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RESEARCH ARTICLE

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Relevant SARS-CoV-2 viremia is associated with COVID-19 severity: Prospective cohort study and validation cohort

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Abstract

Early kinetics of SARS-CoV-2 viral load (VL) in plasma determined by quantitative reverse-transcription polymerase chain reaction (RT-PCR) was evaluated as a predictor of poor clinical outcome in a prospective study and assessed in a retrospective validation cohort. Prospective observational single-center study

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including consecutive adult patients hospitalized with COVID-19 between November 2020 and January 2021. Serial plasma samples were obtained until discharge. Quantitative RT-PCR was performed to assess SARS-CoV-2 VL. The main outcomes were in-hospital mortality, admission to the Intensive Care Unit (ICU), and their combination (Poor Outcome). Relevant viremia (RV), established in the prospective study, was assessed in a retrospective cohort including hospitalized COVID-19 patients from April 2021 to May 2022, in which plasma samples were collected according to clinical criteria. Prospective cohort: 57 patients were included. RV was defined as at least a twofold increase in VL within ≤2 days or a VL > 300 copies/ml, in the first week. Patients with RV ($N = 14$; 24.6%) were more likely to die than those without RV (35.7% vs. 0%), needed ICU admission (57% vs. 0%) or had Poor Outcome (71.4% vs. 0%), ($p < 0.001$ for the three variables). Retrospective cohort: 326 patients were included, 18.7% presented RV. Patients with RV compared with patients without RV had higher rates of ICU-admission (odds ratio [OR]: 5.6 [95% confidence interval [CI]: 2.1–15.1); $p = 0.001$), mortality (OR: 13.5 [95% CI: 6.3–28.7]; $p < 0.0001$) and Poor Outcome (OR: 11.2 [95% CI: 5.8–22]; $p < 0.0001$). Relevant SARS-CoV-2 viremia in the first week of hospitalization was associated with higher in-hospital mortality, ICU admission, and Poor Outcome. Findings observed in the prospective cohort were confirmed in a larger validation cohort.

KEYWORDS

COVID-19, disease severity, poor outcome, SARS-CoV-2, viremia

1 | INTRODUCTION

Nearly 2 years after the pandemic broke out, coronavirus disease 2019 (COVID-19) is still conditioning our way of life, health, and economy. To date, approximately 412 million people have been infected and 5.8 million have died.[1] One of the pending challenges is the prediction of COVID-19 severity in hospitalized patients. Several parameters have been proposed as biomarkers due to their association with severity, such as lymphocyte count, ferritin, D-dimer, interleukin-6 serum levels, among others.[2–4] Nevertheless, the factors implicated in disease worsening are still uncertain.

Some authors have proposed SARS-CoV-2 RNA detection in peripheral blood as a prognostic biomarker.[5,6] Nonetheless, its usefulness in clinical practice is controversial. First, the prevalence of SARS-CoV-2 viremia in hospitalized patients varies considerably between studies (1%–92% in different series).[7–9] Second, while some studies showed a correlation between viremia and inflammation, disease severity, and mortality,[10–12] others did not find such association.[13]

Some studies[14–16] considered a viral load threshold to define viremia in critically ill patients and related this parameter to the risk of mortality; however, viremia was not associated with extrapulmonary organ failure.[16]

Recent studies evaluating SARS-CoV-2 viremia during the course of illness and its relationship to disease severity found that the viral load was higher in those patients with more severe disease and mortality.[14,15,17–19]

The aim of this study was to prospectively assess the predictive capacity for clinical worsening of the early kinetics of SARS-CoV-2 viremia determined by quantitative reverse-transcription polymerase chain reaction (RT-PCR) in plasma, and its usefulness for a rapid risk stratification of COVID-19 patients that might help improve management. A composite variable, relevant viremia (RV), associated with poor prognosis was established and assessed in a retrospective validation cohort.

2 | MATERIAL AND METHODS

2.1 | Study design, population, and data collection

This is a prospective observational study conducted at Hospital Universitario La Princesa (HUP) between November 1, 2020 and January 15, 2021. The patient's inclusion flowchart is shown in Supporting Information: Figure 1.

The inclusion criteria were: (a) positive RT-PCR for SARS-CoV-2 in nasopharyngeal and throat swabs, at most 48 h before hospitalization; (b) acceptance to participate in the study and oral or written informed consent; (c) age higher than 18 years; (d) need for hospitalization. The exclusion criteria were: (a) patients without a

baseline viremia determination in the first 24–36 h after admission; (b) patients who could not be followed-up because they were candidates to be referred to other facilities.

Clinical, laboratory, and therapeutic data were collected from electronic clinical records and then, included in an anonymised database as previously described.[3] Baseline clinical and laboratory data are those obtained at admission day.

The need for hospitalization was decided by the physicians at the emergency room based on clinical criteria, without the intervention of the research team. Patient's treatment and management was decided by attending physicians based on the hospital protocols and their clinical judgment. Attending physicians were blind to the viremia results.

Results obtained with the prospective cohort were validated in a retrospective cohort. This validation cohort retrospectively included all COVID-19 patients hospitalized from April 2021 to May 2022, if they had viremia determinations in plasma samples during the first week of hospitalization, always by decision of their treating physicians based on clinical criteria.

Samples from the prospective cohort were used in a previous study to assess the usefulness of commercially available RT-PCR techniques to determine SARS-CoV-2 viral load kinetics in peripheral blood from hospitalized COVID-19 patients.[20]

2.2 | Sample size

For the prospective cohort, the sample size required to find significant differences in the outcome in-hospital mortality between patients with and without SARS-CoV-2 viremia was calculated based on the results of our previous retrospective study.[6] Using the GRANMO sample size calculator,[21] 29 patients were estimated to be required in the negative viremia group and 27 in the positive viremia group to detect significant differences regarding COVID-19 clinical worsening.

For the validation cohort, all patients who met the study criteria from April 2021 to May 2022 were included.

2.3 | Sample collection

Serial plasma samples were collected during the whole hospitalization in the prospective cohort. The first sample was collected within the first 24–36 h from admission. During the first week of hospitalization, samples were obtained every 48 h. After the 7th day of hospitalization, samples were collected twice a week until discharge. In the retrospective, cohort samples were obtained at time points based on clinical criteria. Surpluses of samples collected at admission were used to analyse the viral load in nasopharyngeal swabs. All plasma and nasopharyngeal samples were frozen at −80°C.

The samples included in the validation cohort were collected at time points according to the criteria of the attending physician.

2.4 | SARS-CoV-2 viral load

SARS-CoV-2 viral load was determined by quantitative RT-PCR with the TaqPath™ COVID-19 CE IVD RT-PCR kit (Thermo Fisher Scientific) from serial plasma samples, using a standard quantification curve. Viral load was assessed using a previously described method[20] and was expressed as copies/ml and log10 of viral load. The ability of the TaqPath™ Kit for the detection of SARS-CoV-2 viremia was evaluated in a previous retrospective study.[22]

Nucleic acid extraction from plasma and nasopharyngeal swab (NP-S) samples was performed by the automatic eMAG® Nucleic Acid Extraction System (Biomerieux), according to manufacturer's indications. An initial volume of NP-S or plasma samples of 400 µl was inactivated with 400 µl of NUCLISENS® easyMag® Lysis Buffer (Biomerieux). Purified nucleic acids were obtained in 60 µl of elution buffer. The RT-PCR assay was performed, adding 10 µl of the eluate obtained according to the manufacturer's instructions, by a QuantStudio™ 5 Real-Time PCR System (Applied Biosystems). Amplification curves were analysed with QuantStudio™ Design and Analysis software version 2.4.3 (Applied Biosystems).

Plasma and NP-S samples were analysed in duplicate and viral load quantification of samples was obtained by plotting Ct values through the standard curve. Samples were considered quantifiable when mean Ct in the duplicate test for each gene was ≤37 and standard deviation (SD) was <0.5; all results not fulfilling these criteria and/or those with detection in only one duplicate, were considered positive, but not quantifiable.

2.5 | SARS-CoV-2 viral load kinetics in blood

Analysis of SARS-CoV-2 viremia kinetics in plasma was performed in the prospective group, because successive samples until discharge for each patient were only available in this group. Time-course curves were obtained plotting viral load change over time and their relationship with clinical evolution was analysed.

2.6 | Variables

SARS-CoV-2 viremia was referred to the detection of viral RNA in plasma and was analysed as a quantitative variable, expressed as viral load in copies/ml and in log10 viral load.

The composite variable RV was defined based on the cut-off determined by the receiver operating characteristic (ROC) analysis (see below) and SARS-CoV-2 viral load increases observed within the first hospitalization week. Patients were classified as having RV when viremia levels exceeded 300 copies/ml or, alternatively, when SARS-CoV-2 viral load increased at least twofold within ≤2.0 days, during the first week.

Three main outcomes in the study were considered to assess RV as a prognostic biomarker: in-hospital all-cause mortality, Intensive Care Unit (ICU) admission and the combination of both

(Poor outcome). Survival time and length of hospital stay were also analysed as secondary outcomes.

Baseline clinical and analytical variables were considered at the day of admission.

2.7 | Statistical analysis

Stata 14.0 for Windows (Stata Corp LP) was used for all the analysis described below. All quantitative variables followed a nonnormal distribution; they were represented as median and interquartile range (IQR), and the Mann–Whitney tests was used to assess significant differences. Qualitative variables were described as counts and proportions and χ^2 or Fisher's exact test was used for comparisons.

ROC curve analyses were performed to estimate the best cut-off point of baseline viremia for the composite endpoint of ICU admission and mortality during hospital admission (Poor outcome). A cut-off value for RV was selected based on the best trade-off between specificity and sensitivity.

Survival time and time to discharge were analysed by the Kaplan–Meier method. Differences in time to death or to discharge between different variables were analysed by log-rank test.

2.8 | Ethics

This study was approved by the Research Ethics Committee of Hospital Universitario La Princesa, Madrid, (register number 4267 for the prospective cohort and 4746 for the retrospective group). As proposed by The Spanish Agency for Medicines and Medical Devices (AEMPS for its acronym in Spanish), all included patients (or their representatives) only gave oral informed consent, due to the COVID-19 emergency.[23] Oral consent was registered in the electronic clinical chart.

This article was written following the STROBE initiative (Strengthening the Reporting of Observational studies in Epidemiology).

3 | RESULTS

3.1 | PROSPECTIVE COHORT

3.1.1 | Demographic and clinical characteristics of the study population

A total of 57 patients were included in the prospective cohort, their median age was 63 years (IQR = 52–81), 61% were male and 68% were caucasian. Regarding comorbidities, 75% had previous pathologies, being dyslipidaemia and hypertension the most frequent ones (42% and 40%, respectively). A total of 300 plasma samples were collected. The median number of viremia determinations per patient was three (IQR = 2–5).

Ten patients (17.5%) had at least one of the two main outcomes: five (8.8%) patients died and eight (14.0%) needed ICU admission during their hospital stay. Some patients had both outcomes during hospitalization. Two the five patients with in-hospital mortality died in the ward without ICU admission. Three out of eight patients admitted to ICU died. No patients were lost to follow-up. Baseline clinical characteristics are shown in Table 1. Baseline analytical variables are shown in Supporting Information: Table 1. At the time of study, no SARS-CoV-2 variants of concern (VOC) were identified in the locations where the patients were enrolled.

3.1.2 | Analysis of SARS-CoV-2 viremia kinetics during admission

Longitudinal viremia curves of all the patients included in this cohort, considering all the plasma samples studied until discharge, were previously analysed,[20] showing that 19 patients presented quantifiable viremia during their hospital admission. Several parameters of viremia kinetics were determined in the present study. Median viremia duration, defined as the number of days from first quantifiable viremia until the last one, was 5 days (IQR = 3–9). Median time from admission to first positive plasma sample with quantified viremia was 1 day (IQR = 1–2) corresponding to a median time from symptom onset of 7 days (IQR = 6–9). Only one patient had quantifiable viremia after day 18. Correlation between viremia and time since onset of symptoms or from admission is shown in Supporting Information: Figure 2.

Clearance of viremia was observed during admission in all but one patient, who progressed rapidly to death. Viral clearance time was considered at the day after the last positive plasma sample. Median time from admission to viral clearance was 9 days (IQR = 5–11).

3.1.3 | SARS-CoV-2 viral load in the first week of hospitalization and COVID-19 severity

Quantifiable viremia was mainly detected during the first week of admission, as shown in Supporting Information: Figure 2. We only considered viremia from the first week of hospitalization (three determinations) for further analysis.

According to the ROC analysis of viremia for the composite endpoint variable (Figure 1), the cut-off value favouring specificity (58% sensitivity and 88% specificity) was a viral load >2.5 $\log_{10}$ (>300 copies/ml).

Some patients with viremia below the cut-off showed a considerable viremia increase in the first week. Accordingly, to better assess the association between viremia kinetics and clinical outcomes, RV was defined as viremia above the cut-off in the first sample or at least a twofold increase of viremia within ≤2.0 days, whichever comes first.

TABLE 1 Baseline clinical characteristics

	Study population ($n = 57$)	Viremia in the first week		
		No relevant viremia ($n = 43$)	Relevant viremia ($n = 14$)	p value
Age; median (IQR)	63 (52–81)	66 (50–81)	60 (55–76)	0.87
Male sex; *n* (%)	35 (61)	24 (56)	11 (79)	0.21
Caucasian; *n* (%)	39 (68)	30 (70)	9 (64)	0.61
Age-adjusted Charlson comorbidity index; median (IQR)	3 (1–5)	4 (1–5)	2.5 (1–4)	0.88
Comorbidities; *n* (%)	43 (75)	32 (74)	11 (78)	1.0
Dyslipidaemia	24 (42)	17 (40)	7 (50)	0.54
Hypertension	23 (40)	18 (42)	5 (36)	0.76
Cardiovascular disease	12 (21)	7 (16)	5 (36)	0.14
COPD	8 (14)	4 (9)	4 (28)	0.09
Diabetes mellitus	7 (12)	6 (14)	1 (7)	0.67
Hypothyroidism	7 (12)	6 (14)	1 (7)	0.067
Duration of symptoms at admission (days); median (IQR)	6 (2–9.5)	6 (2–10)	5.5 (4–7)	0.68
Baseline $SatO_2$; median (IQR)	93 (90–95)	93 (91–96)	92 (84–93)	0.03
Treatment during hospitalization n (%)				
Glucocorticoids	53 (93)	40 (93)	13 (93)	1.0
Methylprednisolone bolus	31 (54)	18 (42)	13 (93)	0.001
Remdesivir	5 (9)	4 (9)	1 (7)	1.0
Tocilizumab	9 (16)	5 (12)	4 (29)	0.2
Hyperimmune plasma	6 (10.5)	2 (5)	4 (29)	0.027
Colchicine	4 (7)	2 (5)	2 (14)	0.25
Ruxolitinib	1 (2)	0 (0)	1 (2)	0.25

Abbreviations: COPD, chronic obstructive pulmonary disease; IQR, interquartile range; $SatO_2$, oxygen saturation.

Analysis of early SARS-CoV-2 RNA viral load in the 14 patients with RV showed that 64.3% of them (9/14) had a viral load above the cut-off value (>300 copies/ml) on their first plasma sample. On the other hand, 25.7% (5/14) showed a 30-fold median viral load increase (range = 3.2–210), between the first and the second quantifiable sample, within an interval of 2 days.

3.1.4 | RV is associated with poor outcomes

Within the study population, 14 patients (24.6%) had RV. Baseline clinical and analytical characteristics are shown in Table 1 and Supporting Information: Table 1. Of the 14 patients with RV, 10 (71.4%) had Poor Outcome (eight needed ICU admission and five died during hospitalization), whereas none of the patients without RV showed Poor Outcome, $p < 0.0001$. Likewise, RV was associated with higher probability of in-hospital death ($p < 0.0001$) or ICU admission ($p < 0.0001$) as shown in Figure 2.

RV showed a sensitivity of 100% for the three main outcomes whereas specificity was 91.5% for the variable Poor Outcome, 87.8% for ICU admission and 82.7% for mortality.

Patients without RV showed a significantly lower viral load in the first week than those with RV: 87 copies/ml (IQR = 47–150 copies/ml) versus 639 copies/ml (IQR = 238–1493 copies/ml), respectively ($p = 0.013$) (Figure 3).

In nasopharyngeal swabs collected at hospital admission, significant lower viral load was detected in those patients without RV compared with those with RV ($p = 0.034$) (Supporting Information: Figure 3).

3.1.5 | Survival is decreased in patients with RV

Patients without RV had significantly better survival than those with RV in the first week ($p < 0.0001$) (Figure 4A). Hazard ratio (HR) could not be calculated, because the incidence of in-hospital mortality was 0 for the group without RV. Patients older than 75 years had higher in-hospital mortality (HR = 14.1, 95% confidence interval [CI]: 1.4–140.1; $p = 0.02$).

Time to hospital discharge was also evaluated. The mean length of stay was 10 days. Patients with RV had significantly longer hospital stay (23 days, IQR = 18–51) compared with those without RV (9 days [IQR = 7–13]; $p > 0.0001$) as shown in Figure 4B.

3.2 | Retrospective validation cohort

RV was also analysed in a retrospective cohort of 326 patients. The recruitment period includes the emergence and prevalence of 4 VOC in Madrid: alpha, delta, omicron, and omicron BA.2 (Supporting Information: Figure 4).[24] Their median age was 73 years (IQR = 60–85 years), 56% of them were men. A total of 265 (81.3%) did not present RV and 61 (18.7%) had RV. No significant differences regarding age and sex distribution were observed between both groups of patients ($p > 0.05$).

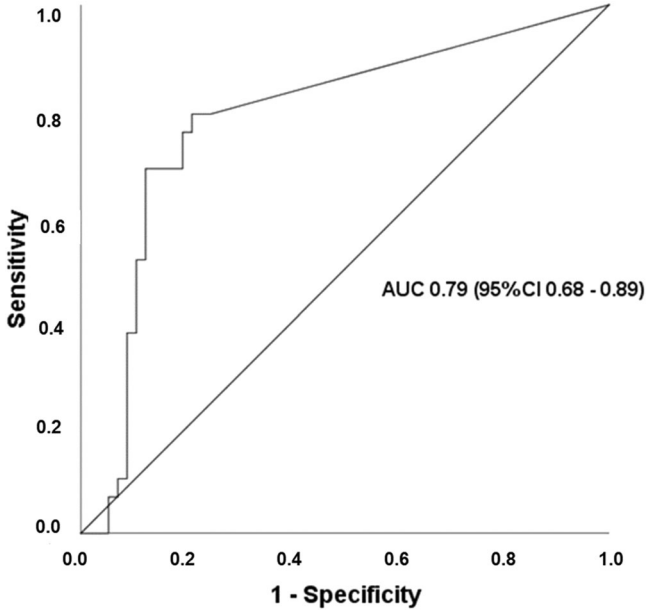

FIGURE 1 ROC analysis curve for the combined endpoint of ICU admission and in-hospital mortality. ICU, intensive care unit; ROC, receiver operating characteristic.

In the retrospective cohort, 17 (5.2%) patients required ICU admission; 38 (11.7%) died during hospitalization and 51 (15.6%) had Poor outcome.

When the three main outcomes were analysed according to RV status, this variable was significantly associated with the risk for ICU-admission (odds ratio [OR]: 5.6 [95% CI: 2.1–15.1]; $p = 0.001$), in-hospital mortality (OR: 13.5 [95% CI: 6.3–28.7]; $p < 0.0001$), and Poor Outcome (OR: 11.2 [95% CI: 5.8–22]; $p < 0.0001$). Distribution of main outcomes according to the presence or absence of RV is detailed in Figure 5. Table 2 summarizes the results obtained in both cohorts.

Furthermore, patients with RV showed a significant longer hospitalization ($p < 0.0001$), although survival did not show significant differences between both types of patients in this cohort (Figure 6).

Comparison of the prospective and retrospective cohorts did not show statistically significant differences in terms of percentages of patients with RV, mortality, and Poor Outcome ($p > 0.05$). However, the percentage of patients who needed ICU admission was significantly higher ($p = 0.02$) in the prospective cohort (14% of the patients) with respect to the retrospective group (5.2% of the patients) (Supporting Information: Table 2).

4 | DISCUSSION

In the present study, SARS-CoV-2 viral load was monitored in plasma of COVID-19 patients throughout hospitalization using quantitative RT-PCR. Early kinetics of viremia was evaluated as a predictor of poor clinical outcome in a prospective study and further assessed in a retrospective validation cohort. RV was described as a composite variable that reflects viral load kinetics in the first week of hospitalization. Patients present RV when they show viremia values above a cut-off (300 copies/ml) or show a viral load increase of at least twofold between consecutive samples within 48 h. RV in the

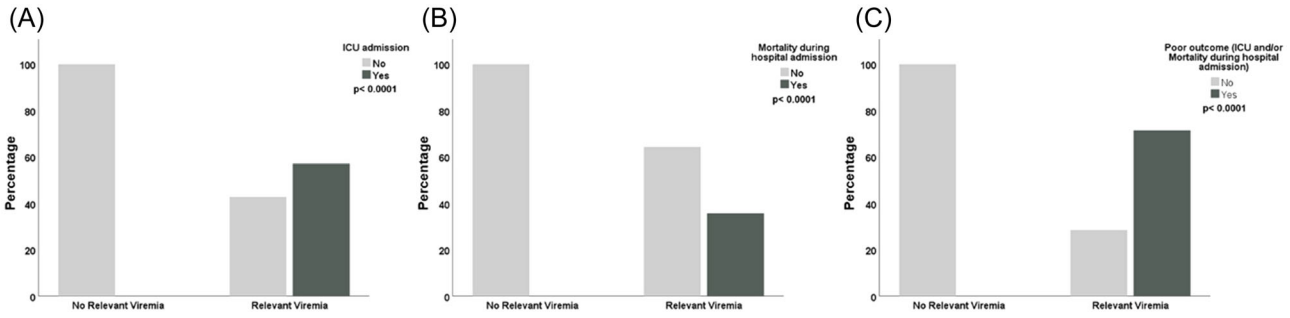

FIGURE 2 Percentage of patients in the prospective cohort with the main clinical outcomes according to the presence or absence of relevant viremia. (A) Intensive Care Unit (ICU) admission, (B) in-hospital mortality and (C) at least one of these variables (Poor Outcome). Differences were analysed by the χ^2 test or Fisher exact test, as appropriate.

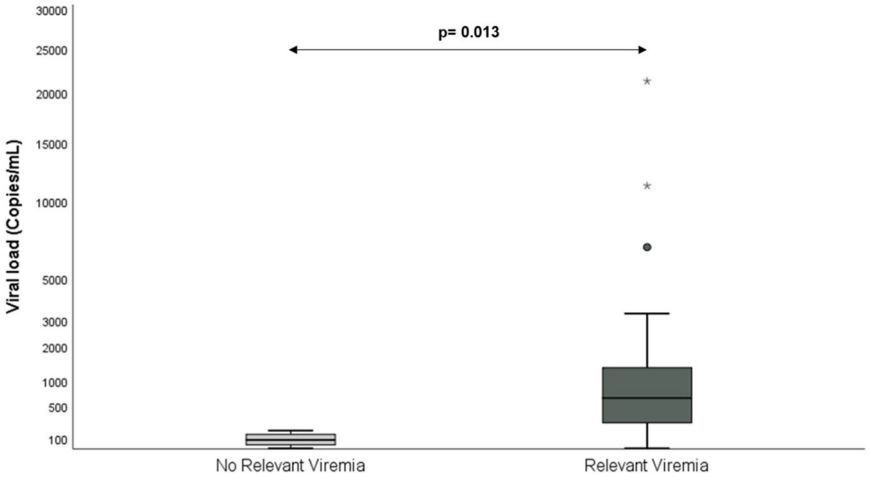

FIGURE 3 Viral load in plasma of patients with relevant viremia was significantly higher than in those patients without relevant viremia. Differences were analysed with Mann–Whitney test.

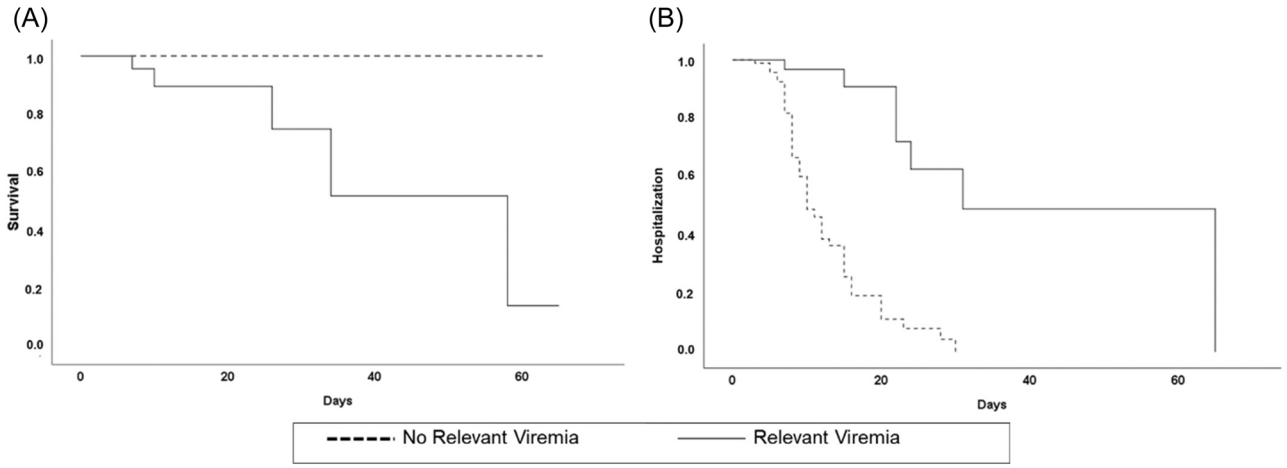

FIGURE 4 Relevant viremia in the first week was associated with survival and time to discharge. (A) Survival analysis with Kaplan–Meier estimator of patients without relevant viremia (dashed line) and patients with relevant viremia (solid line). (B) Time to discharge analysis with Kaplan–Meier estimator of patients with relevant viremia (solid line) and without relevant viremia (dashed line).

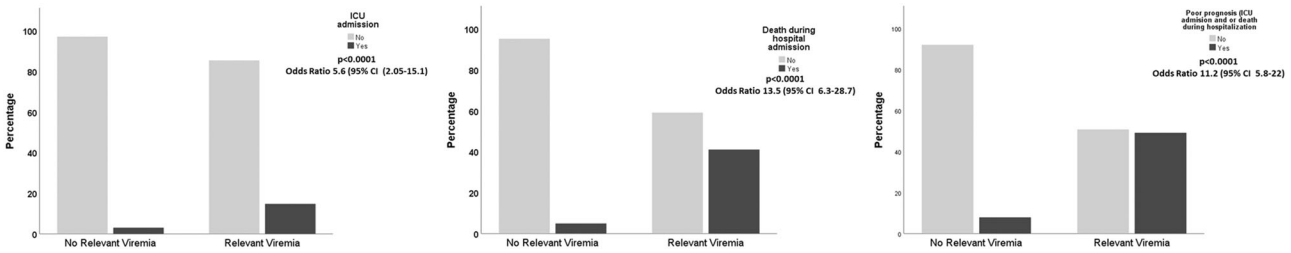

FIGURE 5 Percentage of patients in the retrospective cohort with the main clinical outcomes according to the presence or absence of relevant viremia. (A) Intensive Care Unit (ICU) admission, (B) in-hospital mortality and (C) at least one of these variables (Poor prognosis) Comparison was performed by χ^2 test or Fisher exact test, as appropriate.

first week of hospitalization was associated with a significantly higher in-hospital mortality, ICU admission, and longer hospital stay. Findings observed in the prospective cohort were confirmed in a larger validation cohort.

COVID-19 natural history, viral dynamics in body fluids of patients with different COVID-19 severity and outcomes, is not completely understood. Most studies available to date have focused on the detection and quantification of the virus in the upper respiratory tract and based on this parameter, exhaustive follow-up studies of SARS-CoV-2 time-course have been performed; however, results do not seem to consistently support a role as an indicator of poor prognosis.[5,14,17] In the present study significant association was

TABLE 2 Distribution of patients admitted to Intensive Care Unit (ICU), with death during hospitalization (mortality) and with at least one of both events (Poor outcome) according to their viremia pattern in the prospective and retrospective cohorts.

Viremia pattern	ICU admission				Mortality				Poor outcome			
	Prospective		Retrospective		Prospective		Retrospective		Prospective		Retrospective	
	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No
RV	8 (57.1%)	6 (42.9%)	9 (14.8%)	52 (85.2%)	5 (35.7%)	9 (64.3%)	25 (41%)	36 (59%)	10 (71.4%)	4 (28.6%)	30 (49.2%)	31 (50.8%)
No RV	0	43 (100%)	8 (3.0%)	257 (97%)	0	43 (100%)	13 (4.9%)	252 (95.1%)	0	43 (100%)	21 (7.9%)	244 (92.1%)
Total	8 (14%)	49 (86%)	17 (5.2%)	309 (94.8%)	5 (9%)	52 (91%)	38 (11.7%)	288 (88.3%)	10 (17.5%)	47 (82.5%)	51 (15.6%)	275 (84.4%)
p value	<0.0001		$p = 0.001$		<0.0001		<0.0001		<0.0001		<0.0001	
Odds ratio (95% CI)	NC		5.6 (2.05–15.1)		NC		13.5 (6.3–28.7)		NC		11.2 (5.8–22)	

Note: Association of clinical outcomes with relevant viremia pattern was analysed by χ^2 test or Fisher exact test, as appropriate.
Abbreviations: CI, confidence interval; NC, not calculated; RV, relevant viremia.

found between the viral load in the nasopharyngeal swab and RV (Supporting Information Material).

There is growing evidence for two distinct phases of COVID-19. The first phase is characterized by the replication of SARS-CoV-2, while the second phase is predominantly inflammatory.[25,26] Assessment of SARS-CoV-2 viremia can help in risk stratification of hospitalized COVID-19 patients, distinguishing patients in the replication phase, who could benefit from early antiviral treatment, from those found in the inflammatory phase, who could benefit more from other treatments.[3,27,28]

SARS-CoV-2 detection in plasma of COVID-19 patients is associated with severe disease and unfavorable outcome,[8,19,29,30] but in many studies, viremia is measured in a single sample or not quantified. To date, it has not been explored whether certain specific SARS-CoV-2 viremia kinetics may be associated with an increased risk of poor outcomes in COVID-19 patients. This study, to our knowledge, is the first to analyse the predictive value of the early kinetics of SARS-CoV-2 viral load in plasma samples, during the first week of hospitalization, and its relationship with disease severity. A twofold or greater increase in viral load, in a short period of time, seems to indicate an adverse impact on clinical outcome.[27]

Some studies propose cut-off points in different populations of critical patients, those admitted to the ICU or those who die.[6,14,15] It is known that the quantitative value of viremia is affected by the type and volume of the sample studied, the sensitivity of the technique used (ultrasensitive RT-PCR, digital drop PCR, quantitative RT-PCR) as well as the population studied.[14,15,18] The cut-off point of 300 copies/ml is consistent with other published studies (range from 1000 to 6000 copies/ml), although its value is lower than this range, likely due to specific differences of the qRT-PCR techniques used or different timing of sample collection across studies.[14–16,31]

Sequential assessment of viral load kinetics in individual patients during the first hospitalization week was used to define a composite variable, RV, reflecting both viral load and viremia kinetics. RV was associated with higher rates of ICU admission, death, poor outcomes, and longer hospital stay in our prospective cohort. These results were assessed in a retrospective cohort with 326 patients. The odds ratio values found in the retrospective cohort are relevant; these data could not be obtained in the prospective study, because no adverse outcomes were found in the group without RV. Therefore, the present results support the idea that the presence of RV could be considered as a prognostic biomarker[8,9,27] and could help physicians in making clinical decisions. Nonetheless, SARS-CoV-2 viremia could be only quantified in plasma for a short period of time, therefore a close follow-up from hospital admission is required to properly assess viremia in COVID-19 patients.

This study has some limitations. First, the prospective cohort has a moderate number of patients ($n = 57$). Second, the epidemiological situation in Spain at the time when patients were recruited was different for both cohorts. In the case of the prospective cohort, Spain was between the second and the third wave of the pandemic, while the retrospective cohort included the peaks of the fourth, fifth, and six waves. The different burden of the pandemic on the hospital

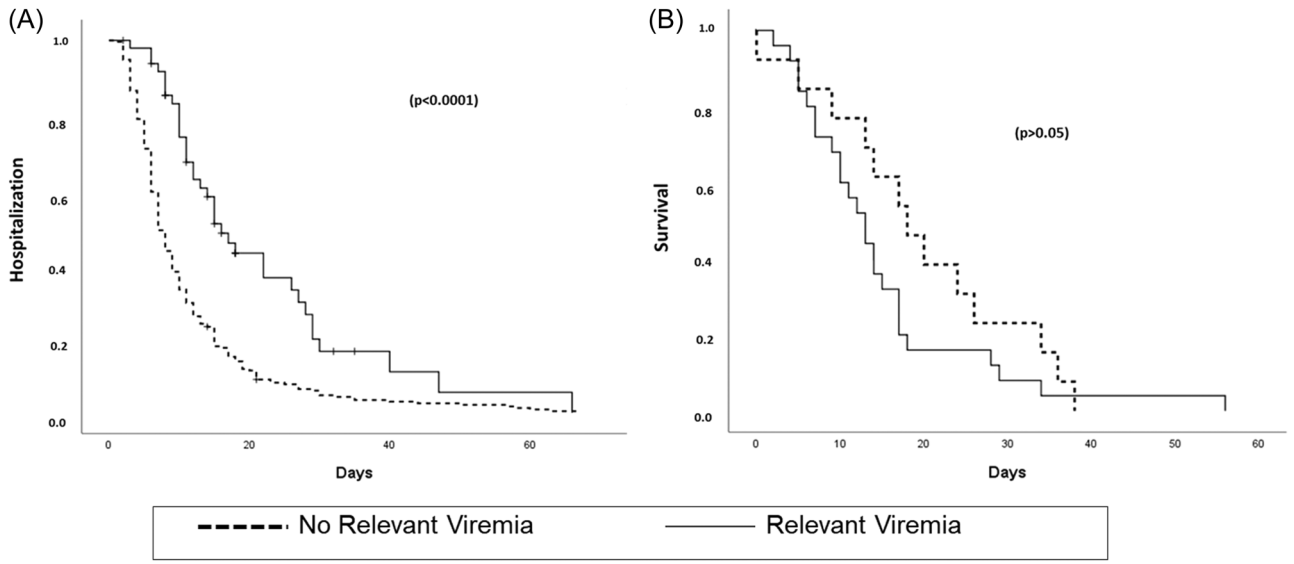

FIGURE 6 Kaplan–Meier curves for duration of hospital admission and survival. (A) Hospital admission was longer in patients with relevant viremia compared to those without relevant viremia. (B) The two groups of patients in this cohort did not show significant differences in survival. Analysis was performed with the long-rank method.

could have affected some variables, such as ICU admission criteria. On the other hand, during the period of the prospective study, there was no circulation of VOC in our zone, while in the period of the retrospective study, four VOC emerged and became predominant: alpha, delta, omicron, and omicron BA.2.[24] However, the consistency in the results suggests that the viremia quantification technique is robust and yields consistent results despite the appearance of new variants, since the use of three targets avoids losing precision, despite mutations occurring in any of them as other authors have reported.[32,33] Third, blood samples in the retrospective cohort were not taken at time points following a protocol and were rather collected at times based on clinical criteria. Finally, although four patients of the prospective cohort had RV, they were not admitted to the ICU or died. As mentioned elsewhere,[8,11] patients with nonsevere COVID-19 may have SARS-CoV-2 RNA in their blood.

In conclusion, detection of RV in the first week of hospitalization in patients with COVID-19 is associated with an increased risk of ICU admission, longer hospital stay, and mortality. Early high viral loads and/or the rate of viral load increase in plasma allows us to predict a poor clinical outcome. Further studies are needed to determine the contribution of monitoring viremia kinetics in COVID-19 patients, its impact on treatment decision-making, and prevention of disease progression.

AUTHOR CONTRIBUTIONS

Laura Cardeñoso conceived the idea and planned the study. Laura Cardeñoso, Emilia Roy Vallejo, Nelly Daniela Zurita, Isidoro González Álvaro and Diego Anibal Rodríguez Serrano wrote the first draft of the manuscript. Emilia Roy Vallejo, Marta Chicot Llano, Elena Ávalos, Ana Barrios, Julia Hernando, Javier Ortiz, Sebastián C. Rodríguez García, Marianela Ciudad, Celeste Marcos, Elena García Castillo, Begoña González, Rosa Méndez, Isabel Iturrate, Almudena Villa, Ana Sánchez Azofra, Begoña Quicios, David Arribas, Ana Triguero Martínez, Cristina Arevalo, Jesús Álvarez Rodríguez, Pablo Patiño, Marina Trigueros, Miren Uriarte, José María Galván Román, Rosario García Vicuña, Julio Ancochea, Joan Soriano, Alfonso Canabal, Cecilia Muñoz Calleja, Rafael de la Cámara and Carmen Suarez Fernández included patients in the study and collected data. Laura Cardeñoso, Nelly Daniela Zurita, Alexandra Martín Ramírez, and Leticia Fontán García-Rodrigo. Methodology, data curation, investigation, and validation of viremia. Laura Cardeñoso, Emilia Roy Vallejo, Nelly Daniela Zurita, Ancor Sanz and Isidoro González Álvaro analysed data. Laura Cardeñoso and Nelly Daniela Zurita finalized the draft for submission. All authors read and approved the final version of the manuscript.

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CONFLICTS OF INTEREST

The authors of this manuscript have the following competing interests: Julio Ancochea reports grants and personal fees from GlaxoSmithKline and Boehringer Ingelheim; grants from Linde Healthcare; and grants, personal fees, and nonfinancial support from Roche and from Chiesi, outside the submitted work. Diego A. R. Serrano reports personal fees from MSD and Pfizer, outside the submitted work. Rafael de la Cámara reports personal fees from MSD, ASTELLAS, Clinigen, Janssen, Roche, and IQONE Health Care outside the submitted work. Rosario G. Vicuña reports grants, personal fees, and nonfinancial support from Abbvie, BMS, Lilly, Novartis, Sanofi, Sandoz, and MSD; personal fees from Biogen and Celltrion and from Mylan, outside the submitted work; personal fees and nonfinancial support from Pfizer; grants from Roche; and grants and personal fees from Janssen. Carmen S. Fernández reports personal fees from Bayer, BMS, Daichi Sankyo, MSD, and Pfizer, outside the submitted work. Cecilia M. Calleja reports competitive grants from ISCIII during the conduct of the study. Isidoro G. Álvaro reports grants from Instituto de Salud Carlos III, during the course of the study; Personal fees from Lilly and Sanofi; personal fees and nonfinancial support from BMS and Abbvie; research support, personal fees, and nonfinancial support from Roche Laboratories; and nonfinancial support from MSD, Pfizer, and Novartis, not related to the submitted work. The rest of the authors declare that they have no relevant conflicts of interests.

DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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