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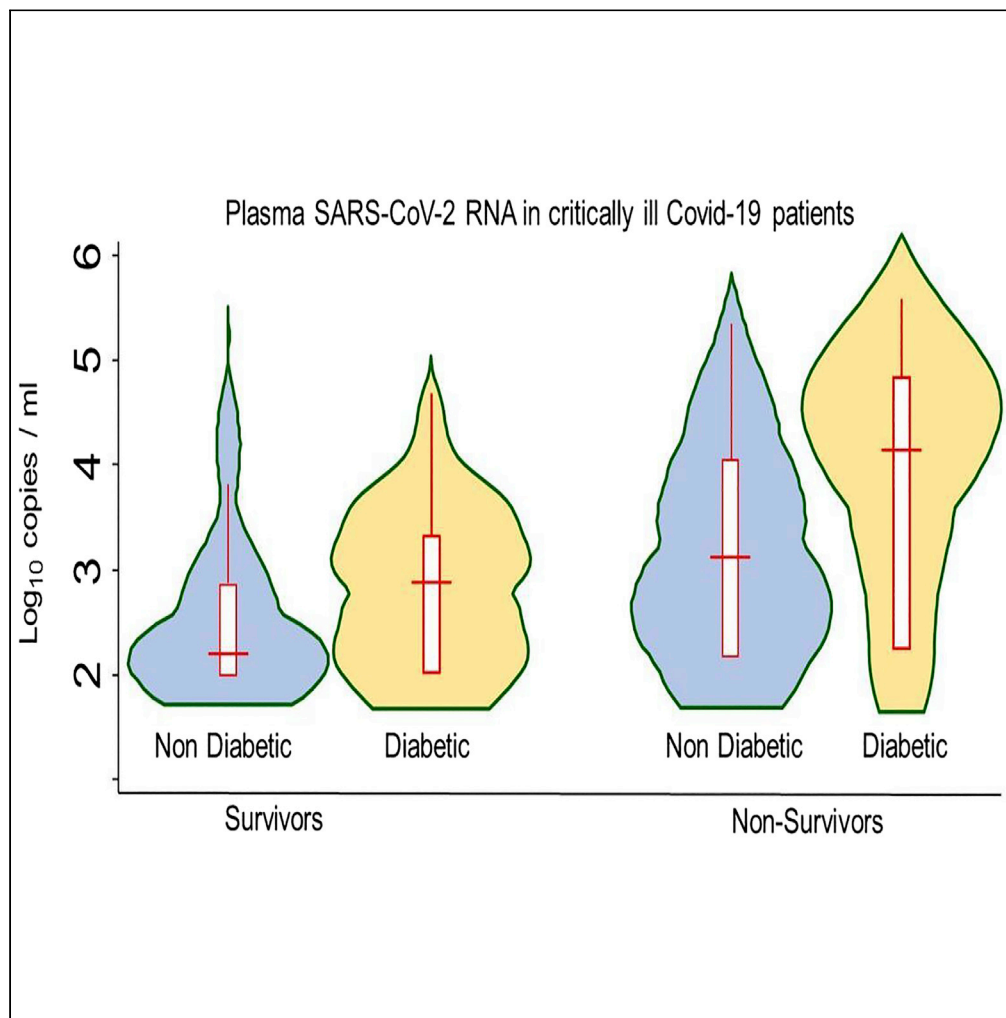
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Article

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Highlights

Plasma SARS-CoV-2 RNA was detected in 90 of 122 COVID-19 critically ill patients

Plasma SARS-CoV-2 RNA
was high (>1000 copies
per mL) in 46 patients

Diabetic patients had significantly higher levels of plasma SARS-CoV-2 RNA

Plasma SARS-CoV-2 RNA levels were independently associated with day-60 mortality

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Article

Association of high SARS-CoV-2 RNAemia with diabetes and mortality in critically ill COVID-19 patients

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SUMMARY

It has been suggested that during the period of respiratory worsening of severe COVID-19 patients, viral replication plays a less important role than inflammation. Using the droplet-based digital PCR (ddPCR) for precise quantification of plasma SARS-CoV-2 viral load (SARS-CoV-2 RNAemia), we investigated the relationship between plasma viral load, comorbidities, and mortality of 122 critically ill COVID-19 patients. SARS-CoV-2 RNAemia was detected by ddPCR in 90 (74%) patients, ranging from 70 to 213,152 copies per mL. A high (>1 000 copies/mL) or very high (>10,000 copies/mL) SARS-CoV-2 RNAemia was observed in 46 patients (38%), of which 26 were diabetic. Diabetes was independently associated with a higher SARS-CoV-2 RNAemia. In multivariable logistic regression models, SARS-CoV-2 RNAemia was strongly and independently associated with day-60 mortality. Early initiation of antiviral therapies might be considered in COVID-19 critically ill patients with high RNAemia.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for the development of COVID-19, has been global public health problem since January 2020. Although the majority of cases of patients with COVID-19 disease have mild symptoms, a proportion of cases progress to severe pneumonia, acute respiratory distress syndrome (ARDS), and death (Zhu et al., 2020).

In severe cases, clinical observations typically describe a disease starting with a mild-to-moderate presentation followed by a secondary respiratory worsening 9 to 12 days after onset of first symptoms (Grasselli et al., 2020). Initial analysis of host response to SARS-CoV-2 suggested a unique and inappropriate inflammatory response to the virus, which is imbalanced with regard to controlling virus replication versus activation of the adaptive immune response (Blanco-Melo et al., 2020). It is defined by low levels of type I and III interferons juxtaposed to elevated cytokines such as IL-6. Given this dynamic, it has been suggested that during the secondary period of respiratory worsening occurring in severe patients, treatments for COVID-19 have less to do with virus replication and more to do with controlling inflammation. In fact, in severe COVID-19 patients, anti-inflammatory treatments such as systemic glucocorticoids (Rochwerg et al., 2020), anakinra (Kyriazopoulou et al., 2021), Janus kinase inhibitors (Marconi et al., 2021), and Interleukin-6 receptor antagonists (RECOVERY Collaborative Group, 2021; REMAP-CAP Investigators et al., 2021) reduced short-term mortality.

In the SARS-CoV-2 pathogenesis, after the worsening period and admission to the intensive care unit, the importance of viral replication rate is still unclear. It has been suggested that the detection of SARS-CoV-2 RNA in plasma at the time of respiratory deterioration is associated with a higher severity of the disease (Eberhardt et al., 2020; Hogan et al., 2021; Kawasuji et al., 2022; Ram-Mohan et al., 2021; The Massachusetts Consortium for Pathogen Readiness et al., 2020; Veyer et al., 2020; Zheng et al., 2020), with circulating SARS-CoV-2 viral load showing a large amplitude. Fajnzylber et al., (2020) also analyzed the association and predictability of SARS-CoV-2 RNAemia with mortality and showed a direct association of RNAemia with mortality (The Massachusetts Consortium for Pathogen Readiness et al., 2020). However, the association between the amplitude of SARS-CoV-2 RNA in plasma and mortality of the most severe

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Table 1. Patients' characteristics

Characteristic	Total population	Survivors	Non survivors	p value
Number of patients	122	84	38	
Age	60 (54–68)	57 (53–65)	65 (59–73)	0.0002
Male sex	82 (67%)	54 (64%)	28 (73%)	0.31
BMI	30.4 (25.8–35.2)	30.3 (26.4–34.5)	30.4 (25.1–35.8)	0.93
Diabetes	45 (37%)	30 (36%)	15 (40%)	0.69
Hypertension	78 (64%)	48 (57%)	30 (79%)	0.020
Immunosuppression	10 (8%)	5 (6%)	5 (13%)	0.18
SAPS-2 severity score	36 (29–44)	35 (28–41)	39 (34–48)	0.005
SOFA severity score	4 (3–5)	4 (3–5)	5 (4–7)	0.019
PaO ₂ on FiO ₂ ratio	108 (85–152)	112 (86–156)	105 (77–148)	0.54
Neutrophil count (10 ⁹ /L)	6.9 (4.8–9.4)	7.0 (4.9–9.3)	6.8 (4.8–10.1)	0.71
Lymphocyte count (10 ⁹ /L)	0.76 (0.57–1.20)	0.86 (0.59–1.42)	0.67 (0.45–0.86)	0.0025
D-dimer, mg/L	1.3 (0.7–3.1)	1.2 (0.7–3.3)	1.6 (1.0–2.9)	0.34
Serum Creatinine (μmol/L)	67 (54–84)	64 (50–77)	82 (62–95)	0.001
eGFR (MDRD) (Levey, 1999)	99 (77–125)	109 (83–133)	81 (63–108)	0.0001
CRP (mg/L)	61 (32–114)	58 (31–112)	76 (34–131)	0.40
Alpha Variant (%)	78 (64%)	54 (64%)	24 (63%)	0.34
Anti-Spike antibodies	23 (4–148)	21 (4–148)	36 (1–157)	0.83
Treated by Remdesivir	13 (11%)	9 (11%)	4 (11%)	0.98
Treated by Sarilumab	46 (38%)	34 (41%)	12 (32%)	0.29
Days from symptom onset to ICU admission	12 (9–15)	12 (9–15)	11 (9–16)	0.55
Plasma RNA load (log 10 copies/ml)	2.63 (<2.0 to 3.45)	2.47 (<2.0 to 3.19)	3.35 (<2.0 to 4.16)	0.0062

eGFR: estimated glomerular filtration rate.

PaO₂ on FiO₂ ratio is the ratio of arterial oxygen partial pressure (PaO₂ in mmHg) to fractional inspired oxygen.

SAPS-2: simplified acute physiology score 2 (Le Gall, 1993).

SOFA: sequential organ failure assessment (Vincent et al., 1996).

patients after adjustment on other predictors of mortality in specific critically ill patients remains undetermined (Bermejo-Martin et al., 2020).

This relationship between plasma viral load and mortality may be of major importance, because, until now, anti-viral therapies have been recommended mainly in the early stages of the disease and in noncritically ill patients.

The availability of precise quantification of the plasmatic SARS-CoV-2 RNA by droplet-based digital PCR (ddPCR) led us to evaluate systematically the SARS-CoV-2 RNAemia as an estimation of whole body viral replication on admission in our intensive care unit (ICU) during the Covid-19 “third wave,” mainly induced by the alpha variant of SARS-CoV-2 in France.

The aim of this retrospective study is to describe the relationship between SARS-CoV-2 Plasma RNA levels, comorbidities, and mortality of a COVID-19 critically ill patients' cohort, after adjustment on other predictors of mortality.

RESULTS

A total of 122 COVID-19 patients were admitted in our ICU during the “third-wave” period of the pandemic, from March 04 to June 22, 2021. Thirteen patients (11%) were treated by remdesivir and 46 (38%) with sarilumab.

Patient characteristics according to day-60 survival status and plasma SARS-CoV-2 viral loads were shown in Tables 1 and 2. Median age was 60 (IQR 54 to 68), and plasmatic RNA levels were positive by ddPCR in 90 (74%) patients, ranging from 70 to 213,152 copies per mL. Plasmatic RNA categories were low in 45 patients

Table 2. Characteristics according to plasma SARS-CoV-2 RNA categories

Plasma SARS-CoV-2 RNA levels (copies/ml)	≤ 100 (low)	>100 to ≤ 1000 (medium)	>1000 to 10,000 (high)	>10,000 (very high)	P
Number of patients	45	31	27	19	
Age	61 (57–68)	55 (48–66)	61 (56–68)	61 (47–69)	0.23
Male sex	28 (62%)	21 (67%)	21 (78%)	12 (63%)	0.57
BMI	29.7 (26.3–36.2)	30.1 (25.8–31.7)	30.9 (25.1–34.2)	31.3 (26.7–3.8)	0.92
Diabetes	14 (31%)	6 (19%)	15 (56%)	10 (53%)	0.013
Hypertension	29 (64%)	16 (52%)	20 (74%)	13 (68%)	0.33
Immunosuppression	3 (7%)	1 (3%)	3 (11%)	3 (16%)	0.40
SAPS-2 severity score	37 (31–44)	34 (28–40)	36 (33–47)	36 (28–46)	0.41
SOFA severity score	4 (4–6)	4 (3–4)	4 (3–6)	5 (3–5)	0.02
PaO ₂ on FiO ₂ ratio	105 (85–157)	121 (87–171)	108 (80–142)	107 (78–140)	0.86
Neutrophil count (10 ⁹ /L)	7.3 (5.0–10.1)	7.1 (5.8–8.9)	6.6 (5.4–9.4)	5.0 (3.8–7.4)	0.05
Lymphocyte count (10 ⁹ /L)	0.9 (0.7–1.5)	0.8 (0.7–1.0)	0.7 (0.5–1.1)	0.6 (0.3–0.9)	0.0046
D-dimer mg/L	1.0 (0.6–2.0)	1.0 (0.7–3.7)	2.8 (1.5–4.1)	1.3 (1.0–1.9)	0.0045
Serum Creatinine (μmol/L)	66 (52–77)	62 (52–79)	71 (58–91)	81 (61–100)	0.19
CRP (mg/L)	58 (30–104)	54 (32–89)	82 (26–145)	76 (48–190)	0.18
Alpha Variant (%)	22/28 (78%)	24/29 (83%)	19/25 (76%)	13/18 (72%)	0.54
Anti-Spike antibodies (U/ml)	159 (45–297)	13 (3–96)	20 (4–62)	1 (0–8)	0.0001
Treated by Remdesivir	2 (4%)	5 (16%)	3 (11%)	3 (16%)	0.34
Treated by Sarilumab	13 (29%)	14 (45%)	14 (44%)	7 (37%)	0.43
Days from symptom onset to ICU admission	14 (12–20)	12 (9–15)	11 (9–12)	9 (7–12)	0.0001
Mortality	12 (26%)	4 (13%)	11 (41%)	11 (58%)	0.005
Days alive out of ICU at day 60	46 (0–54)	52 (36–55)	28 (0–46)	0 (0–37)	0.0003

PaO₂ on FiO₂ ratio is the ratio of arterial oxygen partial pressure (PaO₂ in mmHg) to fractional inspired oxygen.

SAPS-2: simplified acute physiology score 2.

SOFA: sequential organ failure assessment.

(37%), medium in 31 patients (25%), high in 27 patients (22%), and very high in 19 patients (16%). The alpha SARS-CoV-2 variant was detected in 78 (64%) of patients, without a significant association with mortality or plasmatic SARS-CoV-2 RNA levels.

Hypertension was significantly more frequent in non-survivors (79 vs 57%, $p = 0.02$). Survivors had lower ICU severity scores, lower serum creatinine levels and higher lymphocyte counts. The level of median plasmatic SARS-CoV-2 RNA was almost 8 times higher in non-survivors ($p = 0.0062$).

Patients with high and very high plasmatic SARS-CoV-2 RNA levels were diabetic in more than 50% of cases ($p < 0.0001$ vs low and medium RNA categories). Median (IQR) plasmatic SARS-CoV-2 RNAemia were 265 (0–1156) and 1544 (70–7518) copies/mL in nondiabetic and diabetic patients ($p = 0.03$), respectively. Plasmatic SARS-CoV-2 RNA levels according to the diabetic status and survival are shown in the graphical abstract.

Increasing plasma RNA categories were significantly associated with decreasing Neutrophil ($p = 0.05$) and Lymphocyte ($p = 0.0046$) counts (Table 2).

Increasing plasmatic SARS-CoV-2 RNA levels were associated with lower neutrophil and lymphocyte counts. Patients with higher SARS-CoV-2 RNAemia had shorter intervals from symptom onset to ICU admission and lower levels of Anti-spike protein antibodies.

In the ordered logistic regression analysis including variables significantly associated with increasing plasmatic SARS-CoV-2 RNA categories (Table 3), diabetes, shorter intervals from symptom onset to ICU, higher

Table 3. Ordered logistic regression for variables associated with increasing plasmatic SARS-CoV-2 RNA levels

Variable	OR for a 10 fold (1 log ₁₀) increase in plasmatic SARS-CoV-2 RNA copies per mL	95% Confidence Interval for odds ratio	P
Diabetes	3.30	1.51 to 7.21	0.003
Days from symptom onset to ICU (per day)	0.83	0.76 to 0.91	<0.001
SOFA severity score	1.03	0.86 to 1.23	0.75
Neutrophil count (per 1 × 10 ⁹ /L)	0.96	0.85 to 1.08	0.46
lymphocyte count (per 1 × 10 ⁹ /L)	0.74	0.47 to 1.17	0.19
D-dimer (per mg/L)	1.06	1.00 to 1.12	0.042
Anti-Spike antibodies (per 1 log ₁₀ increase above 1 U/ml)	0.44	0.29 to 0.67	<0.001

SOFA: sequential organ failure assessment.

Variables having a significant association with higher plasmatic SARS-CoV-2 RNA levels in univariate analysis (Table 2) were included in the multivariable model.

d-dimer levels, and lower anti-spike antibodies were significantly related to higher levels of plasmatic SARS-CoV-2 RNA.

In both multivariable logistic regression models, including previously published parameters associated with mortality (Table 4) or those observed in our cohort (Table 5), plasmatic SARS-CoV-2 RNA levels were independently associated with day-60 mortality.

DISCUSSION

In this study of patients admitted to our ICU for severe COVID-19 disease, we found that plasmatic viral RNA level assessed by ddPCR was measurable in 74% of patients; high or very high in nearly 40% of patients; besides, the intensity of SARS-CoV-2 RNAemia was independently associated with day-60 mortality with a very strong relationship. We also observed that plasmatic SARS-CoV-2 RNA levels were notably higher in diabetic patients, even after adjustment on the interval between symptom onset and ICU admission (Table 3).

The inverse relationship between plasmatic SARS-CoV-2 RNA levels and anti-spike protein antibodies (Table 2) seems, at least partially, related to the higher interval between symptom onset and ICU admission in patients with higher levels of antibodies.

Several comorbidities including diabetes mellitus can result in a more severe course of COVID-19 that leads to higher mortality (Fleming et al., 2021; Guo et al., 2020; Kamyshnyi et al., 2020). However, to our knowledge, until now, a higher viral load in COVID-19 diabetic patients was not clearly described. Factors contributing to a higher severity of COVID-19 in diabetic patients may include increased inflammation and other comorbidities including hypertension, but the underlying pathophysiology of this increased risk is yet to be fully determined. It has been suggested that changes in the immunometabolism of lymphocytes, as is observed in diabetes, directly affect the course of the disease and thus point out the importance of glycemic control in people with COVID-19 infection (Pozzilli et al., 1987). Unlike bacterial infections, the physiological response in normal individuals to viral replication is activation of transcription factors including interferon regulatory factors and nuclear factor kappa B (NF-κB) (tenOever, 2016). This in turn leads to antiviral response by induction of type I interferon (IFN-I) and IFN-III (Lazear et al., 2019). One study showed that patients with diabetes had decreased antiviral response demonstrated by a diminished production of IFN-α (Summers et al., 2006). Another study performed on human peripheral blood mononuclear cells and human monocyte cell lines *in vitro* showed that hyperglycemia suppressed IFN-I production and that pre-exposure to high glucose concentrations rendered monocytes more sensitive to IFN-α stimulation with heightened signaling (Hu et al., 2018). Therefore, it is probable that patients with diabetes may have a defective host defense system against viral infections by impeding IFN-I production and signaling.

SARS-CoV-2 has a high affinity toward ACE2, and upon binding the virus, the complex is internalized resulting in intracellular replication of the virus (Jin et al., 2020). Hence, ACE2 acts as the "receptor" for

Table 4. Multivariable logistic regression model 1 for day-60 mortality, including previously published variables associated with mortality of COVID-19 critically ill patients (COVID-ICU Group on behalf of the REVA Network)

Variable	Odds ratio for day 60 mortality	95% Confidence Interval for Odds Ratio	P
Plasmatic SARS-CoV2 RNA, per 1 log ₁₀ increase above 2.5 (10 fold increase above 316 copies/mL)	3.53	1.66 to 7.53	0.001
Age, per year above 50	1.11	1.04 to 1.20	0.003
eGFR, per ml above 40 (ml/min per 1.73 m ²)	0.98	0.96 to 0.99	0.028
Immunosuppression	1.52	0.25 to 9.22	0.61
Body mass index ≥ 35	1.88	0.59 to 6.06	0.35
Diabetes	0.50	0.16 to 1.57	0.24
Days from symptom onset to ICU (per day)	1.07	1.00 to 1.14	0.048
absolute lymphocyte count (per 1.0 × 10 ⁹ /L)	0.36	0.10 to 1.32	0.12
PaO2 on FiO2 ratio ≤ 100	1.54	0.58 to 4.17	0.39
Hemodynamic component of SOFA score ≥ 3	0.84	0.23 to 3.07	0.80

eGFR: estimated glomerular filtration rate.

PaO2 on FiO2 ratio is the ratio of arterial oxygen partial pressure (PaO2 in mmHg) to fractional inspired oxygen.

SOFA: sequential organ failure assessment.

SARS-CoV-2 and is the dominant path of the virus's entry into the lungs and other cells in the body (Wan et al., 2020). Expression of ACE2 is increased in patients with diabetes and in response to elevated glucose levels (Bindom and Lazartigues, 2009).

These facts in part may explain the higher susceptibility of diabetic patients to contract a severe form of the disease. Unfortunately, we do not have data on the previous glycemic control of the patients included in our study and we have not measured glycated hemoglobin A1c on ICU or hospital admission. Thus, we cannot evaluate the relationship between previous glycemic levels and SARS-CoV-2 RNAemia. The association between diabetes and disease severity has already been highlighted in several studies (Huang et al., 2020). However, this association has been described mainly in infected populations before any selection of a severity justifying admission to intensive care. When patients with diabetes are compared to a nondiabetic population admitted to intensive care, the nondiabetic population having been selected on criteria of respiratory severity, the differences in mortality are probably attenuated, explaining the fact that diabetes is not associated with mortality in our ICU. Moreover, there is an interaction between diabetes, hypertension, and impaired renal function, and in fact, in our cohort, glomerular filtration is a more powerful predictor of mortality in both logistic regression models than diabetes.

Some prior studies have reported that cycle threshold values of nasopharyngeal swab samples were associated with the severity of the disease (Magleby et al., 2020; Pujadas et al., 2020), but these findings have been challenged by other groups (Le Borgne et al., 2021). In a recent randomized study, Remdesivir was associated with a significant reduction of Covid-19-related hospitalizations (0.7 vs 5.3%, $p = 0.008$) without any effect on nasopharyngeal viral load (Gottlieb et al., 2021); besides, in rhesus macaques infected with SARS-CoV-2, remdesivir reduced viral replication in the lower respiratory tract without any effect in the upper respiratory tract (Williamson et al., 2020). These data support the hypothesis that nasopharyngeal viral load may be not representative of the viral load in the lower respiratory tract. Plasmatic samples are easier to standardize and may be more representative of the viral replication rate throughout the whole body. Several published studies have already suggested that detection of SARS-CoV-2 RNA in the blood was predictive of the severity of the disease, without showing a clear relationship between the levels of plasmatic SARS-CoV-2 RNA copies and the mortality (Prebensen et al., 2021; Veyer et al., 2020). The relationship that we have observed may be explained by the greater severity of our patients and the precision provided by ddPCR technology. In fact, ddPCR technology is able to detect plasma SARS-CoV-2 RNA in a higher proportion of hospitalized patients than real-time PCR (Veyer et al., 2020).

Table 5. Multivariable logistic regression model 2 for day-60 mortality, including the variables associated with mortality of our cohort in univariable analysis (Table 1)

Variable	Odds ratio for day 60 mortality	95% Confidence Interval for Odds Ratio	P
Plasmatic SARS-CoV-2 RNA, per 1 log ₁₀ increase above 2.5 (10 fold increase above 316 copies/mL)	2.45	1.30 to 4.65	0.006
Age, per year above 50	1.10	1.03 to 1.17	0.003
eGFR, per ml above 40 (ml/min per 1.73 m ²)	0.99	0.97 to 1.00	0.09
SOFA severity score (per 1 point)	1.10	0.90 to 1.36	0.36
Hypertension	1.75	0.60 to 5.11	0.30
Lymphocyte count (per 1.0 × 10 ⁹ /L)	0.39	0.12 to 1.28	0.12

eGFR: estimated glomerular filtration rate.

PaO₂ on FiO₂ ratio is the ratio of arterial oxygen partial pressure (PaO₂ in mmHg) to fractional inspired oxygen.

SOFA: sequential organ failure assessment.

The association between plasmatic SARS-CoV-2 RNAemia and mortality leads to several questions.

First, so far, antiviral therapies have been reserved for the early stages of the disease, mainly in noncritically ill patients. This is because of the lack of studies describing a clear efficacy of antiviral therapies in the most severe patients. However, as shown by our data, ICU COVID-19 patients have highly variable viral loads, and it is likely that the inclusion of a significant proportion of patients with a low level of viral replication in antiviral treatment studies may decrease the observed efficacy of these therapies. Therefore, it is reasonable to think that in the most severe forms of COVID-19, antiviral treatments should be evaluated in patients with a high level of SARS-CoV-2 RNAemia. Indeed, plasma SARS-CoV-2 RNA levels could be a reasonable estimation of whole body viral replication even if it only quantitates viral genome and not infectious viral particles. Given the relative limited efficiency of antiviral therapies, it is also reasonable to assume that combined therapies could be necessary in patients with high viral replication levels. Repeated measures of plasmatic SARS-CoV-2 RNA levels by a precise method like ddPCR may help to define the best combination of antiviral therapies for these patients in preliminary studies before larger trials on mortality.

Second, the efficacy of anti-inflammatory therapies such as systemic glucocorticoids (Rochwerg et al., 2020), anakinra (Kyriazopoulou et al., 2021), Janus kinase inhibitors (Marconi et al., 2021), and Interleukin-6 receptor antagonists (RECOVERY Collaborative Group, 2021; REMAP-CAP Investigators et al., 2021) on short-term mortality of COVID-19 patients has been described in large studies without any evaluation of viral replication rate or plasmatic SARS-CoV-2 RNA levels. The benefit observed with these treatments could be very different depending on the patient's viral replication rate.

Limitations of the study

Our study has some limitations. We analyzed only the plasmatic viral RNA levels at the time of ICU admission and we could not assess viral load dynamics over time before and after ICU admission. However, we found that this single parameter at admission had important prognosis value and might lead to new therapeutic strategies.

Another limitation is that our study was retrospective and relied on data that were documented in the electronic medical record. However, the quality of the data collected is fairly robust, all data were double checked.

Last, as we previously evoked, we cannot be certain that plasmatic SARS-CoV-2 RNA level is a reliable reflection of the viral replication rate. However, plasma RNA seems to be a reasonable parameter for the evaluation of viral replication rate in other viral infections such as HIV (Antela et al., 2021) and may be more reliable than nasopharyngeal swabs.

Conclusion

In conclusion, we found that in severe COVID-19 patients, ICU admission plasmatic SARS-CoV-2 RNA levels, as determined by ddPCR, are notably higher in diabetic patients and are strongly associated with

day-60 mortality. These findings could lead to a better selection of patients who could benefit from antiviral and anti-inflammatory therapies.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.isci.2022.104075>.

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AUTHOR CONTRIBUTIONS

M.M. initiated the project and drafted the manuscript. T.B., D.V., A.P., M.P., and V.T. conceptualized and performed plasma measurements by ddPCR. S.J., O.E., O.S., and S.M. processed, analyzed, and interpreted the data. H.P. supervised the project and edited the manuscript.

DECLARATION OF INTERESTS

H.P., D.V., and V.T. have a patent related to the present work to declare: PCT/EP2021/064575 "QUANTIFICATION OF CORONAVIRUS RNAemia".

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
anti-Androgen Receptor (441)	Santa Cruz	Cat# sc-7305
anti-BAG1 (CC9E8)	Santa Cruz	Cat# sc-33704
anti- β -actin	Santa Cruz	Cat# sc-47778
Bacterial and virus strains		
<i>E. coli</i> strain BL21 (DE3)	Invitrogen	Cat# EC0114
<i>E. coli</i> strain DH5 α	Invitrogen	Cat# EC0112
Chemicals, peptides, and recombinant proteins		
(FITC-VQRKQKLMPLRLKLRNKKLENPLDYGSAAAAAQCRY)	Peptide 2.0 Inc.	N/A
Control FITC-labeled NLS-CPP peptide	Peptide 2.0 Inc.	N/A
(Dulbecco's Modified Eagle's Medium) DMEM	Gibco	Cat# 41966-029
Roswell Park Memorial Institute (RPMI) 1640	Gibco	Cat# 11875-085
Without phenol red		Cat# 11835030
Fetal Bovine Serum (FBS)	Gibco	Cat# 10270-106
5 α -Androstan-17 β -ol-3-on (Dihydrotestosterone)	Merck AG	Cat# A8380
Penicillin- Streptomycin	Gibco	Cat# 15140122
L-glutamine	Gibco	Cat# 25030081
Fish water 60 μ g/ml f.c. sea salts	Instant Ocean Spectrum Brands	Cat# SS15-10
Dimethyl sulfoxide	Carl Roth	Cat# A994.2
Passive lysis buffer	Promega	Cat# E1941
FuGENE HD	Promega	Cat# E2311
Dulbecco's phosphate buffered saline	Gibco	Cat# 14190094
Glyglycin	Carl Roth	Cat# 3794.4
Magnesium sulphate heptahydrate	Carl Roth	Cat# P027.1
1,4-Dithiothreitol	Carl Roth	Cat# 6908.2
Adenosine-5'-triphosphate-disodium salt	Carl Roth	Cat# HN35.2
D-Luciferin Firefly	Biosynth/Carbosynth	Cat# L-8200
EGTA	Carl Roth	Cat# 3054.1
Potassium dihydrogen phosphate	Carl Roth	Cat# P018.1
di-Potassium hydrogen phosphate	Carl Roth	Cat# P749.1
Sodium chloride	Carl Roth	Cat# 3957.2
Ethylenediamine tetraacetic acid disodium salt dihydrate	Carl Roth	Cat# 8043.1
Coelenterazine	Biosynth/Carbosynth	Cat# C-7002
Hoechst stain 33258	abcam	Cat# ab228550
Hoechst stain 33342	Invitrogen	Cat# 62249
3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)	Sigma-Aldrich	Cat# 1.11714
2-propanol	Carl Roth	Cat# 9866.1
Crystal Violet	SERVA	Cat# 27335.01
Methanol	Carl Roth	Cat# 8388.3

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
2'7-dichlorofluorescein diacetate	Thermo Fisher	Cat# D399
R1881 (Metribolone)	Sigma-Aldrich	Cat# R0908
Enzalutamide (MDV3100)	Selleckchem	Cat# S1250
Ammonium Chloride (15N)	Cambridge Isotope Laboratories	Cat# NLM-467-10
2-amino-4-(trifluoromethyl)benzenethiol; hydron; chloride	Sigma-Aldrich	Cat# 365734
4-fluorobenzoyl chloride	Sigma-Aldrich	Cat# 119946
dioxane	Fisher Scientific	Cat# 10141470
methylene chloride	Fisher Scientific	Cat# 10616642
NaHCO ₃	Fisher Scientific	Cat# 10553325
Na ₂ SO ₄	Fisher Scientific	Cat# 10032590
Celite	Fisher Scientific	Cat# 10316691
cyclohexane	VWR	Cat# 23224.327
ethyl acetate	VWR	Cat# 23882.330
Silica gel	Interchim	Cat# OV004A
corn oil	Sigma Aldrich	Cat# C8267
Matrigel	Corning	Cat# 354248

Critical commercial assays

Venor@GeM Classic Mycoplasma Detection Kit	Minerva Biolabs	Cat# 11-1250
InnuPrep RNA Mini Kit 2.	Analytik Jena	Cat# 845-KS-2040250
NEBNext@ UltraTM RNA Library Prep Kit	New England Biolabs	Cat# E7530L
PE Cluster Kit cBot-HS	Illumina - Novogene	
M-MLV Reverse Transcriptase	Promega	Cat# M1701
SYBR Green GoTaq PCR mix	Promega	Cat# A6002

Deposited data

The RNA-seq data deposited at the GEO repository	This study This study	GSE89939 GSE159606 GSE182278
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Experimental models: Cell lines

LNCaP	ATCC	Cat# CRL-1740
LNCaP-95		
LAPC-4	ATCC	Cat# CRL-13009
HeLa	ATCC	Cat# CCL-2
MCF-7	ATCC	Cat# HTB-22
ZR75-1	ATCC	Cat# CRL-1500
MDA-MB-231	ATCC	Cat# HTB-26
LNCaP control and BAG1LKO cells		N/A

Experimental models: Organisms/strains

Zebrafish wild-type AB strain	EZRC	#1175
Male athymic nude-Foxn1 ^{nu} mice	Envigo	#6901M

Oligonucleotides

Rib36B4 For	5'-CTCCTGAGCGCAAGTACTCC-3'	metabion
Rib36B4 Rev	5'-GTCACCTTCACCGTTGTCCA-3'	metabion
KLK3 For	5'-CCCGGTTGTCTTCTCACC-3'	metabion
KLK3 Rev	5'-GCCTCCCAATCCGAGACA-3'	metabion

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
F5 For	5'-TCCAGGCCGAGAATACACCTA-3'	metabion
F5 Rev	5'-CGATTGCTTGTCAAACGTCTTC-3'	metabion
DUOX1 For	5'-GTGCTCCCTCTGTTGTCGT-3'	metabion
DUOX1 Rev	5'-GCTTCTCAGACACGATGCTCT-3'	metabion
MICAL1 For	5'-ATGGGCAGCCTGATGTCTCT-3'	metabion
MICAL1 Rev	5'-GGCGCCATGCTTCTCTTG-3'	metabion
NNT For	5'-TGGTCAAGCAGGGTTTAAATGT-3'	metabion
NNT Rev	5'-TCCTTTGCCCTTGGAATTTGG-3'	metabion
BAG1 For	5'-GGTGACCAGGGAGGAAATGG-3'	metabion
BAG1 Rev	5'-GTGCTGACAACGGTGTTC-3'	metabion
AR For	5'-AGCGACTTCACCGCACCT-3'	metabion
AR Rev	5'-GTTTCCCTTCAGCGGCTCTTT-3'	metabion
FKBP5 For	5'-TTCAAGGGAGGCAAATACATG-3'	metabion
FKBP5 Rev	5'-TCCAAGGGCCTTGTCACAG-3'	metabion
SLC7A11 For	5'-ATGCAGTGGCAGTGACCTTT-3'	metabion
SLC7A11 Rev	5'-CATGGAGCCAAAGCAGGAGA-3'	metabion
HMOX1 For	5'-ACTGCGTTCCTGCTCAACAT-3'	metabion
HMOX1 Rev	5'-GGGGCAGAATCTTGCACTTT-3'	metabion
S100P For	5'-CATGGGCATGATCATAGACGTCTTT-3'	metabion
S100P Rev	5'-AATTATCCACGGCATCCTTGCTTTT-3'	metabion
FTH1 For	5'-CGCCAGAACTACCACCAG-3'	metabion
FTH1 Rev	5'-TTCAAGCCACATCATCG-3'	metabion
OSGIN For	5'-AGAAGAAGCGAAGAGGTC-3'	metabion
OSGIN Rev	5'-CGGACACAAAGTTATGCC-3'	metabion

Recombinant DNA

pG5 ΔE4 luc		N/A
Ubi-Renilla luciferase		N/A
pM AR τ5		N/A
pcDNA3.1	Thermo Fisher	Cat# V79020
pcDNA3.1 BAG1L		N/A
VP-16	Clontech	Cat# 630305
VP-16 BAG1L	Katia Jehle	N/A
AR-mEos2	Emmanuel Oppong	N/A

Software and algorithms

ImageJ		https://imagej.nih.gov/ij/download.html
ColonyArea		N/A
GraphPad Prism8	GraphPad	https://www.graphpad.com/
SoftMax Pro 7	Molecular Devices	N/A
GSEA Software v4.1.0		http://www.gsea-msigdb.org/gsea/login.jsp;jsessionid=3B86CA472E2D1D71844F8EC8F4872140
Image Lab	Bio-Rad	https://www.bio-rad.com/de-de/product/image-lab-software?ID=KRE6P5E8Z

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Quantification of SARS-CoV-2 RNAemia

Plasma samples were extracted using the Cellfree200 V7 DSP 200 protocol with the QIAAsymphony® DSP virus/pathogen mini kit (QIAGEN, UK). Samples loaded onto the QIAAsymphony® SP as instructed by the manufacturer, with a 200 µl sample input volume (composed of 150µl of plasma and 50µl of NaCl) and 60 µl elution output volume of AVE buffer, unless stated (QIAGEN, UK). SARS-CoV-2 RT-ddPCR assays were performed using the One-Step RT-ddPCR Advanced Kit for 90 Probes (Bio-Rad Laboratories, Hercules, CA, USA) and the QX200 ddPCR platform (Biorad). A 2-plex RT-ddPCR assay was developed, which targets the Nucleocapside (N1) gene of the SARS-CoV-2 positive-strand RNA genome with specific FAM- probe and primers Cy5-labeled probe for the detection of a human housekeeping gene (RNaseP). RNaseP positivity was necessary to validate the RT-PCR assay prior to any further analysis. Briefly, 9.9 µL of extracted RNA was diluted in a 22 µL final reaction volume containing 5.5 µL of One Step SuperMix, 2.2 µL of Reverse Transcriptase, 1.1 µL of 300mM DTT (One-Step RT-ddPCR Advanced Kit for Probes, Bio-Rad), 1.1 µL of primers and probes mix (final probe concentration: 200 nM each, final primer concentration: 600 nM each) and 2.2 µL (QS) of nuclease-free water. Then, each sample was primarily partitioned into 13000 to 20000 droplets using the QX200™ Droplet Generator™ (Bio-Rad). PCR amplification was then performed on a C1000 Touch™ thermal cycler (Bio-Rad). This protocol included an initial retro-transcription step (60 min, 50°C, and 10 min, 95°C) followed by 45 cycles of cDNA amplification, each cycle including a denaturation step (94°C for 30 s) and a step of annealing and extension at (55.6°C for 1 min). A final denaturation step was realized at 98°C for 10 min. Then the final step of the run was performed at 4°C for 30 min for droplet stabilization. The droplet reading and quantification were performed using the QX200™ Droplet Reader™ and data analysis was performed using the QuantaSoft™ Analysis Pro software (Bio-Rad).

Plasmatic samples are considered of positive SARS-CoV2 RNA if RNaseP concentration was detected above 3.4 log copies per mL and SARS-CoV-2 RNA concentration above the assay limit of detection of 70 copies per mL.

Plasmatic SARS-CoV-2 levels were classified according to base 10 logarithm values and considered as low (≤ 100 copies/mL, including values below the limit of detection), medium (101–1000), high (1001–10,000) and very high ($>10,000$).

QUANTIFICATION AND STATISTICAL ANALYSIS

Continuous variables were represented with medians and interquartile ranges (IQR) and categorical variables were represented as proportions.

We compared baseline characteristics association with day-60 survival status and Plasmatic RNA categories. For these analyses, we used nonparametric Mann-Whitney or Kruskal-Wallis tests for continuous variables and chi-square tests for categorical data.

Variables significantly associated with increasing plasma RNA ordered categories (low, medium, high, very high) were included in a multivariable ordered logistic regression model and adjusted odds for each 10-fold increase of plasma SARS-Cov-2 RNA levels were determined for each parameter.

We then considered plasmatic SARS-Cov-2 RNA and variables previously described to be associated with COVID-19 outcome in ICU patients (COVID-ICU Group on behalf of the REVA Network and the COVID-ICU Investigators, 2021) for their association with day-60 mortality using a multivariable logistic regression. The previously described variables were age, immunodeficiency, BMI ≥ 35 , diabetes, days from symptoms to ICU admission, hemodynamic component of SOFA score ≥ 3 , renal insufficiency, PaO₂ on FiO₂ ratio ≤ 100 and lymphocyte count $\leq 1.0 \times 10^9/L$. For this analysis, to assess the relationship between each numerical variable and mortality, we first plotted continuous variables against mortality at day 60, using the lowest smoothing function with locally weighted least squares. The results of these analysis indicated that age (above 50), eGFR (above 40 mL/min per 1.73 m²), plasmatic SARS-Cov-2 RNA (above 2.5 base 10 logarithmic copies per mL) and lymphocyte counts were near linearly associated with mortality (Figures S1–S4). These variables were then entered into a multivariable logistic regression model and adjusted odds ratios of mortality were calculated for each of these variables with 95% confidence intervals (CIs) (Hosmer et al., 2013). A second multivariable logistic regression model was calculated using SARS-Cov-2 RNAemia and variables associated with day-60 survival status in univariable

analysis of our cohort. For this second multivariable analysis, we choose to include the SOFA score instead of the SAPS-2 score because the SAPS-2 score includes an age component and age was included in the model.

Analyses were conducted using Stata, version 13.0. (Stata corp, college station, Texas). p values less than 0.05 were considered statistically significant. All statistical tests were two-tailed.