



# Persistent Antibody Responses to SARS-CoV-2 Infection in Cancer Patients: A Single-Center Retrospective Observational Study

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## Abstract

**Introduction** There is limited literature available regarding the prevalence and durability of immune response to infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)/coronavirus disease 2019 (COVID-19) in cancer patients.

**Objective** The aim of this study was to analyze the seroconversion rate in cancer patients recovered from SARS-CoV-2 infection.

**Materials and Methods** We retrospectively analyzed antibody levels and seroconversion rates in serum samples from 135 cancer patients who had recovered from SARS-CoV-2 infection. Chemiluminescent immunoassay using Roche Cobas e801 analyzer (Roche Diagnostics, Rotkreuz, Switzerland) was performed to identify Pan Ig antibody against nucleocapsid antigen. Reports of first, third, and sixth month were analyzed. Seroconversion was also compared with health-care workers (HCW) of our institute who had recovered from COVID-19 infection.

**Results** Seroconversion rate in cancer patients was 81.2% at 1 month, 95% at 3 months, and 94.6% at 6 months post reverse transcriptase–polymerase chain reaction positivity. There was no difference in seroconversion rate among different age groups, gender, comorbidities, severity of COVID-19 symptoms, cancer disease status, and treatment with chemotherapy. Seroconversion rate in cancer patients is comparable to HCW (90.4 vs. 96%,  $p = 0.82$ ) and is durable.

**Conclusion** Humoral response to COVID-19 infection in cancer patients is comparable to general population and sustained. Such responses suggest that cancer patients are likely to benefit from COVID-19 vaccination.

## Keywords

- ▶ COVID-19
- ▶ SARS-CoV-2
- ▶ anti-SARS-CoV-2-antibody
- ▶ cancer
- ▶ seroconversion
- ▶ chemotherapy
- ▶ India

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## Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causing coronavirus disease 2019 (COVID-19), emerged in December 2019 and has spread around the world causing several cases and deaths.<sup>1</sup> Patients suffering from cancer are thought to be at a higher risk of developing a severe course of COVID-19 and have higher mortality risk as their immune system is generally compromised.<sup>2,3</sup> Due to this reason, adjuvant chemotherapies, surgeries, and other therapies are either postponed or altered during the current pandemic.

According to literature, seroconversion of most patients with COVID-19 occurs between 7 and 14 days after diagnosis.<sup>4-6</sup> Earlier studies suggested that 40 to 60% of those infected become antibody-negative early in the convalescence period,<sup>7-10</sup> while later studies reported sustained levels for at least 4 months<sup>7,9,11,12</sup> (**Supplementary Table S1**).

There is a paucity of data regarding SARS-CoV-2 seroconversion in cancer patients.<sup>13,14</sup> (**Supplementary Table S1**) To the best of our knowledge, there is no data in literature that has studied longitudinal seroconversion of SARS-CoV-2 antibodies in this subset of patients. It is also not known if these antibodies have long-term persistence and whether they provide protective immunity against reinfection in cancer patients.

Response to pneumococcal and influenza vaccination has shown good response in patients on chemotherapy.<sup>15,16</sup> However, COVID-19 vaccine trials have excluded cancer/immunocompromised patients.<sup>17-19</sup> Hence, there is no data on the safety and efficacy of COVID-19 vaccine in cancer patients.

The aim of this study was to assess SARS-CoV-2 seroconversion in the cancer patients and longitudinal changes of antibody levels in first 6 months after SARS-CoV-2 infection. We also assessed its correlation with sex, age, anticancer treatment, and severity of SARS-CoV-2 infection. Comparison was done with seroconversion in normal population comprising of 100 recovered frontline health-care workers (HCW) with confirmed COVID-19 infection.

## Materials and Methods

### Study Design

This study was a retrospective analysis evaluating serial estimation of anti-SARS-CoV2-antibody in cancer patients who recovered from SARS-CoV2 infection.

### Study Setting

Study was performed at a tertiary care hospital, The study period was from April 30 to December 25, 2020.

### Data Collection

All the demographic data, clinical information, laboratory parameters, and complications during the hospital stay were retrieved from hospital medical records. Cases of acute

leukemia were not included in the study. All the data was analyzed at the cutoff date of December 25, 2020.

### Inclusion Criteria

All cancer patients who tested positive for SARS-CoV-2 by reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) on nasal/throat swab and were tested at least once for SARS-CoV2-antibodies were enrolled in this retrospective analysis.

### Exclusion Criteria

1. All leukemia patients.
2. All cancer patients below age 18 years.

### Primary Outcome

Seroconversion rate in cancer patients recovered from SARS-CoV-2 infection at first, third, and sixth month.

### Secondary Outcome

Comparison of seroconversion rate among cancer patients and healthy HCWs (internal control).

### Diagnostic Criteria

SARS-CoV-2 infection: The severity of the disease was defined according to the World Health Organization (WHO) definition.<sup>20</sup>

### Detection of SARS-CoV-2 RNA by RT-PCR

Presence of SARS-CoV-2 on nasopharyngeal/throat swab specimens was determined by real-time RT-PCR. Allplex 2019 n-CoV Assay (Seegene Inc., Seoul, South Korea) was used to detect SARS-CoV-2 by amplification of RdRp gene, E gene, and N gene according to the Indian Council of Medical Research (ICMR) recommendations.<sup>21</sup>

### Detection of SARS-CoV-2 Antibodies

We tested our patient samples using Roche Elecsys anti-SARS-CoV-2 immunoassay (Roche Diagnostics, Rotkreuz, Switzerland) on a Cobas e801 analyzer. Elecsys anti-SARS-CoV-2 electrochemiluminescence immunoassay detects antibodies (including immunoglobulin M [IgM], immunoglobulin A [IgA], and immunoglobulin G [IgG]) to SARS-CoV-2 in human serum. The immunoassay utilizes a double-antigen sandwich test principle and a recombinant protein representing the nucleocapsid antigen for the determination of antibodies to SARS-CoV-2. Tests were performed according to the manufacturer's instructions, and assay results were interpreted as follows: cutoff index  $\geq 1.0$  was considered as positive/reactive.<sup>22</sup>

In a previous study done at our institute (COVID-19 recovered HCW cohort),<sup>23</sup> we compared the performance of four high-through put commercial chemiluminescence immunoassays [Abbott Architect SARS-CoV-2 IgG assay which detects antinucleocapsid IgG, Roche Elecsys anti-SARS-CoV-2 total assay antinucleocapsid (IgG, IgM & IgG), Ortho-Clinical Diagnostics VITROS anti-SARS-CoV-2 IgG (S1) and Ortho-Clinical Diagnostics VITROS anti-SARS-CoV-2 total antispike

{including IgA, IgM & IgG (S1)} to SARS-CoV-2] most frequently used for the detection of SARS-CoV-2 antibodies worldwide. Comparisons were done in terms of various statistical parameters like sensitivity, specificity, and Cohen's kappa agreement (► Fig. 1).

Results from antinucleocapsid antibody (IgG, IgM, and IgA) on Roche platform (used subsequently for our study) were in complete agreement with IgG (S1) and total antispikes done on Ortho-Clinical Diagnostics platform (Supplementary Tables S2 and S3).

For the purpose of analysis, we divided first month data in three subsets from the day of COVID-19 RT-PCR positivity, that is, less than 15 days, 15 to 22 days, 23 to 30 days, then at 3 and 6 months.

### Sample Size

All consecutive cancer patients fulfilling the inclusion criteria during the study period were included. A total of 135 patients were included in the study.

### Statistical Analysis

To describe patient's characteristic's, demographic, clinical, laboratory investigations, the data were summarized and analyzed using STATA (version 14, StataCorp., College Station, Texas, United States) software. Quantitative data was expressed as median (min, max). Qualitative data was reported as in numbers and percentages. Data was tested for normality using the Kolmogorov–Smirnov test. Student's *t*-test was used to observe the differences between demographic factors, cancer history, clinical findings, laboratory parameters and disease outcome, between patients who developed anti-SARS-CoV2-antibodies after COVID-19 infections versus those who did not. Comparison of baseline and treatment characteristics was performed using Student's *t*-test. To establish the association between patients who developed antibodies versus those who did not, with other qualitative characteristics, chi-squared test/Fisher's exact test was used. A value of *p* less than 0.05 was considered to represent statistical significance of the study.

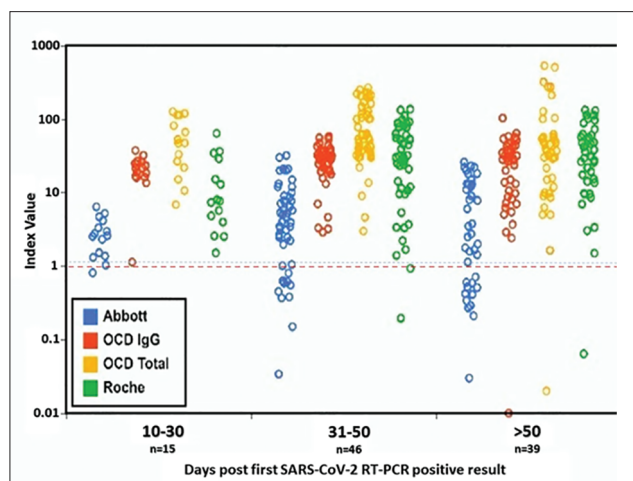


Fig. 1 Comparison of chemiluminescent immunoassay platforms.

### Ethics

The study protocol was reviewed and approved by institute ethics committee (Dr. BL Kapur Memorial Hospital Ethics Committee) on January 7, 2021. The approval letter number is ETHICS COMMITTEE/AARCE/LETTER/JAN/2021/12.

Consent waiver was granted given retrospective nature of analysis confidentiality was maintained by the deidentification of data. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1964, as revised in 2013.

## Results

### Patient Characteristics and Disease Status

A total of 135 patients were enrolled in the study. Their median age was 56 years (range: 24–80 years) and median follow-up was 92 days (range: 12–239).

Details of site of tumor, stage, disease status, and preceding cancer treatment before COVID-19 infection are shown in ►Table 1. Overall, 26 (19%) patients were classified with moderate or severe symptoms, and 41 (30%) patients were asymptomatic. Median duration of symptoms was 2 days (range: 0–10).

### COVID-19 Antibody

Seroconversion rate of overall population was 90.4% (122 of 135). This was 75% at 15 days, 78% at 15 to 22 days, and 84% at 23 to 30 days. It increased to 95% at third month and maintained at 94.6% at sixth month (►Table 2).

Multiple clinical and laboratory covariates were assessed for association with antibody production (age, sex, stage, type of cancer, COVID-19 severity, etc.) and none were found to be correlating with antibody presence. Notably, type, and treatment of cancer and severity of COVID-19 symptoms did not affect production of antibody and its durability (►Table 1).

### Seroconversion in Cancer Patients as Compared with HCWs

A total of 96 of 100 HCWs had antinucleocapsid antibodies positivity. Overall seroconversion rate in cancer patients was 90.4% that was comparable with HCW (96%; *p* = 0.82).

### Loss of Seroconversion

Only three (2.4%) patients became seronegative in subsequent evaluation. All three had borderline positivity for antibody (1.3–2.88 index value) and loss happened at 33, 112, and 127 days, respectively.

## Discussion

Studies evaluating seroconversion in COVID-19 infected individuals have shown results approaching 90%<sup>7-12</sup> (Supplementary Table S1). There are studies showing its subsequent loss,<sup>7-10</sup> whereas a large study from Iceland

**Table 1** Baseline demographics and cancer therapy details

Variable	n(%)	COVID-19 antibody positive patients	COVID-19 antibody negative patients	p-Value
<b>Age</b>				
Median (range)	56 (24–80)			
≤ 60 y	93 (61)	85	8	
> 60 y	42 (39)	37	5	0.376
<b>Sex</b>				
Male	39 (29)	35	4	
Female	96 (71)	87	9	0.55
<b>Type of tumor</b>				
Breast	50 (37)	46	4	
Lung	8 (5.7)	5	3	
Head and neck	5 (3.7)	4	1	
Hepato-Pancreato-Biliary	11 (7.4)	11	0	
Gastrointestinal	20 (15.5)	18	2	
Gastrointestinal	8 (5.7)	8	0	
Genitourinary	14 (10.3)	14	0	
Gynecological	8 (5.7)	8	0	
Others				
<b>Hematological tumors</b>				
Lymphoma	8 (5.7)	5	3	0.03
Multiple myeloma	3 (2.2)	3	0	
<b>Cancer status</b>				
Cured + remission	40 (29.6)	36	4	
Response to active disease or treatment naïve or relapse/refractory disease	95 (70.4)	86	9	0.57
<b>Comorbidity</b>				
None	81 (60)	74	7	
One	29 (21.5)	25	4	
≥ two	25 (18.5)	23	2	0.71
<b>Cancer stage</b>				
I–III	55 (40.7)	49	6	
IV	80 (59.3)	73	7	0.77
<b>Chemotherapy (received) within last 4 weeks (n = 98)</b>				
Cytotoxic	67 (49.6)	60	7	
Targeted	9 (6.7)	9	0	
Hormonal	18 (13.3)	17	1	
Immunotherapy	5 (3.7)	5	0	0.86
<b>Symptoms</b>				
Fever	76 (56.3)	70	6	0.31
Cough	55 (40.7)	50	5	0.55

(continued)

**Table 1** (continued)

Variable	n (%)	COVID-19 antibody positive patients	COVID-19 antibody negative patients	p-Value
Sore throat	46 (34)	43	3	0.29
Myalgia	75 (55.6)	70	5	0.15
Anosmia	34 (25.2)	33	1	0.11
Dysguesia	36 (26.7)	35	1	0.09
<b>Symptoms duration (d)</b>				
Median	2	71	7	
≤ 2 d	78 (57.8)	51	6	0.59
> 2 d	57 (42.2)			
<b>Severity</b>				
Asymptomatic + mild	109 (80.7)	98	11	
Moderate + severe	26 (19.3)	24	2	0.52
<b>Survivors</b>	123 (91)	113	10	
<b>Nonsurvivors</b>	12 (9)	9	3	0.09

Abbreviation: COVID-19, coronavirus disease 2019.

**Table 2** COVID-19 antibody positivity

COVID-19 antibody test time (d)	Positive test/test done (%)
< 1–30	65/80 (81.25)
<15 d	9/12 (75)
15–22	25/32 (78)
23–30	32/38 (84)
61–90	39/41 (95)
151–180	35/37 (94.6)

Abbreviation: COVID-19, coronavirus disease 2019.

showed persistence of antibodies for at least 4 months in ≥90% of recovered patients<sup>11</sup> (**Supplementary Table S1**). Persistence of antibodies has also been documented by other studies.<sup>9,12</sup>

### Anti-COVID-19 Antibody in Cancer Patients

Seroconversion rate in our study population was 81.25% by first month, 95% at third month, and 94% by six month.

There are two published studies addressing positivity of antibody in cancer patients with COVID-19 infection. Solodky et al reported 30% antibody positivity rate (3 out of 10 patients) in cancer patients at day 15 or later after clinical symptoms as compared with 71% in noncancer patients (30 vs. 71%,  $p = 0.04$ ).<sup>13</sup> However, this was a small study with only 10 cancer patients. A study from Wuhan showed anti-COVID-19 antibody prevalence of 72.5% in 40 cancer patients (**Supplementary Table S1**).<sup>14</sup> In this study, the antibodies were tested at 21 days from symptom onset, whereas our study calculated time from RT-PCR positivity (often

performed after appearance of symptoms). Our 15 days' data of 75% seroconversion is comparable to this study. Our larger study reinforces early appearance of antibodies in majority of cancer patients.

In our study cohort, overall, 90.4% seropositivity was seen at the median follow-up of 92 days, which was similar to general population reported in previous studies.<sup>10,11</sup> We also compared overall seroconversion rate of cancer patients with HCW at our institute and found it similar (90.4 vs. 96%). Patients suffering from cancer have equivalent humoral responses compared with general population.

In our study, 35 out of 37 patients showed persistence of COVID-19 antibodies at sixth month. No loss of antibodies was observed between third and sixth month. Persistence of antibodies up to 6 months has been shown for the first time in cancer patients.

In the study, 67 patients received chemotherapy within 4 weeks prior to COVID-19 infection. Seroconversion rate among these patients was 90% (at a median of 33 days) that is similar to the overall cohort of cancer patients. We did not observe any poor antibody responses in patients on chemotherapy.

Protective nature of these antibodies has been documented by studies on pseudoneutralization assay<sup>24-26</sup> and clinical data on reinfection rates.<sup>27,28</sup> Though the titer of antibody that can be considered to be protective is not known, maintained antibody levels are reassuring. Low incidence of reinfection worldwide is another indirect evidence of its protective nature. Sustained responses can possibly prevent reinfection in cancer patients thus avoiding treatment delays, morbidity, and mortality. None of our patients had

reinfection till the date of censoring. This is the first such study to report this in patients suffering from cancer.

### Low Levels May Become Negative

Of 135 patients, 10 patients had antibody levels between 1 and 3 index values at time of first test. Of these 10, three patients (30%) lost their antibody levels in subsequent testing. The patients with low index value need serial testing. Such cases may represent false positive or lower immune responses. These are small numbers and more studies are required to assess true incidence and implication of this subset.

### Rituximab

In the cohort, eight patients were of non-Hodgkin lymphoma. Six out of these eight patients had received rituximab in the past. Among these six patients, three patients did not sero-convert and the other three patients had very low titer of antibodies. Rituximab may thus be playing a role in blunted humoral responses.<sup>29</sup> Lower immune responses possibly can increase the risk of reinfection and also can impact response to vaccination.

### Vaccination in Cancer Patients

Pfizer and AstraZeneca trials have excluded patients of cancer/immunosuppressed states from vaccine trial,<sup>17,18</sup> whereas Moderna<sup>19</sup> does not have clear information regarding the same. Hence, we do not know how the cancer patients would develop immune response to the same. Our data so far supports for the vaccination of cancer patients as there is no adverse impact on antibody production in them even on chemotherapy. The same cannot be said regarding patients on rituximab.

Limitations of the study are its retrospective nature and unadjusted demographic data. Reference date for calculation was taken as date of COVID-19 RT-PCR positivity but patients had symptoms before that. So, the date of antibody positivity may not be the actual date from COVID-19 infection. This data is most mature for 3 months as the median follow-up is 92 days and inference on stability of antibody at 6 months is based on a small number of patients (27.4%).

### Future Research Directions

The study is ongoing and we would come up with 9 months follow-up. Longer follow-up will provide the information regarding the durability of COVID-19 antibody response in cancer patients.

### Conclusion

To conclude, cancer patients even on chemotherapy produce sustained and equivalent antibody response to COVID-19 as general population. Their reinfection rates are low. Cancer patients are therefore likely to respond well to COVID-19 vaccine and should be considered as priority group due to known high case fatality. However, antibody response in rituximab treated patients may be suboptimal and needs further assessment.

### Disclaimers

The views expressed in the submitted article are my own and not an official position of the institution or funder.

The manuscript has been read and approved by all the authors, that the requirements for authorship as stated in the instruction manual have been met, and that each author believes that the manuscript represents honest work.

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### Conflict of Interests

No financial and personal conflict of interest.

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