High Viral Load in Upper Respiratory Specimens of Persons With SARS-CoV-2 Infection in a Northern Italian Area

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Background: An assessment of viral load in biologic specimens of subjects with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection may have important implications for public health planning. The aim of this study was to estimate the prevalence of high viral load in upper respiratory specimens of patients with SARS-CoV-2 infection during the first Italian wave (spring) and at the beginning of the second wave (summer) of the COVID-19 epidemic, through the measurement of cycle threshold (Ct) values from real-time reverse transcription-polymerase chain reaction tests conducted at the University Hospital of Udine, Italy, serving 530,000 inhabitants.

Methods: We compared the prevalence of high viral load, defined as Ct ≤ 20 at the first positive test result, in 262 subjects from the spring and 453 from the summer period. Logistic regression was used to account for potential confounding due to sex, age, and severity of infection.

Results: In the spring, 9.2% of subjects had Ct ≤ 20 versus 21.4% in the summer. After adjusting for confounders, the likelihood of having high viral load was 2.9 times higher in the summer than in the spring (95% confidence interval, 1.7–5.0).

Conclusions: In this Italian area, more COVID-19 patients had high viral load in the spring epidemic wave than at the beginning of the second, during the summer. Cycle threshold values may represent useful information to monitor viral load at a population level in subjects with SARS-CoV-2 infection.

Key Words: SARS-CoV-2, cycle threshold, viral load, prevalence, Italy

In December 2019, a new outbreak of pneumonia of unknown origin occurred in the city of Wuhan, Hubei Province, China.[1] The infection rapidly spread at a global level, and on January 2020, a global public health emergency was declared.[2] The first 2 cases in Italy was confirmed on January 30, 2020, at the Spallanzani Institute in Rome, and the following day, a state of emergency was declared in Italy. On March 11, 2020, the World Health Organization (WHO) declared that the international outbreak due to the new virus, a coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), had to be considered as a pandemic.[3]

To contain the spread of the SARS-CoV-2 and the quick increase of contagion, prompt identification and isolation of infected persons is crucial. International guidelines recommend the use of tests based on nucleic acid amplification, such as real-time reverse transcription-polymerase chain reaction (RT-PCR or RT-qPCR), which is considered the standard for the diagnosis of SARS-CoV-2 infection.[4]

This analytic method allows for a quantitative analysis of the nucleic acid, through the reverse transcription of viral RNA into DNA (RT) and then the quantitative PCR (in which a fluorescent signal increases proportionally to the amount of amplified nucleic acid). The test result is positive if fluorescence reaches a threshold defined within a given number of PCR cycles (cycle threshold, or Ct). The Ct value is inversely related to the viral load,[5] and each increase in Ct of approximately 3.3 reflects a 10-times reduction of the starting material.

La Scola et al[6] found a correlation between the Ct value and infectivity and observed that low values of Ct are associated with a higher probability of a positive viral culture.

A correlation between Ct values and severity of the disease caused by SARS-CoV-2, named COVID-19, was reported.[7] Zheng et al[8] found significantly higher viral load in patients with severe disease than in those with milder disease, suggesting that viral load might be considered as a prognostic factor. Analogously, Liu et al[9] observed an association between disease severity and Ct value.

Veronesi et al[10] assessed the trend of Ct values during different periods of the COVID-19 outbreak in Italy, showing a significant increase in the Ct values from the first period of the study (February 23 to March 25, 2020, including the first 14 days of the Italian lockdown) to the third (May 19 to June 15, 2020, at the end of the Italian lockdown). However, that study assessed Ct values on the whole pool of specimens collected in the 3 time periods, including those of patients who were clinically healed or collected after 14 days since the beginning of symptoms, which were more frequent in the second and third time periods than in the first.

On the other hand, others suspect that viral load among COVID-19 cases identified after the first Italian wave (ie, after the spring) may be higher than in the first outbreak wave,[11] but this hypothesis has never been confirmed by the scientific literature.

As in the rest of Italy, in the 1,200,000-inhabitant Friuli Venezia Giulia Region, located in the northeast of the country, the incidence of the disease has dropped since the end of April 2020 and new cases were sporadic during the month of June.[12] Since July, nonetheless, the incidence started rising again, favored by the international mobility of immigrant people living in the Friuli Venezia Giulia Region to and from their countries of origin and by the exchange of people leaving the region or coming from other regions or countries for vacation.[12] In addition, various touristic sites (beaches, mountains) in Friuli Venezia Giulia are very

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attractive in the summer, and certainly, there was some tourist influx during that season even in 2020.

An assessment of viral load in biologic specimens of different subjects with SARS-CoV-2 infection may have important implications for public health planning for the upcoming months, for increasing our knowledge on the biology of the infection, and as a possible role as a marker of disease severity and prognosis[13] and of infectivity of subjects.[14]

The aim of this study was to estimate the prevalence of high viral load in respiratory specimens of patients with SARS-CoV-2 infection in the first Italian wave (spring) and at the beginning of the second wave (summer) of the COVID-19 epidemic, through the measurement of Ct values from RT-qPCR tests conducted at the Virology Laboratory of the University Hospital of Udine, in the Friuli Venezia Giulia Region. We also aimed at investigating whether there was an association of Ct values with disease severity and with age and sex of patients.

MATERIALS AND METHODS

This observational retrospective study was conducted at the University Hospital of Udine, whose Virology Laboratory serves a population of 530,000 inhabitants. Two samples of subjects with a first positive result of a RT-PCT test for SARS-CoV-2 conducted according to the WHO guidance[4] at the Virology Laboratory on upper respiratory specimens collected through nasopharingeal swabs were extracted from the electronic database of swab results of the hospital, one for the spring period ("wave 1") and one from the summer period ("wave 2"). Since July, when the incidence of SARS-CoV-2 infection started increasing again after reaching the lowest value in June, Ct values were routinely downloaded from the RT-PCR instruments of the Virology Laboratory; thus, information was available for all first positive test results of patients tested in July, August, and September. On the other hand, Ct values had not been downloaded directly during wave 1, so to limit access to the Laboratory, we sampled examinations

conducted only during the last decade of March, when the maximum incidence of SARS-CoV-2 infection was registered in the area. We considered subjects with Ct ≤ 20 at the first positive test result as having a high viral load.[15]

We compared the prevalence of subjects with high viral load in the 2 periods using the χ^2 test, as well as the prevalence of subjects with high viral load in males and females, the prevalence across age groups (0–14, 15–44, 45–64, 65–74, and ≥75 years) and the prevalence across different disease severities. Disease severity was classified into mild disease if the subject was not admitted to a hospital, moderate if the subject was hospitalized in a nonintensive care unit, and severe if the subject was hospitalized in an intensive care unit (ICU). The decision not to follow up subjects to assess mortality was based on the fact that it could be affected by multiple other factors that we could not control for.

The statistical significance of the difference in the distribution of the actual Ct values in the 2 groups was assessed using the Wilcoxon rank sum test (the distribution of Ct values was not normal according to the Kolmogorov-Smirnov test). The correlation between the subjects' age and Ct values was assessed using the Spearman correlation coefficient. In all statistical tests, P values <0.05 were considered statistically significant.

A multivariate logistic regression analysis was conducted to assess the association between high viral load (ie, Ct ≤ 20) and time period, adjusting for the potentially confounding effect of sex, age, and disease severity. The odds ratio and 95% confidence intervals were calculated. The area under the receiver operating characteristic curve was calculated to summarize the discrimination ability of the model, where a value of 1 corresponds to perfect discrimination and 0.5 to a model with no discrimination ability. A multivariate linear regression analysis was also conducted to assess the association between the actual Ct value and time period, adjusting for the other factors. The association was expressed through the β coefficient.

All the analyses were conducted using SAS v9.4 (SAS Institute Inc., Cary, North Carolina).

TABLE 1. Prevalence of High Viral Load (Ct ≤ 20) Among 337 Subjects With SARS-CoV-2 Infection Stratified by Sex, Age Category, Disease Severity, and Time Period

| | High Viral Load | | | | | |
| | No | | Yes | | | |
	n	%	n	%	Total	P Value of χ^2 Test
Period						<0.0001
Wave 1	238	90.8	24	9.2	262	
Wave 2	356	78.6	97	21.4	453	
Sex						0.2754
Female	317	84.5	58	15.5	375	
Male	277	81.5	63	18.5	340	
Age category, y						0.1141
0–14	42	89.4	5	10.6	47	
15–44	215	79.0	57	21.0	272	
45–64	202	85.6	34	14.4	236	
65–74	63	88.7	8	11.3	71	
≥75	72	80.9	17	19.1	89	
Severity						0.0303*
Mild (no hospitalization)	541	82.5	115	17.5	656	
Moderate (hospitalization, no ICU)	51	92.7	4	7.3	55	
Severe (hospitalization with ICU)	2	50.0	2	50.0		

*P value of Fisher exact test.

Power Considerations

Considering that 262 newly positive subjects were recorded in the last decade of March and 453 in the whole period July–September, we estimated that we had a 99% power to detect a hypothesized prevalence of subjects with high viral load of 15% in wave 1 and of 5% in wave 2 (assumption suggested by results of the Italian study[10]) with a 0.05 α error.

RESULTS

The demographic characteristics of the 262 subjects with a first positive RT-PCR test result in the last decade of March 2020 (wave 1) and of the 453 with a first positive test result in July to September 2020 (wave 2) and the prevalence of high viral load by demographic characteristics, infection severity, and time period are shown in Table 1. Prevalence of high viral load was much higher in wave 2 than in wave 1. No significant association was observed between high viral load and either sex or age category. Prevalence of high viral load was much higher in subjects admitted to ICU than in the others, although they were few cases.

Positive subjects at the beginning of wave 2 were younger than those in wave 1 (mean [median] age, 41.3 ± 21.6 [42] years vs 56.0 ± 21.3 [57] years, respectively; *P* value of Wilcoxon rank sum test < 0.0001). No significant association between high viral load and age group was observed after stratifying the analysis by time period (data not shown). A small inverse correlation between the actual Ct value and age in years was observed in wave 1 (Spearman correlation coefficient = −0.1553, *P* = 0.0118), whereas no correlation emerged in wave 2 (Spearman correlation coefficient = 0.0057, *P* = 0.9043).

Mean Ct values were 28.1 ± 5.4 (median, 29; interquartile range, 24.1–32.8) in wave 1 and 26.4 ± 5.9 (median, 27; interquartile range, 21.0–31.5) in wave 2 (*P* value of Wilcoxon rank sum test = 0.0003). Figure 1 shows the distribution of Ct values in the 2 periods.

Mean Ct values were 26.9 ± 5.8 (median, 27.8; interquartile range, 21.6–32.0) in nonhospitalized patients, 28.4 ± 5.0 (median, 29; interquartile range, 24.6–33.5) in hospitalized patients (no ICU), and 23.6 ± 7.0 (median, 24.4; interquartile range, 17.7–29.5) in ICU patients (*P* value of Kruskal-Wallis test = 0.1268). Figure 2 shows the distribution of Ct values in the 3 groups of patients.

Table 2 shows the association between high viral load and time period, adjusting for sex, age, and disease severity. The likelihood of having high viral load in wave 2 was almost 3 times higher than in wave 1. Patients needing an ICU treatment were 10 times more likely to have high viral load upon the first positive swab result. These results were consistent with those of the multivariable linear regression. Adjusting for all the other factors considered in the analyses, the β coefficient for wave 2 was −1.91 (*P* = 0.0001). Compared with adult subjects, the elderly 75 years or older had a β coefficient of −1.93 (*P* = 0.0081), whereas children aged 0 to 14 years had a β coefficient of 1.73 (*P* = 0.0615). Severe disease (need of ICU care) had a β coefficient of −4.46 (*P* = 0.1239). All the other factors were not associated with the Ct value.

DISCUSSION

In this study, we show that Ct values of the RT-PCR tests for the detection of SARS-CoV-2 in upper respiratory specimens collected through nasopharyngeal swabs were significantly smaller during the first 3 months of the so-called COVID-19 wave 2 (ie, August to September 2020) in the northeastern area around the city of Udine than during the wave 1 epidemic peak (ie, the last 10 days of March 2020), indicating higher viral loads in the upper airways of patients.
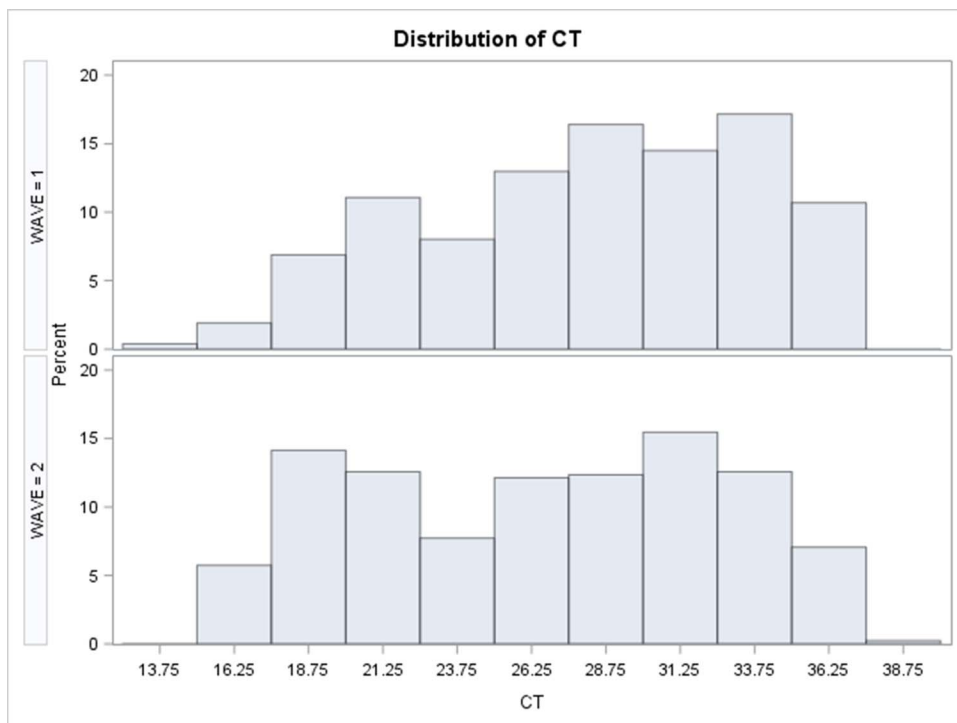

FIGURE 1. Cycle threshold values distribution in 262 Italian subjects who tested positive for SARS-CoV-2 infection in the last decade of March 2020 (wave 1) and in 543 who tested positive from July to September 2020 (beginning of wave 2).

FIGURE 2. Box plots of Ct values distribution in 262 Italian subjects who tested positive for SARS-CoV-2 infection in the last decade of March 2020 (wave 1) and in 453 who teste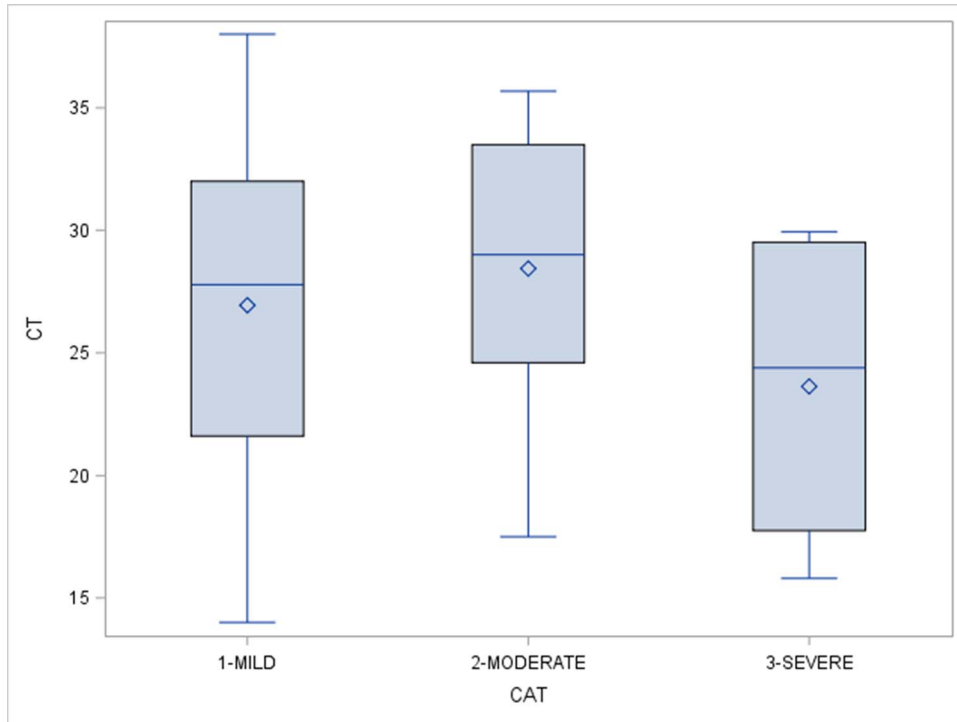d positive from July to September 2020 (beginning of wave 2) by severity of infection (mild, no hospitalized subject; moderate, no-ICU hospitalization; severe, ICU admission). The length of the box represents the interquartile range (the distance between the 25th and 75th percentiles). The symbol in the box interior represents the group mean. The horizontal line in the box interior represents the group median. The vertical lines ("whiskers") issuing from the box extend to the group minimum and maximum values.

Our final results contradicted our initial assumptions, which had been made based on the Italian research by Veronesi et al,[10] which had suggested that viral loads had progressively decreased in specimens from nasopharyngeal swabs since the beginning of the outbreak. Knowledge of COVID-19 is still limited and controversial, and our study contributes new information.

Our finding is very important. In fact, the belief that viral loads were progressively declining or that attenuated SARS-CoV-2 variants were circulating might have contributed to relaxing the restrictive measures that were in place to contrast the spread of COVID-19 during the summer 2020 in Italy to the point that, after having very few new COVID-19 cases by June 2020 (in the whole Friuli Venezia Giulia Region with 1,200,000 inhabitants; according to the Regional Laboratory database, there were 1808 cases in March, 1265 in April, 198 in May, and only 42 in June), the incidence started increasing again (89 in July, 386 in August, 909 in September), reaching 6280 new cases in November.

Previous research produced different findings on the association between Ct values or viral load and infection severity. Some studies found no association between disease severity and initial viral load.[16] Others showed that admission SARS-CoV-2 viral load estimated through Ct among hospitalized patients was associated with the risk of intubation and in-hospital mortality.[17] In our study, although this result is based on very few observations, Ct values of patients requiring ICU care were significantly smaller than the others, indicating that patients with high viral load at the first positive swab result are more likely to develop severe forms of the disease. Knowing the Ct value in addition to just the positive versus negative RT-PCR result might be important to identify subjects who should be more closely monitored or more aggressively treated because of the higher risk to develop serious disease. On the contrary, we fund no significant difference in the Ct value between patients admitted to other hospital wards and

TABLE 2. Results of Multivariable Logistic Regression Analysis* for the Likelihood of Having High Viral Load

	OR	95% CI
Period		
Wave 1	1.0	—
Wave 2	2.9	1.7–5.0
Sex		
Female	0.8	0.6–1.3
Male	1.0	—
Age category, y		
0–14	0.5	0.2–1.5
15–44	1.4	0.8–2.2
45–64	1.0	—
65–74	0.8	0.3–1.9
≥75	1.8	0.9–3.6
Severity		
Mild (no hospitalization)	1.0	—
Moderate (hospitalization, no ICU)	0.7	0.2–2.4
Severe (hospitalization with ICU)	10.6	1.3–83.7

*Area under the ROC curve for the model = 0.6552.

CI indicates confidence interval; OR, odds ratio; ROC, receiver operating characteristic.

those not needing hospitalization. Our finding that elderly patients have lower Ct values is consistent with previous findings of a direct correlation between increasing age and viral load.[16]

This study has some limitations that must be considered when interpreting results. First, we could not retrieve Ct values from all the subjects who had a positive test result between March and June. In fact, given the massive number of RT-PCR tests that had to be processed since October 2020, the Laboratory instruments were not accessible for downloading data because they were busy with the analyses almost 24 hours a day. Our findings might be biased if the missing Ct values were systematically different from the others. Thus, our results may be valid only with reference to the 10 days with the maximum number of new cases and the peak of incidence of the spring outbreak.

Another limitation is that, using only laboratory data, we could not study the association between Ct value at the time of infection diagnosis and long-term outcome because we had no information on potential confounders such as comorbidities or treatment prescribed to the patient.

Caution has been advised when using Ct values from real-time qRT-PCR as direct measures of viral load.[18] We are aware that the actual Ct values that we also explored as a continuous variable may not be directly related to viral load. However, the main measure of interest of our study was the prevalence of subjects with high viral load that we defined as Ct ≤ 20. If, on one hand, this dichotomization is a simplification of the real spectrum of situations, on the other hand, the categorization seems more robust and interpretable.

This study has the great advantage that we only analyzed the first positive test result of each COVID-19 patient, thus excluding the bias originating from the inclusion of follow-up tests. According to the study by To et al,[16] in fact, patients with COVID-19 had the highest viral load near presentation.

Another advantage of our study is that, in both the periods that we analyzed, non-ICU and ICU bed occupancy was not an issue at the hospitals of the Udine area. Thus, using hospital admission as a proxy of disease severity was a valid choice.

Finally, we included in the analyses only tests conducted with the method recommended by the WHO.[4]

In conclusion, we show that in this northeastern Italian area, SARS-CoV-2 viral load increased from the first spring epidemic wave to the beginning of the second, during the summer. At the end of year 2020, Italy is facing a second difficult challenge with more than 17,000 new cases and almost 700 deaths in one single day[19] and more than 67,000 deaths since the beginning of the epidemic.[19] Cycle threshold values may represent useful information to monitor viral load at a population level in subjects with SARS-CoV-2 infection and to identify patients who are at high risk of developing serious forms of the disease.

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