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SHORT COMMUNICATION

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Mucosal antibody response and SARS-CoV-2 shedding in patients with COVID-19 related olfactory dysfunction

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Abstract

Olfactory dysfunction (OD) was one of the most common symptom of infection with the Wuhan strain of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and could persist for several months after symptom onset. The pathogenesis of prolonged OD remains poorly understood but probably involves sustained viral replication associated with limited mucosal immune response to the virus. This prospective study was conducted to investigate the potential relationship between nasal SARS-CoV-2 viral load and antibody levels in patients with loss of smell. One hundred and five patients were recruited 2 weeks after presenting with confirmed coronavirus disease 2019 associated OD. Based on the identification sniffing test performed at enrollment, 52 patients were still anosmic or hyposmic and 53 were normosmic. SARS-CoV-2 was detectable in nasal wash of about 50% of anosmic and normosmic patients. Higher viral load was detected in anosmic patients with lower levels of SARS-CoV-2 specific nasal immunoglobulins (Ig) IgG and IgA. This association was not observed in normosmic patients. No relationship between nasal viral load and antibodies to endemic coronaviruses was observed. SARS-CoV-2 replication in the nasal cavity may be promoted by defective mucosal antibody responses in patients with OD. Boosting mucosal immunity may limit nasal SARS-CoV-2 replication and thereby help in the control of persistent OD.

KEYWORDS
anosmia, coronavirus, COVID-19, olfactory dysfunction, SARS-CoV-2, viral load

Abbreviations: anti-NP, anti-nucleocapsid protein; anti-S-RBD, anti-spike protein receptor binding domain; ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; Ct, cycle threshold; hCOV, human coronaviruses; MFI, mean fluorescence intensity; OD, olfactory dysfunction; RT-qPCR, quantitative reverse transcription polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TDI, threshold (T), discrimination (D) and identification (I); VL, viral load; −ve, negative; +ve, positive.

Arnaud Marchant and Sven Saussez share senior authorship.

1 | INTRODUCTION

During the first wave of the coronavirus disease 2019 (COVID-19) pandemic, olfactory dysfunction (OD) was one of the most common (50%–85%) and long-lasting symptoms of COVID-19 patients.[1,2] Between 5% and 10% of patients, presented a persistent OD beyond a year after onset.[3] Long-term OD appears to be related to the destruction of the neuroepithelium but the mechanisms underlying this destruction remain poorly understood.[4,5] Persistence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replication in the neuroepithelium, potentially promoted by defective mucosal immunity, may be involved.[6] We previously reported lower nasal and salivary immunoglobulin G (IgG) levels in patients with persistent anosmia as compared to patients with more transient symptoms.[7] On the other hand, studies indicated that OD is associated with increasing levels of serum antibodies in the first month following onset of symptoms, suggesting persistent antigen stimulation.[8] Together, these data suggest that viral replication in the nasal cavity may be promoted by low antibody responses and that persistent replication may further stimulate antibody production over prolonged periods of time.[9] More insights are needed on the relationship between viral replication and mucosal antibody response to decipher their interactions in patients with COVID-19 OD.[10]

A potentially important determinant of adaptive immune responses to SARS-CoV-2 is preexisting immunity to endemic coronaviruses, including HKU1, OC43, 229E, and NL63, to which patients have been commonly exposed. Studies suggest that pre-existing immunity to endemic coronaviruses may reduce the severity of COVID-19.[11] Whether an impact on SARS-CoV-2 replication in the nasal cavity can also be observed is currently unknown. This prospective study was conducted to investigate the potential relationship between nasal SARS-CoV-2 viral load and antibody levels in patients with OD.

2 | MATERIALS AND METHODS

2.1 | Study population

This study was approved by institutional review boards of the CHU Saint-Pierre, Brussels, Belgium, and Epicura Hospital, Baudour, Belgium. From October 2020 to November 2020 patients with a diagnosis of COVID-19 and OD confirmed by psychophysical olfactory tests were recruited after 2 weeks of onset of symptoms at the Epicura Hospital. We choose a follow-up period of 2 weeks to investigate the postacute phase of the disease and a time at which we hypothesized that some patients would still be PCR positive. SARS-CoV-2 infection was diagnosed by nasal swab and reverse transcriptase polymerase chain reaction (RT-PCR). Symptoms were evaluated during the clinical course of the disease with the COVID-19 Symptom Index.[7] Subjective olfactory functions were evaluated with the smell and taste component of the National Health and Nutrition Examination Survey.[12] Fourteen to 15 days after onset of OD, patients were invited to perform a sniffing test and to donate blood and nasal secretion samples (nasal washing with physiological saline solution was carried out and nasal secretions were sterile aspirated). Subjects were categorized into anosmic, hyposmic and normosmic according to the results of the Sniffin' sticks test.[13] Psychophysical olfactory assessments were performed with the identification component of Sniffin' Sticks tests (Medisense, Groningen, Netherlands), which is a validated psychophysical olfactory test using 16 smell pens. The final score ranges from 0 (no odor correctly identified) to 16 (all odors correctly identified). Normative values established normosmia as a score ranging between 12 and 16, hyposmia between 9 and 11, and anosmia between 0 and 8.[13] It must be emphasized that at that time COVID-19 infections were associated with significant mortality and vaccines were not available; it is for this reason that only the identification part of the TDI [threshold (T), discrimination (D) and identification (I)] was carried out to reduce the risk of contamination of the research team.

Inclusion criteria consisted of adults with SARS-CoV-2 infection identified through nasal swabs and positive RT-PCR and COVID-19 related OD. Patients with a history of pre-COVID-19 pandemic OD, chronic or self-reported acute rhinosinusitis (with regard to the European Position Paper on Rhinosinusitis and Nasal Polyps guidelines) and dementia at the time of evaluation were excluded. Socio-demographic and clinical data were collected through a standardized online questionnaire or medical records.

2.2 | SARS-CoV-2 viral load

Quantitative detection of SARS-CoV-2 RNA from the nasal wash (NW) of all the clinical samples was done by performing rtRT-PCR using Simplexa™ COVID-19 Direct kit (DiaSorin) as per the manufacturer's instructions. Reactions were run on the LIAISON® MDX instrument (DiaSorin). The DiaSorin Molecular Simplexa™ COVID-19 Direct assay system is a real-time RT-PCR system that enables the direct amplification of Coronavirus SARS-CoV-2 RNA from NW specimens. The data is analyzed with LIAISON® MDX Studio Software. The assay targets two different regions of the SARS-CoV-2 genome, ORF1ab S gene.[14] The sample was considered SARS-CoV-2 positive when one of the target genes in the same sample was positive and negative if both the genes were negative. The Ct values for both the genes were nearly similar. In this study, we used the Ct value of ORF1ab from NW for the comparisons. The Ct value is inversely correlated with RNA copy number of the virus. Ct value <40 was considered COVID-19 positive.

2.3 | Antibody assays

Recombinant SARS-CoV-2 spike antigens (S1, S2, RBD) and spike antigens from hCOVs (HKU1, OC43, NL63, 229E) were purchased from SinoBiological and nucleocapsid protein was kindly provided by André Matagne, University of Liège. All antigens were covalently

coupled to fluorescent magnetic beads (Luminex Corporation) and were used to measure antigen-specific antibodies using the Fc array assay as described previously.[7,15] Data was acquired on BioPlex-200 equipment (Bio-Rad) and the results were expressed as median fluorescence intensity. See the list of reagents in Table SI.

2.4 | Statistical analyses

Statistical analyses were performed using the GraphPad Prism software, version 9.3.1. Antibody titers between the groups were compared with two-tailed Mann–Whitney *U* test and significance is indicated as $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. If there is no significant difference, it is not depicted in the figure. Correlations between the viral load (1/Ct) value of the viral RNA and antibody levels in each compartment are represented using Heatmaps. Heatmaps depicting the magnitude and statistical significance between the 1/Ct value of viral RNA and antibody (IgG, IgA) were analyzed with a two-tailed Spearman test. Each box represents an antigen-specific antibody response. Color indicates correlation coefficient magnitude and direction. Positive correlation of the viral load (1/Ct) values with antibody feature is indicated in blue and negative correlation in red.

3 | RESULTS AND DISCUSSION

One-hundred and five patients presenting with COVID-19-related OD were recruited. Based on the identification-sniffing test, 28 patients were anosmic, 24 were hyposmic, and 53 had recovered from their olfactory symptoms (normosmic), respectively. Clinical and demographic characteristics are available in Table 1. Patients included in the three study groups were comparable. Anosmic and hyposmic patients were grouped for the virological and immunological analyses presented below and are referred to as anosmic (Figure 1).

3.1 | Relationship between nasal viral load and SARS-CoV-2 antibody levels

SARS-CoV-2 viral load was measured in NW collected at the first visit. SARS-CoV-2 was detected in about 48% (25/52) of anosmic and 45% (24/53) of normosmic patients. Among SARS-CoV-2 positive patients, viral load was not significantly different in anosmic and normosmic patients (data not shown). Anosmic patients tended to have higher levels of SARS-CoV-2 specific nasal and serum IgG and this difference reached significance for RBD IgG in NW (Figure 2A). No significant difference was observed for nasal or serum IgA. These results contrast with our previous report indicating that patients with prolonged anosmia had lower levels of nasal, but not serum IgG, 2 months after onset of symptoms.[7] This difference suggests that anosmia is associated with dynamic changes in antibody levels that may be related to the changes in SARS-CoV-2 antigen load.[16] The association with serum and

TABLE 1 Clinical characteristics of the study population.

	Anosmic (*N* = 28)	Hyposmic (*N* = 24)	Normosmic (*N* = 53)
Mean age (SD)—yo	38.4 (13.8)	38.8 (14.2)	40.3 (10.6)
Gender (male/female)	6/20	7/16	22/30
Smoker	1 (3.8)	1 (4.3)	3 (5.8)
Patients with seasonal allergy	5 (19.2)	2 (8.7)	12 (23.1)
Main comorbidities (*N*, %)			
Chronic rhinitis	5 (19.2)	3 (13.0)	7 (13.5)
Hypertension	0 (0)	3 (13.0)	3 (5.8)
Asthma	1 (3.8)	1 (4.3)	5 (9.6)
Hypothyroidism	3 (11.5)	2 (8.7)	3 (5.8)
Auto-immune disorder	0 (0)	0 (0)	2 (3.8)
Reflux	4 (15.4)	1 (4.3)	9 (17.3)
Heart disease	1 (3.8)	0 (0)	1 (1.9)
Psoriasis	5 (19.2)	1 (4.3)	0 (0)
Objective dysfunction: sniffing stick test			
Self-reported olfactory dysfunction			
Complete	24 (92.3)	19 (82.6)	43 (82.7)
Partial	2 (13.0)	4 (17.4)	6 (11.5)
No olfactory dysfunction	0 (0)	0 (0)	3 (5.8)
Onset of smell dysfunction			
Before the other symptoms	0 (0)	4 (17.4)	7 (13.5)
Concurrent with other symptoms	7 (26.9)	4 (17.4)	8 (15.4)
After the other symptoms	18 (69.2)	13 (56.5)	36 (69.2)
Did not remember	1 (3.8)	2 (8.7)	1 (1.9)
Self-reported taste dysfunction	8 (30.8)	9 (39.1)	21 (40.4)
Ear, nose, and throat symptoms (*N*, %)			
Nasal obstruction	15 (57.7)	13 (56.5)	40 (76.9)
Sore throat	15 (57.7)	15 (65.2)	32 (61.5)
Rhinorrhea	17 (65.4)	13 (56.5)	34 (65.4)
Postnasal drip	5 (19.2)	4 (17.4)	10 (19.2)
Face pain/heaviness	9 (34.6)	6 (26.1)	16 (30.8)
SNOT-22 (mean, *SD*)	38.2 (18.6)	38.0 (15.2)	38.0 (16.6)

(Continues)

TABLE 1 (Continued)

	Anosmic (*N* = 28)	Hyposmic (*N* = 24)	Normosmic (*N* = 53)
General symptoms (*N*, %)			
Headache	21 (80.8)	18 (78.3)	42 (80.8)
Asthenia	25 (96.2)	22 (95.7)	50 (96.2)
Myalgia	22 (84.6)	17 (73.9)	44 (84.6)
Cough	19 (73.1)	18 (78.3)	35 (67.3)
Anorexia	15 (57.7)	14 (60.9)	32 (61.5)
Dyspnea	10 (38.5)	12 (52.2)	30 (57.7)
Fever (>38°C)	16 (61.5)	15 (65.2)	33 (63.5)
Arthralgia	15 (57.7)	14 (60.9)	33 (63.5)
Symptom duration (days, *SD*)	8.3 (5.2)	8.7 (3.7)	8.9 (5.0)

Abbreviations: N, number, *SD*, standard deviation; SNOTT, sino-nasal outcome test; yo, years old.

nasal IgG, and not IgA, levels suggests that antigen challenge occurs at the systemic level. To further explore the relationship between viral replication and antibody levels, nasal SARS-CoV-2 PCR-positive and negative patients were compared. Among anosmic patients, SARS-CoV-2 PCR-positive patients had lower levels of nasal and serum IgG as compared to SARS-CoV-2 PCR-negative patients (Figure 2B). Similar results were observed with IgG1 and IgG3 levels in serum whereas signals measured in nasal fluids were relatively low (Figure S1a). Nasal IgA and IgA1 levels showed trends similar to total nasal IgG (Figure 2B and Figure S1a). In serum, SARS-CoV-2 negative patients had higher levels of SARS-CoV-2 specific IgA and IgA1 (Figure 2B and Figure S1a), contrasting with IgG levels. Similar opposite trends for serum IgG and IgA levels have been observed in previous studies of COVID-19 convalescent patients, although the mechanism involved has not been determined.[17]

Among SARS-CoV-2 PCR-positive patients, a negative correlation was observed between SARS-CoV-2 viral load and nasal and serum IgG and IgA (Figure 2C). Together these data suggest that, among patients with more severe OD, SARS-CoV-2 replication in the nasal cavity may be controlled by the presence of high levels of IgG and IgA. The most significant negative correlations were observed with antibodies to RBD and S1 subunit, suggesting that neutralizing antibodies may play an important role (Figure 2C). As the relationship between viral load and levels of antibodies was observed in both NW and in serum, both mucosal immunity and systemic immunity transferred to the mucosa may participate in the control of nasal SARS-CoV-2 replication.[18] Surprisingly, no significant association between nasal SARS-CoV-2 viral load and antibody levels was observed in normosmic patients (Figure 2B,C). This intriguing observation suggests an interaction between more severe OD and antibody-mediated control of SARS-CoV-2 replication in the nasal cavity. Such interaction may involve other immune effectors,

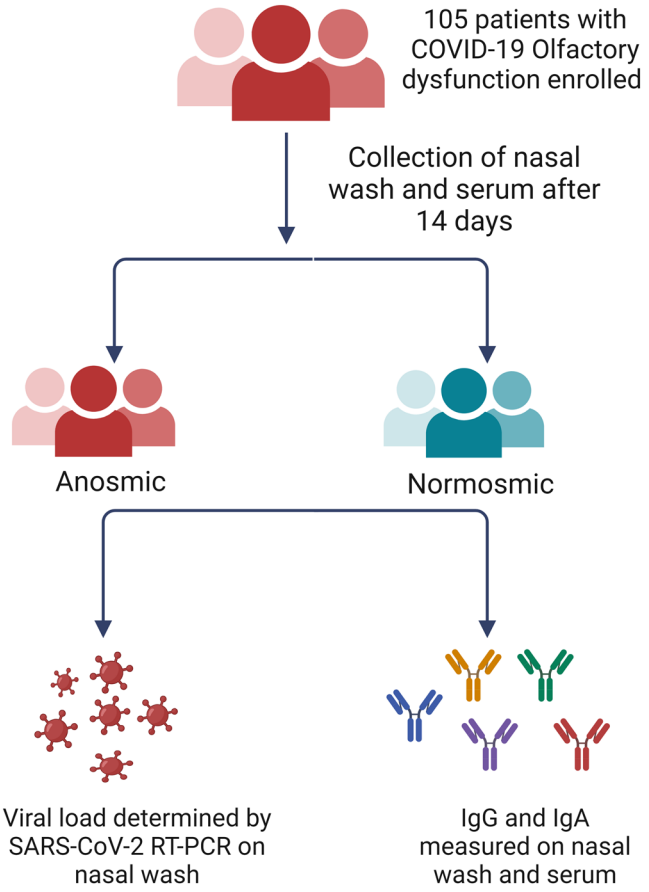

FIGURE 1 Outline of the study. Patients with a confirmed subjective diagnosis of COVID-19 related olfactory dysfunction were recruited 2 weeks after the onset of symptoms. Sniffing test (identification part of the TDI) was performed and the SARS-CoV-2 viral load was measured in the nasal wash 2 weeks after the first day of symptom reported. Antibody levels to SARS-CoV-2 and to endemic coronaviruses were measured in nasal wash and serum. Based on their sniffing test, anosmic and hyposmic patients have been regrouped in one group called "anosmic". COVID-19, coronavirus disease 2019; Ig, immunoglobulin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

including innate immune cells, participating in the antiviral activity of antibodies.[19]

3.2 | Relationship between nasal SARS-CoV-2 viral load and endemic coronavirus antibody levels

To explore the potential basis for the higher antibody response to SARS-CoV-2 in patients with undetectable or lower SARS-CoV-2 viral load, the levels of antibodies to the S protein of endemic coronaviruses were measured (Figure 3). First, we observed that anosmic and normosmic patients had similar levels of nasal and serum IgG and IgA to the S protein of 229E, OC43, HKU1, and NL63 viruses, except for serum levels of IgG to 229E that were slightly lower among anosmic patients (Figure 3A). We then observed that

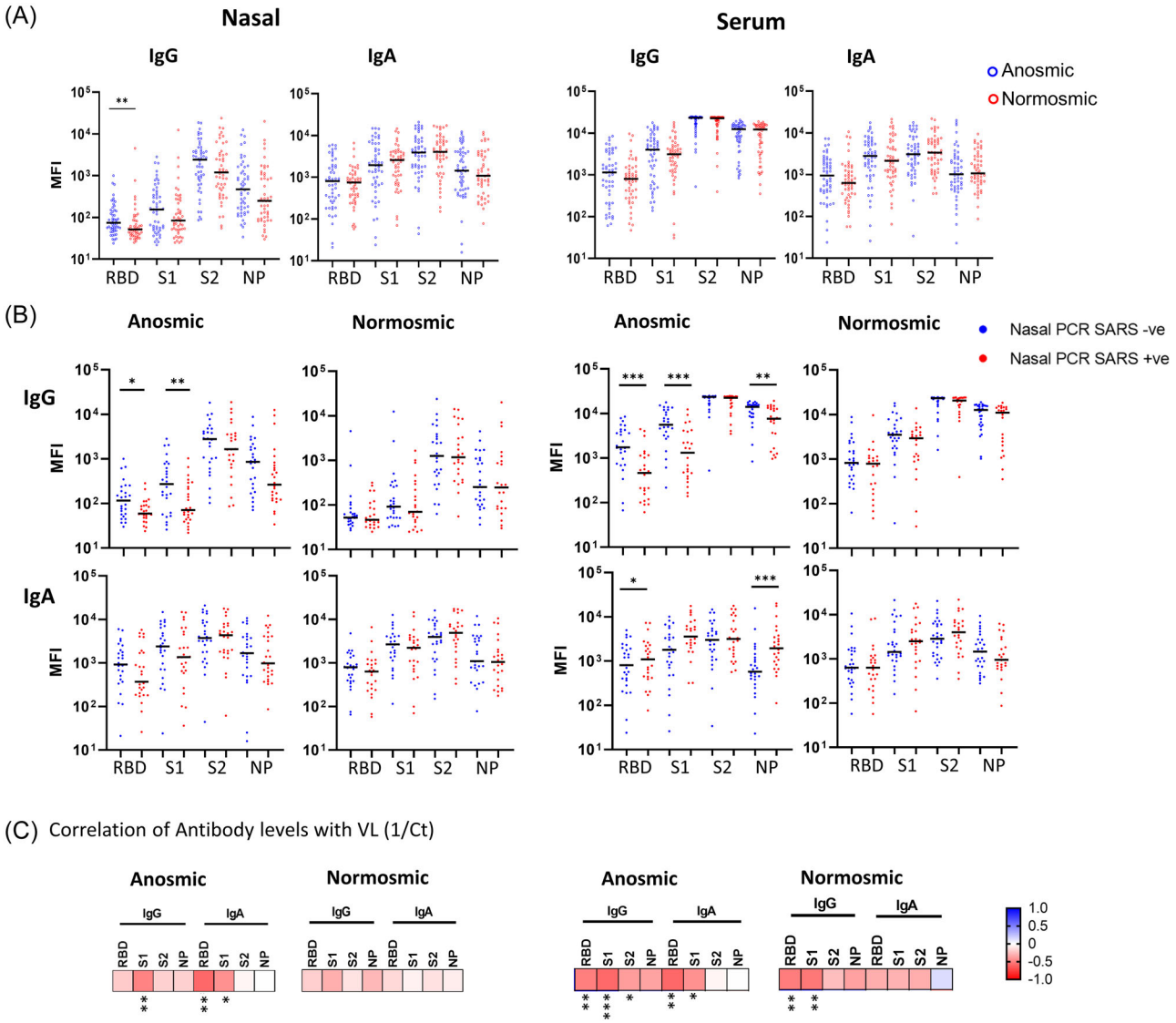

FIGURE 2 SARS-CoV-2 antibody levels and nasal viral load. Levels of IgG and IgA to SARS-CoV-2 antigens in nasal wash (left panels) and serum (right panels). (A) Antibody levels in anosmic (blue) and normosmic (red) patients. (B) Antibody levels in nasal SARS-CoV-2 PCR negative (blue) and positive (red) patients. (C) Correlations between nasal SARS-CoV-2 viral load (1/Ct) and antibody levels. The results are expressed as median fluorescence intensity (MFI). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, VL- viral load, Ct-cycle threshold. Ig, immunoglobulin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

antibody levels to the four endemic coronaviruses were comparable in SARS-CoV-2 PCR positive and negative patients, whether anosmic or normosmic (Figure 3B). Similar results were obtained with IgG1, IgG3, and IgA1 subclasses (Figure S1b). A moderate negative correlation was observed between SARS-CoV-2 viral load and nasal levels of IgG to OC43 and HKU1 but no other correlations were observed (Figure 3C). Together these data indicate that overall antibody levels to endemic coronaviruses are not associated with nasal SARS-CoV-2 viral load in our study population. Immunity to endemic coronaviruses can therefore not explain the association between SARS-CoV-2 viral load and SARS-CoV-2-specific antibodies. However, the fact that OC43 and HKU1 have the closest homology with SARS-CoV-2 among endemic coronaviruses suggests that the moderate negative correlation observed between SARS-CoV-2 viral load and serum levels of IgG to these two coronaviruses may indicate some role for cross-reactive immunity in the control of SARS-CoV-2 replication in anosmic patients.

To the best of our knowledge, this is the first study showing an inverse association between nasal SARS-CoV-2 viral load and the levels of antibodies. The specificity of this observation in patients with COVID-19 OD suggests an interaction with other immune components involved in the pathogenesis of the olfactive disorder. The data suggests that boosting local immunity to SARS-CoV-2 may be beneficial to patients with OD and may reduce the duration of symptoms. Systemic vaccination with currently available vaccines provides limited mucosal immunity. The development of mucosal vaccines may offer

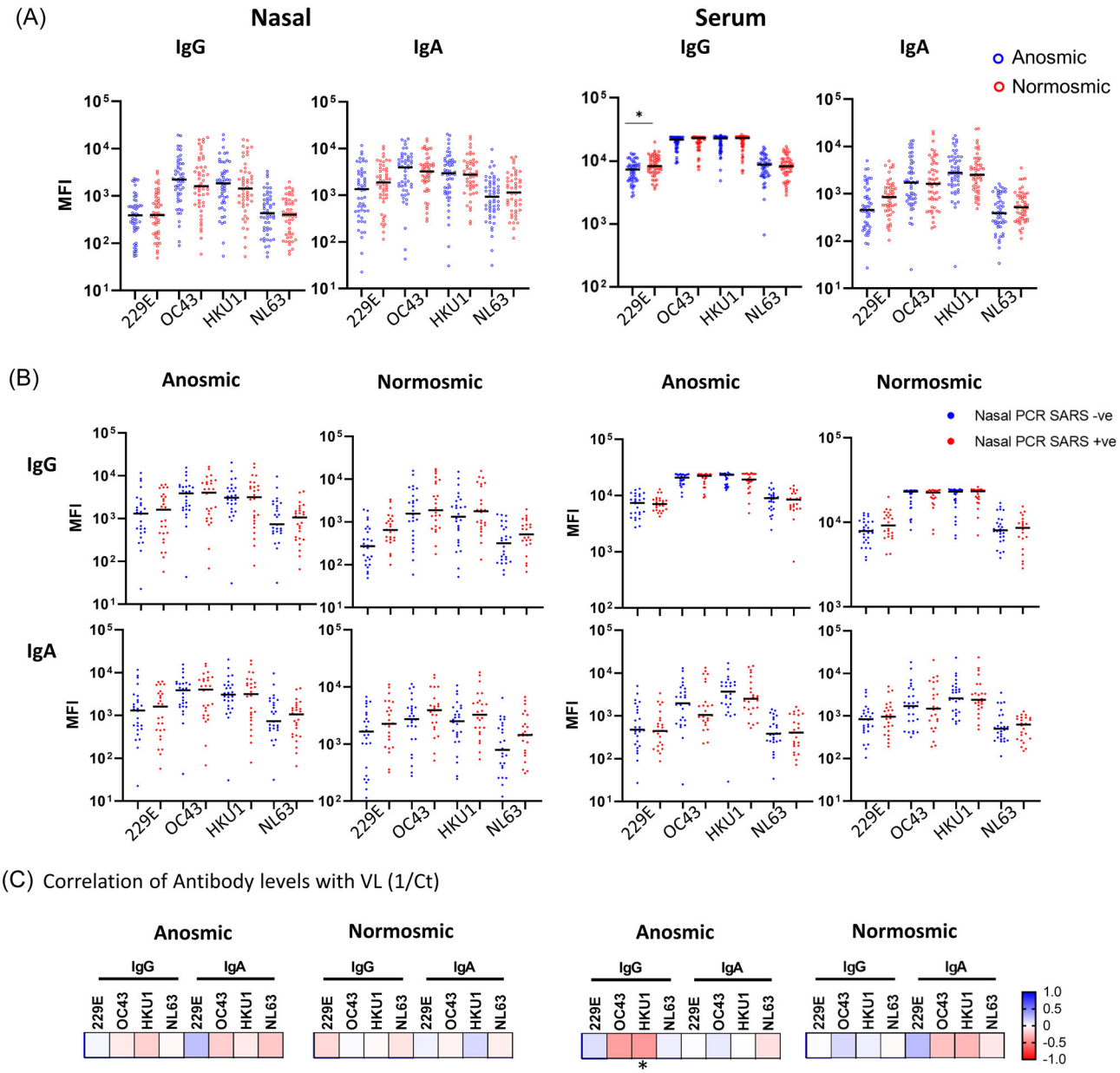

FIGURE 3 Endemic coronaviruses antibody levels and nasal SARS-CoV-2 viral load. Levels of IgG and IgA to SARS-CoV-2 antigens in nasal wash (left panels) and serum (right panels). (A) Antibody levels in anosmic (blue) and normosmic (red) patients. (B) Antibody levels in nasal SARS-CoV-2 PCR negative (blue) and positive (red) patients. (C) Correlations between nasal SARS-CoV-2 viral load (1/Ct) and antibody levels. The results are expressed as median fluorescence intensity (MFI). *$p < 0.05$, Ct, cycle threshold; Ig, immunoglobulin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VL, viral load.

opportunities for the prevention of local symptoms, in addition to the reduction of SARS-CoV-2 shedding and transmission.[20]

The relatively large number of patients and the prospective design are the main strengths of the study. Moreover, patients were recruited and followed-up within relatively narrow time windows, increasing the likelihood of measuring dynamic virological and immunological events at similar times between patients. Some limitations can be mentioned as well. Measurements were performed at a single time point, preventing analysis of the dynamics of the relationship between viral replication and antibody responses. Also, the neutralizing capacity of nasal antibodies was not assessed and the levels of nasal IgM that could contribute to viral control were not measured. As explained above, the study was conducted from October to November 2020 (second wave of SARS-CoV-2 in Europe) when the dominant strain of SARS-CoV-2 was the Wuhan strain. The interactions between variants of concern and antibodies at the mucosal level could be significantly different, limiting the potential extrapolation of our study results to the current stage of the pandemic. The lack of fully objective methods to assess the OD is another limitation. The use of Identification Sniffin'Sticks Test

(16 items) and not the complete Threshold, Discrimination, and Identification test was made according to the quarantine restrictions of physician-patient contact in the first waves of the pandemic.

AUTHOR CONTRIBUTIONS

Shilpee Sharma, Anaïs Thiriard, Véronique Olislagers, and Marie-Hélène Jurion: performed the experimental work. **Marie-Luce Delforge and Arnaud Marchant**: supervised the experimental work. **Sven Saussez**: recruited the study population. **Shilpee Sharma, Sven Saussez, Jerome R. Lechien, and Arnaud Marchant**: wrote the manuscript. **Shilpee Sharma and Anaïs Thiriard**: performed data analysis. **Sven Saussez and Arnaud Marchant**: conceptualized the study and secured the funding. All authors contributed to the manuscript review and approved the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

A.M. is Research Director at the F.R.S.-FNRS, Belgium. The other authors declare no conflicts of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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