Highly sensitive quantification of plasma SARS-CoV-2 RNA sheds light on its potential clinical value

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Summary:

Plasma SARS-CoV-2 RNAemia quantified by the new technology of droplet-based digital PCR is correlated with clinical severity of COVID-19 and constitutes a promising prognosis biomarker to monitor in hospitalized COVID-19 patients.
Abstract

**Background.** Coronavirus disease 2019 (COVID-19) is a global public health problem that has already caused more than 662,000 deaths worldwide. Although the clinical manifestations of COVID-19 are dominated by respiratory symptoms, some patients present other severe damage such as cardiovascular, renal and liver injury or/and multiple organ failure, suggesting a spread of the SARS-CoV-2 in blood. Recent ultrasensitive polymerase chain reaction (PCR) technology now allows absolute quantification of nucleic acids in plasma. We herein intended to use the droplet-based digital PCR technology to obtain sensitive detection and precise quantification of plasma SARS-CoV-2 viral load (SARS-CoV-2 RNAaemia) in hospitalized COVID-19 patients.

**Methods.** Fifty-eight consecutive COVID-19 patients with pneumonia 8 to 12 days after onset of symptoms and 12 healthy controls were analyzed. Disease severity was categorized as mild-to-moderate in 17 patients, severe in 16 patients and critical in 26 patients. Plasma SARS-CoV-2 RNAaemia was quantified by droplet digital Crystal Digital PCR™ next-generation technology (Stilla Technologies, Villejuif, France).

**Results.** Overall, SARS-CoV-2 RNAaemia was detected in 43 (74.1%) patients. Prevalence of positive SARS-CoV-2 RNAaemia correlated with disease severity, ranging from 53% in mild-to-moderate patients to 88% in critically ill patients (p=0.036). Levels of SARS-CoV-2 RNAaemia were associated with severity (p=0.035). Among nine patients who experienced clinical deterioration during follow-up, eight had positive SARS-CoV-2 RNAaemia at baseline while only one critical patient with undetectable SARS-CoV-2 RNAaemia at the time of analysis died at day 27.

**Conclusion.** SARS-CoV-2 RNAaemia measured by droplet-based digital PCR constitutes a promising prognosis biomarker in COVID-19 patients.
Introduction

Coronavirus disease 2019 (COVID-19)\(^1\) is a global public health problem that has already caused more than 662,000 deaths worldwide. Severe respiratory syndrome coronavirus 2 (SARS-CoV-2)\(^2\), responsible for the development of COVID-19, was first isolated and sequenced in early January 2020\(^3\). Although most patients present mild-to-moderate disease, 5 to 10% progress to severe or critical disease\(^4\), including pneumonia and acute respiratory failure. Based on data from patients with laboratory-confirmed COVID-19 from mainland China, admission to intensive care unit (ICU), invasive mechanical ventilation or death occurred in 5.0%, 2.3% and 1.4% of cases, respectively\(^1\). In severe cases, clinical observations typically describe a two-step disease progression, starting with a mild-to-moderate presentation followed by a secondary respiratory worsening 9 to 12 days after onset of first symptoms\(^4-6\). Clinical deterioration is dominated by worsening of respiratory symptoms, potentially concomitant with severe systemic damage including cardiovascular, renal and liver injury and/or multiple organ failure\(^4,7\). In addition, coagulopathy has been reported in severe COVID-19 cases\(^8,9\). These systemic clinical features strongly suggest the spread of SARS-CoV-2 within extra-pulmonary sites via the blood flow.

Detection of circulating SARS-CoV-2 RNA by conventional real-time reverse transcription-polymerase chain reaction assay (RT-PCR) which is used for routine SARS-CoV-2 molecular diagnosis from respiratory tract samples was previously reported in COVID-19 patients in few studies\(^4,10,11\). As there is no certainty as whether this blood RNA belongs to entire infectious viral particles or come from infected-cell lysis, "SARS-CoV-2 RNAemia" was preferred to "viraemia" when referring to this circulating SARS-CoV-2 RNA. In their retrospective series of 57 COVID-19 patients, Chen W. et al. found only 6 patients with positive SARS-CoV-2 RNAemia by RT-PCR and suggested an association between
detectable serum SARS-CoV-2 RNA with disease severity\textsuperscript{11}. However, conventional RT-PCR for SARS-CoV-2 RNA may lack sensitivity in patients showing low viremia. Furthermore, in the absence of adequate quantitative standards availability, RT-PCR does not allow precise quantification of low SARS-CoV-2 viral loads. Ultrasensitive PCR technology is now available for absolute quantification of nucleic acids in plasma, such as droplet-based digital PCR (ddPCR), a more sensitive technique for virus detection than RT-PCR\textsuperscript{12-14}. ddPCR was primarily developed for cancer research to detect and quantify mutated circulating tumoral DNA among cell-free DNA in liquid biopsies by optimizing sensitivity and absolute quantification compared with classical q(RT-)PCR. This innovative method is based on the realization of thousands of single-molecule PCRs in parallel in independent compartment, here droplets of an induced emulsion with nucleic acid extracts. Therefore, every single PCR is performed separately and consequently avoids the bias seen in conventional PCR. Every single droplet containing targeted DNA or RNA will become fluorescent after (RT-)PCR and is detected as a positive droplet by laser excitation. In that way, droplet-based digital PCR allows the generation of quantitative and accurate data without standard curves. As the raw data fits the Poisson distribution, it allows absolute quantification of the amplified target. Besides higher sensitivity over qPCR, ddPCR also presents many advantages for the analysis of biological samples that are complex in nature. Indeed, performing every single PCR in an isolated droplet provides higher resistance to a variety of inhibitors that could be contained within biological samples and consequently significantly increases reproducibility of the obtained data and comparability of the results between different laboratories\textsuperscript{15}. The increased tolerance of ddPCR to PCR inhibitory molecules makes it an attractive alternative to qPCR for medical applications including viral diagnostics\textsuperscript{16}. Recently, ddPCR was used to quantify SARS-CoV-2 RNA in lower respiratory
tract samples of COVID-19 patients, and showed higher sensitivity than RT-PCR, especially with low viral loads\textsuperscript{17}.

In the present study, ddPCR was used to precisely quantify circulating SARS-CoV-2 RNA in the blood from COVID-19 patients hospitalized with different clinical severity.

Methods

Study design and patients
COVID-19 patients admitted to the Cochin Hôpital, Paris, France, at the time of respiratory deterioration and between days 8 and 12, were included between March 19, 2020 and April 3, 2020, in the setting of the local RADIPEM biological samples collection derived from samples collected in routine care. The present work focused on SARS-CoV-2 RNAemia is part of another study primarily designed to explore innate inflammatory responses in hospitalized COVID-19 patients\textsuperscript{18}, and used the same inclusion criteria. Our study is an ancillary study focused on circulating SARS-CoV-2 RNA from another more global study regarding Inclusion criteria for COVID-19 inpatients were: age between 18 and 80 years, diagnosis of COVID-19 according to WHO interim guidance (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/infection-prevention-and-control), and positive SARS-CoV-2 RT-PCR testing on a respiratory sample (nasopharyngeal swab or invasive respiratory sample). Inpatients with pre-existing unstable chronic disorders (such as uncontrolled diabetes mellitus, severe obesity defined as body mass index greater than 30, unstable chronic respiratory disease or chronic heart disease) were excluded. SARS-CoV-2 RNAemia was performed on samples collected between 8 to 12 days after onset of first symptoms. The choice to sample patients 9 to 12
days after disease onset was related to early Chinese clinical observations reporting a two-step disease progression, starting with a mild-to-moderate presentation followed by a secondary respiratory worsening few days after onset of first symptoms in severe cases. The mean interval between the onset of illness and the admission to ICU was of 9-12 days in these early reports\textsuperscript{4-6,18}.

Healthy controls were RT-PCR SARS-CoV-2 negative asymptomatic adults, matched with cases on age and sex.

The clinical severity of COVID-19 was described according to the adaptation of the Sixth Revised Trial Version of the Novel Coronavirus Pneumonia Diagnosis and Treatment Guidance published on February 19th, 2020 (http://www.kankyokansen.org/uploads/uploads/files/jsipc/protocol_V6.pdf). Mild cases were defined as patients with mild clinical symptoms (fever, myalgia, fatigue, and diarrhea) and no sign of pneumonia on thoracic computed tomography (CT) scan. Moderate cases were defined as patients with clinical symptoms associated with dyspnea and radiological findings of pneumonia on thoracic CT scan, and requiring a maximum of 3 L/min of oxygen. Severe cases were defined as respiratory distress patients requiring over 3 L/min of oxygen with no other organ failure. Critical patients were those with mechanical ventilation, shock and/or multiple organ failure and hospitalized in an intensive care unit (ICU). Clinical evolution was evaluated during hospital stay and clinical deterioration defined as: increased oxygen intake requiring introduction of high-flow nasal oxygen therapy or mechanical ventilation, transfer to the intensive care unit or death.

Biological collection and informed consent were approved by the Direction de la Recherche Clinique et Innovation (DRCI) and the French Ministry of Research (N°2019-3677). The study conforms to the principles outlined in the Declaration of Helsinki, and received
approval by the appropriate Institutional Review Board (Cochin-Port Royal Hospital, Paris, France; number AAA-2020-08018).

Quantification of SARS-CoV-2 RNAemia

Plasma SARS-CoV-2 RNA (140 µL) was extracted using QIAamp® Viral RNA Mini Kit (QIAGEN®, Hilden, Germany), according to manufacturer’s instructions. The elution volume was 35 µL and we added 10.5 µL of the elution to the RT-PCR mix for amplification. SARS-CoV-2 RNAemia was quantified at each time point by droplet-based Crystal Digital PCR™ (Stilla Technologies, Villejuif, France) on the Naica™ System (Stilla Technologies, Villejuif, France) using the following commercial RT-PCR amplification kit (Novel Coronavirus (2019-nCoV) Digital PCR Detection Kit, Apexbio™, Beijing, China) following manufacturer's instruction. The kit includes primers and FAM- and HEX- labeled probes specific to two distinct regions [ORF1ab and Nucleocapside (N) genes] of the SARS-CoV-2 positive strand RNA genome and primers and a Cy5-labeled probe for the detection of a human housekeeping gene (RNaseP) detected on the 3rd channel of the Naica™ system. RNaseP positivity was necessary to validate the RT-PCR assay prior any further analysis. This single assay design permits the simultaneous detection of two independent SARS-CoV-2 sequences reported as conserved, while concurrently monitoring PCR effectiveness using the third channel of detection. Plasma samples with one of the two ORF1 or N genes or both genes detected were considered as positive samples and results were automatically analyzed using "Crystal reader" (Stilla) and "Crystal Miner" software (Stilla) based on the most amplified gene positive droplets. SARS-CoV-2 RNA concentrations (cp/mL) were finally calculated considering the extracted volume of plasma.
Statistical analysis

Descriptive statistics were computed for the population at baseline. Quantitative variables were described as mean ± standard deviation (SD) if normally distributed, or median and inter-quartile range (IQR) otherwise. Categorical variables were described as group sizes and percentages.

Bivariate comparisons between clinical classes were computed using the Fisher test for categorical variables and the Kruskal-Wallis one-way analysis of variance for continuous variables. When comparing control and COVID-19 patients, the Mann-Whitney rank-sum test was used for quantitative variables.

An ordinal regression multivariate model was used to evaluate the association between the clinical class and circulating viral DNA quantification (log values), adjusted for clinical predictors. Patients' clinical outcomes are presented using Kaplan-Meier curves. We did not perform a formal statistical survival analysis, since the study was not primarily designed for survival analysis, the patients belonging to different clinical classes at baseline, and the outcome being a composite criterion of mechanical ventilation requirement or death.

Computations were performed using the R software, and the ordinal package for the ordinal regression model. P values < 0.05 were considered significant.

Role of the funding source. The funder of the study had no role in study design, data collection, data analysis, data interpretation, or report writing. The corresponding author had full access to all the data in the study and shared with all co-authors the final decision to submit the paper for publication.
Results

Patients characteristics

Fifty-eight COVID-19 patients and 12 healthy controls were analyzed. Demographic and clinical characteristics of the patients are shown in Table 1. Median age of the patients was 55.1 years (interquartile range, 15) and 81% were male.

At inclusion, the degree of severity of COVID-19 was categorized as mild-to-moderate in 17 patients (median oxygen requirement 2L/min), severe in 15 patients (median oxygen requirement 5L/min) and critical in 26 patients. The median time from symptoms onset to inclusion was of 9.5 days (interquartile range [IQR], 7.0-12.5): respectively 9 days (IQR: 9-11) 10 days (IQR: 9-11) and 10 (IQR: 9-11) in the mild to moderate, severe and critical categories (anova test=0.43). All of 55 CT-scans available at time of admission were abnormal, showing ground-glass opacities (100%) with bilateral patchy distribution (95%). During patients’ clinical monitoring (median time of follow-up of 7 days, IQR: 3.0-14.0), no patient in the mild-to-moderate group secondly required admission to ICU or use of mechanical ventilation, compared to 5 out of 15 in the severe group. None of the healthy controls and 55% of the COVID-19 patients had any comorbidity. In the remaining COVID-19 patients (45%), comorbidities were: non-insulin diabetes mellitus (n=7), solid cancers (n=6), hematological cancer (n=3), asthma (n=3), autoimmune diseases (n=3) and chronic obstructive pulmonary disease (n=1). Fourteen COVID-19 patients received an antiviral treatment at time of their hospitalization for disease progression by hydroxychloropquine +/- azithromycin (n=5), lopinavir/ritonavir (n=8) and remdesivir (n=1).
SARS-CoV-2 RNAemia results by ddPCR

At inclusion, 43 (74.1%) of COVID-19 patients were positive for SARS-CoV-2 RNA in plasma, with at least the N gene detected. Only 28% were positive for ORF1 gene, systematically associated with N gene positivity. Proportion of positive SARS-CoV-2 RNAemia was significantly different between patients from different clinical classes, from 53% in mild-to-moderate patients to 88% in critically ill patients (p=0.036) (Table 1).

Overall, SARS-CoV-2 RNAemia were associated with COVID-19 severity (p=0.035, Kruskal-Wallis), as depicted in Figure 1, with median SARS-CoV-2 RNAemia of 89, 279 and 301 cp/mL, in mild-to-moderate, severe and critically ill patient groups, respectively. Raw plasma SARS-CoV-2 RNA value for each patient in the three different clinical classes were detailed in supplementary table 1.

In multivariate analysis adjusted on age, sex and comorbidities, SARS-CoV-2 RNAemia was strongly associated with the clinical class (adjusted hazard ratio 2.2, 95% CI 1.39–3.9; p=0.0018) (Table 2).

All healthy controls showed negative SARS-CoV-2 RNAemia with no detection of both specific SARS-CoV-2 N and ORF-1 genes.
Clinical monitoring and correlation with baseline SARS-CoV-2 RNAaemia

Clinical deterioration was observed in nine COVID-19 patients during follow-up: introduction of high-flow nasal oxygen therapy (n=3), introduction of mechanical ventilation (n=3), or death (n=3).

Eight of the nine COVID-19 patients with clinical deterioration had positive SARS-CoV-2 RNAaemia at baseline, while only one critical patient with undetectable SARS-CoV-2 RNAaemia at the time of analysis died at day 27 (Figure 2). Of note, the patient with the highest SARS-CoV-2 RNAaemia (65,476 cp/ml) died from COVID-19 one day after plasma sampling.

Discussion

In the present study, SARS-CoV-2 RNAaemia was measured by ddPCR in a large cohort of hospitalized COVID-19 patients at time of disease worsening. SARS-CoV-2 RNAaemia was detectable in the majority of patients, confirming that the SARS-CoV-2 may invade the systemic compartment beyond the lungs. Circulating SARS-CoV-2 viral load showed a large amplitude spanning almost 4 log, with an overall significant difference in viral load levels between clinical classes, showing higher viral loads in severe/critical patients, compared to the mild to moderate ones. The median SARS-CoV-2 RNAaemia was very similar between severe and critical patients and such a small difference in SARS-CoV-2 RNAaemia cannot be used to distinguish them. The clinical classification was made accordingly to the Sixth Revised Trial Version of the Novel Coronavirus Pneumonia Diagnosis and Treatment Guidance and the similar viral loads would rather reflect a strong continuum between these two groups of patients. The proportion of patients with detectable SARS-CoV-2 RNAaemia was also strongly correlated with disease severity. Furthermore, viraemic COVID-19 patients showed a
tendency to have a higher risk of poor outcome, unlike non-viraemic patients. Taken together, these observations strongly suggest that SARS-CoV-2 RNAaemia is associated with disease severity, making it a potential biomarker of COVID-19 worsening.

Few studies have previously reported the detection of SARS-CoV-2 viral RNA within COVID-19 patients’ blood\textsuperscript{4,10,11}. In these studies, SARS-CoV-2 RNAaemia was assessed using classical RT-PCR considered as the gold standard for SARS-CoV-2 RNA molecular diagnosis in nasopharyngeal swabs, and detectable SARS-CoV-2 RNA in plasma was a rare event. RT-PCR did not allow precise quantification of the level of circulating SARS-CoV-2 RNA, as adequate quantitative standards were not available. Thus, only semi-quantitative evaluation of SARS-CoV-2 RNAaemia could have been established by reference to the cycle threshold (Ct) of amplification curves in one limited series\textsuperscript{11}. Furthermore, low viral loads are likely to be inaccurately quantified by classical RT-PCR as it was recently shown in lower respiratory samples in which ddPCR showed significantly better performance to detect and quantify low viral load\textsuperscript{17,19,20}. Therefore, SARS-CoV-2 RNAaemia could have been underdetected by using conventional RT-PCR technology in first studies.

Droplet-based digital PCR primarily developed in cancer research field was optimized to enhance sensitivity and obtain absolute quantification compared with classical q-PCR. This new technology is based on the realization of millions of single-molecule PCRs in parallel in independent compartments (droplets of an emulsion), and the resulting amplification products reflect more closely the original composition of nucleic acid mixtures than conventional PCR\textsuperscript{12–14} as every single targeted DNA or RNA molecule will be amplified and detected separately. The use of ddPCR for the analysis of biological samples that are complex in nature presents many advantages over conventional PCR procedures. In addition to a higher sensitivity over quantitative PCR or RT-PCR and not needing calibration curves, ddPCR also demonstrates substantially higher resistance to a variety of inhibitors that could be contained
within biological samples\textsuperscript{15}. This increased tolerance of ddPCR to PCR inhibitory molecules makes it an attractive alternative to quantitative PCR or RT-PCR for medical applications including viral diagnostics\textsuperscript{16} and cancer research\textsuperscript{13}. Finally, digital RT-PCR was described as presenting high potentiality over conventional real time RT-qPCR\textsuperscript{21}. In the present study, SARS-CoV-2 RNAaemia assessed by ddPCR was detected in roughly 75\% of hospitalized COVID-19 patients, and ddPCR was highly specific for the detection of SARS-COV-2 RNAaemia. Compared to recent studies reporting positive blood SARS-CoV-2 viral load by RT-PCR in less than a half of hospitalized patients for COVID-19\textsuperscript{11,22}, our results indicate that ddPCR is more efficient and more precise to detect and quantify SARS-CoV-2 RNAaemia as compared to classical RT-PCR.

In one previous study, SARS-CoV-2 RNAaemia indirectly assessed with the cycle threshold of RT-PCR was correlated with the COVID-19 severity\textsuperscript{11}. We show here that precise quantification of SARS-CoV-2 RNAaemia by ddPCR confirms these previous observations. In our study, detectable SARS-CoV-2 RNAaemia was also more frequently detected in patients with increasing severity. These data suggest that the systemic spread of SARS-CoV-2 could be of importance in the different clinical features observed. It has been clearly shown that immunopathologic mechanisms are involved in distant clinical deterioration of COVID-19 patients\textsuperscript{23}; however, it cannot be ruled out that an uncontrolled replication of SARS-CoV-2 could be at the origin of this immune deregulation.

During follow-up, nine patients experienced clinical deterioration, from important respiratory worsening in severe patients (n=6) to death in critical patients (n=3). Strikingly, eight out of these nine patients had positive SARS-CoV-2 RNAaemia. Considering this engaging finding, it could be henceforth relevant to quantify RNAaemia in patients before clinical deterioration and it would be interesting to prospectively assess the predictive power of plasmatic SARS-CoV-2 RNAaemia for clinical deterioration as the second step of
COVID-19 generally occurring between days 8 and 12 from the first symptoms. Therefore, longitudinal measurements starting at the onset of COVID-19 symptoms would add very interesting information on viral dynamics at different stages of the disease. However, such data were not available due to the cross-sectional design of our study but should be investigated in further protocols.

Detectable SARS-CoV-2 RNAemia observed in most hospitalized COVID-19 patients could begin to explain systemic damage and/or multiple organ failure observed in some cases. However, we cannot be certain that detected SARS-CoV-2 RNAemia corresponds to circulating infectious viral particles and is a marker for an infectious systemic spread of SARS-CoV-2. Finally, as we did not report any secondary extra-pulmonary damage during the follow-up of our patients, we could not confirm the hypothesis that SARS-CoV-2 systemic spread could be at the origin of other systemic organ failure. We will address this question in further studies by quantifying SARS-CoV-2 RNAemia by ultrasensitive ddPCR in COVID-19 patients with systemic damage. It will be also necessary to determine how this SARS-CoV-2 RNAemia could represent an easy-to-use and useful biomarker in monitoring COVID-19 patients.
NOTES

Authors' contributions. DV, SK, BT and HP have conceived and designed the research; GP, NR, FR, ASH and JH have performed the experiments; MW performed statistical analyses; DV, SK, MW, VT, PLP and HP analyzed the results; DV, SK, MW, VT, PLP, LB, JH, BT and HP drafted the manuscript. All authors validated the final version of the manuscript.

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Table 1: Clinical characteristics of hospitalized COVID-19 patients

|                        | N  | %   | Med | IQR |
|------------------------|----|-----|-----|-----|
| **Sexe**               |    |     |     |     |
| Female                 | 11 | 19  |     |     |
| Male                   | 47 | 81  |     |     |
| **Age**                | 58 | 55.14 | 15  |     |
| **Clinical classes**   | 58 |     |     |     |
| Mild/Moderate          | 17 | 29  |     |     |
| Severe                 | 15 | 26  |     |     |
| Critical               | 26 | 45  |     |     |
| **Clinical Deterioration** | 58 |       |     |     |
| Death                  | 3  | 5.2 |     |     |
| Mechanical Ventilation | 3  | 5.2 |     |     |
| Optiflow               | 3  | 5.2 |     |     |
| Absence                | 49 | 84  |     |     |
| **Monitoring delay**   | 58 | 7   | 11  |     |
| **Pulmonary radiography showing ground-glass opacities** | 55 |     |     |     |
| No                     | 0  | 0   |     |     |
| Yes                    | 55 | 100 |     |     |
| **Pulmonary radiography showing bilateral patchy distribution** | 55 |       |     |     |
| No                     | 3  | 5.5 |     |     |
| Yes                    | 52 | 95  |     |     |
| **Controlled comorbidities** | 58 |       |     |     |
| No                     | 32 | 55  |     |     |
| Yes                    | 26 | 45  |     |     |
| **Oxygeno-requerance** | 58 |       |     |     |
| No                     | 3  | 5   |     |     |
| Yes                    | 29 | 50  |     |     |
| Mechanical Ventilation | 26 | 45  |     |     |
| **Mechanical Ventilation** | 26 |       |     |     |
| **Antiviral treatment** | 58 |       |     |     |
| No                     | 43 | 74  |     |     |
| Yes                    | 15 | 26  |     |     |

IQR: Interquartile range
Table 2: Univariate and multivariate analysis of demographic, clinical and virological data

| Clinical classes          | Mild/moderate | Severe | Critical |
|---------------------------|---------------|--------|----------|
|                           | N  | %  | Median | IQR | N  | %  | Median | IQR | N  | %  | Median | IQR |
| Sex                       |    |    |        |     |    |    |        |     |    |    |        |     |
| Female                    | 17 | 15 |        |     | 26 | 0.52 | 2.6 [0.63 - 11] | 0.19 |
| Male                      | 12 | 71 |        |     |    |    |        |     |    |    |        |     |
| Age                       | 17 | 51 | 24     | 15  | 15 | 57.54 | 12 | 55.16 | 12 | 0.24 | 1 [0.98 - 1.1] | 0.23 |
| Clinical Deterioration    |    |    |        |     |    |    |        |     |    |    |        |     |
| Death                     | 0  | 0  | 0      | 0   | 3  | 12 |
| Mechanical Ventilation    | 0  | 0  | 3      | 20  | 0  | 0  |
| Optiflow                  | 0  | 0  | 3      | 20  | 0  | 0  |
| Absence                   | 17 | 100| 9      | 60  | 23 | 88  |
| Controlled comorbidities  |    |    |        |     |    |    |        |     |    |    |        |     |
| No                        | 15 | 88 | 8      | 53  | 9  | 35 |
| Yes                       | 2  | 12 | 7      | 47  | 17 | 65 |
| Positive SARS-CoV-2 RNAemia | 17 | 15 | 26     |     |    |    |        |     |    |    |        |     |
| SARS-CoV-2 RNAemia (cp/mL)|    |    |        |     |    |    |        |     |    |    |        |     |
| No                        | 8  | 47 | 4      | 27  | 3  | 12 |
| Yes                       | 9  | 53 | 11     | 73  | 23 | 88 |
| Log(SARS-CoV-2 RNAemia) (cp/mL) | 17 | 2  | 2,2    | 15  | 26 | 2,48 | 1,2 |

OR: Odd ratio; IQR: Interquartile range
Legends for the figures

**Figure 1.** Plasmatic SARS-CoV-2 RNAemia (cp/mL) measured by droplet-based digital PCR according to clinical classes.

**Figure 2.** Kaplan–Meier curves showing the prognostic value of circulating SARS-CoV-2 RNAemia as assessed by frequency of deterioration events at time of plasma viral detection by droplet-based digital PCR. The grey and hatched black lines represent patients without and with detectable SARS-CoV-2 RNAemia, respectively. The hatched line at 0.5 represents the median survival.
