SARS-CoV-2 antibody vaccine response in Inflammatory Bowel Disease patients with positive anti-nucleocapsid serology or history of COVID-19 infection

A. Hoyois[1,2], C. Gulkilik[1], L. Mekkaoui[3], H. Dahma[3], V. Wambacq[1], C. Minsart[1], N. Rosewick[4], C. Liefferinckx[1], L. Amininejad[1], A. Van Gossum[1], A. Cremer[1], O. Vandenberg[5], D. Franchimont[1]

(1) Department of Gastroenterology, Hepatopancreatology, and Digestive Oncology, HUB Hôpital Erasme, Université Libre de Bruxelles, Brussels Belgium; (2) Department of Hepato-Gastroenterology, CHU Saint-Pierre, Université Libre de Bruxelles, Brussels, Belgium; (3) Department of Microbiology, Laboratoire Hospitalier Universitaire de Bruxelles, Universitair Laboratorium Brussel (LHUB-ULB), Université Libre de Bruxelles, Brussels, Belgium; (4) Laboratory of Experimental Gastroenterology, Université Libre de Bruxelles, Brussels, Belgium; (5) Innovation and Business Development Unit, Laboratoire Hospitalier Universitaire de Bruxelles-Universitair Laboratorium Brussel (LHUB-ULB), Université Libre de Bruxelles (ULB), Brussels, Belgium.

Abstract

Background: **Previous history of COVID-19 infection is a natural booster of the vaccine response in the general population. The response to COVID-19 vaccines is lessened in Inflammatory Bowel Disease patients on selected class of immunosuppressive treatments.**

Aims: **The study was to assess anti-SARS-CoV-2 spike-specific IgG antibody response in Inflammatory Bowel Disease patients with a history of COVID-19 infection.**

Patients and methods: **This single-center prospective study involved 504 Inflammatory Bowel Disease patients. Demographic data and clinical data were gathered through questionnaires and patient charts. Anti-SARS-CoV-2 spike-specific and anti-nucleocapsid antibody levels were measured at T1, T2 (after the 2-dose series), and T3 or T4 (booster vaccine).**

Results: **This study included 504 Inflammatory Bowel Disease patients, and 234 completed one year follow-up with blood tests. Positive anti-nucleocapsid serology or history of COVID-19 infection was significantly associated with increased median anti-SARS-CoV-2 spike-specific IgG titers after the 2-dose series (1930 BAU/mL vs. 521 BAU/mL $p < 0.0001$) and the booster vaccine (4390 BAU/mL vs. 2160 BAU/mL, $p = 0.0156$). Multivariate analysis showed that higher anti-SARS-CoV-2 spike-specific IgG levels were independently associated with anti-nucleocapsid antibodies at T2 (OR=2.23, $p < 0.0001$) and T3 (OR=1.72, $p = 0.00011$). Immunosuppressive treatments did not impact the antibody response or levels in patients with a history of COVID-19 infection or positive anti-nucleocapsid serology.**

Conclusions: **In Inflammatory Bowel Disease, prior COVID-19 infection or positive anti-nucleocapsid serology leads to increased anti-SARS-CoV-2 spike-specific IgG levels after vaccination, regardless of immunosuppressive treatments. This emphasizes the significance of accounting for previous infection in vaccination approaches.** (Acta gastroenterol. belg., **2024,** 87, **263-273**)

Keywords: Inflammatory Bowel Disease, COVID-19 vaccine, anti-nucleocapsid antibody, anti-SARS-CoV-2 spike-specific IgG antibody.

Introduction

The coronavirus disease 2019 (COVID-19) pandemic began in China in December 2019 and has led to severe acute respiratory syndrome in some patients (1). The World Health Organization report over 774 million confirmed cases and over seven million deaths in March 2024. Vaccinations have prevented 14.4 million deaths from COVID-19 in 185 countries between December 8, 2020 and December 8, 2021 (2).

Inflammatory Bowel Disease (IBD) patients treated by systemic corticosteroids, and methotrexate marginally, were associated with an increased risk of severe illness or poorer outcomes from COVID-19 infection (3). Age and increased number of comorbidities are associated with adverse COVID-19 outcomes among IBD patients to the same extent as in the general population (4). Biologic medications are not associated with severe COVID-19 infections (5). However, it is important to maintain appropriate vaccination status as the effectiveness of vaccinations can be altered by immunosuppressive therapies (5,6,7). Current data on two-dose COVID-19 vaccinations have shown that immunogenicity to vaccination varies according to immunosuppressive drug exposure and is attenuated in patients treated with infliximab, infliximab plus thiopurines, and tofacitinib (8). In the VARIATION study, IBD patients had attenuated serological responses to SARS-CoV-2 vaccination independently of their treatment. The use of anti-TNF (9) therapy negatively impacted anti-Spike IgG levels further. These data supported the administration of booster vaccines to improve the effectiveness of vaccinations. In the general population, vaccine efficacy following two mRNA vaccine doses is lower against Omicron-related severe disease phenotype, but its efficacy is regained in individuals who received a third dose (10). The booster of the mRNA vaccine is effective in protecting individuals against severe COVID-19-related outcomes, providing 95.3% efficacy, compared to those receiving only two doses (11,12) and hence, immunity was boosted (13). Recent IBD studies about booster vaccines have shown a stronger response to SARS-CoV-2 vaccine additional doses among immuno-suppressed patients with IBD (14,15) but these responses were attenuated in patients taking infliximab, infliximab plus thiopurine, and tofacitinib (14,16,17). However, IBD patient had a sustained humoral immune response 6 months after a third dose of a COVID-19 mRNA vaccine

Correspondence to: Alice Hoyois, MD, Department of Gastroenterology, Erasme University Hospital, Route de Lennik, 808 1070 Bruxelles, Belgium, Phone: +325354663.
Email: alice.hoyois@erasme.ulb.ac.be

Submission date: 10/01/2024
Acceptance date: 28/04/2024

dose (18). Most IBD studies included patients without prior history of COVID-19 infection. However, in the general population, the additional antigen exposure from natural infection substantially boosts the quantity, quality, and breadth of humoral immune response regardless of whether it occurs before or after vaccination (19). To distinguish between a previous history of COVID-19 infection and vaccination, two types of antibodies can be measured. Anti-nucleocapsid antibodies are a type of antibody that the immune system produces in response to the nucleocapsid protein of a virus. These antibodies specifically target and bind to the nucleocapsid protein, helping to neutralize the virus and prevent it from infecting cells. They are commonly used as biomarkers for viral infections, including COVID-19. In the context of vaccination, spike-specific antibodies are generated in response to a vaccine that includes an antigen, such as a protein or peptide, that mimics the spike protein of a virus (20). Very few studies have described the impact of COVID-19 infection on the 2-dose series and booster vaccine response in IBD patients.

The aim of this prospective cohort is to evaluate anti-SARS-CoV-2 spike-specific IgG antibody response in IBD patients with a history of COVID19 infection.

Material and methods

Study Design

This is a prospective, monocentric, single-center, observational cohort study including patients with IBD followed in Erasme University Hospital from January 2021 to May 2022. Patients were recruited by answering an online questionnaire received by email or by postal mail. Data were collected on REDCap (Research Electronic Data capture), a secure web application for managing online surveys and databases. Patients who agreed to participate, signed an informed consent, and were invited to complete a questionnaire and to take a blood sample for anti-SARS-CoV-2 serology prior to the vaccination at the baseline (T1), 6 months (T2) and 1 year (T3) after the first questionnaire. Anti-SARS-CoV-2 spike-specific IgG serology was conducted in T1 before vaccination while both anti-SARS-CoV-2 spike-specific IgG serology and anti-nucleocapsid serology were performed at T2 and T3, post-vaccination. The nucleocapsid protein cannot be expressed by the viral vaccines used in our cohort, as both ChAdOx1 nCoV-19 (1) and Ad26.COV2.S (2) are designed to elicit an immune response based on the expression of the spike protein of the SARS-CoV-2 virus. ChAdOx1 nCoV-19 utilizes a replication-deficient chimpanzee adenovirus vector ChAdOx1, while Ad26. COV2.S employs a replication-incompetent human adenovirus type 26 vector: Viral vector vaccines, such as those using adenoviruses, intro-duce genetic material (DNA) that encodes the spike protein, not the nucleocapsid protein. Thus, in our cohort, the presence of nucleocapsid response can in fact be used to identify natural infection (21,22). The inclusion criteria were as follows: Patient with IBD and over 18 years of age. Participants who completed the 3 questionnaires and took the 3 blood samples were included in this present analysis.

Demographic and clinical characteristics of the patients

Demographic data and clinical characteristics of the patient's data were collected from the questionnaires and include demographics (age, sex), body mass index (BMI), IBD subtype (Crohn's Disease or Ulcerative colitis), ongoing IBD treatment, COVID-19 vaccination, type of vaccine (mRNA vaccines or viral-vector vaccines) and date of vaccination, and symptoms related to the COVID-19 infection, and positive or negative SARS-CoV-2 Reverse Transcription-Polymerase Chain Reaction (RT-PCR).

Immunoassays

Humoral response after vaccination was investigated with the LIAISON® SARS-CoV-2 TrimericS IgG (DiaSorin, Still-water, USA), a chemiluminescence immunoassay (CLIA) measuring IgG antibodies against the SARS-CoV-2 trimeric spike specific protein of the SARS-CoV-2 performed before the initial vaccination series, after the administration of the second vaccine dose (6-months after the first blood sample) and after the booster vaccine (6-months after the second blood sample). This serology assay was performed on the Liaison XL platform (DiaSorin, Saluggia, Italy) according to the manufacturer's instructions at the University-Hospital Laboratory Brussels (LHUB-ULB). The measuring ranges were between 4.81 and 2080 binding antibody units (BAU)/ mL). Seropositivity was defined as IgG titres ≥33.8 BAU/mL. Patients with IgG titres ≥2080 BAU/mL were diluted using the LIAISON® TrimericS IgG Diluent Accessory with a dilution factor of 1:20 to know the antibodies kinetics.

Humoral response after contact with COVID-19 infection was investigated with the Anti-nucleocapside Euroimmun Anti-SARS-CoV-2 NCP ELISA (IgG) (Euroimmun, Lübeck, Germany). The assay was performed according to the manufacturer's instructions for the ELISA automated system ETI-MAX 3000 (DiaSorin, Saluggia, Italy) at the University-Hospital Laboratory Brussels (LHUB-ULB). The microplate wells are coated with modified nucleocapsid protein (NCP) that only contains diagnostically relevant epitopes. The results are evaluated semi-quantitatively by calculation of the ratio of the samples' extinction value over the calibrator's extinction. The ratio interpretation was as follows: < 0.8 = negative, ≥ 0.8 to < 1.1 = borderline, ≥ 1.1 = positive.

Statistical analyses

Descriptive statistics were used, and results were reported as medians and interquartile ranges (IQRs).

Categorical variables were summarized as counts and percentages. Mann-Withney U test was used to compare independent nonparametric variables. Kruskal-Wallis test was used to compare multiple independent non parametric variables. The Wilcoxon signed rank test was used to compare paired groups of serological responses after 2-doses regimen and the booster of COVID-19 vaccination. The statistically significant threshold value was fixed at 5%. Statistical analyses were performed with prism 9 software and R 4.2.0.

Ethical considerations

This study was approved by the Ethics Committee (CCB B4062020000091) of Erasme University Hospital in accordance with the Declaration of Helsinki, and written informed consent was obtained from the patients.

Results

Clinical characteristics of IBD population

From January 2021, the first questionnaire was sent to 2,349 patients. Five hundred and four patients responded, and among them, 370 patients did the first blood test. Six months and one year later, questionnaires and blood tests were conducted in 294 and 234 patients, respectively (Fig 1). All demographic data of the 234 patients who completed their follow-up are shown in Table 1. The median age of study participants was 47 years (IQR: 35-57), and 56% were female. Sixty-seven-point five percent (158/234) of patients had Crohn's disease, 28.6% (67/234) had ulcerative colitis, and 3.8% (9/234) had unclassified IBD. Sixty-five percent (152/234) of patients were currently treated by at least one or more immunosuppressants. Participants were treated with Thiopurine monotherapy (n=11), Anti-TNF (Adalimumab or Infliximab) alone (n=59), Anti-TNF with Thiopurine (n=11), Ustekinumab or Risankizumab (n=30), Vedolizumab (n=25), Tofacitinib (n=6), 5-amino-salicylic acid (5-ASA) or no treatment (n=82), and others (n=10).

The seroprevalence of COVID-19 infection, determined by the presence of anti-SARS-CoV-2 Spike-specific IgG, was 17% (40/234) prior to vaccination (T1). Six percent (15/234) of patients were not included in the seroprevalence estimate because serology was done after the first dose of vaccine. Ninety-two percent (216/234) of patients received the initial two-dose series of an mRNA vaccine (BNT162b2 [Pfizer-BioNTech], mRNA-1273 [Moderna], or two-dose series of a vector-based vaccine (ChAdOx1 nCoV-19 [AstraZeneca]), or a dose of vector-based vaccine (Ad26.COV2.S [Johnson & Johnson]). Eighty-eight percent (189/216) of patients received an additional mRNA booster vaccine, and 9.5% (18/189) received a second booster vaccine. Blood samples for anti-SARS-CoV-2 spike-specific IgG titers were available at a median of 101 days (IQR: 77-138.5) after the two-dose series vaccine (T2) and 95 days (IQR: 63-125.8) after the booster vaccine (T3) (p=0.03). The median time between the two-dose series and the booster vaccine was six months (IQR: 4-6).

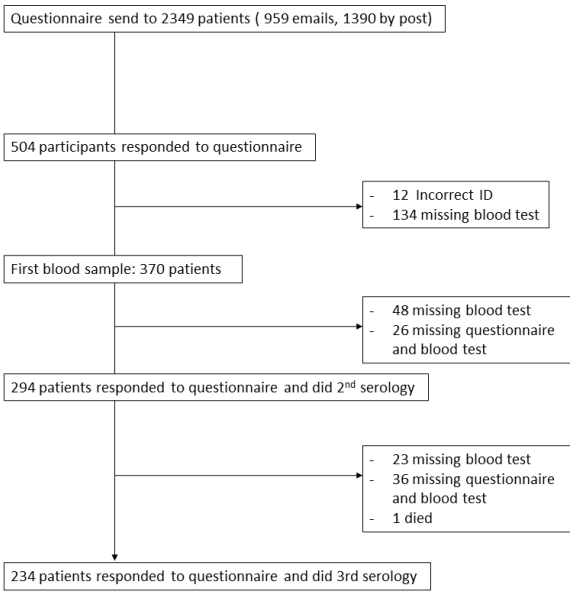

Figure 1. — Flow-chart illustrating enrolment of IBD patients.

Ninety-four percent (204/216) of patients were immunized after the two-dose series vaccine. Of the 12 non-immunized patients, 75% (9/12) were treated with Anti-TNF, 17 % (2/12) with thiopurine, 8 % (1/12) with anti-TNF plus thiopurine. All patients were seropositive after the booster vaccine dose.

During the one-year follow-up, symptoms related to COVID-19 infection were reported by 52 % (121/234) of patients in T1, by 20 % (47/234) of patients between T1 and T2 and 52.7% (123/234) of patients between T2 and T3. Among patients who reported symptoms in T1, SARS-CoV-2 RT-PCR tests were positive in 22% (26/121), negative in 41% (50/121) while 37% (45/121) did not undergo PCR testing between T1 and T2, 11% (5/47) tested positive, 42% (20/47) tested negative with 47% (22/47) not having undergone PCR testing. Similarly, between T2 and T3, 36% (44/123) of patients had positive PCR, 37% (4/123) had negative PCR while 27% (33/123) did not undergo PCR.

Among the infected patients in January 2022, 9% (4/44) had not been vaccinated and 72.7% (32/44) had received a booster vaccine. To objectively assess COVID-19 infection during the vaccination period, anti-nucleocapsid serologies were used. 18.5% (40/216) of vaccinated patients had a history of COVID-19 infection in T2. This included patients who were previously infected before vaccination and those who developed anti-nucleocapsid antibodies during the second assessment (T2). 29.6 % (56/189) of vaccinated patients had a history of COVID-19 infection in T3. This included patients with history of COVID-19 infection in T2 and those who developed anti-nucleocapsid antibodies during the third

Table 1. — **Clinical characteristics of IBD patients**

Patients, n (%)			234 (100)
Age, years, median (IQR)			47 (35 – 57)
Sex, n (%)			
female			132 (56)
male			102 (44)
Body mass index, kg/m2 (IQR)			24,4 (21.7 – 28)
Ethnicity, n (%)			
Belgian/European			199 (85)
African			15 (6.5)
Asian/Russian			8 (3.4)
American			8 (3.4)
Others			4 (1.7)
Diagnosis,n (%)			
Crohn's disease			158 (67.5)
Ulcerative colitis			67 (28.6)
Unclassified IBD			9 (3.8)
Montreal classification			
Crohn's disease, n (%)			
	Age at diagnosis	A1 (below 16 years)	22 (14)
		A2 (between 17 and 40 years)	107 (67.7)
		A3 (above 40 years)	27 (19.1)
	Location	L1 (ileal)	53 (33.5)
		L2 (colonic)	24 (15.4)
		L3 (ileocolonic)	72 (45.6)
		L1 + L4 (with upper disease)	2 (1.3)
		L2 + L4	1 (0.6)
		L3 + L4	4 (2.5)
	Behaviour	B1 (nonstricturing, nonpenetrating)	77 (48.7)
		B2 (stricturing)	48 (30.4)
		B3 (penetrating)	29 (18.4)
		B2 +B3	2 (1.3)
	p (perianal disease modifier)		52 (22.2)
	Unknown		2 (1.3)
Ulcerative colitis, n (%)			
	E1 (ulcerative proctitis)		7 (10.5)
	E2 (left-side UC)		36 (53.7)
	E3 (extensive)		21 (31.3)
	Unknown		3 (4.5)
Comorbidities, n (%)			
Heart disease			9 (3.8)
Arterial hypertension			33 (14.1)
Diabete			12 (5.1)
Lung disease			28 (12)
Kidney disease			3 (1.3)
Liver disease			9 (3.8)
Smoker, n (%)			
Yes			38 (16.2)
Not Currently			75 (32.1)
Never			121 (51.7)
IBD Treatment, n (%)			
5-ASA or no treatment			82 (35)
Thiopurine			11 (4.7)
Anti-TNF alone			59 (25.2)

Table 1. — **Clinical characteristics of IBD patients** (cont.)

IBD Treatment, n (%)	
5-ASA or no treatment	82 (35)
Thiopurine	11 (4.7)
Anti-TNF alone	59 (25.2)
Anti-TNF plus Thiopurine	11 (4.7)
Ustekinumab/Risankizumab	30 (12.8
Ustekinumab/Risankizumab plus Thiopurine	4 (1.7))
Vedolizumab	25 (10.7)
Vedolizumab plus Thiopurine	2 (0.9)
Tofacitinib	6 (2.6)
Combotherapy	2 (0.9)
Other	2 (0.8)
Serology SARS-CoV-2 spike-specific IgG before vaccine, n (%)	
Positive	40 (17.1)
Negative	179 (76.5)
Unknown	15 (6.4)
Vaccine, n (%)	216 (92,3)
mRNA	
BNT162b2 mRNA	162 (75)
2-dose interval, days, median (IQR)	29 (21-35)
Days between vaccine and Anti-Sars-CoV-2 IgG Titers, median (IQR)	103,5 (80.5-140.5)
mRNA-1273	17 (7.9)
2-dose interval, days, median (IQR)	28 (26.5-28.5)
Days between vaccine and Anti-Sars-CoV-2 IgG Titers, median (IQR)	142 (85 -157)
Vector-based vaccine	
ChAdOx1 nCoV-19	1 (14.4)
2-dose interval, days, median (IQR)	80 (70 - 84)
Days between vaccine and Anti-Sars-CoV-2 IgG Titers, median (IQR)	85 (42-109)
Ad26.COV2.S	6 (3.2)
Days between vaccine and Anti-Sars-CoV-2 IgG Titers, median (IQR)	108,5 (70-136)
Seroconversion after 2 doses	203 (94)
Booster vaccine, third dose, n (%)	
mRNA	189 (80.8)
BNT162b2 mRNA	159 (84.2)
Interval with 2nd dose, month, median (IQR)	6 (4-6)
Days between vaccine and Anti-Sars-CoV-2 IgG Titers, median (IQR)	98 (65.5-131)
mRNA-1273	30 (15.9)
Interval with 2nd dose, month, median (IQR)	6 (4-6)
Days between vaccine and Anti-Sars-CoV-2 IgG Titers, median (IQR)	83 (48-104)
Seroconversion after booster vaccine	189 (100)
Booster vaccine, fourth dose, n (%)	20 (8,5)
BNT162b2 mRNA	20 (100)

IQR, interquartile range; IBD, Inflammatory bowel disease; 5-ASA, 5-aminosalicylic acid; anti-TNF, anti-tumor necrosis factor; SARS-CoV2, severe acute respiratory syndrome coronavirus 2, mRNA, messenger ribonucleic acid; IgG, Immunoglobulin G.

assessment (T3). No severe disease was reported and no hospitalization for COVID-19 was required.

Serological Responses in IBD patients according to history of COVID-19 infection

We first compared anti-SARS-CoV-2 spike-specific IgG titers in IBD vaccinated patients. Median anti-SARS-CoV-2 spike-specific IgG titers significantly increased after the 2-dose series (698.5 BAU/mL (IQR, 190.3-1515) and after the booster vaccine 2480 BAU/mL (IQR, 1590-6820), respectively ($p < 0.0001$)) **(**Fig 2a). Among patients without the booster vaccine (n=27), no significant difference in anti-SARS-CoV-2 spike-specific IgG titers was observed, with median titers of 1920 BAU/mL (IQR, 464-2840) at T2 and 1370 BAU/mL (IQR, 490-1930) at T3, respectively. Positive anti-nucleocapsid serology or a history of COVID-19 infection significantly increased

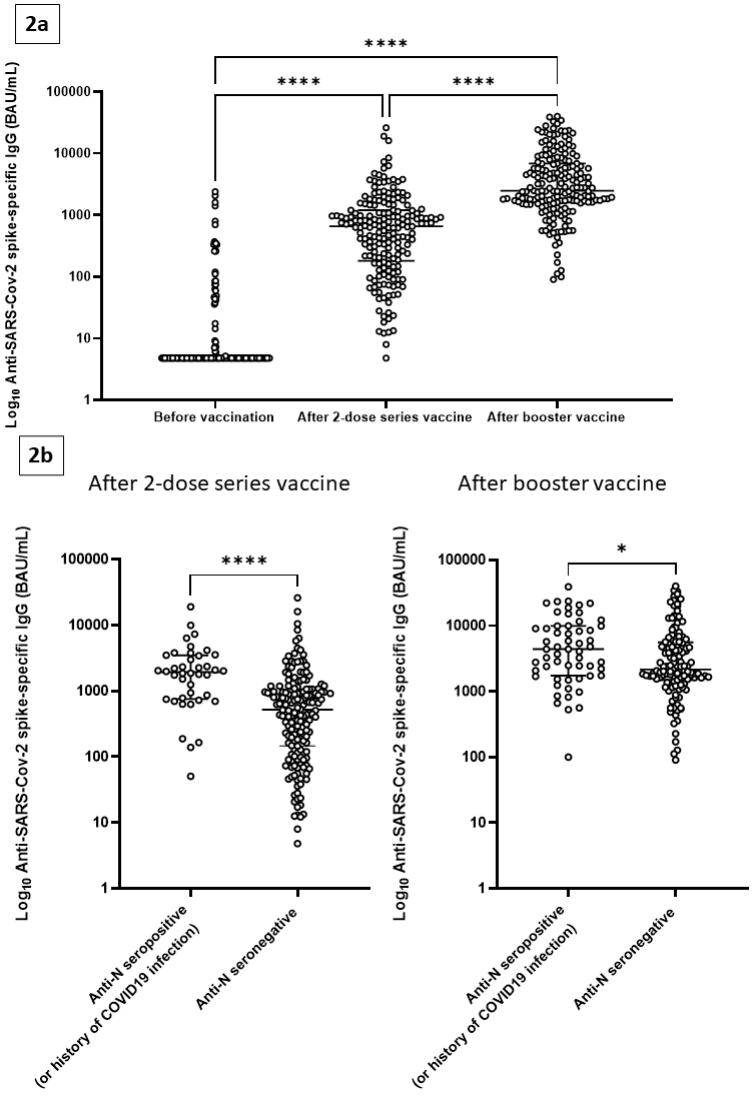

Figure 2. — Serological Responses in IBD patients according to history of COVID-19 infection. 2a. Anti-SARS-CoV-2 spike-specific IgG antibody levels prior to COVID-19 vaccination, after a 2-dose series, and after a booster vaccine. Statistical analysis was performed using the Kruskal-Wallis test. 2b. Comparison of anti-SARS-CoV-2 spike-specific IgG antibody levels between patients with positive anti-nucleocapsid antibodies and patients with negative anti-nucleocapsid antibodies after a two-dose series and booster vaccine. Statistical analysis was performed using the Mann-Whitney test.

median anti-SARS-CoV-2 spike-specific IgG titers after the 2-dose series vaccine (1930 BAU/mL (IQR,756.5-3483) versus 521 BAU/mL (IQR, 146.5-1048), $p < 0.0001$) and after the booster vaccine (4390 BAU/mL (IQR, 1730- 9900) versus 2160 BAU/mL (IQR, 1500-5600), $p = 0.0156$) (Fig 2b).

Serological Responses in IBD patients according to treatments

The median anti-SARS-CoV-2 spike-specific IgG titer was lower in patients treated with anti-TNF (243 BAU/mL (IQR, 70-665.3)) compared to patients without treatment or 5-ASA (889.5 BAU/mL (IQR, 507.5-2180) $p < 0.0001$), compared to Ustekinumab/Risankizumab group (971.5 BAU/mL (IQR, 493-1825), $p = 0.0004$) and compared to Vedolizumab group (966 BAU/mL (IQR, 338-2020) $p = 0,0011$) after a 2-dose series of the vaccine (Fig 3a).

The median anti-SARS-CoV-2 spike-specific IgG titer was also lower in patients treated with anti-TNF (1885 BAU/mL (IQR, 1150-3275), $p = 0.026$) and anti-TNF plus thiopurine (562 BAU/mL (IQR, 226-3705), $p = 0.029$) compared to patients without treatment or 5-ASA (3780 BAU/mL (IQR, 1818-8170)) after a booster vaccine. The median anti-SARS-CoV-2 spike-specific IgG titer was lower in patients treated with anti-TNF plus thiopurine (562 BAU/mL (IQR, 226-3705)) compared to anti-TNF

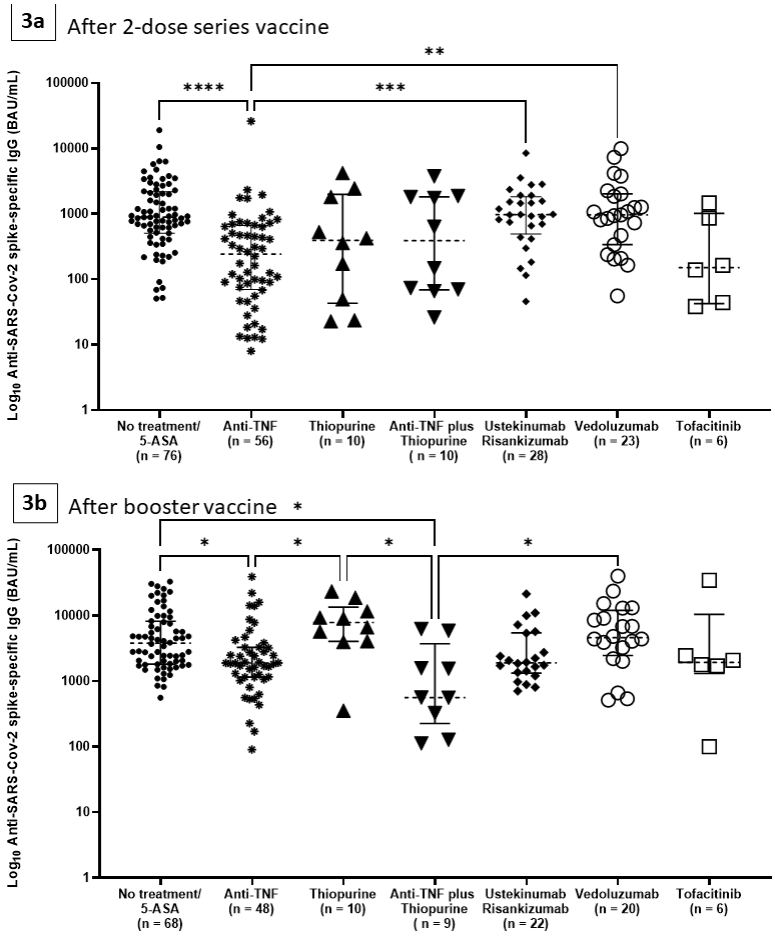

Figure 3. — Serological Responses in IBD patients according to treatments. 3a. Anti-SARS-CoV-2 spike-specific IgG antibodies after 2-dose series vaccine related to the treatment. 3b. Anti-SARS-CoV-2 spike-specific IgG antibodies after booster vaccine related to the treatment. Statistical analysis was performed with Kruskal-Wallis test with comparison of the mean of each column with the mean of every other column.

group (1885 BAU/mL (IQR, 1150-3275), p = 0.045), compared to Thiopurine alone (7830 BAU/mL (IQR, 4035-13400) p = 0.011) and compared to Vedolizumab group (4605 BAU/mL (IQR, 2463-12020) p = 0.023) after a booster vaccine (Fig 3b).

However, in immunocompromised patients, anti-SARS-CoV-2 spike-specific IgG antibody levels were significantly higher following a booster vaccine than following a 2-dose series vaccine. Anti-SARS-CoV-2 spike-specific IgG titers were significantly higher following the booster vaccine in the No treatment/5-ASA group, anti-TNF alone group, and Vedolizumab group ($p < 0.0001$) but also in the thiopurine group (p=0.004) and Ustekinumab/Risankizumab group (p=0.002) than following a 2-dose series vaccine (Fig 4).

Serological Responses in IBD patients according to treatments and history of COVID-19 infection

Interestingly, immunosuppressive treatments did not reduce the antibody response and levels in patients with a history of COVID-19 infection or positive anti-nucleocapsid serology. After a 2-dose series of the vaccine, the median anti-SARS-CoV-2 spike-specific IgG titer was 2150 BAU/mL (IQR, 771.5-3575) in patients without treatment or 5-ASA (n=18) and 1780 BAU/mL (IQR, 717.8-2630) in immunocompromised patients (n=22) (p = 0.352). After a booster vaccine, the median was 4230 BAU/mL (IQR, 1548-9910) in patients without treatment or 5-ASA (n=20) and 4225 BAU/mL (IQR, 1823-9070) in immunocompromised patients (n=36) (p = 0.976). Indeed, anti-TNF treatment did not reduce the antibody response and levels in patients with history of COVID-19 infection or positive anti-nucleocapsid serology. After a 2-dose series of the vaccine, the median anti-SARS-CoV-2 spike-specific IgG titer was 2150 BAU/mL (IQR, 771.5-3575) in patients without treatment or 5-ASA (n= 18) and 1312 BAU/mL (IQR, 759-2180) in patients with anti-TNF (n= 4) (p = 0.342). After a booster vaccine, the median was 4230 BAU/mL (IQR, 1548-9910) in patients without treatment or 5-ASA (n=20) and 4610 BAU/mL (IQR, 2228 -17425) in patients with anti-TNF (n=10) (p = 0.74) (Fig 5).

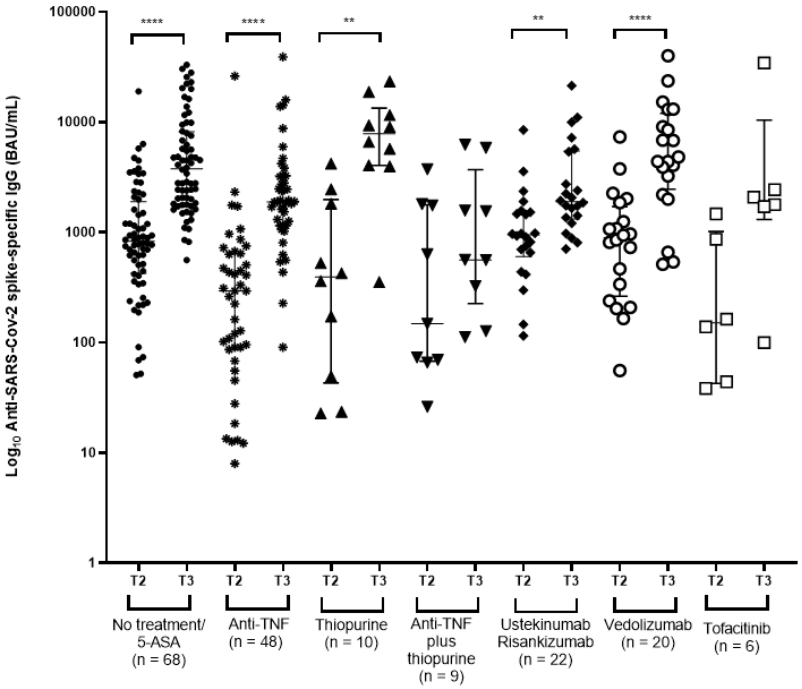

Figure 4. — Serological Responses in IBD patients according to treatments after 2-dose series and booster vaccine. Levels of anti-SARS-CoV-2 spike-specific IgG antibodies after a 2-dose series vaccine and after a booster vaccine, stratified by treatment group. Statistical analysis was performed using the Wilcoxon signed-rank test.

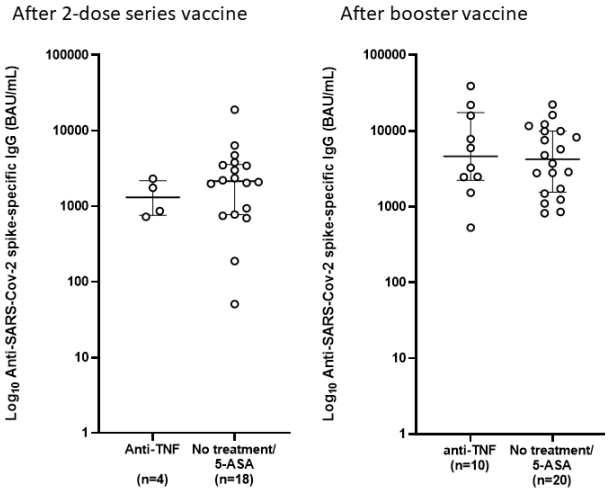

Figure 5. — Serological Responses in IBD patients according to treatments and history of COVID-19 infection. Comparison of anti-SARS-CoV-2 spike-specific IgG antibodies between patients with anti-TNF treatment and no treatment or 5-ASA in patients with positive anti-nucleocapsid antibodies or history of COVID-19 infection. Statistical analysis was performed using the Mann-Whitney test.

Multivariate analysis of the serological responses in IBD patients

In our multivariate models in Table 2, lower antibody concentrations were independently associated with anti-TNF treatment (OR=0.57 (95% CI 0.45-0.71) $p < 0.0001$) in T2 and associated with anti-TNF treatment (OR=0.77 (95% CI 0.64 -0.93) $p = 0.0064$) and anti-TNF plus thiopurine treatment (OR=0.51 (95% CI 0.37-0.71) $p = 0.00013$) in T3. Higher antibody levels were found to be independently associated with the presence of anti-nucleocapsid antibodies (OR=2.23 (95% CI 1.55-3.19) $p < 0.0001$) and receiving an mRNA vaccine (OR=2.22 (95% CI 1.81-2.72) $p < 0.0001$) in T2. Additionally, in T3, higher antibody levels were independently associated with the presence of anti-nucleocapsid antibodies (OR=1.72 (95% CI 1.32-2.26) $p = 0.00011$).

Discussion

This is one of the few studies in IBD patients where the monitoring of anti-nucleocapsid antibodies has allowed us to determine the prevalence of infection in a vaccinated cohort of IBD patients and to understand the impact of this infection on the vaccine response. Our results indicate that individuals who have previously been infected with COVID-19 or have been tested positive for anti-nucleocapsid antibodies during vaccination experience a significantly stronger boost in their antibody response compared to those who receive only the two-dose series and booster vaccine. Indeed, this had been reported in the general population, but this is the first time, to our knowledge, in IBD population.

In our cohort, the seroprevalence of COVID-19 infection was 17% before vaccination in a Belgian

Table 2. — **Predictors factors of Anti-SARS-CoV-2 spike-specific IgG levels**

T2	OR (95% CI)	P-value
Age	1 (0.99-1.01)	0.705
Male (versus female)	0.97 (0.81-1.16)	0.725
Crohn disease (versus unclassified IBD) Ulcerative colitis (versus unclassified IBD)	0.95 (0.62-1.45) 1.12 (0.73-1.74)	0.796 0.6
Anti-TNF (versus no treatment/5-ASA) Thiopurine (versus no treatment/5-ASA) Anti-TNF plus Thiopurine (versus no treatment/5-ASA) Ustekinumab/Risankizumab (versus no treatment/5-ASA) Vedolizumab (versus no treatment/5-ASA) Tofacitinib (versus no treatment/5-ASA)	0.57 (0.45-0.71) 0.67 (0.45-1) 0.58 (0.38-0.88) 0.98 (0.74-1.3) 1.01 (0.74-1.38) 0,58 (0,3-1)	< 0.0001 0.05 0.01 0.88 0.96 0,052
mRNA vaccine (versus Vector-based vaccine)	2.22 (1.81-2.72)	< 0.0001
Anti-nucleocapsid seropositive (versus seronegative)	2.23 (1.55-3.19)	< 0.0001
T3	OR (95% CI)	P-value
Age	1 (0.99-1)	0.588
Male (versus female)	1.06 (0.92-1.22)	0.457
Crohn disease (versus indeterminate) Ulcerative colitis (versus indeterminate)	0.89 (0.65-1.21) 0.97 (0.71-1.34)	0.447 0.862
Anti-TNF (versus no treatment/5-ASA) Thiopurine (versus no treatment/5-ASA) Anti-TNF plus thiopurine (versus no treatment/5-ASA) Ustekinumab/Risankizumab (versus no treatment/5-ASA) Vedolizumab (versus no treatment/5-ASA) Tofacitinib (versus no treatment/5-ASA)	0.77 (0.64-0.93) 1.1 (0.8-1.51) 0.51 (0.37-0.71) 0.84 (0.66-1.07) 1.06 (0,83-1,36) 0,4 (0,3-0,75)	0.006 0.572 0.00013 0.166 0,634 0,001
mRNA vaccine (versus Vector-based vaccine) After 2-dose series vaccine	1,2 (1-1, 44)	0,052
Days between booster vaccine and blood sample T3	1 (1-1)	0,022
Anti-nucleocapsid seropositive in T3 (versus seronegative)	1.72 (1.32-2.26)	0,00011

First Multivariate model is referred to the anti-SARS-CoV-2 spike-specific IgG levels in vaccinated patients in T2 (number of observations, 216) and second Multivariate model is referred to the anti-SARS-CoV-2 spike-specific IgG levels in vaccinated patients in T3 (number of observations, 189). Factor were taken in account were age, sex, disease, immunosuppressive treatments (reported to the group "No treatment/5-ASA"), type of vaccine and anti-nucleocapsid antibodies. Log10 median anti-SARS-CoV-2 spike-specific IgG levels are exponentiated in the text for interpretability. T2, follow-up after 6-months; T3, follow-up after 1-year; 5-ASA, 5-aminosalicylic acid; anti-TNF, anti-tumor necrosis factor.

IBD population. It is higher than the one reported by ICARUS-IBD Consortium, which was 6.7% (23) while local general population seroprevalence was 15.1 to 18.7% (24), suggesting perhaps variations in the timing of screenings across the country. Ninety-two percent of patients received a two-dose series of the COVID-19 vaccine and 88 % of them also received a booster vaccine. This is a significant proportion of vaccinated individuals. Some studies have reported that only half or a third of hesitant patients are willing to be vaccinated (25,26). Volunteering to participate in the study likely contributes to selecting patients who want to get vaccinated. Ninety-four percent of patients in our cohort were immunized after receiving the two-dose series vaccine, and all patients tested positive for antibodies after receiving the booster vaccine dose. As the pandemic progressed, breakthrough infections or reinfections with new variants became more frequent. The proportion of patients who tested positive for anti-nucleocapsid antibodies increased in January 2022, which suggests a possible post-vaccination breakthrough infection or reinfection linked to the "Omicron" wave. In our study, we found that IBD patients with a positive anti-nucleocapsid serology or a history of COVID-19 infection had higher levels of anti-SARS-CoV-2 spike-specific IgG antibodies following a two-dose series and booster vaccination. This was further highlighted in multivariate analysis where higher anti-SARS-CoV-2 spike-specific IgG concentrations were independently associated with a positive anti-nucleocapsid antibody test result**.** Thus, additional antigen exposure from natural infection substantially boosts the quantity, quality, and breadth of the humoral immune response, regardless of whether it occurs before or after vaccination.

In our study population of individuals with IBD, we observed that levels of anti-SARS-CoV-2 spike-specific IgG antibodies were reduced in patients taking anti-TNF and anti-TNF plus thiopurine after receiving the 2-dose series vaccine and booster vaccine, respectively. Our multivariate models showed that lower antibody

concentrations were independently associated with anti-TNF therapy at 6 months of follow-up, and with both anti-TNF and anti-TNF plus thiopurine at one year of follow-up. These findings are consistent with previous studies, such as the VIP study, which found that COVID-19 vaccine-induced antibody response is attenuated in IBD patients taking Infliximab, Infliximab plus Thiopurine or Tofacitinib (8). However, in immunocompromised patients, anti-SARS-CoV-2 spike-specific IgG antibody levels were significantly higher despite immunosuppressive treatment following the booster vaccine than following the 2-dose series vaccine, as reported in the PREVENT-COVID (15) HERCULES (14) and VIP studies (8). Remarkably, our study suggests that immunocompromised (mostly anti-TNF) patients with positive anti-nucleocapsid serology or previous history COVID19 infection do not experience a dampened antibody response (after the 2-dose and booster vaccine) as compared to immunocompromised patients without anti-nucleocapsid antibody or a previous history COVID19 infection. This is consistent with the result of a recent study, indicating that patients with IBD who previously contracted COVID-19 had exhibited comparable anti-spike IgG levels after the third vaccination, similar to those observed in healthy controls with a history of prior COVID-19 infection (27). In a recent longitudinal study, the efficacy of immunity induced by primary SARS-CoV-2 infection, characterized by positive anti-SARS-CoV-2 spike or anti-SARS-CoV-2 Nucleocapsid antibodies, was compared between vaccinated patients with prior infection and those lacking a pre-existing infection before vaccination. Notably, individuals exposed to three antigens-comprising two vaccine doses and natural infection-failed to demonstrate a statistically significant difference in immune response to spike protein when contrasted with individuals who exclusively received two vaccine doses (28). It is important to acknowledge that this study does not take account infections that may have occurred during the vaccination period, introducing potential biases that could impact antibody dynamics. Aligning with observations in the general population, it is known that infection breakthrough during vaccination were found to elicit the highest peak IgG response. This underscores the pivotal role of recurrent exposures to SARS-CoV-2 antigens in heightening IgG responses. Moreover, this study reveals that the temporal relationship between infection and vaccination, rather than the severity of the initial infection, positively correlates with an augmented post-vaccination response (29). Despite the limited sample size within our study's subgroup of patients experiencing infections before or during vaccination, the antecedent history of COVID-19 infection, breakthrough infections, or re-infections emerges as a potential natural booster.

Finally, in our multivariate model, higher anti-SARS-CoV-2 spike-specific IgG concentrations were independently associated with a 2-dose series of mRNA vaccine, supporting the use of mRNA vaccines as a booster. This is also the case in the general population, where mRNA vaccine boosters in individuals vaccinated can be highly beneficial, as they markedly increase the humoral and cellular immune responses against the virus, including against the Omicron variant (30).

However, our study has some limitations. We enrolled non-immunosuppressed patients with a diagnosis of IBD as a control group, which could underestimate the antibody level. Some subgroups of treatment, such as tofacitinib-treated patients, have a small sample size, which could explain the differences with literature data. We don't know the impact of disease activity on this vaccine response. Unfortunately, the use of corticosteroids was not considered in this study. Only 9 patients reported the use of corticosteroids. The sample size was too small and too heterogeneous to draw conclusive analysis. Additionally, by using positivity for anti-nucleocapsid IgG serology as a marker for breakthrough infection or reinfection, we may still underestimate the number of COVID-19 infected patients.

In conclusion, our study found that IBD patients with a history of COVID-19 infection during vaccination have higher levels of anti-SARS-CoV-2 spike-specific IgG antibodies following 2-dose series and booster vaccination. This reflects that the additional antigen exposure from natural infection boosts the quantity of anti-SARS-CoV-2 spike-specific IgG antibodies. Our study adds to the growing body of literature on the response to COVID-19 vaccines in immunocompromised IBD patients and highlights the importance of considering COVID19 infection in vaccination strategies. Further investigations should explore the optimal timing for administering the booster dose by examining the interval between infection and theoretical time for booster vaccination.

Authors' contribution

AH and DF participated in the conception and design of the study. AH, CG, CL, LA, AC and DF contributed to the acquisition and analysis of the data, AH and NR performed the statistical analyses, VW, CM and EQ participated in acquisition/collection of the blood samples, HD, LM, and OV coordinated and processed serological analyses, AH made the draft of the manuscript with contribution from DF and CG; all authors carried out critical revision of the manuscript for important intellectual content; all authors read and approved the final version of the manuscript.

Declaration of personal interests

None declared.

Conflicts of interests

None declared.

Funding

This work was supported by LHUB-ULB, Laboratoire Hospitalier Universitaire Bruxelles, Universitair Laboratorium Brussel, Belgium for the serologic analyses.

References

1. Richardson S, Hirsch JS, Narasimhan M, et al. Presenting Characteristics, Comorbidities, and Outcomes among 5700 Patients Hospitalized with COVID-19 in the New York City Area. *JAMA - Journal of the American Medical Association*. 2020;323(20):2052-2059. doi:10.1001/jama.2020.6775
2. Watson OJ, Barnsley G, Toor J, Hogan AB, Winskill P, Ghani AC. Global impact of the first year of COVID-19 vaccination: a mathematical modelling study. *Lancet Infect Dis*. 2022;22(9):1293-1302. doi:10.1016/S1473-3099(22)00320-6
3. Ungaro RC, Brenner EJ, Agrawal M, et al. Impact of Medications on COVID-19 Outcomes in Inflammatory Bowel Disease: Analysis of More Than 6000 Patients From an International Registry. *Gastroenterology*. 2022;162(1):316-319.e5. doi:10.1053/j.gastro.2021.09.011
4. Parekh R, Zhang X, Ungaro RC, et al. Presence of Comorbidities Associated with Severe Coronavirus Infection in Patients with Inflammatory Bowel Disease. *Dig Dis Sci*. 2022;67(4):1271-1277. doi:10.1007/s10620-021-07104-0
5. Brenner EJ, Ungaro RC, Gearry RB, et al. Corticosteroids, But Not TNF Antagonists, Are Associated With Adverse COVID-19 Outcomes in Patients With Inflammatory Bowel Diseases: Results From an International Registry. *Gastroenterology*. 2020;159(2):481-491.e3. doi:10.1053/j.gastro.2020.05.032
6. Jones JL, Tse F, Carroll MW, et al. Canadian Association of Gastroenterology Clinical Practice Guideline for Immunizations in Patients With Inflammatory Bowel Disease (IBD)—Part 2: Inactivated Vaccines. *Gastroenterology*. 2021;161(2):681-700. doi:10.1053/j.gastro.2021.04.034
7. Melmed GY, Agarwal N, Frenck RW, et al. Immunosuppression impairs response to pneumococcal polysaccharide vaccination in patients with inflammatory bowel disease. *American Journal of Gastroenterology*. 2010;105(1):148-154. doi:10.1038/ajg.2009.523
8. Alexander JL, Kennedy NA, Ibraheim H, et al. COVID-19 vaccine-induced antibody responses in immunosuppressed patients with inflammatory bowel disease (VIP): a multicentre, prospective, case-control study. *Lancet Gastroenterol Hepatol*. 2022;7(4):342-352. doi:10.1016/S2468-1253(22)00005-X
9. Doherty J, Morain NO, Stack R, et al. Reduced Serological Response to COVID-19 Vaccines in Patients with IBD is Further Diminished by TNF Inhibitor Therapy; Early Results of the VARIATION study [VAriability in Response in IBD Against SARS-COV-2 ImmunisatiON]. *J Crohns Colitis*. Published online February 18, 2022. doi:10.1093/ecco-jcc/jjac029
10. Thompson MG, Natarajan K, Irving SA, et al. *Morbidity and Mortality Weekly Report Effectiveness of a Third Dose of MRNA Vaccines Against COVID-19-Associated Emergency Department and Urgent Care Encounters and Hospitalizations Among Adults During Periods of Delta and Omicron Variant Predominance-VISION Network, 10 States*. https://papers.ssrn.com/sol3/papers.cfm?abstract_id=3961378
11. Barda N, Dagan N, Cohen C, et al. Effectiveness of a third dose of the BNT162b2 mRNA COVID-19 vaccine for preventing severe outcomes in Israel: an observational study. *The Lancet*. 2021;398(10316):2093-2100. doi:10.1016/S0140-6736(21)02249-2
12. Moreira ED, Kitchin N, Xu X, et al. Safety and Efficacy of a Third Dose of BNT162b2 Covid-19 Vaccine. *New England Journal of Medicine*. 2022;386(20):1910-1921. doi:10.1056/nejmoa2200674
13. Munro APS, Janani L, Cornelius V, et al. Safety and immunogenicity of seven COVID-19 vaccines as a third dose (booster) following two doses of ChAdOx1 nCov-19 or BNT162b2 in the UK (COV-BOOST): a blinded, multicentre, randomised, controlled, phase 2 trial. *The Lancet*. 2021;398(10318):2258-2276. doi:10.1016/S0140-6736(21)02717-3
14. Schell TL, Knutson KL, Saha S, et al. Humoral Immunogenicity of 3 COVID-19 Messenger RNA Vaccine Doses in Patients With Inflammatory Bowel Disease. *Inflamm Bowel Dis*. 2022;28(11):1781-1786. doi:10.1093/ibd/izac082
15. Long MD, Weaver KN, Zhang X, et al. Strong Response to SARS-CoV-2 Vaccine Additional Doses Among Patients With Inflammatory Bowel Diseases. *Clinical Gastroenterology and Hepatology*. 2022;20(8):1881-1883.e1. doi:10.1016/j.cgh.2022.01.056
16. Shiga H, Kakuta Y, An K, et al. Response to COVID-19 vaccine is reduced in patients with inflammatory bowel disease, but improved with additional dose. *Journal of Gastroenterology and Hepatology (Australia)*. 2023;38(1):44-51. doi:10.1111/jgh.16001
17. Liu Z, Alexander JL, Lin KW, et al. Infliximab and Tofacitinib Attenuate Neutralizing Antibody Responses Against SARS-CoV-2 Ancestral and Omicron Variants in Inflammatory Bowel Disease Patients After 3 Doses of COVID-19 Vaccine. *Gastroenterology*. 2023;164(2):300-303.e3. doi:10.1053/j.gastro.2022.10.010
18. Caldera F, Richard L, Almasry M, et al. Persistence of Antibodies Six Months after Three COVID-19 mRNA Vaccine Doses in Patients with Inflammatory Bowel Disease. *Inflamm Bowel Dis*. 2023;29(10):1662-1666. doi:10.1093/ibd/izad019
19. Bates TA, Mcbride SK, Leier HC, et al. *Vaccination before or after SARS-CoV-2 Infection Leads to Robust Humoral Response and Antibodies That Effectively Neutralize Variants*. Vol 7.; 2022. https://www.science.org
20. Meyer B, Drosten C, Müller MA. Serological assays for emerging coronaviruses: Challenges and pitfalls. *Virus Res*. 2014;194:175-183. doi:10.1016/j.virusres.2014.03.018
21. Voysey M, Clemens SAC, Madhi SA, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *The Lancet*. 2021;397(10269):99-111. doi:10.1016/S0140-6736(20)32661-1
22. Sadoff J, Gray G, Vandebosch A, et al. Safety and Efficacy of Single-Dose Ad26.COV2.S Vaccine against Covid-19. *New England Journal of Medicine*. 2021;384(23):2187-2201. doi:10.1056/nejmoa2101544
23. Wong SY, Wellens J, Helmus D, et al. Geography Influences Susceptibility to SARS-CoV-2 Serological Response in Patients With Inflammatory Bowel Disease: Multinational Analysis From the ICARUS-IBD Consortium. *Inflamm Bowel Dis*. 2023;29(11):1693-1705. doi:10.1093/ibd/izad097
24. Leclercq V, Van den Houte N, Gisle L, et al. Prevalence of Anti-SARS-CoV-2 Antibodies and Potential Determinants among the Belgian Adult Population: Baseline Results of a Prospective Cohort Study. *Viruses*. 2022;14(5). doi:10.3390/v14050920
25. Caron B, Neuville E, Peyrin-Biroulet L. Inflammatory Bowel Disease and COVID-19 Vaccination: A Patients' Survey. *Dig Dis Sci*. 2022;67(6):2067-2073. doi:10.1007/s10620-021-07040-z
26. Clarke K, Pelton M, Stuart A, et al. COVID-19 Vaccine Hesitancy in Patients with Inflammatory Bowel Disease. *Dig Dis Sci*. 2022;67(10):4671-4677. doi:10.1007/s10620-021-07377-5
27. Doherty J, O'Morain N, Stack R, et al. Reduced Serological Response to COVID-19 Booster Vaccine is Associated with Reduced B Cell Memory in Patients With Inflammatory Bowel Disease; VARIATION [VAriability in Response in IBD AgainsT SARS-COV-2 ImmunisatiON]. *J Crohns Colitis*. 2023;17(9):1445-1456. doi:10.1093/ecco-jcc/jjad065
28. González Cueto E, Edmans M, Wellens J, et al. Antibody, not Cellular, Immune Responses to SARS-CoV-2 Vaccination Outperform Infection in Inflammatory Bowel Disease Patients. *Inflamm Bowel Dis*. Published online November 3, 2023. doi:10.1093/ibd/izad265
29. Epsi NJ, Richard SA, Lindholm DA, et al. Understanding "Hybrid Immunity": Comparison and Predictors of Humoral Immune Responses to Severe Acute Respiratory Syndrome Coronavirus 2 Infection (SARS-CoV-2) and Coronavirus Disease 2019 (COVID-19) Vaccines. *Clinical Infectious Diseases*. 2023;76(3):E439-E449. doi:10.1093/cid/ciac392
30. Zuo F, Abolhassani H, Du L, et al. Heterologous immunization with inactivated vaccine followed by mRNA-booster elicits strong immunity against SARS-CoV-2 Omicron variant. *Nat Commun*. 2022;13(1). doi:10.1038/s41467-022-30340-5