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Hot Topic

Seroprevalence of SARS-CoV-2–specific antibodies in cancer outpatients in Madrid (Spain): A single center, prospective, cohort study and a review of available data

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ABSTRACT

Background: Coronavirus disease in 2019 (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has emerged as a global pandemic. Published data suggests that patients with a history of or active malignancy are at increased risk of infection and developing COVID-19 related complications. To date, the published data has analyzed the seroprevalence of COVID-19 infection in the general population, but not in cancer patients. Here we present the results of prevalence of IgG and IgM antibodies against SARS-CoV-2 in cancer patients from the University Hospital of Torrejón (Torrejón de Ardoz, Madrid, Spain).

Methods: SARS-CoV-2 IgG and IgM antibodies was assessed using a commercially available rapid test (Testsealabs® IgG/IgM Rapid Test Cassette) and collect the result from cancer outpatients who attended the medical oncology consult at University Hospital of Torrejón between June 1st and June 19th, 2020.

Findings: We analyzed the serological test results of 229 cancer patients. We estimated an overall seroprevalence (IgG or IgM positive) of 31.4%. The probability of SARS-CoV-2 seropositivity was similar between men and women, type of treatment and cancer stage. The probability of seropositivity was significantly higher in cancer patients with pneumonia compared with cancer patients without pneumonia (Odds Ratio (OR) 7.65 [95% confidence interval (CI) 1,85–31,58]).

Interpretation: Our results show a higher rate of SARS-CoV-2 antibodies in cancer patients than in the general population. The role of those antibodies in the immune response against the virus infection is unclear.

Introduction

Coronavirus disease in 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has emerged as a global pandemic [1]. COVID-19 was first reported in Wuhan, China, in December 2019, among a group of individuals presenting with atypical pneumonia of unknown etiology [2]. Published data suggests that patients with a history of or active malignancy are at increased risk of infection and developing COVID-19 related complications [3,4]. Data from China have shown that cancer patients infected with COVID-19 are at 3.5 times the risk of requiring mechanical ventilation or intensive care unit (ICU) admission, compared to the general population [3]. In a recent cohort study, 928 cancer patients (39% were on active anticancer treatment, and 43% had active cancer) were analyzed, with a 30-day all-cause mortality of 13%, associated with general risk factors and risk factors unique to patients with cancer [1].

Seroprevalence surveys are of utmost importance to assess the proportion of the population that has already developed antibodies against the virus and might potentially be protected against subsequent infection [5].

SARS-CoV-2 IgM/IgG tests have been developed for the diagnosis and management of COVID-19 patients, identifying convalescent cases and sero-epidemiological surveillance [6]. In patients infected with SARS-CoV-2, IgM antibodies are detectable around 7 days postinfection and IgG antibodies usually take 2 weeks to develop [7].

Patients with cancer and COVID-19 have a low prevalence of IgG

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antibodies to SARS-CoV-2. Liu T et al [8] found that only 72.5% had IgG antibodies to SARS-CoV-2 after 21 days post-symptom onset, much lower than patients without cancer. Currently, the role of IgG antibodies to SARS-CoV-2 in the immune response against the virus infection is unclear. It is hard to interpret the finding of the low prevalence of IgG antibodies to SARS-CoV-2 in cancer patients. Since all the above serological tests have been developed rapidly and under urgent market demands, they are poorly validated with clinical samples in everyday practice. Within several studies, these tests show divergence in sensitivity and specificity that may deviate from what the manufacturers report. Recent meta-analysis concludes that all methods yield high specificity with some of them (Enzyme-linked immunosorbent assay (ELISA) and Lateral Flow Immunoassays (LFIA)) reaching levels around 99% [9].

It is unknown whether there is a difference in the prevalence of antibodies to SARS-CoV-2 between cancer patients and other patients in the COVID-19 era.

To date, the published data has analyzed the seroprevalence of COVID-19 infection in the general population [10–12], with values ranging between 1.8% and 10.9%, without specifying the cancer population.

Since the start of the alert by COVID-19 and until June 21 st, 2020 (health alert center), 246,272 cases of SARS CoV-2 infection have been diagnosed by PCR in Spain [1], with 71,223 cases (28.92% cases registered in the country) diagnosed in the region of Madrid (Spain) [13].

ENE-Covid19 is a large population-based seroepidemiological longitudinal study, whose objectives are to estimate the prevalence of SARS-Cov2 infection by determining antibodies against the virus in Spain and evaluating its temporal evolution. The results presented in the first round (April 27 to May 11) included 60,983 participants. The estimated prevalence of IgG antibodies against SARS-Cov2 in Spain is 5% (95% confidence interval (CI): 4.7–5.4%) and in Madrid is 11.3% (95% CI: 9.8–13%) [14].

At the University Hospital of Torrejón on June 16th, 2020, 1098 cases of COVID-19 were hospitalized, with a total cumulative incidence of 835 cases per 100,000 habitants.

Here, we presents the results of the prevalence of IgG and IgM antibodies against SARS-CoV-2 in cancer patients from University Hospital of Torrejón (Torrejón de Ardoz, Madrid, Spain).

Material & methods

Study design and participants

The seroprevalence study is a population-based study of Torrejón de Ardoz, in Madrid, Spain, and included all citizens over 1 year of age. From a total of 139,452 registered citizens in the city of Torrejón de Ardoz, the study was carried out on 104,299 volunteers (participation rate of 74.8%) in the period from May 29th to June 5th, 2020. The study was approved by the institutional ethics board at Elche-Vinalopó and Torrevieja Hospital (Comunidad de Valencia, Spain).

Using this information, authorization was requested to collect the test result from oncology outpatients who attended the medical oncology consultation of the University Hospital of Torrejón between June 1st and June 19th, 2020. The main objective was to estimate the seroprevalence of SARS-CoV-2 infection in part of the cancer population of the center. A total of 229 patients were included. All participants gave written informed consent before participation in the study.

Laboratory analysis

We assessed anti-SARS-CoV-2 IgG and IgM antibodies using a commercially available rapid test (Testsealabs® IgG/IgM Rapid Test Cassette, Hangzhou Testsea Biotechnology Co., Ltd) targeting the S1 domain of the spike protein of SARS-CoV-2. The manufacturer reported sensitivity of 96% for IgG and 88% for IgM and specificity of 100% for both IgG and IgM, using RT-PCR as the gold standard.

The test is based on reliability studies carried out in various hospitals of the Spanish National Health System, and has the approval of the European community and ISO13485 certificate.

The following studies were carried out to validate the test as an instrument for measuring health: a validation study of the selected test, before carrying out the study in a sample of randomly selected health workers, comparing the results with the ELISA technique, with the aim of obtaining a gross agreement of over 80% for both measures; a concordance study performed on a randomly selected sample, which is performed (in addition to the serological test) for the detection of antibodies using ELISA techniques, with the aim of obtaining a kappa index > 0.7 and a concordance study conducted with patients diagnosed at the Torrejón Hospital by PCR before the start of the study and who have attended serological tests (the required objective was to obtain a crude concordance greater than 95%).

The validation study showed a diagnostic agreement of 93.6% between Testsealabas® and ELISA test.

Statistical analysis

After collection, data were coded and entered on the computer using SPSS version 26. To identify risk factors in cancer patients associated with SARS-COV2 exposure, we performed a cross sectional study based on serologic test results. Frequency tables, cross-tabulation, chi-squared tests, and two-sided Fisher's tests were carried out at first place to correlate categorical data. The variables included were age, sex, cancer stage, tumor type, cancer treatment, and pneumonia diagnosis. The association between different variables were calculated by logistic regression. All variables with a p-value of < 0.6 were considered independent in the logistic regression analysis; the dependent variable was the stable infection: infected (seropositive) /non-infected (seronegative). Goodness of fit was checked using the Hosmer-Lemeshow test, unless otherwise reported, had $p > 0.05$. p-values < 0.05 were considered statistically significant.

Results

A total of 229 cancer patients were analyzed. 124 (54.1%) were women; and 35 (15.3%) were aged < 50 years, 122 (53.3%) were aged 51–70 years and 72 (31.4%) were older than 70 years. Seventy-nine patients (34.5%) had stage IV and 150 (65.5%) had a localized / locally advanced stage. Of these, 92 patients (40.2%) were without treatment and 137 (59.8%) were on active antitumor treatment (25.8% chemotherapy, 18.8% hormone therapy, 4.3% immunotherapy and 10.9% target therapy). The most frequent tumor locations were: breast (29.3%), digestive tract (27,9%), thoracic (15,3%) and urinary tract and male genital organs (14.4%, Table 1).

Sixty-four of 229 individuals tested positive for SARS-CoV-2 IgG antibodies (27.9%) and 22 patients were positive for SARS-CoV-2 IgM antibodies (9.6%). We estimated an overall seroprevalence (IgG or IgM positive) of 31.4% (Table 2).

The probability of seropositivity was similar between men and women (Odds ratio (OR) 2.53 [95% CI 0.934–6.61]); between type of treatment (none or active treatment) (OR 1.28 [95% CI 0.72–2.28]) and between cancer stage (I-III versus IV) (OR 1.01 [95% CI 0.56–1.82]). The probability of seropositivity was significantly higher in cancer patients with pneumonia compared with cancer patients without pneumonia (OR 7.65 [95% CI 1,85–31,58], Tables 3 and 4).

There are no statistically significant differences ($p = 0.25$) in seroprevalence based on the therapy received by patients, 27.1% of patients who developed SARS-COV2 antibodies were treated with chemotherapy during the pandemia, 38.5% with therapies other than chemotherapy (hormone therapy, immunotherapy, and targeted treatment) and 28.3% did not receive any antineoplastic treatment.

According to our multivariate logistic regression results, only the

Table 1
Clinical features of patients.

	All patients (n = 229)
Age, years	64 (22-88)
Age group, years	
• < 50	35 (15,3%)
• 51–70	122 (53,3%)
• > 70	72 (31.4%)
Sex	
• Male	105 (45,9%)
• Female	124 (54,1%)
Cancer type	
• Respiratory and Intrathoracic organs	35 (15,3%)
• Digestive organs	64 (27,9%)
• Breast	67 (29.3%)
• Urinary tract and male genital organs	33 (14,4%)
• Female genital organs	15 (6,6%)
• Other	15 (6,6%)
Cancer stage	
• Primary tumour localised/locally advanced	150 (65.5%)
• Metastatic	79 (34.5%)
Cancer treatment during covid-19 pandemic	
• Chemotherapy	59 (25,8%)
• Hormone therapy	43 (18,8%)
• Immunotherapy	10 (4,3%)
• Targeted therapy	25 (10,9%)
• None	92 (40,2%)

Table 2
Rates of the serological test results.

Results	N	%
IgM − / IgG −	157	68,6%
IgM − / IgG +	50	21,8%
IgM + / IgG −	8	3,5%
IgM + / IgG +	14	6,1%

N: number of patients. % Percentage.

Table 4
Multivariante regression model.

	Odds ratio (95% CI)	P value
Sex		
- Female	1 (ref)	0.066
- Male	2.530 (0.934-6.612)	
Cancer type		
- Respiratory and Intrathoracic organs	1.466 (0.314-6,843)	0.453
- Digestive organs	0.721 (0.168-2.768)	
- Breast	1 (ref)	
- Urinary tract and male genital organs	1.079 (0.257-0.4529)	
- Female genital organs	3.012 (0.549-16.521)	
- Other	0.623 (0.140-2.768)	
Cancer treatment durign covid-19 pandemic		
- Chemotherapy	0.527 (0.226-1.229)	0.306
- Non-chemotherapy treatment	1 (ref)	
- None	0.619 (0.272-1.411)	
Pneumonia		
- None	1(ref)	**0.005**
- Yes	7.653 (1.854-31,583)	

presence of pneumonia was related to the positivity of the antibodies (Table 4).

PCR was performed on a total of 41 cancer patients due to the presence of symptoms, 12 had pneumonia, of which 4 were positive, 5 negative and 3 had no PCR (clinical-radiological diagnosis). The 22 patients with IgM + underwent PCR, and the result was negative in all. Of these, 4 had had pneumonia and had previously had positive PCR on admission, but when repeated in the study they were negative.

Of the 12 patients (5.2%) with pneumonia; 33.3% (4/12) were polymerase chain reaction (PCR) positive for SARS-CoV-2, 41.6% (5/12) had radiological suspicion of COVID-19-related pneumonia and 25% (3/12) had pneumonia not compatible with COVID-19. All patients (9 out of 9 cases) with COVID-19-related pneumonia were seropositive.

5 patients (41,6%) did not present any risk factor (41.6%), 3 were obese (25%), 3 (25%) were dyslipidemic (25%), 3 had chronic bronchopathy (25%) and 1 (8%) ischemic heart disease.

Table 3
Demographic characteristics of the patients based on seroprevalence.

Characteristic	SARS-COV2 seropositive (n = 72)		SARS-COV2 seronegative (n = 157)		P value
	n	%	n	%	
Age group					
• < 50 (n = 35)	11	31,4%	24	68,6%	0.7
• 51-70 (n = 122)	41	33,6%	81	66,4%	
• > 70 (n = 72)	20	27,8%	52	72,2%	
Sex					0.317
• Male (n = 105)	37	35,2%	68	64,8%	
• Female (n = 124)	35	28,2%	89	71,8%	
Cancer type					
• Respiratory and Intrathoracic organs (n = 35)	14	40%	21	60%	
• Digestive organs (n = 64)	15	23,4%	49	76,6%	
• Breast (n = 67)	21	31,3%	46	68,7%	
• Urinary tract and male genital organs (n = 33)	11	33,3%	22	66,7%	0.426
• Female genital organs (n = 15)	7	46.7%	8	53,3%	
• Other (n = 15)	4	26,7%	11	73,3%	
Cancer stage					
• Primary tumour localised/locally advanced (n = 150)	47	31,3%	103	68,7%	
• Metastatic (n = 79)	25	31,6%	54	68,4%	0.961
Cancer treatment during covid-19 pandemic					
• Chemotherapy (n = 59)	16	27,1%	43	72,9%	
• Non-chemotherapy treatment (n = 78)	30	38,5%	48	61,5%	0.256
• None (n = 92)	26	28,3%	66	71,7%	
Pneumonia					
• No (n = 217)	63	29%	154	71%	**0.002**
• Yes (n = 12)	9	75%	3	25%	

Seroprevalence of SARS-COV-2-specific antibodies in cancer patients

To date, there are no data on seroprevalence in the cancer population. Published data do not provide subgroup analyzes of cancer patients. We only know the seroprevalence of cancer patients diagnosed with COVID-19 or of cancer staff.

Epstude J et al [15] conducted a single center study (German federal state of Thuringia) in 45 members of the cleaning staff and 20 members of the oncology ward. Antibody titers (IgA and IgG) against COVID-19 were measured. Significantly elevated IgA antibody titers were detected in 1 person in the first group (2,2%) and in 1 person (5%) in the second group. Significantly elevated IgG antibody titers were not detected in the first group and in 1 person (5%) of the second group. In case of positive or indeterminate testing, swabs for direct virus detection were taken, but were negative in all cases.

Liu T et al [8] assessed prevalence of IgG antibodies against SARS-CoV-2 in cancer patients with COVID-19 and other hospitalized COVID-19 patients from Zhongnan Hospital of Wuhan University, Wuhan No. 7 Hospital, and Leishenshan Hospital in Wuhan, China. There were no differences in age and sex in cancer patients with COVID-19 and other COVID-19 patients. IgG prevalence was 72.5% (95% CI 58.0–87.0%) in cancer patients with COVID-19 compared to 90.3% (95% CI 88.7–91.8%) in other patients ($P < 0.001$). IgM prevalence was 20.0% (95% CI 7.0–33.0%) in cancer patients with COVID-19 and 31.7% (95% CI 29.3–34.1%) in other patients.

Discussion

Liu T et al [8] reporting a seroprevalence of 72.5% in cancer patients with COVID-19. This is much lower than patients without cancer. Our study evaluates cancer patients seen in routine clinical practice, not previously selected for COVID-19 infection, hence the seroprevalence is lower (31.4%). However, it is probably much higher than expected, especially considering the data published in the general population, with values ranging from 1.8% to 10.9% [10–12], being 11.3% in the city of Madrid [14] (Table 5). This could be explained because of the high infection rate in Torrejón de Ardoz region, with a total cumulative incidence of 835 cases per 100,000 habitants, with a prevalence of IgG in the general study population of 20.18%.

Although there are many questions about the degree of immunity generated by COVID-19 in cancer patients, in our study the only statistically significant and differentiating variable found was the presence or absence of pneumonia (75% vs. 29% seroprevalence; $p = 0.002$). If we exclude patients with non-COVID pneumonia, 100% of them (9/9) developed antibodies, which would cast doubt on the lack of ability to generate antibodies from the cancer population (immunosuppressed), although the limitations of the study and the small number of patients with pneumonia do not allow definitive conclusions to be drawn.

There are no statistically significant differences between seroprevalence and the different treatments received by patients. However, it is interesting to note that 27.1% of patients receiving chemotherapy and 38.5% with other treatments (hormone therapy, immunotherapy and targeted treatment) developed antibodies. It is striking to see how it is a higher value than that presented by cancer patients who were not receiving treatment (seroprevalence of 28.3%). These are interesting data that once again reveal to the scientific community the long way to go regarding the knowledge of the relationship between the immune system and the SARS-CoV-2 virus, above all in cancer patients.

Our study had several limitations. Because the study population was not drawn by random sampling, the estimation of the seroprevalence was subject to potential sampling bias. By including outpatients in a 2-week period, a significant number of patients were excluded, especially those most critical requiring hospital admission. Samples collected from infected individuals outside the time window of antibody response

Table 5
Seroprevalence in differences studies.

Study	N	Population	Country	Prevalence IgG	Prevalence IgM	Prevalence IgM or IgG	Sensitivity and specificity test
Sood N et al. [11]	865	Global	USA (California)	NR	NR	4,65%	Senstivity of 82,7% and a specificity of 99,5%
Xu X el al. [10]	17,368	Global	China	Hospital settings 2% Community settings 0,5%	Hospital settings 0,6% Community settings 0,4%	Hospital settings 2,5% Community settings 0,8%	Specificity of 99.3% and 100% for IgG and IgM
Stringhini S et al. [12]	2766	Global	Switzerland (Geneva)	10,8%*	NA	10,8%*	Sensitivity of 93% and a specificity of 100%.
ENE-COVID19 [14]	60,983	Global	Spain	5%	NR	5% (11,3% in Madrid)	Sensitivity of 79% (IgG) and 73% (IgM) and a specificity of 100% (IgG) and 98% (IgM)
Current study	229	Cancer patients	Spain (Madrid)	27,9%	9,6%	31,4%	Sensitivity of 96% (IgG) and 88% (IgM) and a specificity of 100% (IgG) and 100% (IgM)

N: number of patients; USA: United States of America; NR: not reported. NA: not analyzed; *Seroprevalence in the fifth week.

could produce false negatives, and therefore the observed seroprevalence in our study could potentially underestimate the true prevalence rate of the disease. Owing to the cross-sectional design of the current study, the dynamic changes of antibody titer in infected individuals over time were not evaluated. Prevalence estimates could change with new information on the accuracy of test kits used. Also, the study was limited to one country and one city.

Conclusions

Currently, the role of antibodies to SARS-CoV-2 in the immune response against the virus infection is unclear. It is hard to interpret the finding of the high prevalence of IgG/IgM antibodies to SARS-CoV-2 in cancer patients, especially when limiting the study to a single region, which has also been especially affected by the disease.

CRediT authorship contribution statement

Luis Cabezón-Gutiérrez: Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Supervision. **Sara Custodio-Cabello:** Methodology, Formal analysis, Investigation, Writing - original draft. **Magda Palka-Kotlowska:** Supervision. **Eduardo Oliveros-Acebes:** Methodology. **María José García-Navarro:** Writing - original draft, Writing - review & editing. **Parham Khosravi-Shahi:** Formal analysis, Investigation, Supervision.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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