Dynamics of the Immune Response in Hospitalized SARS-CoV-2-infected Cancer Patients

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ABSTRACT

Seroconversion rates were compared between oncological and nononcological patients infected with SARS-CoV-2 during a 14-day hospitalization time. All COVID-19 non-oncological and solid malignancies patients reached 100% seroconversion at day 14, while less than half of the hematological patients were seroconverted at the same time point. Despite the limited number and variability of the patient's cohort, we conclude that there is a delayed seroconversion in the hematological malignancies group, which may be linked to changes in the hematological parameters, immune suppression and/or oncological treatments that are typically associated with these patients.

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Introduction

The knowledge about SARS-COV-2 has progressively and rapidly increased since the emergence of the COVID-19 pandemic, with the development and production of diagnostic protocols and vaccines. In this context, FDA approved Pfizer-BioNTech, Moderna and Janssen SARS-CoV-2 vaccines for emergency use based on scientific reports (1–3). Meanwhile, Brazilian health regulation agency ANVISA approved Pfizer-BioNTech, AstraZeneca and Sinovac for local and emergency use (4–6).

The COVID-19 pandemic resulted in delays in cancer diagnosis and surgeries. People with cancer experienced reduction in routine activity of cancer services and of the number of cancer surgeries; there were delays in radiotherapy and the need to reschedule or cancel outpatient visits (7). Overall, in developing countries, cancer patients encounter more serious problems during an outbreak, due to limited resources and lack of prioritization of cancer patients in these countries (8). Despite this observation, it was demonstrated that COVID-19 pandemic also had a significant impact on surgical oncology in Europe (9).

The worldwide demand for hospital services, drugs and vaccines evidenced the need to define priority groups, which should be enrolled for special care. In general, cancer patients are a high-risk group for infections. Indeed, some COVID-19 clinical studies have already indicated poorer outcomes for these patients, especially for those with hematological cancers. The SOAP study (Sars-CoV-2 fOr cAncer Patients) provided evidence of similar seroconversion rates in solid cancer patients, as compared to non-oncological COVID-19 cases. In contrast, the seroconversion was heterogeneous in hematological cancer patients, with a high rate of failure to mount an antibody response against SARS-CoV-2 (10). Cancer patients diagnosed with COVID-19 were reported to be at higher risk of mortality, with nine times increased risk of death when compared to nononcological patients. This increased risk was independent of the demographic data, comorbidities, and treatments. Cancer patients may also present higher false-negative rates of
PCR results for SARS-COV-2 and atypical symptoms, leading to a delay in COVID-19 diagnosis (11). A few studies have reported the lower prevalence of IgG antibodies to SARS-CoV-2 in cancer patients as compared to patients without cancer (12,13). The safety and efficacy of the COVID-19 vaccines was described recently, with variability in the seroconversion rates (14,15).

In this report, we described a temporal analysis of seroconversion during 14 days of hospitalization due to COVID-19 of solid and hematological cancer patients, compared to nononcological patients in the same period. Slower rates of seroconversion were observed in solid and hematological malignancies patients when compared to non-oncological ones. Despite this delay, all patients in the solid malignancies group showed seroconversion at day 14, while less than half of the onco-hematological patients reached seroconversion at the same time point. The results described here are important to reinforce previous studies, given the high heterogeneity commonly found in studies about the impact of SARS-CoV-2 on cancer patients, especially regarding their serological response.

Methods

Samples and ethical statement

This manuscript is part of a prospective study, but the results presented here were retrospectively analyzed from data obtained from May to December 2020, hence before the start of vaccination in Brazil. The patients included were admitted to the Erasto Gaertner Hospital with suspected SARS-COV-2 infection. All patients signed a written consent form, and the study was approved by the Local Ethics Committee (CAAE 31592620.4.3001.5248 and 31592620.4.1001.0098). Sample collection and experimental procedures were carried out in accordance with relevant guidelines and Brazilian regulations. Erasto Gaertner Hospital assists patients as part of the Brazilian Unified Health System (referred to as SUS, in Portuguese) in the city of Curitiba, state of Paraná, South Brazil. By the end of 2020, the year in which this study was conducted, the total number of SARS-COV-2 cases in Brazil was 7,714,819, while the number of deaths from COVID-19 was 195,742. The geographic regions of Brazil were differentially affected by COVID-19 along the year of 2020. South and Southeast regions showed a peak in the number of COVID-19 in November and December of 2020, while North and Northeast regions of Brazil had a peak in the number of cases between June and July (16).

From the 466 total number of patients that were initially included for COVID-19 investigation, 260 were excluded because they were negative SARS-CoV-2 infection according to RT-PCR results (17). From the remaining 206 SARS-CoV-2-positive patients, we excluded those that were under treatment for bone marrow transplantation and with less than two plasma samples collected within the 14 days of hospitalization. These exclusion criteria resulted in the final number of 92 patients that were included in this report. All the selected patients were classified as moderate (medium risk) or severe (high risk), according to COVID-GRAM risk critical illness (18). Patients were divided into three groups according to the oncological status, nononcological (n = 31) solid (n = 35) and hematological malignancies group (n = 26). On the first day of hospitalization, two nasal and one oral rayon-swabs were collected from the patients and used for RNA isolation with QIAmp Viral RNA Mini Kit (Qiagen), as described by the manufacturer. Peripheral blood was collected in EDTA tubes on the first day of hospitalization, and then subsequently collected at days three, five, seven, 10 and 14. The plasma was separated from the whole blood and used for the serological assays, as described in the next sections.

SARS-CoV-2 molecular detection: Real-time PCR

RNA collected from patients in day 1 of hospitalization was used for the detection of SARS-CoV-2 using RT-qPCR protocol (17) as a gold-standard. Primers and probes for SARS-CoV-2 E-gene (FAM) and human RNase P (HEX) were acquired from Integrated DNA Technologies (IDT, United States). Reactions were performed in duplicates with SuperScript™ III Platinum™ One-Step qRT-PCR Kit.
(ThermoFisher, USA) and 5 μL of RNA at 50°C for 30 minutes, 95°C for 5 minutes, 45 cycles of 95°C for 15 seconds and 58°C for 30 seconds in a LightCycler96 platform (Roche, Germany). Positive control was RNA isolated from supernatants of in vitro cultured Vero cells infected with SARS-CoV-2. Samples with a Ct value lower than 35 for the E-gene were considered SARS-CoV-2 positive, but only for those samples showing positive amplification of the internal control (RNase P) at a maximum Ct of 35.

Serological method

The levels of IgG reactive to SARS-CoV-2 Nucleocapsid protein were determined using an ultrafast magnetic bead immunoassay, described previously by our group (19). Briefly, t²⁺ magnetic particles (Promega) were coated with purified SARS-CoV-2 N terminal 6x His tagged Nucleocapsid protein. The beads were blocked in TBST buffer containing 1% skimmed milk for 1 minute and incubated with plasma samples for 2 min in TBST buffer containing 1% skimmed milk under constant agitation. The beads were then washed twice with TBST for 30 seconds and incubated for 2 minutes with goat anti human IgG – HPR (Thermo Fisher) at 1/1,500 dilution in TBST. Beads were washed again twice for 30 seconds in TBST and incubated for 5 minutes in HPR chromogenic substrate TMB (Life Technologies). The beads were removed and the OD650nm measured using a tecan infinity microplate reader. Raw data was converted to percentage (%) of the signal of a calibrant. Samples with signal ≥11% were considered seroconverted.

Receiver operating characteristic (ROC) analysis revealed an area under curve (AUC) of 0.996. A sensitivity of 97% could be achieved at a cost of 99.5% specificity, with high intra assay/inter assay reproducibility (20).

Flow cytometry

Fresh peripheral blood samples were incubated for membrane staining with monoclonal antibodies for 15 minutes, protected from light, at room temperature. The BD Multitest™️ (CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC) was used for the staining. After that, red blood cells were lysed with FACS Lysing solution (Becton–Dickinson Biosciences™️, San Jose, CA, USA), centrifuged and the pellets were washed with PBS/0.2% albumin. A minimum of 50,000 events was acquired using BD FACSDiva (Becton–Dickinson Biosciences) in a FACSCanto II BD equipment. Data were analyzed with Infinicyt software (Cytognos SL, Salamanca, Spain). For flow cytometry, we compared nonseroconverted (NSC) and seroconverted (SC) patients from the groups. Non-oncological (NSC= 9; SC= 26); solid malignancies (NSC= 20; SC= 17) and hematological malignancies (NSC = 18; SC = 4).

Statistical analyses

Statistical data were analyzed with GraphPad Prism, using a Chi-Square test for gender distribution, seroconversion, and death rate. For age, seroconversion days and clinical data, t-tests were used (One-way ANOVA with Tukey multiple comparisons test). A p-value < 0.05 was considered significant. As the number of days of hospitalization differed among patients, some of data may be censored. The serological test was converted to percentage (%) of the signal of a calibrant and samples, where signals ≥11% were considered seroconverted for SARS-COV-2. Death/discharge were used to define the clinical outcome, while serological outcome was the day of seroconversion (first day with ≥11% IgG for SARS-COV-2). multiple regression analysis was performed to assess the strength of the relationship between an outcome (day of seroconversion) and several predictor variables as well as the importance of each of the predictors to the relationship.

Results

Demographic and epidemiological data

Table 1 shows the different groups of patients, their gender, age, systemic treatment, and the number of plasma consecutive samples used for SARS-COV-2 seroconversion analyses. There was no significant difference in gender, while the mean age was significantly higher in the solid malignancies group as compared to non-
oncological group. Table 2 shows the patients by cancer type, distinguishing solid and hematological malignancies.

**Seroconversion**

Table 3 shows the percentage of SARS-COV-2 IgG seroconverted patients for each group on the day of outcome. Chi-Square test showed a significantly lower percentage of seroconverted patients for both solid and hematological malignancies groups when compared to the reference non-oncological group. The average time from symptom onset to seroconversion (sum of DASO until the first positive SARS-COV-2 IgG test) and death rates was not significantly different between the groups, according to Chi-Square test. Figure 1 shows the dynamics of SARS-COV-2 IgG seroconversion in the three groups of samples for each day over the 14-days of hospitalization (days 1, 3, 5, 7, 10 and 14). Less than half of the oncologic/hematological patients achieved seroconversion at day 14, while nononcological and solid patients reached 100% of seroconversion by day 14. The comparison of oncological and nononcological (reference) was significantly different only between hematological patients and nononcological group, on days D1 (p = 0.046), D7 (p = 0.015) and D14 (p = 0.034), according to ANOVA test. It is important to notice that the number of patients varied for each day analyzed, since some of them were discharged or deceased before the end point (day 14) of the analysis. The table

| Table 1. Number of patients, age and number of sequential samples analyzed in each group of patients included in the study. |
|---------------------------------------------------------------|
| **Non-oncological** | **Solid Malignancies** | **Hematological Malignancies** |
| Patients number | 31 | 35 | 26 |
| Systemic chemotherapy | – | 10 | 17 |
| Hormone therapy | – | 1 | 0 |
| Plasma samples | 105 | 120 | 108 |
| Male (%) | 0.613 | 0.471 | 0.5 |
| Female (%) | 0.387 | 0.529 | 0.5 |
| Age (Mean ± SD) | 54.42 (14.9) | 63.53 (11.3) | 57.7 (14.04) |

*p < 0.05 for the comparison between solid malignancies and non-oncological patients, according to One-way ANOVA test, followed by a Tukey multiple comparisons test.

| Table 2. Patients grouped by cancer type, divided into solid and hematological malignancies. |
|---------------------------------------------------------------|
| **Solid malignancies** | **Hematological malignancies** |
| Type | Samples | Type | Samples |
| Breast | 6 | CLL | 4 |
| Prostate | 5 | ALL | 1 |
| Gastric/digestive | 13 | CML | 2 |
| NCS | 1 | AML | 3 |
| Lung | 1 | MM | 4 |
| Excretory system | 4 | Lymphomas | 9 |
| Skin | 1 | MDS | 2 |
| Liver | 1 | ET | 1 |
| Liposarcoma | 1 | | |
| Female reproductive | 2 | | |

CLL, Chronic Lymphocytic Leukemia; ALL, Acute Lymphocytic Leukemia; CML, chronic myeloid leukemia; AML, Acute Myeloid Leukemia; MM, multiple myeloma; MDS, myelodysplastic syndrome, ET, essential thrombocytosis.

| Table 3. Seroconversion rate data and death rates in non-cancer, solid malignancies and hematological malignancies SARS-COV-2 infected patients. |
|---------------------------------------------------------------|
| **Non-cancer** | **Solid Malignancies** | **Hematological Malignancies** |
| Seroconversion on outcome | 100% | 65.7%* | 42.3%* |
| Days from symptom onset to seroconversion (Mean ± SD) | 12.5 ± 4.78 | 10.46 ± 5.95 | 15.36 ± 7.63 |
| Death rate | 32.3% | 51.4% | 53.8% |

*p < 0.0001, Chi-square test, solid malignancies versus non-cancer group, hematological malignancies versus non-cancer group.

Figure 1. Dynamics of SARS-COV-2 IgG seroconversion in the three groups of samples for each day over the 14 days of hospitalization (days 1, 3, 5, 7, 10 and 14). ANOVA analysis assessing days D1, D7 and D14 was performed, with nononcological group as reference to compare solid and hematological neoplasia.
below Figure 1 shows the patient numbers for each of the 14 days of hospitalization.

Table 4 depicts the numbers and percentages of seroconverted and nonseroconverted for each group of patients, according to the day of hospitalization. In this table it is observed that non-oncological samples reached 100% of SARS-CoV-2 IgG seroconversion by day 10, while solid malignancies reached 75% and hematological malignancies only 30.77%. At the endpoint (day 14), non-oncological and solid malignancies were 100% seroconverted, while hematological malignancies group remained with only 40% of seroconversion. Figure 2 shows the overall seroconversion along the days in the three groups of patients. A Chi-Square test showed a significant difference (p < 0.0001) between solid and hematological malignancies group and non-cancer group. Yes, seroconverted samples. No, non-seroconverted patients. Number of patients (n) and percentage of patients (%).

![Figure 2. Seroconversion rates for the groups of COVID-19 positive patients, subdivided in seroconverted and non-seroconverted samples. Chi-Square test showed a significant difference (p < 0.0001) in the comparison between solid and hematological malignancies to the reference (non-oncological group). SC, seroconverted; NSC, nonseroconverted.](image)

The seroconverted samples, while the number of lymphocytes did not significantly change (Figure 3(B)). In the hematological malignancies group, there was a significant difference only in neutrophils, which were increased in seroconverted samples (p = 0.016). However, it is important to observe that there was an enormous variation in the cell counts for the hematological malignancies group, which included lymphocytic and myeloid neoplasias.

Figure 4 shows the comparison of hematological parameters between groups of patients, considering only the samples obtained after seroconversion. Lymphocyte’s count was significantly lower (p = 0.0014) in seroconverted samples from solid malignancies as compared to those from nononcological patients. The number of lymphocytes was significantly higher (p = 0.015) in hematological malignancies as compared to non-oncological patients, but these numbers were extremely variable, with lymphocytes percentages
ranging from as low as 2% to as high as 100% in the hematological samples.

To further investigate lymphocyte’s role in SARS-CoV-2 seroconversion we performed flow cytometry for CD3, CD4 and CD8 in non-seroconverted and seroconverted samples of the three groups (Table 5). No difference was seen within the groups. Again, despite a tendency to lower CD4/CD8 ratio in oncological patients, no difference was observed when we compared seroconverted samples among three groups.

**Discussion**

Previous or current history of cancer is considered an important parameter to establish degrees of severity in COVID-19, but few studies have been published about the impact of cancer in the effective seroconversion rates in COVID-19. Seroconversion may have an impact not only in disease recovery but also to guarantee immunity to the affected individuals. Cancer patients often receive chemotherapy and immunosuppressive treatments, which may impact their immune response and increase the severity of COVID-19. In this report, the production of SARS-CoV-2 IgG antibodies and the temporal seroconversion rates during a 14-day hospitalization time are described for SARS-COV-2 positive individuals, divided in three groups, non-oncological, solid, and hematological malignancies patients.

Despite the undeniable heterogeneity of our cohort, which is a weakness of our study and should be considered to interpret all the presented results, the most frequent type of solid cancer were the digestive ones, while lymphomas were the most frequent type of hematological malignancy. The heterogeneity is a recurrent and common feature in this kind of study, as reported in a systematic review and meta-analysis of cohort studies, which have shown that hospitalized COVID-19 cancer patients were highly heterogenous even after subdividing hematological patients and those receiving active therapy (21).

The seroconversion rate in non-oncological and solid malignancies groups of patients was very high, corroborating recent reports (13). Significantly lower SARS-COV-2 IgG seroconversion rates were observed in hematological malignancies group at the end of the 14 days of hospitalization, indicating a slower seroconversion process in this group. This slower time to
build an immunological response might be related to the disease or to the treatment, as both of which can influence the immune response. This finding agrees with Abdul-Jawad and colleagues (10). Despite this, another study (22) found no differences between solid and hematological cases. The dynamics of seroconversion was assessed by temporal and sequential IgG serological tests during the 14 days of hospitalization, showed a slower rate of seroconversion in solid malignancies group as compared to non-oncological patients. Despite this, at the endpoint, on day 14 of hospitalization, both groups had 100% of seroconverted patients, so solid malignancies patients’ seroconversion was equivalent to non-oncological patients at this time point, which then agrees with Abdul-Jawad et al (10), who described similar seroconversion rates in solid cancer and non-oncological patients. Onco-hematological neoplasia group had significantly lower rates of seroconversion as compared to non-oncological group at days D1, D7 and D14. The lower seroconversion of onco-hematological patients is in accordance with Abdul Jawad (10).

Figure 4. Hematological parameters: comparison between different groups of seroconverted patients (SARS-Cov-2 IgG positive). White blood cells (WBC), neutrophil counts, lymphocyte counts and neutrophil-to-lymphocyte ratio (NLR).

Table 5. CD3, CD4, CD8 and CD4/CD8 ratios.

| Group            | Before Seroconversion | After Seroconversion |
|------------------|-----------------------|----------------------|
|                  | CD3 | CD4 | CD8 | CD4/CD8 | CD3 | CD4 | CD8 | CD4/CD8 |
| Non-oncological  | 626.1 (±386.8) | 379.9 (±231.9) | 217.5 (±259.7) | 2.72 (±1.5) | 683.8 (±375.2) | 412.8 (±247.6) | 237.2 (±169.9) | 2.20 (±1.4) |
| Solid            | 572.1 (±478.2) | 285.7 (±227.4) | 242.1 (±233.6) | 1.33 (±0.7) | 751.9 (±699.1) | 422.1 (±381.8) | 280.5 (±251.2) | 1.76 (±0.8) |
| Hematological    | 801.3 (±1153.1) | 409.4 (±693.9) | 277.5 (±289.9) | 1.42 (±1.0) | 632.7 (±147.7) | 316.2 (±172.6) | 291.3 (±41.2) | 1.14 (±0.7) |

The number of samples analyzed in each group was: nononcological, n = 9 before seroconversion and n = 26 after seroconversion; solid malignancies, n = 20 before seroconversion and n = 17 after seroconversion; hematological malignancies, n = 18 before seroconversion and n = 4 after seroconversion.
A recent meta-analysis reported the seroconversion rates following COVID-19 vaccination in cancer patients and concluded that seroconversion rates in patients with hematologic malignancies are lower than in patients with solid tumors (23).

COVID-19 severity correlates with neutrophil-to-lymphocyte ratio (NLR), a laboratory parameter of immune-cell composition that acts as a surrogate marker of systemic inflammation. The NLR is a poor prognostic factor in COVID-19 and positively correlates with advanced age and obesity, especially in the context of metabolic syndromes and type 2 diabetes. The immunological condition of individuals with increased NLR may result in less productive antiviral immune responses or exacerbated responses upon infection, which lead to hyperinflammation and acute respiratory distress syndrome, characteristic of severe COVID-19 (24). In the present study, the average NLR did not significantly change between seroconverted and non-seroconverted patients from all groups of patients. Despite this, lymphocyte count was significantly increased after seroconversion in non-oncological patients, which was not observed in solid and hematological patients. Solid malignancies patients showed significantly higher average WBC and neutrophil counts in seroconverted samples, while hematological patients showed only higher neutrophils counts in seroconverted samples. The lower and delayed SARS-CoV-2 seroconversion rates in cancer patients may be at least partly associated with the decreased lymphocyte numbers observed here but can also reflect functional deficiencies of these cells in these patients, caused either by the disease itself or by the oncological treatments.

Limitations

The heterogeneity of our cohort, the small number of patients in each group, the short course of seroconversion analysis can be listed as some of the limitations of this study. The different admission criteria and the distinct severity grades in the moment of hospitalization, as well as the small number of subjects in each group are additional limitations, that should be considered to interpret the results.

Conclusions

This study shows that seroconversion was similar in non-cancer and solid malignancies SARS-CoV-2 patients at day 14 of hospitalization, while hematological malignancies group showed a delayed time and decreased rates of seroconversion at the same timepoint. These results led us to hypothesize that this delay could be related with the pathological features of the immune system of patients with hematological malignancies or by the oncological therapies to which they are submitted, although we could not address these data. Nevertheless, our results may indicate that individuals with hematological malignancies could represent a higher-risk cohort for SARS-CoV-2 infection. This manuscript demonstrate strength in correlate SARS-CoV-2 seroconversion in non-oncological and oncological patients, especially due to temporal analysis. But the clear weakness is the great heterogeneity in the patient’s cohort, mainly in the hematological malignancies’ ones. However, this weakness is a widely common and hard to overcome limitation in similar studies. Hence, the results presented here are important to increase the evidence of a higher risk and a limited response of hematological patients to COVID-19.

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Ethical approval

The project was approved by the Local Ethics Committee of Erasto Gaertner Hospital (Curitiba - Brazil) (CAAE 31592620.4.3001.5248 and 31592620.4.1001.0098). All the enrolled patients or the patient’s legal guardian or representative signed a consent form.
Author contributions

MNA and DLZ performed molecular biology procedures, compiled the results, and wrote the manuscript. LB and LFH worked on funding acquisition. LFH and MSC developed and performed the serological tests and revised the manuscript. JMN, MEAM, BF and HMPM were responsible for the enrollment of patients and signature of the consent form. JMN and MEAM also collected and revised clinical and laboratory data. DLZ revised the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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