

Different dynamics of mean SARS-CoV-2 RT-PCR Ct values between the first and second COVID-19 waves in the Madrid population

Cristina Rodríguez-Grande^{1,2} | Pilar Catalán^{1,2,3} | Luis Alcalá^{1,2,3} | Sergio Buenestado-Serrano^{1,2}  | Javier Adán-Jiménez^{1,2} | Sandra Rodríguez-Maus^{1,2} | Marta Herranz^{1,2,3} | Jon Sicilia^{1,2} | Fermín Acosta^{1,2} | Laura Pérez-Lago^{1,2}  | Patricia Muñoz^{1,2,3,4} | Darío García de Viedma^{1,2,3}  |
On behalf of Gregorio Marañón Microbiology-ID COVID 19 Study Group*

¹Servicio de Microbiología Clínica y Enfermedades Infecciosas, Hospital General Universitario Gregorio Marañón, Madrid, Spain

²Instituto de Investigación Sanitaria Gregorio Marañón (IISGM), Madrid, Spain

³CIBER Enfermedades Respiratorias (CIBERES), Madrid, Spain

⁴Departamento de Medicina, Universidad Complutense, Madrid, Spain

Correspondence

Darío García de Viedma, Servicio de Microbiología Clínica y Enfermedades Infecciosas, Hospital General Universitario Gregorio Marañón, Madrid, Spain.
Email: dgvedma2@gmail.com

Abstract

SARS-CoV-2 RT-PCR cycle threshold values from 18,803 cases (2 March–4 October) in Madrid define three stages: (i) initial ten weeks with sustained reduction in viral load (Ct: 23.4–32.3), (ii) stability with low viral loads (Ct: 31.9–35.5) in the next nine weeks and (iii) sudden increase with progressive higher viral loads until reaching stability at high levels in the next twelve weeks, coinciding with an increased percentage of positive cases and reduced median age. These data indicate differential virological/epidemiological patterns between the first and second COVID-19 waves in Madrid.

KEYWORDS

COVID-19, Cts, dynamics, first wave, RT-PCR, SARS-CoV-2, second wave

1 | INTRODUCTION

COVID-19 infection laboratory diagnosis is based on the detection of SARS-CoV-2 RNA by RT-PCR. Besides determining the presence of viral genetic material, RT-PCR provides semi-quantitative information on viral load from clinical specimens. This may be estimated from the cycle threshold (Ct) value, defined as the RT-PCR cycle at which the fluorescent signal reaches the detection threshold. The higher the Ct value, the lower the viral load in the specimen.

Several studies in different contexts have identified different added values for Ct values. For example, correlations have been

found between the severity of COVID-19 and Ct values either at diagnosis (Magleby et al., 2020) or associated with the chronological changes throughout infection (Li et al., 2020; Liu et al., 2020), thus indicating its potential usefulness as a prognostic marker (Rao et al., 2020). Cts have also been used as reference to determine infectiousness (He et al., 2020; Singanayagam et al., 2020) or decide when healthcare personnel with a diagnosis of COVID-19 can return to work (Shrestha et al., 2020).

We aimed to determine other possible uses of Ct values beyond patient management, oriented to a population-based approach. That is, we examined the evolution of SARS-CoV-2 viral load along the

*Gregorio Marañón Microbiology-ID COVID 19 Study Group: Adán-Jiménez (Adán), Alcalá (Luis), Aldámiz (Teresa), Alonso (Roberto), Álvarez (Beatriz), Álvarez-Uría (Ana), Arias (Alexi), Arroyo (Luis Antonio), Berenguer (Juan), Bermúdez (Elena), Bouza (Emilio), Burillo (Almudena), Candela (Ana), Carrillo (Raquel), Catalán (Pilar), Cercenado (Emilia), Cobos (Alejandro), Diez (Cristina), Escribano (Pilar), Estévez (Agustín), Fanciulli (Chiara), Galar (Alicia), García (M^a Dolores), García de Viedma (Darío), Gijón (Paloma), González (Adolfo), Guillén (Helmuth) Guinea (Jesús), Haces (Laura Vanessa), Herranz (Marta), Kestler (Martha), López (Juan Carlos), Losada (Carmen Narcisa), Machado (Marina), Marín (Mercedes), Martín (Pablo), Montilla (Pedro), Moure (Zaira), Muñoz (Patricia), Olmedo (María), Padilla (Belén), Palomo (María), Parras (Francisco), Pérez-Granda (María Jesús), Pérez-Lago (Laura), Pérez (Leire), Pescador (Paula), R Maus (Sandra), Reigadas (Elena), Rincón (Cristina), Rodríguez (Belén), Rodríguez (Sara), Rodríguez-Grande (Cristina), Rojas (Adriana), Ruiz-Serrano (María Jesús), Sánchez (Carlos), Sánchez (Mar), Serrano (Julia), Tejerina (Francisco), Valerio (Maricela), Veintimilla (M^a Cristina), Vesperinas (Lara), Vicente (Teresa), de la Villa (Sofía).

COVID-19 pandemic, covering the first and second waves, in the population of 650,000 inhabitants covered by a tertiary hospital in Madrid, Spain.

2 | MATERIALS AND METHODS

The study period included the 18,803 cases, with a Ct value available, diagnosed in our laboratory from Week 10 to Week 40 (2 March–4 October). The first COVID-19 case was diagnosed by RT-PCR on 29 February. We stopped at Week 40 of our observation period because antigenic rapid tests started to be applied at the emergency room in our hospital and at the health centres covering our population. It meant a marked reduction in the number of COVID-19 cases which were diagnosed by RT-PCR which would have biased the data if prolonging our study beyond Week 40 (a reduction of 41.7% in the number of RT-PCRs was registered for Week 41).

2.1 | RNA purification

RNA was extracted and purified from 300 μ l of nasopharyngeal exudates by using for most of the specimens the KingFisher (Thermo Fisher Scientific, Waltham, Massachusetts) system and, alternatively, the EasyMag (Biomérieux, France) equipment. 20 μ l of purified RNA was used as template for the RT-PCR.

2.2 | Diagnostic SARS-CoV-2 RT-PCRs

Along the first COVID wave, due to frequent shortages of reagents, different RT-PCR systems were used: TaqMan 2019-nCoV Assay Kit v1 (Thermo Fisher Scientific, USA), Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (Sansure Biotech, China) and TaqPath 1-step Multiplex Master Mix (Thermo Fisher Scientific, USA). For the second wave, TaqPath 1-step Multiplex Master Mix was the kit mainly used. RT-PCRs were run in the thermocyclers Stratagene, QuantStudio 5 Real-Time PCR system (Fisher Scientific, New Hampshire, USA) and 7,500 Fast Real-Time PCR system (Fisher Scientific, New Hampshire, USA).

In all reactions, we included as controls a SARS-CoV-2 RT-PCR-positive specimen and a negative clinical specimen, which were re-extracted and tested in parallel with the clinical problem specimens. Samples were considered positive if the cycle threshold (Ct) value was ≤ 40 .

3 | RESULTS AND DISCUSSION

The geometric mean for the Ct values (for FAM channel in RT-PCR, corresponding to the detection of the N gene) for the positive cases diagnosed weekly was calculated (considering only the first positive RT-PCR for each patient). The weekly progress over time of Ct mean values was put into context with the total number of patients tested per week and the percentage of positive SARS-CoV-2 cases (Figure 1).

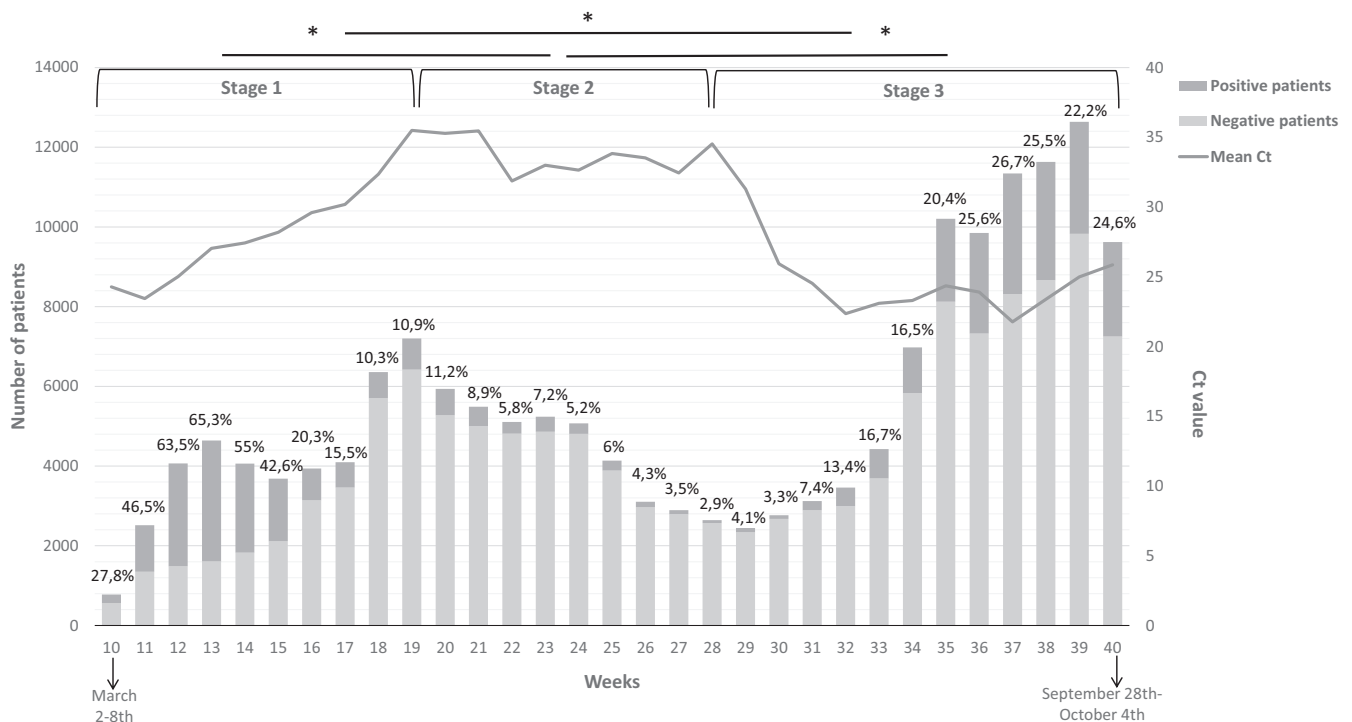


FIGURE 1 Weekly (line) distribution of Ct values (geometric means). A total number of patients with a first RT-PCR diagnostic result for each week are shown as bars, indicating the number of RT-PCR-positive (dark section of the bar) and RT-PCR-negative (clear section of the bar) cases. The value at the top of each bar represents the percentage of positive cases. *Statistically significant differences ($p < .01$) between each of the three periods as per the ANOVA and Tukey HSD tests

Based on mean Ct values, three different stages were clearly differentiated (Weeks 10–19, 20–28 and 29–40), with statistically different behaviours (the ANOVA test was applied for the comparison between geometric means for the three periods; $p < .01$ for all comparisons between the three stages) (Figure 1). In Weeks 10 to 19, a sustained increase in mean Ct values was observed, raising from 24.3 to 35.5. This last value was the highest for the whole study period, suggesting a constant and progressive reduction in SARS-CoV-2 viral load in newly diagnosed cases during the first weeks of the pandemic, at a population-based perspective. This first stage included the weeks with the highest percentages of positive SARS-CoV-2 RT-PCRs (10.3%–65.3%, with percentages $> 40\%$ for five consecutive weeks).

A second stage between Weeks 20 and 28 was defined. Ct values, corresponding to low viral loads, remained stable throughout this stage (Ct: 31.9–35.5, with five weeks stabilized at the 34–35 range) that coincided with a significant decrease in the rate of positive SARS-CoV-2 RT-PCRs (from 11.2% in Week 20 to 2.9% in Week 28). The percentage of positive cases in Week 28 was the lowest for the whole study period, suggesting the final phase of the first wave.

From Week 29 to Week 40, the third stage determined in this study, defining the starting of the second wave, a sudden change occurred in comparison with the stability at high Ct values observed in the second stage. A dramatic reduction in Ct mean values, from 31.3 to 21.8, was observed, followed by a period of stability at low Ct values

(23.4–25.9). For the same period, the percentage of positive RT-PCRs increased from 4.1% to 26.7% and stayed in the 22.2%–25.5% range for the last three weeks in the study. Ct values for this third stage were the lowest registered since the beginning of the pandemic (at ranges corresponding to the highest viral loads). Specifically, for Week 37, Ct values for 45% of the positive RT-PCRs were ≤ 20.0 with $12\% \leq 15.0$.

Several factors may be considered regarding the dynamics of our descriptive analysis. On the one hand, certain virus–host interaction-related features, such as the attenuation described for other RNA viruses due to genetic drift (Armengaud et al., 2020), could explain the progressive reduction in viral load and subsequent stabilization at values associated with low viral burden seen for the two first stages. However, the sharp increase in viral load observed in the last stage rules out this hypothesis. Alternatively, the dynamics may be explained by an increased efficiency in early diagnosis not only of the most severe cases, but also progressively the mild ones. The dynamics for the third current stage are worrying. The low viral load value plateau observed in the previous weeks was interrupted, and a new stage emerged characterized by an alarming progressive increase towards the highest viral load ever registered amongst our population.

The median age of patients showed a decreasing trend throughout the study period. Linear regression analysis revealed that this trend adjusted to the function $\text{Age} = -0.9 \cdot \text{Week} + 73.5$, which corresponds to a mean reduction of 0.9 years per analysed week (Figure 2).

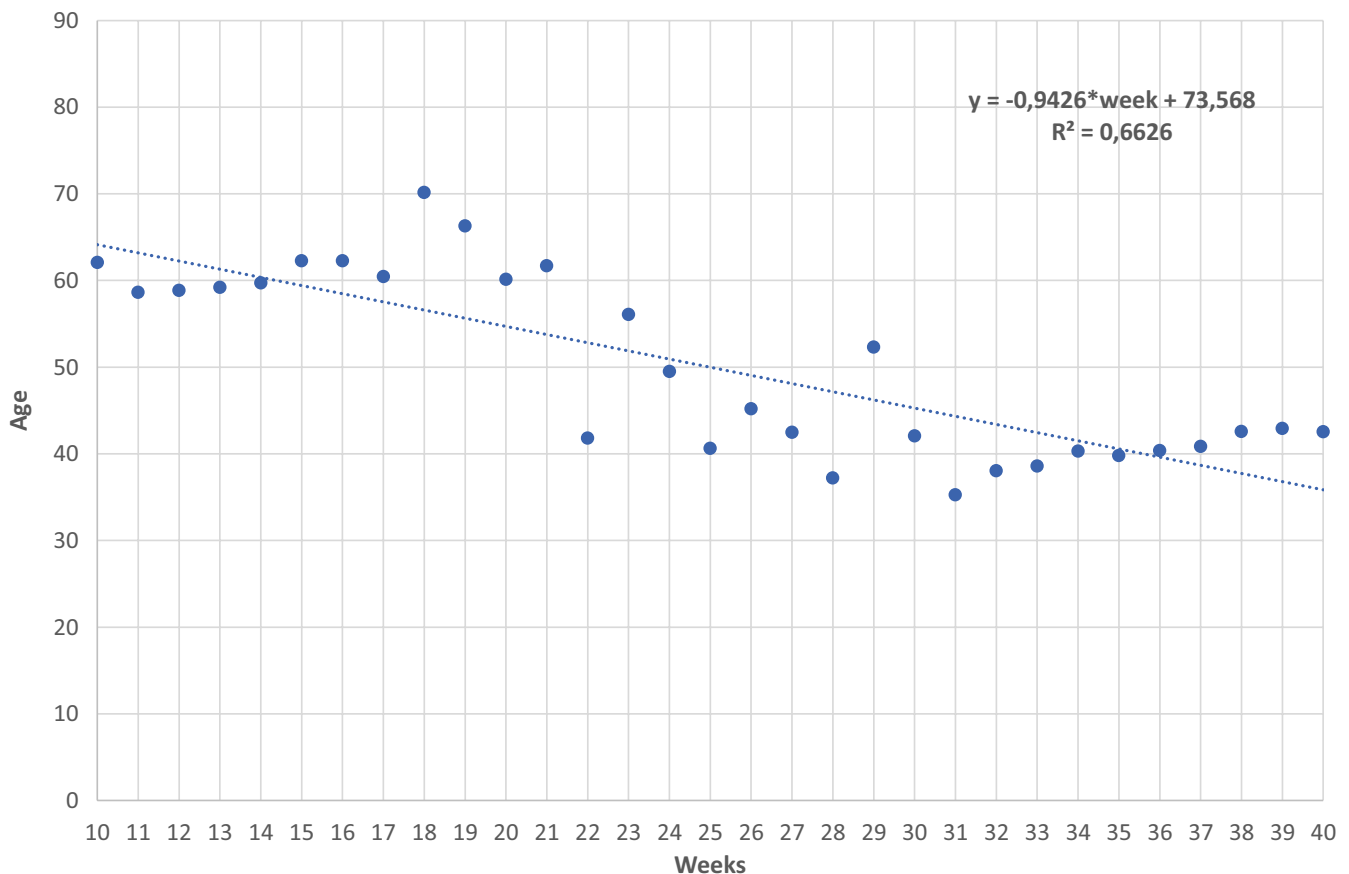


FIGURE 2 Linear regression showing the relationship between median age and weeks. The regression function is shown at the upper right corner

The combination of high viral loads plus younger ages was not observed in the two first stages. Higher transmission rates are expected due to this new pattern and consequently a cause of alarm.

We must assume the limitations of using Ct values as a proxy to estimate viral load. Samples used for COVID-19 diagnosis are mainly nasopharyngeal; thus, different biases, related to the quality and efficiency of the sampling, may influence Ct values, besides the viral load itself (Mathers, 2020). However, our sample size, providing data of nearly 10,000 cases diagnosed in the whole pandemic, should compensate this potential bias. We believe that, despite not being very precise, the information extracted from Ct data may be useful at the population level, similar to the alternative uses given to Ct values for the clinical management of patients.

The analysis of the evolution of the mean Ct values throughout the pandemic in a large population in Madrid illustrates relevant time events: on the one hand, for the first wave, two consecutive stages, an initial progressive reduction in mean viral load followed by a prolonged plateau with stabilized low viral load values; on the other hand, for the second wave, a notorious change in the preceding situation, with marked increase in mean viral load of the diagnosed cases that coincides with higher percentages of positive COVID-19 cases in younger subjects. These two points seem to point to marked differential epidemiological features characterizing the consecutive COVID-19 waves in Madrid.

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CONFLICT OF INTEREST

No author declared a conflict of interest.

ORCID

Sergio Buenestado-Serrano  <https://orcid.org/0000-0003-1739-4459>

Laura Pérez-Lago  <https://orcid.org/0000-0001-5249-7619>

Darío García de Viedma  <https://orcid.org/0000-0003-3647-7110>

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