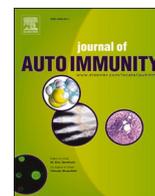




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## Glucocorticoid use as a cause of non-cellular immune response to SARS-Cov2 Spike in patients with immune system diseases

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### ABSTRACT

Disease modifying therapies compromise immune response to SARS-Cov2 or its vaccine in patients with immune system diseases (ISD). Therefore, analysis of the humoral and cellular responses against Spike is of utmost importance to manage ISD patients. A single-center retrospective study was conducted to evaluate the impact of COVID-19 immunization in 87 ISD patients and 81 healthy controls. We performed a whole blood interferon gamma release assay using SARS-Cov2 Spike and Nucleocapsid recombinant proteins in order to evaluate T-cell memory response, and an IgG anti-Spike ELISA to evaluate humoral response. Cellular (26.4%) and humoral (44.8%) responses were negative against Spike in ISD patients following COVID-19 immunization. In univariate analysis, an anti-Spike T cell defective response was associated with the use of glucocorticoids (Odds ratio [OR] = 10.0;  $p < 10^{-4}$ ), serum albumin level  $\leq 40$  g/L (OR = 18.9;  $p < 10^{-4}$ ), age over 55 years old (OR = 3.9,  $p = 0.009$ ) and  $\leq 2$  vaccine injections (OR = 4.9;  $p = 0.001$ ). The impact of glucocorticoids persisted after adjustment for age and number of vaccine injections (OR = 8.38,  $p < 0.001$ ). In contrast, the humoral response was impacted by the use of anti-CD20 mAb (OR = 24.8,  $p < 10^{-4}$ ), and an extended time since immunization ( $\geq 75$  days; OR = 4.3,  $p = 0.002$ ). Double defective cellular/humoral responses (6.9%) were typically encountered in glucocorticoids and/or anti-CD20 mAb treated ISD with a serum albumin level  $\leq 40$  g/L (OR = 17.5;  $p = 0.002$ ). Glucocorticoid usage, B cell depleting therapies, and a low serum albumin level were the main factors associated with a non-response to COVID-19 immunization in ISD patients. These results need further confirmation in larger studies.

### 1. Introduction

Patients with immune system diseases (ISD) receive disease modifying therapies (DMT), which can have variable impact on the immune functions and, in turn, can lead to increased risk of infections. As a consequence, and in the context of SARS-Cov2 pandemic, ISD patients under DMT face increased risks for severe coronavirus-disease-19

(COVID-19) through a defective capacity to respond to the vaccine and/or to control SARS-Cov2 infection. Indeed, it has been established that glucocorticoids (GC), antimetabolite therapies such as methotrexate (MTX) or mycophenolate mofetil (MMF), and anti-CD20 B cell targeted monoclonal antibody (mAb) increase the risk of hospitalization, and severe/fatal outcomes resulting from SARS-Cov2 infections [1, 2]. It has further been shown that there is an association between DMT

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<sup>1</sup> contributed equally to this work.

**Table 1**

Clinical and COVID-19 characteristics of the patients with immune system diseases and healthy controls studied.

	N	Sex F: M	Age, years (SEM)	GC, n (%)	$\alpha$ CD20, n (%)	$\alpha$ ME, n (%)	Bio, n (%)	HCQ, n (%)	No, n (%)	Vaccine: COVID19	Time from immunization, days (SEM)	Vaccin, N (SEM)
SLE/SjS	24	23:1	49 $\pm$ 3	10 (42)	1 (4)	14 (58)	3 (12)	14 (58)	1 (4)	22:2	97 $\pm$ 12	2.8 $\pm$ 0.1
MS	25	15:10	49 $\pm$ 2	1 (4)	24 (96)	2 (8)	0	2 (8)	1 (4)	19:6	92 $\pm$ 11	3.3 $\pm$ 0.1
AI-CNS	8	4:4	53 $\pm$ 5	8 (100)	7 (88)	0	0	0	0	8:0	155 $\pm$ 39	2.9 $\pm$ 0.2
Vasculitis	11	9:2	69 $\pm$ 4	7 (64)	2 (18)	3 (27)	1 (9)	0	1 (9)	10:1	66 $\pm$ 17	2.9 $\pm$ 0.1
Myositis	5	4:1	62 $\pm$ 4	4 (80)	1 (20)	2 (40)	0	0	1 (20)	3:2	55 $\pm$ 8	3.0 $\pm$ 0.0
Sarcoidosis	4	2:2	54 $\pm$ 11	1 (24)	0	2 (50)	1 (25)	1 (25)	0	2:2	40 $\pm$ 10	2.8 $\pm$ 0.2
Others	10	6:4	60 $\pm$ 6	4 (40)	4 (40)	1 (10)	1 (10)	0	3 (30)	7:3	50 $\pm$ 9	2.9 $\pm$ 0.1
HC	81	37:44	48 $\pm$ 2	–	–	–	–	–	–	68:13	82 $\pm$ 9	2.3 $\pm$ 0.1

**Abbreviations:** SLE: systemic lupus erythematosus; SjS: Sjögren's syndrome; MS: multiple sclerosis; AI-CNS: non-MS autoimmune central nervous system disease; HC: healthy controls; N: number; F: female; M: male; GC: glucocorticoids;  $\alpha$ CD20: anti-CD20 monoclonal antibodies (rituximab n = 31 or ocrelizumab n = 8);  $\alpha$ ME: antimetabolite; Bio: other biotherapies; HCQ: hydroxychloroquine; No: no medication; Immun: days from last immunization against COVID-19 vaccine or infection; SEM: standard error of the mean.

exposure and a lower humoral response after COVID-19 vaccination in ISD patients [3], which led to the proposal to stop MTX or delaying anti-CD20 mAb therapy to increase patients' chances of responding well to the vaccine [4,5]. Furthermore, the French health authorities recommend anti-SARS-Cov2 prophylactic therapies to vaccinated ISD patients when the titer of anti-Spike antibodies is above 264 binding antibody units (BAU). This threshold was selected since it provides 80% protection against the variant of concern (VOC) alpha [6]. However, such a strategy does not take into account the protection due to memory T lymphocytes. To explore the latter, we have recently developed a whole blood interferon gamma release assay (IGRA) using SARS-Cov2 purified proteins from Spike and Nucleocapsid (Nuc) to evaluate the T cell immune response to COVID-19 vaccine and natural infection, respectively [7]. In the present study, we describe the humoral and T-cell response of various ISD patients following COVID-19 vaccination or infection.

## 2. Material and methods

### 2.1. Patients

From July 2021 to March 2022, investigations were performed on 87 patients with various ISD diseases. Patients suffered from systemic lupus erythematosus/Sjögren's syndrome (SLE, n = 22 and SjS, n = 2), multiple sclerosis (MS, n = 25), non-MS autoimmune central nervous system diseases (AI-CNS, n = 8), vasculitis (n = 11), myositis (n = 5), sarcoidosis (n = 4), and other immune diseases (n = 10). Information collected from medical records included age, sex, vaccine injection numbers and types (BNT162b2/Pfizer-BioNTech, AZD1222/Astrazeneca-Oxford, and mRNA-1273/Moderna) in vaccinated ISD (n = 84), time from the last SARS-Cov2 antigenic challenge (vaccine injection or infection), polymorphonuclear neutrophil (PMN) count, lymphocyte count, serum albumin level, and current treatment.

The vaccinated healthy control (HC) group comprised 81 staff members from the medical laboratory of the University Hospital of Toulouse (CHU de Toulouse, Occitania, France) and blood bank donors (EFS Toulouse, Occitania, France), part of them were previously described [7]. Among the 81 vaccinated HC, 13 had developed a COVID-19 infection.

Blood was collected during a routine care visit in the Neurology and Internal Medicine departments to control immunization after vaccination or infection (see Table 1) as recommended by the French health authorities to determine if patients were eligible or not for administration of preventive mAb against SARS-Cov2. Participants were informed and gave their oral or written consent according to the French reference MR003 methodology. This study was conducted under the authorization numbers 21.04719.00068 b y the French Committee of Persons Protection (CPP) Ile-de-France-I and DC20162804 by the French Ethical Southwest and Overseas Committee (SOOM2).

### 2.2. Whole blood interferon gamma release assay (IGRA-covid)

As previously described [7], the blood samples were drawn into heparinized tubes (Becton Dickinson, Heidelberg, Germany), and 1 mL of whole blood was distributed in 4 tubes with: (i) 20  $\mu$ L of SARS-Cov2 full-length Spike protein (2  $\mu$ g/tube); (ii) 2  $\mu$ L of SARS-Cov2 Nuc protein (2  $\mu$ g/tube); (iii) 20  $\mu$ L of RMPI (negative control); and (iv) 20  $\mu$ L of phytohemagglutinin (PHA, 40  $\mu$ g/mL). Endotoxin free Spike and Nuc recombinant proteins were produced by INVIVOGEN® (Toulouse, France) based on the initial strain protein sequences [8]. After 18–24 h incubation at 37 °C, tubes were centrifuged, and the concentration of IFN- $\gamma$  in supernatants quantified by the QuantIFERON Monitor ELISA technique (Qiagen, Hilden, Germany), and results were expressed as international units (IU) of IFN- $\gamma$ /mL (1 IU IFN- $\gamma$ /mL = 2  $\times$  10<sup>4</sup>  $\mu$ g IFN- $\gamma$ ). For analysis, data from the negative control tube was subtracted from the signal obtained after stimulation with recombinant proteins. IGRA-Spike and IGRA-Nuc thresholds for positivity were fixed at 0.040 IU IFN- $\gamma$ /mL. The test is recorded as indeterminate when the negative control is > 8 IU IFN- $\gamma$ /mL or when the mitogen control <0.5 IU IFN- $\gamma$ /mL, but such cases were not observed in this study.

### 2.3. Serological tests

The serological tests were carried out on serum and the level of IgG antibodies to SARS-Cov2 Spike mammalian cell-expressed recombinant protein was assessed by using the SARS-CoV-2 IgG II Quant assay (Abbott Laboratories, IL, USA). ELISA total values are expressed in BAU/mL, using the WHO international standard NIBSC 20/136, and with an assigned cutoff at 7.14 BAU/mL, as previously described [9,10].

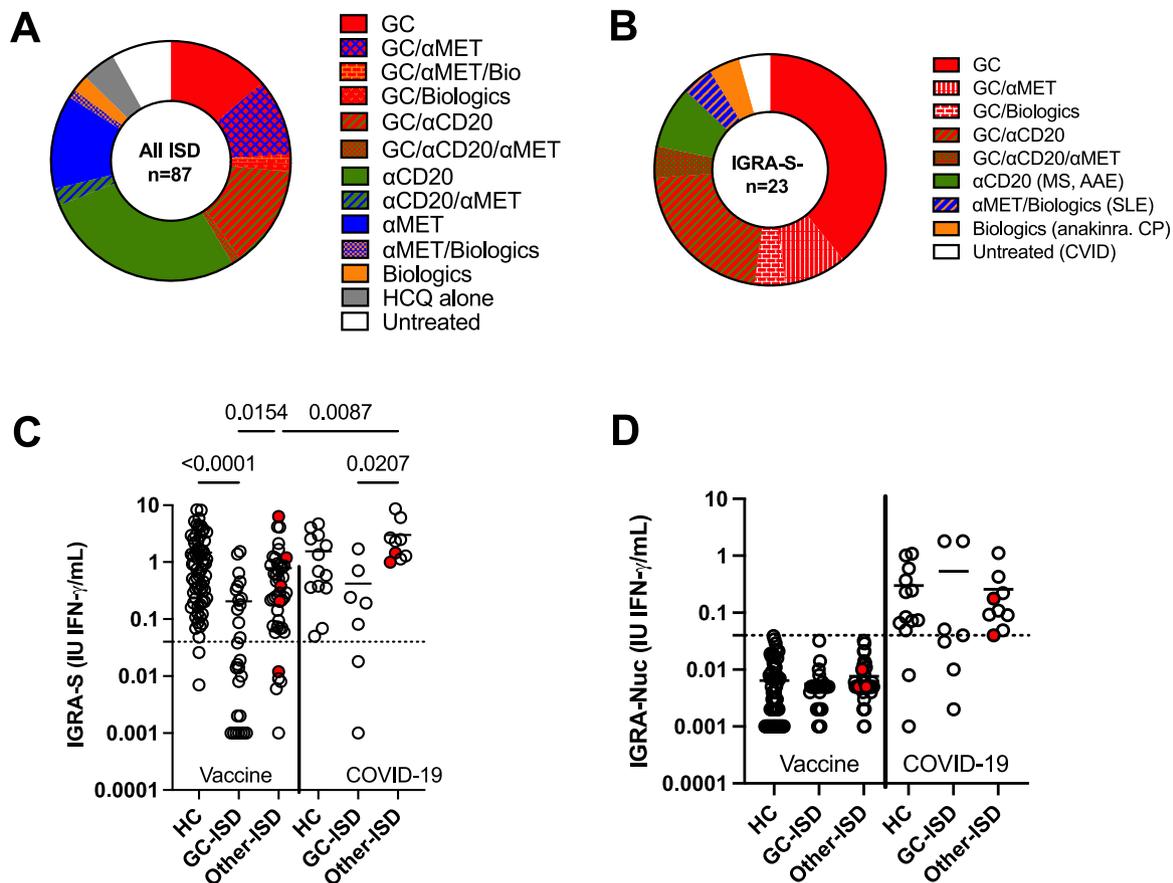
### 2.4. Statistics

Quantitative data presented as mean  $\pm$  standard error of the mean (SEM) or as median and interquartile range (IQR) 25th–75th percentile when analyzed using one-way ANOVA non-parametric assay, and Tukey's test was used for post-hoc comparison. Receiver operating characteristic (ROC) curves were generated to determine the area under the curve (AUC) and the Youden's index was calculated. Categorical data were analyzed using Fisher's exact test, and the Odds ratio (OR) with confidence interval (CI)95% calculated when appropriate. Statistics were conducted using GraphPad Prism 9.2 (La Jolla, CA) and SAS 9.4 (SAS on demand for academics) softwares, p-values <0.05 were considered significant.

## 3. Results

### 3.1. Patients

Eighty-seven patients with ISD were included in the study (Table 1),



**Fig. 1.** Glucocorticoids (GC) influence SARS-Cov2 memory T cell response in patients with immune system diseases (ISD, n = 87) as compared to healthy volunteers (HC, n = 81). **A-** Disease modifying therapies (DMT) repartition in ISD patients included in the study. **B-** DMT repartition in ISD patients with a negative whole blood IFN-γ release assay (IGRA) in response to the full-length Spike recombinant protein (IGRA-S). Characteristics of the non-GC ISD patients are indicated in brackets. **C-** Whole blood IGRA-S results. **D-** Whole blood IGRA-nucleocapsid (Nuc). For IGRA-S and IGRA-Nuc results, documented SARS-Cov2 infection was individualized from vaccination, and, within ISD patients, GC intake was further considered and untreated patients (5 in the vaccine group and 2 in the covid-9 infection group) are represented by red circles. Abbreviations: αCD20: anti-CD20 monoclonal antibodies; αME: antimetabolite; Biologics: other biotherapies; HCQ: hydroxychloroquine; MS: multiple sclerosis; AAE: acquired angioedema; SLE: systemic lupus erythematosus; SLE: systemic lupus erythematosus; CP: chronic pericarditis; CVID: common variable immunodeficiency. Cut-off (dot lines) and p values < 0.05 are indicated when significant (ANOVA).

and demographic analysis retrieved a mean age of  $54 \pm 2$  years old (range: 25–85 years) with a majority of females (63/87, 72.4%). Compared with ISD patients, age and time from last COVID-19 vaccine/infection at inclusion were similar in the HC individuals, while females and males were almost equally distributed (37:44, 45.7%). Ten ISD patients had received 4 doses of COVID-19 vaccine, 59 three doses, 15 two doses (Pfizer: n = 70; Moderna n = 12; Astrazeneca n = 2), and 3 unvaccinated ISD had previously developed COVID-19. In this real-life population, SARS-Cov2 infection was documented among 16/87 (18.4%) ISD patients and 13/81 (16.0%) HC (either by RT-PCR/antigenic assays and/or anti-Nuc Ab positivity). Infection occurred in ISD patients at the time of the variant of concern (VOC) beta (n = 4, January/August 2021) before vaccination, or during the delta/omicron waves (n = 12, November 2021/March 2022).

Regarding DMTs and as described in Fig. 1A, 35/87 (40.2%) received GC alone (n = 11) or in combination with anti-metabolites (n = 10; 7 MTX, 3 azathioprine [Aza]), anti-CD20 mAb (n = 12), other biotherapies (n = 3; 2 belimumab and tocilizumab), and/or hydroxychloroquine (HCQ, n = 5). Non-GC patients were treated with anti-CD20 mAb (n = 26), anti-metabolites (n = 14; 8 MTX, 4 Aza and 2 MMF), other biotherapies (n = 3, belimumab, adalimumab, and anakinra), HCQ (n = 12), or were untreated (n = 7).

### 3.2. Glucocorticoids (GC) influence cellular response to spike

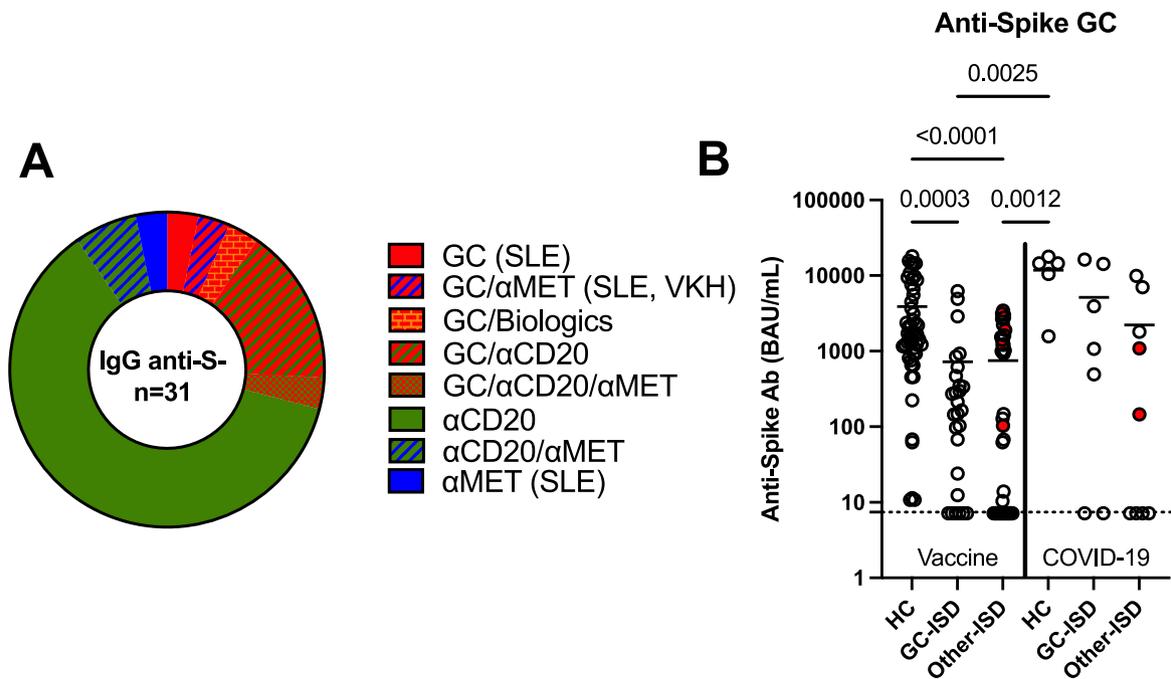
Memory T cell response against Spike (IGRA-S) was first evaluated showing a negative IGRA-S response in 23/87 (26.4%) ISD patients as compared to 2/81 (2.5%) HC ( $p < 10^{-4}$ ). Next and as presented in Fig. 1B, analysis of the 23 IGRA-S negative ISD patients revealed that they were predominantly treated with GC (18/23 versus 35/87 in all ISD,  $p < 10^{-4}$ ). Five ISD patients had a IGRA-S negative test despite vaccination and no exposure to GCs. They suffered from SLE (treated with MTX/Belimumab/HCQ), MS (on anti-CD20 mAb), common variable immunodeficiency (CVID, untreated), chronic pericarditis treated with the anti-IL1 receptor antagonist anakinra, and acquired angioedema (AAE) treated with an anti-CD20 mAb. Considering the 65 patients included less than 4 months after the last antigenic challenge (either vaccine or infection), 9 (mean age:  $57.6 \pm 6.1$  years) were on prednisone  $\geq 10$  mg/d (median: 20; range: 10–80), 1 being also on MTX and 1 on AZA, and 4/9 (44.4%) had no cellular IGRA-S response; 14 (mean age:  $64.4 \pm 3.1$  years) were on prednisone 5–10 mg/d (median dose: 6 mg; range 5–10) and 7/14 (50%) had no IGRA-S response, 2 of them being also on MTX.

It was observed that there was (i) in vaccinated but non-infected patients (Fig. 1C, left), a lower IGRA-S level within the 23 GC-1SD patients as compared to the 42 other-1SD patients ( $p = 0.015$ ) and 68 HC ( $p < 10^{-4}$ ); (ii) in COVID-19 infected ISD (Figure 1C, right), a reduced

**Table 2**  
Glucocorticoids (GC) and T-cell/humoral responses against SARS-Cov2-Spike.

	IGRA-Spike (IFN- $\gamma$ /mL)		Statistics	IgG anti-Spike (BAU)		Statistics
	Neg (n = 18)	Pos (n = 17)		Neg (n = 9)	Pos (n = 26)	
Age (years)	59.5 [48–71]	54 [39–69]	0.477	44 [29–66]	59.5 [51.8–71.2]	0.095
Sex (F:M)	13:5	13:4	1.00	7:2	19:7	1.00
GC dose at immunization, mg/day [IQR]	10 [5.0–20]	8 [5.0–12.5]	0.289	10 [5.0–12.5]	8 [7.75–20]	0.991
GC dose at sample collection, mg/day [IQR]	10 [5.75–20]	8 [5.0–12.5]	0.355	10 [6.2–12.5]	8 [5.0–20]	1.00
Cumulative GC dose, mg [IQR]	7662 [4704–21,491]	9982 [5168–23,275]	0.683	16,260 [4973–31,050]	7662 [4904–20,179]	0.342
GC exposure, days [IQR]	856 [163–1642]	759 [454–1073]	0.968	1152 [558–1871]	697 [199–1034]	0.289
Prednisone:prednisolone	15:3	17:0	0.229	8:1	24:2	1.00

Abbreviations: IGRA: interferon gamma release assay, IQR: interquartile range; F: female; M: male; GC: glucocorticoids; BAU: binding antibody units; IFN: interferon.



**Fig. 2.** Anti-CD20 monoclonal antibody ( $\alpha$ CD20) and glucocorticoids (GC) influence SARS-Cov2 immunization in patients with immune system diseases (ISD,  $n = 87$ ) as compared to non-vaccinated volunteers (HC,  $n = 81$ ). A- Disease modifying therapies (DMT) repartition in patients with ISD with a negative anti-SARS-Cov2 Spike IgG antibody result. Characteristics of the non-*anti*-CD20 mAb ISD patients are indicated in brackets. B- Anti-SARS-Cov2 Spike IgG antibody (Ab) titers (BAU/mL) by ELISA. SARS-Cov2 infection was individualized from vaccination, and, within ISD patients, anti-CD20 mAb, GC without aCD20 association (GCwoCD20), and other DMT were further considered. Untreated patients (5 in the vaccine group and 2 in the covid-9 infection group) are represented as red circles. Abbreviations:  $\alpha$ ME: antimetabolite; Biologics: other biotherapies; HCQ: hydroxychloroquine; SLE: systemic lupus erythematosus; and VKH: Vogt-Kaoyanagi-Harada's syndrome. Cut-off (dot lines) and p values  $< 0.05$  are indicated when significant (ANOVA).

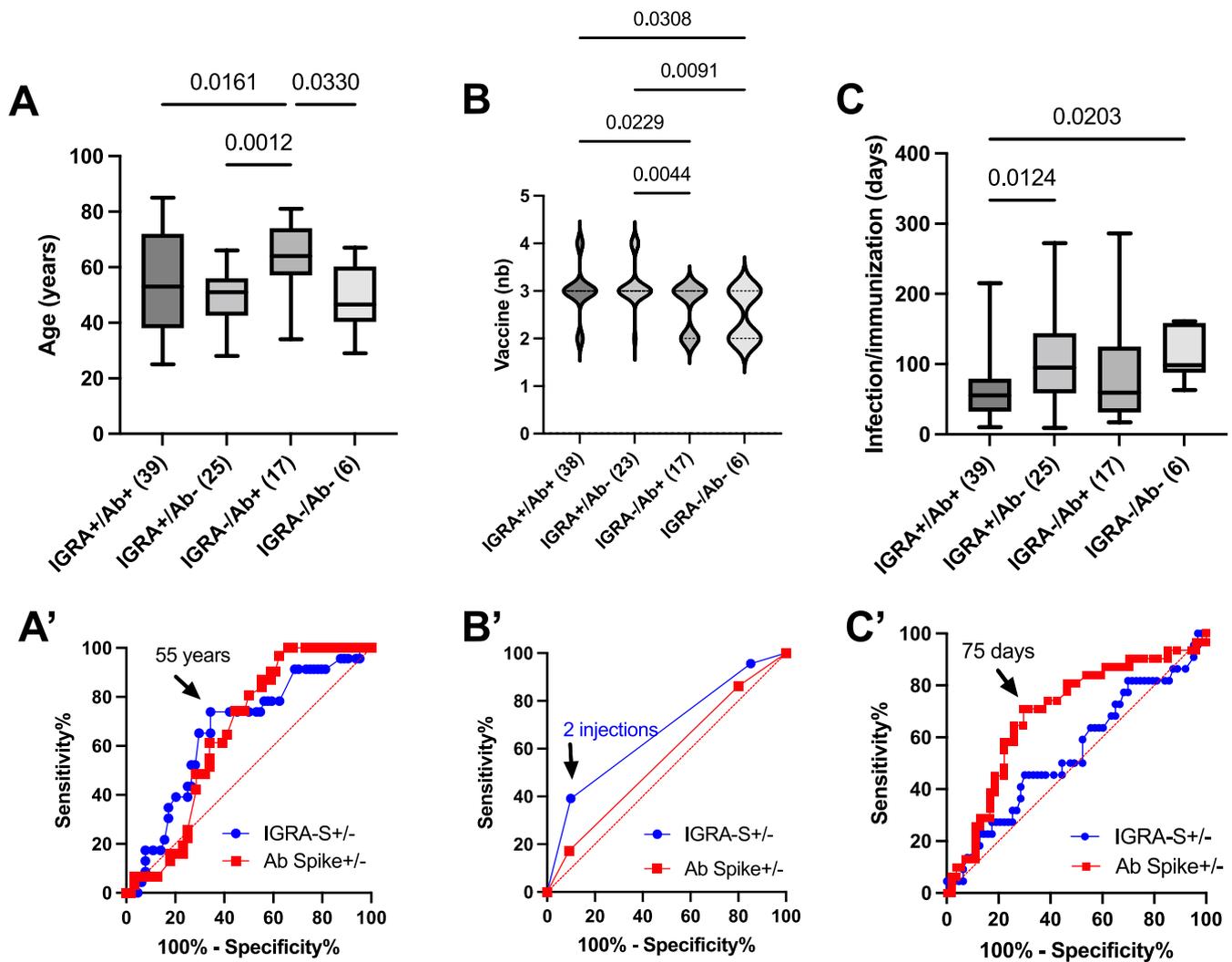
IGRA-Spike level within GC-ISD patients as compared to other-ISD patients ( $p = 0.02$ ); (iii) no difference in IGRA-S level between COVID-19 vaccinated GC-ISD patients and infected GC-ISDs patients ( $p = 0.99$ ) and (iv) in the absence of GC, a difference between COVID-19 vaccinated ISD patients and infected ISD patients ( $p = 0.009$ ). Among the ISD patients receiving GC (Table 2), GC capacity to inhibit T-cell mediated immune response was independent from dose/day, cumulative dose, time from GC initiation, and the synthetic GC that was used. The multivariate analysis testing the effects of GC, age, number of antigenic challenges and time elapsed since last antigenic challenge, identified only 2 independent predictors of lack of T cell response: exposure to GC (OR = 8.38 [2.58–27.2],  $p < 0.001$ ) and age  $\geq$  median age (OR = 4.24 [1.33–13.6],  $p < 0.05$ ).

A defective T cell response coupled with GC usage may suggest a fatal evolution of COVID-19. Such a situation was observed in a 74 years old woman, included in the study, with myositis and who was treated with GC at 7.5 mg/day (IGRA-Spike  $< 0.04$  IFN- $\gamma$ /mL and IgG anti-Spike 1345 BAU/mL). Next, GC were increased to 60 mg/day and one month later she developed acute respiratory distress syndrome and died thereafter from an infection with the VOC Omicron (data not shown).

With regards to the memory T cell response against Nuc (a target not present in the COVID-19 vaccine), the vaccinated COVID-19 ISD patients and HC individuals were all IGRA-Nuc negative, while 24/29 (82.8%) ISD and HC were IGRA-Nuc positive in the SARS-Cov2 infected subgroup, which validate our classification of patients according to clinical criteria. However, the influence of GC on IGRA-Nuc failed to reach significance in this limited population of ISD patients infected with SARS-Cov2. We conclude from the IGRA-S/Nuc analysis that GC usage affects T cell memory immunization response against Spike following both COVID-19 vaccine and infection.

### 3.3. Anti-CD20 mAb therapy influences the humoral response to spike

As reported by others [11,12] and presented in Fig. 2A, a negative IgG anti-Spike Ab response ( $< 7.14$  BAU/mL,  $n = 31$ ) was retrieved within 27/31 (87.1%) ISD patients having received anti-CD20 mAb therapy (versus 39/87 in all ISDs,  $p < 10^{-4}$ ). The remaining 4 cases were related to 2 SLE cases and a Vogt-Koyanagi-Harada's syndrome treated with GC in association with antimetabolites in 2 cases, and 1 SLE patient treated with MMF. Accordingly, COVID-19 vaccinated and infected



**Fig. 3.** Individual and vaccine characteristics influencing cellular (IGRA-S) and/or humoral (Ab) negativity to Spike. A- Age. B- COVID-19 vaccine injection number. C- Time from last immunization. A'/B'/C'- Receiving operating characteristic (ROC) curve to establish IGRA-Spike (IGRA-S, red) and anti-Spike Ab (blue) cut-offs at which sensitivity and specificity are optimal using the Youden's index. ISD patients were subdivided into IGRA-S+/Ab+, IGRA-S+/Ab-, IGRA-S-/Ab+, IGRA-S-/Ab-, and the number of patients analyzed is indicated in brackets. p values < 0.05 are indicated when significant (ANOVA). Abbreviations: IGRA: interferon gamma release assay; Ab: IgG anti-Spike antibodies. p values < 0.05 are indicated when significant (ANOVA).

patients were subdivided into 3 groups whether or not they had received an anti-CD20 mAb, GC in the absence of an anti-CD20 mAb, or another drug regimen ("other ISD"). As presented in Fig. 2B, the IgG anti-Spike Ab level against COVID-19 Spike following vaccination and infection was affected by the anti-CD20 mAb therapies (Fig. 2B;  $p < 10^{-4}$  and  $p = 0.01$ , respectively) and by GC following vaccination ( $p = 0.03$ ). GC effects were independent from dose used, cumulative dose, and time from GC initiation (Table 2).

Among the 6 ISD patients defective for T cell and humoral responses, 2 patients had received a combination of GC and anti-CD20 mAb (1 SLE and one AI-CNV with Susac's syndrome), 2 SLE patients were treated with GC in the absence of anti-CD20 mAb, and 2 patients were treated with anti-CD20 mAb (1 MS and 1 AAE). This supports the concept that GC and anti-CD20 mAb are major factors implicated in the defective T cell and humoral responses, respectively.

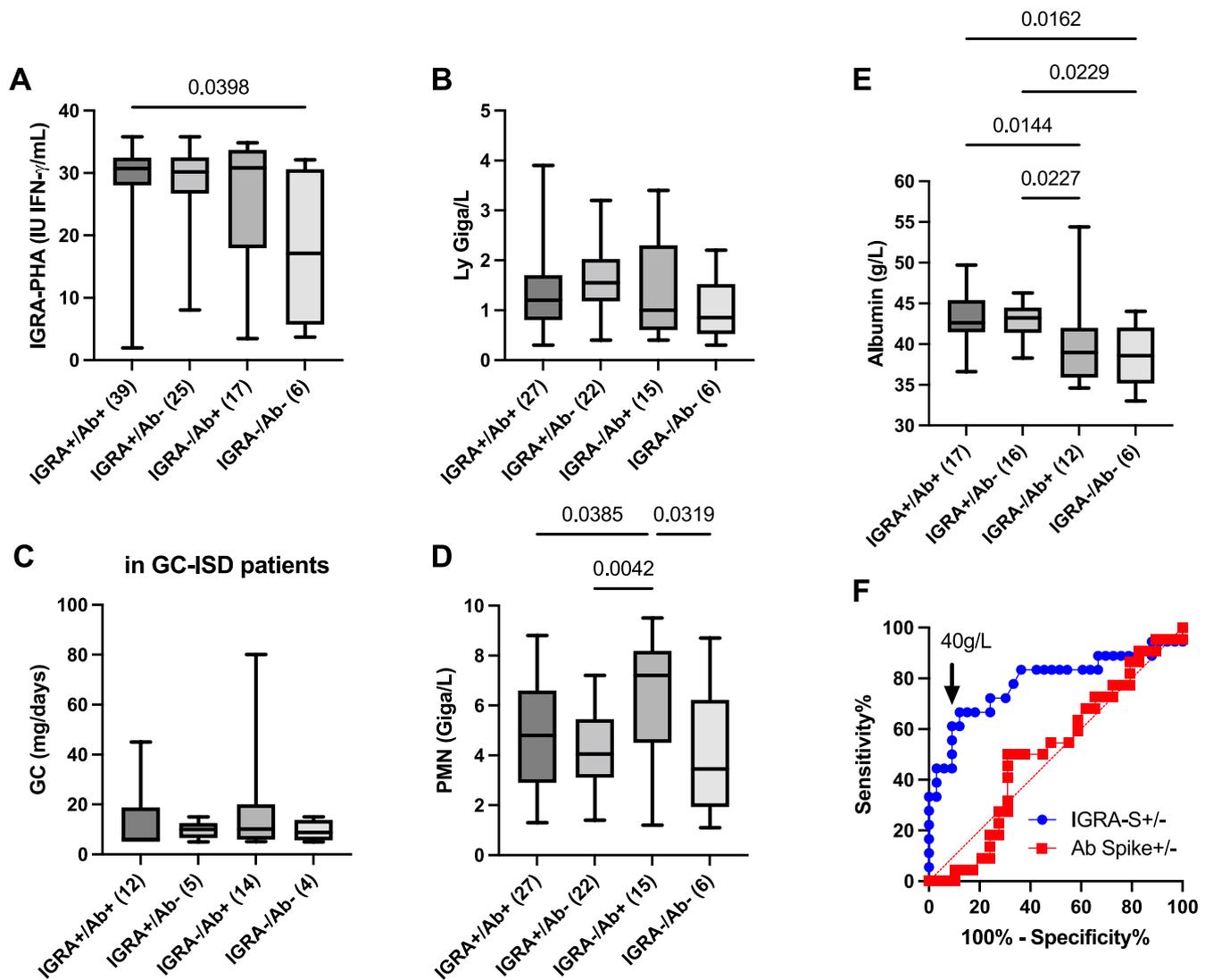
### 3.4. T cell and humoral non-responder characteristics

To delve further into the characterization of factors associated with a defective T cell and/or humoral response, ISD patients were subdivided

into IGRA-S+/Ab+ ( $n = 39$ ), IGRA-S+/Ab- ( $n = 25$ ), IGRA-S-/Ab+ ( $n = 17$ ), and IGRA-S-/Ab- ( $n = 6$ ). Five parameters were considered corresponding to demographic factors (age, sex), and factors associated with COVID-19 immunization (vaccine type, vaccine dose number, and time since the last vaccine injection or SARS-Cov2 infection).

Regarding demographic factors (Fig. 3A/A'), ISD patients negative for IGRA-S and positive for IgG anti-Spike Ab were older ( $p < 0.04$ ). The ROC curve coupled with the Youden's index was further used to fix the threshold age at 55-years old to dichotomize IGRA-S in ISD patients ( $AUC = 0.662$ ;  $p = 0.02$ ) and IgG anti-Spike Ab in ISD patients ( $AUC = 0.653$ ;  $p = 0.02$ ).

Analysis of the vaccine characteristics (Fig. 3B/C) retrieved an association between the number of COVID-19 vaccine doses and the T cell response ( $3.0 \pm 0.1$  doses in IGRA-S positive versus  $2.6 \pm 0.1$  doses in IGRA-S negative ISD patients;  $p = 0.003$ ), with a threshold fixed at  $\leq 2$  vaccine doses from the ROC analysis ( $AUC = 0.672$ ;  $p = 0.02$ ). Moreover, time from immunization was discriminant for the humoral response ( $73 \pm 8$  days in IgG anti-Spike Ab + versus  $108 \pm 12$  days in IgG anti-Spike Ab-,  $p = 0.01$ ), and the cut-off delay was fixed at 85 days for the IgG anti-Spike Ab decline. In contrast, no difference was observed



**Fig. 4.** Factors influencing the interferon gamma release assay (IGRA). **A-** Memory T cell capacity to produce interferon in the presence of phytohemagglutinin (IGRA-PHA), a non-specific mitogen. **B-** Peripheral blood lymphocyte (Ly) count. **C-** Glucocorticoid (GC) dose among patients with immune system diseases (ISD). Of note, ISD patients not treated with GC were excluded from the analysis. **D-** Peripheral blood polymorphonuclear neutrophil (PMN) count. **E-** Serum albumin level. **F-** Receiving operating characteristic (ROC) curve to establish IGRA-Spike (IGRA-S, red) and anti-Spike Ab (blue) cut-offs at which serum albumin sensitivity and specificity are optimal (Youden's index = 40 g/L). ISD patients were subdivided into IGRA-S+/Ab+, IGRA-S+/Ab-, IGRA-S-/Ab+, and IGRA-S-/Ab-. p values < 0.05 are indicated when significant.

according to the sex and vaccine type (data not shown).

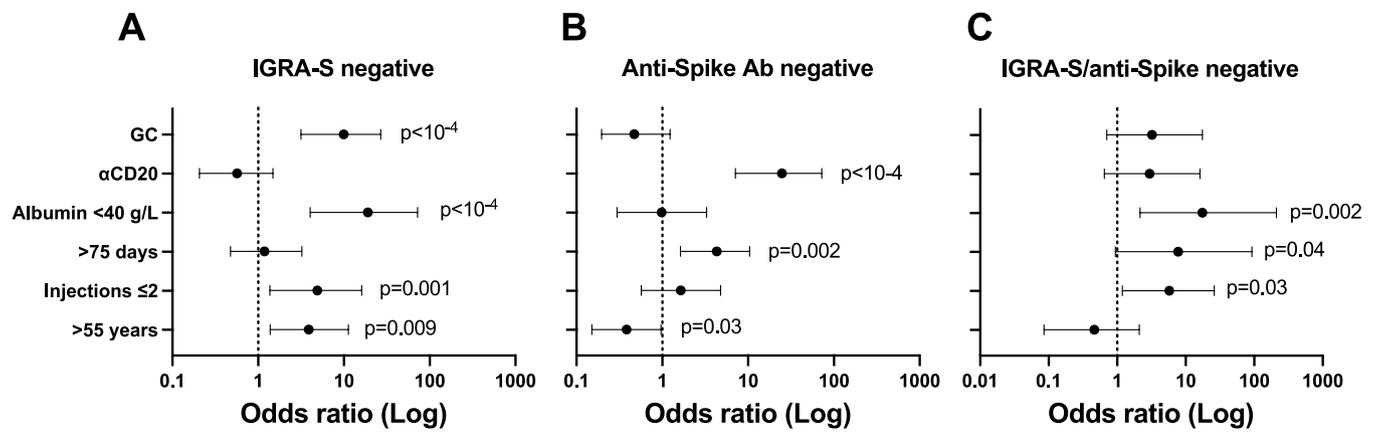
76.4–96.9%).

### 3.5. Factors associated with an impaired T cell response

Several parameters were previously demonstrated to influence the T cell response in the IGRA assay [13–15]. They included PMN and lymphocyte counts, a defective T cell capacity to respond to PHA stimulation (IGRA-PHA), GC dose, and serum albumin level. As presented in Fig. 4, IGRA-PHA levels were significantly lower in the IGRA-S-/Ab-subgroup (p = 0.04), and this difference could not be ascribed to a reduced lymphocyte count or GC dose in the subset of GC-ISD patients. PMN levels were elevated in the IGRA-S-/Ab + subgroup as compared to the other subgroups (p < 0.04). Moreover, serum albumin levels were lower in the IGRA-S-/Ab ± subgroups (p < 0.02) and in the ROC curve analysis (AUC = 0.783; p = 0.0009) the optimal sensitivity and specificity using the Youden's index to discriminate IGRA-Spike positive from IGRA-Spike negative ISD patients was fixed at 40 g/L (Sensitivity: 60%, 95% CI: 38.6–79.7%; Specificity 91%, 95% CI:

### 3.6. Logistic regression analysis

Finally, the contribution of the different parameters associated with T cell and/or humoral response against Spike (age >55 years old, vaccine injections ≤2, time from last immunization >75 days, serum albumin ≤40 g/L, anti-CD20 mAb, and GC use) were evaluated. Using an univariate approach (Fig. 5A), the main factor implicated in a defective memory T cell IGRA-S response was a serum albumin level ≤40 g/L (OR = 18.9; 95% CI: 4.0–72.3), followed by GC intake (OR = 10.0; 95% CI: 3.2–26.9), COVID-19 vaccine number ≤2 (OR = 4.9; 95% CI:1.4–16.1), and age over 55 years old (OR = 3.9; 95% CI:1.4–11.3). As presented in Fig. 5B, a negative humoral response against Spike (<7.14 BAU/mL) was associated with the use of anti-CD20 mAb therapy (OR = 24.75; 95% CI:7.1–72.1), with a time elapsed from last immunization over 75 days (OR = 4.3; 95% CI:1.6–10.4). Paradoxically, an age over 55 years old was also protective (OR = 0.38; 95% CI:0.15–0.97) but this



**Fig. 5.** Univariate logistic regression analysis for negative cellular (IGRA-S) and/or humoral (anti-Spike antibody [Ab]) response to Spike. **A:** Odds ratio (OR) to predict a negative interferon gamma release assay (IGRA-S) to Spike. **B:** OR to predict a negative IgG anti-Spike Ab result. **C:** OR to predict both a negative IGRA-S and IgG anti-Spike Ab result. Abbreviations: GC: glucocorticoids; αCD20: anti-CD20 monoclonal antibodies (rituximab or ocrelizumab); days: days from the last immunization in response to COVID-19 vaccine or infection; injection: vaccine injection number. Odds ratio, 95% confidence interval, and p values < 0.05 are indicated when significant (Fisher's exact test).

counterintuitive result may be explained by a shorter time elapsed since last immunization after 55 years old ( $98 \pm 10$  days when  $\leq 55$  years old versus  $70 \pm 8$  days when  $>55$  years old,  $p = 0.04$  data not shown). For IGRA-S/Ab double negative patients, risk factors were a serum albumin level  $\leq 40$  g/L (OR = 17.5; 95% CI:2.2–210),  $>75$  days from last immunization (OR = 7.7; 95% CI:0.95–92.8), and COVID-19 injections  $\leq 2$  (OR = 5.8; 95% CI:1.2–26.1).

#### 4. Discussion

In this retrospective study aimed at depicting the humoral and cellular responses to SARS-Cov2 in a cohort of ISD patients, we found strong evidence that GC affects not only the humoral response as previously reported [16] but also the cellular response. In contrast, anti-CD20 mAb affects only the humoral responses. Furthermore, decreased albumin level was associated with a weaker cellular response.

GC remains a mainstay of ISD treatment since their discovery in the 1940s, indeed GC induce important anti-inflammatory and immunosuppressive effects on both innate and adaptive immune cells. T cell and to a lesser extent B cell responses are affected by long term GC usage, as retrieved in our study, and these abnormalities are resolved within months or years including when low-dose GC are used [17–18]. GC exerts multiple actions on lymphocytes by controlling differentiation, migration, polarization into IFN $\gamma$  producing TH1 cells, memory T cell formation, while regulatory T cells are increased [19]. Other studies conducted in ISD patients have shown qualitative and quantitative decreases in the COVID-19 humoral response [3,20]. In contrast, T cell responses on GC treated patients have been described only in small cohorts of patients. T cell exploration was limited to ISD patients showing a defective humoral response or after the exclusion of previously SARS-Cov2 infected patients [5,11,21,22]. We confirm that T cell response to COVID-19 vaccine is defective among ISD patients receiving DMT as reported in MS and rheumatoid arthritis (RA) [22]. Anti-CD20 mAb most likely affects the humoral anti-Spike response but has no strong effect on the T cell response [22]. From our knowledge, our study is the first to incriminate GC as the main DMT to dampen the T cell response against Spike, and such an effect is observed both following COVID-19 vaccine and infection. This negative impact exists also for 'low' GC doses ( $<10$  mg/d) often considered to be safe regarding the risk of severe COVID infection. However and after excluding GC characteristics (dose, cumulative dose, and time from initiation), aging and prescription of other immunosuppressive drugs may have contributed to the defective T cell response observed among our patients on low GC

doses. GC-induced suppression of mitogen capacity to drive IFN $\gamma$  from memory T cells has been demonstrated in vitro [23] and in vivo in GC treated patients [14]. Accordingly, the capacity of GC to affect cellular immunity against Spike protein provides a possible explanation for worse COVID-19 outcomes associated with chronic GC in ISD patients [24]. Conversely, the COVID-19 vaccine humoral response remains marginally affected by GC as was observed in our study after removing associations with anti-CD20 mAbs [3].

Systemic inflammation, which is frequent in ISD patients, is a common cause of low albumin levels and can lead to a protein-energy malnutrition, which in turn affects innate and acquired immune responses to pathogens and vaccines [25]. Our study supported an association between a low serum albumin level ( $\leq 40$  g/L) and a negative IGRA-S response as well as a reduced IGRA-PHA response in the IGRA-S-/Ab-subgroup. This result is in line with recent reports showing an association between lower serum albumin concentrations ( $\leq 35$  g/L) and a defective IGRA response to PHA used as mitogen [26, 27]. The interdependence in the IGRA-PHA assay between GC and low level serum albumin was tested and excluded by Kaur et al. [28]. A low level of serum albumin level was further described to be associated with a defective cellular COVID-19 vaccine response in hemodialysis patients [29], as well as with acute COVID-19 prognosis and hospitalization [30].

Recently, we tested the humoral and cellular response to COVID-19 vaccine in healthy controls and we observed a marked reduction in anti-Spike Abs 100 days after the vaccination, while the reduction of IGRA-S T-cell response was significantly lower [7]. Time from immunization was also discriminant in ISD patients with a cut-off fixed at 75 days to observe a significant decline in IgG anti-Spike Ab response contrasting with a persistence of the IGRA-Spike T-cell response. Poorly affected by the delay from the last immunization, the cellular IGRA-Spike response was found to be influenced by aging and the number of antigenic challenges.

Strengths of our real-life study were the inclusion of many patients suffering from a large spectrum of ISD, of healthy controls and of patients vaccinated and/or infected with SARS-Cov2. We use both Spike and Nuc proteins in the IGRA assay and observed that this may help to discriminate vaccination from infections. A limit of our study was lack of albumin dosage and other common biomarkers (such as PMN ratio, C-reactive protein ...) in some patients not allowing to include these variables in a multivariable analysis, and the relatively small number of patients limiting the conclusions of the multivariable analysis as well. Accordingly, these results need further confirmation in larger studies, and by other methods to study the T cell response.

In conclusion, our study identified GC use as a cause of non-response to IGRA-S together with a moderate effect on the humoral response, while B cell depletion only affected IgG anti-Spike Ab production. Consequently, we think that prospective studies should evaluate the risk of symptomatic or severe COVID-19 infection among patients according to their ability to develop both T-cell and humoral responses after vaccination. This may help to select the best candidates for prophylactic or curative drugs proposed to prevent severe COVID-19 infection.

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### Author statement

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### Declaration of competing interest

None.

### Data availability

Data will be made available on request.

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