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SARS-CoV-2 systemic infection in a kidney transplant recipient: sequence analysis in clinical specimens

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Abstract. – OBJECTIVE: Herein we report clinical and virological data in a patient with COVID-19 infection and a prior history of kidney transplantation who had a good clinical recovery despite systemic infection.

PATIENTS AND METHODS: Reverse transcriptase quantitative PCR analysis for the RdRp, N and E target genes detected viral RNA in different types of biological specimens. Whole viral genome sequences were obtained and analyzed from respiratory tract, feces and blood.

RESULTS: Viral sequences showed high (~99.9%) homology with the Wuhan seafood market pneumonia virus. Phylogenetic analysis assigned of the SARS-CoV-2 strains to clade G. A rare variant in the orf1ab gene was present in both sequences, while a missense variant was detected only in viral RNA from stool.

CONCLUSIONS: The evolution of the COVID-19 systemic infection in the patient presented here was favorable to the hypothesis that immunosuppressive therapy in organ transplant recipients might be involved in viral dissemination. A missense mutation was present in only one specimen from the same patient implying the occurrence of a mutational event in viral RNA, which is suggestive for the presence of an active virus, even though viral isolation is necessary to demonstrate infectivity.

Key Words:
COVID-19, SARS-CoV-2 sequence, Kidney transplantation.

Abbreviations
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019; CPAP: Continuous Positive Airway Pressure.

Introduction

Corona virus 2019 (COVID-19) is a new respiratory disease with an evolving understanding of its pathophysiology and clinical course. While the immunocompromised status of solid organ transplant recipients raises the concern for atypical presentations of viral infection, only limited data regarding risk, presentation, and outcomes in transplant patients with systemic infection of SARS- COV-2 are found[1,2]. SARS-CoV-2 RNA detection in the respiratory tract is essential for COVID-19 diagnosis, but viral genes have also been detected from blood, feces, and other biological fluids[3,4]. Viral shedding is reported to occur for long time after negative conversion on the nasal-oropharyngeal swab, but the clinical effects of SARS-CoV-2 persistence in blood and gastrointestinal tract remains to be elucidated[3,5-7]. In this study, we describe a case of a patient with a prior history of kidney transplantation with COVID-19 infection with good clinical recovery despite the presence of viral (COVID-19) RNA from different types of biological specimens. Sequences of the entire viral genome were obtained and analyzed from respiratory tract, stool, and blood samples.

Patients and Methods

On April 3, 2020, a 62-year-old female was admitted to the hospital with a 5-day history of high-grade fever, dry cough, and diarrhea. Ten years before admission she had undergone a kidney

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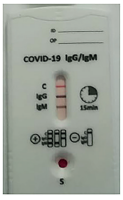
transplantation for end-stage renal failure. The patient's medical history revealed post-transplant treatment with everolimus and mycophenolate mofetil as maintenance immunosuppressive therapy. Hypertension under olmesartan medoxomil treatment was also present as a comorbid disease. Renal function was stable at her most recent follow-up on December 4, 2019.

At admission, physical examination of the body temperature was 38.5°C, the blood pressure 161/86 mm Hg, the respiratory rate of 22 breaths per minute and the oxygen saturation 95% while breathing ambient air. Initial laboratory work up (Table I) showed lymphocytopenia displaying absolute count 800 lymphocyte, C-reactive protein 12.50 mg/L, procalcitonin 0.32 ng/ml, D-dimer 16.5 ng/ml, white blood cell counts 6.23x10^{9}/L. A postero-anterior chest X-ray showed typical signs of COVID-19 pneumonia with a diffuse bilateral infiltrates. Nasopharyngeal swab test for SARS-CoV-2 resulted RT-qPCR positive using the Allplex 2019-nCoV assay (Seegene Inc., Arrow Diagnostics S.r.l, Genoa, Italy). Immunosuppressive therapy was withdrawn and a treatment according to a local protocol based on lopinavir/ritonavir, methylprednisolone, hydroxychloroquine, low-molecular-weight heparin and azithromycin with the addiction of broad-spectrum antibiotic therapy (amoxicillin-clavulanate) was started.

On April 8, she developed hypokalemia with subsequent QT interval prolongation reversed for three days upon correction of serum K+; on April 10, the patient was supported with red blood cell transfusion for a moderate grade anemia (Hb 7g/dL). Azithromycin was discontinued. Nasopharyngeal swab RT-qPCR test was repeated and gastrointestinal symptoms led to search for viral presence also in stool, urine and blood samples. All analyzed specimens, but urine, resulted in SARS-CoV-2 RT-qPCR positive results with *RdRp* Ct values of 28.6, 25.6 and 32.7 in nasal, feces and serum, respectively.

Table I. Laboratory results.

	Admission	Day 5	Day 10	Day 17	Discarge
White blood cell, x10^{9}/µl	4.64	5.62	11.43	11.7	3.94
Hemoglobin g/dL	10.8	7		9	9.7
Neutrophil, x10^{3}/µl	3.7	5	10.4	10.2	3.6
Lymphocyte, x10^{3}/µl	0.8	0.5	0.7	0.6	0.2
Platelets 10^{3}/µl	196	101	90	84	36
Lactic dehydrogenase UI	-	1050	619	568	540
Procalcitonin, ng/mL	-	0.32	-	0.13	0.29
C-reactive protein,mg/L	-	12.5	-	0.98	9.25
D-dimer, ug/ML	-	16.5	-	3.75	-
Fibrinogen, g/L	-	780	-	251	-
Creatinine mg/dL	1.9	2.3	-	-	0.9
eGFR (mL/min/1.73 m^{2})	34	27	-	-	85
Aspartate aminotransferase, UI	70	136	-	81	64
Alanine aminotransferrase, UI	45	65	-	116	185
Sodium mmol/L	133	149	-	135	136
Potassium mmol/L	3.2	2.3	-	4.9	3.2
2019-nCoV2* nasopharyngeal swab	+	+	+	+	-
2019-nCoV2* serum	na	+	+	+/-	-
2019-nCoV2* plasma	na	+	+	+/-	-
2019-nCoV2* urine	na	-	-	-	na
2019-nCoV2* feces swab	na	+	+	+	+
SARS-CoV-2 antibodies detection (IgG and IgM)					

na = not analyzed.; + SARS-CoV-2 RNA detected; - SARS-CoV-2 RNA not detected; *2019nCoV-2 RNA assay for E, RdRp, N gene.

On April 11 the patient started worsening dyspnea and was transferred to the Intensive Care Unit (ICU). Search for cytomegalovirus DNA and parvovirus B19 antibodies returned negative; a repeated high-resolution chest X-ray showed a worsening pneumonia with extension of parenchymal thickening, obliteration of the cost-phrenic sinuses due to the presence of bilateral pleural effusion. Oxygen saturation was 89% with a fraction of inspired oxygen (FiO_2) of 60%. Oxygen was administered to the patient at 60L/min *via* a helmet CPAP at 12 cm H_2O PEEP. Biological specimens tested at this time point revealed the presence of viral RNA in nasopharyngeal and stool swabs, as well as in serum and plasma. Anti-SARS-CoV-2 IgM and IgG antibodies were detected in blood using the NADAL® COVID-19 IgG/IgM Test Cassette flow chromatographic immunoassay[8].

On day 14 from admission the patient's clinical condition, routine blood test, and C-reactive protein began to improve. A high-resolution chest CT scan showed resolving patchy infiltrates and the patient was discharged from the intensive care unit.

On May 14, following two sequential nasopharyngeal swabs testing negative, she was successfully transferred to a rehabilitation clinic. The viral RNA was detectable at low level in blood and was still present in stool sample.

Results

Sequencing analysis from different specimens was performed to validate RT-qPCR results and to obtain an overview of any genomic SARS-CoV-2 mutations and/or the presence of different viral strains. Viral RNA obtained from nasopharyngeal swab, stool and serum were massively sequenced using the amplicon approach. The consensus sequences from nasopharynx and feces (EPI_ISL_458085, EPI_ISL_458084) were submitted to GISAID[9]. The sequences, although not identical, showed high (~99.9%) homology with the Wuhan seafood market pneumonia virus (NC_04551.2). Phylogenetic analysis allowed the assignment of the SARS-CoV-2 strains to clade G[10]. A rare variant A13703G in the *orf1ab* gene was present in both sequences, while the missense variant p.His4918Tyr was detected in viral RNA from stool, but was absent in the sequence from nasopharyngeal swab. Serum SARS-CoV-2 viral RNA was only partially sequenced likely due to the low viral load in blood.

Molecular analysis demonstrated a systemic infection with viral RNA found throughout the disease course; seroconversion occurred after two weeks from the onset of symptoms but was not followed by a rapid decline in viral load. It has been reported[11,12] that there is an association between severity of illness, RNAemia and duration of viral shedding. In our case, we observed a positive rate of SARS-CoV-2 RT-qPCR for nasopharyngeal swabs and blood with a gradually decreasing trend thereafter, with feces remaining positive after resolution of symptoms. The persistence of viral RNAemia throughout the hospitalization contrasts a relatively early seroconversion and clinical improvement and is in line with the concept that the immunosuppression could play a role in triggering the virus dissemination[13,14].

Sequences shared 7 mutations that allowed to assign the viral strains to SARS-CoV-2 clade G lineage B.1.1, as like other sequences from EU countries, including Italy. Interestingly, a rare variant N79S in the *orf1ab* gene was present in both nasal and stool specimens, while the A526L mutation was found only in stool sample. However, none of the observed mutations so far have been associated with changes in viral pathogenicity or transmissibility.

Discussion

Solid organ transplant patients with COVID-19 infection are theoretically at higher risk of severe clinical course due to immunosuppression; underlying comorbidities, such as diabetes mellitus and hypertension may add further risk. However, it is still uncertain whether the immunocompromised hosts are *per se* at higher risk of more severe systemic disease[1,2,15].

We describe the case of COVID-19 affecting a kidney transplanted patient who presented with mild symptoms at admission, a secondary progression to pneumonia and had a good clinical outcome despite the presence of viral RNA in respiratory tract, blood and feces. This patient did not experience neurological symptoms, such as headache, anosmia and ageusia that are commonly reported in COVID19 immunocompromised patients[16-18].

In the case presented here, the RNAemia and impaired immune response did not induce particularly severe complications. Sequence analysis of SARS-Cov-2 RNA revealed the presence of a mutation in stool and not in the nasopharyngeal specimen from the same patient, indicating the

occurrence of a mutational event in viral RNA which is suggestive for the presence of a replicating virus, even though virus isolation is necessary to demonstrate infectivity. It is important to note that while the sequence data deposited in GISAID are very helpful in tracking inter-personal variation of the virus, we still do not know much about intra-personal viral evolutionary dynamics.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Contributors

Contributions of each author can be summarized as follows: MM, MF, GM, GP involved in the conceptual development, writing the paper, analysis, and editing. GP, TF, RA performed sequencing, collected and analyzed sequencing data. VF, FS, AT, AF, GM contributed to the section on virological testing. All authors contributed to the writing of the final version of the article.

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