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ORIGINAL ARTICLE

Impact of viral load at admission on the development of respiratory failure in hospitalized patients with SARS-CoV-2 infection

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Abstract

The aim of our study was to elucidate if SARS-CoV-2 viral load on admission, measured by real-time reverse transcriptase–polymerase chain reaction (rRT-PCR) cycle threshold (Ct) value on nasopharyngeal samples, was a marker of disease severity. All hospitalized adult patients with a diagnosis of SARS-CoV-2 infection by rRT-PCR performed on a nasopharingeal sample from March 1 to March 18 in our institution were included. The study population was divided according to the Ct value obtained upon admission in patients with high viral load (Ct < 25), intermediate viral load (Ct: 25–30) and low viral load (Ct > 30). Demographic, clinical and laboratory variables of the different groups were analyzed to assess the influence of viral load on the development of respiratory failure during admission. Overall, 455 sequential patients were included. The median Ct value was 28 (IQR: 24–32). One hundred and thirty patients (28.6%) had a high viral load, 175 (38.5%) an intermediate viral load and 150 (33%) a low viral load. Advanced age, male sex, presence of cardiovascular disease and laboratory markers such as lactate dehydrogenase, lymphocyte count and C-reactive protein, as well as a high viral load on admission, were predictive of respiratory failure. A Ct value < 25 was associated with a higher risk of respiratory failure during admission (OR: 2.99, 95%IC: 1.57–5.69). SARS-CoV-2 viral load, measured through the Ct value on admission, is a valuable tool to predict the development of respiratory failure in COVID-19 inpatients.

Introduction

The 2019 novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epidemic has quickly spread worldwide. As of December 2020, the worldwide outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for the viral pneumonia known as COVID-19, has caused more than 71 million infections and 1,597,000 deaths [1]. Most patients experience only minor symptoms that can be monitored at home. However, some individuals will present a severe respiratory distress syndrome, needing intensive care unit (ICU) admission and early respiratory support with significant morbidity and mortality [2]. Taking into account the current limited efficacy of pharmacological therapy, an adequate timely respiratory support is a cornerstone of COVID-19 management. Therefore, identification of early markers, ideally obtained on admission, able to predict respiratory failure, is essential to face the SARS-CoV-2 in a pandemic situation, where respiratory support facilities may be on the verge of being overwhelmed. Previous studies have shown that lymphopenia, elevated serum levels of lactate dehydrogenase (LDH) or proinflammatory cytokines such as interleukin (IL)-6 or IL-1 [3, 4] are markers of disease severity. On the other hand, several works have reported that patients with severe pneumonia needing mechanical ventilation have very high viral loads for extended periods of time [5, 6]. Despite

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this, to date, few studies have related the initial viral load to the outcome of COVID-19 patients [7].

The aim of our study was to assess the association between the viral load measured by real-time reverse transcriptase–polymerase chain reaction (rRT-PCR) cycle threshold (Ct) value on upper respiratory tract samples on admission and development of respiratory failure in patients who were admitted to our institution with a confirmed SARS-CoV-2 infection.

Methods

Study population

We performed a retrospective analysis from prospective automated medical records of all hospitalized adult patients (aged over 18) with a confirmed diagnosis of SARS-CoV-2 infection by rRT-PCR assay performed on nasopharyngeal exudate on admission, from March 1 to March 18. The study was developed at University Hospital "12 de Octubre", a large 1300-bed hospital that serves a population of 450,000 inhabitants in southern Madrid (Spain). The study protocol was approved by the University Hospital 12 de Octubre Clinical Research Ethics Committee (reference 20/117), and a waiver of informed consent was granted, due to the retrospective nature of the observational design.

Microbiological methods

Nasopharyngeal samples were collected with flocked swabs in UTM™ viral transport medium (Copan Diagnostics, Brescia, Italy) and processed by automatized extraction and specific polymerase chain reaction methods [8]. Nucleic acid extraction was performed using the MicrolabStarlet IVD platform and the STARMag 96 × 4 Universal Cartridge Kit (Seegene, Seoul, South Korea) or NucliSENS EasyMAG instrument (bioMerieux, Marcy l'Etoile, France). For rRT-PCR the LightCycler 480 System instrument II (Roche Life Science, Indianapolis, IN, USA) performing the test TaqMan 2019nCoV assayKit v1, provided by Thermosfisher Scientific that amplifies three different viral regions in singleplex reactions was used. As a measure of relative quantification, the cycle threshold value (Ct) obtained in the amplification of the N gene was recorded. The RT-PCR Ct value represents the first PCR cycle in which the fluorescent signal for the target is greater than the minimal detection level [9]; thus, the lower Ct value represents the higher viral load in the sample.

Study definitions

For the purpose of the study, we arbitrarily divided the study population into tertiles based on the quantitative value of Ct. A high viral load was defined by a Ct < 25, intermediate viral load between 25 and 30 and low viral load if Ct was > 30.

The primary endpoint was development of respiratory failure, which was defined as the need for mechanical ventilation, non-invasive positive pressure ventilation or invasive mechanical ventilation. This was also the consideration with patients who developed a PaO_2/FiO_2 level < 200 during admission even if mechanical ventilation was not initiated. Cardiovascular disease was defined as the presence of coronary heart disease, heart failure, stroke and hypertension. Chronic lung disease was defined as the presence of chronic obstructive pulmonary disease, asthma or severe obstructive sleep apnea. Immunosuppression was defined as the presence of any of the following: active malignant neoplasia, autoimmune disease, solid organ transplantation, HIV infection, use of steroids or chemotherapy. Sepsis, septic shock and organ dysfunction were defined according to the terms proposed recently by the Third International Consensus Definitions for Sepsis and Septic Shock [10], including the Sequential Organ Failure Assessment (SOFA) score and the National Early Warning Score (NEWS). Acute respiratory distress syndrome (ARDS) was defined according to the American-European Consensus Conference on ARDS [11].

Statistical methods

Continuous variables were described with mean ± standard deviation (SD) or median and interquartile range (IQR) and compared with analysis of variance (ANOVA), Kruskal-Wallis test, Student's *t* test or Mann-Whitney *U* test, as appropriate. Categorical parameters were notes in absolute numbers and percentage and compared with the Chi2 test or the Fisher exact test. The association of the viral load and the primary endpoint was assessed by multivariate analysis with logistic regression.

Principal component analysis was performed to obtain an overall idea of the data and the interrelationships among the different categorical variables (supplemental material).

Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 25.0 (Armonk, NY: IBM Corp and SAS/STAT 10.1; SAS Institute Inc., Cary, NC).

Results

We included 455 patients with a confirmed SARS-CoV-2 infection. Mean age was 64.9 (SD: 18.1) and 255 (56%) were male. The median Ct value was 28 (IQR: 24–32). One hundred and thirty patients (28.6%) had a high viral load, 175 (38.5%) an intermediate viral load and 150 (33%) a low viral load.

Baseline comorbidities and clinical findings present on admission are shown in Table 1, as well as the differences between the three groups according to their viral load. Patients

Table 1 Baseline demographic, clinical characteristics and laboratory parameters at admission

Characteristics	All patients ($n = 455$)	Ct value (viral load)			P value[a]	P value[b]
		< 25 ($n = 130$)	25–30 ($n = 175$)	> 30 ($n = 150$)		
Age (mean ± SD)	64.9 ± 18.1	69.1 ± 18.5	65.8 ± 17.4	60.1 ± 17.7	< 0.001	0.002
Sex male (n, %)	255 (56)	79 (60.8)	102 (58.3)	74 (49.3)	0.051	0.199
Caucasian race (n, %)	344 (75.6)	112 (86.2)	128 (73.1)	104 (69.3)	0.001	0.001
Hispanic race (n, %)	92 (20.2)	15 (11.5)	38 (21.7)	39 (26)	0.003	0.004
Charlson index score (median, IQR)	3 (1–5)	4 (2–6)	3 (1–5)	2 (0.75–4)	< 0.001	< 0.001
Previous comorbid conditions						
Cardiovascular disease[1]	205 (45.1)	70 (53.8)	79 (45.1)	56 (37.3)	0.006	0.017
Chronic lung disease[2]	98 (21.5)	37 (28.5)	34 (19.4)	27 (18)	0.037	0.023
Diabetes mellitus	76 (16.7)	28 (21.5)	29 (16.6)	19 (12.7)	0.048	0.080
Immunosuppression[3]	89 (19.6)	37 (28.5)	39 (22.3)	13 (8.7)	<0.001	0.002
Chronic renal disease	29 (6.4)	10 (7.7)	15 (8.6)	4 (2.7)	0.075	0.466
Chronic liver disease	22 (4.8)	4 (3.1)	11 (6.3)	7 (4.7)	0.571	0.339
Obesity [4]	136 (33.4)	42 (35.6)	50 (32.5)	44 (32.6)	0.624	0.552
Current or former smoker	115 (25.3)	36 (27.7)	49 (28)	30 (20)	0.128	0.453
Days of symptoms prior to positive rRT-PCR (median, IQR)	5 (3–7)	4 (2–6)	5 (3–7)	7 (4–8.25)	< 0.001	< 0.001
Clinical findings						
Fever (T > 38 °C)	218 (47.9)	59 (45.4)	87 (49.7)	72 (48)	0.683	0.495
Cough	358 (78.7)	102 (78.5)	136 (77.7)	120 (80)	0.741	0.942
Dyspnoea	240 (52.7)	66 (50.8)	94 (53.7)	80 (53.3)	0.679	0.593
Tachypnea [5]	120 (27.6)	36 (29)	51 (30.7)	33 (22.9)	0.244	0.684
SpO2 < 90% air room	92 (20.3)	31 (23.8)	33 (19)	28 (18.7)	0.294	0.229
Diarrhoea	82 (18)	22 (16.9)	32 (18.3)	28 (18.7)	0.709	0.700
Myalgia	159 (34.9)	39 (30)	55 (31.4)	65 (43.3)	0.017	0.162
Vomiting	39 (7.9)	11 (8.5)	16 (9.1)	9 (6)	0.427	0.784
SOFA score	1 (0–2)	2 (0–3)	1 (0–2)	1 (0–2)	0.041	0.086
NEWS score	3 (1–5)	3 (1–5)	3 (1–5)	2 (1–4.25)	0.198	0.311
Chest X-ray abnormal findings	366 (80.4)	94 (72.3)	138 (78.9)	134 (89.3)	< 0.001	0.006
Laboratory parameters						
Lymphocytes ($\times 10^3$ cells/μl)	0.9 (0.6–1.2)	0.8 (0.6–1.2)	0.8 (0.6–1.2)	0.9 (0.7–1.2)	0.310	0.671
LDH (U/l) [6]	326.5 (265–408)	317 (245.2–383)	322 (265–426)	348 (282–432)	0.004	0.002
GOT (U/l)	32 (24–50)	31 (23–45)	32 (25–50)	37 (25–58)	0.049	0.026
GPT (U/l)	25 (17–40)	23 (15–31)	25 (17–39)	29 (19–50)	0.001	0.001
CPK (U/l)	86 (52–176.7)	101 (61.7–180)	87 (48–194)	78.5 (47–173)	0.239	0.119
TnT (U/l) [7]	10.5 (5.8–21.4)	12.7 (7–30)	10.6 (6.6–18)	7.4 (5–20)	0.012	0.011
C-reactive protein (mg/dl)	7.7 (3.1–14.9)	6.9 (2.7–15)	7.5 (3–14)	8.3 (3.8–15)	0.503	0.453
Ferritin (mg/dl) [8]	699 (335–1357)	665 (314–1335)	675 (328–1263)	794 (365–1466)	0.376	0.488
D-dimers (ng/ml) [9]	664 (418–1220)	611 (350–1175)	684 (457–1346)	667 (441–1308)	0.206	0.085

Results are expressed as mean ± standard deviation (SD) or median with interquartile range (IQR) or as absolute value (percentage); *Ct* cycle threshold, *rRT-PCR* real-time reverse transcription polymerase chain reaction, *SOFA* Sequential Organ Failure Assessment, *NEWS* National Early Warning Score

[1] Cardiovascular disease was defined as the presence of coronary heart disease, heart failure, stroke and hypertension

[2] Chronic lung disease was defined as the presence of chronic obstructive pulmonary disease, asthma or severe obstructive sleep apnea

[3] Immunosuppression was defined as the presence of any the following: active malignant neoplasia, autoimmune disease, solid organ transplantation, HIV infection, use of steroids, or chemotherapy. Use of steroids was defined as (1) more than 20 mg/day of oral prednisone during 7 days or longer or (2) less than 20 mg/day during a minimum of 3 months

[4] Obesity was defined as body mass index ≥ 30, data available in 407 patients

[5] Tachypnea was defined as breathing rate > 20 beats per minute; data available for 434 patients

[6] *LDH* lactate dehydrogenase; data available for 364 patients (upper limit of LDH in the local laboratory is 225 mg/dl)

[7] Troponin T: data available for 257 patients

[8] Ferritin: data available for 402 patients

[9] D-dimers: data available for 270 patients

[a] Across all Ct value groups. *P* value for trend is used when appropriate

[b] Between high viral load group (Ct < 25) and other groups combined

Table 2 Outcome of patients with SARS-CoV-2 infection according viral load at diagnosis

	All patients ($n = 455$)	Ct value (viral load)			P value[a]	P value[b]
		< 25 ($n = 130$)	25–30 ($n = 175$)	> 30 ($n = 150$)		
Need for supplemental oxygen	311 (68.4)	94 (72.3)	121 (69.1)	96 (64)	0.134	0.251
ARDS	132 (29)	43 (33.1)	53 (30.3)	36 (24)	0.092	0.227
ICU admission	48 (10.5)	8 (6.2)	21 (12.3)	19 (12.7)	0.084	0.054
Length of ICU stay (days)	13 (7–18)	15 (11–18)	13 (5.5–17)	12 (7–23)	0.533	0.420
Non-invasive mechanical ventilation	29 (6.4)	8 (6.2)	12 (6.9)	9 (6)	0.946	0.903
Invasive mechanical ventilation	46 (10.1)	8 (6.2)	19 (10.9)	19 (12.7)	0.075	0.077
Days of invasive ventilation	12.5 (7–17)	15 (10–17.2)	13 (7–16)	11 (7–20)	0.488	0.258
Prone position	39 (8.6)	7 (5.4)	19 (10.9)	13 (8.7)	0.360	0.125
Septic shock	12 (2.6)	3 (2.3)	8 (4.6)	1 (0.7)	0.346	0.781
Acute kidney injury (AKI)	69 (15.2)	23 (17.7)	32 (18.3)	14 (9.3)	0.045	0.342
Venous thrombosis	8 (1.8)	2 (1.5)	4 (2.3)	2 (1.3)	0.873	0.822
Hepatitis [1]	32 (7)	6 (4.6)	16 (9.1)	10 (6.7)	0.544	0.202
MACE event [2]	5 (1.1)	3 (2.3)	1 (0.6)	1 (0.7)	0.203	0.118
Length of hospital stay (days)	9 (6–14)	10 (6–17)	9 (5–13)	8.5 (6–13.2)	0.214	0.085
Respiratory failure	161 (35.4)	55 (42.3)	64 (36.6)	42 (28)	0.012	0.051
Days of symptoms to RF [3] (median, IQR)	9 (6–11)	8 (6–10)	9(6–11)	9(6.7–12.2)	0.426	0.280
Overall in-hospital mortality	120 (26.4)	44 (33.8)	44 (25.1)	32 (21.3)	0.019	0.022

Results are expressed as median with interquartile range (IQR) or as absolute value (percentage). *ICU* intensive care unit, *ARDS* acute respiratory distress syndrome, *RF* respiratory failure, *rRT-PCR* real-time reverse transcription polymerase chain reaction

[1] Hepatitis was defined by AST and/or ALT levels > 5 times the ULN

[2] Major adverse cardiovascular event (MACE) was defined as nonfatal stroke, nonfatal myocardial infarction and cardiovascular death

[3] Days from the onset of symptoms to development of respiratory failure

[a] Across all Ct value groups. *P* value for trend is used when appropriate

[b] Between high viral load group (Ct < 25) and other groups combined

with high viral load at admission were of advanced age and mostly Caucasian and had more comorbidities. The time from symptom onset to positive rRT-PCR ranged from 0 to 19 days with a median of 5 days (IQR: 3–7); and was lower in patients with high viral load compared to patients with intermediate and low viral load (4 [IQR: 2–6]) vs. 6 [4–8], respectively; $P < 0.001$). There were no differences between the groups regarding the presence of fever, cough or dyspnoea. Treatments administered during admission were mainly lopinavir/ritonavir in 60.4% of patients, hidroxicloroquine in 74.1%, antibiotics in 91.4% and corticosteroids in 25.1%; only 36 patients (7.9%) received tocilizumab and one patient (0.2%) remdesivir. Corticosteroids were more frequently prescribed in patients with high viral load than in patients with intermediate and low viral load (31.8% vs. 23.4% and 16.7%, respectively; $P = 0.026$). Treatment with lopinavir/ritonavir was more frequently prescribed in patients with low and intermediate viral load than in patients with high viral load (69.9% and 64 .5% vs. 51%, respectively; $P = 0.010$).

Laboratory values in the first 24 h from admission are shown in Table 1. Most patients presented lymphopenia as well as elevated acute phase reactants such as ferritin, C-reactive protein (CRP) and D-dimers, without significant differences between groups.

Respiratory failure occurred in 161 patients (35.4%) after a median of 9 days (IQR 6–11), and 120 (26.4%) patients died during hospitalization (Table 2). According to the viral load on admission (Table 2), the patients with high viral load developed respiratory failure more often (42.3% vs. 32.6%, $P = 0.051$) and experienced higher mortality at 30 days (33.8% vs. 23.4%, $P = 0.022$). Intensive care unit (ICU) admission rate, however, was higher in the group of patients with intermediate and low viral load (12.3% vs. 6.2%, $P = 0.054$). There were no differences in the presence of septic shock, acute kidney injury, venous thrombosis, hepatitis or major adverse cardiovascular events (MACE) between groups.

We performed an analysis of the variables associated with the development of respiratory failure during admission (Table 3). Parameters associated with a higher likelihood of respiratory failure were age, the presence of comorbidities, current or former smoking habit, the presence of dyspnoea or tachypnea on admission, pathological chest X-ray, lymphopenia, a high CR*P* value and LDH. Patients who developed respiratory failure had a high viral load on admission in greater proportion than those who did not (34.2% vs. 25.5%, $P = 0.05$). In a multivariate analysis (Table 4) adjusted for age, sex, cardiovascular disease, chronic lung disease, immunosuppression, current or former smoker, the presence of

Table 3 Risk factors for respiratory failure during admission

Characteristics	Respiratory failure $n = 161$	Non-respiratory failure $n = 294$	*P* value	OR	IC 95%
Age ≥ 60 years	132 (76.4)	158 (53.7)	< 0.001	2.78	1.81–4.28
Sex male (n, %)	109 (67.7)	146 (49.7)	< 0.001	2.12	1.42–3.17
Caucasian race (n, %)	127 (78.9)	217 (73.8)	0.228		
Hispanic race (n, %)	31 (19.3)	61 (20.7)	0.704		
Charlson index score ≥ 2	135 (83.9)	172 (58.5)	< 0.001	3.68	2.28–5.95
Previous comorbid conditions					
Cardiovascular disease [1]	96 (59.6)	109 (37.1)	< 0.001	2.51	1.69–3.72
Chronic lung disease [2]	43 (26.7)	55 (18.7)	0.047	1.58	1.00–2.50
Diabetes mellitus	34 (21.1)	42 (14.3)	0.062		
Immunosuppression [3]	43 (26.7)	46 (15.6)	0.004	1.96	1.23–3.14
Chronic renal disease	14 (8.7)	15 (5.1)	0.133		
Chronic liver disease	10 (6.2)	12 (4.1)	0.311		
Obesity [4]	60 (39.7)	76 (29.7)	0.038	1.56	1.02–2.38
Current or former smoker	57 (35.4)	58 (19.7)	<0.001	2.23	1.45–3.44
Days of symptoms prior to positive PCR (median, IQR)	5 (3–7)	5 (3–7)	0.896		
Clinical findings at admission					
Fever (T > 38 °C)	81 (50.3)	137 (46.6)	0.449		
Cough	131 (81.4)	227 (77.2)	0.301		
Dyspnoea	112 (69.6)	128 (43.5)	<0.001	2.96	1.97–4.45
Tachypnea [5]	70 (45.8)	50 (17.8)	<0.001	3.90	2.51–6.06
Diarrhoea	24 (14.9)	58 (19.7)	0.201		
Myalgia	45 (28)	114 (38.8)	0.021	0.61	0.40–0.93
Vomiting	17 (3.7)	19 (4.2)	0.122		
Chest X-ray abnormal findings	141 (87.6)	225 (76.5)	0.005	2.16	1.30–3.71
Baseline laboratory findings					
Lymphocytes ≤ 0.7×10^3 cells/μl	87 (54)	80 (27.3)	<0.001	3.13	2.09–4.68
LDH ≥ 350 U/l [6]	103 (64.8)	80 (27.5)	<0.001	4.85	3.20–7.35
C-reactive protein ≥ 6 mg/dl	133 (82.6)	126 (43.3)	<0.001	6.21	3.89–9.90
High viral load (Ct < 25)	55 (34.2)	75 (25.5)	0.051	1.51	0.99–2.30
Intermediate viral load (Ct 25–30)	64 (39.8)	111 (37.8)	0.676		
Low viral load (Ct > 30)	42 (26.1)	108 (36.7)	0.021	0.61	0.40–0.93

Results are expressed as mean with standard deviation (SD), median with interquartile range (IQR) or as absolute value (percentage). *LDH* lactate dehydrogenase, *Ct* cycle threshold

[1] Cardiovascular disease was defined as the presence of coronary heart disease, heart failure, stroke and hypertension

[2] Chronic lung disease was defined as the presence of chronic obstructive pulmonary disease, asthma or severe obstructive sleep apnea

[3] Immunosuppression was defined as the presence of any the following: active malignant neoplasia, autoimmune disease, solid organ transplantation, HIV infection, use of steroids or chemotherapy. Use of steroids was defined as (1) more than 20 mg/day of oral prednisone during 7 days or longer or (2) less than 20 mg/day during a minimum of 3 months

[4] Obesity was defined as body mass index ≥ 30, data available in 407 patients

[5] Tachypnea was defined as breathing rate > 20 beats per minute; data available for 434 patients

[6] LDH: data available for 450 patients

dyspnoea, abnormal chest x-ray findings on admission, severe lymphopenia ≤ 0.7×10^3 cells/μl, LDH ≥ 350 U/l and C-reactive protein ≥ 6 mg/dl, a high viral load was independently associated with increased risk of respiratory failure (adjusted

Table 4 Multivariate analysis of patients for the occurrence of respiratory failure

Variable $n = 450$	Multivariate analysis		
	OR	95% IC	*P* value
Age ≥ 60 years	2.02	1.16–3.53	0.013
Sex male	1.66	1.02–2.72	0.042
Cardiovascular disease [1]	1.93	1.16–3.21	0.011
Dyspnoea	2.46	1.50–4.03	<0.001
Lymphocytes ≤ 0.7×10^3cells/μl	2.26	1.41–3.63	0.001
LDH ≥ 350 U/l [3]	3.00	1.79–5.05	<0.001
C-reactive protein ≥ 6 mg/dl	3.39	1.93–5.95	<0.001
Low viral load (Ct > 30)	Ref.		
Intermediate viral load (Ct 25–30)	1.81	1.02–3.22	0.044
High viral load (Ct < 25)	2.99	1.57–5.69	0.001

OR odds ratio, *IC* confidence interval, *LDH* lactate dehydrogenase, *Ct* cycle threshold

[1] Cardiovascular disease was defined as the presence of coronary heart disease, heart failure, stroke and hypertension

[3] LDH: Data available for 450 patients

odds ratio 2.99; 95% CI: 1.57–5.69; $P = 0.001$). Likewise, a high viral load was significantly associated with in-hospital mortality (OR: 2.04, 95%IC: 1.44–4.00, $P = 0.037$) (data shown in supplemental material).

Discussion

According to our results, patients with a high viral load of SARS-CoV-2 on admission, measured by a Ct value of the rRT-PCR below 25, were more than twice as likely to develop in-hospital respiratory failure compared to patients with lower viral loads, regardless of other previously described severity parameters.

Viral nucleic acid detection by RT-PCR assays is the gold standard for the diagnosis of COVID-19. Using this technique, we can obtain an indirect viral load value (Ct) easily and immediately after diagnosis. This parameter has been correlated in previous studies with the viral ability of spread and with a longer persistence of the virus in respiratory samples [12, 13]. Also, it has been shown that patients with severe disease have significantly higher viral loads than patients with mild disease [14, 15], and Zou and cols. reported that patients admitted to ICU had detectable viral RNA in nasopharyngeal exudate after 10 days from symptom onset [16]. However, few studies have attempted to elucidate whether there is a relationship between viral load at diagnosis and a worse evolution of the disease.

In our study, patients with a high viral load at admission were of advanced age and had more comorbidities than those with lower viral loads, something that has already been reported in previous studies with SARS-CoV-2 [12, 14]. Indeed, elderly, fragile patients may present a higher expression of ACE2 receptors which serve as a cell entry for SARS-CoV-2 [17] and may also have an impaired immunity, all this contributing to higher viral loads on admission and a more severe disease. Likewise, patients with a higher viral load presented lymphopenia, elevated LDH and CPR more frequently, events that have been related with greater severity of the disease [6, 18]. Finally, patients with a Ct < 25 had a significantly shorter period of symptoms prior to diagnosis suggesting greater severity of the illness.

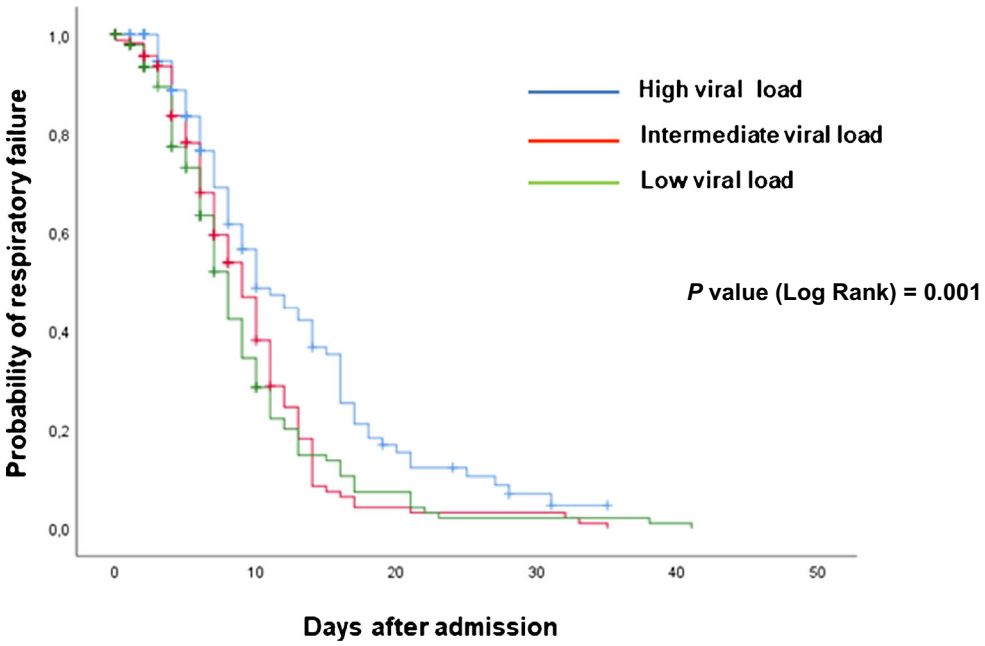

Fig. 1 Probability of respiratory failure during hospitalization among patients with high, intermediate and low viral load

Besides a high viral load at admission, other factors such as advanced age, comorbidity, lymphopenia, LDH or C-reactive protein were associated with a higher risk of respiratory failure. Remarkably, despite that our patients with a high viral load developed respiratory failure more frequently than those with a lower viral load, the rates of admission to the ICU and mechanical ventilation are lower due to the limited access of these patients to the ICU at the time of maximum healthcare pressure in our centre. Although this fact probably contributed to increase mortality in group, a high viral load remained as an independent factor after adjusting for age and comorbidity in the multivariate analysis.

Our findings align with other previously published studies. In the study by Magleby et al. [7], the presence of a high viral load measured by Ct was independently associated with mortality and with the risk of intubation during hospitalization. They also found that patients with higher viral loads developed myocardial infarction, congestive heart failure and acute kidney injury more frequently than those with lower viral loads. However, this is a fact that may be caused by multiple factors related to hospitalization, and this finding is not replicated in our work. A recently published work also found a relationship between high viral load on admission and a composite outcome of death, intubation or ECMO during hospitalization [19], and Westblade et al. reported a higher mortality in patients with high viral load in the oncological population [20]. Yu X. et al. [21] reported in a limited number of patients that those with high viral loads in sputum had more severe disease than those with lower viral loads. On the other hand, a recently published study [22] did not find an association between initial viral load and clinical outcome in a cohort of patients, mostly non-hospitalized with mild disease, a population not comparable with ours (Fig. 1).

An important question to be answered is whether the viral load measured in nasopharyngeal exudate is a good indicator of that present in lung tissue, since the lower respiratory tract specimens have significantly higher SARS-CoV-2 viral RNA levels than nasal and throat swab specimens [23, 24]. However, there seems to be a good correlation as previously reported [25], and the nasopharyngeal sample is easier, quicker and widely available.

Our study presents several limitations. Firstly, this is a single-centre observational cohort study and includes only patients from the first days of the pandemic in Spain, with high hospital occupancy rates; therefore, mortality and respiratory failure rates could decrease in the current situation. However, it does not necessarily signify that the relationship between higher viral load and worse prognosis is modified. Secondly, it only includes nasopharyngeal samples, whose quality is determined by the ability to collect the sample and may not adequately reflect viral load in lung tissue, as previously noted. However, this sample is much easier to obtain than sputum or lower respiratory tract samples and is accessible in all centres and provides quick results for decision-making.

Thirdly, SARS-CoV-2 RT-PCR assays are multiple and vary between centres; some authors have questioned whether the relationship between viral load and worse evolution is consistent with different diagnostic assays. However, studies using different diagnostic platforms have corroborated this relationship, although there are variations in the cut-off points of Ct [20].

In addition, the rRT-PCR does not distinguish between viable and non-viable virus. Some studies [26] have shown that positive viral culture rate decreases with increasing Ct values. Despite these data, RT-PCR is the gold-standard for diagnosis worldwide due to its sensitivity and specificity and the impossibility of performing viral culture techniques in most centres [27].

In conclusion, in our experience, SARS-CoV-2 viral load, measured by the Ct value of the rRT-PCR in nasopharyngeal swabs on admission, is a viable prognostic marker for the development of respiratory failure. It is easy to obtain and a widely available tool that could help to select those patients who would benefit from a closer follow-up and an in-time and appropriate respiratory support.

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Authors' contributions All authors meet the requirements for authorship. Cristina De la Calle, Antonio Lalueza, and Carlos Lumbreras designed the study. Cristina De la Calle, Antonio Lalueza, Mikel Mancheño-Losa, Jaime Lora-Tamayo, Guillermo Maestro-De la Calle, Ana García-Reyne, Estibaliz Arrieta, Raquel Díaz-Simón, Irene Losada, Borja De Miguel, Francisco López-Medrano, Rafael San Juan, and Octavio Carretero acquired data. Cristina De la Calle and Antonio Lalueza performed the statistical analysis. Cristina De la Calle, Antonio Lalueza, Carlos Lumbreras, and José María Aguado participated in data interpretation and edited the article. Cristina De la Calle, Antonio Lalueza, and Carlos Lumbreras created the first draft of the article. Cristina De la Calle, Antonio Lalueza, Mikel Mancheño-Losa, Jaime Lora-Tamayo, Guillermo Maestro-De la Calle, Ana García-Reyne, Estíbaliz Arrieta, Raquel Díaz-Simón, Borja De Miguel, Irene Losada, Rafael San Juan, Mario Fernández-Ruiz, Octavio Carretero, Francisco López-Medrano, José María Aguado, and Carlos Lumbreras revised and gave the final approval of the manuscript.

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Data availability Data archiving is not mandated, but data will be made available on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study protocol was approved by the University Hospital 12 de Octubre Clinical Research Ethics Committee (reference 20/117) in view of the retrospective nature of the study, and all the procedures being performed were part of routine care.

Consent to participate A waiver of informed consent was granted due to its retrospective observational design.

Consent to publish All authors have read the manuscript and agree with its current form and with its publication.

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