JIM Brief Report

doi: 10.1111/joim.13237

Chronic rhinosinusitis is associated with prolonged SARS-CoV-2 RNA shedding in upper respiratory tract samples: A case-control study

B. Recalde-Zamacona[1,2], A. Tomás-Velázquez[1,3], A. Campo[1,2], B. Satrústegui-Alzugaray[4], M. Fernández-Alonso[1,5], M. Iñigo[1,5], M. Rodríguez-Mateos[1,5], M. Di Frisco[1,2], C. Felgueroso[1,2], J. Bertó[1,2], M. Marín-Oto[1,2], A. B. Alcaide[1,2], J. J. Zulueta[1,2], L. Seijo[1,2,†] & M. F. Landecho[1,6,†]

From the [1]Covid19 Department; [2]Pulmonary Medicine Department, Clinica; [3]Dermatology Department, Clinica Universidad de Navarra; [4]Primary Care, Health Center of San Juan; [5]Microbiology and Infectious Diseases Division; and [6]Internal Medicine department, Clinica Universidad de Navarra, Pamplona, Spain

Abstract. Recalde-Zamacona B, Tomás-Velázquez A, Campo A, Satrústegui-Alzugaray B, Fernández-Alonso M, Iñigo M, Rodríguez-Mateos M, Di Frisco M, Felgueroso C, Bertó J, Marín-Oto M, Alcaide AB, Zulueta JJ, Seijo L, Landecho MF (Clinica Universidad de Navarra; Health Center of San Juan, Pamplona, Spain). Chronic rhinosinusitis is associated with prolonged SARS-CoV-2 RNA shedding in upper respiratory tract samples: A case-control study. *J Intern Med* 2021; **289**: 921–925. https://doi.org/10.1111/joim.13237

Background. SARS-CoV-2, the COVID-19 causative agent, has infected millions of people and killed over 1.6 million worldwide. A small percentage of cases persist with prolonged positive RT-PCR on nasopharyngeal swabs. The aim of this study was to determine risk factors for prolonged viral shedding amongst patient's basal clinical conditions.

Methods. We have evaluated all 513 patients attended in our hospital between 1 March and 1 July. We have selected all 18 patients with prolonged viral shedding and compared them with 36 sex-matched randomly selected controls. Demographic, treatment and clinical data were systematically collected.

Results. Global median duration of viral clearance was 25.5 days (n = 54; IQR, 22–39.3 days), 48.5 days in cases (IQR 38.7–54.9 days) and 23 days in controls (IQR 20.2–25.7), respectively. There were not observed differences in demographic, symptoms or treatment data between groups.Chronic rhinosinusitis and atopy were more common in patients with prolonged viral shedding (67%) compared with controls (11% and 25% respectively) ($P < 0.001$ and $P = 0.003$). The use of inhaled corticosteroids was also more frequent in case group ($P = 0.007$). Multivariate analysis indicated that CRS (odds ratio [OR], 18.78; 95% confidence interval [95%CI], 3.89–90.59; $P < 0.001$) was independently associated with prolonged SARS-CoV-2 RNA shedding in URT samples, after adjusting for initial PCR Ct values.

Conclusion. We found that chronic rhinosinusitis and atopy might be associated with increased risk of prolonged viral shedding. If confirmed in prospective trials, this finding might have clinical implications for quarantine duration due to increased risk of pandemic spread.

Keywords: Chronic rhinosinusitis, SARS-CoV-2 shedding, upper respiratory tract, atopy, inhaled corticosteroids.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the COVID-19 causative agent, has infected millions of people and killed over 1.6 million worldwide. Transmission of COVID-19 depends on SARS-CoV-2-loaded microdroplets generated mainly in the upper respiratory tract (URT), by coughing, sneezing and/or talking. These microdroplets can be aerosolized and remain suspended in air currents drifting away considerable distances. Finally, they settle on a surface. However, human activity can re-aerosolize it. Medical procedures such as tracheal intubation, non-

[†]L. Seijo and M.F. Landecho have equally contributed to the development of the document.

invasive ventilation, or bronchoscopy can also aerosolize SARS-CoV-2 particles. SARS-CoV-2 in the microdroplets can be detected by real-time polymerase chain reaction (RT-PCR) tests or by the ability to replicate the virus in cultured cells. This last detection technique is a better surrogate for infectivity, but it is not used in routine practice as it is time-consuming and can only be performed by specialized laboratories [1]. A positive PCR test result does not necessarily imply the presence of viable virus with potential to infect, although cycle threshold (Ct) values may be a useful proxy for infectivity [1]. The Ct value (number of PCR cycles required for the sample fluorescence to exceed a predefined threshold) is inversely related to the initial viral load, and every 3.3 increase in the Ct value reflects a 10-fold reduction in viral RNA [2].

Previous observational studies have reported some risk factors associated with prolonged viral shedding in the setting of COVID-19, including old age, severe illness and need for invasive mechanical ventilation amongst others [3, 4]. Many patients with prolonged admission achieve viral clearance during hospitalization and have lower risk of pandemic spread. However, little is known regarding the clinical characteristics of patients with mild to moderate disease and prolonged viral shedding, who are more likely to be the main transmitters of the disease. The aim of this study was to determine risk factors for prolonged viral shedding amongst patient's basal clinical conditions.

Methods

We selected all survivors older than 18 years with prolonged positive RT-PCR on nasopharyngeal swabs amongst 513 hospitalized patients during the first wave of the COVID-19 pandemic in the University Clinic of Navarra, Pamplona, Spain (from March to June, 2020). Prolonged viral shedding was defined as a positive RT-PCR result for more than 28 days, and 18 cases were included. We selected a control population consisting in 36 sex-matched COVID-19 patients who turned RT-PCR negative in less than 28 days after admission. In both groups, a cycle threshold value (Ct) of less than 40 was defined as a positive test result, whilst a Ct value of 40 or more was defined as a negative result [2]. Despite presenting late clearance of SARS-CoV-2, patients who died were excluded.

Characteristics of patients (age, sex, smoking condition, comorbidities or respiratory diseases) and infection (severity, time from onset to admission, days of admission, symptoms and treatments) were obtained retrospectively from electronic medical records. Atopy was defined as the presence of allergic underlying diseases such as rhinosinusitis, asthma and atopic dermatitis. Chronic rhinosinusitis (CRS) was defined as the presence of rhinorrhoea, cough, sneezing and/or nose itching for more than 3 months in the previous 12 months, suggesting persistent mucosal inflammation of the nose and paranasal sinuses [5]. The study was approved by the institutional Ethics Committee. All patients gave informed consent.

Statistical analysis was performed using Stata 12 (StataCorp. 2011. *Stata Statistical Software: Release 12*. College Station, TX: StataCorp LP). Continuous variables were expressed as means (standard deviation (SD)) or medians (interquartile range (IQR)) and were compared by t-test or parametric tests as appropriate. Categorical variables were expressed as number (%) and compared by chi-square (χ^2) or Fisher exact test as appropriate. Significant risk factors for late clearance of SARS-CoV-2 identified on univariate analyses were further analysed by multivariate logistic regression. The significance level of the hypothesis tests was set at 0.05 (2-sided).

Results

A total of 54 patients (18 cases and 36 controls) were included. Demographic and clinical characteristics are summarized in Table 1.

Amongst basal and previous conditions, there were no significant differences in sex, age or smoking status. CRS and atopy were more common in patients with prolonged viral shedding (67% each) compared with controls (11% and 25% respectively) ($P < 0.001$ and $P = 0.003$). The use of inhaled corticosteroids was also more frequent in patients with prolonged viral shedding (33.3%) compared to the control group (5.6%) ($P = 0.007$).

In respect to COVID-19 infection, no differences were observed in symptoms or treatment between cases and controls (Table 1). The median time from the onset of symptoms to admission was 7 days (IQR 5–10, $P = 0.977$). No significant difference was found in disease severity (55.6% vs. 36.1%; $P = 0.173$), although prolonged hospital stay was more common in cases (14 vs. 10 days; $P = 0.006$).

Table 1. *Demographic and clinical characteristics. Bold* P-*values indicate statistically significant results*

Variable	Case ($N = 18$) N (%)	Control ($N = 36$) N (%)	P-value
Age (years)	Mean: 66.7 SD: 18.8	Mean: 61.3 SD:14.8	0.25
Sex			1
Male	8 (44.4)	16 (44.4)	
Female	10 (55.6)	20 (55.6)	
Smoking			0.697
Never	11 (61.1)	20 (55.6)	
Current/Former	7 (38.9)	16 (44.4)	
Comorbidities			
High Blood Pressure	8 (44.4)	18 (50)	0.700
Diabetes	1 (5.5)	10 (27.7)	0.056
Dyslipemia	6 (33.3)	13 (36.1)	0.840
Obesity	4 (22.2)	7 (19.4)	0.811
Cardiovascular disease	3 (16.7)	6 (16.7)	1
Cancer	3 (16.6)	5 (13.9)	0.786
Immunodeficiency	3 (16.6)	3 (8.3)	0.358
Atopy	12 (66.7)	9 (25)	**0.003**
Respiratory diseases			
COPD	1 (5.5)	4 (11.1)	0.507
Asthma	4 (22.2)	3 (8.3)	0.152
Pneumonia	4 (22.2)	9 (25)	0.822
Chronic pharyngitis	1 (5.5)	4 (11.1)	0.507
Chronic rhinosinusitis	12 (66.7)	4 (11.1)	**<0.001**
Severity of disease			0.173
Mild or Moderate	8 (44.4)	23 (63.9)	
Severe or Critical	10 (55.6)	13 (36.1)	
Time from onset to admission	Median: 7 IQR: 4–10	Median: 7 IQR: 5–8	0.554
Days of admission	Median: 14 IQR: 8–22	Median: 10 IQR: 7–12	**0.006**
Symptoms			
Fever	16 (88.9)	32 (88.9)	1
Dyspnoea	8 (44.4)	19 (52.8)	0.564
Cough	10 (55.6)	23 (63.9)	0.554
Asthenia	8 (44.4)	20 (55.6)	0.441
Anosmia	9 (50)	16 (44.4)	0.700
Ageusia	9 (50)	17 (47.2)	0.847
Rhinorrhoea	4 (22.2)	5 (13.9)	0.439
Diarrhoea	7 (38.9)	20 (55.6)	0.248
Cephalea	5 (27.8)	18 (50)	0.120
Dermatological	5 (27.8)	7 (19.4)	0.487
Previous treatment			
Systemic Corticosteroids	0 (0)	0 (0)	
Inhaled Corticosteroids	6 (33.3)	2 (5.6)	**0.007**
COVID-19's Treatment			
Hydroxychloroquine	16 (88.9)	36 (100)	0.107
Azithromycin	16 (88.9)	28 (77.8)	0.322
Lopinavir/Ritonavir	12 (66.7)	17 (47.2)	0.177
Tocilizumab	3 (16.6)	6 (16.7)	1
Interferon beta	1 (5.5)	4 (11.1)	0.655
Systemic Corticosteroids	7 (38.9)	10 (27.7)	0.407

Median duration of viral clearance was 48.5 days in cases (IQR 38.7–54.9 days) and 23 days in controls (IQR 20.2–25.7), respectively. The longest duration until SARS-CoV-2 RT-PCR became negative was 85 days.

The Ct values of SARS-CoV-2 RT-PCR at diagnosis did not show significant differences between groups (gen N, $P = 0.38$) (gen E, $P = 0.34$). However, when comparing patients with and without chronic rhinosinusitis, significant differences were found in the E gene ($P = 0.036$) whilst no differences were observed in the N gene ($P = 0.084$). In addition, 86% cases with Ct values below 35 on day 30 of infection presented CRS.

Multivariate analysis indicated that CRS (odds ratio [OR], 18.78; 95% confidence interval [95% CI], 3.89–90.59; $P < 0.001$) was independently associated with prolonged SARS-CoV-2 RNA

shedding in URT samples, after adjusting for initial PCR Ct values.

Discussion

We report a preliminary case–control study which suggests that CRS and atopic phenotypes are associated with prolonged SARS-CoV-2 viral shedding. If corroborated by larger, multicentre, prospective studies, these findings may condition quarantine policies [6, 7].

Current World Health Organization (WHO) guidelines for COVID-19 symptomatic patients recommend isolation during at least 10 days after symptom onset plus at least 3 additional days without symptoms [7]. In line with the WHO, the Centers for Disease Control and Prevention (CDC) recommend similar quarantine time frames for most people and specific recommendations for patients suffering from severe illness who may produce replication-competent virus beyond 10 days extending the duration of isolation and precautions for up to 20 days after symptom onset [6]. These recommendations are based on available data showing that the median incubation period is estimated to be 5.1 days, and 97.5% of persons who develop symptoms do so within 11.5 days of infection [8]. Viral RNA levels are detectable in the respiratory tract 2–3 days before symptoms appearance, with a peak at symptom onset, and decline over the following 7–8 days in most patients. However, some patients maintain significant viral loads for prolonged periods of time [9, 10]. Contagion is supposed to decrease rapidly to near-zero after about 10 days from symptom onset in mild to moderately ill patients [1]. SARS-CoV-2 RNA shedding from upper respiratory tract specimens may persist for more than 60 days from the onset of symptoms [11, 12]. That notwithstanding, a persistently positive PCR does not necessarily imply the presence of viable or transmissible virus, especially in recovered patients. Given the uncertainty, cycle threshold (Ct) values may be a useful proxy for infectivity [1]. All our positive cases had a dual-target lower Ct count below 40, which does not necessarily mean contagiousness but translates a prolonged viral load.

We identified a subgroup of 3.5% of our hospitalized patients with prolonged SARS-CoV-2 viral shedding in nasopharyngeal swabs. This subgroup may be of high risk of pandemic spread if international guidelines are strictly adhered to, in the absence of follow-up PCR testing. In fact, the reason for a prolonged length of stay in some of our cases was precisely the persistent finding of a positive PCR prior to hospital discharge. As the aim of the present study was to identify variables related to prolonged viral shedding in survivors of COVID-19, we excluded patients who died. Most of them had SARS-CoV-2 RNA detectable until death [13], were older, and required mechanical ventilation, consistently with other reports [3, 4].

CRS is a prevalent upper airway disease affecting 10% of the general population. Our results suggest that patients with mild-to-moderate COVID-19 and previous CRS are at higher risk of having a significant viral load (below 40 Ct), during a prolonged period of time. Atopy and the use of inhaled corticosteroids also confer higher risk of prolonged SARS-CoV-2 RNA shedding with low Ct count in URT samples, suggesting that upper airway of certain patients may act as a viral reservoir regardless of symptoms. Further studies are required to evaluate clinical and epidemiological role played by late viral clearance, as well as the mechanisms underlying prolonged viral shedding in URT specimens of patients with atopy and CRS.

Our study has some limitations. First, the presence of SARS-CoV-2 RNA does not necessarily correlate with infectivity. Secondly, we excluded fatal cases because most of them had detectable SARS-CoV-2 RNA until death and the time to death could not accurately reflect the duration of infectivity. Finally, interpretation of our findings was limited by the sample size. Further large-scale cohort studies are still needed to better define the risk factors for prolonged viral shedding, including CRS and atopy.

In conclusion, prolonged SARS-CoV-2 RNA shedding in URT specimens was associated with CRS, atopy and chronic use of inhaled corticosteroids in this preliminary, case–control study. Premature release of still infective patients from isolation should be consistently avoided. Policies that permit a timely but safe return to work for infected workers are critical. If confirmed by larger, prospective, multicentre reports, patients with CRS, atopy or those treated with inhaled corticosteroids could require prolonged quarantines to prevent community transmission.

Conflict of interests

None to declare.

Author Contribution

Borja Recalde Zamacona: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (lead); Writing-original draft (lead). **Alejandra Tomás-Velázquez:** Conceptualization (supporting); Data curation (supporting); Investigation (supporting); Methodology (supporting); Writing-review & editing (supporting). **Aránzazu Campo:** Data curation (supporting); Formal analysis (supporting); Methodology (supporting). **Blanca Satrustegui:** Investigation (supporting). **Mirian Fernández-Alonso:** Data curation (supporting). **Melania Iñigo:** Data curation (supporting). **Mariano Rodriguez:** Data curation (supporting). **Madelein Di Frisco:** Investigation (supporting). **Carmen Felgueroso:** Investigation (supporting). **Juan Bertó:** Methodology (supporting); Visualization (supporting); Writing-review & editing (supporting). **Marta Marín-Oto:** Visualization (supporting). **Javier J. Zulueta:** Writing-review & editing (supporting). **Luis Seijo:** Methodology (supporting); Project administration (supporting); Writing-original draft (supporting); Writing-review & editing (supporting). **Manuel Fortún Landecho:** Methodology (supporting); Project administration (supporting); Writing-original draft (supporting); Writing-review & editing (supporting).

References

1 Rhee C, Kanjilal S, Baker M, Klompas M. Duration of SARS-CoV-2 infectivity: when is it safe to discontinue isolation? *Clin Infect Dis* 2020 Aug 25 [cited 2020 Aug 27]; Available from: https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciaa1249/5896916.

2 Tom MR, Mina MJ. To Interpret the SARS-CoV-2 test, consider the cycle threshold value. *Clin Infect Dis* 2020;**71:**2252–4.

3 Xu K, Chen Y, Yuan J *et al.* Factors associated with prolonged viral RNA shedding in patients with coronavirus disease 2019 (COVID-19). *Clin Infect Dis* 2020;**71:**799–806.

4 Chen X, Zhu B, Hong W *et al.* Associations of clinical characteristics and treatment regimens with the duration of viral RNA shedding in patients with COVID-19. *Int J Infect Dis* 2020;**98:**252–60.

5 Kucuksezer UC, Ozdemir C, Akdis M, Akdis CA. Chronic rhinosinusitis: pathogenesis, therapy options, and more. *Expert Opin Pharmacother* 2018;**19:**1805–15.

6 CDC. *Duration of Isolation and Precautions for Adults with COVID-19*. CDC. [cited 2020 Sep 4]. Available from: https://www.cdc.gov/coronavirus/2019-ncov/hcp/duration-isolation.html.

7 WHO. *Criteria for releasing COVID-19 patients from isolation.* [cited 2020 Sep 4]. Available from: https://www.who.int/news-room/commentaries/detail/criteria-for-releasing-covid-19-patients-from-isolation.

8 Lauer SA, Grantz KH, Bi Q *et al.* The incubation period of coronavirus disease 2019 (CoVID-19) from publicly reported confirmed cases: Estimation and application. *Ann Intern Med* 2020;**172:**577–82.

9 To KKW, Tsang OTY, Leung WS *et al.* Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis* 2020;**20:**565–74.

10 He X, Lau EHY, Wu P *et al.* Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nat Med* 2020;**26:**672–5.

11 Lee S, Kim T, Lee E *et al.* Clinical course and molecular viral shedding among asymptomatic and symptomatic patients with SARS-CoV-2 infection in a community treatment center in the Republic of Korea. *JAMA Internal Med* 2020;**180:**1447.

12 Young BE, Ong SWX, Kalimuddin S *et al.* Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. *JAMA - J Am Med Assoc* 2020;**323:**1488–94.

13 Recalde B, García-Tobar L, Argueta A *et al.* Histopathological findings in fatal COVID-19 severe acute respiratory syndrome: preliminary experience from a series of 10 Spanish patients. *Thorax* 2020 Aug 24 [cited 2020 Aug 28]; Available from: http://www.ncbi.nlm.nih.gov/pubmed/32839288.

Correspondence: B. Recalde-Zamacona, Covid19 Department, Clinica Universidad de Navarra, Avenida Pio XII numero 36. CP: 31008. Pamplona, Navarra, Spain.
(e-mail: brecalde@unav.es).