Qualitative Subgenomic RNA to Monitor the Response to Remdesivir in Hospitalized Patients with COVID-19: impact on the length of hospital stay and mortality

Rodrigo ALONSO-NAVARRO[1], Genoveva CUESTA[2,3] Marta SANTOS[2,3], Celia CARDOZO[1], Verónica RICO[1], Nicole GARCIA-POUTON[1], Montse TUSET[4], Marta BODRO[1], Laura MORATA[1], Pedro PUERTA-ALCALDE[1], Sabina HERRERA[1], Dafne SORIA[2], Marta ALDEA[5], Josep MENSA[1], José Antonio MARTÍNEZ[1,6], Ana DEL RIO[1], Jordi VILA[2,3,6], Felipe GARCIA[1], Carolina GARCIA-VIDAL[1] M. Angeles MARCOS[2,3,6]† and Alex SORIANO[1,6]†

[1]Department of Infectious Diseases, Hospital Clinic of Barcelona-IDIBAPS, University of Barcelona, Barcelona, Spain; [2]Department of Microbiology, Hospital Clinic of Barcelona, University of Barcelona, Barcelona, Spain; [3]Institute for Global Health (ISGlobal), Barcelona, Spain; [4]Department of Pharmacy, Hospital Clinic, IDIBAPS, University of Barcelona, Barcelona, Spain, [5]Department of Preventive Medicine, Hospital Clinic of Barcelona, [6]CIBERINF

†Contributed equally.

Corresponding author: Dr. Alex Soriano

Department of Infectious Diseases, Hospital Clinic of Barcelona. C/ Villarroel 170, 08036 Barcelona, Spain. Email: asoriano@clinic.cat.

Running head: Subgenomic RNA dynamics under Remdesivir

Abstract

Background: There is no reliable microbiological marker to guide the indication and the response to antiviral treatment in patients with COVID-19. We aim to evaluate the dynamics of subgenomic RNA (sgRNA) in patients with COVID-19 before and after receiving treatment with remdesivir.

Methods: We included consecutive patients admitted for COVID-19 who received remdesivir according to our institutional protocol and accepted to participate in the study. A nasopharyngeal swab for qRT-PCR was collected at baseline, and after 3 and 5 days of treatment with remdesivir. Genomic and sgRNA were analyzed in those samples and main co-morbidities and evolution were collected for the analyses. The main outcomes were early discharge (≤10 days) and 30-day mortality.

Results: A total of 117 patients were included in the study, from which 24 had a negative sgRNA at baseline with a 62.5% (15/24) of early discharge (≤10 days) and no deaths in this group. From the 93 remaining patients, 62 of them had a negative sgRNA at day 5 with 37/62 (59.6%) of early discharge and a mortality of 4.8% (3/62). In the 31 patients subgroup with positive sgRNA after 5 days of RDV, the early discharge rate was 29% (9/31) and the mortality rate was 16.1% (5/31). In the multivariable analyses, the variables associated with early discharge were negative sgRNA at day 3, and not needing treatment with corticosteroids or ICU admission.

Conclusions: Qualitative sgRNA could help monitoring the virological response in patients who receive remdesivir. Further studies are needed to confirm these findings.

Keywords: COVID-19, subgenomic RNA, remdesivir, early display

Introduction

Remdesivir (RDV) has a potent in vitro activity against different variants of SARS-CoV-2 [1] and animal models have shown a rapid clearance of viable virus from the respiratory samples [2]. However, clinical trials have shown conflicting results [3–5] probably because the indication of RDV was based on patients' clinical status instead of considering the viral load or the number of days from symptoms onset. A higher viral load is present mainly at early stages of the disease and it explains why the major benefit of RDV has been observed in the sub-groups of patients with shorter duration of symptoms [3,6–8]. This is in line with retrospective studies showing that RDV reduces the mortality when it is given within the first days from symptoms onset [9–11]. However, we need additional virological markers that help to identify patients who most benefit from antiviral therapy but also to monitor the virological response. Although there is a good correlation between RNA viral load and positive viral culture [12,13], in a recent clinical trial RDV was associated with an 85% reduction in the risk of hospital admission and death at 28 days in outpatients with mild COVID-19, but no difference in RNA copies/mL was observed between RDV and placebo [14]. This finding supports that the number of RNA copies/mL is not a good surrogate marker for monitoring the response to RDV and, therefore; alternative measurements are necessary.

At the beginning of the pandemic, Wölffel et al [15] showed a good correlation between qualitative subgenomic RNA (sgRNA) and positive viral culture. More recently, our Microbiology Laboratory confirmed the good correlation between these two parameters in more than 100 samples [16]. Accordingly, sgRNA could be a good parameter to identify patients that require RDV and to evaluate the response to antivirals. The aim of the present study was to prospectively follow-up consecutive patients treated with RDV and to determine genomic RNA

(gRNA) and sgRNA by reverse transcriptase-real time polymerase chain reaction (qRT-PCR) in nasopharyngeal swabs at baseline and on day 3 and 5 after starting RDV, and to correlate the results with the time to discharge and mortality.

Patients and methods

Patients

Consecutive hospitalized patients with confirmed SARS-CoV-2 infection and criteria for receiving remdesivir according to our protocol from February 2021 to May 2021 were prospectively followed up. A nasopharyngeal swab for virological analysis was performed at baseline (before RDV), day 3 and 5 after starting RDV. The indication for RDV in our institution includes: 1) COVID-19 confirmed by qRT-PCR, 2) ≤7 days from symptoms onset or ≤10 days in immunosuppressed patients, 3) radiological signs of pneumonia, and 4) requiring supplemental oxygen support or respiratory rate ≥24 breaths per minute or $Pa0_2/FiO_2$<300 mmHg. The RDV dose was 200 mg as a loading dose and 100 mg/24h for the next 4 consecutive days. The other treatments including anti-inflammatory drugs, and heparin were decided by the physician in charge and according to our institutional protocol. Since there was no prior data about the dynamics of sgRNA in patients under RDV treatment, the sample size was decided as an exploratory analysis and to capture the different types of patients that were admitted to the hospital (age ranges, and different comorbidities). The baseline and follow-up samples were frozen and were analyzed all together after including all patients, therefore, this information was not available for physicians to make any clinical decision based on these results.

Variables and Outcomes

Variables gathered were age, gender, comorbidities, respiratory rate and oxygen saturation at admission, biochemical parameters including C-reactive protein, LDH, ferritin, creatinine, and D-dimer as well as lymphocyte count. The qualitative result of the qRT-PCR of gRNA and the cycle threshold (Ct) of the 3 nasopharyngeal swabs (before, after 3 and 5 days of RDV) and the qualitative result of the qRT-PCR of the sgRNA were gathered.

The main outcome was to be discharged from the hospital within the first 10 days from admission (median value of hospital duration in the total population included in the study) and mortality at 30 days. The Ethical Committee of our institution accept the protocol (HCB/2021/0080) and the included patients signed the informed consent to participate in the study.

Microbiological methods

All the samples (baseline, 3, and 5 days) were gathered at -80ºC, and the qRT-PCR of gRNA and sgRNA were performed after all patients were included.

The presence of SARS-CoV-2 genomic RNA was determined by qRT-PCR in the automatic system Cobas 6800 (Roche, Barcelona) according to the manufacturer's instructions. For the detection of sgRNA of SARS-CoV-2, the total nucleic acid from all samples was extracted using MagNA Pure Compact (Roche, Switzerland). All samples were analyzed for the presence of Envelope (E) sgRNA using the leader-specific primer described by Wölfel et al [15] as well as primers and probes targeting sequences downstream of the start codons of the E gene [17]. qRT-PCRs were performed using the SuperScript™ III Platinum™ One-Step qRT-PCR kit (Invitrogen) with 400nM primers concentration and 200nM probe concentration. Cycling involved 15 min at 50ºC for reverse transcription, 3 min at 95ºC for Taq activation and 45 cycles of 10s at 95ºC, 15s at 60ºC (where the fluorescence was quantified), and 5s at 72ºC in the

thermocycler StepOne (Applied Biosystems). Ct values >40 were considered gRNA and sgRNA negative.

Statistical analysis

Categorical variables were described as absolute numbers and percentages and continuous ones as median and IQR. For the analysis, continuous variables were dichotomized according to the median value. Percentages were compared using Chi^2-squared or Fisher's exact tests and the median (IQR) values using a U-Mann Whitney test. Variables independently associated with early discharge (≤10 days) and positive sgRNA at day 5 were identified using a multivariable analysis. Variables with a P value ≤0.10 in the univariable analysis were subjected to further selection by using logistic regression method. The calibration of the model was assessed by means of the Hosmer–Lemeshow goodness-of-fit test and the area under the receiver operating characteristic (ROC) curve was used to measure the predictive ability of the model. Statistical significance was defined as a two-tailed P value <0.05.

Results

During the study period a total of 117 patients signed the inform consent and were included in the study. Samples at all 3 time points were available in all patients except one sample at day 3 in one patient. Only 7 out of 117 (6%) had a negative qRT-PCR of gRNA after 5 days of RDV. In Figure 1, we show the outcomes of patients according to the qualitative (positive or negative) sgRNA result. In 24 (20.5%) cases, the baseline sgRNA was negative. In this group, 15 (62.5%) patients were discharged from the hospital in ≤10 days and no patient died. The characteristics of patients according to the baseline sgRNA are depicted in Table 1. The other 93 (79.5%) cases with positive sgRNA had different outcomes according to the evolution of sgRNA. Those 62

patients (66.6%) with a negative sgRNA after 5 days of RDV had a similar percentage of early (≤10 days) discharge (37 out of 62, 59.6%) than those with negative sgRNA (62.5%), but there were 3 deaths (4.8%).

On the other hand, those 31 cases (33.3%) with persistent positive sgRNA after 5 days of RDV had a significantly lower proportion of early discharge (9 out of 31, 29%) and a significantly higher mortality rate (5 out of 31, 16.1%) compared to the rest of the cohort including those with baseline negative sgRNA and those who became negative within 5 days (52 out of 86, 60.5% for early discharge, P=0.003, and 3 out of 86, 3.5% for mortality, P=0.03).

We analyzed the variables associated with early (≤10 days) hospital discharge among those with positive sgRNA (n=93). Having at least one comorbidity, a Ct of gRNA ≤ 21 (median Ct value) at baseline (high viral load), a qualitative positive sgRNA result at day 3 and 5, the need to receive tocilizumab or corticosteroid therapy, and ICU admission or the need of mechanical ventilation showed a significant inverse association with early discharge (Table 2). In the multivariable analysis, a negative sgRNA after 3 days under RDV was strongly associated with early discharge (OR: 7.540, 95% CI: 2.330-24.401), while ICU admission and receiving corticosteroid treatment were significantly associated with late discharge (Table 2). The goodness-of-fit of the model was assessed with the Hosmer–Lemeshow test (P>0.05) and the area under the ROC curve was 0.832 (95% CI=0.749–0.915, P=0.0001), showing a good ability to predict early discharge.

We evaluate the baseline characteristics of patients that did not clear the sgRNA after 5 days of RDV (Table 3). In the univariable analysis only a CRP > 6 mg/dL, and a baseline Ct of qRT-PCR of gRNA ≤ 21 (high viral load) were associated with a positive sgRNA at day 5. There was

also a trend among patients with lymphoma. The multivariable analysis did not identify independent predictors of positive sgRNA at day 5.

Discussion

The results from our study showed that 20.5% (24 out of 117) of patients with documented SARS-CoV-2 pneumonia had negative sgRNA in the initial respiratory sample, 62% were discharge within the first 10 days from admission and no patient died (Figure 1). These results are in line with previous studies showing that the mortality is associated with the viral load at admission [18]. Since we only included patients that received RDV, further studies comparing with placebo should be made to evaluate the use of sgRNA as a tool to prescribe RDV. Indeed, a recent study from Spain shows that the major benefit of antivirals is among patients with a baseline Ct value < 25 [19]. Due to the low numbers, the difference in mortality rates among groups according to the sgRNA (Figure 1) should be interpreted cautiously, and future studies are needed to confirm this result.

On the other hand, the rate of early discharge was 49.5% (46 out of 93) among patients having a positive sgRNA at admission and their mortality rate was 8.6% (8 out of 93). However, these outcomes were different according to the dynamics of sgRNA during RDV treatment. The 59.6% (37 out of 62) of the patients with negative sgRNA at day 5 were early discharged and their mortality rate was 4.8% (3 out of 62) while the rate of early discharge was only 29% (9 out of 31) and the mortality 16.1% (5 out of 31) for those who remained positive (Figure 1). As expected, ICU admission and the need of corticosteroids therapy were associated with late discharge but negative sgRNA at day 3 from starting RDV was a potent independent predictor of early discharge (OR 7.540, CI95%: 2.330-24.401, P=0.001). These data strongly support that a

rapid clearance of viral load is associated with faster patient's recovery. Interestingly, this benefit was observed even after adjusting for corticosteroid treatment. In other viral infections [20] the use of steroids has been associated with worse outcome, but in COVID-19 the data is conflicting. In critically ill patients, dexamethasone reduces mortality. On the other hand, in non-mechanically ventilated patients a recent quasi-experimental study comparing 2 consecutive cohorts, one receiving corticosteroid alone and the other in combination with RDV [21], showed a significant reduction in the mortality rate in the RDV arm (1.3% vs. 16%, P=0.005). Our study confirms that the beneficial effect of RDV is independent of the use of corticosteroid therapy.

From our results, 33.3% of patients with positive sgRNA at the moment of starting RDV did not clear the sgRNA and they had longer hospitalizations and the highest mortality rate (16.1%). In this study no patients without RDV were included, therefore, it is impossible to make interpretations about its efficacy. One previous study determined the presence of sgRNA of E gene at hospital admission in 185 patients and correlate it with symptoms duration. They showed that 50% of patients became negative for sgRNA after 14 days from symptoms onset [22]. In our study 66.6% were negative after 5 days of RDV, with a median duration of symptoms of 6 days; therefore, the higher proportion of negative patients in a shorter period of time suggests a beneficial effect.

On the other hand, the reason for virological failure could be the selection of resistant variants or a slow virological response. Although RDV resistance has been documented, there is only one report to date [23], therefore; the second option seems the most likely one. Patients at a higher risk of remain with positive sgRNA at day 5 were those with a high baseline viral load (Ct of gRNA $\leq$21) and those with lymphoma. Accordingly, it is reasonable to speculate that these patients require longer courses of RDV or to combine RDV with a second antiviral agent with a

different mechanism of action including monoclonal antibodies or other antiviral agents (nirmatrelvir/ritonavir or molnupiravir).

The role of sgRNA as a surrogate marker of viral viability is not yet clear [15,24]. All the studies revealed that sgRNA become undetectable sooner than gRNA but recent analysis suggests that this could be attributable to the lower concentration of sgRNA rather than a true correlation with viral viability, not adding new information to viral load measurement [22,25]. However, even in this case, a qRT-PCR for the detection of sgRNA is faster, and easier to perform than the measurement of viral load in heterogenous matrices like respiratory samples. Therefore, we consider that the qualitative determination of sgRNA is an attractive test to make clinical decisions like when to start or to stop an antiviral therapy.

The major limitation of the present study is that this is a single arm study and there was no control without antiviral treatment, however, RDV was the standard of care in our institution, and it would not have been ethical not to treat these patients. In contrast, this was a prospective collection of data, and the result of sgRNA and Ct value of qRT-PCR were not available to the physicians, so the decision of patient's discharge was not biased by this result but based on clinical improvement giving strength to our analysis. Additionally, viral culture would be ideal, but samples were frozen, and this precluded optimal viral culture results.

In conclusion, sgRNA could help to decide when to start antiviral therapy, particularly in doubtful cases (e.g. duration of symptoms around 7 days), to monitor the virological response, and to decide the duration of RDV. Our results showed that early microbiological response (negative sgRNA at day 5) is associated with shorter hospital stay, and indicates that 5 days of RDV would be enough. In contrast, slower microbiological response (positive sgRNA at day 5) is associated with a slower clinical response and it should be considered to prolong the antiviral

therapy until sgRNA turns negative. In the future, it is necessary to validate this strategy in a prospective cohort of patients under antiviral therapy. Finally, our study has implications for the development of clinical trials directed to evaluate different antiviral strategies.

NOTES

Authors' contributions

Rodrigo Alonso has participated in the inclusion of patients, data collection statistical analysis, drafting the article and revising the bibliography.

Genoveva Cuesta, Marta Santos, Dafne Soria and Jordi Vila have contributed in the microbiological analyses of the samples and the redaction of the laboratory aspects within the methods section.

Celia Cardozo, Veronica Rico, Nicole Garcia-Pouton, Marta Bodro, Laura Morata, Pedro Puerta-Alcalde, Sabina Herrera and Marta Aldea have helped in the recruitment of patients and collection of data.

Josep Mensa, Jose Antonio Martínez, Ana del Rio, Felipe García and Carolina Garcia-Vidal have collaborated in the interpretation of the data and revising the final draft.

M Angeles Marcos and Alex Soriano are responsible for de conception and design of the study, the statistical analysis and interpretation, drafting the article and are responsible for the final supervision of the draft.

Funding

This work was supported by a grant from Gilead Sciences (IN-ES-540-6089)

This work was financed by ad hoc patronage funds for research on COVID-19 from donations from citizens and organizations to the Hospital Clínic de Barcelona-Fundació Clínic per a la Recerca Biomèdica.

Transparency declarations

Carolina Garcia-Vidal has received support for attending meetings and/or travel and honoraria for talks on behalf of Gilead Sciences, MSD, Novartis, Pfizer, Janssen and Lilly, as well as grants from Gilead Sciences and MSD. Laura Morata has received honoraria for talks on behalf of Merck Sharp and Dohme, Pfizer and Angelini. Pedro Puerta-Alcalde has received honoraria for talks on behalf of Gilead Sciences and MSD. Montse Tuset has received grants from Janssen, Gilead, ViiV and Merck Sharp and Dohme, honoraria from Janssen, Gilead, ViiV,MSD, TheraTechnologies and Shionogi for HIV andantimicrobial related presentations, and support for attending meetings and/or travel fromViiv and Gilead. Josep Mensa has received honoraria for talks on behalf of Merck Sharp and Dohme, Pfizer, Novartis and Angelini. Alex Soriano has received consulting fees and honoraria for talks on behalf of Merck Sharp and Dohme, Pfizer, Novartis, Gilead, Shionogi, Menarini and Angelini, as well as grant support from Pfizer and Gilead; including support for attending meetings from Pfizer. Dafne Soria reports support for attending meetings and/or travel from Pfizer (contribution to costs of events-registration fees). Angeles Marcos reports receipt of materials of laboratory from Gilead (reactive - IN-ES-540-6089). Nicole García Poutón reports support for attending meetings and/or travel from Gilead. Pedro Puerta-Alcalde reports grants or contract from Rio Hortega contract, Juan Rodes contract, and National competitive grant (to Instituto de Salud Carlos III), Honoraria for lectures from Pfizer, Gilead S.A., ViiV Healthcare, and MSD, support for attending meetings and/or travel from Pfizer (National Congress), MSD (National Congress), and Gilead S.A. (International

Congress), and Participation on a Data Safety Monitoring Board or Advisory Board for Gilead S.A. (personal payment). Rodrigo Alonso reports support for attending meetings and/or travel from Pfizer (contribution to costs of events-registrations fees). Sabina Herrera reports payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from MSD and Shire, and support for attending meetings and/or travel from Pfizer. All other authors: none to declare.

References

1. Vangeel L, Chiu W, Jonghe SD, et al. Remdesivir, Molnupiravir and Nirmatrelvir remain active against SARS-CoV-2 Omicron and other variants of concern. Antivir Res 2022; 198:105252–105252.

2. Williamson BN, Feldmann F, Schwarz B, et al. Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2. Nature 2020; 585.

3. Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the Treatment of Covid-19 — Final Report. New Engl J Med 2020; 383:1813–1826.

4. Arch B, Kovacs D, Scott J, et al. Evaluation of the effectiveness of remdesivir in treating severe COVID-19 using data from the ISARIC WHO Clinical Characterisation Protocol UK: a prospective, national cohort study. Medrxiv 2021; :2021.06.18.21259072.

5. Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet 2020; 395:1569–1578.

6. Ader F, Bouscambert-Duchamp M, Hites M, et al. Remdesivir plus standard of care versus standard of care alone for the treatment of patients admitted to hospital with COVID-19 (DisCoVeRy): a phase 3, randomised, controlled, open-label trial. Lancet Infect Dis 2022; 22:209–221.

7. Ali K, Azher T, Baqi M, et al. Remdesivir for the treatment of patients in hospital with COVID-19 in Canada: a randomized controlled trial. Cmaj 2022; 194:cmaj.211698.

8. Paules CI, Gallagher SK, Rapaka RR, et al. Remdesivir for the Prevention of Invasive Mechanical Ventilation or Death in Coronavirus Disease 2019 (COVID-19): A Post Hoc Analysis of the Adaptive COVID-19 Treatment Trial-1 Cohort Data. Clin Infect Dis Official Publ Infect Dis Soc Am 2021; 74:1260–1264.

9. Garcia-Vidal C, Alonso R, Camon AM, et al. Impact of remdesivir according to the pre-admission symptom duration in patients with COVID-19. J Antimicrob Chemoth 2021; :dkab321-.

10. Olender SA, Perez KK, Go AS, et al. Remdesivir for Severe COVID-19 versus a Cohort Receiving Standard of Care. Clin Infect Dis Official Publ Infect Dis Soc Am 2020; 73:ciaa1041.

11. Benfield T, Bodilsen J, Brieghel C, et al. Improved Survival Among Hospitalized Patients With Coronavirus Disease 2019 (COVID-19) Treated With Remdesivir and Dexamethasone. A Nationwide Population-Based Cohort Study. Clin Infect Dis Official Publ Infect Dis Soc Am 2021; 73:ciab536.

12. Huang C-G, Lee K-M, Hsiao M-J, et al. Culture-Based Virus Isolation To Evaluate Potential Infectivity of Clinical Specimens Tested for COVID-19. J Clin Microbiol 2020; 58:e01068-20.

13. Kampen JJA van, Vijver DAMC van de, Fraaij PLA, et al. Duration and key determinants of infectious virus shedding in hospitalized patients with coronavirus disease-2019 (COVID-19). Nat Commun 2021; 12:267.

14. Gottlieb RL, Vaca CE, Paredes R, et al. Early Remdesivir to Prevent Progression to Severe Covid-19 in Outpatients. New Engl J Med 2021; 386:NEJMoa2116846.

15. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020; 581:465–469.

16. Bravo MS, Berengua C, Marín P, et al. Viral Culture Confirmed SARS-CoV-2 Subgenomic RNA Value as a Good Surrogate Marker of Infectivity. J Clin Microbiol 2021; 60:e01609-21.

17. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Eurosurveillance 2020; 25:2000045.

18. Miller EH, Zucker J, Castor D, et al. Pretest Symptom Duration and Cycle Threshold Values for Severe Acute Respiratory Syndrome Coronavirus 2 Reverse-Transcription Polymerase Chain Reaction Predict Coronavirus Disease 2019 Mortality. Open Forum Infect Dis 2021; 8:ofab003.

19. Padilla S, Polotskaya K, Fernández M, et al. Survival benefit of remdesivir in hospitalized COVID-19 patients with high SARS-CoV-2 viral loads and low-grade systemic inflammation. J Antimicrob Chemoth 2022;

20. Moreno G, Rodríguez A, Reyes LF, et al. Corticosteroid treatment in critically ill patients with severe influenza pneumonia: a propensity score matching study. Intensive care medicine 2018; 2:395.

21. Marrone A, Nevola R, Sellitto A, et al. Remdesivir plus dexamethasone versus dexamethasone alone for the treatment of COVID-19 patients requiring supplemental O2 therapy: a prospective controlled non-randomized study. Clin Infect Dis Official Publ Infect Dis Soc Am 2022; :ciac014.

22. Dimcheff DE, Valesano AL, Rumfelt KE, et al. Severe Acute Respiratory Syndrome Coronavirus 2 Total and Subgenomic RNA Viral Load in Hospitalized Patients. J Infect Dis 2021; 224:1287–1293.

23. Gandhi S, Klein J, Robertson A, et al. De novo emergence of a remdesivir resistance mutation during treatment of persistent SARS-CoV-2 infection in an immunocompromised patient: A case report. Medrxiv 2021; :2021.11.08.21266069.

24. Binnicker MJ. Can Testing Predict SARS-CoV-2 Infectivity? The Potential for Certain Methods To Be Surrogates for Replication-Competent Virus. J Clin Microbiol 2021; 59:e00469-21.

25. Alexandersen S, Chamings A, Bhatta TR. SARS-CoV-2 genomic and subgenomic RNAs in diagnostic samples are not an indicator of active replication. Nat Commun 2020; 11:6059.

Tables

Table 1. Variables associated with negative subgenomic RNA at admission (continuous variables are dichotomized by the median value).

Variable (%)	**Negative sgRNA (N=24)**	**Positive sgRNA (N=93)**	**P-value**
Demographics			
Male gender	12 (50)	58 (62.4)	0.27
Age > 65 years	6 (25)	45 (48.4)	**0.04**
Comorbidities			
At least one	16 (66.7)	71 (76.3)	0.33
Hypertension	7 (29.2)	46 (49.5)	0.07
Diabetes mellitus	3 (12.5)	24(25.8)	0.28
Obesity	5 (20.8)	14 (15.1)	0.49
Heart disease	3 (12.5)	29 (31.2)	0.77
Chronic obstructive pulmonary disease	1 (4.2)	12 (12.9)	0.3
Chronic kidney disease	0 (0)	2 (2.2)	1
Liver disease	0 (0)	1 (1.1)	1
Solid neoplasia	1 (4.2)	3 (3.2)	1
Solid neoplasia with metastasis	0 (0)	4 (4.3)	0.58

Lymphoma	0 (0)	10 (10.8)	0.12
Leukemia	1 (4.2)	5 (5.4)	1
Solid organ transplantation	0 (0)	4 (4.3)	0.58
Parameters at admission			
Respiratory rate >20 bpm[1]	10 (47.6)	33 (37.5)	0.46
Oxygen saturation ≤ 95%	25 (54.3)	29 (61.7)	0.472
C-reactive protein >6 mg/dL	16 (66.7)	47 (50.5)	0.16
LDH >296 U/L[2]	16 (66.7)	46 (56)	0.14
Ferritin >570 ng/mL[3]	12 (50)	44 (49.5)	0.96
Creatinine >0.97 mg/dL	6 (25)	46 (49.5)	**0.03**
Lymphocyte count >700 cells/mm[3]	13 (54.2)	41 (44.1)	0.37
D-dimer >700 ng/mL[4]	6 (25)	42 (45.7)	0.067
Virological parameters			
Baseline Ct of qRT-PCR of gRNA ≤ 21	2 (8.3)	49 (52.7)	**<0.001**
Anti-inflammatory therapy			
Tocilizumab	13 (54.2)	45 (48.4)	0.61
Baricitinib	13 (54.2)	44 (47.3)	0.55
Corticosteroids	15 (62.5)	63 (67.7)	0.63
Outcomes			
ICU admission	7 (29.2)	28 (30.1)	0.93
Invasive mechanical ventilation	2 (8.3)	9 (9.7)	1
Mortality	0 (0)	8 (8.6)	0.2
Discharge within 10 days	15 (62.5)	46 (49.5)	0.25

1 Bpm, breaths per minute. Ct, cycle threshold. gRNA, genomic RNA. sgRNA, subgenomic RNA.

2 RDV, remdesivir.

[1] data from 88 patients; [2] data from 92 patients; [3] data from 89 patients; [4] data from 92 patients.

Table 2. Variables associated with discharge ≤10 days from admission (continuous variables are dichotomized by the median value). Independent factors associated with discharge ≤10 days from admission (multivariable analyses).

Variable (%)	**≤10 days (N=46)**	**>10 days (N=47)**	**P-value**	**OR (CI 95%)**	**P-value**
Demographics					
Male gender	27 (58.7)	31 (66)	0.47		
Age > 65 years	20 (47.8)	25 (53.2)	0.35		
Comorbidities					
At least one	29 (63)	42 (89.4)	**0.03**		
Hypertension	24 (52.2)	22 (46.8)	0.6		
Diabetes mellitus	13 (28.3)	11(23.4)	0.59		
Obesity	8 (17.4)	6 (12.8)	0.53		
Heart disease	12 (26.1)	17 (36.2)	0.29		
Chronic obstructive pulmonary disease	5 (10.9)	7 (14.9)	0.56		
Chronic kidney disease	1 (2.2)	1 (2.1)	1		
Liver disease	0 (0)	1 (2.1)	1		
Solid neoplasia	2 (4.3)	1 (2.1)	0.62		
Solid neoplasia with metastasis	1 (2.2)	3 (6.4)	0.62		
Lymphoma	5 (10.9)	5 (10.6)	0.97		
Leukemia	2 (4.3)	3 (6.4)	1		
Solid organ transplantation	2 (4.3)	2 (4.3)	1		

Parameters at admission					
Respiratory rate >20 bpm[1]	16 (37.2)	17 (37.8)	0.96		
Oxygen saturation ≤ 95%	25 (54.3)	29 (61.7)	0.472		
C-reactive protein >6 mg/dL	23 (50)	24 (51.1)	0.92		
LDH >296 U/L[2]	21 (45.7)	25 (54.3)	0.4		
Ferritin >576 ng/mL[3]	20 (45.5)	24 (53.3)	0.46		
Creatinine >0.97 mg/dL	21 (45.7)	25 (53.2)	0.47		
Lymphocyte count >700 cells/mm[3]	19 (58.7)	22 (53.2)	0.59		
D-dimer >700 ng/mL[4]	18 (39.1)	24 (52.2)	0.21		
Virological parameters					
Baseline Ct of qRT-PCR of gRNA ≤ 21	19 (41.3)	30 (63.8)	**0.03**		
Day 3 of Ct of qRT-PCR of gRNA ≤ 26	18 (39.1)	28 (60.9)	0.14		
Positive sgRNA[5] after 3 days on RDV	21 (46.6)	39 (82.9)	**<0.001**	0.136 (0.041-0.429)	0.001
Positive sgRNA after 5 days on RDV	9 (19.6)	22 (46.8)	**0.005**		
Anti-inflammatory therapy					
Tocilizumab	16 (34.8)	29 (61.7)	**0.009**		
Baricitinib	25 (54.3)	19 (40.4)	0.18		
Corticosteroids	24 (52.2)	39 (83)	**0.01**	0.299 (0.094-0.947)	0.04
Outcomes					
ICU admission	4 (8.7)	24 (51.1)	**<0.001**	0.117 (0.031-	0.002

				0.441)	
Invasive mechanical ventilation	1 (2.2)	8 (17)	**0.03**		
Mortality	0 (0)	8 (17)	**0.006**		

Bpm, breaths per minute. Ct, cycle threshold. gRNA, genomic RNA. sgRNA, subgenomic RNA. RDV, remdesivir.

[1] data from 88 patients; [2] data from 92 patients; [3] data from 89 patients; [4] data from 92 patients; [5] data from 92 patients.

1 Table 3. Variables associated with positive sgRNA after 5 days of remdesivir (continuous

2 variables are dichotomized by the median value).

Variable (%)	**Negative d5 sgRNA (N=62)**	**Positive d5 sgRNA (N=31)**	**P-value**
Demographics			
Male gender	36 (58.1)	22 (71)	0.23
Age > 65 years	30 (48.4)	15 (48.4)	1
Comorbidities			
At least one	45 (72.6)	26 (83.9)	0.23
Hypertension	29 (46.8)	17 (54.8)	0.46
Diabetes mellitus	14 (22.6)	10(32.3)	0.31
Obesity	7 (11.3)	7 (22.3)	0.15
Heart disease	17 (27.4)	12 (38.7)	0.27
Chronic obstructive pulmonary disease	6 (9.7)	6 (19.4)	0.19
Chronic kidney disease	2 (3.2)	0 (0)	0.55
Liver disease	0 (0)	1 (3.2)	0.33
Solid neoplasia	2 (3.2)	1 (3.2)	1
Solid neoplasia with metastasis	3 (4.8)	1 (3.2)	1
Lymphoma	4 (6.5)	6 (19.4)	0.08
Leukemia	2 (3.2)	3 (9.7)	0.33
Solid organ transplantation	3 (3.2)	1 (1.1)	1
Parameters at admission			
Temperature >37ºC	31 (50)	9 (29)	0.54

Respiratory rate >20 bpm[1]	23 (39.7)	10 (33.3)	0.56
Oxygen saturation ≤ 95%	33 (53.2)	21 (67.7)	0.18
C-reactive protein >6 mg/dL	36 (58.1)	11 (35.5)	**0.04**
LDH >296 U/L[2]	30 (49.2)	16 (51.6)	0.82
Ferritin >570 ng/mL[3]	29 (49.2)	15 (50)	0.94
Creatinine >0.97 mg/dL	28 (45.2)	18 (58.1)	0.24
Lymphocyte count >700 cells/mm^3	26 (41.9)	15 (48.4)	0.55
D-dimer >700 ng/mL[4]	30 (49.2)	12 (38.7)	0.34
Virological parameters			
Baseline Ct of qRT-PCR of gRNA ≤ 21	34 (54.8)	10 (32.3)	**0.04**

Bpm, breaths per minute. Ct, cycle threshold. gRNA, genomic RNA. sgRNA, subgenomic RNA. RDV, remdesivir.

[1] data from 88 patients; [2] data from 92 patients; [3] data from 89 patients; [4] data from 92 patients.

Figure 1. Flowchart of outcomes (≤10 days discharge and mortality) according to the results of subgenomic RNA

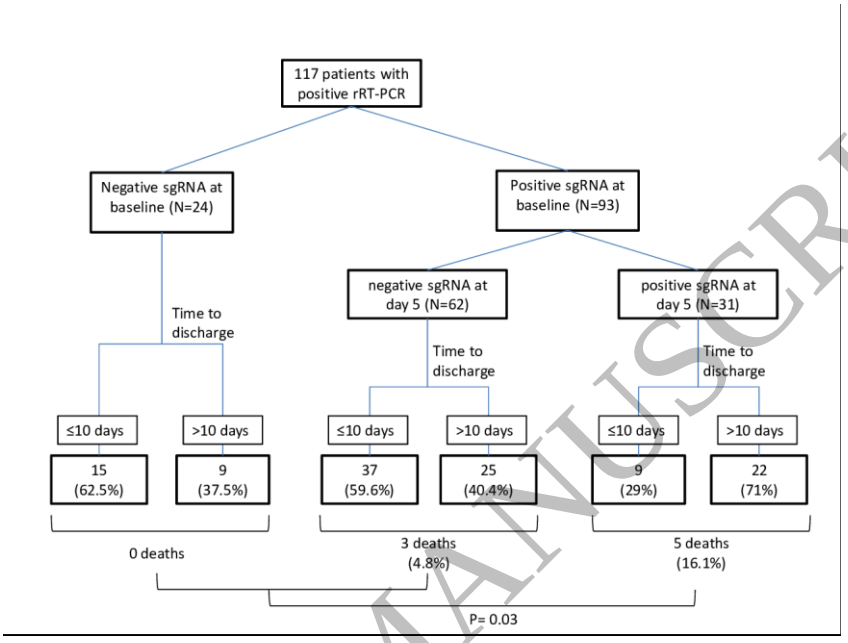

Figure 1
111x84 mm (x DPI)