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Research Article

Rare SARS-CoV-2 Antibody Development in Cancer Patients

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ABSTRACT

SARS-CoV-2 antibody development and immunity will be crucial for the further course of the pandemic. Until now, it has been assumed that patients who were infected with SARS-CoV-2 develop antibodies as it is the case with other coronaviruses, like MERS-CoV and SARS-CoV. In the present study, we analyzed the antibody development of 77 oncology patients 26 days after positive RT-qPCR testing for SARS-CoV2. RT-qPCR and anti-SARS-CoV2-antibody methods from BGI (MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit) and Roche (Elecsys Anti-SARS-CoV-2 immunoassay) were used, respectively, according to the manufacturers' specifications.

Surprisingly, antibody development was detected in only 6 of 77 individuals with a confirmed history of COVID-19. Despite of multiple testing, the remaining patients did not show measurable antibody concentrations in subsequent tests. These results undermine the previous hypothesis that SARS-CoV2 infections are regularly associated with antibody development and cast doubt on the provided immunity to COVID-19. Understanding the adaptive and humoral response to SARS-CoV2 will play a key-roll in vaccine development and gaining further knowledge on the pathogenesis.

Keywords: Antibodies; SARS-CoV-2; RT-qPCR; Infection

INTRODUCTION

SARS-CoV2 and its underlying disease, COVID-19, has spread around the world, so far causing over 11 million infections and 528,000 deaths, according to the numbers of WHO. Coronaviruses are a subgroup in a spectrum of viruses that are phenotypically and genotypically diverse and have provoked recent epidemics [1,2]. Coronaviruses are enveloped viruses containing single-stranded positive-sense RNA with a viral genome of about 27-32 kb, which encodes for structural and non-structural proteins [3-5]. The novel SARS-CoV2 consists of four structural proteins, namely: the Spike protein (S), the Envelope protein (E), the Membrane glycoprotein (M) and the Nucleocapsid protein (N) [3,6]. The majority of produced antibodies are formed against the Nucleocapsid, which are therefore considered to be highly sensitive for antibody testing, even though it has to be noted that there is a sequence of homologies which could lower the sensitivity [3,7]. So far, 10 million cases have been registered with positive RT-qPCR result whereas antibody testing has just recently become a factor.

Patients suffering from chronic diseases are generally thought to be at higher risk of developing a severe course of COVID-19, which could lead to intensive care treatment [8] has shown in a recent study, that cancer patients treated in oncological outpatient settings, who tested positively for SARS-CoV2 in RT-qPCR, remained mostly asymptomatic virus carriers without an impact on the applied systemic cancer therapy (submitted manuscript). Nevertheless, measures are made to counter and minimize the risk of SARS-CoV2 infection and severe complications. Due to this reason, adjuvant chemotherapies, surgeries and other compromising therapies were eventually postponed or changed [9].

As the symptoms and course of COVID-19 vary broadly, tests

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by nasopharyngeal or throat swabs were recently also taken from asymptomatic patients to identify virus carriers. It is estimated that over 50% of the cases are asymptomatic [10], and there is also a risk of false negative results because of poor swab techniques or a sparse amount of virus-RNA. However, an antibody test with high sensitivity and specificity could provide epidemiological information on the actual rate of infection. So far, it is unclear whether the majority of SARS-CoV2 infected patients produce a sufficient quantity of antibodies that sustains immunity. Until now, it has been assumed that antibodies are formed after the viral infection, as it is the case with other coronaviruses, namely MERS-CoV and SARS-CoV [11-14]. Numerous studies also describe antibody production after infection with SARS-CoV2 [15,16] were able to detect positive rates of IgG and IgM at a median of 13 days after the onset of symptoms. IgG was detected at a constant level in 100% of the 19 tested patients within 6 days. The authors recommended a simultaneous detection of IgG and IgM at an early stage of infection. Zhao et al. analyzed the samples of 173 patients, detecting the presence of antibodies <40% among patients within 1-week after onset, and showed a rapid increase of up to 94.3% for IgM, and 79.8% for IgG from day-15 after onset [17] described antibody development even earlier, on the 4th day after symptom onset, which showed that antibodies against SARS-CoV2 can be detected in the middle and later stages of infection [18].

Until recently, there was a lack of a widespread availability of valid test kits making antibody testing in routine clinical care challenging. In May 2020, an Elecsys antibody-test was released by Roche Diagnostics to detect anti-SARS-CoV2 immunoglobulins, with the ability to bind the viral nucleocapsid antigen [19]. According to the manufacturer, the sensitivity 14 days after a positive SARS-CoV2 test is up to100% and the specificity 99.91%, respectively. Currently there are no studies available to confirm these numbers. Moreover, studies describing antibody production in oncologic patients after SARS-CoV-2 infection are lacking. The aim of our study was to observe the course of antibody development and analyze the seroprevalence of antibodies against SARS-CoV2 in oncologic patients with a history of COVID-19.

MATERIALS AND METHODS

From 15th April 2020, all patients visiting one of the seven participating outpatient clinics were tested for SARS-CoV2 infection by throat swab and RT-qPCR, regardless of symptoms. A total of 77 oncology patients who were tested positive for SARS-CoV2 by RT-qPCR were enrolled for the analysis of anti-SARS-CoV2-antibodies. Clinical characteristics and demographics of the enrolled patients are shown in Table 1.

The distribution of age of the enrolled patients is shown in Figure 1.

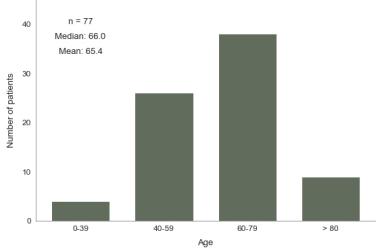

Figure 1: Age distribution of SARS-CoV-2 antibody positive tested patients.

For RNA isolation, the MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit was used on MGI SP-960 instruments. The extracted RNA was analyzed by RT-qPCR using the BGI Real-time fluorescent RT-PCR kit for detecting 2019-nCoV2. RT-qPCR and signal interpretation were performed on Applied Bioscience ABI 7500 Fast machines according to the instruction manual. The target sequences in RT-qPCR were ORF1ab for SARS-CoV2 and human GADPH, which served as an internal reference for effective RNA isolation. Positive and negative controls were included on each plate. The average number of days between a patient's positive RT-qPCR result and the first subsequent negative result was 14 days (SD 7,9).

After the confirmation of the SARS-CoV2 infection, blood was drawn from the patients according to the individual therapy algorithm, within a median time interval of 26 days (SD 13,6) after a positive test result in RT-qPCR. The samples were taken at different intervals, expecting the presence of antibodies at least at day 14 after SARS-CoV2 detection.

For measurements anti-SARS-CoV2 antibodies (IgM and IgG), the Elecsys anti-SARS-CoV2 immunoassay from Roche was used on a Cobas e801 according to the vendor's instructions. The assay targets a recombinant protein representing the Nucleocapsid (N) antigen for the determination of antibodies against SARS-CoV2.

Differences of clinical characteristics of the patients between the two subgroups (positive and negative anti-SARS-CoV2 antibody results) were tested for statistical significance using the Chi-square test. Due to multiple testing, test results were adjusted using the Bonferroni method. Adjusted p-values <0.05 were regarded as statistically significant.

The study was approved by the Ethics Committee of the Bavarian Chamber of Physician (BLÄK) with the ethic committee´s approval No. 20037.

RESULTS

Out of 77 patients with a positive SARS-CoV2 RT-qPCR result enrolled in the study, only 6 patients showed measurable antibodies development for SARS-CoV2 after 14 days or longer, whereas 71 of the tested patients were below the assay's cut-off value, even after multiple testing later in the course.

The first antibody test was performed on average 26 (SD 13,61) days after a positive SARS-CoV2 RT-qPCR result (Figure 2).

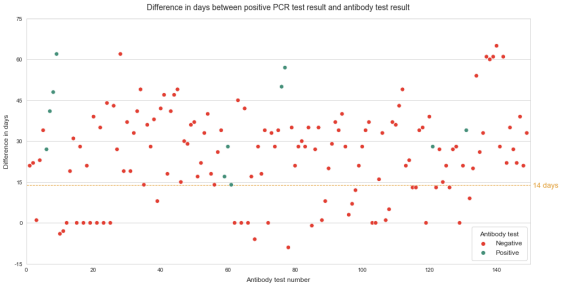

Figure 2: Difference in days between positive RT-qPCR test result and first anitbody test result.

A second measurement was performed in 45 patients after 35 days (median, SD 9,9). 30 patients received a third measurement at day 41 (mean, SD 10,8). 13 patients were tested multiple times (<6). The patients who tested negative for antibodies in the first sample did not show any increase in antibody concentration signal (COI) in subsequent tests. However, in 3 out of 6 patients who tested positive for antibodies, an increase in COI was observed over the

Table 1: Clinical characteristics and demographics of the enrolled patients.

	Antibody positive (n=6)	Antibody negative (n=71)	All patients (n=77)	p value	Adjusted p value
Age in years[1]	68 (± 13)	65 (± 14)	65 (± 14)	0.6141	0.6141
Sex				0.9814	0.9814
Male	2 (8%)	24 (92%)	26		
Female	4 (8%)	47 (92%)	51		
Mortality				1.0000	1.0000
Survived	6 (8%)	71 (92%)	77		
Died	0 (0%)	0 (0%)	0		
Disease type					
Solid cancer	5 (10%)	43 (90%)	48	0.269	0.269
M0	0 (0%)	12 (100%)	12	0.2731	0.8192
M1	1 (7%)	13 (93%)	14	0.9202	1.0000
MX	4 (18%)	18 (82%)	22	0.0315	0.0944
Cancer type					
Lip, oral cavity, and pharynx	0 (0%)	1 (100%)	1	0.7698	1.000
Digestive organs	1 (10%)	9 (90%)	10	0.7801	1.000
Respiratory and intrathoracic organs	0 (0%)	4 (100%)	4	0.5504	1.000
Melanoma (skin)	0 (0%)	2 (100%)	2	0.677	1.000
Breast	1 (8%)	12 (92%)	13	0.9882	1.000
Female genital organs	1 (14%)	6 (86%)	7	0.5014	1.000
Male genital organs	0 (0%)	7 (100%)	7	0.4199	1.000
Urinary tract	2 (50%)	2 (50%)	4	0.0012	0.0122
Thyroid and other endocrine glands	1 (50%)	1 (50%)	2	0.024	0.2405
Hematological/lymphatic malignancies	4 (21%)	15 (79%)	19	0.013	0.1297
Unknown or unspecified site[2]	0 (0%)	2 (100%)	2	–	–
No information	0 (0%)	15 (100%)	15	–	–
Cancer treatment[3]					
Chemotherapy	3 (14%)	18 (86%)	21	0.193	1.0000
Chemoimmunotherapy	0 (0%)	11 (100%)	11	0.2977	1.0000
Antihormone therapy	1 (10%)	9 (90%)	10	0.7801	1.0000
Immunotherapy	1 (25%)	3 (75%)	4	0.1873	1.0000
Bisphosphonate	0 (0%)	2 (100%)	2	0.677	1.0000
TKI	0 (0%)	1 (100%)	1	0.7698	1.0000
Surgery	2 (17%)	10 (83%)	12	0.2119	1.0000
No systemic oncological therapy	0 (0%)	10 (100%)	10	0.3244	1.0000
No information	2 (10%)	19 (90%)	21	–	–
Comorbidities					
Hypertension	2 (11%)	17 (89%)	19	0.6085	1.0000
Diabetes	2 (14%)	12 (86%)	14	0.3163	1.0000
Nicotine abuse	0 (0%)	6 (100%)	6	0.4584	1.0000
Chronic obstructive	1 (17%)	5 (83%)	6	0.3984	1.0000
Pulmonary disease					
Cerebral infarction, stroke	1 (20%)	4 (80%)	5	0.2923	1.0000
Hypercholesterolemia	0 (0%)	5 (100%)	5	0.5014	1.0000
Peripheral artery disease	1 (33%)	2 (67%)	3	0.0923	0.9228
Heart failure	1 (33%)	2 (67%)	3	0.0923	0.9228
Myocardial infarction	1 (50%)	1 (50%)	2	0.024	0.2405
Rheumatoid arthritis	0 (0%)	1 (100%)	1	0.7698	1
No information	2 (5%)	42 (95%)	44	–	–
Comedication					
Glucocorticoid	3 (6%)	48 (94%)	51	0.3812	1.0000
Bisoprolol	0 (0%)	12 (100%)	12	0.2731	1.0000
Ramipril	0 (0%)	12 (100%)	12	0.2731	1.0000
Simvastatin	0 (0%)	10 (100%)	10	0.3244	1.0000
Furosemid	0 (0%)	10 (100%)	10	0.3244	1.0000
Zometa	0 (0%)	8 (100%)	8	0.3851	1.0000
ASS	1 (13%)	7 (87%)	8	0.4199	1.0000
Others	3 (8%)	35 (92%)	38	0.9736	1.0000
No information	2 (20%)	8 (80%)	10	–	–

Mean (± SD); [2]ICD-10-Codes C76.- and C80.-; [3]Treatments in the past 6 months

course of the study (Figure 3).

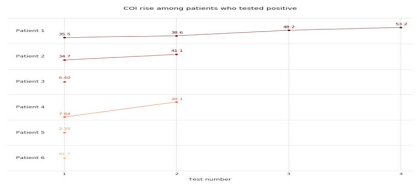

Figure 3: Rise of cut off index among SARS-CoV-2 antibody positive tested patients.

3 patients who developed antibodies showed mild symptoms like shortness of breath and common cold symptoms. These patients had slightly elevated temperature (median 37.5°C). 2 of the 6 patients with positive SARS-CoV2 antibody results experienced severe forms of COVID-19. One of the patients who was tested positive, developed pneumonia (CURB-65 score of 2) and had to be hospitalized, but was not admitted to ICU and did not require assisted ventilation according to a low CURB-65 index. This patient suffered from an active tumor disease and received immunotherapy with Revlimid at the time of testing. Due to the critical medical condition of the patient, tumor therapy had to be aborted. Recently, this patient tested negative on SARS-CoV2 PCR (7 days after first positive RT-qPCR result) with complete remission of pneumonia and has continued immunotherapy. One additional patient suffering from ARDS was hospitalized and had to be treated at ICU using Extra Corporeal Membrane Oxygenation (ECMO). This patient did not receive systemic oncological therapy within the last 6 months. After 5 days of treatment at ICU, using ECMO followed by a two week stay in hospital her condition stabilized.

In the antibody positive group 4 patients suffered from hematological or lymphatic malignancies compared to 15 patients in the antibody negative group (Table 1). The second most common malignancies were tumors of the urinary tract 2/6 in the positive tested group. Within the antibody negative tested patients most solid cancer types were breast tumors 12/71, tumors of digestive organs 9/71 and tumors of male genital organs 7/71 (Table 1).

The most common comorbidities in both the antibody positive tested individuals and the antibody negative tested individuals were hypertension and diabetes. Therapies carried out up to 6 months before the positive RT-qPCR result are taken into account in the evaluation. Glucocorticoids were applied in 3 of the 6 antibody positive patients compared to 48 patients in the group without detectable antibodies. In the antibody negative group 12 patients were treated with Bisoprolol and 12 with Ramipril (Figure 4).

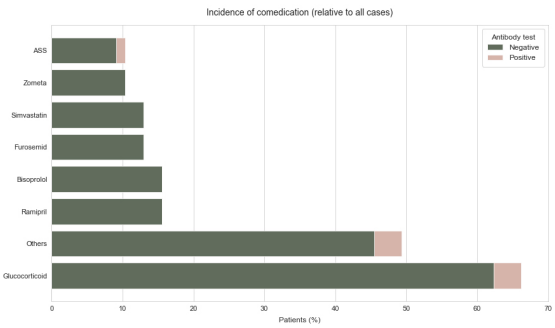

Figure 4: Incidence of comedication of SARS-CoV-2 anitbody positive tested and SARS-CoV-2 negative tested patients relative to all cases.

The most common applied systemic cancer therapies in the antibody positive group were chemotherapy in 3 patients, antihormonal therapy in 1 patient and immunotherapy in 1 patient (Figure 4). Within the negative tested patients 18 received chemotherapy, 11 received chemoimmunotherapy, and 9 received antihormone therapy (Figure 5).

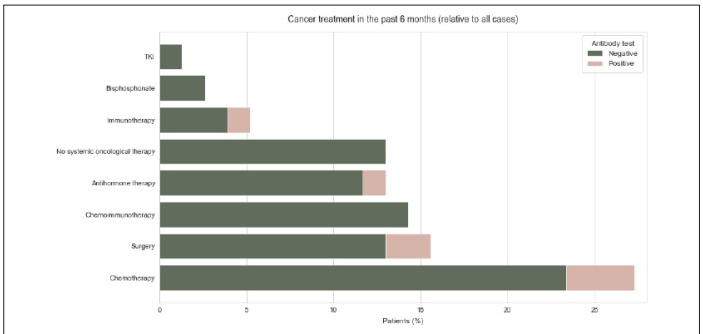

Figure 5: Cancer treatment in the past 6 month of SARS-CoV-2 anitbody positive tested and SARS-CoV-2 negative tested patients relative to all cases.

DISCUSSION

So far, COVID-19 has globally led to more than 500,000 deaths and to enormous socio-economic damage due to shutdowns worldwide [20]. Even though the numbers of newly diagnosed COVID-19 cases in Europe are decreasing compared to numbers in April and May, there are still new infections. With these decreasing infection numbers some governments are starting to reservedly open again even if comprising data is missing of how many people already have been infected with the virus. Importantly, our data suggest that an infection with SARS-CoV2 is not automatically accompanied by antibody development. After the peak of positive SARS-CoV2 PCRs in Bavaria between April 15 and March 30, there should now be a peak phase of antibody development in those patients (Figure 6).

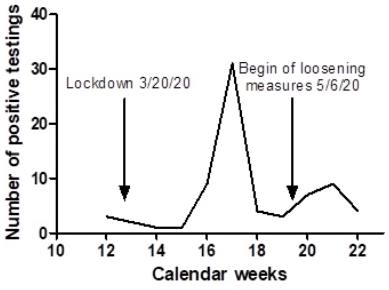

Figure 6: Course of the RT-qPCR tests for SARS-CoV-2.

Our results clearly indicate that far from all patients develop antibodies, as has shown multiple testing at regular intervals. This is particularly interesting in view of the therapies administered during this period. Most of the patients tested positive in RT-qPCR received the first negative RT-qPCR result 13 days (in median, SD 8,1) after confirmation of the positive test. The timeframe of positive PCR detection of the virus was therefore only a few days. The reason for the short time interval of positive RT-qPCR results might be a low virus load to which patients are exposed. Based on patient survey we presume that the patients consistently adhered to the requirements of social distancing and may therefore only been exposed to low virus concentrations. Since we assume that patients with a low viral load may not be infectious, the viral load determination could be used to enable selective isolation measures, which would make a decisive socioeconomic contribution. The

patient cohort of oncological patients is particularly suitable for this purpose, as they are predominantly asymptomatic SARS-CoV2 carriers (manuscript submitted).

A further explanation for the mild cases could be special oncological therapies that inhibit virus replication and thus have a positive effect on the course of the infection. So far it is largely unclear why many infected tumor patients remain asymptomatic or show only mild symptoms, whereas previously healthy individuals can develop a fatal infection. This illustrates the importance of determining the viral load in addition to RT- qPCR test. The observed lower incidence of COVID-19 disease in oncology patients offers a completely new perspective on the possible underlying pathomechanisms of the disease.

The limitations of the study are the sample size of only 77 patients, even if they have been followed up over a relatively long period of time. In addition, only one test (Roche) was used to test antibody development, even though the test has a sensitivity of up to 100% and a specificity of 98% according to the manufacture´s specification. Furthermore, our cohort consisted of only oncology patients, including immunosuppressed patients and thus represents a special cohort. However, focus on non-hospitalized cases of COVID-19 is a strength and represent real-world data of outpatient oncology medical care.

CONCLUSION

For the further management of the pandemic and the socio-economic impact on society, a strategy that allows selective isolation measures is particularly important. So far, it has been assumed that patients suffering from COVID-19 develop antibodies that provide immunity and are thus protected from a reinfection with SARS-CoV2. This also forms the basis of the assumption that rapid vaccine development will lead to rapid control of the pandemic.

Our study indicates that only a part of SARS-CoV2 infected patients develop anti-SARS-CoV2 antibodies. Thus, it has to be noted that RT-qPCR only shows a test result at a certain point in time, whereas antibody tests can provide information about an infection that has occurred in the past. Moreover, it could be that antibody tests detect patients who were infected earlier, without being tested by RT-qPCR. However, further investigations are needed to determine which patients infected by SARS-CoV2 develop antibodies and if this provides immunity. Antibody tests cannot replace the RT-qPCR but could provide further information on immunity. According to our assumption, a negative test cannot rule out an infection that has already occurred. A positive antibody development, however, indicates that the patient has been infected. Comprehensive testing of the population could provide important information on the number of infected persons. In our opinion, antibody tests should be widely available, but in combination with RT-qPCR, solely due to our data which demonstrates that some infected individuals do not develop antibodies. In so far as our understanding goes, on how the mechanism works but determines who develops antibodies and who does not, both tests should be comprehensive. This is particularly important, as it is assumed that people who have suffered from the infection will automatically become immune. Even though our data shows that this is not the case and that these patients could be reinfected. This could prove to be a special challenge for those countries that pursue the strategy of herd immunity. Due to the novelty of SARS-SoV2, there are still no long-term studies on answering the question whether people who have experienced the disease are protected from new infections; therefore it is important to follow an antibody development through long-term studies to find out how long they provide immunity to COVID-19. This underlines the urgent need to validate the antibody detection approaches to support diagnosis, vaccine development and safety.

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