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SARS-CoV-2 RNA in plasma samples of COVID-19 affected individuals: a cross-sectional proof-of-concept study

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Abstract

Background: Recent studies showed that plasma SARS-CoV-2 RNA seems to be associated with worse COVID-19 outcome. However, whether specific population can be at higher risk of viremia are to date unexplored.

Methods: This cross-sectional proof-of-concept study included 41 SARS-CoV-2-positive adult individuals (six affected by haematological malignancies) hospitalized at two major hospital in Milan, for those demographic, clinical and laboratory data were available. SARS-CoV-2 load was quantified by ddPCR in paired plasma and respiratory samples. To assess significant differences between patients with and patients without viremia, Fisher exact test and Wilcoxon test were used for categorical and continuous variables, respectively.

Results: Plasma SARS-CoV-2 RNA was found in 8 patients (19.5%), with a median (IQR) value of 694 (209–1023) copies/mL. Viremic patients were characterized by an higher mortality rate (50.0% vs 9.1%; $p = 0.018$) respect to patients without viremia. Viremic patients were more frequently affected by haematological malignancies (62.5% vs. 3.0%; $p < 0.001$), and had higher viral load in respiratory samples (9,404,000 [586,060-10,000,000] vs 1560 [312–25,160] copies/mL; $p = 0.002$).

Conclusions: Even if based on a small sample population, this proof-of-concept study poses the basis for an early identification of patients at higher risk of SARS-CoV-2 viremia, and therefore likely to develop severe COVID-19, and supports the need of a quantitative viral load determination in blood and respiratory samples of haematologic patients with COVID-19 in order to predict prognosis and consequently to help their further management.

Keywords: Viremia, SARS-CoV-2, COVID-19, ddPCR, Molecular diagnosis, Hematological malignancies

Background

Severe Acute Respiratory Coronavirus 2 (SARS-CoV-2) has been detected in specimens from multiple sites [1–3], including plasma. Its frequency ranges from 1 to 73% [2–11] in COVID-19 patients, and it seems to be significantly

associated with adverse clinical outcomes [5, 7–10]. Preliminary data aside, the relationship between patients' characteristics, plasma SARS-CoV-2 RNA, and the severity and outcome of COVID-19, remains largely unexplored.

In this study, we systematically quantified SARS-CoV-2 load in paired plasma and respiratory samples of 41 well characterized patients, with the aim of defining whether the presence of viremia could be correlated with clinical and/or laboratory characteristics, and COVID-19 outcome.

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Methods

Data collection

This proof-of-concept cross-sectional study included 41 SARS-CoV-2-positive adult patients hospitalized at Grande Ospedale Metropolitano Niguarda and at Fondazione IRCCS Policlinico Ca' Granda (Milan, Italy) since March 01, 2020, and who have either died ($N=7$) or been discharged ($N=34$) by July 01, 2020, for those paired respiratory (nasopharyngeal swabs or bronchoalveolar lavages) and plasma samples were available. Demographic and clinical data were retrospectively collected thanks to pseudonymized electronic forms. The severity of the disease was classified in mild, moderate, severe and critical in line with WHO scale [12].

SOFA score and Charlson Comorbidity index were computed from the patients' electronic medical records.

The study protocol was approved by the Ethical Committees of ASST Grande Ospedale Metropolitano Niguarda and IRCCS Ca' Granda Ospedale Maggiore Policlinico Foundation (Prot. Numbers 92–15,032,020 and 0008489) and conducted in accordance with the principles of the 1964 Declaration of Helsinki. Study participation was by written informed consent. The requirement for informed consent was waived for ICU patients for those communication of information was impossible or characterized by disproportionate efforts [13].

SARS-CoV-2 quantification

Total RNA was extracted from 280 μ l of respiratory samples or plasma samples using QIAamp viral RNA mini kit (Qiagen) following manufacturer's instruction. SARS-CoV-2 genomic RNA was quantified by means of the QX200™ Droplet Digital™ PCR System (ddPCR, Biorad) using an home-made protocol targeting the RNA dependent RNA polymerase (RdRp) of SARS-CoV-2 and the housekeeping gene RNase P [14, 15]. All results were further confirmed by using a second assay adapted for ddPCR and targeting two different portions of RdRp (https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteur-paris.pdf?sfvrsn=3662fcb6_2).

SARS-CoV-2 quantification was finally expressed in number copies/mL.

Statistical analysis

Descriptive statistics are expressed as median values and interquartile range (IQR) for continuous data and number (percentage) for categorical data. To assess significant differences between patients with and patients without viremia, Fisher exact test and Wilcoxon test were used for categorical and continuous variables, respectively.

SPSS statistical software (version 23.0, IBM, Armonk, NY, USA) was used for statistical analysis.

Results

Patients' characteristics

From March 01 through July 01, 2020, a total of 1801 patients received a diagnosis of SARS-CoV-2 infection at two of major hospital in Milan, Lombardy. Forty-one patients with available paired respiratory and plasma samples for SARS-CoV-2 detection were included in the analysis. Six of them (14.6%) were affected by haematological malignancies. Demographic, clinical, and laboratory, findings at admission, stratified by presence of SARS-CoV-2 in plasma, are reported in Table 1.

Thirteen patients (30.2%) were male, and median age was 59 (IQR: 50–67) years. Chest radiographs or CT scan confirmed a classical bilateral interstitial pneumonia in 95.1% of cases (39/41). Looking at the outcome available, 7 patients (17.1%) died in a median time of 28 (27–36) days from symptoms onset, and 34 (82.9%) were discharged in a median time of 54 (30–76) days from symptoms onset at the study endpoint.

Sars-CoV-2 load in respiratory and plasma samples

The presence of SARS-CoV-2 RNA in respiratory specimens was confirmed for all 41 patients, with a median (IQR) viral load of 3680 (595–166,800) copies/mL, with no difference between the bronchoalveolar lavages and nasopharyngeal swabs (median, IQR: 3180 [312–166,800] vs 9040 [671–6,800,000] copies/mL, $p=0.149$). SARS-CoV-2 load was found in 8 plasma samples (19.5%), with a median (IQR) value of 694 (209–1023) copies/mL (Table 1).

Stratifying the population by plasma SARS-CoV-2 RNA, viremic patients were characterized by an higher mortality rate (50.0% vs 9.1%; $p=0.018$) respect to no viremic patients. Respiratory failure was the leading cause of death in 3 out of 4 patients with SARS-CoV-2 viremia and in the 2 out 3 patients without evidence of SARS-CoV-2 in plasma. The underlying disease found in the remaining death with viremia was a T-cell lymphoma at advanced stage.

By looking at the characteristics of patients with viremia, we found that they were more frequently affected by haematological malignancies (5 [62.5%] vs. 1 [3.0%]; $p<0.001$), and had higher viral load in respiratory samples (9,404,000 [586,060–10,000,000] vs 1560 [312–25,160] copies/mL; $p=0.002$).

SARS-CoV-2 dynamics in the 8 patients with SARS-CoV-2 viremia

SARS-CoV-2 loads in plasma and respiratory samples of the 8 viremic patients in relation to day of symptoms onset was monitored until viremia clearance (defined as two consecutive negative plasma samples, Fig. 1, number of samples per patient median, [IQR]: 4 [2–6]). Viremia was detected since 12 (9–14) days after symptom onset, and viral clearance occurred while SARS-CoV-2 in

Table 1 Demographic, clinical, viral, and laboratory findings of 41 COVID-19 patients for those blood and respiratory specimens were available for SARS-CoV-2 detection

	Overall, N = 41	Sars-CoV-2 in the blood		P-value
		Present, N = 8	Absent, N = 33	
Demographics and clinical characteristics				
Age, years	59 (50–67)	65 (41–69)	59 (50–66)	0.469
Sex				
Male	13 (30.2)	5 (62.5)	23 (69.7)	0.692
Symptoms				
Fever	35 (85.4)	5 (62.5)	30 (90.9)	0.077
Cough	24 (58.5)	3 (37.5)	21 (63.6)	0.172
Dyspnea	28 (68.3)	5 (62.5)	23 (69.7)	0.692
Evidence of interstitial pneumonia	39 (95.1)	7 (87.5)	32 (97.0)	0.356
Chronic comorbidities	27 (65.9)	6 (75.0)	21 (63.6)	0.435
Solid malignancy	5 (12.2)	1 (12.5)	4 (12.1)	0.683
Haematological malignancies ^a	6 (14.6)	5 (62.5)	1 (3.0)	< 0.001
Diabetes	5 (12.2)	2 (25.0)	3 (9.1)	0.246
Obesity	9 (22.0)	0 (0.0)	9 (23.7)	0.116
Cardiovascular disease	3 (7.3)	2 (25.0)	1 (3.0)	0.092
Chronic obstructive lung disease	4 (9.8)	1 (12.5)	3 (9.1)	0.596
Hypertension	8 (19.5)	1 (12.5)	7 (21.2)	0.503
Chronic kidney disease	1 (2.4)	0 (0.0)	3 (9.1)	0.512
Chronic liver disease	1 (2.4)	0 (0.0)	1 (3.0)	0.805
Other ^b	3 (7.3)	0 (0.0)	3 (9.1)	0.512
Immunosuppressive therapy	27 (65.8)	4 (50.0)	23 (69.9)	0.411
SOFA score ^c	3 (1–4)	2 (1–4)	3 (1–4)	
0	5 (12.8)	0 (0.0)	5 (15.6)	0.828
1–2	12 (30.8)	4 (57.1)	8 (25.0)	
3	8 (20.5)	1 (14.3)	7 (21.9)	
≥ 4	14 (35.9)	2 (28.6)	12 (37.5)	
CHARLSON comorbidity index	2 (1–4)	4 (2–6)	2 (1–3)	
0	7 (17.1)	1 (12.5)	6 (18.2)	0.298
1–2	19 (46.3)	2 (25.0)	17 (51.5)	
3–4	8 (19.5)	2 (25.0)	6 (18.2)	
≥ 5	7 (17.1)	3 (37.5)	4 (12.1)	
COVID-19 manifestation				
Critical	15 (36.6)	4 (50.0)	11 (33.3)	1.000
Severe	14 (34.1)	2 (25.0)	12 (36.4)	0.692
Moderate	11 (26.8)	2 (25.0)	9 (27.3)	1.000
Mild	1 (2.4)	0 (0)	1 (3.0)	1.000
Time from symptoms-onset to hospital admission, days	5 (3–7)	3 (1–5)	6 (3–8)	0.024
SARS-CoV-2 load, ddPCR				
Respiratory samples ^d				
Viral load, copies/mL	3680 (595–166,800)	9,404,000 (586,060–10,000,000)	1560 (312–25,160)	0.002
Blood samples				
Viral load, copies/mL	0 (0–0)	694 (209–1023)	0 (0–0)	–

Table 1 Demographic, clinical, viral, and laboratory findings of 41 COVID-19 patients for those blood and respiratory specimens were available for SARS-CoV-2 detection (Continued)

	Overall, N = 41	Sars-CoV-2 in the blood		P-value
		Present, N = 8	Absent, N = 33	
Laboratory findings^e				
White blood cell count, × 10 ⁹ per L	8.43 (5.59–11.58)	9 (2.70–9.90)	8.30 (5.70–12.50)	0.295
Lymphocytes count, × 10 ⁹ per L	0.74 (0.55–1.06)	0.50 (0.40–1.00)	0.80 (0.60–1.10)	0.230
Platelet count, × 10 ⁹ per L	155 (78–274)	108 (77–285)	202 (78–274)	0.567
Total bilirubin, mg/mL	0.47 (0.28–0.66)	0.40 (0.20–0.80)	0.50 (0.30–0.60)	0.574
Estimated glomerular filtration rate, mL/min	83 (57–99)	57 (46–105)	88 (62–98)	0.806
Creatinine, μmol/L	85 (70–107)	107 (76–132)	82 (69–98)	0.264
D-dimer, μg/mL	1.08 (0.70–2.11)	1.20 (0.80–3.20)	1.10 (0.70–1.90)	0.629
IL-6, pg/mL	53 (16–203)	62 (28–134)	44 (15–251)	0.968
Lactate dehydrogenase, U/L	320 (220–422)	236 (220–332)	344 (217–552)	0.252
Outcome				
Death	7 (17.1)	4 (50.0)	3 (9.1)	0.018
Time from symptoms-onset to death, days	36 (27–83)	31 (21–42)	83 (28–98)	0.400
Discharged	34 (82.9)	4 (50.0)	30 (90.9)	0.018
Time from symptoms-onset to discharge, days	50 (33–73)	38 (35–39)	54 (30–77)	0.621

Data are expressed as median (interquartile range), or N (%). P-values comparing COVID-19 patients with evidence of RNAemia to patients without RNAemia were calculated by Mann-Whitney U test, or Fisher's exact test, as appropriate. $P < 0.05$ was considered statistical significant. ALT = alanine aminotransferase. AST = aspartate transaminase. COVID-19, coronavirus disease 2019. ^aIncluding: Large cell lymphoma (n = 4), T-cell lymphoma (n = 1), Hodgkin's Lymphoma (n = 1).

^bIncluding: Sarcoidosis (n = 1), Hashimoto's thyroiditis (n = 1), renal transplantation (n = 1). ^cInformation available for 39 individuals. ^d20 Bronchoalveolar lavages and 21 nasopharyngeal swabs. SARS-CoV-2 load did not differ between the two different samples (median, IQR: 3680, 366–166,800 vs 1530, 249–11,230, $P = 0.419$). ^eInformation available for 40 individuals

respiratory samples was still detectable (SARS-CoV-2 load copies/mL median, [IQR]: 1,607,260 [1418–8,303, 100]). Two patients died while still viremic.

Focus on hematological malignancies

As mentioned before, 5 out of 6 patients affected by hematological malignancies were viremic for SARS-CoV-2 (Supplementary Table 1). Three of these 5 viremic

patients were affected by large B-cell lymphoma (60.0%) and 4 received chemotherapy during COVID-19 (80.0%), with one patient receiving Bendamustine in combination with Gemcitabine and Vinorelbine (BEGEV). The median time to SARS-CoV-2 clearance in respiratory tract and plasma was of 6.5 (4.4–6.5) and 1.7 (1.5–3.0) weeks, respectively. The longer time for viral clearance in respiratory and peripheral tracts was observed in one patient

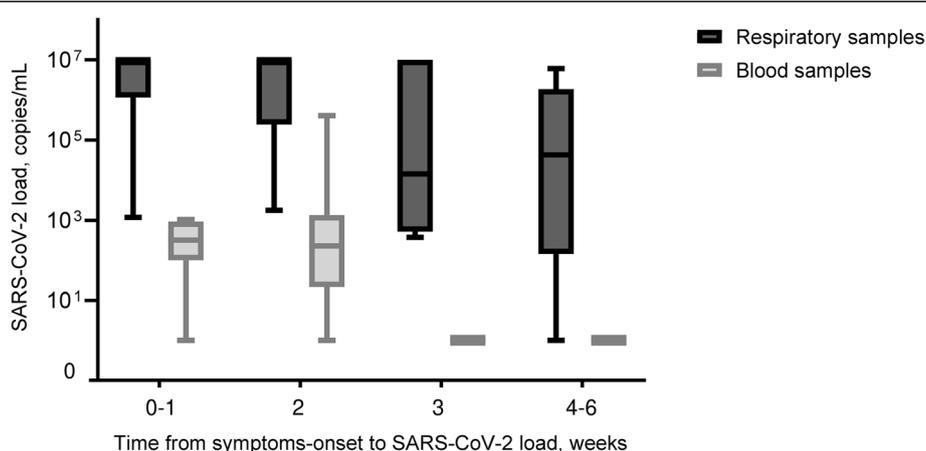


Fig. 1 Decay of SARS-CoV-2 load in respiratory and blood samples of the 8 viremic patients, against time from symptoms onset. Box and whiskers plots indicates the distribution of SARS-CoV-2 load according to sample (respiratory vs. blood) and time from symptoms-onset. The upper and lower quartiles are represented by boxes, while the variability outside this range by whiskers. The median value is represented by a short line within the box

affected by Hodgkin lymphoma and treated with BEGEV, followed by one patient affected by diffuse large B-cell lymphoma with a recent treatment history of high-dose methotrexate.

Discussion

In our study plasma SARS-CoV-2 RNA was found in 8 (19.5%) individuals, 6 (75.0%) of whom had a severe or critical COVID-19 manifestation and 4 (50.0%) died. This is in line with studies published so far, describing plasma SARS-CoV-2 RNA detectable in different percentage of COVID-19 patients, ranging from 0 to 73%, and frequently and significantly associated with worse clinical outcomes [2–11]. It should be noted that SARS-CoV-2 was not the first respiratory viral agent to be detected in blood of critical individuals. The viremic phase was frequently correlated with the severity of the disease and worse outcome in most viral infections, like those sustained by Rhinoviruses, Respiratory syncytial virus, Adenovirus, SARS-CoV, and Metapneumovirus [16–18].

Now that more evidence suggests a close relationship between viremia and severe COVID-19, the identification of specific factors or conditions that can define a population at risk of viremia is of utmost importance. In our study, plasma SARS-CoV-2 load was frequently detected in patients affected by hematological malignancies (62.5% vs. 3.0%, $p < 0.001$), and with higher viral load in respiratory samples (9,404,000 [586,060–10,000,000] vs. 1560 [312–25,160] copies/mL, $p = 0.002$), as compared to patients without viremia (Table 1). Previous studies on series of hospitalised patients showed that the concurrent haematological malignancies and COVID-19 are strongly related to mortality [19], and to prolonged viral shedding in the respiratory tract and serum samples [1, 20]. An episode of prolonged plasma SARS-CoV-2 RNA started prior to the onset of symptoms and diagnosis of COVID-19 was also recently described in a 51-year-old patient with acute myeloid leukemia, and thus with a heavily compromised immune status [21]. Consistent with these earlier reports, we found that viremia was detected since 12 (9–14) days from symptom onset, and viral undetectability in the blood occurred while SARS-CoV-2 in respiratory samples was still present. All these evidences may pose this fragile population at higher risk of developing a systemic infection, suggesting a potential utility of plasma SARS-CoV-2 RNA testing as a prognostic indicator in severe immunocompromised patients.

Our proof-of-concept study has some limitations. a) The small sample size and the high proportion of individuals with chronic comorbidities (80.5% of patients) prevented to appreciate a clear association between viremia and severe COVID-19, that preliminary data have already shown [7–10], as well as correlations

between viremia and IL-6 or other known COVID-19 prognostic markers (i.e. lymphocytes count, lactate dehydrogenase or D-dimer level) [5, 22, 23]. b) The correlation between viremia and the outcome was based only on 4 definitive events, requiring a confirmation on larger sample size. c) The real infectivity of the virus found in plasma was not assessed. Thus, it cannot be determined at present whether detectable RNA represents intact infectious virus or inactive, non-replicating nucleic acid. d) The study design, the heterogeneity of hematological malignancies and chemotherapies prevented to draw certain conclusions about correlations between these parameters and time to SARS-CoV-2 clearance. Even if the prolonged respiratory shedding and persistent viremia observed in one patient treated with the bendamustine is in line with recent findings, [24, 25] this observation requires further evaluation by ad hoc designed studies.

Conclusion

Our cross-sectional proof-of-concept study provides evidence for plasma SARS-CoV-2 load detection in the 19.5% of patients ($n = 8$), 50% of whom died, confirming the association between viremia and worse outcome [7–11]. Patients with SARS-CoV-2 in plasma were frequently characterized by haematological malignancies, and elevated viral load in respiratory samples. Although the associations here observed are based on a small population and need to be confirmed on a larger sample size, they pose the basis for an early identification of patients at higher risk of SARS-CoV-2 viremia, and therefore likely to develop severe COVID-19. This proof-of-concept study also supports the need of a quantitative viral load determination in blood and respiratory samples of haematologic patients with COVID-19 in order to predict prognosis and consequently to help their further management.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12879-021-05886-2>.

Additional file 1.

Abbreviations

SARS-CoV-2: Severe Acute Respiratory Coronavirus 2; COVID-19: CoronaVirus Disease 2019; ARDS: Acute Respiratory Distress Syndrome; ddPCR: Droplet Digital PCR; RdRp: RNA dependent RNA polymerase

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Authors' contributions

LC performed data collection, data analysis, data interpretation, writing; MA, SR, EM, CV, DF processed samples and collected data; SNM performed data processing; MM, GT, MV, JC, AM, GG, VS, EC, AB, AG, MP recruited samples and enrolled patients; CA conceived the study design and data

interpretation; CA, VC, AB, AG, and CFP critically revised the manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

Data are described in Table 1, Supplementary Table 1 and Fig. 1. The datasets used in this study will be available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study protocol was approved by the Ethical Committees of ASST Grande Ospedale Metropolitano Niguarda and IRCCS Ca' Granda Ospedale Maggiore Policlinico Foundation (Prot. Numbers 92–15032020 and 0008489) and conducted in accordance with the principles of the 1964 Declaration of Helsinki. Study participation was by written informed consent. The requirement for informed consent was waived for ICU patients for those communication of information was impossible or characterized by disproportionate efforts (EDPB - Linee-guida 03/2020 sul trattamento dei dati relativi alla salute a fini di ricerca scientifica nel contesto dell'emergenza legata al COVID-19).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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