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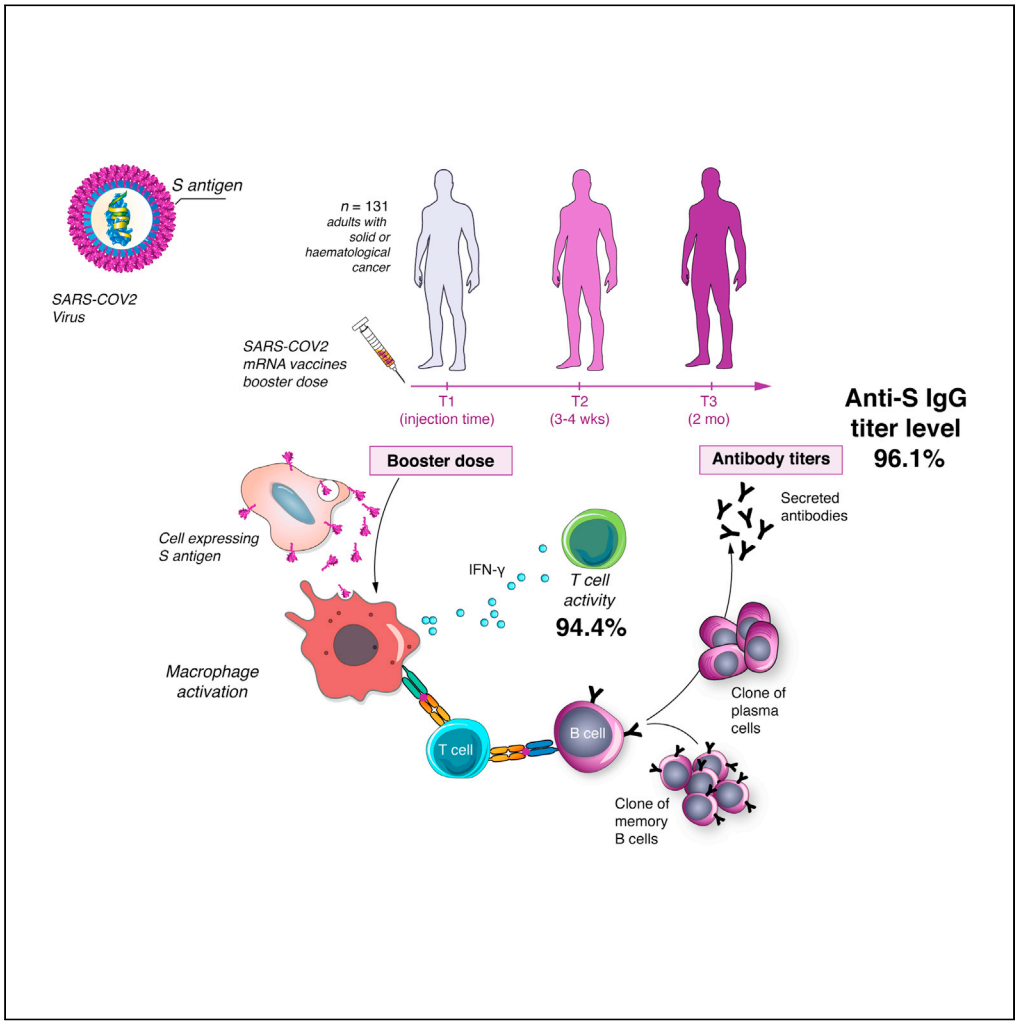

Natacha Bordry, Alfredo Addeo, Cyril Jaksic, ..., José Sandoval, Pierre-Yves Dietrich, Nicolas Mach

alfredo.addeo@hcuge.ch

Highlights

Seroconversion remains high at two months after the second vaccine dose in patients with cancer

Two doses of mRNA vaccine allow effective protection, with a low infection incidence in our cohort

mRNA vaccination induces T cell activation especially among patients who seroconverted

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Humoral and cellular immunogenicity two months after SARS-CoV-2 messenger RNA vaccines in patients with cancer

Natacha Bordry,[1,6] Alfredo Addeo,[1,6,7,*] Cyril Jaksic,[2] Valérie Dutoit,[1] Pascale Roux-Lombard,[3] Dimpy P. Shah,[4] Pankil K. Shah,[4] Angèle Gayet-Ageron,[2] Alex Friedlaender,[1,5] Sébastien Bugeia,[1] Garance Gutknecht,[1] Anna Battagin,[1] Maragrazia Di Marco,[1] Pierre-Francois Simand,[1] Intidhar Labidi-Galy,[1] Sarah Fertani,[1] José Sandoval,[1] Pierre-Yves Dietrich,[1] and Nicolas Mach[1]

SUMMARY

Little is known on the long-lasting humoral response and the T cell activation induced by SARS-CoV-2 mRNA vaccines in patients with cancer. The study assessed the efficacy of the SARS-CoV-2 mRNA vaccines through measuring the seroconversion rate at pre-specified time points and the effect on the T cell immunity in patients with cancers. The study included 131 adult patients with solid or hematological cancer, who received SARS-CoV-2 mRNA vaccines. 96.2% of them exhibited adequate antibody response to the SARS-CoV-2 mRNA vaccines 2 months after the booster dose. SARS-CoV-2 mRNA vaccines could induce T cell activation; however, this is more likely in patients who have a positive seroconversion (94%) compared with the patients who did not (50%). Further research into the clinical relevance of low antibodies titers and lack of T cell activity is required to set up an effective vaccination strategy within this group of patients.

INTRODUCTION

Messenger RNA (mRNA)-based vaccines have changed the outlook of the COVID-19 pandemic (Regev-Yochay et al., 2021). However, clinical trials leading to regulatory authority approval of these vaccines included very few patients with cancer, and patients on active therapy were excluded (Polack et al., 2020).

Several studies in patients with solid and hematological malignancies (Addeo et al., 2021; Goshen-Lago et al., 2021; Thakkar et al., 2021) have shown that the seroconversion rate, at a minimum of 3 weeks after the second dose, ranged from 90% to 95% in patients with solid tumors and 70% to 75% in patients with hematological malignancy (Polack et al., 2020; Voysey et al., 2021). Studies of acute and convalescent COVID-19 patients highlighted that SARS-CoV-2-specific T cell responses are significantly associated with milder disease (Grifoni et al., 2020; Sekine et al., 2020).

Here, we report data on humoral immune responses after SARS-CoV-2 mRNA vaccination among patients with solid and hematological malignancy. We measured seroconversion rates and antibody titers 60 days after the booster dose, as well as T cell responses in two pre-specified cohorts of patients.

RESULTS

Clinical characteristics of the study cohort

We recruited 131 patients with cancer who received either BNT162b2 or mRNA-1273 vaccines at the Geneva University Hospital (HUG). All patients were SARS-CoV-2 naive, as determined by a negative anti-SARS-CoV-2 nucleocapsid (N) protein IgG test at baseline. One patient developed COVID-19 4 weeks after the second dose. That patient did not show any seroconversion at T2 and was on anti-CD20 treatment. Thus, 130 patients were included in the final analysis.

Patient characteristics are described in Table S1. There was almost equal proportion of males (56.2%) and females (43.8%). Most malignancies were solid tumors (82.2%), with gastrointestinal (17.7%), thoracic

[1]Department of Oncology, Geneva University Hospital, University of Geneva and Swiss Cancer Center Leman, Rue Perret-Gentil 4, 1205 Geneva, Switzerland

[2]CRC& Division of Clinical Epidemiology, Department of Health and Community Medicine, University of Geneva and Geneva University Hospital, Geneva, Switzerland

[3]Immunology and Allergology Laboratory, Geneva University Hospital, University of Geneva, 1205 Geneva, Switzerland

[4]Mays Cancer Center at UT Health San Antonio MD Anderson, San Antonio, TX, USA

[5]Oncology Department, University Hospital of Geneva, Switzerland, Clinique Générale Beaulieu, Geneva, Switzerland

[6]These authors contributed equally

[7]Lead contact

*Correspondence: alfredo.addeo@hcuge.ch

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Table 1. Seroconversion after SARS-CoV-2 mRNA vaccination at 3 different time points

	Time point 1		Time point 2		Time point 3	
	n (%)	p value (chi2)	n (%)	p value (chi2)	n (%)	p value (chi2)
Overall	101/130 (77.7)		121/130 (93.1)		125/130 (96.2)	0.009[b]
Genre		0.213		0.515[a]		0.999[a]
Female	49/57 (86.0)		55/57 (96.5)		55/57 (96.5)	
Male	52/73 (71.2)		66/73 (90.4)		70/73 (95.9)	
Age		0.035		0.153[a]		0.350[a]
65 and older	35/54 (64.8)		47/54 (87.0)		50/54 (92.6)	
younger Than 65	66/76 (86.8)		74/76 (97.4)		75/76 (98.7)	
Type of malignancy		0.371		0.009[1]		0.021[a]
Solid tumor	86/107 (80.4)		104/107 (97.2)		106/107 (99.1)	
Hematologic malignancy	15/23 (65.2)		17/23 (73.9)		19/23 (82.6)	
Anti-cancer therapy		0.009[a]		0.280[a]		0.371[a]
Anti-CD20	0/2 (0)		1/2 (50.0)		1/2 (50.0)	
Chimio-immunotherapy	7/9 (77.8)		7/9 (77.8)		8/9 (88.9)	
Clinical surveillance	32/38 (84.2)		36/38 (94.7)		37/38 (97.4)	
Cytotoxic chemotherapy	20/36 (55.6)		32/36 (88.9)		34/36 (94.4)	
Endocrine therapy	14/14 (100)		14/14 (100)		14/14 (100)	
Immunotherapy	10/11 (90.9)		11/11 (100)		11/11 (100)	
Kinase inhibitor	13/15 (86.7)		15/15 (100)		15/15 (100)	
Monoclonal antibody	5/5 (100)		5/5 (100)		5/5 (100)	
Stage		0.689		0.515[a]		0.999[a]
Localized	43/53 (81.1)		51/53 (96.2)		51/53 (96.2)	
Metastatic	58/77 (75.3)		70/77 (90.9)		74/77 (96.1)	
Vaccine		0.999[a]		0.999[a]		0.999[a]
BNT162b2	16/21 (76.2)		20/21 (95.2)		21/21 (100)	
mRNA-1273	85/109 (78.0)		101/109 (92.7)		104/109 (95.4)	

[a]Fisher's exact test.
[b]Comparison among the three time points.

(15.4%), and breast cancers (14.6%) being the most common solid tumor types. Twenty-three (17.8%) patients had hematological malignancy. Thirty-eight patients (29.2%) did not receive anti-cancer therapy within 6 months prior to COVD-19 vaccination. The most common anti-cancer therapy received by this cohort of patients was cytotoxic chemotherapy (27.7%), followed by kinase inhibitors (11%), endocrine therapy (10.8%), immunotherapy (8.5%), chimio-immunotherapy (6.9%), monoclonal antibody (3.8%), and anti-CD20 therapy (1.5%).

Median (IQ1/IQ3) anti-S IgG titer at baseline, T1 (time of second vaccine dose), T2 (3–4 weeks after the second vaccine dose), and T3 (60 days after second vaccine dose) were 18.2 (1.5/94.3), 2285.5 (407.5/2501), and 1327.5 U/mL (405.0/2501.0), respectively. Twenty-seven patients (20.7%) had QuantiFERON T cell analysis (Table S2).

Serological outcome after SARS-CoV-2 mRNA vaccination at three different time points

There was no statistically significant difference in the seroconversion rates by gender, age, vaccine, or tumor stage (Table 1). The seroconversion rate was 93% (121/130) at T2 and rose to 96.2% (125/130) at T3. Among the 9 patients who were seronegative at T2, 4 (44.4%) showed spontaneous seroconversion at T3 without any sign of contact with SARS-CoV-2, as anti-N IgG remained undetectable.

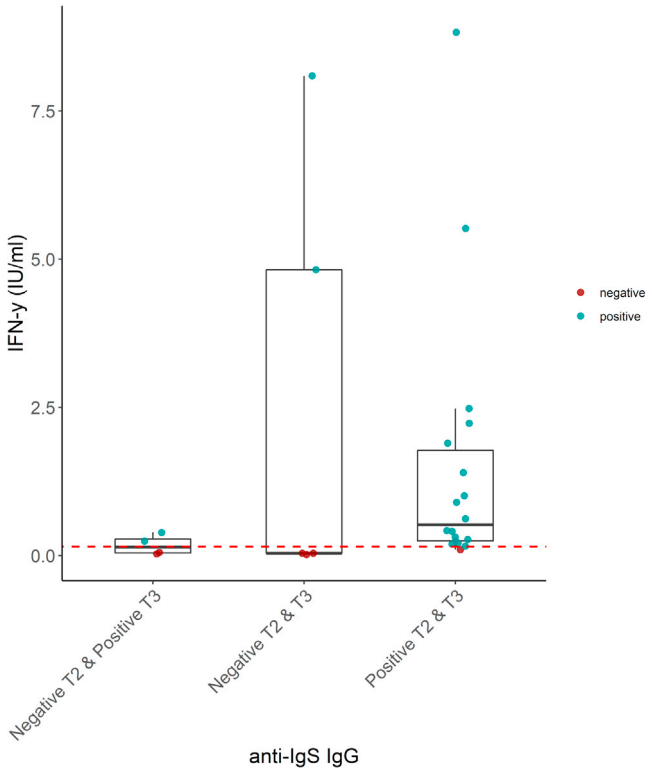

Figure 1. Interferon-gamma (IFN-γ) concentration with regard to serology results at time points 2 and 3

The threshold of 0.15 UI/mL is represented by the dashed line. Green dots represent patients with positive QuantiFERON values (COVT2IFN-γ>0.15 IU/mL) and red dots represent patient with negative QuantiFERON values (COVT2IFN-γ>0.15 IU/mL). Boxplot showing median (horizontal bar), 25th and 75th quartiles, and error bars depicting highest and lowest values.

The seroconversion rate was significantly lower in patients with hematological malignancy, 73.9% (17/23) at T2 and 82.6% (19/23) at T3 as compared to 97.2 (104/107) and 99.1% (106/107) in patients with solid tumors ($p = 0.009$ and 0.02, respectively). Only 1 out of 2 patients receiving anti-CD20 therapy (50%) produced anti-S IgG antibodies at T3. Other treatments, including chemotherapy, endocrine therapy, or immunotherapy (immune checkpoint inhibitors), had no discernible impact on the seropositivity rate at T3.

IFN-γ concentration in two pre-specified cohorts

We then measured T cell activity, by positive IFN-γ levels, in two pre-specified cohorts of patients. Cohort 1 consisting of patients who did not seroconvert 3–4 weeks after second vaccine dose (T2) (n = 9) and cohort 2 consisting of patients who had the highest antibody titers at T2 (n = 18). T cell activity was detected in 4/9 patients (44.6%) within cohort 1 at T3. Within cohort 2, 17/18 patients (94.4%) showed T cell activation at T3 through a strong QuantiFERON signal (Figure 1). Higher QuantiFERON values were seen in hematological patients once compared to solid cancer patients although this difference was not significant ($p = 0.664$) (Figure 2).

DISCUSSION

We showed high humoral immune response to mRNA SARS-CoV-2 vaccines among patients with solid and hematological malignancies even at 60 day from the second dose. Solid tumors were associated with significantly higher seroconversion rates than hematological malignancies at T2 and T3. The study showed that the seroconversion rate increased from 93.1% (T2) to 96.1% at T3 highlighting that mRNA vaccines can induce rapid and delayed durable seroconversion.

Within our study, T cell activation occurred in the vast majority of the cases (77.7%), more frequently in patients who had high anti-S IgG titer level (94.5%) compared to patients who had no antibodies or

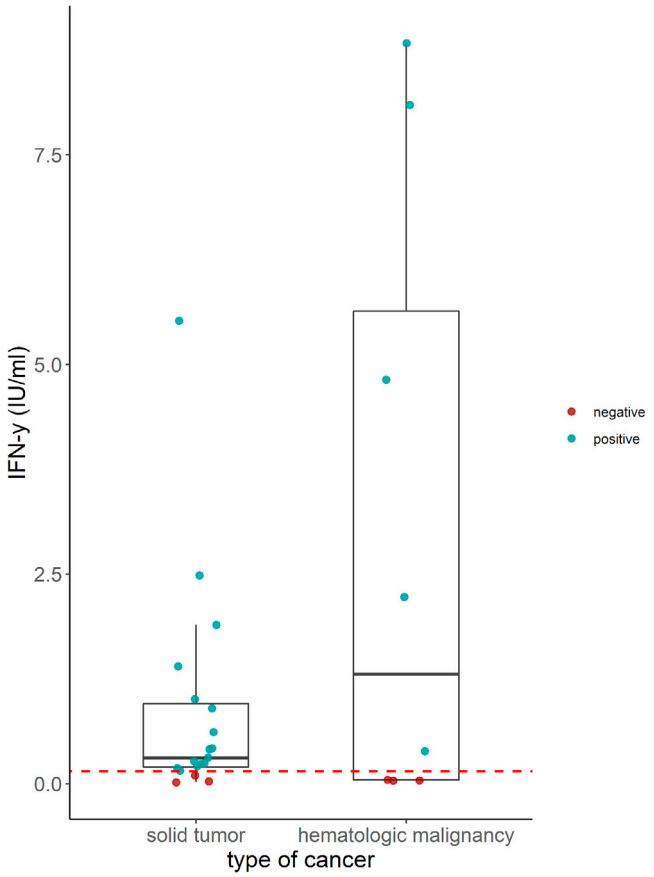

Figure 2. Interferon-gamma (IFN-γ) concentration in solid and hematological malignancy at time point 3

The threshold of 0.15 is represented by the dashed line. Green dots represent patients with positive QuantiFERON values (COVT2IFN-γ>0.15 IU/mL) and red dots represent patient with negative QuantiFERON values (COVT2IFN-γ>0.15 IU/mL). Boxplot showing median (horizontal bar), 25th and 75th quartiles, and error bars depicting highest and lowest values.

seroconverted at T3 (44.6%) Furthermore, despite the fact that the delta SARS-CoV-2 variant has become predominant in Geneva, only 1 patient within our study group (1/131) was infected and had mild COVID-19.

In conclusion, our data show that seroconversion rates remain high 60 days after the second vaccine dose in patients with cancer, that T cell activation is present in most of cases, and that the level of protection remains very high (only 1 case of COVID-19 in the whole study group). Given the recent FDA and EMA authorization for third doses in immunocompromised patients, we believe more data on the relevance and impact of cellular immunity are warranted to set up new guidance on vaccination strategies for patients with cancer.

Limitations of the study

Despite these strengths, the present study had several limitations. Firstly, one of the possible limitations might be how to interpret the anti-S-IgG antibodies level and T cells activity and degree of protection they could offer to patient with cancers. We have no clear data so far to claim that patients with higher antibodies level are more protected than the others and whether a certain cutoff should be used, for example, to consider a third dose of vaccine. Equally, we have no data to claim that T cell activation on its own could offer a real protection from SARS-COV-2 infection. However, only 1 patient out of 131 (0, 76%) in our fully vaccinated patients got mild COVID-19.

Secondly, the sample size of the seronegative cohort (patient who did not develop any SARS-CoV-2 IgG antibody) in our study is relatively small due to the efficacy of the vaccines. T cell response has therefore been evaluated on a small subgroup of patients.

Finally, the SARS-CoV-2 specific T cell assay has not yet been approved for clinical use but it provides reliable data on the T cell response although it does not evaluate the full T cell repertoire.

Larger validation prospective cohort studies assessing humoral and cellular immunity on non-vaccine responders are needed.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.103699.

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AUTHOR CONTRIBUTIONS

Concept and design: NB, PYD, AA, NM. Acquisition, analysis, or interpretation of data: NB, AA, NM, PRL. Statistical analysis: AGA, CJ. Drafting of the manuscript: NB, AA, NM. Critical revision of the manuscript for important intellectual content: All co-authors. Obtained funding: NB, PYD, AA, NM. Administrative, technical, or material support: PRL. Supervision: AA, NM.

DECLARATION OF INTERESTS

AA reported receiving personal fees for attending advisory from Bristol-MyersSquibb, AstraZeneca, Roche, Pfizer, Merck Sharp and Dohme, Astella, Eli Lilly, and Boehringer-Ingelheim and receiving fees for speaking bureau for Eli Lilly, AstraZeneca, MSD for work performed outside of the current study. NM is a founder and minority shareholder of MaxiVAX SA, a private biotech company based in Geneva, Switzerland, working on personalized cancer immunotherapy and infectious disease vaccines, with no impact on the current manuscript. PS reported receiving grant from the Biomedical Advanced Research and Development Authority outside of this work. All other co-authors reported no competing interests.

INCLUSION AND DIVERSITY

We worked to ensure gender balance in the recruitment of human subjects. We worked to ensure ethnic or other types of diversity in the recruitment of human subjects.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Serum sample	Patients recruited in this study	In this study
Critical commercial assays		
Elecsys® Anti-SARS-CoV-2 Nucleocapsid	Roche	Catalognumber 7304
Elecsys® Anti-SARS-CoV-2 Spike	Roche	Catalognumber 3608
QuantiFERON ELISA, Human IFN-γ SARS-CoV-2	Quiagen	Catalognumber 626410
Software and algorithms		
R 4.0.5	https://www.r-project.org/	https://www.r-project.org/
Other		
Clinical data	Electronic medical record	Study ID

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Alfredo Addeo, alfredo.addeo@hcuge.ch.

Materials availability

This study did not generate new unique reagents.

Data and code availability

The published article includes all data generated and analysed during this study. Data will be made available freely from the corresponding authors upon request.

All analyses were conducted with built-in and freely available R packages.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Patient data collection

This study was approved by institutional review board. We performed a prospective observational study on patients with cancer who received mRNA-1273 or BNT162b2 vaccine at University Hospital of Geneva (HUG) between January 29, 2021, and June 31, 2021.

Vaccination series was administered as per the manufacturer guidelines (gap between first and second dose was 21 days for mRNA-1273 and 28 days for BNT162b2). Participants were enrolled in the study by signing an informed consent. The inclusion criteria consisted of adult patients (age 18 years or older), eligible to receive COVID-19 vaccination, diagnosed with any malignancy with the exception of early-stage squamous cell skin cancer, early-stage basal cell skin carcinoma and non-invasive pathology such as Ductal Carcinoma in-situ (DCIS). Patients who were currently receiving anti-cancer treatment or had received active treatment within the last 5 years, were eligible. Exclusion criteria included a laboratory confirmed diagnosis of SARS-CoV-2 exposure either by polymerase chain reaction or serology, pregnancy or breast-feeding, and unable to comply with study-related procedures. Clinical characteristics were collected by clinical chart review. Blood samples are collected at the different time points: time of the first vaccine dose (baseline), at the time of the second vaccine dose which was equivalent to 3 weeks after first dose of BNT162b2, 4 weeks after first dose of mRNA-1273 (time point 1), at 3 weeks after second dose of mRNA-1273 or 4 weeks after second dose of BNT162b2 (time point 2). Finally a blood sample is collected

2 months after the 2nd dose (T3). Further blood sample are collected at T3 to measure INFy as way to measure T cellular response.

Data on humoral activity at T1 and T2 were previously reported and published. Here we report the data on seroconversion at T3 and the data about T cellular active in 2 pre-specified cohorts at T3. These samples were tested for both anti-SARS-CoV-2 spike (S) IgG and nucleocapsid (N) IgG titers. The samples at T3 were tested for INFy level.

The current study has two primary outcomes: 1) rates of seroconversion to the SARS-CoV-2 S protein at T3; and 2) Level of T cell activation in 2 pre-specified cohorts (Cohort 1 that includes patients who didn't show any seroconversion at T2 and cohort 2 that includes patients who showed high antibody level at T2).

METHOD DETAILS

Anti-SARS-CoV-2 spike IgG and nucleocapsidIgG assays

Blood samples collected using standard sampling tubes were directly centrifuged, and serum was stored at −80°C until batch analysis. The immunogenicity of mRNA vaccines was assessed by Elecsys® Anti-SARS-CoV-2S immunoassay for the *in vitro* quantitative determination of antibodies (including IgG) to the SARS-CoV-2 spike (S) protein receptor binding domain (RBD) in human serum and plasma (Elecsys® Anti-SARS-CoV-2 S. Package Insert 2020-09, V1.0; Material Numbers 09289267190 and 09289275190). The assay uses a recombinant protein representing the RBD of the S antigen in a one-step double antigen sandwich (DAGS) assay format, which favours detection of high affinity antibodies against SARS-CoV-2. The test is intended as an aid to assess the adaptive humoral immune response to the SARS-CoV-2 S protein. Briefly, patient samples are incubated with a mix of biotinylated and ruthenylatedRBD antigen. After addition of streptavidin-coated microparticles, the DAGS complexes bind to the solid phase via interaction of biotin and streptavidin. The reagent mixture is transferred to the measuring cell, where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are subsequently removed. Electrochemiluminescence is then induced by applying a voltage and measured with a photomultiplier. The signal yield increases with the antibody titer. Using internal Roche standard for anti-SARS-CoV-2-S consisting of monoclonal antibodies, 1 nM antibodies correspond to 20 U/mL of the Elecsys Anti-SARS-CoV-2 S assay. The cutoff value for this assay is 0.8 U/mL with <0.8 U/mL values reported as negative, and the maximum value is 2500 U/mL. This threshold resulted in a sensitivity of 98.8% (95% CI: 98.1–99.3%) in 1,610 samples from a cohort of 402 symptomatic patients with PCR confirmed SARS-CoV-2 infection and a specificity of 99.98% (95% CI: 99.91–100%) in a cohort of 5991 samples from pre-pandemic routine diagnostics and blood donors (Elecsys Anti-SARS-CoV-2 S. Package Insert, 2020-09, V1.0; Material Numbers 09289267190 and 09289275190). IgG antibodies against the N antigen of SARS-CoV-2 were measured on a Cobase801 analyzer (Roche Diagnostics, Rotkreuz, Switzerland) according to the manufacturer's instructions. Results are reported as numeric values in form of a cut-off index (signal sample/cut-off or signal calibrator ratio) and are considered as positive when equal to or above 1.

Determination of interferon-γ in plasma

All patients who did not have a positive seroconversion (anti-IgSIgG titers <0.8 U/mL) at T2 (9/130 patients) were identified and tested on T3 for T cell immunity through QuantiFERON analysis as part of cohort 1. We used as a control group (cohort 2) 18 patients who showed high antibody titers at T2 with anti-IgSIgG titers above 2000 U/mL). They were identified and subsequently tested at T3 for T cell immunity through QuantiFERON analysis. SARS-CoV-2-specific T cell responses were assessed by QuantiFERON ELISA (Qiagen, Hilden, Germany) which is a whole blood Interferon-Gamma Release immuno Assay (IGRA) that uses two combinations of specific peptides from the spike antigen (S1, S2, RBD subdomains) eliciting CD4+ (COVT1) and CD4+ and CD8+ (COVT2) T cell responses. The performance of this assay has been internally tested and validated using clinical and biological criteria for control (no prior SARS-COV 2 infection and no vaccination received) and vaccinated patient cohorts (Jaganathan S. et al., Infect. Dis. Ther. 2021).

Briefly, venous blood samples were collected directly into the QuantiFERON® tubes containing Spike peptides as well as positive and negative controls (626715 QFN SARS-CoV-2 Starter Pack). Whole blood was incubated at 37°C for 16–24 hours and centrifuged to separate plasma. IFN-γ (IU/mL) was measured in these plasma samples using ELISA (626410 QuantiFERON ELISA, Human IFN-γ SARS-CoV-2, Qiagen) tests. A cut-off value of 0.15 IU/mL was used to discriminate positive from negative cell-mediated immune responses to SARS-CoV-2, as reported previously. We used here the COVT2 values for Figures 1 and 2.

QUANTIFICATION AND STATISTICAL ANALYSIS

Continuous variables are presented as mean with standard deviation (SD) and/or median with interquartile range (25th percentile, IQ1/75the percentile, IQ3). Categorical variables are presented as absolute counts and relative percentages. We reported 95% confidence intervals (95% CI) for seroconversion rates (positive) using the Clopper-Pearson method. Group comparisons on seroconversion rate used chi-square test, or Fisher's exact test, when appropriate. A Wilcoxon rank sum test was used to compare the maximal interferon-gamma concentration between patients with solid tumours and those with hematologic malignancy. With a sample size of 8 (haematological malignancies) and 19 (solid tumors), we could detect, at an alpha of 0.05 (two-tailed) and with a power of 0.8, a difference between these groups of Cohen's d = 1.23. To account for the increased rate of type I error due to multiple testing, a Benjamini-Hochberg correction was applied to all p-values. No missing data imputation were conducted. Statistical analyses were carried out using the R software, version 4.1.0.