate a decrease in IgG antibodies over time.

Our study has several limitations. First, we should mention the non-random sampling scheme used to recruit participants and that the recruitment strategy had the lower odds of including institutionalized individuals which may have led to selection bias. Individuals of low socio-economic status may also have lower accessibility to health care and therefore may be less likely to perform blood tests and consequently to participate in this study. Another limitation of this study is the possible memory bias, given that all the collected data is self-reported by the participants. Third, due to quick waning of the IgG anti-NP antibodies below the detectable levels we were not able to provide a reliable estimate of the cumulative number of SARS-CoV-2 infections in Portugal. Due to collinearity between age, time since vaccination and vaccine brand used associated with the vaccination campaign roll-out, we were not able to estimate differences in IgG anti-S median concentrations by including all variables in the model simultaneously. To overcome this limitation, we restricted the analysis to those without previous infection and with complete vaccination scheme or to a single brand, which reduced the sample size and the study power, leading to gains in internal validity.

CONCLUSION

The data from the third national COVID-19 serological survey showed a significant increase in the total SARS-CoV-2 seroprevalence rate following the mass vaccination campaign. Besides the universal and free of charge vaccination, some population subgroups (lower level of education, Algarve region, male individuals) had lower seroprevalence. Higher IgG anti-S titers in individuals vaccinated with the booster dose of the vaccine support the implementation of this measure in the Portuguese population. Continuous monitoring of the population-level IgG response after vaccination remains important to guide further public health measures (recommendation for vaccination, duration of certificates among others).

AUTHOR CONTRIBUTIONS

IK collaborated in conceptualization of the study, performed statistical analysis of the data, and wrote the first draft of the manuscript.

PG, SR, MB, ART, VG, VG, RS, RG collaborated in the conceptualization and development of the study, interpretation of results and critically reviewed the manuscript.

CM, JAS, SS, AM, CH performed laboratory testing, collaborated in the interpretation of results and critically reviewed the manuscript.

APR coordinated the study, interpreted results, and critically reviewed the manuscript. ISNCOVID-19 group members participated in the data collection, laboratory testing and provided critical comments on the manuscript.

All authors read and approved the final version of the manuscript.

PROTECTION OF HUMANS AND ANIMALS

The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the Helsinki Declaration of the World Medical Association updated in 2013.

DATA CONFIDENTIALITY

The authors declare having followed the protocols in use at their working center regarding patients' data publication.

COMPETING INTERESTS

PG is a member of the Disease Network Coordinating Committee of the European Legionnaire's Disease Surveillance Network.

All other authors have declared that no competing interests exist.

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