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SARS-CoV-2 in saliva, viremia and seroprevalence for COVID-19 surveillance at a single hematopoietic stem cell transplantation center: a prospective cohort study

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ABSTRACT

This prospective cohort study aims to analyze the surveillance of COVID-19 at a single hematopoietic stem cell transplantation (HSCT) center in Brazil, in 29 patients undergoing allogeneic HSCT and 57 healthcare workers (nurses and dentists), through viral shedding of SARS-CoV-2 in saliva and plasma and seroprevalence of anti-SARS-CoV-2 IgG. In addition, we report two cases with prolonged persistent detection of SARS-CoV-2 without seroconversion. The sample collection was performed seven times for patients and five times for healthcare workers. Only two patients tested positive for SARS-CoV-2 in their saliva and plasma samples (6.9%) without seroconversion. All healthcare workers were asymptomatic and none tested positive. Two patients (6.9%) and four nurses (8%) had positive serology. No dentists had positive viral detection or positive serology. Our results reflect a low prevalence of positive RT-PCR and seroprevalence of SARS-CoV-2 in patients and healthcare workers at a single HSCT center. Results have also corroborated how the rigorous protocols adopted in transplant centers were even more strengthened in this pandemic scenario.

KEYWORDS: COVID-19. SARS-CoV-2. Hematopoietic stem cell transplantation. Healthcare workers.

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is considered a complex and aggressive procedure and demands specific care from a multidisciplinary team working in the same therapeutic context. Complications of treatment should be closely observed to avoid life-threatening problems or conditions that affect the survival and quality of life of patients[1].

HSCT recipients are at an increased risk for viral infections due to underlying disease and immunosuppression. Respiratory viral infections are prevalent before and post-HSCT[2,3]. Most patients develop upper and lower respiratory tract infections, with an average mortality rate of 32%[4-6]. In addition, prolonged neutropenia and lymphopenia, as well as graft-versus-host disease (GVHD), increase the risk of viral infections and these patients often have prolonged viral shedding. The initial laboratory evaluation of these patients for COVID-19 (Coronavirus disease 2019) is part of the protocol for evaluating active infection and management during the pandemic[7-9].

Recent studies have shown that saliva samples are just as sensitive to molecular diagnosis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as those collected by nasopharyngeal swabs (NPS). Saliva can be an advantageous alternative compared to the NPS as it generates less discomfort for patients and reduces the exposure of healthcare workers (HCW) due to the possibility of self-collection[10-14].

Patients with hematologic malignancies, including HSCT recipients, have an increased risk of severe outcomes and mortality from COVID-19, as several studies have shown[15-21] and, as far as we know, only one study has reported the surveillance (only seroprevalence) for COVID-19 in pediatric HSCT recipients[22]. Therefore, our study is among the first to evaluate the surveillance for COVID-19 in the specific setting of an HSCT service, including adult patients (≥ 18 years old) and HCW, using the molecular identification of SARS-CoV-2 in saliva and plasma, as well as the seroprevalence of anti-SARS-CoV-2 IgG.

Therefore, this study aims to conduct a prospective cohort for the surveillance of COVID-19 from a single HSCT center, in allo-HSCT receptors patients and HCW through viral shedding of SARS-CoV-2 in saliva and plasma and the seroprevalence of anti-SARS-CoV-2 IgG. In addition, two cases with prolonged persistent detection of SARS-CoV-2 with no seroconversion were also reported.

MATERIALS AND METHODS

Sample and data collection

This is a prospective cohort study with a sample composed, for convenience, of 86 individuals who are patients or HCW from the Bone Marrow Transplantation Department of the Clinical Hospital Complex of the Federal University of Parana (STMO-CHC/UFPR).

The participants were divided into three groups: patients undergoing allo-HSCT (day -7 to day +180 after HSCT), nurses and dentists. All participants signed an informed consent form. The study was approved by the Ethics Committee of the institution, protocol Nº 4.414.355 and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2013.

All participants answered a questionnaire with sociodemographic information and medical history. The questionnaire covered respiratory symptoms, fever, history of underlying diseases acting as risk factors for COVID-19, exposure to biological material, or living with people diagnosed with COVID-19 in the 14 days before the sample collection.

Saliva, plasma and serum samples were collected from each participant. Unstimulated saliva was collected in a sterile Falcon tube for 5 min, and 500 µL aliquots were frozen at -80 ºC and stored until analysis. Whole blood samples for plasma and serum separation were collected by central venous catheters in hospitalized patients or by peripheral venipuncture in HCW and patients under outpatient follow-up. Vacuum blood collection was performed in a K2EDTA tube to obtain plasma and in a tube with gel separator in addition to a clot activator to obtain serum. Blood tubes were centrifuged for 10 min at 2000 x g. After centrifugation, 500 µL aliquots were frozen at -80 ºC and stored until analysis.

Collections were performed monthly, seven times (June to December 2020) for patients, and five times (June to October 2020) for HCW, totaling 331 samples of each specimen (saliva, plasma and serum). For the patients, sample collections were performed pre-HSCT and monthly (30, 60, 90, 120, 150, and 180 days after HSCT) in routine consultations at the dental clinic. For HCW, sample collections were performed in the first week of each month.

Data processing and analysis

For saliva specimens, 200 µL from each specimen were used for RNA extraction using an automated magnetic EXTRACTA - RNA and DNA Viral kit (Loccus Biotecnologia, Brazil). The analysis was performed with a pool of five saliva specimens. If the pool result was positive, individual analyses of the five specimens were performed to identify which one (or ones) was (were) positive. Amplification was performed on a QuantStudio5™ instrument (Thermo Fisher Scientific Waltham, MA, USA) using the AllPlex nCov-2019 RT-PCR Master Mix Kit (Seegene Inc., Seoul, Republic of South Korea). The kit includes the detection of the nucleocapsid (N), envelope (E), RNA-dependent RNA polymerase (RdRP) viral genesand an MS2-phage added to the reagents master mix as an internal control gene. We followed the protocols previously described by Adamoski *et al.*[23].

For plasma specimens, 200 µL of each sample were used for RNA extraction, following the same procedures observed for saliva. Only plasma specimens from individuals who had positive results in saliva were analyzed.

For serum specimens, a magnetic bead-based immunoassay was performed to identify IgG reacting to SARS-CoV-2 nucleocapsid protein, following the protocols previously described by Huergo *et al.*[24].

Statistical analysis

Descriptive analysis was performed using the SPSS (New York, IBM, Armonk, NY, USA) version 20.0

software. Due to the small number of positive cases, it was not possible to perform inferential statistics by variable association.

RESULTS

Sociodemographic data and medical history

The sample consisted of 29 patients undergoing allo-HSCT (day -7 to day +180 after HSCT), 50 nurses and seven dentists. The groups of patients and of dentists had a higher number of males (58.6% and 57.1%, respectively), while the group of nurses was mostly composed of females (96%). The mean age in the groups was 37.2±13.8 years in patients, 41.2±9.8 years in nurses, and 29.5±6.5 years in dentists. Regarding the pre-existence of underlying diseases acting as risk factors for COVID-19, 100% of the patients were at risk for HSCT-induced immunosupression (Table 1). In the nurse and dentist groups, 24% and 28.6% reported risky diseases, respectively (Table 2).

Table 1 - Demographic and clinical characteristics of the patients.

Parameter	N (%)
Sex	
Female	12 (41.4)
Male	17 (58.6)
Underlying disease	
Acute lymphoblastic leukemia	4 (13.8)
Acute myeloblastic leukemia	9 (31)
Non-Hodgkin's lymphoma	3 (10.3)
Myelodysplastic syndrome	1 (3.4)
Severe aplastic anemia	10 (34.5)
Paroxysmal nocturnal hemoglobinuria	1 (3.4)
Pure red cell aplasia	1 (3.4)
Allograft type	
Related	17 (58.6)
Unrelated	12 (41.4)
Matched	15 (51.7)
Mismatched	14 (48.3)
Stem cell source	
Bone marrow	21 (72.4)
Peripheral blood	8 (27.6)
Conditioning regimen	
Myeloablative	23 (79.3)
Reduced intensity	5 (17.2)
Non-myeloablative	1 (3.4)
GVHD prophylaxis regimen	
Cyclosporine A + methotrexate	25 (86.2)
Cyclosporine A + mycophenolate mofetil	4 (13.8)
GVHD	
Yes	19 (65.5)
No	10 (34.5)

GVHD = Graft vs Host Disease.

Saliva and plasma RT-PCR

Only two patients tested positive for SARS-CoV-2 in their saliva (6.9%). A male patient (A), came out positive on his second test, and a female patient (B) on her fourth and fifth tests (Figure 1a). Patients A and B also performed

Table 2 - Demographic and clinical characteristics of healthcare workers.

Parameter	**N (%)**
Nurses	
Sex	
Female	48 (96)
Male	2 (4)
Risk group	
Yes	12 (24)
No	38 (76)
Respiratory symptoms or fever (14 days before collection)	
Yes	0 (0)
No	50 (100)
Exposure to biological material with SARS-CoV-2 (14 days before collection)	
Yes	11 (22)
No	39 (88)
Dentists	
Gender	
Female	3 (42.9)
Male	4 (57.1)
Risk group	
Yes	2 (28.6)
No	5 (71.4)
Respiratory symptoms or fever (7 days before collection)	
Yes	2 (28.6)
No	5 (71.4)
Exposure to biological material with SARS-CoV-2 (14 days before collection)	
Yes	3 (42.9)
No	4 (57.1)

Risk group = age, systemic arterial hypertension, diabetes, obesity, immunosuppression.

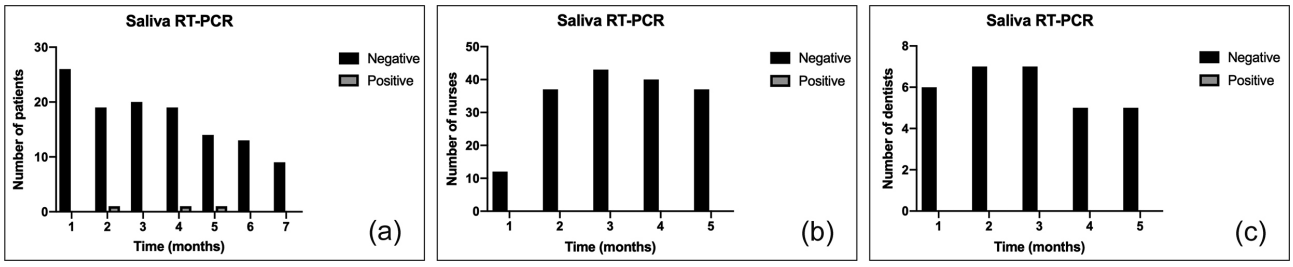

Figure 1 - Distribution of positive and negative results in the detection of SARS-CoV-2 by RT-PCR in saliva at different times during the follow-up of patients (a), nurses (b), and dentists (c).

RT-PCR tests on NPS samples (hospital internal protocol) and showed positive results for more than 40 consecutive days in weekly collections. Only three plasma specimens, corresponding to the times that the saliva specimens had positive results, were analyzed, one samples from patient A and two samples from patient B. In patient A, SARS-CoV-2 was detected in plasma on the second test performed, while in patient B it was only detected on the fourth test. These two cases are reported below in detail to better elucidate their characteristics.

No asymptomatic HCW (nurses and dentists) tested positive for SARS-CoV-2 in their saliva (Figures 2b and 2c). Therefore, no plasma specimens were evaluated. The hospital's internal flow for HCW who presented any respiratory symptoms or fever greater than 37.8 °C were oriented not to go to work and attend the hospital's collection laboratory, in addition to isolating themselves until the reception of a negative result, or, in case of a positive result, comply a 14-days quarantine. For this reason, it was not possible to collect samples from symptomatic professionals, as they should not go to work in these situations. However, 8% of the nurses reported having symptoms and a positive result for SARS-CoV-2 after NPS collection performed in accordance with the hospital's internal flow. No dentist had a positive result after NPS collection for having symptoms associated with COVID-19.

Anti-SARS-CoV-2 IgG

Only two patients had positive serology (6.9%) (Figure 2a). One female patient (C) on the fifth time tested and one male patient (D) on the fourth and fifth times tested. These two patients were asymptomatic and did not show viral detection in saliva specimens before or concomitantly with the NPS collection. The infection likely occurred in the interval between monthly collections, thus, no virus was detected at the time of collection. As they were asymptomatic, no NPS samples were collected in the context of the hospital's internal flow.

Four nurses had positive serology (8%) (Figure 2b). A male nurse (a) on his third, fourth, and fifth times tests, a female nurse (b) on her fourth and fifth times tests, a male nurse (c) on his fifth time tests and a female nurse (d) on her fourth time test. The four nurses were diagnosed by detecting the SARS-CoV-2 virus on NPS samples (hospital internal flow). Saliva collection was not performed at the time of diagnosis, as they were in quarantine, as required by the hospital's internal flow. No dentist had positive serology (Figure 2c).

CASE REPORT

It is the STMO-CHC/UFPR's protocol to hospitalize post-HSCT patients with high fever to monitor and identify the origin of the symptom. In the COVID-19 pandemic scenario, the hospital's internal flow required that before admission, patients should undergo an RT-PCR test for SARS-CoV-2. Since the beginning of the pandemic, the service has divided the inpatient sector into three wards: ward A (patients negative for COVID-19), ward B (patients suspected or awaiting test results for COVID-19), and ward C (patients diagnosed with COVID-19). Patients

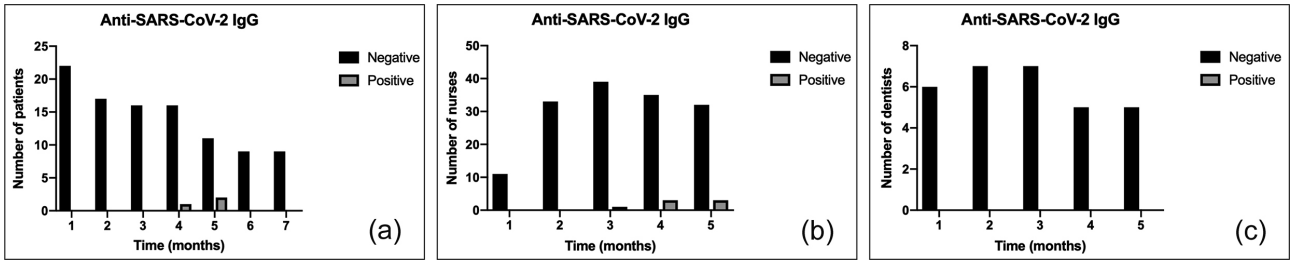

Figure 2 - Distribution of positive and negative results in the detection of anti-SARS-CoV-2 IgG antibodies in serum at follow-up times of patients (a), nurses (b), and dentists (c).

were discharged from hospital and referred for weekly outpatient follow-up with molecular and serological tests. Patients were instructed to perform to home isolation while the RT-PCR test results were positive.

Patient A

A 21-year-old male patient with non-Hodgkin's lymphoma as the underlying disease, day +90 post-allo-HSCT and manifestation of GVHD on skin and liver, in use of cyclosporine A, attended the STMO-CHC/UFPR service with a fever higher than 37.8 ºC. The patient was admitted to ward B, and the next day, with a positive result for COVID-19, he was transferred to ward C. The patient had no other symptoms besides fever, was hospitalized for 15 days and after his clinical improvement, was transferred to a weekly outpatient follow-up.

Twenty-one days after the diagnosis of COVID-19, saliva was collected (for the second time) for this study, concurrently with the collection of NPS following the hospital protocol. On that day, the patient had lesions in the oral mucosa (hard palate and floor of mouth) as shown in Figure 3. The hard palate lesion (Figure 3a) was a painful ulcer compatible with a viral manifestation of herpes simplex, probably caused by the patient's immunosuppression state. He was using a prophylactic acyclovir dose that was increased to the therapeutic dose. Lesion remission was observed in 10 days. On the other hand, the floor of mouth lesion (Figure 3b) was composed of asymptomatic reddish petechiae. We do not believe that oral mucosal lesions are directly associated with SARS-CoV-2[25]. However, previous studies[26,27] have shown vascular involvement and detection of the SARS-CoV-2 spike protein in inflammatory endothelial cells and keratinocytes, as well as in acinar and ductal cells of the minor salivary glands. The petechiae had complete remission at 15 days of follow-up.

The tests were performed weekly with positive results 7, 14, 21, 28, 35 and 42 days after the diagnosis. Then, 48 days after the first diagnosis, another saliva collection (third time) was performed concurrently with the NPS collection, but the results were negative. On days 0, 7, 14, 21, 28, 35 and 42 serological tests for IgG and IgM were non-reactive, with IgG being also non-reactive on the following collection days of this study: 48 (third time), 78 (fourth time) and 108 (fifth time). The genotyping analysis showed that positive saliva samples were from the wild-type SARS-CoV-2 lineage.

Patient B

A 19-year-old female patient with acute myeloid leukemia as the underlying disease, day +60 post-allo-HSCT, attended the STMO-CHC/UFPR service with a fever higher than 37.8 °C. The patient was admitted to ward B, and the following day, with a positive result for COVID-19, she was transferred to ward C. In addition to the fever, the patient had episodes of dry cough for a few days and was hospitalized for 13 days. After her clinical improvement, she was transferred to a weekly outpatient follow-up. The saliva collection that was performed eight days before (fourth time) the fever symptoms came out positive for the presence of SARS-CoV-2. NPS samples were weekly collected with positive results on days 0, 7, 14, 21, 28 and 35. On day 21 (fifth time), after the symptoms, saliva was collected concomitantly with NPS, with a positive result. The results of the sixth and seventh tests were negative. Serological tests performed on days -8, 0, 7, 14, 21, 28 and 35 were non-reactive for IgG and IgM, being also non-reactive for IgG on days 48 (time 6) and 80 (time 7). The genotyping analysis showed that positive saliva samples were from the wild-typelineage.

DISCUSSION

As a vulnerable group, surveillance for COVID-19 in HSCT recipients, from pre-hospitalization to the onset of signs of immunodepression and also of HCW directly

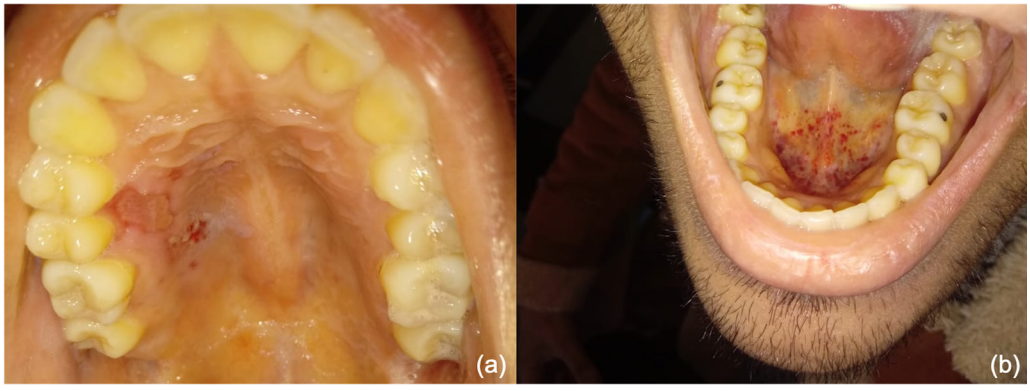

Figure 3 - Oral mucosa lesions of patient A: hard palate (a) and floor of mouth (b).

exposed through the contact with these patients, was essential to understand and control the infection and obtain a response to the protective measures installed as a protocol in the first months of the COVID-19 pandemic in Brazil. Through detection of SARS-CoV-2 in saliva and plasma and serology performed on serum samples, it was possible to assess the persistence of the virus in body fluids, the viral load and the identification of anti-SARS-CoV-2 IgG antibodies. In addition, given the greater distance between appointments after HSCT due to the pandemic, the results of this study showed that protection and surveillance strategies for monitoring patients with immunosuppression, possible symptoms of COVID-19 and other systemic manifestations, mainly GVHD, viral and fungal infections, led to a favorable outcome for the low number of patients and HCW who had positive results for SARS-CoV-2 and positive IgG serology.

We decided to use saliva samples for the molecular detection of SARS-CoV-2 because of its advantages when compared to NPS samples, mainly less discomfort, less aerosoland reduced exposure to HCW, as patients can perform self-collection. Furthermore, several studies have shown that the sensitivity of SARS-CoV-2 detection is similar between saliva and NPS samples[10-14].

Other studies from different countries have also reported surveillance through the molecular detection of SARS-CoV-2 and the seroprevalence of anti-SARS-CoV-2 antibodies in healthcare professionals and patients in general hospitals[28-30] and cancer centers[18,22,31-33]. However, only one pediatric study[23] evaluated the seroprevalence in patients undergoing HSCT. As far as we know, our study is the first to report surveillance for SARS-CoV-2 and also its seroprevalence in a prospective cohort, specifically conducted in adult patients and healthcare professionals from a single HSCT center.

Our positive RT-PCR results corroborate results from other studies that cross-sectionally evaluated HCW, with a prevalence ranging from 2.4% to 11% and a low mortality rate (0.5%)[18,28,30]. Our seroprevalence results in HCW also corroborated the low prevalence found in other studies, which ranged from 0.43% to 24.4%[18,28,30,32,33].

The results of RT-PCR and seroprevalence of patients also corroborated other studies conducted in cancer patients with hematological malignancies, although they did not specify if patients underwent HSCT or not, except for Jimenez-Kurlander *et al.*[22] who reported a low prevalence ranging between 0.7% and 7.8%[18,22,32]. Other studies showed high mortality[21] rates and poor overall survival[20] in allo-HSCT recipients with COVID-19.

These low prevalence results can also be justified by the fact that HSCT centers, regardless of the pandemic scenario, have always had strict biosafety and isolation protocols, with a trained and qualified multidisciplinary team, due to the increased risk of contamination and opportunistic infections in these immunocompromised patients[2-6].

Our findings regarding the detection of SARS-Cov-2 viremia in plasma samples of immunocompromised individuals (patients A and B) also corroborated the findings from other studies, which showed a low prevalence of SARS-Cov-2 viremia ranging from 5.6% to 19.5% in infected patients[34-36], however, representing a minority when compared to saliva and NPS findings, but these results may not represent the acute phase of infections.

Prolonged persistence of viral load in patients in general (not necessarily immunocompromised ones) has also been reported in some studies[37,38], in which prolonged persistence ranged from more than 40 days to more than 90 days after the first diagnosis. Other studies have shown coinfections with different variants in the prolonged persistence of viral load in immunocompromised patients[39], in addition to being potential hosts for the emergence of new mutations. In our study, the genotyping analysis showed that positive saliva samples were from the wild-type lineage. Furthermore, as with patients A and B in our study, many patients did not seroconvert during or after the presence of the virus was evidenced, indicating that the prolonged persistence of SARS-CoV-2 can compromise the immune response of individuals, or the immunosuppressive status of some allo-HSCT receptors may prevent a sufficient immune response against SARS-CoV-2[40].

There are important limitations in our study. Post-HSCT outpatient appointments were postponed and took place more widely. Telemedicine consultations were also used to avoid displacements and reduce the risk of infection for patients who lived further away from the HSCT center. Thus, we did not have information about symptoms or positive patients who underwent exams at care facilities close to their residence. Due to this, it was not possible to carry out a monthly collection on some patients during this 7-month follow-up period. In addition, collections for HCW were conducted on fixed days, and for workers who were on vacation or leave, or for some other reason, did not show up on the day of collection at the HSCT center, collections were not carried out. It should be noted that our results represent a specific single HSCT center study in South Brazil and that the number of cases related to the time and pandemic scenario in the region may have influenced our results.

CONCLUSION

To conclude, our results reflect a low prevalence of positive RT-PCR and seroprevalence for SARS-CoV-2 IgG

antibodies in patients and HCW from a single HSCT center. These results also reflect the rigorous protocols followed by transplant centers, which were even more strengthened during the pandemic. Future studies in different regions are encouraged so that they can provide answers to the reality of different HSCT centers and thus help in the management and control of hospital infection and outcomes of COVID-19 in this population.

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CONFLICT OF INTERESTS

None to declare.

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