# Prolonged viral replication in patients with hematologic malignancies hospitalized with COVID-19

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has emerged as a major cause of mortality worldwide. Immunocompromised hosts, especially patients with hematologic malignancies, have suffered greatly due to this pandemic, with reported mortality rates reaching between 28-44%.[1–3]

Multiple studies have described clinical features of coronavirus disease 2019 (COVID-19) to be similar in both immunocompromised hosts and other patients.[1,4] Similarly, the broad spectrum of COVID-19 clinical phenotypes described in immunocompetent patients, including complications due to immunological hyperactivation, co-infection, and/or coagulopathy, has been also observed in hematologic patients. However, information about viral evolution is scarce.

Only a few case reports have documented that immunosuppressed patients may have prolonged viral replication.[5–12] Yet, such viral shedding poses a major threat to both patients and public health. It can delay treatment for hematologic malignancies, increase duration of a patient's infectivity, and potentially favor selection of mutant strains. This series of patients would, therefore, require a personalized approach in response.

We aimed to describe the incidence, clinical features, risk factors and outcomes of prolonged SARS-CoV-2 viral shedding in a cohort of consecutive patients with hematologic malignancies admitted to our hospital. For this purpose, we performed a prospective study including all consecutive adults with hematological malignances hospitalized ≥48 hours for COVID-19 at Hospital Clinic (Barcelona, Spain) between March 2020 and March 2021. Real-time reverse transcription polymerase chain reaction (rRT-PCR) testing performed on nasal and oropharyngeal swab specimens confirmed COVID-19 diagnoses. Hematologic patients with a prolonged positive rRT-PCR underwent further testing for viral sub-genomic mRNA (sgRNA) identification, which is better correlated with active viral replication.[13] Prolonged viral replication was defined as patients with a prolonged positive rRT-PCR and positive sgRNA at ≥3 weeks since initial positive rRT-PCR. Only these patients who underwent rRT-PCR testing at ≥3 weeks since diagnosis were eligible for evaluation in this study. In this sense, we discarded all those patients who either died within the first 3 weeks of diagnosis or did not undergo follow-up rRT-PCR testing.

High-quality data on characteristics of all patients hospitalized for COVID-19 were directly collected from electronic health records with an intelligent system (SILDv1.0 system). We performed rRT-PCR in most samples (85%) using Cobas®6800 (Roche Diagnostic, Germany) which detected the E and ORF1b genes. In the remaining samples, rRT-PCR was performed using the LightMix ModularDx SARS-CoV (COVID19) E-gene kit (Tib Molbiol, Roche Diagnostics). A positive result was defined when the cycle threshold (Ct) value of the E gene was ≤38. Presence of sgRNA was tested with primers and probes targeting sequence downstream of the start codons of the E gene with a specific forward leader primer, using SuperScript™ III Platinum™ One-Step qRT-PCR Kit (Invitrogen) in the thermocycler StepOne (Applied Biosystems). A positive result was defined when the Ct value of the E gene was ≤39.

Factors associated with prolonged viral shedding were evaluated using a multivariate analysis (step-forward procedure), including all significant variables ($P$<0.05) obtained from univariate analysis. A two-tailed $P$<0.05 was considered as significant.

A total of 3,216 consecutive adults with COVID-19 were admitted to our hospital for ≥48 hours during the study period, of whom 124 (3.9%) had hematologic malignancies. Of those, 67 (54%) were eligible for viral persistence assessment due to survival status and follow-up rRT-PCR at ≥3 weeks since initial diagnosis. Prolonged viral replication was documented in 17 (25.4%) evaluable patients, representing 17.3% (17 of 98) of all hematologic patients alive after 3 weeks. *Online Supplementary Figure S1* details the study flowchart, while *Online Supplementary Table S1* compares patient characteristics on the basis of eligibility for viral persistence assessment. The most common hematologic malignancies in the study population were lymphoma and chronic lymphocytic leukemia. Overall, 20.9% of patients had received a hematopoietic stem cell transplant (HSCT), and median C-RP at admission was 6.1 (interquartile range [IQR], 2.2-11.2) mg/dL. Table 1 details epidemiological and clinical characteristics of the cohort.

*Online Supplementary Table S2* summarizes the main characteristics, clinical features, and outcomes of those 17 patients with prolonged viral replication. In 12 (70.6%) of those patients, sgRNA was negative before genomic rRT-PCR. Median time of sgRNA positivity was 54 (IQR, 32.5-100) days compared to 77 (IQR, 56.5-107) days for rRT-PCR positivity ($P$<0.001). Six (35.3%) patients died after a median time of 72 (IQR, 53-144) days of receiving a COVID-19 diagnosis.

Table 2 shows univariate and multivariate risk factors for prolonged viral replication. In the multivariate analysis, lymphoma (odds ratio [OR] 5.44, 95% confidence interval [CI]: 1.24-23.84); hypogammaglobulinemia (OR 4.64, 95% CI: 1.10-19.60); and prior chemotherapy (OR 27.21, 95% CI 2.88-257.40) were independently associated with prolonged viral replication. The discriminatory power of the score, as evaluated by the area under the receiver operating characteristic curve, was 0.884 (95% CI: 0.803-0.965), demonstrating a good ability to predict prolonged viral replication.

Some case reports have described the possibility of pro-

**Table 1. Main epidemiological and clinical characteristics of hematologic patients.**

| | Patients N=67 (%) |
|---|---|
| **Patient's characteristics** | |
| Median (IQR) age, in years | 65 (54-77) |
| Age > 65 years (%) | 35 (52.2) |
| Sex male, N (%) | 42 (62.7) |
| **Hematologic malignancy** | |
| Lymphoma* | 30 (44.8) |
| Chronic lymphocytic leukemia | 10 (14.9) |
| Multiple myeloma | 7 (10.4) |
| Acute leukemia | 6 (9) |
| Myelodysplastic syndrome | 5 (7.5) |
| Others | 6 (9) |
| Prior HSCT | 14 (20.9) |
| Allogenic HSCT# | 4 (6) |
| Autologous HSCT# | 12 (17.9) |
| Prior CAR-T cell therapy | 3 (4.5) |
| **Other comorbidities** | |
| Hypertension | 26 (38.8) |
| Diabetes mellitus | 13 (19.4) |
| Chronic heart disease | 21 (31.3) |
| Chronic lung disease | 7 (10.4) |
| Chronic renal failure | 12 (17.9) |
| Chronic liver disease | 9 (13.4) |
| Solid organ transplant | 5 (7.5) |
| **Other clinical features** | |
| Prior corticosteroid use (3 months) | 35 (52.2) |
| Prior chemotherapy (3 months) | 36 (53.7) |
| Prior rituximab use (12 months) | 15 (22.4) |
| Current neutropenia (< 500/mm$^3$) | 6 (9.0) |
| Long-term lymphopenia (> 1 month; < 900 lymphocytes/mm$^3$) | 32 (47.8) |
| Hypogammaglobulinemia | 23/62 (34.3) |
| Active hematologic disease | 40 (59.7) |
| Median (IQR) days from symptom onset to hospital admission | 4 (2-6) |
| **Vital signs at admission; median (IQR)** | |
| Temperature -Median (°C) | 37.0 (36.2-37.9) |
| Respiratory rate -Median (rpm) | 20 (18-24) |
| Oxygen saturation -Median | 95 (94-97) |
| **Laboratory values at admission; median (IQR)** | |
| Ferritin (ng/mL) | 207 (487-973) |
| C-RP (mg/dL) | 6.1 (2.2-11.2) |
| D-dimer (ng/mL) | 800 (400-1800) |
| LDH (U/L) | 291 (207-365) |
| Lymphocyte count (cells/mm$^3$) | 800 (400-1350) |

IQR: interquartile range; HSCT: hematopoietic stem cell transplant; CAR: chimeric antigen receptor; rpm: respirations per minute; C-RP: C-reactive protein; LDH: lactate dehydrogenase. *Diffuse large B-cell lymphoma, 11 patients; follicular lymphoma, 7; Hodgkin lymphoma, 5; T-cell lymphoma, 2; other, 4. #Two allogenic HSCT recipients had undergone a prior autologous transplant.

longed COVID-19 infection in immunosuppressed patients.[5–12] We documented that such a prolonged SARS-CoV-2 infection is quite frequent. It affected more than 25% of patients with hematologic malignancies, at least in our cohort of patients requiring hospitalization due to COVID-19 and surviving more than 3 weeks.

We agree with prior case reports that the clinical spectrum of these infections range from chronic asymptomatic infection to severe presentation and death. This fact, therefore, implies that physicians should actively screen for this complication by performing repeated rRT-PCR after a COVID-19 diagnosis until the patient tests negative, particularly among those with risk factors for prolonged viral replication. In immunocompetent patients, viral culture was positive only in samples with a cycle threshold value of 28 or less.[14] Therefore, to preclude the diagnosis of a persistent viable virus, it seems reasonable that physicians perform a sgRNA and/or viral culture in all immunosuppressed patients in whom the rRT-PCR value is persistently positive with Ct values below 28.[13]

Hospitalized hematologic patients with COVID-19 and hypogammaglobulinemia, recent chemotherapy, and/or lymphoma, especially after rituximab use, face a higher risk of viral persistence. It is understandable that these risk factors are present among patients with prolonged SARS-CoV-2 infection. Such factors deeply impair B-cell response and the production of antibodies with neutralizing activity against the virus, which comprise the main factors of the host immune response against the virus.[15,16] In order to compensate this deficit, physicians could consider administering convalescent plasma or specific monoclonal antibodies on a repeated basis. Conversely, in this setting, administering dexamethasone as COVID-19 treatment without the presence of an inflammatory phenotype may prove deleterious.

Prolonged SARS-CoV-2 replication represents a huge challenge in patient care, public health and medicine. First, viral load persistence could cause delays in required treatment for hematologic malignancies and thereby, significantly contribute to a worsening in a patient's prognosis. Moreover, mandatory prolonged isolation due to such persistence impacts a patient's daily social life and decreases quality of life. Second, from a public health perspective, prolonged viral replication is a tremendous problem since it is conceptually feasible that prolonged infections may facilitate the progressive emergence of mutants. However, this is rather theoretical and mainly supported by single case reports. Further studies demonstrating this association are needed. Fi-

**Table 2. Univariate and multivariate risk factors for prolonged viral shedding.**

| Risk factor | Non-prolonged viral shedding N=50 (%) | Prolonged viral shedding N=17 (%) | Univariate odds ratio (95% CI) | *P*-value | Multivariate odds ratio (95% CI) | *P*-value |
|---|---|---|---|---|---|---|
| Age > 65 years | 27 (54.0) | 4 (23.5) | 0.26 (0.08-0.91) | **0.047** | 0.47 (0.08-2.61) | 0.387 |
| Male sex | 33 (66.0) | 9 (52.9) | 0.58 (0.19-1.77) | 0.336 | - | - |
| Lymphoma | 18 (36.0) | 12 (70.6) | 4.27 (1.30-14.06) | **0.013** | 5.44 (1.24-23.84) | **0.025** |
| Acute leukemia | 5 (10.0) | 1 (5.9) | 0.56 (0.61-5.19) | 1.000 | - | - |
| Myelodysplastic syndrome | 5 (10.0) | 0 (0) | - | 0.319 | - | - |
| Multiple myeloma | 4 (8.0) | 3 (17.6) | 2.46 (0.49-12.35) | 0.358 | - | - |
| Chronic lymphocytic leukemia | 9 (18.0) | 1 (5.9) | 0.56 (0.07-4.51) | 0.432 | - | - |
| Chronic myeloid leukemia | 3 (6.0) | 0 (0) | - | 0.565 | | |
| Others | 6 (12.0) | 0 (0) | - | 0.325 | - | - |
| Hematopoietic stem cell transplant | 10 (20.0) | 4 (23.5) | 1.23 (0.33-4.60) | 0.740 | - | - |
| CAR-T cell therapy | 0 (0) | 3 (17.6) | - | **0.014** | - | - |
| Hypertension | 22 (44.0) | 4 (23.5) | 0.39 (0.11-1.37) | 0.160 | - | - |
| Diabetes mellitus | 11 (22.0) | 2 (11.8) | 0.47 (0.09-2.39) | 0.490 | - | - |
| Chronic heart disease | 17 (34.0) | 4 (23.5) | 0.60 (0.17-2.12) | 0.550 | - | - |
| Chronic lung disease | 6 (12.0) | 1 (5.9) | 0.46 (0.05-4.11) | 0.669 | - | - |
| Chronic renal failure | 11 (22.0) | 1 (5.9) | 3.18 (1.35-7.49) | 0.270 | - | - |
| Chronic liver disease | 5 (10.0) | 4 (23.5) | 2.77 (0.65-11.83) | 0.216 | - | - |
| Solid organ transplant | 4 (8.0) | 1 (5.9) | 0.72 (0.08-6.92) | 1.000 | - | - |
| Prior corticosteroid use | 22 (44.0) | 13 (76.5) | 4.14 (1.18-14.47) | **0.026** | 1.39 (0.23-8.31) | 0.721 |
| Hypogammaglobulinemia | 11 (22.0) | 11 (64.7) | 6.50 (1.96-21.56) | **0.001** | 4.64 (1.10-19.60) | **0.037** |
| Active hematologic malignancy | 25 (50.0) | 15 (88.2) | 7.50 (1.55-36.27) | **0.009** | 3.61 (0.53-24.36) | 0.188 |
| Prior rituximab (12 months) | 5 (10.0) | 10 (58.8) | 12.86 (3.38-48.94) | **<0.001** | 2.52 (0.26-24.60) | 0.427 |
| Prior chemotherapy (3 months) | 20 (40.0) | 16 (94.1) | 24.0 (2.95-195.60) | **<0.001** | 27.21 (2.88-257.40) | **0.004** |
| Current neutropenia | 4 (8.0) | 2 (11.8) | 1.53 (0.26-9.23) | 0.639 | - | - |
| Long-term lymphopenia | 20 (40.0) | 12 (70.6) | 3.60 (1.10-11.80) | **0.029** | 2.87 (0.52-15.85) | 0.227 |

nally, guidelines regarding diagnostic approaches and treatments in this particular setting are lacking. In our practice, we observed three patterns: i) some of our patients had no response to standard treatment with a 5-day course of remdesivir, and convalescent plasma and prolonged antiviral treatment were necessary at times for improvement; ii) some patients experienced a clinical response after receiving remdesivir but either fell ill again or had an increase in SARS-CoV-2 load after antiviral therapy completion; and iii) some patients did not respond to antiviral strategies at all. That stated, each of these situations requires a personalized approach that would perhaps necessitate the inclusion of prolonged antiviral treatment with remdesivir, combined with monoclonal antibodies and/or plasma.

Our study has some limitations that should be noted. We retrospectively described the incidence of prolonged viral replication. However, we may have missed some patients with prolonged viral replication, especially at the beginning of the pandemic when we did not perform follow-up testing. Another limitation is the lack of genetic tests performed in some patients to confirm the same linage. However, we would like to highlight that we have this information for four patients and were able to confirm pro-

longed infection. Finally, viral culture was only available (and positive) in two of the 17 patients; we used sgRNA as a surrogate marker of viral viability. Although there is an open debate, our experience has shown that sgRNA has a good correlation with viral culture.[15]

In summary, prolonged viral replication is frequent in patients with hematologic malignancies, especially in those with recent chemotherapy, lymphoma and hypogammaglobulinemia. This represents a major threat for both, patients and the public health. A personalized diagnostic approach and specific therapeutic strategies are necessary to address this concerning clinical scenario.

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**Contributions**

CG-V, PP-A, M-AM and AS developed the concept; CG-V, PP-A, AS, and M-AM developed the methodology; GC-C, MS-B, M-AM performed the software analysis.; CC-V, PP-A and AS performed the formal analysis; PP-A, MC, NG-P, CG-V, EG carried out the investigation; all authors provided resources and took care of data; CG-V, PP-A and AS wrote the original draft; all authors reviewed and edited the manuscript; CG-V, M-AM, AS supervised the research; PP-A and CG-V were responsible for project adminstration.

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