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RESEARCH ARTICLE

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Presence of SARS-CoV-2 RNA in COVID-19 survivors with post-COVID symptoms 2 years after hospitalization: The VIPER study

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Abstract

The SARS-CoV-2 VIrus PERsistence (VIPER) study investigated the presence of long-lasting SARS-CoV-2 RNA in plasma, stool, urine, and nasopharyngeal samples in COVID-19 survivors. The presence of SARS-CoV-2 RNA reverse transcription polymerase chain reactions (RT-PCR) were analyzed within plasma, stool, urine, and nasopharyngeal swab samples in COVID-19 survivors with post-COVID symptoms and a comparison group of COVID-19 survivors without post-COVID symptoms matched by age, sex, body mass index and vaccination status. Participants self-reported the presence of any post-COVID symptom (defined as a symptom that started no later than 3 months after the initial infection). Fifty-seven (57.9% women, age: 51.1, standard deviation [SD]: 10.4 years) previously hospitalized COVID-19 survivors with post-COVID symptoms and 55 (56.4% women, age: 50.0, SD: 12.8 years) matched individuals who had a past SARS-CoV-2 infection without post-COVID symptoms were evaluated 27 (SD 7.5) and 26 (SD 8.7) months after hospital discharge, respectively. The presence of SARS-CoV-2 RNA was identified in three

nasopharyngeal samples of patients with post-COVID symptoms (5.2%) but not in plasma, stool, or urine samples. Thus, SARS-CoV-2 RNA was not identified in any sample of survivors without post-COVID symptoms. The most prevalent post-COVID symptoms consisted of fatigue (93%), dyspnea, and pain (both, 87.7%). This study did not find SARS-CoV-2 RNA in plasma, stool, or urine samples, 2 years after the infection. A prevalence of 5.2% of SARS-CoV-2 RNA in nasopharyngeal samples, suggesting a potential active or recent reinfection, was found in patients with post-COVID symptoms. These results do not support the association between SARS-CoV-2 RNA in plasma, stool, urine, or nasopharyngeal swab samples and post-COVID symptomatology in the recruited population.

KEYWORDS
long-COVID, plasma, post-COVID-19, RNA, stool, urine, viral persistence

1 | INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the agent responsible for the spread of coronavirus disease 2019 (COVID-19) has led to over 776 million confirmed cases and 7 million reported deaths globally.[1] To date, the spread and virulence of the COVID-19 outbreak have been controlled due to rapid advances in the pathogenesis and the development of preventive strategies such as vaccines.[2]

The COVID-19 has also provoked a second healthcare problem, the development of long-lasting symptoms once the acute phase of the infection has passed. The presence of long-lasting symptoms after a SARS-CoV-2 infection is referred to as long-COVID[3] or post-COVID-19 condition.[4] Different meta-analyses reported that up to 25% of COVID-19 survivors report long-lasting symptoms one[5,6] and two[7,8] years after an acute SARS-CoV-2 infection. Individuals experiencing post-COVID symptoms reported worse health-related quality of life[9] and tend to use greater healthcare resources with the consequent increase in direct and indirect medical costs.[10]

The presence of long-lasting post-COVID symptoms is highly heterogeneous and more than 100 post-COVID symptoms affecting cardiovascular, respiratory, neurological, or musculoskeletal systems have been described.[11] Thus, it has been hypothesized that this plethora of post-COVID symptoms can be related to a predominant pathophysiological mechanism.[12] Therefore, better understanding of those mechanisms behind long-COVID is crucial for proper management. Among the different underlying mechanisms proposed, viral persistence is one of the potential factors that could be involved in the presence of long-lasting post-COVID symptoms.[13] This hypothesis is based on the presence of a persistent remnant of SARS-CoV-2 which may be able to generate pathogen-associated molecular patterns (PAMPs), for example, viral RNA or bacterial cells wall, and engage host pattern recognition receptors (PRRs) triggering innate host immune activation. In fact, most published studies have investigated the adaptative immune response associated with the presence of SARS-CoV-2 persistence rather than the long-lasting presence of RNA remnants itself.[14] A recent systematic review identified evidence regarding the presence of SARS-CoV-2 RNA in plasma, stool, urine, or nasal/oral swab samples in people with post-COVID symptoms.[15] This review found that published studies included follow-up periods shorter than 60 days after the infection and they did not include a comparative group of patients without post-COVID symptoms.[15] Thus, the prevalence of SARS-CoV-2 RNA was heterogeneous ranging from 5% to 59% depending on the sample tested.[15]

We present the SARS-CoV-2 VIrus PERsistence (VIPER) study which aimed to investigate the presence of long-lasting SARS-CoV-2 RNA in nonrespiratory (plasma, stool, and urine) as well as nasopharyngeal samples in previously hospitalized COVID-19 survivors with and without post-COVID symptoms. We hypothesized that COVID-19 survivors who develop post-COVID symptoms would exhibit higher prevalence of SARS-CoV-2 RNA than those COVID-19 survivors who do not develop post-COVID symptoms.

2 | METHODS

2.1 | Participants

The VIPER is a case-control study comparing the presence of SARS-CoV-2 RNA in plasma, stool, urine, and nasopharyngeal swab samples between COVID-19 survivors with post-COVID symptoms (cases) and a comparison group of COVID-19 survivors without post-COVID symptoms matched by age (±3 years), sex, body mass index (±3 units) and vaccination status (controls).

Individuals who had been hospitalized due to SARS-CoV-2 infection at two urban hospitals in Madrid (Spain) between September 2020 and March 2021 were screened for eligibility. The predominant variants that circulated at the time of hospitalization were the historical strain (20A.EU2) between September and

December 2020 and the Alpha variant (B.1.1.7) between January and March 2021. The diagnosis of SARS-CoV-2 infection was confirmed in all cases by reverse transcription-polymerase chain reaction (RT-PCR) assay of nasopharyngeal/oral swab samples as well as clinical/radiological findings at hospital admission The study was approved by the Institutional Ethics Committees of all institutions (H12OCT23/418; HUIL/092-20; URJC0907202015920). All participants provided their informed consent prior collecting data.

2.2 | SARS-CoV-2 RNA analysis

SARS-CoV-2 reverse transcription polymerase chain reaction (RT-PCR) was performed in plasma, stool, urine, and nasopharyngeal samples. The microbiological analyses were carried out at Synlab laboratory centers. For SARS-CoV-2 RT-PCR detection the diagnostic method implemented in this laboratory testing was as follows: RNA extraction was carried out using the QIAamp Viral RNA Mini QIAcube Kit (Qiagen), an RT-PCR was performed with the coviNplex™ CE-IVD rt-PCR kit (Molgentix) in a QuantStudio5 Real-Time PCR (Thermofisher®).

RNA extractions from blood and nasopharyngeal samples, and urine and stool samples with some modifications were performed with a QIAmp Viral RNA kit. The coviNplex™ assay is a molecular in vitro diagnostic test being based on widely used nucleic acid amplification technology. The kit contains oligonucleotide primers and dual-labeled hydrolysis probes (TaqMan®) used in RT-PCR for qualitative detection of 2019-nCoV RNA in upper respiratory specimens. Oligonucleotide primers and probes for specific detection of SARS-CoV-2 are designed to target the nucleocapsid gene (N) of the SARS-CoV-2 genome. The kit includes primers/probes for the N1 gene and N2 genes of SARS-CoV-2. An endogenous internal control is also included for the human RNase P gene to confirm RNA integrity and the absence of PCR inhibitors. Samples were considered positive if the cycle threshold (Ct) value was ≤37, as specified by the manufacturer.

2.3 | Clinical data collection

Demographic (age, gender, height, weight), clinical data (medical comorbidities, vaccination status), and clinical data (COVID-19 onset symptoms, intensive care unit, ICU admission, days at hospital) were collected from medical records.

Individuals who agreed to participate in the study were scheduled for a telephone interview performed by experienced healthcare researchers. Participants were asked to self-report the presence of symptoms that appeared the three consecutive months after hospitalization due to SARS-CoV-2 infection and whether the symptom(s) persisted at the time of the study. A predefined list of symptoms including fatigue, dyspnea, anosmia, ageusia, pain, brain fog, hair loss, pain, or concentration loss was systematically used, but individuals were free to report any symptom that they experienced. We used the definition of post-COVID-19 condition as proposed by Soriano et al.[4]: "post-COVID-19 condition occurs in people with a history of probable or confirmed SARS-CoV-2 infection, usually 3 months from the onset of infection, with symptoms that last for at least 2 months and cannot be explained by an alternative medical diagnosis."

Anxiety and depressive symptoms as well as sleep quality were evaluated with the Hospital Anxiety and Depression Scale (HADS) and the Pittsburgh Sleep Quality Index (PSQI) were completed since both questionnaires can be evaluated by telephone.[16] Both the anxiety (HADS-A, 7-items, 0–21 points) and depressive (HADS-D, 7-items, 0–21 points) scales of the HADS were included.[17] The HADS has demonstrated good validity in people with long-COVID.[18] A cut-off score of ≥8 points on each scale has shown good sensitivity and specificity for identifying the presence of anxiety or depressive symptoms.[19] The PSQI (0–21 points) assesses sleep quality during the previous month, where a cut-off of ≥8 points is considered indicative of poor sleeper.[20]

Finally, the Functional Impairment Checklist (FIC), an 8-item disease-specific questionnaire showing good psychometric properties in individuals with long-COVID,[21] was used for evaluating symptoms' severity and function. Four items assess symptoms severity, for example, breathlessness at rest, breathlessness on exertion, general fatigue, and muscle weakness (FIC symptoms, 0–12 points), whereas other four assess limitations in occupational daily living activities, leisure/social activities, basic daily living activities, and instrumental activities of daily living (FIC disability, 0–12 points).[21]

2.4 | Statistical analysis

Data was collected with STATA 16.1 and processed using Python's library statsmodels 0.13.2; Scipy 1.7.3 was used for conducting the statistical tests and statsmodels 0.11.0 for performing p value correction. Data are presented as means (standard deviations, SDs) for quantitative data or as number of cases (percentage) for categorical data. We compared the differences in demographic, clinical, and hospitalization data between COVID-19 survivors with/without post-COVID symptoms with χ^2 or analysis of variance tests as appropriate. The level of significance was set at a priori 0.05, with p-values being corrected by means of the Holm-Bonferroni correction. The analysis was performed using Python's library statsmodels 0.13.2 and Scipy 1.7.3. A priori, the level of significance was set at 0.05. No Type I error correction was used for this analysis.

3 | RESULTS

From 75 hospitalized COVID-19 survivors experiencing post-COVID symptoms invited to participate, a total of 57 (mean age: 51.1, SD: 10.4 years, 57.9% women) were enrolled. In addition, 55 (mean age: 50.0, SD: 12.8 years, 56.4% women) hospitalized patients with microbiological confirmed SARS-CoV-2 infection but without reporting

post-COVID symptoms matched by sex, age, BMI, and vaccination status were also included. Patients who experienced post-COVID symptoms reported a higher number of COVID-19 onset symptoms (mean: 2.6, SD: 1.2) than those not developing post-COVID symptoms (mean: 2.05, SD: 1.35), particularly the presence of dyspnea ($p = 0.03$, Table 1). No other significant difference in hospitalization data was observed (Table 1). Thus, both groups exhibited similar pre-existing comorbidities such as hypertension, obesity, asthma, or diabetes before the infection (Table 1).

The results revealed the presence of SARS-CoV-2 RNA just in three nasopharyngeal samples of COVID-19 survivors with post-COVID symptoms but not in the other samples including plasma, stool, or urine. Accordingly, the prevalence of long-lasting SARS-CoV-2 RNA in nasopharyngeal samples in COVID-19 survivors with post-COVID symptoms 2 years after infection was 5.25%. Finally, SARS-CoV-2 RNA was not identified in any sample of the group of COVID-19 survivors without post-COVID symptoms. Participants with post-COVID symptoms were

TABLE 1 Clinical and hospitalization data according to the presence or absence of post-COVID symptoms.

	Post-COVID symptoms ($n = 57$)	No post-COVID symptoms ($n = 55$)	p Value
Female, *n* (%)	33 (57.9%)	31 (56.4%)	0.915
Age (years)	51.1 ± 10.4	50.0 ± 12.8	0.582
Weight (kg)	77.0 ± 16.0	75.0 ± 15.2	0.392
Height (cm)	167.2 ± 8.5	170.0 ± 9.1	0.112
Body mass index (kg/m^2)	27.5 ± 6.0	26.0 ± 5.0	0.301
Number of pre-existing comorbidities	0.8 ± 0.9	0.6 ± 0.7	0.155
Obesity (pre-existing)	4 (7.0%)	2 (3.5%)	0.439
Hypertension (pre-existing)	9 (15.6%)	9 (16.4%)	0.939
Diabetes (pre-existing)	4 (7.9%)	4 (7.3%)	0.956
Asthma (pre-existing)	4 (7.0%)	7 (12.7%)	0.335
COPD (pre-existing)	1 (1.75%)	0 (0.0%)	0.326
Musculoskeletal pain (pre-existing)	18 (31.6%)	15 (27.2%)	0.674
Cardiac diseases (pre-existing)	4 (7.0%)	2 (3.5%)	0.439
Other diseases (pre-existing)*	19 (33.3%)	7 (12.7%)	0.025*
Number of COVID-19 symptoms at hospital admission*	2.6 ± 1.2	2.05 ± 1.35	0.01*
Fever (COVID-19 onset)	30 (52.6%)	28 (50.1%)	0.899
Dyspnea (COVID-19 onset)*	22 (38.6%)	5 (9.1%)	0.03*
Myalgias (COVID-19 onset)	23 (40.35%)	16 (29.1%)	0.312
Cough (COVID-19 onset)	19 (33.3%)	15 (27.3%)	0.560
Headache (COVID-19 onset)	20 (35.1%)	14 (25.45%)	0.355
Diarrhea (COVID-19 onset)	5 (8.8%)	1 (1.8%)	0.112
Anosmia (COVID-19 onset)	12 (21.1%)	10 (18.2%)	0.731
Ageusia (COVID-19 onset)	5 (8.8%)	7 (12.7%)	0.522
Throat pain (COVID-19 onset)	10 (17.55%)	14 (25.5%)	0.367
Dizziness (COVID-19 onset)	4 (7.0%)	2 (3.65%)	0.439
Days at hospital	12.9 ± 12.6	16.6 ± 12.4	0.365
ICU admission (yes)	28 (12.9%)	29 (13.4%)	0.816
Days at ICU	5.0 ± 11.5	5.8 ± 15.0	0.861

Note: *Significant differences between groups.

Abbreviations: COPD, chronic obstructive pulmonary disease; ICU, intensive care unit.

assessed 27 (SD 7.5) months after the infection, whereas those without post-COVID symptoms were assessed 26 (SD 8.7) months after the infection. At the time of study, the most prevalent post-COVID symptom was fatigue (93%) followed by dyspnea on exertion and musculoskeletal pain (87.7%, Table 2). Each patient exhibited a mean of 2 (SD 2.05) post-COVID symptoms.

In addition, almost 25% of COVID-19 survivors with post-COVID symptoms also exhibited anxiety ($n = 13$, 22.8%) and depressive ($n = 12$, 21.05%) symptomatology against just one (2%) COVID-19 survivors without post-COVID symptoms ($p < 0.001$). Finally, a significantly ($p = 0.01$) greater proportion of COVID-19 survivors with post-COVID symptoms ($n = 38$, 66.6%) reported poor sleep (PSQI ≥ 8 points) as compared with those without post-COVID symptoms ($n = 20$, 36.6%).

TABLE 2 Post-COVID symptomatology in the case group ($n = 57$).

	Post-COVID symptoms
Fatigue	53 (93.0%)
Dyspnea at exertion	50 (87.7%)
Pain	50 (87.7%)
Dyspnea at rest	14 (24.5%)
Memory loss	30 (52.6%)
Cognitive blurring-brain fog	26 (45.6%)
Concentration loss	12 (21.0%)
Hair loss	5 (8.8%)
Palpitations-tachycardia	11 (19.3%)
Skin rashes	2 (3.5%)
Gastrointestinal problems	8 (14.0%)
Diarrhea	7 (12.3%)
Voice problems	6 (10.5%)
Ageusia	2 (3.5%)
Anosmia	7 (12.3%)
Ocular problems	17 (29.8%)
Throat pain	6 (10.5%)
FIC symptoms (0–12)	5.45 ± 2.0
FIC disability (0–12)	4.75 ± 2.9
HADS-A (0–21)	4.6 ± 3.5
Anxiety (HADS-A ≥ 8 points)	13 (22.8%)
HADS-D (0–21)	4.8 ± 3.7
Depression (HADS-D ≥ 8 points)	12 (21.05%)
Sleep quality (0–21)	11.0 ± 4.1
Poor sleep quality (PSQI ≥ 8 points)	38 (66.6%)

Abbreviations: FIC, feline idiopathic cystitis; HADS, Hospital Anxiety and Depression Scale.

4 | DISCUSSION

This study found the absence of SARS-CoV-2 RNA in plasma, stool, or urine samples, 2 years after an acute SARS-CoV-2 infection in previously hospitalized COVID-19 survivors. We identified a prevalence of 5.25% of SARS-CoV-2 RNA in nasopharyngeal samples, suggestive of a potential active or recent reinfection, in those with post-COVID symptoms. These results cannot support an association between the presence of persistent SARS-CoV-2 RNA in the analyzed samples and long-term post-COVID symptomatology in the studied population.

The hypothesis of viral persistence explaining the development of long-COVID is conflicting and three aspects must be considered: sample tested, post-COVID symptoms, and follow-up period.[15] For instance, previous results on the presence of SARS-CoV-2 RNA in nasopharyngeal samples are heterogeneous. Zhang et al.[22] identified that 5.3% of patients with post-COVID symptoms had SARS-CoV-2 RNA shedding in nasal/oral swab samples 45 days after infection. On the contrary and similar to our results Natarajan et al.[23] did not find SARS-CoV-2 RNA in nasal/oral swab samples 4 months after infection. We identified the presence of SARS-CoV-2 RNA in 5.25% of nasopharyngeal samples 2 years after infection. The presence of SARS-CoV-2 RNA in nasopharyngeal sample should not be considered as viral persistence since it could underly an active or recent reinfection, but no correlation neither with post-COVID symptomatology nor with any new COVID-19 acute symptom was identified.

Tejerina et al. identified the presence of SARS-CoV-2 RNA in stool and urine in 15% of COVID-19 survivors with post-COVID symptoms 4 months after infection,[24] but this prevalence dropped to 3.8% at 7 months after the infection.[23] We did not find SARS-CoV-2 RNA neither in stool nor urine in our study up to 2 years on average after the infection, similar to as Natarajan et al. who also did not find viral persistence in stool samples almost 1 year after the infection.[23] Current results would support the hypothesis that viral persistence can be time-dependent suggesting the virus or viral particles may disappear with time.[14]

Previous results on the presence of SARS-CoV-2 RNA in plasma seem to be more consistent since Tejerina et al. who found viral persistence in 44.8% ($n = 13$) of patients 55 days after the infection[24] whereas Craddock et al. observed viral persistence in 59% ($n = 20$) of the patients 7 months after.[25] It is important to consider that Tejerina et al. did not include a group of COVID-19 survivors without post-COVID symptomatology.[24] Thus, Craddock et al. included a small group ($n = 14$) of COVID-19 survivors without post-COVID symptoms and reported the presence of SARS-CoV-2 RNA in plasma in 28% ($n = 4$) of the patients.[25] Our study did not identify SARS-CoV-2 RNA in plasma at a follow-up of 2 years after the acute infection, further supporting the hypothesis that viral persistence decreases with longer follow-up periods.

It has been also proposed that viral persistence could be tissue-specific, that is, the virus would be present in those tissues potentially associated with particular post-COVID symptoms.[14] In

fact, SARS-CoV-2 RNA has been found in different human tissues such as the lungs,[26] the gastrointestinal tract,[27] or the brain[28] which may explain the presence of respiratory (e.g., dyspnea), gastrointestinal (e.g., diarrhea) or cognitive (e.g., brain fog) post-COVID symptomatology, respectively. This hypothesis would be supported by Su et al. who reported an association between the presence of SARS-CoV-2 RNA in nasal/oral swab samples and post-COVID ageusia/anosmia the first month after the infection.[29] Again, the presence of SARS-CoV-2 RNA in nasal/oral swabs could be also interpreted as persistent infection, and not as a viral reservoir. Further studies are needed to support or refute this hypothesis. Thus, the analysis of viral persistence grouping patients with post-COVID symptoms by current identified clusters, for example, cardiorespiratory (fatigue, dyspnea, chest pain, muscle pain, headache, palpitations), systemic inflammatory (dizziness, muscle pain, gastrointestinal symptomatology, hair loss, muscle weakness, sleep disorders) or neurological (headache, anosmia, paresthesia, neuropathy, balance problems, memory problems, visual problems, poor concentration)[30] can lead to more consistent findings.

The results of the current study should be considered according to its strengths and limitations. A first strength is the study design, it should be noted that this is the first case-control study including a comparative group of COVID-19 survivors without reporting post-COVID symptom matched to the case group by age, sex, body mass index, and vaccination status. In addition, both groups exhibited similar pre-existing medical comorbidities, which could not explain the results. Second, the VIPER study has included the longest follow-up period of studies published to date, 2 years after hospitalization. Nevertheless, some limitations should be recognized. First, due to the heterogeneity in the published literature related to biological samples and follow-up periods, we were unable to conduct an "a priori" sample size calculation, accordingly, the sample size could be considered small. However, the consistency of the identified results suggests that a larger sample would not alter the direction of the findings. It should also be considered that these results can only be applied to previously hospitalized COVID-19 survivors, so we do not currently know the presence of SARS-CoV-2 RNA in nonhospitalized individuals. Second, we did not evaluate the presence of SARS-CoV-2 spike protein since the presence of these proteins represents the immune response of the host potentially derived from the viral reservoir, but they do not directly evaluate the presence of SARS-COV-2 RNA.[14] Swank et al.[31] found that persistent SARS-CoV-2 spike proteins in plasma were associated to post-COVID fatigue, accordingly, the lack of presence of persistent SARS-CoV-2 RNA does not exclude the presence of SARS-CoV-2 spike proteins as a response of the host's immune system against the initial infection. Furthermore, this study also identified a fluctuating nature of spike proteins, suggesting that viral persistence can exhibit periods of inactivity associated with the host immune response.[31] In such a scenario, the cross-sectional nature of our study did not permit us to identify the longitudinal evolution of SARS-CoV-2 RNA in the analyzed samples and this potential fluctuating nature of viral persistence. A fluctuating nature of viral persistence would agree with current evidence showing higher prevalence of viral persistence in the first months after an acute infection.[15]

5 | CONCLUSION

This study found the absence of SARS-CoV-2 RNA in plasma, stool, or urine samples in patients with a previous SARS-CoV-2 infection with an average of two post-COVID symptoms 2 years after infection. We identified a prevalence of 5.25% of SARS-CoV-2 RNA in nasopharyngeal samples, suggestive of a potential active or recent reinfection, in individuals with post-COVID symptoms. These results cannot support an association between the presence of persistent SARS-CoV-2 RNA in the analyzed samples and long-term post-COVID symptomatology in our study population.

AUTHOR CONTRIBUTIONS

César Fernández-de-las-Peñas: Conceptualization; visualization; methodology; validation; data curation; writing—original draft; writing—review and editing. **Juan Torres-Macho**: Methodology; validation; data curation; writing—original draft; writing—review; editing. **Maria Ruiz-Ruigómez**: Methodology; validation; data curation; writing—original draft; writing—review and editing. **Estibaliz Arrieta-Ortubay**: Methodology; validation; data curation; writing—original draft; writing—review and editing. **Carolina Rodríguez-Rebollo**: Validation; writing—original draft; writing—review and editing. **Míriam Akasbi-Moltalvo**: Validation; writing—original draft; writing—review and editing. **Virginia Pardo-Guimerá**: Validation; writing—original draft; writing—review and editing. **Pablo Ryan-Murua**: Validation; writing—original draft; writing—review and editing. **Carlos Lumbreras-Bermejo**: Validation; writing—original draft; writing—review and editing. **Oscar J. Pellicer-Valero**: Validation; data curation; writing—original draft; writing—review and editing. **Rocco Giordano**: Validation; writing—original draft; writing—review and editing. **Lars Arendt-Nielsen**: Validation; writing—original draft; writing—review and editing. **Anabel Franco-Moreno**: Methodology; validation; data curation; writing—original draft; writing—review and editing. All the authors cited in the manuscript had substantial contributions to the concept and design, the execution of the work, or the analysis and interpretation of data; drafting or revising the manuscript, and have read and approved the final version of the paper.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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