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Original Article

COVID-19 epidemiological waves cannot be predicted with SARS-CoV-2 viral load

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ABSTRACT

Background: SARS-CoV-2 infection has caused more than 6.6 million deaths worldwide, and its transmission model has been questioned. The viral load of the virus was probably the main driver of the transmission of COVID-19. Therefore, this study assessed the impact of the viral load of SARS-CoV-2 on the epidemiological wave of COVID-19. **Methods:** Samples for COVID-19 were analyzed using RT-qPCR at the Molecular Biology Laboratory of the Hospital Santa Casa de Misericórdia de Porto Alegre. Epidemiological data were drawn from prefectural and state websites. Wavelet analyses with a 3-days filter were used. **Results:** A total of 11.302 positive COVID-19 samples from patients residing in Porto Alegre were evaluated; most patients were female, and the mean age was 44.6 years-old. The median relative viral load (RVL) median was 9.98 copies/mL. **Conclusions:** The relative viral load could not predict the waves of COVID-19, due to the virus mutation. There were periods of high RVL where transmission occurred within 32 days and periods of low RVL where transmission occurred up to 16 days.

Keywords: *viral load; wave analysis; wavelet; RT-qPCR; infectiousness; SARS-CoV-2; COVID-19.*

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BACKGROUND

SARS-CoV-2 is a positive-sense, single-stranded RNA virus that caused several outbreaks of respiratory syndrome in China in 2019, which was followed by a global epidemic[1]. As determined by the World Health Organization (WHO) as a pandemic, COVID-19 has spread rapidly, causing numerous fatal cases. More than 6.6 million deaths and 656 million confirmed cases have been recorded worldwide[2].

The diagnosis of COVID-19 is usually established by real-time reverse transcriptase polymerase chain reaction (RT-qPCR), most commonly using an assay

standardized by the Centers for Disease Control and Prevention (CDC) in the United States[3]. Analysis of RT-qPCR results relies on cycle threshold (Ct) readings, that is, the cycle at which fluorescence accumulates exponentially to cross the threshold, which separates background noise from genomic amplification[4]. In this regard, there is an inverse relationship between Ct and viral load, such that samples with a higher viral load produce lower Cts, and vice versa.

The SARS-CoV-2 viral load was probably the main driver of COVID-19 transmission[5]. In vitro studies have demonstrated that the rate of infectivity by COVID-19 is significantly lower when Ct is higher than 24 cycles, and that for each unit increase in Ct, the odds ratio for infectivity decreases by 32%[6]. Understanding the epidemiological waves of COVID-19 is a more complex process, in which viral transmission is related to evolutionary changes in the virus as well as changes in host immunity and its behaviors[7]. To the best of our knowledge, this is the first study to evaluate the impact of the SARS-CoV-2 viral load on the epidemiological waves of COVID-19.

METHODS

Study design

Retrospective transversal study.

Clinical samples

Patients included in the study tested positive for SARS-CoV-2 using RT-qPCR at Santa Casa de Misericórdia de Porto Alegre, a large tertiary hospital (1,200-beds) and a reference health care institution in Porto Alegre, Southern Brazil. Only patients residing in Porto Alegre were eligible for the study. This study was conducted between March 2020 and March 2022. Clinical samples included nasopharyngeal and oropharyngeal swab samples, bronchoalveolar lavage, and tracheal aspirates. Inpatients and outpatients, adult and pediatric patients were included in the study. Only positive samples from residents of Porto Alegre were considered for this project.

Epidemiologic data

In March 2020, the first case of COVID-19 was confirmed in the state of Rio Grande do Sul (Southern Brazil). Since then, local authorities have implemented a system of flags to provide recommendations on the mobility of people in the state. The flag system was used as a controlled distancing model, involving rules that were adopted according to the region's flag and economic sector, providing four stages of control: yellow, orange, red, and black. It measured two major metric groups: propagation and serviceability from each region of the state. It started on April 30, 2020, and lasted until April 2021. After this, 3As monitoring was used, with the same methodology as flags, but giving each region their own ability to use it or not, with recommendations for Warning, Alert, and Action.

For the purpose of this study, we recorded epidemiologic data, protective measures (flag system), genomic reports of circulating variants, and social distancing in the city of Porto Alegre, Rio Grande do Sul (southern Brazil). WHO's website was also consulted for different moments of the epidemic. According to the sequencing state genomic bulletin, the entry of variant of concern (VOC) Gamma occurred in March 2021, VOC Delta in August 2021, and VOC Omicron in late December 2021[8]. We have previously reported on VOC Alpha and 614G in the state.

Relative viral load (RVL) was associated with the number of COVID-19 hospitalizations, vaccine doses administered in the community, and deaths during the study period.

RT-qPCR for SARS-CoV-2

RNA was extracted from clinical samples using Maxwell RSC Viral Total Nucleic Acid Purification kits (Promega, Wisconsin, USA), and RT-qPCR was performed using the following thermocyclers: Step One Plus, Step One, and 7500 Real-Time (Applied Biosystems, Foster City, CA, USA). Primers and probes were used according to the Centers for CDC protocol for COVID-19 detection[3]. Even though the CDC COVID-19 RT-qPCR test is a qualitative assay, the SARS-CoV-2 viral load was inferred by considering the CT value of the COVID-19 N1 gene and internal control RNAse P, using the following delta calculation: relative viral load (RVL) = 2(-ΔCT)(9), in which ΔCT = CTN1 – CTRNA.

Statistics

Descriptive statistics were used to analyze the data. Qualitative data were analyzed with Chi-square or Fisher's exact test, according to the data distribution. Quantitative data were analyzed using Student's t-test or the Mann-Whitney test, as appropriate. Comparison of viral load according to Variable categories was performed using Mann-Whitney and Kruskal-Wallis tests with Dunn's test for multiple comparisons. Results with a p-value of <0.05 were considered statistically significant. Data analysis was performed using SPSS software (version 25.0; International Business Machines Corporation, New York, United States).

Wavelet analysis

The Wavelet analyses were carried out according to Torrence and Compo[10] and Morettin[11] to analyze the collected data in the energy and Frequency spectra[12]. In this study, only one-dimensional wavelets were applied to identify the time scales of the most relevant processes acting in the data filtered with high- and low-pass Lanczos-cosine filters[12], with a time window of three days (in order to include or avoid incubation period by COVID-19).

The temporality of COVID-19 cases associated with the relative viral load (RVL) was analyzed using the wavelet method with a time series of RVL filtered with a highand low-pass filter. In the wavelet method, a global wavelet power spectrum was used to demonstrate the occurrence of events with high energy over the global spectra over time, thus enhancing moments with a high dominance of the studied variable (RVL).

Ethical approval

This study was approved by the ethical committee of Santa Casa Hospital and Universidade Federal de Ciências da Saúde de Porto Alegre (UFCSPA) (49313421.8.0000.5335).

RESULTS

During the study period, 126,945 samples were tested for COVID-19 using qPCR in Santa Casa de Misericórdia de Porto Alegre. Of these, 11,302 samples were positive from Porto Alegre residents and were therefore included in the study. Most of these patients were female (55.4%), and the mean age was 44.6 years-old (+ standard deviation, sd, 20.8 years-old).

Most samples were combined from oral and nasopharyngeal swabs (99.3%), mostly (96%), representing the first sample tested for qPCR. Along the 666 days analysed, the highest positivity rates occurred in August 2020 (9.3%), March 2021 (9.9%) and January 2022 (11.6%).

Median RVL for samples tested in the study was 9.98 copies/mL (lower range value was <0.001 and upper range value was 4.10^8). The 25th percentile was 0.86, and the 75th percentile was 184.73, as measured using the Livak method (9).

Table 1 shows the association between RVL and demographic data in the study. Regarding age, a significant difference in RVL occurred: RLV was higher in the 0-17 years-old group ($p<0.001$), with a median RVL of 20.8, in comparison to other age groups. The RVL did not vary significantly according to sex. A total of 433 patients were tested more than once, and these had a higher median RVL on the first positive test than on the second ($p>0.001$). Regarding the flag systems, the black flag showed the highest RLV (median of 27.8), the second highest was warning (RVL median of 27.4), and the third was orange (RVL median of 15.5), while the others were lower than unmonitored (RVL median 10). There was no association between median RVL and the number of COVID-19 hospitalizations, vaccine doses, or deaths ($p>0.05$).

Table 1: Demographic variables of patients with COVID-19 and their association with SARS-CoV-2 relative viral load.

Total (n=11.302)		n	%	Median	P25	P75	p-value
Age (years)	0-17	766	6.8	20.8	1.00	170.7	**<0.001**
	18-39	4300	38.0	13.6	1.00	236.8	
	40-59	3431	30.4	10.5	0.80	180.8	
	>=60	2805	24.8	4.4	0.30	115.1	
Sex	Male	5018	44.4	10.7	0.9	199.1	0.129
	Female	6284	55.6	9.3	0.8	172.6	
Flags system	Yellow	0	0	0	0	0	**<0.001**
	Orange	1592	14.1	15.5	0.8	300.8	
	Red	5028	44.5	4.1	0.7	160.3	
	Black	1793	15.9	27.8	1.00	394.3	
	Warning	889	7.9	27.4	1.00	329.9	
	Unmonitored	2000	17.7	10.0	0.6	67.8	

P25: Percentile 25; P75: Percentile 75.

Applying wavelet analysis to the RVL time series, with a low-pass filtered RVL treated with a 3 days window (Figure 1, upper panel), it can be noted that there was a close relationship between the presence of positive cases and the increase in viral load. In addition, the local power spectrum (Figure 1, bottom left panel) showed that positive correlations (i.e., the red contours) were enhanced with RVL values above 600, as well as low correlation with less RVL, enhancing the correlation of RVL within a window of up to 3 days, which are able to exert influence towards high period, reaching 128 days of influence in Nov/20, 64 days in Feb/22. However, despite extreme events related to pandemic peaks, on average, this influence was maintained for approximately 16-32 days. Interestingly, the figure also shows that despite a period of low incidence of occurrence (Apr/21 to Jan/22) the viral charge maintained high values with a decreasing tendency. During this period, there was a peak in the local energy spectra for 64 days on Jul/21.

The most energetic period of occurrence was 8 days, dominating the energy spectra, which was corroborated by Fourier analysis (Figure 1, bottom right panel). A general result from the wavelet analysis is that among the RVL data, there was an average influence of 32 days of occurrence cycles. This result showed that the first COVID-19 variant was able to gradually increase the viral load in patients during 2020, reaching its peak on Nov/20, with 128 days of influence period; after the first wave of vaccines (after Apr/21), the cases reduced as well as the viral charge that oscillated.

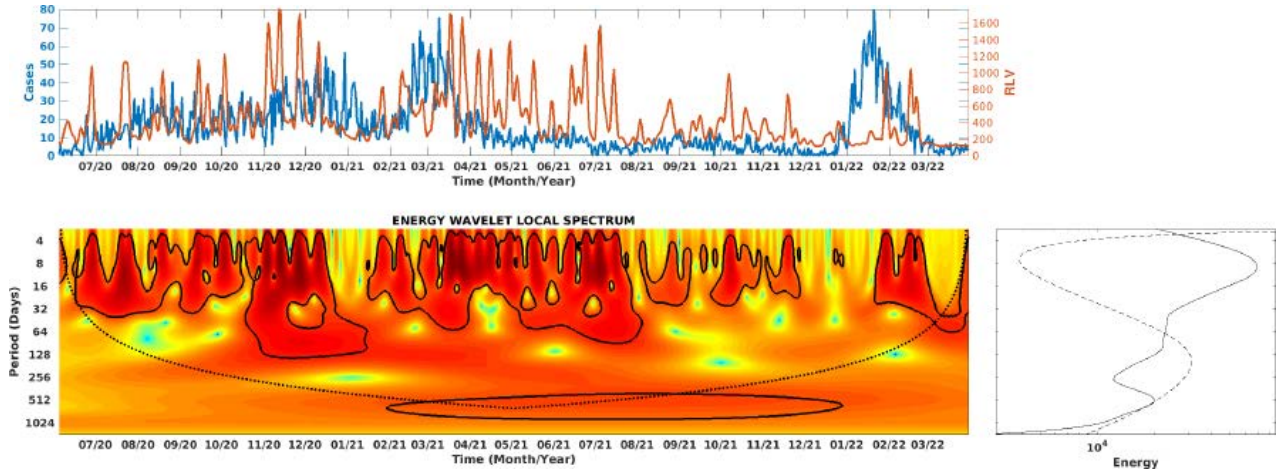

Figure 1: (**Upper panel**) Time series of positive samples (blue line) and relative viral load, RVL, (orange line) treated with a low pass filter of 3 days. (**Bottom left panel**) Local wavelet power spectrum for the time series using Morlet wavelet. Thick contour lines enclose regions of greater than 95% confidence for a red-noise process with a lag-1 coefficient of 0.87. Cross-hatched regions indicate the cone of influence where edge effects become important. (**Bottom right panel**) Global wavelet power spectrum identify period values above the tendency dashed line that represent 95% of confidence. (for interpretation of color in this Figure, the reader is referred to the web version of the article).

High-frequency filtered RVL analysis was performed, showing that the time series (Figure 2 upper panel) has an alternating pattern towards high and low values of RVL resulting from the high variance of RVL within 3 days. The local power spectrum of the wavelet analysis (Figure 2, bottom left panel) confirmed with 95% confidence for cycles occurring from 1 to 3 days that positive samples can exert influence on temporal scales of occurrence shorter than 16 days, meaning that a positive case could be spreading COVID for up to 16 days, thus enforcing the idea of high transmissibility of COVID variants (Figure 2, bottom right panel).

In addition, after the first vaccination period for alpha variation (from Jan/2021), the period of influence of the viral charge decreased, regarding the cycles of 1 to 3 days, reaching no correlation or influence less than 4 days, indicating a significant reduction in RVL and the period of influence that a positive patient can spread COVID. At the beginning of the omicron variation (Feb/22), it is possible to confirm an increase in the viral load, as COVID-19 is immune to the previous vaccine.

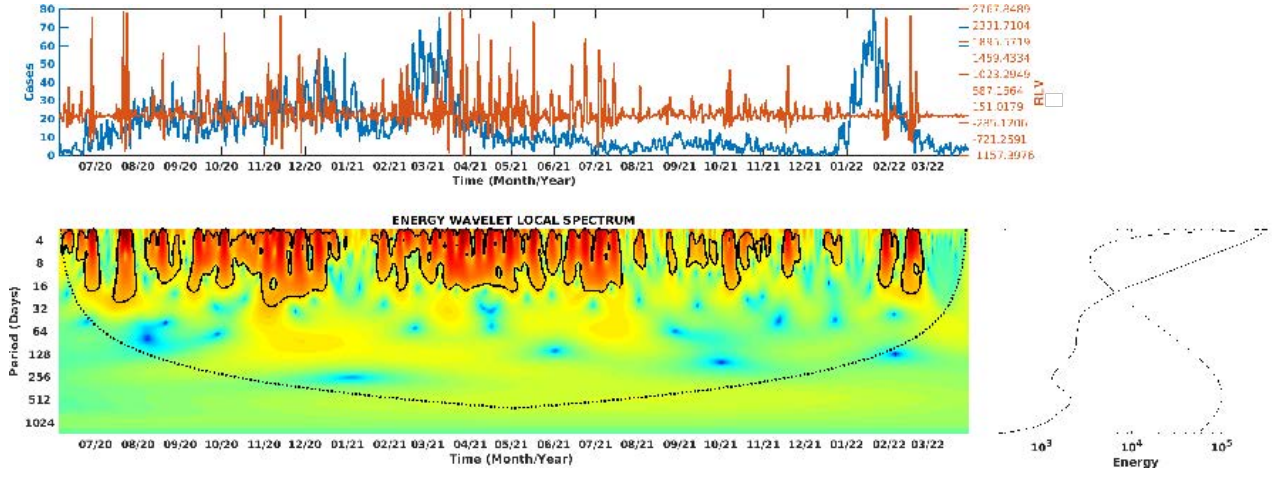

Figure 2: (**Upper panel**) Time series of positive samples (blue line) and relative viral load,RVL, (orange line) treated with a high pass filter of 3 days. (**Bottom left panel**) Local wavelet power spectrum for the time series of power converted at SP site using Morlet wavelet. Thick contour lines enclose regions of greater than 95% confidence for a red-noise process with a lag-1 coefficient of 0.87. Cross-hatched regions indicate the cone of influence where edge effects become important. (**Bottom right panel**) Global wavelet powerspectrum identify period values above the tendency dashed line that represent 95% of confidence (for interpretation of color in this Figure, the reader is referred to the web version of the article).

DISCUSSION

So far, the viral load of SARS-CoV-2 has been related to shedding dynamics of the virus, transmission, immunity, and virus mutations[5,13], but there have been no studies relating the viral load to the emergence of new epidemic waves.

Our findings, based on wavelet analysis with RVL and considering the chronological order of events, revealed an increasing number of COVID-19 cases (the first epidemiological wave) between Jul/20 and Jan/21. After that, the second wave occurred between Mar/21 and Apr/21, following Carnival in Brazil, and the third wave between Jan/22 and Mar/22, following important holidays such as New Year's eve and Carnival. The introduction of novel VOCs and the failure to promote social distancing, including clandestine parties during the epidemic, showed an increase in cases.

It is important to note that in Brazil, 614G and Alpha were the predominant VOCs in the first wave, and the Gama variant already reached its peak of infection during the second wave. Even though the Delta variant entered the state in August 2021, it had a higher RVL, but not so many cases of positive people. The Omicron variant reached its state in December 2021, and is the predominant variant of the third wave[8].

Regarding the sample type, we found a higher RVL in combined swabs of tracheal and nasopharyngeal aspirates than in other types, in accordance with published literature[5].

On a low-frequency graph of RVL, for three days of cases, we note a higher viral load, and the positive cases can still be infectious for up to 32 days; however, there are periods when there is a reduction in this pattern without a high viral load, which we can call a vaccine effect. On a high frequency graph of RVL, 1-3 days of cases, we noted a lower viral load, and the positive cases could be infectious for up to 16 days. Despite the vaccine's effect, it still maintained the existence of new infections in short periods, even with a low viral load.

Although the evolution of variants shows that they are more transmissible, this does not mean that their virulence is greater, such as Omicron, which is less clinically serious than its previous variants[7]. Transmission models have shown that agematched contacts are associated with an increased risk of infection[14], which contributes to the agglomeration rate and typical holidays in Porto Alegre.

The high-frequency result is much more reliable if the sample is controlled, as if all residents of a given neighborhood were evaluated to understand the transmission, as we have transmission data for up to 16 days, but we do not know if this spread occurs in the same location, that is, the infected patient influences the sample globally, but not necessarily locally.

Our study has several limitations. Missing data on prefecture and state websites due to technical problems in the collection. As it is a quantitative qPCR assay, we could not assess which strains were predominant in the hospital for each positive sample. If the sample is controlled for, the high-frequency graph would be even more reliable.

CONCLUSION

In conclusion, we demonstrated that the study of RVL does not allow for the prediction of novel epidemiological waves of COVID-19 in the context of evolving variants. SARS-CoV-2 does not cause a regular illness that maintains the same pattern of infection because many variables can interfere with the SARS-CoV-2 transmission cycle. However, we can notice a pattern of outbreaks, where it occurs again from 500 to 1000 days, not caused by RVL but from its virus cycle, not necessarily deadly or so clinically serious, due to human behavior, host immunity enhancement, evolution of the virus, and vaccination.

Unlike other viruses, SARS-CoV-2 stands out for not following the same pattern of dissemination by viral load, because even at times when we had a high viral load, we had low dissemination, demonstrating that the strains of the virus determine its dissemination.

There are epidemiological periods in which the viral load increases, and it has a prolonged effect on viral transmission for up to 32 days and other periods that have an effect on viral transmission for up to 16 days.

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