Seroprevalence of anti-SARS-CoV-2 IgG antibodies: relationship with COVID-19 diagnosis, symptoms, smoking, and method of transmission

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A B S T R A C T

Aims: The study of SARS-CoV-2 antibodies in the population is a crucial step towards overcoming the COVID-19 pandemic. Seroepidemiological studies allow an estimation of the number of people who have been exposed to the virus, as well as the number of people who are still susceptible to infection.

Methods: In total, 13 560 people from Arganda del Rey, Madrid (Spain) were assessed between January and March 2021 for the presence of IgG antibodies, using rapid tests and histories of symptoms compatible with COVID-19.

Results: 24.2% of the participants had IgG antibodies and 9% had a positive COVID-19 diagnosis. Loss of smell/taste was the most discriminating symptom of the disease. The main transmitters of infection were found to be household members. Unexpectedly, in smokers, the incidence of positive COVID-19 diagnoses was significantly lower. Additionally, it was found that there was a discrepancy between COVID-19 diagnosis and the presence of IgG antibodies.

Conclusions: Rapid anti-IgG tests are less reliable in detecting SARS-CoV-2 infection at an individual level, but are functional in estimating SARS-CoV-2 infection rates at an epidemiological level. The loss of smell/taste is a potential indicator for establishing COVID-19 infection.

Introduction

Since the beginning of the COVID-19 pandemic, many seroepidemiological studies have been conducted in order to determine the presence of antibodies against SARS-CoV-2 (Strinhgini et al., 2020; Eslami and Jalili, 2020; Mack et al., 2021; Figueiredo-Campos et al., 2020). There are two main premises of these studies. The first is that the presence of antibodies allows us to estimate the number of people who have been exposed to the virus in an objective way, and in spite of the presence of asymptomatic cases. The second is the ability to estimate the number of people who remain susceptible to infection. This is important in determining whether the threshold of herd immunity has been reached. The concept of herd immunity refers to the fact that a large part of the population must have been exposed to the virus naturally (infected) or by vaccination. Herd immunity in the SARS-CoV-2 pandemic has been estimated at 50–66% (Neagu, 2020). This would result in a lower probability of infection between individuals.

An added value of seroepidemiological studies is the self-reporting that can accompany the clinical assessment of IgG antibodies. Thanks to these questionnaires it is possible to determine which symptoms have greater predictive value for the diagnosis of COVID-19. On one hand, serological testing can detect cases that have been asymptomatic (Arabkhazaeli et al., 2021; Shakiba et al., 2020). On the other hand, the patients' self-reported data allow us to associate these symptoms with the test results. For example, one of the symptoms that appears to be most associated with SARS-CoV-2 infection is loss of taste and/or smell (Marcgese-Ragona et al., 2020; Aziz et al., 2021). There is also a debate regarding a link between smoking activity and the likelihood

Table 1
General characteristics of the study participants

	Number of participants	Percentage (%)
Data collection stage		
1st stage	4466	32.9
2nd stage	5136	37.9
3rd stage	3958	29.2
Sex		
Female	6289	46.4
Male	7271	53.6
Age, Years		
0–19	583	4.3
20–34	2216	16.3
35–49	5158	38.0
50–64	3433	25.3
≥ 65	2170	16.0
Nationality		
Spanish	11819	87.2
Other	1741	12.8
Occupation		
Active worker	7460	56.5
Retired	2217	16.8
Unemployed	1378	10.4
Student	987	7.5
House person	518	3.9
Other	633	4.8
Presence of COVID-19 at some point		
Positive COVID-19 diagnosis	1224	9.0
Negative COVID-19 diagnosis	12336	91.0
Vaccine		
Vaccinated participant – 1° Dose	390	2.9
Vaccinated participant – 2° Dose	139	1.02

of a worsening COVID-19 disease course, and/or number of hospital admissions in the smoking population (Vardavas and Nikitara, 2020; Farsalinos et al., 2020; Proppers, 2020; Gupta et al., 2021). Intuitively, one would expect that in the smoking population the number of COVID-19 cases would be higher. In addition, epidemiological studies allow us to explore the main routes of infection. It is to be expected that proximity, cohabitation between individuals, and the duration of cohabitation are the main factors facilitating contagion.

This study relied on the national seroepidemiological study in Spain, which has been a reference for the Spanish population and was published in the journal *The Lancet* in July 2020 (Pollán et al., 2020). Our study adapted most of the methodology used in this national study to a local population in Madrid. To increase the extrapolability/comparability across both studies, the same serological rapid test was employed and most of the items of the self-reported questionnaires applied to participants were replicated.

Materials and methods

Study design and participants

The study design included three successive stages of data collection, with a 3-week break between each one of them – from January 18th to 23rd, from February 15th to 20th, and from March 15th to 18th, 2021. Participants were randomly selected based on the town census for Arganda del Rey. In order to have participants of a wide age range, the selection was made by household. All household residents were invited to participate in the study, resulting in a final sample of 13 560 individuals, representing 24.04% of the entire population of Arganda del Rey (National Institute of Statistics, 2021). The main characteristics of this sample of participants are described in Table 1.

Individuals residing in the selected households were contacted by telephone and informed of the objective and characteristics of the study. Once their willingness to participate had been confirmed, participant information was obtained and an appointment for a rapid immunochro-

matographic test was made at the facilities provided by the City Council of Arganda del Rey.

The telephone contact service was provided by the company CTi Soluciones. The inclusion criteria for the study were: (1) being registered in the town of Arganda del Rey; (2) being older than 1 year of age; (3) having knowledge of the Spanish language.

Written informed consent was obtained from all study participants. Different forms of informed consent were used for adults, teenagers, parents of participating children, and guardians of mentally disabled participants. All data collected for the study were identified by a random code to protect the identity of the participants. The study complied with Organic Law 3/2018 of December 5, 2018, on Personal Data Protection and Guarantee of Digital Rights, and Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on Data Protection (General Data Protection Regulation – GDPR). The study was approved by the Research Ethics Committee of the Community of Madrid (Law 14/2007 on Biomedical Research).

General procedure

Once contacted and invited, the participants attended the town sports center 'Príncipe Felipe', where they filled out the informed consent form and answered a questionnaire that included: history of symptoms compatible with COVID-19 (i.e. fever, chills, fatigue, sore throat, cough, shortness of breath, headache, and loss of taste or smell, among others); contact with suspected or confirmed cases; and other risk factors. The epidemiological questionnaires were applied by professional psychologists contracted through the Complutense University of Madrid. Blood samples were then extracted by finger prick. These extractions were performed by 16 nursing professionals. Once the extraction was completed, the participants left the facility and the samples were sent to the Faculty of Medicine of the Complutense University of Madrid, where they were centrifuged before rapid immunochromatographic testing. Transport of samples to the laboratories was carried out in accordance with current regulations for the transport of category B infectious substances (packing instruction P650 and UN3373). Disposal of biological waste was carried out according to the regulations described in the BOE of March 22, 2020.

Detection of SARS-CoV-2 antibodies

The analysis of the SARS-CoV-2 antibodies was carried out by means of a rapid immunochromatographic test (Orient Gene Biotech COVID-19 IgG/IgM Rapid Test Cassette; Zhejiang Orient Gene Biotech, Zhejiang, China), a lateral-flow immunochromatographic assay for qualitative differentiation between IgG and IgM against the receptor-binding domain of SARS-CoV-2 spike (S) protein, which yields results in 10 minutes. The manufacturer reported sensitivity of 97.2% and specificity of 100% for IgG, using RT-PCR as the gold standard. These antibody tests have been shown in previous quality studies to indeed show high sensitivity and specificity (Hanssen et al., 2021), and have been used in several seroprevalence studies (Akpabio et al., 2021; Álvarez-Antonio et al., 2021). Blood samples were extracted by finger prick and centrifuged in order to detect SARS-CoV-2 antibodies in serum samples. Due to the lower sensitivity and specificity of IgM, its shorter duration, and the heterogeneity of results observed in initial IgM readings, results for the point-of-care test reported here are based only on IgG.

Statistical analysis

In total, 13 560 individuals from Arganda del Rey, Madrid, Spain were assessed for the presence of IgG antibodies. All the questionnaires were checked manually and exported to the SPSS version 23 software package (SPSS Inc., Chicago, IL, USA) for analysis. After performing descriptive analysis, the direction and strength of statistical associations
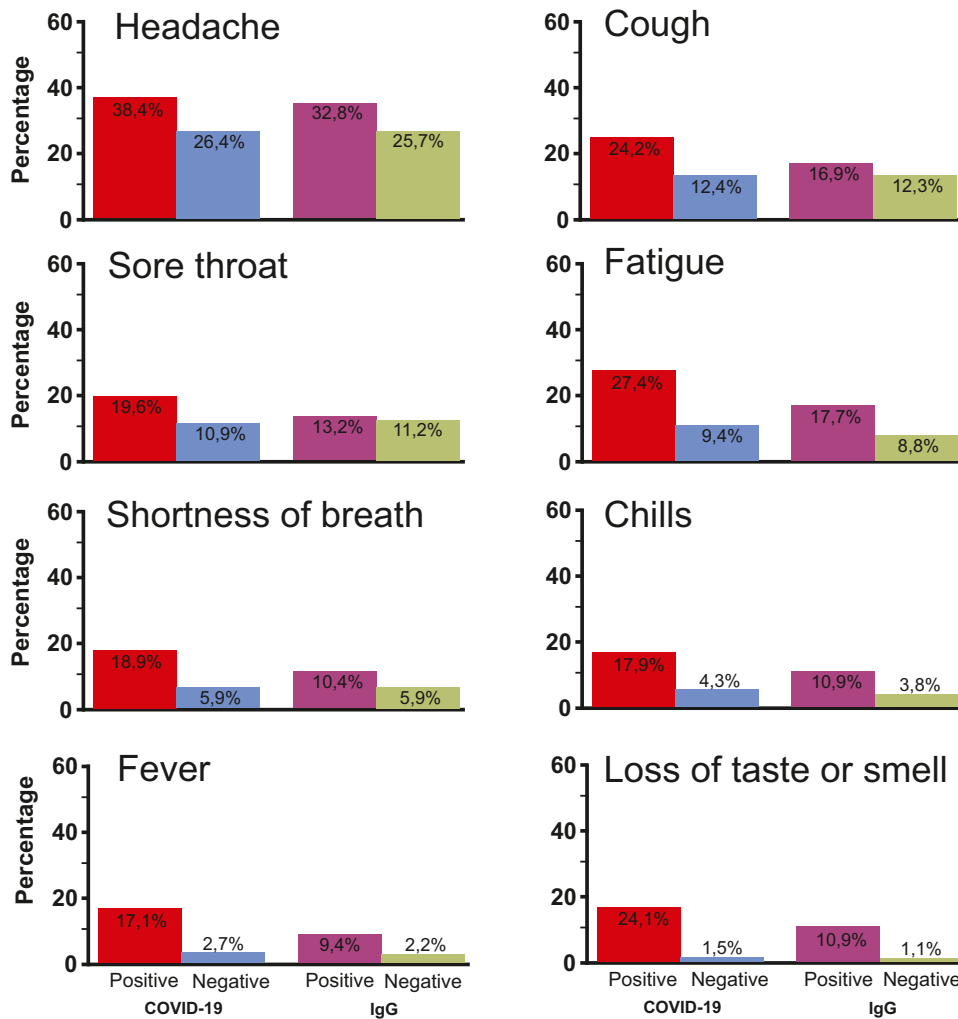
Figure 1. Association between symptoms and either positive COVID-19 diagnosis or presence of IgG antibodies. Bars on the **left** (red and blue) show the frequency percentages of certain symptoms grouped by the presence of a positive test or without COVID-19 diagnosis. Bars on the **right** (green and purple) show the association between symptoms and the presence or absence of IgG antibodies. The larger the orange/purple area, the more specific that symptom is to COVID-19 as it is less prevalent in the general population.

between symptoms and COVID-19 diagnosis or presence of IgG antibodies were measured using odds ratios with 95% CI. For this study, a p-value < 0.05 was considered to represent a statistically significant association.

Results

Relationship between symptoms, presence of a positive COVID-19 diagnosis, and IgG antibodies

Considering data for the total population ($N = 13\,560$), around one in ten participants had a positive COVID-19 diagnosis (9%; $n = 1224$), and around one in four participants showed the presence of IgG antibodies (24.2%; n = 3286). Of those who did not have IgG antibodies, 97% did not have a positive COVID-19 diagnosis. Of those with a positive COVID-19 diagnosis, 74.5% had IgG antibodies, while for those without a positive COVID-19 diagnosis, 19.2% had IgG antibodies.

The symptoms associated with a positive COVID-19 diagnosis were, from most to least frequent: headache (38.4%), fatigue (27.4%), cough (24.2%), loss of smell/taste (24.1%), sore throat (19.6%), chills (17.9%), and fever (17.1%). However, the higher frequency of a symptom should not be confused with that symptom being the most discriminating for having COVID-19. As shown in Figure 1, the symptoms of loss of smell/taste and fever are those that are least frequent in the population without a COVID-19 diagnosis – 1.5% and 2.7%, respectively. These would be the symptoms that most discriminate and support a differential

diagnosis. Similar results are obtained when exploring the association between symptoms and the presence of IgG antibodies.

With regard to the association between more common symptoms and common diseases included in the questionnaire and a positive COVID-19 diagnosis (Figure 2a) and the presence of IgG antibodies (Figure 2b), the association between loss of smell/taste and COVID-19 diagnosis was again extremely significant. Consequently, a strong association between loss of taste/smell and COVID-19 diagnosis became evident – OR 21.43 (95% CI 17.50–26.25). This implied that this symptom was more than 21 times more likely to be found in the COVID-19 population than in the control population. Other symptoms also showed statistically significant ORs, albeit in a more modest fashion.

None of the evaluated diseases, including diabetes, arterial hypertension, cardiovascular disease, and pulmonary disease, among other chronic diseases, showed any association with COVID-19. This indicates that the presence of COVID-19 was independent of any of these diseases.

Relationship between being a smoker and having a positive diagnosis of COVID-19 and IgG antibodies

Approximately one in four study participants were smokers (28.4%; $n = 3768$).

Considering the entire sample, among the smoking population there was a higher percentage of participants who did not have a positive diagnosis for COVID-19 (29%) vs 21.7% of smokers who had a positive diagnosis. Figure 3 shows the percentage of smokers who claimed to have or not to have a positive COVID-19 diagnosis, as well as the
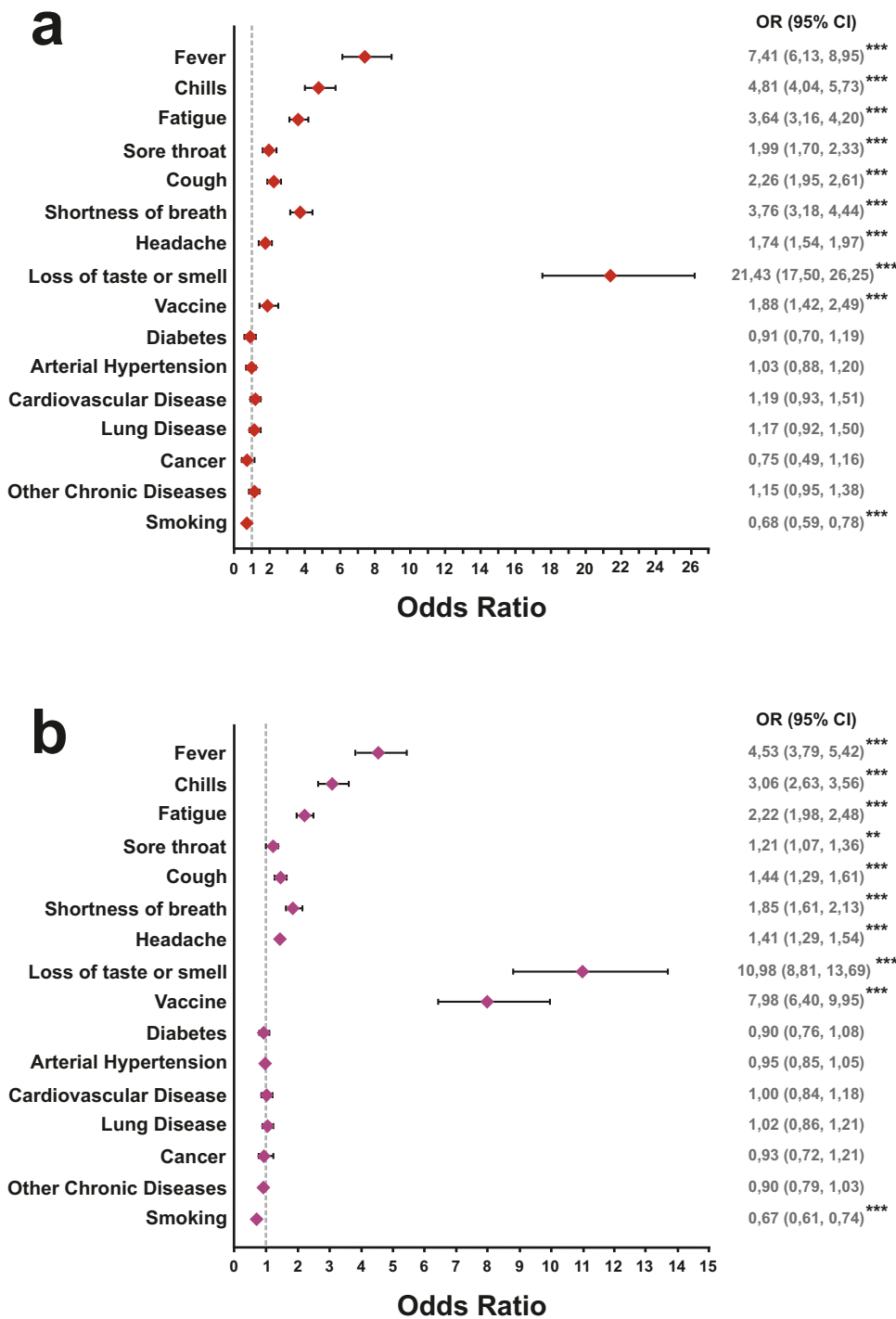
Figure 2. Odds ratios (OR) and 95% confidence intervals (95% CI) for COVID-19 symptoms and other common diseases associated with **(a)** the presence of a positive COVID-19 diagnosis and **(b)** the presence of IgG antibodies. In both cases, the most significant association was shown by loss of smell/taste, followed by fever. **$p < 0.01$; ***$p < 0.001$

presence of IgG antibodies in both populations. As shown, in smokers the probability of being diagnosed with COVID-19 was 33.6% lower. Moreover, the frequency of presence of IgG antibodies differed between smokers and non-smokers. Whereas 26.6% of non-smokers showed IgG antibodies, this value decreased significantly to 19.26% in smokers (χ^2 (1) = 70.36; $p < 0.001$).

Transmission of COVID-19 and relationship to presence of IgG antibodies

Only 4.6% of the participants who had a positive diagnosis stated that they had no contact with anyone diagnosed with COVID-19, or at least that they were aware of, whereas the most frequent source of identified infection was a member of the household, followed by a customer

or patient, and non-cohabiting relative or friend (Figure 4a). When evaluating the relationship between having been in contact with a person diagnosed with COVID-19 and the presence of IgG antibodies, the results followed the same pattern. Here 19.4% of the participants with IgG antibodies stated that they had no contact with anyone diagnosed with COVID-19, or at least to their knowledge. Again, contact with infected customers (including patients) and cohabiting persons was associated with higher level of IgG presence.

Discussion

The main findings of our study were as follows: among the symptoms associated with COVID-19, loss of smell/taste was the most discriminat-
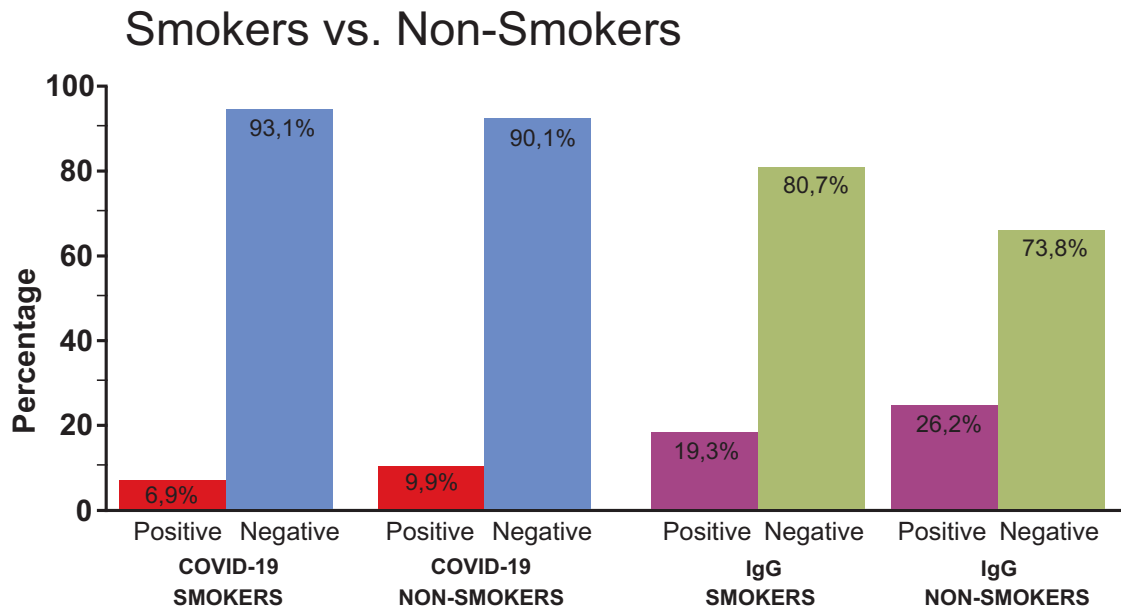
Smokers vs. Non-Smokers

Figure 3. Association between smoking status and having a positive COVID-19 diagnosis or presence of IgG antibodies. Among the smoking population, there was a higher percentage of participants who did not have a positive diagnosis for COVID-19 or positive test for IgG antibodies. The association between smoking status and presence of IgG was significant at $p < 0.001$ (χ^2 (1) = 70.36; $p < 0.001$).

ing; in smokers, the incidence of positive COVID-19 diagnosis was significantly lower; most of those diagnosed with COVID-19 or who had IgG antibodies had identified a person who could have infected them, with a member of the household being the most frequent source; the use of rapid IgG serological tests for the detection of SARS-CoV-2 virus is an effective tool for epidemiological studies of populations, despite the associated deviations in individual measurements.

At the time of writing, there were several reviews stating that most patients who contract COVID-19 are asymptomatic or have mild-to-moderate symptoms of the disease. For example, among those aged under 20 years, 15–42% have been shown to be asymptomatic (Viner et al., 2020). It is interesting to note that loss of smell/taste was among the first symptoms to be associated with COVID-19 (Russell et al., 2020; Lorenzo Villalba et al., 2020). One of the first studies to propose the usefulness of hyposmia and hypogeusia for the diagnosis of COVID-19 was that of Bénézit et al. (2020). Using a sample of 68 patients with COVID-19 and 189 patients without the disease, they found odds ratios of 7.44–13.44. Our study, with a sample of 1224 participants with a positive COVID-19 diagnosis and 12 336 control participants, found a significantly higher odds ratio of 21.43 (95% CI 17.50–26.25). Therefore, our results would support the proposed usefulness of a lack of taste/smell as a predictive tool in the diagnosis of COVID-19 where clinical laboratory tests, such as RT-PCR or rapid antigen tests, are not possible. Other frequent symptoms of COVID-19 have similar incidences in other types of disease or infectious process, and therefore offer a lower capacity for differential diagnosis. Moreover, when taking into account the presence of IgG antibodies as a sign of having been exposed to the SARS-CoV-2 virus, loss of smell/taste was found to be most specifically associated with the presence of IgG antibodies.

One of the most controversial results of our study was the relationship between smoking and a lower incidence of positive COVID-19 diagnosis. As early as March 2020, a first systematic review was published, which concluded that smoking was associated with worse progression and negative consequences of COVID-19 (Vardavas & Nikitara, 2020). New research continues to point in the same direction. It appears that smoking is associated with an increased risk of having severe COVID-19 symptoms in hospitalized patients (Saadatian-Elahi, 2021). However, another set of studies has shown different results. For example, the

prevalence of hospitalized COVID-19 smokers in China was shown to be 10.2%, when the expected prevalence would have been 31.3%. This would go against the argument that being a smoker is a risk factor for hospitalization for COVID-19 (Farsalinos et al., 2020; Propper et al., 2020). In our study, 28.4% were smokers. Therefore, if smoking activity were independent of the number of positive COVID-19 diagnoses, we should have found approximately 28–30% of smoking participants being diagnosed with the disease. However, the incidence of positive tests among smokers was reduced to 21.7%. Our data support the idea that the probability of a positive COVID-19 diagnosis, and possible hospitalization of the patient, is significantly lower in the smoking population. Some studies indicate a link between smoking and changes in the expression of some key genes, like angiotensine-converting enzyme -2 (ACE-2) used by the SARS-CoV-2 virus to infect cells but studies remain contradictory (Gupta et al., 2021). In conclusion, it seems that the rate of hospitalization or positive COVID-19 diagnosis in smokers is lower, but once hospitalized their prognosis is worse.

Regarding the association between being a smoker and the presence of IgG antibodies against the SARS-CoV-2 virus, the data showed that 26.2% of non-smokers had IgG antibodies compared with only 19.29% of smokers. Several explanatory hypotheses can be proposed from these results. One of these could be that smokers are impaired in their ability to produce IgG antibodies against SARS-CoV-2, since the percentage of smokers without antibodies was significantly higher than those with antibodies. However, given that the presence of a positive COVID-19 diagnosis was also taken into consideration, the presence of IgG antibodies served as an indicator of the number of infections. Therefore, since smokers showed reduced IgG antibodies as well as a lower frequency of COVID-19 diagnosis, both sets of data in combination indicate an association between smoking and reduced virus infection. Moreover, this link between IgG and COVID-19 diagnosis supports the use of IgG antibody levels as a proxy in assessing the general population for virus exposure.

The most frequent routes of virus transmission appear to be household members, clients (e.g. patients in the case of healthcare workers), and family members or friends who do not reside in the same household. Only 4.6% of the COVID-19-diagnosed population did not know the source of the infection, suggesting a high percentage of awareness in the population. These results were in line with other epidemiological
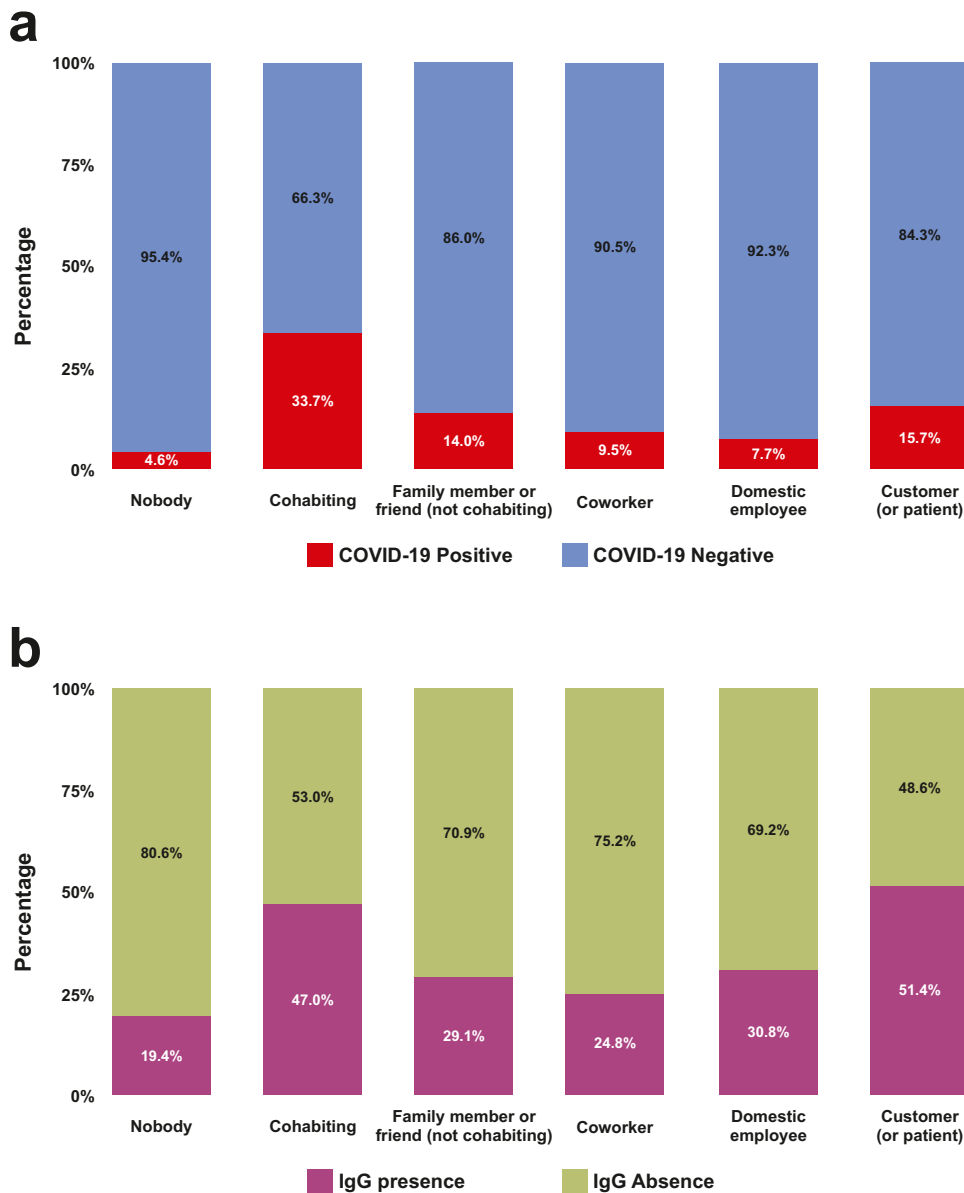
a

b

studies. Thomson et al., in a recent systematic review and meta-analysis, showed that the home was the focus of infection and that, if the duration of contact exceeded 5 days, the rate of transmission was significantly higher (Thompson et al., 2021).

Evaluating IgG antibody levels and contact with a person with a positive COVID-19 diagnosis, one might expect to find percentages similar to those of having been diagnosed with COVID-19 (Figure 4. Panel A). However, according to our results, these percentages were higher, especially in the case of participants who could not identify the source of infection. In our study, 19.4% of the participants who showed IgG antibodies did not know who could have transmitted the virus to them. This is almost four times more than in the case of participants who had a positive COVID-19 diagnosis. This would suggest that a proportion of the population has been exposed to the virus and has not developed COVID-19 symptoms, or have not linked the experienced symptoms to a possible COVID-19 infection.

Regarding the extrapolation of the results shown here, the percentages of infections found here in this specific locality of Madrid were higher than those found in an earlier Spanish national study carried out by Pollán et al. (2020). For example, the IgG seroprevalence in Spain at the time of the study (April–May 2020) was 11.3% in the province of Madrid. By the time of our study in the Madrid town of Arganda del Rey, almost 1 year later (January–April 2021), the IgG seroprevalence was 24.2%, including people who had already been vaccinated ($n = 399$; 2.9%). The latter were not discarded from the study because many of these participants had previously been infected by the virus. Therefore, our study suggests that in the 10–12 month period between studies, the IgG seroprevalence rate in the Madrid population increased by about 16.5%.

Population serological studies have been used mainly to estimate the prevalence of SARS-CoV-2 infection, since in many cases COVID-19 is asymptomatic (Pollán et al., 2020; Le Vu et al., 2021). It has been estimated that 35.1% of those infected by the virus are asymptomatic (Sah et al., 2021). According to our data, self-reports from participants showed that 9% had had a positive COVID-19 diagnosis. Of this population of positives, 25.5% had no IgG antibodies. That is, these participants would not have been detected as infected using serological tests. And yet, when we evaluated the 91% of participants who did not have a positive COVID-19 diagnosis, 19.2% had IgG antibodies. Therefore, to help our estimations, if we combine the 25.5% deviation for non-detected and

the 19.2% deviation for detected, they would roughly cancel each other out. This is not an optimal solution, but functional in terms of epidemiological estimation. This suggests that serological studies can indeed be a valid tool for establishing the prevalence of infection by the virus at the population level, but not at the individual level, since the estimated margin of error would be between 19% and 26%. Considering also that these IgG antibody evaluations were performed with rapid tests, which had previously validated clinically, our study suggests that rapid tests for antibodies against SARS-CoV-2 virus can support population serological studies in an efficient and cost-effective way. At the time of the study, it could be ruled out that herd immunity had been achieved.

Declaration of Competing Interest

The authors declare no commercial or financial conflicts of interest.

Ethical statement

The General Directorate of Public Health of Madrid approved this study (project number: AR-COVID19). Written informed consent was obtained from all participants.

Author contribution

EG and JALM were responsible for the conception, design, and coordination of the study. VEA, JCC, PDG, and LSG were responsible for test analyses and acquisition of laboratory data. ADI, PMA, and FGR were responsible for clinical revision of the study. MPW and JAM were in charge of statistical analyses and table and figure design. JALM, KMB, and JCC wrote the first draft. All remaining authors contributed to data acquisition, laboratory analysis, and interpretation, and critically reviewed the first draft. All authors approved the final version and agreed to be accountable for the work.

Acknowledgments

References

Álvarez-Antonio C, Meza-Sánchez G, Calampa C, et al. Seroprevalence of anti-SARS-CoV-2 antibodies in Iquitos, Peru in July and August, 2020: a population-based study. The Lancet Global Health 2021;9(7):e925–31. doi:10.1016/S2214-109X(21)00173-X.

Akpabio AA, Onukak AE, Udoette SB, Umoh VA. Safety practices and COVID-19 antibody positivity among health care workers in a tertiary hospital in southern Nigeria. West Afr J Med 2021;38(10):930–5. doi:10.1016/j.actbio.2021.12.022.

Arabkhazaeli A, Maghsudlu M, Mohammadi S, Eshghi P. Positive anti-SARS-CoV-2 rapid serological test results among asymptomatic blood donors [published online ahead of print, Aug 21, 2021]. Transfus Clin Biol 2021;S1246-7820(21):00122. doi:10.1016/j.tracli.2021.08.008.

Aziz M, Goyal H, Haghbin H, Lee-Smith WM, Gajendran M, Perisetti A. The association of 'loss of smell' to COVID-19: a systematic review and meta-analysis. Am J Med Sci 2021;361(2):216–25. doi:10.1016/j.amjms.2020.09.017.

Bénézit F, Le Turnier P, Declerck C, et al. Utility of hyposmia and hypogeusia for the diagnosis of COVID-19. Lancet Infect Dis 2020;20(9):1014–15. doi:10.1016/S1473-3099(20)30297-8.

Eslami H, Jalili M. The role of environmental factors to transmission of SARS-CoV-2 (COVID-19). AMB Express 2020;10(1):92 Published May 15, 2020. doi:10.1186/s13568-020-01028-0.

Farsalinos K, Barbouni A, Niaura R. Smoking, vaping and hospitalization for COVID-19. Qeios 2020. doi:10.32388/Z69O8A.13.

Figueiredo-Campos P, Blankenhaus B, Mota C, et al. Seroprevalence of anti-SARS-CoV-2 antibodies in COVID-19 patients and healthy volunteers up to 6 months post disease onset. Eur J Immunol 2020;50(12):2025–40. doi:10.1002/eji.202048970.

Gupta I, Sohail MU, Elzawawi KE, et al. SARS-CoV-2 infection and smoking: what is the association? A brief review. Comput Struct Biotechnol J 2021;19:1654–60. doi:10.1016/j.csbj.2021.03.023.

Hanssen DA, Slaats M, Mulder M, Savelkoul PH, et al. Evaluation of 18 commercial serological assays for the detection of antibodies against SARS-CoV-2 in paired serum samples. European Journal of Clinical Microbiology & Infectious Diseases 2021;40(8):1695–703.

Le Vu S, Jones G, Anna F, et al. Prevalence of SARS-CoV-2 antibodies in France: results from nationwide serological surveillance. Nat Commun 2021;12(1):3025 Published May 21, 2021. doi:10.1038/s41467-021-23233-6.

Lorenzo Villalba N, Maouche Y, Alonso Ortiz MB, et al. Anosmia and dysgeusia in the absence of other respiratory diseases: should COVID-19 infection be considered? Eur J Case Rep Intern Med 2020;7(4) Published Apr 3, 2020. doi:10.12890/2020_001641.

Mack D, Gärtner BC, Rössler A, et al. Prevalence of SARS-CoV-2 IgG antibodies in a large prospective cohort study of elite football players in Germany (May–June 2020): implications for a testing protocol in asymptomatic individuals and estimation of the rate of undetected cases. Clin Microbiol Infect 2021;27(3) 473.e1–473.e4. doi:10.1016/j.cmi.2020.11.033.

Marchese-Ragona R, Restivo DA, De Corso E, Vianello A, Nicolai P, Ottaviano G. COVID-19 e olfatto: revisione critica della letteratura sulla valutazione olfattiva. Acta Otorhinolaryngologica Italica 2020;40(4):241–7.

Neagu M. The bumpy road to achieve herd immunity in COVID-19. J Immunoassay Immunochem 2020;41(6):928–45. doi:10.1080/15321819.2020.1833919.

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(10250):535–44. doi:10.1016/S0140-6736(20)31483-5.

Propper RE. Does cigarette smoking protect against SARS-CoV-2 infection? Nicotine Tob Res 2020;22(9):1666. doi:10.1093/ntr/ntaa073.

Russell B, Moss C, Rigg A, Hopkins C, Papa S, Van Hemelrijck M. Anosmia and ageusia are emerging as symptoms in patients with COVID-19: what does the current evidence say? Ecancermedicalscience 2020;14:ed98 Published Apr 3, 2020. doi:10.3332/ecancer.2020.ed98.

Saadatian-Elahi M, Amour S, Elias C, Henaff L, Dananché C, Vanhems P. Tobacco smoking and severity of COVID-19: experience from a hospital-based prospective cohort study in Lyon, France. J Med Virol 2021;93(12):6822–7. doi:10.1002/jmv.27233.

Sah P, Fitzpatrick MC, Zimmer CF, et al. Asymptomatic SARS-CoV-2 infection: a systematic review and meta-analysis. Proc Natl Acad Sci USA 2021;118(34). doi:10.1073/pnas.2109229118.

Shakiba M, Nazemipour M, Heidarzadeh A, Mansournia MA. Prevalence of asymptomatic COVID-19 infection using a seroepidemiological survey. Epidemiol Infect 2020;148:e300 Published Nov 13, 2020.

Stringhini S, Wisniak A, Piumatti G, et al. Seroprevalence of anti-SARS-CoV-2 IgG antibodies in Geneva, Switzerland (SEROCoV-POP): a population-based study. Lancet 2020;396(10247):313–19. doi:10.1016/S0140-6736(20)31304-0.

Thompson HA, Mousa A, Dighe A, et al. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) setting-specific transmission rates: a systematic review and meta-analysis. Clin Infect Dis 2021;73(3):e754–64. doi:10.1093/cid/ciab100.

Vardavas CI, Nikitara K. COVID-19 and smoking: a systematic review of the evidence. Tob Induc Dis 2020;18:20 Published Mar 20, 2020. doi:10.18332/tid/119324.

Viner RM, Ward JL, Hudson LD, et al. Systematic review of reviews of symptoms and signs of COVID-19 in children and adolescents [published online ahead of print, Dec 17, 2020]. Arch Dis Child 2020 archdischild-2020-320972. doi:10.1136/archdischild-2020-320972.