SARS-CoV-2 RNA in plasma is associated with ICU admission and mortality in patients hospitalized with COVID-19

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Abstract:

The clinical significance of SARS-CoV-2 RNA in the circulation is unknown. In this prospective cohort study, we detected viral RNA in the plasma of 58/123 (47%) patients hospitalized with COVID-19. RNA was detected more frequently, and levels were higher, in patients who were admitted to the ICU and/or died.

Keywords: SARS-CoV-2, COVID-19, RNAemia, viral load, antibodies

Introduction:

While infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) frequently causes severe pulmonary disease, autopsy studies suggest viral dissemination to multiple organs, including heart, kidney, brain and GI tract [1]. The clinical significance of detecting viral RNA outside the respiratory tract is unknown. In this prospective observational study, we aimed to quantify SARS-CoV-2 RNA in the plasma and upper respiratory tract of patients hospitalized with coronavirus disease 2019 (COVID-19) and evaluate the association between RNA loads, disease severity and the SARS-CoV-2-specific antibody response.

Methods:

The COronaVIrus Disease MECHanisms study (COVID-MECH, NCT04314232) recruited consecutive adult patients admitted to Akershus University Hospital with COVID-19 confirmed by RT-PCR between March 18th and May 4th 2020. Written informed consent was granted by study participants, or by next-of-kin if the patient was unable to consent. The study was approved by local regulatory authorities.

The predefined primary endpoint was a composite of intensive care unit (ICU) admission >24 hours and in-hospital mortality. Clinical data were extracted from electronic patient records. Routine biochemistry was taken at admission and study-specific samples of EDTA plasma and serum were taken at three time points; baseline (enrollment), day 3 (±1 day) and day 9 (± 2 days) in patients who were still hospitalized (details in Supplementary Figure 1).

SARS-CoV-2 RNA was detected in plasma samples and upper respiratory tract swabs by RT-PCR and RNA levels were estimated using the cycle threshold (Ct) value. In PCR-positive plasma samples, RNA was quantified and expressed as log10 copies/mL (details in Supplementary Methods File 1). Upper respiratory samples were combined oropharyngeal and nasopharyngeal swabs, taken in the emergency department unless the patient had a positive RT-PCR from another laboratory within the preceding week. All RT-PCRs were performed as described by *Corman et al.*, targeting the viral envelope (E)-gene [2].

Total antibodies against SARS-CoV-2 nucleocapsid (NP) were quantified using the Elecsys Anti-SARS-CoV-2 test on the cobas e801 module (Roche, Penzberg, Germany). IgG antibodies directed against subunits S1 and S2 of the SARS-CoV-2 spike protein were quantified using the LIAISON SARS-CoV-2 S1/S2 IgG assay and the Liaison XL chemiluminescence analyzer (DiaSorin, Saluggia, Italy).

Data are presented as N (%), mean ± SD and median [Q1, Q3]. Differences between groups were assessed by t-tests, Mann-Whitney U tests, chi-square tests and Spearman tests, as appropriate. All statistical analyses were performed in Stata 16 (StataCorp, College Station, TX, USA). P-values <0.05 were considered statistically significant.

Results:

Plasma and serum samples were available from 123/135 (91%) patients, of whom 35 (28%) patients reached the primary endpoint. 31 patients were admitted to the ICU; 29 received mechanical ventilation and four died. Another four patients with do-not-intubate orders died on regular wards. All ICU admissions and deaths were attributable to COVID-19. Clinical, biochemical, virological and

serological data are presented in Table 1 (data on 12 patients without biobank samples in Supplementary Table 1).

SARS-CoV-2 RNAemia was detected in at least one sample in 58/123 (47%) patients, and in a significantly higher proportion of patients who were admitted to the ICU or died (80% vs. 34%, p<0.001). RNAemia was detected in 48/123 (39%) patients at baseline, a median 0 [-1, 3] days before ICU admission. 24 (41%) of patients with detectable RNAemia had Ct values >38, below the quantitation limit of 2.70 $\log_{10}$ copies/mL, and Ct values were therefore used in this analysis. Of note, the lowest Ct value of 25.9 (corresponding to an RNA load of 6.3 $\log_{10}$ copies/mL) was observed in a female ICU patient with a prolonged course of disease. RNAemia was significantly more frequent at all time points in patients who reached the primary endpoint, whereas RNA loads were significantly higher at baseline and day 3 (Table 1, Supplementary Figure 2A). In a logistic regression analysis where nine patients with baseline samples taken after ICU admission were excluded, baseline RNAemia and RNA load were both significantly associated with ICU admission and/or death (Supplementary Table 2). This association persisted after adjusting for age, sex and race (Model #1) and BMI, diabetes mellitus and symptom duration (Model #2). There was no correlation between days from symptom onset and RNAemia frequency or RNA load at baseline.

Ct values from diagnostic upper respiratory swabs taken at admission were available from 102 (83%) patients, including all 35 who were admitted to the ICU or died. There was no association between upper respiratory Ct values and the primary endpoint (Supplementary Figure 2B). There was no correlation between upper respiratory Ct values and RNAemia frequency or plasma RNA loads, nor with the number of days from symptom onset to admission.

Titers of anti-SARS-CoV-2 NP total Ig and anti-spike IgG both rose progressively in the majority of patients (Supplementary Figure 2C). There was no difference in the titers of total Ig or IgG at any timepoint between patients who reached the primary endpoint or not. There was no correlation between baseline RNAemia, RNA loads or upper respiratory Ct values and subsequent antibody titers. While four patients had low or undetectable antibody titers out to 20+ days after symptom onset, there was no association between a lack of an antibody response and the primary endpoint, baseline RNAemia and RNA loads, or admission upper respiratory Ct values.

Discussion:

In this prospective study of patients hospitalized with COVID-19 we detected SARS-CoV-2 RNAemia in 47% of included patients, and a significantly higher frequency of RNAemia and higher RNA loads in patients who required ICU admission and/or died. By contrast, RT-PCR Ct values in upper respiratory swabs obtained at admission yielded no prognostic information. The development of SARS-CoV-2-specific antibodies was independent of RNAemia, RNA loads in plasma, upper respiratory Ct values and the study primary endpoint.

To our knowledge, no prospective cohort studies of this size have detected circulating SARS-CoV-2 RNA in such a high proportion of patients with COVID-19. Our findings confirm and extend those of *Hogan et al.*, who detected RNAemia in 28/85 (32.9%) hospitalized patients in a retrospective study, and similarly found that RNAemia was associated with ICU admission and hospital mortality [3]. Collectively, this supports the possible utility of SARS-CoV-2 RNAemia as a prognostic marker in COVID-19. With potent antiviral agents in the pipeline, early markers of severe disease will facilitate the identification of patients who may benefit the most from these new therapeutics, particularly as early initiation tends to be a critical factor in antiviral drug efficacy [4].

Our data do not support the use of upper respiratory RT-PCR Ct values as a prognostic tool in COVID-19. This contrasts with two previous reports reporting lower ORF1ab Ct values in patients with severe disease and high mortality [5, 6]. On the other hand, negative upper respiratory PCRs are not uncommon in patients with severe COVID-19 [7], and high RNA loads are frequently detected in individuals with few or no symptoms [8]. Furthermore, differences in sampling techniques and specimen type, PCR targets (ORF1ab vs. E gene), and variations in preanalytical conditions may contribute to discrepant findings.

We found no evidence that viral RNA in plasma or the upper respiratory tract influenced development of SARS-CoV-2-specific antibodies during acute infection. Several retrospective studies have found higher levels of IgG in patients with more severe COVID-19 [9, 10], but other investigators, like us, have detected no such association [11]. Larger prospective studies with long-term follow-up data will be required to determine the significance of SARS-CoV-2-specific antibodies in acute infection and subsequent re-challenge.

This was a single center study, which may limit the generalizability of our findings. While patients were recruited consecutively, we were unable to recruit and sample all patients admitted with COVID-19 in the study period and cannot exclude selection bias. The time between baseline sampling and ICU admission was frequently short, largely due to severe clinical disease at presentation. We expect this to be common, which may limit the useful prognostic time window of a marker such as RNAemia. No correction for multiple comparisons was made, potentially increasing the risk of type I statistical errors, and our findings should be replicated in other cohorts.

While we detected RNAemia in a large proportion of patients, quantitative comparisons were limited by frequent RNA loads below the limit of quantitation. Furthermore, the presence of viral RNA does not necessarily represent replication-competent virus. A recent study failed to culture virus from clinical serum samples positive for SARS-CoV-2 RNA by RT-PCR, but Ct values were consistently high, suggesting low RNA copy numbers [12]. Our data cannot determine whether RNAemia represents direct viral involvement in causing extrapulmonary pathology or is merely spill-over from an intense pulmonary infection. Further investigations into the pathogenesis of extra-pulmonary SARS-CoV-2 infection should include viral culture assays and mechanistic studies of viral infection of various tissues and cell types.

In conclusion, we found a high proportion of SARS-CoV-2 RNA in the plasma of patients hospitalized with COVID-19, and a significantly higher frequency and level of plasma RNA in patients who were admitted to the ICU or died. SARS-CoV-2 RNAemia may be a useful prognostic marker in COVID-19. The pathophysiological significance of circulating viral RNA must be ascertained by future studies.

NOTES

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Table 1: Patient and assay data, by study primary endpoint.

	Ward (N=88)	ICU/death (N=35)	p-value
Demographic			
Age, years	57.8 ± 16.3	64.3 ± 10.7	0.031
Male sex, n (%)	47 (53.4)	25 (71.4)	0.07
Caucasian, n (%)	47 (53.4)	21 (60.0)	0.51
Comorbidity			
Body mass index, kg/m^2	28.2 ± 5.3	28.5 ± 6.2	0.79
Type-2 diabetes mellitus, n (%)	11 (12)	10 (29)	0.033
Clinical			
Days of symptoms at admission	9.2 ± 4.8	9.5 ± 4.0	0.79
NEWS score at admission	4 [2.5, 6.0]	8 [6.0, 10.0]	<0.001
Biochemistry at admission			
CRP, mg/L	55 [27, 110]	120 [50, 220]	<0.001
Ferritin, ug/L	547 [234, 982]	1135 [487, 2443]	<0.001
D-dimer, mg/L	0.5 [0.3, 0.8]	0.7 [0.4, 1.1]	0.027
LDH, U/L	280 [220, 350]	390 [290, 520]	<0.001
Virological			
Days from baseline sample to ICU admission		0 [-1, 3]	
Baseline plasma SARS-CoV-2 RNA			
RT-PCR positive, n (%)	24 (27)	24 (69)	<0.001

RT-PCR Ct value*	38.5 [39.3, 37.0]	36.4 [38.4, 35.0]	0.003
log_{10} RNA copies/mL*	< 2.7	3.2	
Day 3 plasma SARS-CoV-2 RNA			
RT-PCR positive, n (%)	11 (17)	19 (59)	<0.001
RT-PCR Ct value*	38.3 [39.2, 37.3]	35.7 [38.5, 34.5]	0.037
log_{10} RNA copies/mL*	< 2.7	3.4	
Day 9 plasma SARS-CoV-2 RNA			
RT-PCR positive, n (%)	4 (17)	10 (38)	0.10
RT-PCR Ct value*	38.5 [38.9, 38.1]	36.3 [37.0, 35.2]	0.024
log_{10} RNA copies/mL*	< 2.7	3.2	
Admission upper respiratory SARS-CoV-2 RNA RT-PCR Ct value	26.1 ± 8.1	27.3 ± 6.3	0.47
Serological			
Baseline serum antibodies			
Anti-SARS-CoV-2 NP total Ig, AU/mL	0.1 [0.1, 2.8]	0.1 [0.1, 2.8]	0.38
Anti-SARS-CoV-2 Spike IgG, AU/mL	5.6 [0, 19.3]	5.8 [0, 20.2]	0.95
Day 3 serum antibodies			
Anti-SARS-CoV-2 NP total Ig, AU/mL	4.5 [0.2, 16.8]	1.9 [0.2, 6.54]	0.36
Anti-SARS-CoV-2 Spike IgG, AU/mL	21.2 [5.0, 61.1]	17.9 [7.2, 75.9]	0.67
Day 9 serum antibodies			

Anti-SARS-CoV-2 NP total Ig, AU/mL	19 [6.1, 32.6]	14.9 [2.1, 20.1]	0.10
Anti-SARS-CoV-2 Spike IgG, AU/mL	113 [32.6, 187]	127 [30.8, 207]	0.82

Table legend: * Values from plasma samples with detectable RNA. P-values are derived from chi-square tests for binary variables, Mann-Whitney tests for skewed continuous variables and student´s t-tests for continuous normally distributed variables. NEWS: National Early Warning Score CRP: C-reactive protein. LDH: lactate dehydrogenase. Ct: cycle threshold. NP: nucleocapsid protein.