



OPEN Cracking the code of a correlate of protection against SARS-CoV-2 breakthrough infection in cancer patients

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The level of protection against SARS-CoV-2 breakthrough infections conferred by the presence of anti-S1 SARS-CoV-2 antibodies (IgGs) in cancer patients is still understudied. This work examines the existence of an anti-S1 immunoglobulin G (IgG) -based correlate of protection (CoP) established by prospectively collected observational data about breakthrough infections with different SARS-CoV-2 variants in a large cohort study with vaccinated cancer patients. 760 cancer patients were longitudinally followed-up, starting before first vaccination until six months after second booster. Anti-S1 SARS-CoV-2 IgGs were quantified in serum samples ($N = 2958$) and breakthrough infections were monitored using questionnaires, routine COVID-19 testing and medical chart review. A Generalized Estimating Equations approach was used to model the binary infection status as endpoint in relation to anti-S1 IgG titers. It is observed that higher anti-S1 IgG titers correspond to a lower probability of breakthrough infection. For the early pandemic phase, a protective anti-S1 IgG titer above 20.42 BAU/mL was observed. However, with the emergence of the Omicron variant, higher anti-S1 IgG titers are required to be protective, but no clear CoP could be identified.

Keywords SARS-CoV-2, COVID-19, Cancer patients, Correlate of protection, COVID-19 vaccination, Antibodies, Breakthrough infection

Coronavirus Disease 2019 (COVID-19), caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is associated with a broad range of clinical symptoms, ranging from asymptomatic infection,

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sensation of a mild cold to severe bilateral pneumonia and even death^{1–3}. Both natural infection and vaccination against COVID-19 induce humoral immunity and provide a certain degree of protection against (re)infections in the general population⁴. However, some individuals display impaired humoral immunity due to underlying illness or immunosuppressing therapy⁵. Cancer patients are immunocompromised due to the cancer itself and as a result of the anti-neoplastic treatment they are receiving^{6,7}. Hence, they are at risk to develop serious illness after infection with SARS-CoV-2⁸. It is therefore of utmost importance to protect these oncological patients by imposing hygienic measures, but also by focusing on deployment of pharmaceutical interventions like vaccination against COVID-19. When vaccines against COVID-19 became available at the end of 2020, an initial protection against severe disease and death up to 90% could be provided in the general population^{9–11}. However, decreased vaccine effectiveness and an increased probability of experiencing SARS-CoV-2 infection has been observed in cancer patients in comparison to healthy individuals^{10,12–14}. Moreover, overall protection drastically dropped with the emergence of the Omicron variant (BA.1). Whereas this variant showed decreased pathogenicity^{10,15}, vaccine effectiveness reduced due to increased immune escape^{16–18}. Originally, this variant was detected in South-Africa in November 2021^{10,19}, but because of an unseen speed of transmission, the Omicron variant rapidly became the worldwide dominant SARS-CoV-2 variant^{10,19}, which was also associated with an increased number of breakthrough infections^{19–21}. Hence, concerns about the longevity of vaccine induced protection against infection and disease were raised, especially for vulnerable cancer patients, leading to administration of additional vaccine doses (booster doses).

A correlate of protection (CoP) is the extent of the immune response associated with protection from an infectious agent following natural infection or vaccination^{22,23}, being important for policy makers to estimate the optimal period between consecutive vaccine doses. In the absence of a CoP reference, serological tests are unable to confirm immunity against a certain virus. Antibody titers are not the only immunological markers that can be used for the determination of a CoP, but because they are much easier to measure than cellular responses and therefore more clinically useful²⁵, most of the accepted CoPs are based on antibody titer concentrations^{24,25}. This work aimed to identify an antibody-based CoP for SARS-CoV-2 in a cohort of vaccinated cancer patients under different treatment regimens which were followed longitudinally throughout their vaccination course. As such both antibody responses and breakthrough infections could be monitored over time to unravel their relationship and define a CoP for this population.

Materials and methods

Study design

This manuscript bundles clinical data and IgG data obtained from serum samples collected according to the protocols of three studies performed in Belgium and investigating the response of cancer patients with regard to COVID-19 vaccination (EudraCT nos. 2021–000300–38, 2021–003573–58 and BUN nr. B3002021000069)^{12,26–28}. Across these clinical trials, a total of 760 cancer patients were included (Fig. 1). In these studies, up to ten blood samples were collected every one–six months for every patient, starting before first vaccine dose administration until 28 days after second booster (Suppl. Figure 1). In total, 2958 serum samples were analyzed. Almost all recruited patients completed primo-vaccination ($N=755$, 99.3%) and a first booster dose was administered to 653 patients (85.9%). A second booster was administered to 389 patients (51.2%). Major reasons for study discontinuation were cancer-related death and individual patient decision.

Analysis of humoral immune response

The humoral immune response against SARS-CoV-2 was assessed in 2958 serum samples by the quantitative detection of anti-S1 IgG antibodies with the use of the Siemens Healthineers Atellica IM SARS-CoV-2 S IgG (sCOVG) assay following the described protocol^{29,30}. Quantitative anti-S1 IgG titers were converted to binding antibody units per mL (BAU/mL). The measuring interval of the immunoassay is 10.90–16350.00 BAU/mL. Values below the lower limit of detection (LLD) were imputed half of it (5.45 BAU/mL). On the other hand, values above the measuring interval were imputed 33% above the upper limit of detection (21800.00 BAU/mL). Patients with detectable antibodies were considered as responders.

Monitoring of breakthrough infections

For all patients, occurrence of breakthrough infections was documented up to 6 months after last vaccination or up to the third booster dose if this was administered earlier in time. A breakthrough infection was defined as a PCR confirmed SARS-CoV-2 infection registered in the Belgian COVID-19 Test Result Database of Sciensano³¹. All patients in active treatment underwent a standardized testing protocol with PCR at regular intervals and upon hospitalization.

Statistical analysis

For descriptive statistics regarding patient characteristics (demographics and oncological information), continuous variables are summarized using mean, standard deviation, median, minimum and maximum values. For categorical variables, both absolute and relative frequencies (expressed in percentages) are shown.

Anti-S1 IgG measurements were transformed using a log₁₀-transformation (hereafter referred to as logIgG) to comply with distributional assumptions in all inferential procedures described below. Geometric Mean Titres (GMTs) between time points are compared using a linear mixed model approach (with posthoc tests and Bonferroni-Holm correction for multiple testing), thereby including patient-specific random intercepts to account for repeated anti-S1 IgG measurements on the same individual. The longitudinal vaccination course was subdivided into different nine intervals according to the vaccination schedule and blood sample collections of the different patients in the study (Fig. 2; Table 4). PCR-confirmed SARS-CoV-2 breakthrough infections in all intervals were documented. LogIgG titers prior to the occurrence of each breakthrough infection were compared

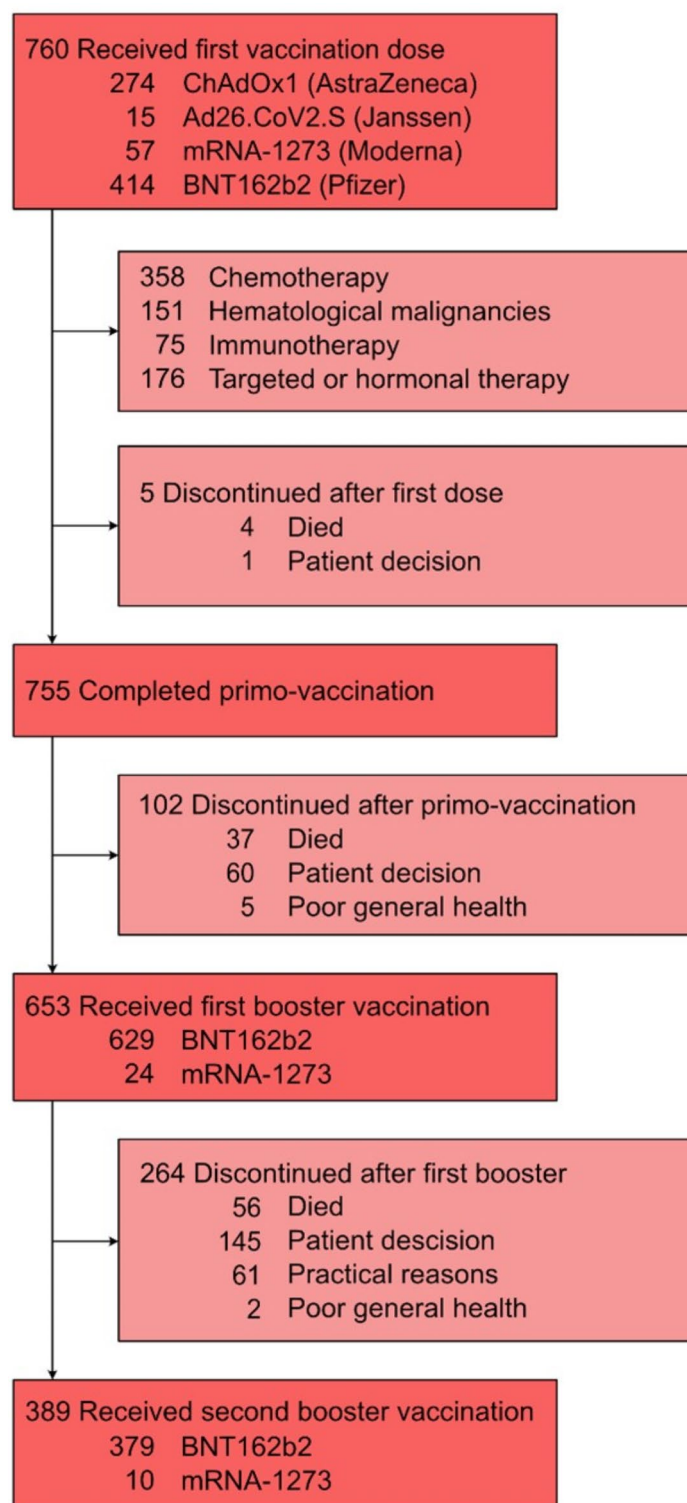


Fig. 1. Patient vaccination flow diagram.

with the titers of individuals that were not infected within corresponding time intervals. To assess whether significant differences in mean logIgG concentrations exist between infected and non-infected individuals, a Tobit regression analysis was performed thereby explicitly accommodating that some of the anti-S1 IgG measurements are left- and right-censored due to the presence of lower and upper limits of detection.

Logistic regression models were used to differentiate between patients with and without breakthrough infections for each of the aforementioned time periods. A Generalized Estimating Equations (GEE) approach was used to account for association between observations coming from the same patients over time when

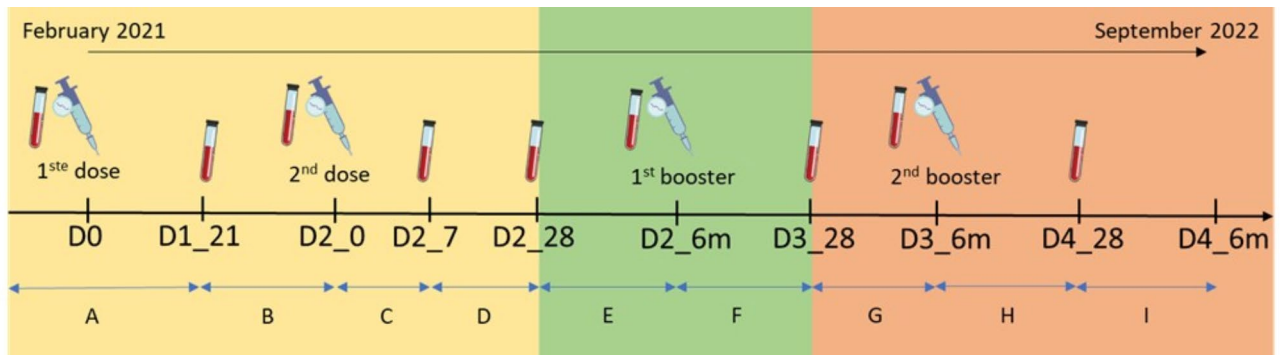


Fig. 2. Classification of the used time periods with regards to vaccine administration and blood sampling. Classification of the different time periods used for statistical analysis. The yellow color defines the period at which few infections are observed, and no threshold estimation could be performed. The green color represents the period before Omicron was dominant in the population and the orange color represents the period at which Omicron was the dominant SARS-CoV-2 variant.

considering a combined analysis of the binary endpoint (i.e., breakthrough infection status) across different time periods.

Probabilistic classification of infected vs. non-infected patients based on their logIgG titers, was performed and performance was evaluated based on sensitivity, specificity and the Area Under the (Receiver Operating Characteristic) Curve (AUC). Sensitivity and specificity were defined based on the infection status: sensitivity corresponds to the proportion of patients classified as infected among the truly infected patients. On the other hand, specificity refers to the proportion of patients classified as non-infected individuals among those not experiencing a breakthrough infection. In our analyses, we examine whether an optimal threshold value in terms of anti-S1 IgG titers can be determined. Optimality is defined in terms of maximizing the Youden's index, thereby maximizing the sum of sensitivity and specificity.

In a next step, an overarching analysis was performed to assess whether a single threshold across time periods can lead to a good classification performance. A GEE approach (with exchangeable working correlation structure) was used to model the binary breakthrough infection status in relation to the logIgG titers for the defined time periods. As only a very limited number of breakthrough infections was reported in time periods A to D, the model was restricted to time periods E up to I. Due to potential differences in probability of breakthrough infection between time periods, caused by vaccine administration and the occurrence of different viral variants, the time period was considered as a main effect in the model. Additionally, an interaction effect between time period and logIgG titers was included to assess whether time-specific differences exist in the relation between logIgG titers and the probability of breakthrough infection.

For all analysis, a two-sided p -value < 0.05 was considered statistically significant. 95% confidence intervals (CI) for sensitivity and specificity are Wilson Score intervals for proportions. All statistical analyses were performed using the statistical software program R (version 4.3.2)³².

Results

Patient characteristics, oncological treatment and vaccine schedule

Among 760 patients, 467 (61.4%) are female. The median age of the patients equals 61 years. Detailed demographics on ECOG, smoking status and comorbidities are available in Table 1. Solid tumors were present in 609 patients (80.1%) (Table 2) and hematologic malignancies were present in a minority of 151 patients (19.9%) (Table 3). From the 609 patients with a solid tumor, 358 (58.8%) were treated with chemotherapy, 75 (12.3%) received immunotherapy and 176 (28.9%) patients were receiving targeted or hormonal therapy at time of first vaccine dose administration. Previous results indicated that the effect of chemotherapy on vaccine-induced antibody production exceeds that of immunotherapy¹². Therefore, patients receiving a combination of both therapies were assigned to the chemotherapy cohort. The most common primary tumor locations were breast (254; 41.7%), lower gastrointestinal (GI) (89; 14.6%) and upper GI (63; 10.3%). Six patients had multiple primary tumors and more than half of the patients (319; 52.4%) had metastatic disease at inclusion (Table 2).

The majority of the patients (414; 54.5%), received BNT162b2 primo-vaccination. Less than half of the patients (274, 36.1%) received ChAdOx1 primo-vaccination. Additionally, 15 and 57 patients received respectively Ad26.CoV2.S (2.0%) and mRNA-1273 (7.5%) primo-vaccination (Fig. 1). Patients receiving Ad26.CoV2.S or mRNA-1273 as primo-vaccination were younger compared to other patients. 54.5% of the patients received a first booster within the recommended interval of 4–6 months after completion of the primary vaccination course^{33,34}. The interval to receive the first booster dose was slightly extended to 194–242 days for 26 patients and 271 patients received a first booster earlier than recommended at 45–111 days. A second booster was administered to 389 patients of whom 95.6% received it within three to seven months after first booster.

	ChAdOx1 (N = 274)	Ad26.CoV2.S (N = 15)	mRNA-1273 (N = 57)	BNT162b2 (N = 414)	Overall (N = 760)
Gender					
Female	168 (61.3%)	12 (80.0%)	27 (47.4%)	260 (62.8%)	467 (61.4%)
Male	106 (38.7%)	3 (20.0%)	30 (52.6%)	154 (37.2%)	293 (38.6%)
Age					
Mean (SD)	60.7 (10.7)	51.2 (12.4)	52.9 (13.2)	61.1 (13.3)	60.2 (12.6)
Median [Min, Max]	61.0 [28.0, 91.0]	51.0 [34.0, 78.0]	57.0 [23.0, 84.0]	63.0 [20.0, 88.0]	61.0 [20.0, 91.0]
ECOG performance status					
0	126 (55.8%)	11 (73.3%)	31 (63.3%)	316 (80.0%)	484 (70.7%)
1	90 (39.8%)	4 (26.7%)	18 (36.7%)	76 (19.2%)	188 (27.4%)
2	6 (2.7%)	0 (0.0%)	0 (0.0%)	3 (0.8%)	9 (1.3%)
3	4 (1.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (0.6%)
Missing	48	0	8	19	75
Smoking status					
Current smoker	40 (15.4%)	2 (13.3%)	8 (14.0%)	34 (8.6%)	84 (11.5%)
Former smoker	85 (32.7%)	2 (13.3%)	18 (31.6%)	152 (38.3%)	257 (35.3%)
Non smoker	135 (51.9%)	11 (73.3%)	31 (54.4%)	211 (53.1%)	388 (53.2%)
Missing	14	0	0	17	31
Comorbidities					
Auto immune disease	12 (4.6%)	0 (0.0%)	0 (0.0%)	16 (3.9%)	28 (3.7%)
Kidney disease	25 (9.5%)	0 (0.0%)	0 (0.0%)	17 (4.1%)	42 (5.6%)
Hypertension	90 (34.4%)	2 (13.3%)	10 (17.5%)	117 (28.3%)	219 (29.3%)
Diabetes	25 (9.5%)	1 (6.7%)	5 (8.8%)	42 (10.2%)	73 (9.8%)
Coronary disease	25 (9.5%)	0 (0%)	6 (10.5%)	44 (10.7%)	75 (10.0%)
Missing	12	0	0	1	13

Table 1. Demographics of the patients enrolled in the clinical trials as mentioned in study design. Demographics of the patients at time of enrolment in one of the described clinical trials. Patients were divided based on the first vaccine they received within their vaccination course against COVID-19; ChAdOx1, Ad26.CoV2.S, mRNA-1273, BNT162b2. Percentages of valid observations (missing observations excluded) are indicated. SD; standard deviation, ECOG; Eastern Cooperative Oncology Group Performance Status: Scale used by healthcare providers to assess a patient's level of functioning and ability to carry out daily activities.

Humoral immune responses

Antibody anti-S1 IgG titers started to increase during the primo-vaccination course after the 2nd dose and a decline was observed approximately six months after the second dose administration (Fig. 3). Administration of a first booster dose mounted significantly higher anti-S1 IgG titers after 28 days as compared to primo-vaccination ($p < 0.05$). Again, a decline was observed six months after first booster dose administration and administration of a second booster dose mounted significantly higher anti-S1 IgG titers after 28 days as compared to the first booster. The percentage responders gradually increased over time as more vaccination doses were administered. After administration of a second booster dose, more than 90% of patients were able to mount a detectable anti-S1 antibody response against SARS-CoV-2. A total of 37 patients (8.0%) had detectable anti-S1 IgG antibodies before administration of the first vaccination dose. Next to the regular measurement moments for most patients, some were measured four, six and twelve months after first vaccine administration as their vaccination schedule followed the one implemented by the national authorities in Belgium. All differences in GMTs across consecutive time points were found to be significant (after Bonferroni-Holm multiplicity correction), except for the difference in GMT 21 days after the first vaccine dose and measurements just prior to the second vaccine dose ($p = 0.216$).

From Supplementary Fig. 2 it is clear that median antibody titer concentrations are generally higher among patients with solid tumors, irrespective of the time point at which measurements are performed. Among patients with hematological malignancies, the median anti-S1 IgG levels show almost no response to vaccination until 6 months after the third vaccine dose, albeit that a humoral response is observed among those patients measured 4 months, 6 months and 12 months after the start of the study. A similar picture is obtained when considering these subgroups for BNT162b2 and ChAdOx1 primo-vaccination with an earlier response following second dose vaccination among those patients with hematological malignancies under ChAdOx1 primo-vaccination (Suppl. Figures 3 and 4). The subgroups who received primo-vaccination with Ad26.CoV2.S or mRNA-1273, were too small to explore these differences in detail. Finally, in general, no differences in median antibody levels were observed across time between patients with different types of solid tumors (Suppl. Figure 5).

	ChAdOx1 (N = 223)	Ad26.CoV2.S (N = 13)	mRNA-1273 (N = 40)	BNT162b2 (N = 333)	Overall (N = 609)
Treatment at first dose administration					
Chemotherapy	129 (57.8%)	11 (84.6%)	30 (75.0%)	188 (56.5%)	358 (58.8%)
Immunotherapy	35 (15.7%)	0 (0.0%)	4 (10.0%)	36 (10.8%)	75 (12.3%)
Targeted/Hormonal therapy	59 (26.5%)	2 (15.4%)	6 (15.0%)	109 (32.7%)	176 (28.9%)
Primary tumor					
Brain	7 (3.1%)	2 (15.4%)	1 (2.5%)	9 (2.7%)	19 (3.1%)
Head/neck	6 (2.7%)	1 (7.7%)	1 (2.5%)	12 (3.6%)	20 (3.3%)
Breast	87 (39.0%)	6 (46.2%)	15 (37.5%)	146 (43.8%)	254 (41.7%)
Lung	13 (5.8%)	0 (0.0%)	1 (2.5%)	17 (5.1%)	31 (5.1%)
Upper gastrointestinal	21 (9.4%)	0 (0.0%)	7 (17.5%)	35 (10.5%)	63 (10.3%)
Lower gastrointestinal	26 (11.7%)	2 (15.4%)	8 (20.0%)	53 (15.9%)	89 (14.6%)
Gynaecological	13 (5.8%)	2 (15.4%)	1 (2.5%)	21 (6.3%)	37 (6.1%)
Urological	23 (10.3%)	0 (0.0%)	3 (7.5%)	14 (4.2%)	40 (6.6%)
Skin	11 (4.9%)	0 (0.0%)	2 (5.0%)	12 (3.6%)	25 (4.1%)
Multiple	5 (2.4%)	0 (0.0%)	0 (0.0%)	1 (0.3%)	6 (1.0%)
Other	11 (4.9%)	0 (0.0%)	1 (2.5%)	13 (3.9%)	25 (4.1%)
Metastases					
Absent	112 (50.2%)	9 (69.2%)	14 (35.0%)	155 (46.5%)	290 (47.6%)
Present	111 (49.8%)	4 (30.8%)	26 (65.0%)	178 (53.5%)	319 (52.4%)

Table 2. Cancer related information of patients with solid tumors. Cancer-specific information of the patients with solid tumors. Cancer patients were assigned to treatment cohorts based on type of treatment received when the first vaccination dose was administered.

	ChAdOx1 (N = 51)	Ad26.CoV2.S (N = 2)	mRNA-1273 (N = 17)	BNT162b2 (N = 81)	Overall (N = 151)
Treatment at first dose administration					
B-cell depletion	20 (39.2%)	0 (0.0%)	4 (23.5%)	36 (44.4%)	60 (39.7%)
Other hematological cancer treatments	31 (60.8%)	2 (100.0%)	13 (76.5%)	45 (55.6%)	91 (60.3%)
Type of hematological malignancies					
Acute lymphoblastic leukemia	1 (2.0%)	0 (0.0%)	0 (0.0%)	2 (2.5%)	3 (2.0%)
Acute myeloid leukemia	7 (13.7%)	0 (0.0%)	4 (23.5%)	5 (6.2%)	16 (10.6%)
Chronic lymphoblastic leukemia	6 (11.8%)	0 (0.0%)	0 (0.0%)	32 (39.5%)	38 (25.2%)
Chronic myeloid leukemia	2 (3.9%)	1 (50.0%)	0 (0.0%)	6 (7.4%)	9 (6.0%)
Hodgkin lymphoma	1 (2.0%)	0 (0.0%)	1 (5.9%)	4 (4.9%)	6 (4.0%)
Multiple myeloma	13 (25.5%)	0 (0.0%)	4 (23.5%)	16 (19.8%)	33 (21.9%)
Myelodysplastic syndrome	1 (2.0%)	0 (0.0%)	1 (5.9%)	4 (4.9%)	6 (4.0%)
Non-Hodgkin lymphoma	14 (27.5%)	0 (0.0%)	5 (29.4%)	6 (7.4%)	25 (16.6%)
Unknown	1 (2.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.7%)
Other	5 (9.8%)	1 (50.0%)	2 (11.8%)	6 (7.4%)	14 (9.3%)

Table 3. Type of hematological malignancies of the participants. Cancer-specific information of the patients diagnosed with hematological malignancies. Cancer patients were assigned to treatment cohorts based on type of treatment received when the first vaccination dose was administered. A distinction was made between patients receiving B-cell depleting therapy and all other treatments.

Breakthrough infections

A total of 174 patients (22.9%) were infected with SARS-CoV-2 during the study period of which 172 patients had detectable SARS-CoV-2 anti-S1 IgG antibodies prior to infection. Seven patients were infected multiple times, resulting in a total of 181 confirmed SARS-CoV-2 breakthrough infections. Breakthrough infections were observed between March 2021 and August 2022 and most frequently during the winter months. Infection numbers increased when the Omicron variant appeared in Belgium (November 2021). A peak of 21% of all reported breakthrough infections occurred in January 2022 alone. Six breakthrough infections were observed before completion of the primo-vaccination. After primo-vaccination, 15 breakthrough infections were reported. Lastly, a total of 107 and 53 breakthrough infections were recorded after respectively first and second booster

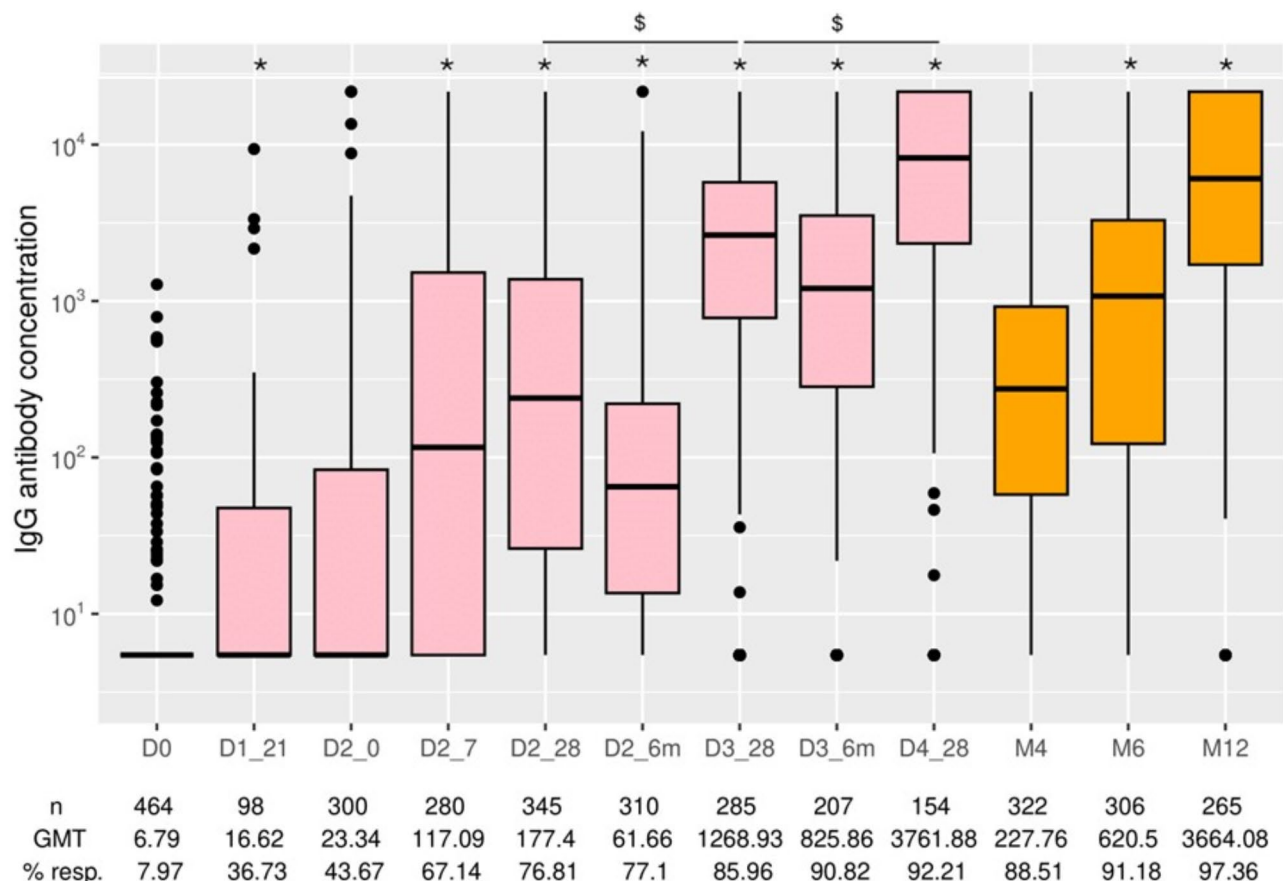


Fig. 3. SARS-CoV-2 anti-S1 IgG antibody titers over the vaccination course. Data is presented as boxplots over time. Inside each boxplot, median values are depicted as a black line and outliers are depicted as black dots outside the boxes. The x-axis shows the time point of sample collection, expressed in terms of days or months since the indicated vaccine dose (e.g. D1_21 refers to 21 days after first vaccine dose, D3_6m refers to 6 months after third vaccine dose). The y-axis represents log-scaled SARS-CoV-2 anti-S1 IgG antibody titers (BAU/mL). Anti-S1 IgG antibody titers were quantified using a SARS-CoV-2 Immunoassay, Siemens Healthineers Atellica IM SARS-CoV-2 S IgG (sCOVG) assay, for the detection of antibodies (BAU/mL). The number of observations per time point (n), geometric mean titer (GMT) and percentage of responders (with IgG titers larger than LLD) are displayed below the figure. * indicates $p < 0.05$ with previous time point. \$ indicates $p < 0.05$ between indicated time points. Note: pink boxes refer to consecutive time points at which measurements of the anti-S1 IgG concentration are taken while orange boxes refer to measurements of a subset of patients at time points 4 months, 6 months and 12 months after start of the study (first vaccine dose administration).

dose administration (Table 4). Five patients deceased as a result of COVID-19. These patients had no detectable anti-S1 IgG SARS-CoV-2 antibodies prior to infection.

The impact of logIgG titers (in each time interval separately) on the probability of breakthrough infection, and consequently on the classification of individuals (infected vs. non-infected), was examined first using a univariable logistic regression approach (Table 4). Median logIgG titers were observed to be lower in infected patients compared to non-infected ones within the time interval of interest. The differences in probability of breakthrough infections in relation to antibody titers are considered in a combined analysis later.

For time periods A to D (up to 28 days after second vaccine dose), see Fig. 2, only a very limited number of breakthrough infections was reported, preventing the estimation of optimal threshold values. In Table 4, the results of the time period specific analyses are shown with regard to classifying infected and non-infected individuals based on logIgG titers. Additionally the corresponding values were presented in binding antibody units per mL (BAU/mL).

The results in Table 4 suggest that the optimal cut-off value for classification of individuals (infected vs. non-infected) is around 150.00 BAU/mL, especially for time periods E and G. However, the optimal threshold value increases for time periods H and I. In addition, for each of the time periods the AUC is found to be higher than 0.5, indicating relatively good predictive ability of the model. Moreover, except for time periods G and H, sensitivity and specificity values are also well above 0.5.

To assess whether significant differences in mean logIgG titers exist between infected and non-infected individuals, a Tobit regression analysis was performed thereby explicitly accommodating that some of the IgG measurements are left- or right-censored due to the presence of both a lower and upper limit of detection.

Period	Description	N	Number of infections	Optimal threshold (log10) (95% CI)	Optimal threshold (BAU/mL) (95% CI)	AUC (95% CI)	Sensitivity	Specificity
A	Prior to the first dose – 21 days after the first dose	464	3	-		-	-	-
B	21 days after the first dose – Prior to the second dose	101	3	-		-	-	-
C	Prior to the second dose – 7 days after the second dose	301	1	-		-	-	-
D	7 days after the second dose – 28 days after the second dose	294	1	-		-	-	-
E	28 days after the second dose – Prior to the first booster	567	13	2.232 (0.736, 2.988)	170.610 (5.450, 972.280)	0.726 (0.600, 0.851)	0.846	0.552
F	Prior to the first booster – 28 days after the first booster	422	8	1.184 (1.054, 2.383)	15.280 (11.336, 241.326)	0.694 (0.542, 0.845)	0.625	0.785
G	28 days after the first booster – 6 months after the first booster	489	99	2.132 (1.993, 3.659)	135.520 (98.318, 4562.958)	0.657 (0.600, 0.715)	0.374	0.862
H	6 months after the first booster (second booster vaccine) – 28 days after the second booster	249	27	3.363 (1.792, 3.982)	2306.750 (61.912, 9593.526)	0.545 (0.429, 0.660)	0.741	0.392
I	28 days after the second booster – end of the study	350	26	3.632 (3.157, 4.008)	4285.490 (1436.184, 10193.680)	0.668 (0.561, 0.776)	0.731	0.627

Table 4. Definition of different time periods with associated sample size and infection occurrence. Optimal threshold for the logIgGs, area under the curve (AUC), BAU/mL sensitivity, and specificity are shown based on the univariable logistic regression approach. The green color represents the period before Omicron was dominant in the population and the orange color represents the period at which Omicron was the dominant SARS-CoV-2 variant. The rows in yellow define the period at which few infections are observed and no threshold estimation could be performed. The 95% confidence intervals (CIs) for the AUC are determined based on the DeLong formula⁶¹. For the optimal threshold, we rely on bootstrap-based 95% percentile CIs based on 1000 nonparametric bootstrap samples.

Evidence for a significant difference in mean logIgG titers between infected and non-infected patients has been found in time periods E, G and I. However other time periods also present a lower estimate for the mean logIgG titers in infected as compared to non-infected patients, these differences were not statistically significant at a 5% significance level (Table 5). Demographic details of infected and non-infected patients are presented in Suppl.

Period	Effect	Estimate (Std. Error)	P-value
E	Infected vs. non-infected	- 1.019 (0.331)	0.008
F	Infected vs. non-infected	- 0.754 (0.417)	0.142
G	Infected vs. non-infected	- 0.682 (0.132)	<0.001
H	Infected vs. non-infected	- 0.177 (0.227)	0.435
I	Infected vs. non-infected	- 0.701 (0.268)	0.027

Table 5. Tobit regression. Tobit regression results when modeling the log10-transformed anti-S1 IgG titers in relation to the infection status of patients by time period (see definition in Table 4). Parameter and standard error estimates together with corresponding two-sided Bonferroni-Holm adjusted *p*-values are presented. Left and right censoring at log10 IgG level equals to 1.037 and 4.214, respectively.

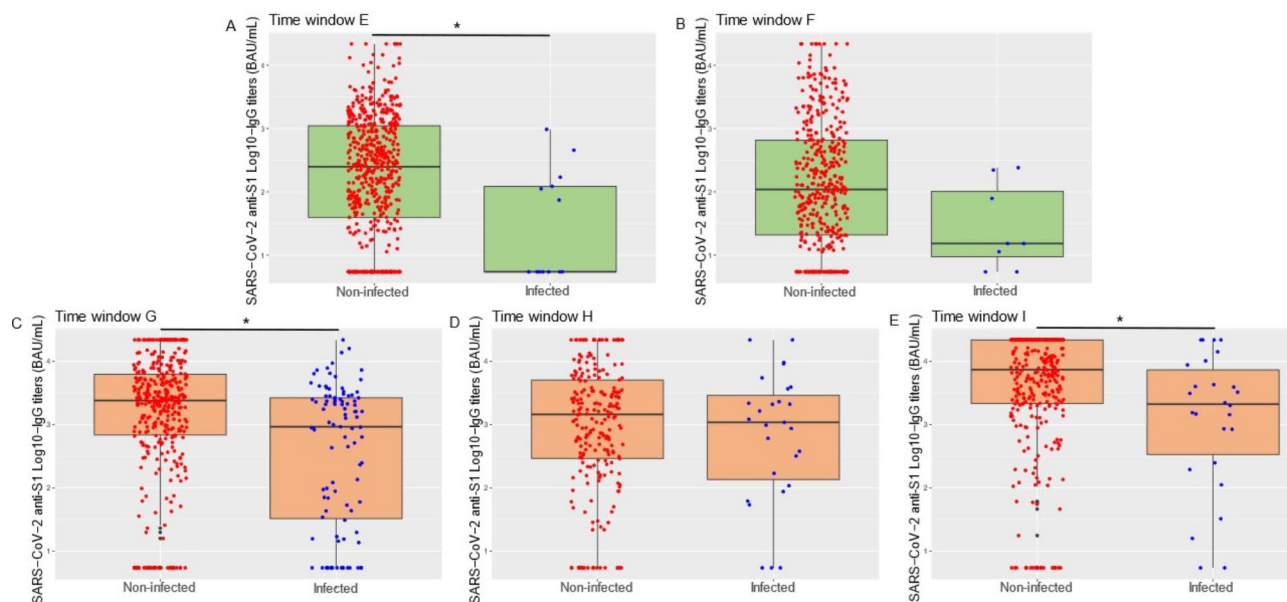


Fig. 4. SARS-CoV-2 anti-S1 IgG antibody titers for infected vs. non-infected cancer patients for different time periods. Boxplots represent log10-transformed SARS-CoV-2 anti-S1 IgG antibody titers for cancer patients infected with SARS-CoV-2 (blue) and non-infected patients (red) during the indicated time periods. A two-sided *p*-value < 0.05 after Bonferroni-Holm correction for multiple testing was considered statistically significant: **p* < 0.05. (A) SARS-CoV-2 anti-S1 IgG antibody titers between 28 days after second vaccine dose and prior to first booster dose (time period E). (B) SARS-CoV-2 anti-S1 IgG antibody titers between first booster administration and 28 days afterwards (time period F). (C) SARS-CoV-2 anti-S1 IgG antibody titers between 28 days and 6 months after first booster dose (time period G). (D) SARS-CoV-2 anti-S1 IgG antibody titers between 6 months after first booster dose and second booster administration (time period H). (E) SARS-CoV-2 anti-S1 IgG antibody titers between 28 days after second booster administration and the end of the study (time period I).

Tables 1 and 2. A total of 15 patients did not have sufficient information, for example as a consequence of dying early on in the study, to be included in the final analysis. Therefore the final analysis contains data of 745 patients.

These data are graphically depicted in Fig. 4. Lower mean logIgG titers were observed in infected as compared to the mean logIgG titers among non-infected patients in the respective time periods. This trend can be observed in all time periods, despite increased mean logIgG titers in both non-infected and infected patients over time. Across the different time periods, there are infected patients mounting high SARS-CoV-2 anti-S1 IgG antibody titers, indicating that breakthrough infections are possible despite the presence of high antibody titers prior to infection.

Model parameter and standard error estimates obtained using the GEE approach with binary endpoint (infection status) and logit-link function are presented in Suppl. Table 1. In general, an increase in logIgG titers leads to a decrease in probability of breakthrough infection. The effect of logIgG titers on the probability of breakthrough infection is not significantly different between period E (reference period) and periods F, G, H and I. These results are in line with earlier results (Suppl. Table 2).

Based on Quasi-likelihood under the Independence model Criterion (QIC), a model without interaction between time and antibody titers is preferred, thereby simplifying the interpretation. Consequently, parameter estimates for the reduced model are presented in Table 6. As expected, a significant increase in probability of

Parameter	Estimate (robust s.e.)	P-value
Intercept	– 2.814 (0.356)	< 0.001
LogIgG	– 0.462 (0.074)	< 0.001
Period E	Reference	–
Period F	– 0.255 (0.467)	0.580
Period G	2.769 (0.317)	< 0.001
Period H	1.987 (0.357)	< 0.001
Period I	1.844 (0.340)	< 0.001

Table 6. Results from final model selected based on QIC implying no interaction effect between antibody titers and time. The probability of SARS-CoV-2 breakthrough infection is modelled in relation to log10-transformed antibody titer and time period (i.e., time period E (reference) up to time period I) without interaction effect between these variables.

Period	Optimal threshold (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Threshold before and after period H (log10) (95% CI)	Threshold (BAU/mL) (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
E	2.332 (0.754, 3.171)	0.846 (0.563, 0.966)	0.525 (0.484, 0.567)	1.310 (1.190, 2.128)	20.420 (15.488, 134.122)	0.538 (0.292, 0.767)	0.796 (0.760, 0.827)
F	1.198 (1.038, 3.305)	0.625 (0.304, 0.862)	0.785 (0.743, 0.822)	1.310 (1.190, 2.128)	20.420 (15.488, 134.122)	0.625 (0.304, 0.862)	0.758 (0.715, 0.797)
G	2.144 (2.027, 3.84)	0.374 (0.285, 0.472)	0.862 (0.823, 0.892)	1.310 (1.190, 2.128)	20.420 (15.488, 134.122)	0.242 (0.169, 0.336)	0.897 (0.863, 0.924)
H	3.379 (1.930, 4.119)	0.741 (0.550, 0.869)	0.392 (0.330, 0.458)	3.640 (3.220, 4.010)	4365.160 (1659.587, 10232.930)	0.815 (0.627, 0.921)	0.261 (0.208, 0.323)
I	3.634 (2.494, 4.125)	0.731 (0.536, 0.864)	0.627 (0.573, 0.578)	3.640 (3.220, 4.010)	4365.160 (1659.587, 10232.930)	0.731 (0.536, 0.864)	0.627 (0.573, 0.678)
		Overall performance				0.445 (0.373, 0.520)	0.717 (0.697, 0.736)

Table 7. Optimal threshold values for the LogIgG and corresponding anti-S1 IgG titers based on the combined QIC approach. Presentation of the optimal threshold values for the log-transformed SARS-CoV-2 anti-S1 IgG values and corresponding anti-S1-IgG antibody titers based on the combined QIC approach when modelling the probability of SARS-CoV-2 breakthrough infection in relation to antibody titers and time period (i.e., time period E (reference) up to time period I), though maximizing the Youden index for each of the periods separately. Different colors are used to indicate the periods for which the same threshold is assumed. For the column “Threshold before and after period H” we explicitly estimate threshold values in the period before and after circulation and dominance of Omicron infections in the population. The 95% CIs for sensitivity and specificity are Wilson Score intervals for proportions. For the thresholds, we rely on bootstrap-based 95% percentile CIs based on 1000 nonparametric bootstrap samples.

breakthrough infection is found with decreasing logIgG titers. Although no significant difference in baseline probability of infection between periods E and F was observed, periods G, H and I show a higher probability of infection as compared to the reference period ($p < 0.001$).

Correlate of protection

Using the final model based on QIC, classification of individuals in non-infected and infected patients led to a sensitivity and specificity of 0.827 (bootstrap-based 95% percentile CI: 0.631, 0.878) and 0.630 (bootstrap-based 95% percentile CI: 0.601, 0.818), respectively. Furthermore, the AUC is equal to 0.787 (bootstrap-based 95% percentile CI: 0.756, 0.818). However, this approach leads to different threshold values across different time periods due to differences in baseline infection risk. Alternatively, thresholds per time period can be derived based on maximizing the Youden index per time period (Table 7).

Thresholds in time periods E and F on one hand, and periods H and I on the other, are very similar leading to sensitivity and specificity values which are moderate to high (Table 7). However, the period with the highest number of breakthrough infections (time period G), shows a suboptimal sensitivity value, at least if classification is based on a common threshold value of 20.42 BAU/mL across time periods E to G (columns 5 to 8 in Table 7). In general, good discrimination between infected and non-infected individuals based on logIgG titers seems impossible (with low to moderate AUC values ranging between 0.544 and 0.726 for the different time windows – see Suppl Fig. 6), which is especially the case in the transition periods G and H (transition between Delta and Omicron variant). Additionally, the threshold values obtained for periods E, F and G are lower as compared to the ones for the later periods, indicating that higher anti-S1 IgG titers are required in periods H and I to be protective against breakthrough infections. These time periods correspond to periods in which breakthrough infections with the Omicron variant were dominant, indicating that a higher anti-S1 IgG titer is required to

Period	Cohort	N	Number of cases	Sensitivity	Specificity	AUC	Optimal threshold (log10)	Optimal threshold (BAU/mL)
E	Chemotherapy	254	2	1.000	0.560	0.738	2.090	123.03
	Hematology	109	7	0.857	0.529	0.709	0.736	5.45
F	Chemotherapy	176	5	1.000	0.509	0.671	2.380	239.88
	Hematology	86	1	1.000	0.506	0.753	0.736	5.45
G	Chemotherapy	198	37	0.838	0.547	0.736	3.400	2511.87
	Hematology	109	30	0.733	0.532	0.547	2.130	134.90
H	Chemotherapy	80	8	0.625	0.500	0.490	3.360	2290.87
	Hematology	59	4	1.000	0.491	0.764	2.230	169.82
I	Chemotherapy	135	9	0.556	0.643	0.593	3.630	4265.80
	Hematology	93	6	0.833	0.598	0.680	2.930	851.14

Table 8. SARS-CoV-2 breakthrough infections in chemotherapy and hematology treatment subgroups. Performance of classification based on univariate logistic regression models stratified by treatment group and time period.

prevent a cancer patient from Omicron infection. Finally, the threshold was determined, maximizing the Youden index across all time periods with the same thresholds before and after initiation of the Omicron dominance in period H. This resembles the change in predictive ability of the logIgG titer about the probability of breakthrough infection.

In order to perform a sensitivity analysis, periods E and F (prior to the emergence of Omicron) as well as time periods G, H and I (with Omicron present) were combined. The overall sensitivity and specificity values were 0.815 and 0.605, respectively. Optimal threshold values were found to be equal to 15.85 and 4570.88 BAU/mL for the two periods considered (Suppl. Table 3). This led to an improvement in sensitivity for period G, however, at the cost of a lower specificity (i.e. 0.321 as compared to 0.897 as reported in Table 7).

Treatment-specific differences

In order to investigate whether threshold values differ upon types of anti-neoplastic treatment, treatment-specific subanalyses were performed for the relevant time periods (E–I) (Table 8). However, this was complicated by the low number of cases in some of the treatment groups. The largest amount of breakthrough infections occurred in the patient groups receiving chemotherapy and in patients with hematological malignancies not receiving B-cell depleting therapy. Therefore, threshold values were determined based on a univariable logistic regression approach per time period for the respective treatment groups (i.e., the subgroups having the largest number of breakthrough infections).

In periods E and F, the observed optimal threshold values for patients receiving chemotherapy are higher compared to the threshold for patients with hematological malignancies. Similar to the observations in the entire patient cohort, threshold values increase from period G onwards (with the largest number of infections in both groups observed in period G) with sensitivity values being high, though specificity levels are around 0.5. In general, upon the emergence of the SARS-CoV-2 Omicron variant, threshold values increase considerably.

Discussion

The current study provides valuable insights in the dynamics of SARS-CoV-2 anti-S1 IgG antibody titers following COVID-19 vaccination and SARS-CoV-2 breakthrough infections in cancer patients. In line with previous observations, this study revealed that cancer patients mount limited humoral immune responses after primo-vaccination^{12,35}. Moreover, SARS-CoV-2 anti-S1 IgG antibody titers significantly waned six months after primo-vaccination in our study population, which was also observed by Chong et al. in a nationwide cohort study, including cancer patients and healthy individuals, performed in Singapore³⁶. To improve protection against SARS-CoV-2 infection, booster doses were broadly administered. Our data indicate the added value of COVID-19 booster vaccinations as both the percentage of vaccine responders and the SARS-CoV-2 anti-S1 IgG antibody titers gradually increased over time as more vaccination doses were administered. This observation is confirmed by other studies in which humoral immunity is boosted by the administration of additional vaccination doses, even in immunocompromised patients^{28,36–40}. More specifically, we observed that the administration of a first booster dose is able to elicit higher SARS-CoV-2 anti-S1 IgG antibody titers compared to primo-vaccination and that the administration of a second booster further increased antibody titers in cancer patients. This is in line with the results of other studies in immunocompromised patients^{26,28,41–43}.

Although COVID-19 vaccination initially provided a solid protection against SARS-CoV-2 infection, we observed a drastic increase in the amount of breakthrough infections with the emergence of the Omicron variant. Several studies also indicated reduced vaccine efficacy against Omicron compared to the wild type Wuhan-1 variant, leading to an increased number of breakthrough infections^{9,10,19,44}. However, besides viral profile, immunity characteristics (mucosal vs. systemic immunity, duration of immunity, etc.) might also play an important role in the occurrence of breakthrough infections¹⁰. Supporting this statement, we clearly observe lower mean logIgG titers in infected individuals prior to SARS-CoV-2 infection as compared to non-infected patients. This observation is confirmed by two studies in hematological cancer patients and one in healthy individuals in which similar findings are described^{45–47}.

Some studies indicated that higher SARS-CoV-2 antibody titers are associated with increased vaccine efficacy and reduced risk for symptomatic COVID-19^{48–50}. These observations indicate the potential of an anti-SARS-CoV-2 antibody-based CoP against SARS-CoV-2 breakthrough infections^{51,52}. However, it was unsure what amount of SARS-CoV-2 anti-S1 IgG antibodies is needed in order to be protected against breakthrough infection. Therefore, we aimed to define an antibody-based CoP (i.e., level of humoral immunity required to prevent infection) against SARS-CoV-2 in cancer patients. Few studies were able to identify a serological CoP. Another study performed in hematological cancer patients stated that a serological cut-off titer of 250 BAU/mL could be predictive for breakthrough infection⁴⁵. However, this study comprises the use of different serological tests, which complicates the interpretation of these results. A study performed by Goldblatt and coworkers in 122 healthy adults identified a protective SARS-CoV-2 IgG anti-Spike antibody titer of 154 BAU/mL against the ancestral Wuhan-1 variant⁵³. The mixed results of different studies regarding a CoP against SARS-CoV-2 infection, with lack of standardization between laboratory methodology, assay targets and time points of sampling, complicate comparison and interpretation. Classification of individuals could be improved based on the inclusion of additional covariate information, including, for example, age, gender, tumor types or primo-vaccination types, that would improve the prediction rule, thereby potentially resembling differences in infection risk induced by differences in risk behavior. Here, we primarily focused on studying whether a clear protective level of antibodies exists, preventing breakthrough infections after COVID-19 vaccination. Threshold values are estimated to be higher after the emergence of Omicron, which is supported by an increased amount of Omicron breakthrough infections despite reduced testing frequency. However no definite CoP could be identified post-Omicron. Despite a high sensitivity based on the optimal cut-off determined for these periods, the specificity associated therewith is low in some periods. Although optimal threshold values are different between time periods in the current study, some evidence exists with respect to a protective level of anti-S1 IgG titers, at least in the period before the emergence and establishment of Omicron breakthrough infections in the population. Before the emergence of the Omicron variant in Belgium, an anti-S1IgG antibody titer above 20.42 BAU/mL was found protective against SARS-CoV-2 breakthrough infection. This observation indicates that cancer patients whom are able to mount a detectable antibody response against SARS-CoV-2 (SARS-CoV-2 anti-S1 IgG antibody titer > 10.9 BAU/mL), might be better protected against SARS-CoV-2 breakthrough infection. Additionally, it needs to be considered that different thresholds across different time periods could be attributed to the emergence of different circulating variants as well as differences in cellular immunity, which are not considered in this work.

In previous research we observed that the type of anti-neoplastic treatment impacts humoral immune outcomes after COVID-19 vaccination^{12,27}. This raises the question whether optimal CoP threshold values are different among types of anti-neoplastic treatment. In order to address this question, we performed a sub-analysis in which we aimed to compare threshold values between different treatment groups. Since this was complicated by the low number of cases in some of the treatment groups, this analysis was only performed in the patient groups with the largest amount of breakthrough infections, respectively chemotherapy and patients with hematological malignancies not receiving B-cell depleting therapy. Interestingly, these are the patient cohorts in which we previously observed reduced antibody titers after primo-vaccination compared to healthy controls and other treatment types¹². Additionally, for all appointed time periods, the CoP in the chemotherapy cohort was found to be higher compared to the hematology group. The studied patients with hematological malignancies not receiving B-cell depleting therapy were largely patients that have received an allogeneic hematopoietic stem cell transplantation (HSCT) in the past. The date of HSCT must have been at least one year prior to study inclusion, in order to be eligible for study participation. One year post HSCT, the immune system is already partially recovered^{54,55}, indicating that these patients are more immunocompetent compared to patients receiving chemotherapy, which is known to significantly affect the immune system^{56,57}. Although the small number of breakthrough infections per treatment group per time period should be taken into account and further research with larger groups is required, this observation might indicate that cancer patients receiving chemotherapy have an increased risk for SARS-CoV-2 breakthrough infection compared to patients with hematological malignancies (not receiving B-cell depleting therapy). Additionally, studying differences between different types of solid tumors is considered an important topic for further research.

Additional analyses are incorporated based on a different selection of time periods under study (Suppl. Table 3). The results are in line with those presented in Table 7 and indicate a clear increase in threshold values after the emergence of the Omicron variant. The performance of the prediction rule, based on the individual-level log antibody concentrations prior to infection, is quantified based on the training data, hence, the predictive performance could be overestimated. Further studies could therefore opt to implement an optimism-adjusted performance measures (e.g., optimism-adjusted AUC) to adjust for this limitation. This analysis covers SARS-CoV-2 breakthrough infections in a large cohort of cancer patients. However, it did not include information about infection severity and self-reported infections. Despite the fact that cancer patients were frequently screened for SARS-CoV-2 infections, the reported numbers might be an underestimation. It needs to be considered that PCR-confirmed SARS-CoV-2 infections prior to vaccination were included in the model and accounted for the first time period, though only if anti-S1 IgG titers were available for subsequent analysis. Although this study aimed to identify an easily measurable antibody-based CoP, the lack of information on neutralizing antibodies and cellular immunity should be considered since several papers highlighted their role in protection^{58–60}. Finally, a limitation of this analysis is the assumption of the predictive value of the anti-S1 IgG titers at some point in time prior to infection without explicitly accounting for waning of humoral immunity and the time in between blood sampling and the occurrence of a breakthrough infection. An alternative modeling study would therefore include a model for the anti-S1 IgG dynamics in combination with, for example, a time-to-event model for the time to breakthrough infection in relation to the time-dependent IgG level. This is an avenue for further research as it could be more reliable to assess whether a protective level of humoral immunity exists.

Conclusion

This study provides evidence with respect to a protective IgG titer against SARS-CoV-2 breakthrough infection in cancer patients. Before the emergence of the Omicron variant in Belgium, an anti-S1 IgG titer above 20.42 BAU/mL was found protective. Protective thresholds are estimated to be higher after the emergence of Omicron, but no clear CoP could be identified. Additionally, the type of anti-neoplastic treatment impacts SARS-CoV-2 anti-S1 IgG antibody threshold values needed for protection against SARS-CoV-2 infection.

Data availability

While these data were generated under license for the current studies and are not publicly available, they can be made accessible upon reasonable request. To obtain access, interested parties should contact the corresponding author Prof. Timon Vandamme.

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Declarations

Competing interests

TV reports consultancy, advisory roles and honoraria from AstraZeneca outside the scope of presented work. The other authors report no conflicts of interest.

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee Antwerp University Hospital (EC nos. 2021–0543 dd. 01/02/2021, 2021–0541 dd. 09/08/2021, 21/12/172 dd. 17/05/2021).

Informed consent

Informed consent was obtained from all subjects involved in the studies.

Additional information

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