



Systemic and T cell-associated responses to SARS-CoV-2 immunisation in gut inflammation (STAR SIGN study): effects of biologics on vaccination efficacy of the third dose of mRNA vaccines against SARS-CoV-2

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Summary

Background: Immunosuppressed patients with inflammatory bowel disease (IBD) experience increased risk of vaccine-preventable diseases such as COVID-19.

Aims: To assess humoral and cellular immune responses following SARS-CoV-2 booster vaccination in immunosuppressed IBD patients and healthy controls.

Methods: In this prospective, multicentre, case-control study, 139 IBD patients treated with biologics and 110 healthy controls were recruited. Serum anti-SARS-CoV-2 spike IgG concentrations were measured 2–16 weeks after receiving a third mRNA vaccine dose. The primary outcome was to determine if humoral immune responses towards booster vaccines differ in IBD patients under anti-TNF versus non-anti-TNF therapy and healthy controls. Secondary outcomes were antibody decline, impact of previous infection and SARS-CoV-2-targeted T cell responses.

Results: Anti-TNF-treated IBD patients showed reduced anti-spike IgG concentrations (geometric mean 2357.4 BAU/ml [geometric SD 3.3]) when compared to non-anti-TNF-treated patients (5935.7 BAU/ml [3.9]; $p < 0.0001$) and healthy controls (5481.7 BAU/ml [2.4]; $p < 0.0001$), respectively. In multivariable modelling, prior infection (geometric mean ratio 2.00 [95% CI 1.34–2.90]) and vaccination with mRNA-1273 (1.53 [1.01–2.27]) increased antibody concentrations, while anti-TNF treatment (0.39 [0.28–0.54]) and prolonged time between vaccination and antibody measurement (0.72 [0.58–0.90]) decreased anti-SARS-CoV-2 spike antibodies. Antibody decline was comparable in IBD patients independent of anti-TNF treatment and antibody

STAR SIGN study investigators are listed in Appendix S1.

Simon Woelfel and Joel Dütschler contributed equally.

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concentrations could not predict breakthrough infections. Cellular and humoral immune responses were uncoupled, and more anti-TNF-treated patients than healthy controls developed inadequate T cell responses (15/73 [20.5%] vs 2/100 [2.0%]; $p = 0.00031$).

Conclusions: Anti-TNF-treated IBD patients have impaired humoral and cellular immunogenicity following SARS-CoV-2 booster vaccination. Fourth dose administration may be beneficial for these patients.

1 | INTRODUCTION

The approval of the first SARS-CoV-2 mRNA vaccine in 2020 marked a turning point in the ongoing coronavirus pandemic. Despite the evolution of highly contagious variants, vaccines have ever since limited the number of SARS-CoV-2 transmission, hospitalisations and deaths.¹

However, conclusive data on the protection of immunocompromised individuals is scarce, since initial vaccine trials excluded such patient groups. The inflammatory bowel diseases (IBD) Crohn's disease (CD) and ulcerative colitis (UC) are immunological disorders of multifactorial origin that affect more than 0.3% of the population in Western countries and often require immunosuppressive treatment with biologics.^{2,3} It has been demonstrated that anti-tumour necrosis factor- α (TNF) antibodies, such as infliximab or adalimumab, are associated with reduced immune responses after vaccination against several viruses such as hepatitis B, hepatitis A and influenza, which sparked concerns on the protection of these patients by SARS-CoV-2 vaccines.^{4–6} Importantly, such effects could not be found in patients treated with vedolizumab or ustekinumab, which antagonise molecular mediators of inflammation distinct to the TNF pathway, namely $\alpha 4\beta 7$ integrin and IL-12/IL-23.^{7,8} Furthermore, IBD patients face an increased risk of requiring hospitalisation following SARS-CoV-2 infection.⁹

Strikingly, several recent studies demonstrated that the concentration of anti-spike antibodies following one or two doses of the SARS-CoV-2 mRNA vaccine BNT162b2 are reduced in IBD patients treated with infliximab compared to patients treated with vedolizumab or ustekinumab and healthy controls.^{10–13} These findings highlight the importance of additional booster vaccines in order to improve the protection of these vulnerable patient groups. This is especially important in consideration of the rapid spread of the B.1.1.529 (omicron) variant which is more contagious than its predecessor and can partly escape immune responses.¹⁴ So far, third dose SARS-CoV-2 vaccination was shown to be efficient in improving immune responses and increasing the breadth of protection.¹⁵ However, little is known about the benefit of a third vaccine dose for IBD patients treated with biologics.

In order to improve guidelines for the fourth SARS-CoV-2 vaccination in immunosuppressed patients, we assessed humoral and cellular immune responses following SARS-CoV-2 booster vaccination with a third dose in immunosuppressed IBD patients and

healthy controls. We hypothesised that anti-SARS-CoV-2 spike IgG levels following a third vaccine dose are reduced in anti-TNF-treated IBD patients compared to healthy controls and IBD patients treated with other biologics such as vedolizumab and ustekinumab. Furthermore, we hypothesised that these patients develop attenuated SARS-CoV-2-directed T cell immunity after receiving a third vaccine dose.

2 | MATERIALS AND METHODS

2.1 | Study design

The STAR SIGN study (Systemic and T cell-Associated Responses to SARS-CoV-2 booster Immunisation in Gut iNflammation) is a national multi-centre case-control study with the aim to investigate the effect of biologics on immunogenicity towards a third dose of SARS-CoV-2 mRNA vaccines in IBD patients. The study is designed as a prospective observational trial. Prospective data were collected through a patient questionnaire and via assessment of blood samples taken at inclusion. Retrospective data were collected manually from electronic medical records. The study protocol was approved by the Ethics Committee of Eastern Switzerland (project-ID 2021-02511).

2.2 | Study population

Participants for the subject group were recruited at the IBD outpatient clinic of the Cantonal Hospital St. Gallen, at the outpatient clinic Rorschach, and at the IBD outpatient clinic of the Inselspital Bern University Hospital during hospital visits scheduled for application of biologic therapy. Healthy participants were recruited from staff of the Cantonal Hospital St. Gallen. Staff who were directly involved in the research project, for example, via project planning, patient recruitment, data collection and handling or evaluation of results were excluded from this study. For all participants, age of at least 18 years and completion of a third dose of a SARS-CoV-2 vaccine 2–16 weeks prior to enrolment were essential inclusion criteria. Patient eligibility criteria included a diagnosis of UC, CD or indeterminate colitis and treatment with either anti-TNF antibodies (infliximab, adalimumab, golimumab and certolizumab pegol) or non-anti-TNF biologics (vedolizumab and ustekinumab). Healthy

control eligibility criteria included the absence of IBD. Study exclusion was prompted upon incapability to answer the questionnaire, absence of signed consent, pregnancy at the time of SARS-CoV-2 booster vaccination or between booster vaccination and inclusion, and administration of a booster vaccine different from BNT162b2 or mRNA-1273. Regarding the control group, study exclusion was also prompted upon use of immunosuppressive medication including steroids, immunomodulators and biologics within 6 months before booster application or between booster application and inclusion. Variables recorded for this study include booster vaccine type (BNT162b2, mRNA-1273), participant demographics (age, gender, ethnicity, education, BMI, smoking status and comorbidities) and specific information about IBD such as diagnosis (CD, UC and indeterminate colitis), disease duration, age at disease diagnosis, concomitant medication and disease activity. Additionally, the presence of a prior SARS-CoV-2 infection based on a positive PCR or rapid antigen test was recorded. Detailed characteristics of study participants can be found in [Table 1](#).

2.3 | Study outcomes

Our primary outcome was to determine if the anti-spike antibody levels in anti-TNF-treated IBD patients differ from non-anti-TNF-treated IBD patients and healthy controls, respectively, after third SARS-CoV-2 (booster) vaccination. This comparison was determined by analysing the ratio of the geometric means of anti-spike protein IgG concentrations 2–16 weeks after receiving a third dose of SARS-CoV-2 mRNA vaccines. We analysed this outcome using a multivariable linear regression model controlling for age, smoking, detectable nucleocapsid antibodies, time between receiving the second and third vaccine doses, time between booster vaccination and measurement of antibody levels, and type of vaccine.

Secondary outcomes were

1. antibody concentrations following SARS-CoV-2 booster vaccination with a third dose over time,
2. antibody concentrations in participants who experienced a SARS-CoV-2 infection before booster vaccination, and
3. cellular immunity status, as indicated by T-cell-mediated interferon- γ (IFN- γ) release after stimulation of whole blood with SARS-CoV-2 antigens.

2.4 | Measurement of SARS-CoV-2 spike protein and nucleocapsid antibody concentrations

Anti-SARS-CoV-2 spike protein and anti-nucleocapsid antibody concentrations were determined in the sera of study participants two to 16 weeks after third dose SARS-CoV-2 booster vaccination. All measurements were performed by trained laboratory staff at the Center of Laboratory Medicine (ZLM) in St. Gallen (Switzerland) which is accredited according to the ISO/IEC 17025 norm. Serum

was isolated after a coagulation time of 20 min by centrifugation for 10 min at 2800g.

Concentrations of IgG antibodies targeting the SARS-CoV-2 spike protein were measured using the LIAISON® SARS-CoV-2 TrimericS IgG assay (DiaSorin Inc.) according to the manufacturer's instructions. This chemiluminescence immunoassay (CLIA) uses recombinant trimeric SARS-CoV-2 spike protein-covered magnet particles (solid phase) and isoluminol-conjugated antibodies directed against human IgG. Briefly, in the first step, SARS-CoV-2 spike protein-targeting antibodies in the sample bind to the trimeric spike protein in the solid phase and unbound antibodies are washed off. In the second step, the isoluminol-conjugated antibodies bind to IgG antibodies that are attached to the solid phase. After removing excess antibody conjugate, the amount of attached isoluminol is quantified by measuring the Relative Light Units (RLU) released by a flash chemiluminescence reaction. RLUs are converted to the WHO international standard BAU/ml (BAU = binding antibody units) using a numerical factor of 2.6, as evaluated by the manufacturer. Samples containing 33.8 BAU/ml or more were considered seropositive. If anti-spike protein IgG levels were above the detectable range of the assay (>2080 BAU/ml), samples were diluted 1:20 in LIAISON® TrimericS IgG Diluent Accessory.

The presence of IgG antibodies against the SARS-CoV-2 nucleocapsid protein was determined using the Biomerica COVID-19 IgG/IgM Rapid Test (Biomerica) according to the manufacturer's instructions. This is a lateral flow chromatographic immunoassay, in which the specimen reacts with a SARS-CoV-2 nucleocapsid antigen-gold conjugate, and then migrates upwards on the membrane chromatographically to react with anti-human IgM and anti-human IgG. A positive result is indicated by the appearance of a band next to the "IgM" or "IgG" in the test window, respectively. Sufficient loading of sample volume and test buffer is indicated by the appearance of a band next to the "C". Participants with a positive test for nucleocapsid IgG antibodies were considered nucleocapsid seropositive. These participants were considered to have experienced a SARS-CoV-2 infection prior to study inclusion.

2.5 | IFN- γ release assay

For the assessment of CD4⁺ and CD8⁺ T cell-mediated immune responses towards SARS-CoV-2, the amount of IFN- γ released by peripheral blood lymphocytes was measured upon stimulation with T cell-directed SARS-CoV-2 antigens. For stimulation, the Qiagen QuantiFERON® SARS-CoV-2 starter and extended sets (Qiagen) were utilised according to the manufacturer's instructions. Briefly, blood was collected in heparinized blood collection tubes, stored overnight at 4°C and transferred to QuantiFERON® SARS-CoV-2 tubes, which contain the following SARS-CoV-2 antigen peptides: CD4⁺-stimulating spike protein peptide derived from the S1 subunit (SARS-CoV-2 Ag1 tube), CD4⁺- and CD8⁺-stimulating spike protein peptides derived from the subunits S1 and S2 (SARS-CoV-2 Ag2 tube), or the latter antigens combined

TABLE 1 Baseline characteristics of study population.

Variable	Level	Anti-TNF (n = 73)	Non-anti-TNF (n = 52)	Healthy controls (n = 100)
Type of vaccine (%)	BNT162b2 (Pfizer-BioNTech)	55 (75.3)	40 (76.9)	91 (91.0)
	mRNA-1273 (Moderna)	18 (24.7)	12 (23.1)	9 (9.0)
Age, years (mean [SD])		44.64 (15.32)	51.87 (18.19)	46.63 (11.25)
Gender (%)	Female	31 (42.5)	20 (38.5)	54 (54.0)
	Male	42 (57.5)	32 (61.5)	46 (46.0)
Ethnicity (%)	European	68 (93.2)	50 (98.0)	98 (98.0)
	Asian	1 (1.4)	1 (2.0)	1 (1.0)
	African	2 (2.7)	0 (0.0)	0 (0.0)
	Other	2 (2.7)	0 (0.0)	1 (1.0)
Education (%)	Primary	12 (16.4)	6 (11.5)	1 (1.0)
	Secondary	34 (46.6)	23 (44.2)	22 (22.0)
	Tertiary	27 (37.0)	23 (44.2)	77 (77.0)
BMI, kg/m ² (mean [SD])		24.48 (3.64)	25.47 (5.39)	24.08 (3.42)
Smoking (%)	Never	28 (38.4)	23 (44.2)	57 (57.0)
	Former	32 (43.8)	21 (40.4)	29 (29.0)
	Current	13 (17.8)	8 (15.4)	14 (14.0)
Diagnosis (%)	Crohn's disease	54 (74.0)	31 (59.6)	
	Ulcerative colitis	19 (26.0)	20 (38.5)	
	Indeterminate colitis	0 (0.0)	1 (1.9)	
Disease duration, years (median [IQR])		11.00 [6.00, 19.50]	12.00 [7.00, 22.00]	
Age at diagnosis, years (median [IQR])		26.00 [20.00, 40.50]	30.00 [22.00, 50.50]	
Steroids (%)	Yes	17 (23.3)	9 (17.3)	
	No	56 (76.7)	43 (82.7)	
Immunomodulator (%)	Yes	1 (1.4)	2 (3.8)	
	No	72 (98.6)	50 (96.2)	
5-ASA (%)	Yes	16 (21.9)	16 (30.8)	
	No	57 (78.1)	36 (69.2)	
Disease activity (PRO2) (%)	Remission	49 (69.0)	33 (63.5)	
	Mild	13 (18.3)	8 (15.4)	
	Moderate	9 (12.7)	10 (19.2)	
	Severe	0 (0.0)	1 (1.9)	
Cancer (%)	No	73 (100.0)	52 (100.0)	
Heart disease (%)	Yes	4 (5.5)	6 (11.5)	
	No	69 (94.5)	46 (88.5)	
Hypertension (%)	Yes	6 (8.2)	10 (19.2)	
	No	67 (91.8)	42 (80.8)	
Pulmonary disease (%)	Yes	1 (1.4)	5 (9.6)	
	No	72 (98.6)	47 (90.4)	
Kidney disease (%)	Yes	2 (2.7)	3 (5.8)	
	No	71 (97.3)	49 (94.2)	
Diabetes (%)	Yes	1 (1.4)	0 (0.0)	
	No	72 (98.6)	52 (100.0)	

TABLE 1 (Continued)

Variable	Level	Anti-TNF (n = 73)	Non-anti-TNF (n = 52)	Healthy controls (n = 100)
Hyperlipidaemia (%)	Yes	1 (1.4)	2 (3.8)	
	No	72 (98.6)	50 (96.2)	
Arthritis (%)	Yes	9 (12.3)	1 (1.9)	
	No	64 (87.7)	51 (98.1)	
Reported infection before booster (%)	Yes	6 (8.2)	2 (3.8)	10 (10.0)
	No	67 (91.8)	50 (96.2)	90 (90.0)
Anti-SARS-CoV-2 nucleocapsid IgG (%)	Positive	6 (8.2)	6 (11.5)	22 (22.4)

with immunodominant CD8⁺ epitopes derived from the whole genome (SARS-CoV-2 Ag3 tube). As negative and positive controls, QuantiFERON® Nil and Mitogen blood collection tubes that contain no antigen (Nil) or mitogen (Mitogen) were used, respectively. For stimulation, antigen tubes were incubated at 37°C for 16–24 h and plasma was harvested by centrifugation for 10 min at 2800g. IFN- γ in serum from stimulated whole blood was quantified using the DiaSorin Liaison® QuantiFERON®-TB Gold Plus assay kit (DiaSorin Inc.) as instructed by the manufacturer. In brief, IFN- γ contained in the sample interacts with anti-IFN- γ antibodies which are coupled to magnetic beads. After washing off unbound proteins, an anti-IFN- γ antibody conjugated with biotin binds to IFN- γ and a streptavidin-isoluminol-conjugate is added. Following an additional washing step, the reaction initiation reagents are added and emitted light is quantified in relative light units (RLU) by a photomultiplier. Samples were considered positive for T cell-mediated immune responses against SARS-CoV-2 when the detected light signal exceeded the cutoff value of 0.15 IU/ml after subtraction of the background value from the Nil tube as described before.¹⁶ Analysis of cellular immune responses was only performed in study participants recruited at the Cantonal Hospital St. Gallen, because inclusion of participants from external centres was limited by the requirement of fresh blood sampling.

2.6 | Statistics

Categorical variables were summarised with absolute and relative frequencies; continuous variables were summarised with mean and standard deviation (SD) or median and interquartile range (IQR). The Kruskal–Wallis test was applied to test for differences in anti-SARS-CoV-2 spike antibody concentration between the three study groups (IBD patients treated with anti-TNF biologics, IBD patient treated with non-anti-TNF biologics and healthy controls). Dunn's post hoc tests were additionally performed while controlling the family-wise error rate with the Holm method.

We performed multivariable linear modelling with log-transformed anti-SARS-CoV-2 spike IgG levels as outcome to analyse the impact of treatment with biologic agents on third dose booster

vaccine-elicited humoral immunity, adjusted for potential confounders which were chosen based on previous studies.^{10,12} In this model, we included anti-TNF treatment, non-anti-TNF treatment, detectable nucleocapsid antibodies, time between third vaccination and determination of antibody levels, age, smoking, time between second and third vaccination, and vaccine type. The coefficients of time between booster vaccination and antibody measurement, and of time between second and third vaccination were expressed per month. The coefficient of age was expressed per decade. All three of these potential confounders were treated as continuous variables in the analysis. Additionally, possible interactions between IBD treatment and time between booster vaccination and measurement of antibody levels or detectable nucleocapsid antibodies were tested. Although no strict linear relationship between the outcome and time between third vaccination and determination of antibody levels was found, the assumptions of linearity seemed reasonable for most of the observations. As residuals were not normally distributed, a non-parametric 95% confidence interval was obtained by simple bootstrapping, using the 2.5% and 97.5% quantiles of 10,000 bootstrapped values.

The number of participants without adequate T cell response was compared between IBD patients treated with anti-TNF antibodies, IBD patients treated with non-anti-TNF antibodies and healthy controls with Holm-corrected Fisher's exact tests. Anti-SARS-CoV-2 spike antibody concentrations were additionally compared between participants with a positive and negative T cell response with a Wilcoxon rank-sum test with continuity correction. T cell negative individuals were assigned to quartiles based on their individual anti-spike IgG concentration and the association between anti-spike IgG concentration and negative T cell response was tested with the chi-squared test. All analyses were performed in the R programming language (version 4.0.2; R Core Team, 2020). The package "tableone" was used to compute descriptive statistics. The package "Dunn's test" was used to calculate Dunn's post hoc tests. The package "boot" was used to bootstrap confidence intervals.

Assuming a similar difference in serological response as in the CLARITY-IBD study, we calculated with a geometric mean (geometric SD) of 6.0 U/ml (5.9) in patients with anti-TNF treatment and 28.8 U/ml (5.4) in patients with non-anti-TNF.¹⁰ We

additionally assumed those immune responses of non-anti-TNF patients are comparable to those of healthy controls. Taking the natural logarithm, these values correspond to 1.8 (1.8) and 3.4 (1.7). To simplify matters, a power calculation was done for a two-sided two-sample equal-variance *t*-test. Group sample sizes of 21 (total 42) achieve 90% power to reject the null hypothesis of equal means when the population mean difference is $\mu_1 - \mu_2 = 1.8 - 3.4 = -1.6$ with an SD for both groups of 1.8 and with a significance level of 0.05 using a two-sided two-sample equal-variance *t*-test. This sample size calculation was done with PASS 2021, v21.0.5. As we planned a multivariable linear model as primary analysis, we assumed that with a minimum sample size of 200, a linear model with 10–15 parameters (including non-linear terms and interactions) would not be over-fitted.¹⁷

3 | RESULTS

3.1 | Study population

Between 17 January 2022 and 4 April 2022, a total of 249 study participants were recruited from the outpatient clinics and hospital staff of Cantonal Hospital St. Gallen, Ambulatorium Rorschach and Inselspital Bern. This includes 139 patients diagnosed with IBD and 110 healthy controls, of which 24 had to be excluded for different reasons (Figure 1). Of the recruited IBD patients, 81 were treated with the TNF antagonists infliximab, adalimumab or golimumab and 58 were treated with the non-anti-TNF biologics vedolizumab or ustekinumab. At the day of study inclusion, baseline parameters were recorded and anti-SARS-CoV-2 spike IgG and anti-nucleocapsid antibodies, as well as anti-SARS-CoV-2 T cell responses were quantified. Detailed characteristics of the study population, excluding participants who were excluded from the analysis for not meeting inclusion criteria, are summarised in Table 1.

3.2 | Anti-TNF treatment attenuates antibody responses following SARS-CoV-2 booster vaccination with a third vaccine dose

We included 225 participants in our primary analysis. IBD patients receiving anti-TNF treatment showed a reduced geometric mean of anti-spike IgG concentrations (geometric mean 2357.4 BAU/ml [geometric SD 3.3]), compared to IBD patients treated with non-anti-TNF biologics (5935.7 BAU/ml [3.9]; $p < 0.0001$) and healthy controls (5481.7 BAU/ml [2.4]; $p < 0.0001$), respectively (Figure 2A). No difference in anti-spike IgG levels was observed between non-anti-TNF treatment and healthy controls ($p = 0.54$). In all participants, antibody concentration surpassed the threshold for seroconversion as defined by a cut-off of 33.8 BAU/ml (Figure 2A), except for one non-anti-TNF-treated IBD patient. Furthermore, in all three study groups, most participants showed antibody concentrations below 10,000 BAU/ml. Interestingly, this proportion was higher in the anti-TNF group (65/73; 89.0%) compared to the non-anti-TNF group (34/52; 65.4%) and healthy controls (75/100; 75.0%), respectively. No participant in the anti-TNF group reached antibody levels higher than 30,000 BAU/ml, while this was found in 9.6% (5/52) and 5% (5/100) of non-anti-TNF-treated patients and healthy controls, respectively (Figure 2B).

Multivariable modelling using log-transformed anti-SARS-CoV-2 spike protein IgG concentrations as dependent variable revealed that anti-TNF treatment had the strongest negative impact on anti-spike IgG concentrations relative to absence of anti-TNF treatment in our study population (geometric mean ratio 0.39 [95% CI 0.28–0.54]) (Figure 3). In contrast, participants treated with the non-anti-TNF drugs vedolizumab or ustekinumab had no significant changes in IgG concentrations, when compared to healthy individuals (1.0 [0.6–1.47]). In addition, time per month since booster vaccination (0.72 [0.58–0.9]) was significantly associated with reduced IgG concentrations. Notably, time since booster vaccination was dissimilar in healthy controls and IBD patients (Figure S1), highlighting the

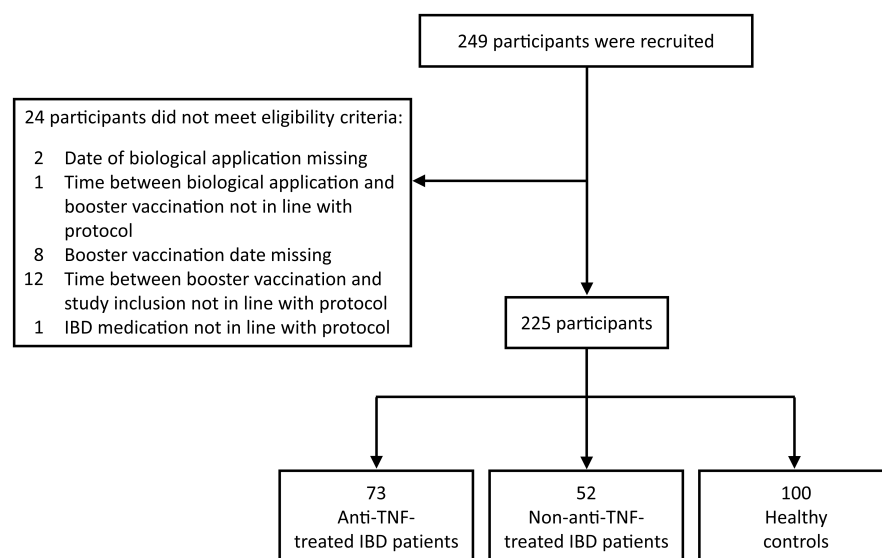


FIGURE 1 Participants included in the STAR SIGN cohort. All included individuals received a third SARS-CoV-2 vaccine dose of either BNT162b2 or mRNA-1273.

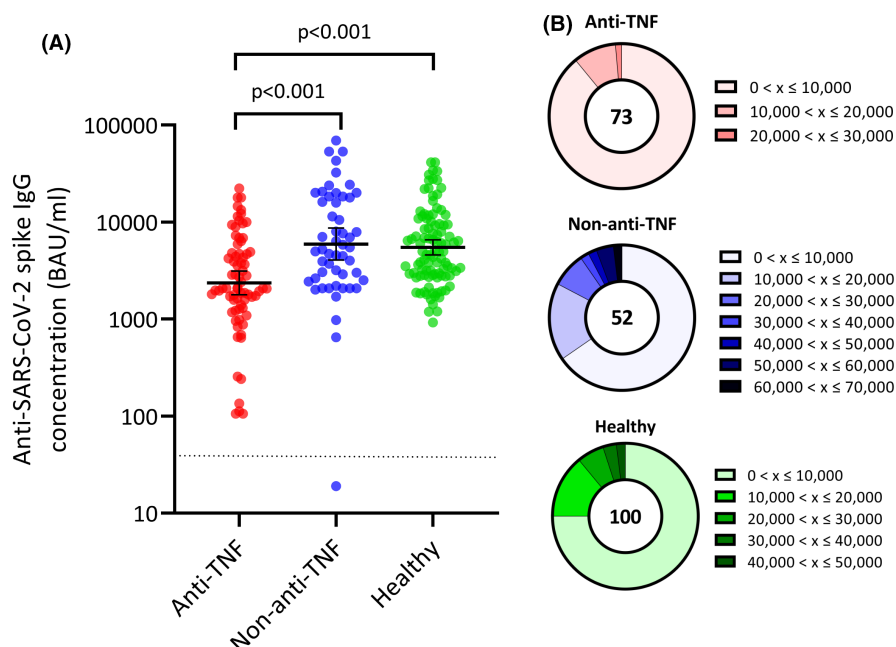


FIGURE 2 Anti-spike protein antibody concentration following SARS-CoV-2 booster vaccination is attenuated by anti-TNF treatment. (A) Concentrations of anti-spike protein IgG antibodies were determined two to 16 weeks after booster vaccination with BNT162b2 or mRNA-1273 in sera from healthy controls and IBD patients treated either with the anti-TNF biologics (anti-TNF: Infliximab, adalimumab, golimumab) or with non-anti-TNF biologics (non-anti-TNF: Vedolizumab, ustekinumab). Geometric means are represented by the bold bar and 95% confidence intervals are displayed as error bars. The threshold for seroconversion of 33.8 binding antibody units (BAU)/ml is indicated by the dotted line. p -values are based on holm-corrected Dunn's post hoc tests following a significant Kruskal-Wallis test ($p < 0.0001$). (B) fractions of participants with anti-SARS-CoV-2 spike IgG concentrations as indicated, stratified by study group. Total number of participants in each study group is displayed in the centre of the donut chart.

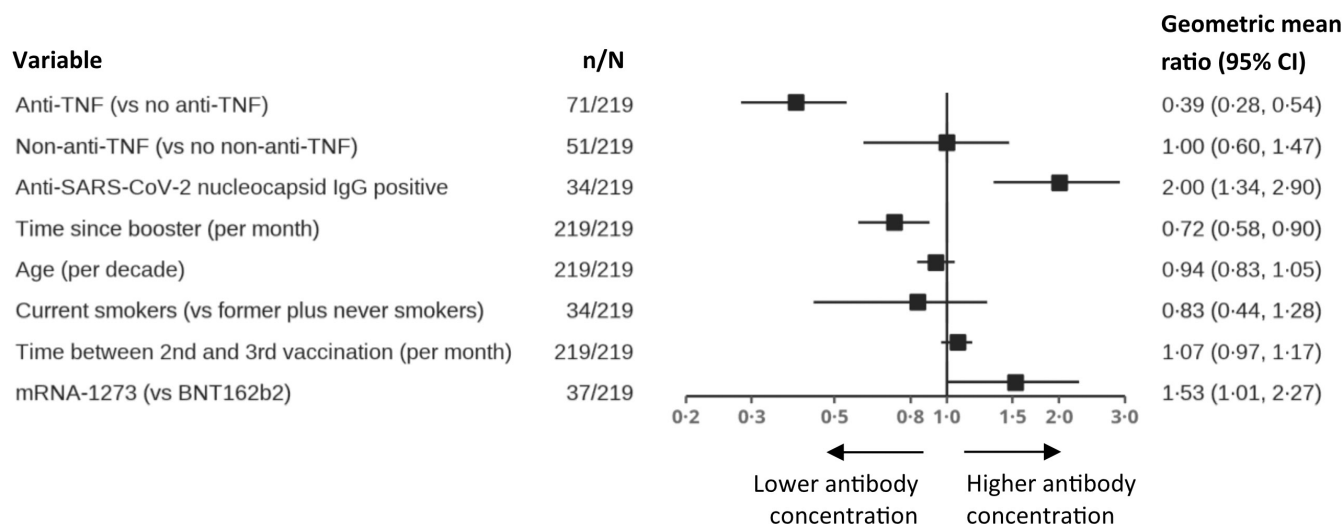


FIGURE 3 Factors associated with serologic response following SARS-CoV-2 booster vaccination. Exponential coefficients of a multivariable linear regression model of log-transformed anti-spike antibody for peer review concentrations are indicated by black squares. Error bars indicate 95% confidence intervals. Geometric mean ratios of antibody concentration associated with each variable are listed together with 95% confidence intervals. Of note, for better visualisation the x-axis scale varies throughout different sections as indicated.

importance of covariate for this variable. Antibody concentrations were significantly increased in participants who received mRNA-1273 as a third dose compared to BNT162b2 (1.53 [1.01–2.27]) and in patients with positive anti-SARS-CoV-2 nucleocapsid IgG seral status, which is an indicator of a previous SARS-CoV-2 infection

(2.00 [1.34–2.90]). Anti-spike protein IgG concentrations for participants with and without previous SARS-CoV-2 infection are visualised in Figure S2. No significant changes were associated with time per month between receiving the second and third vaccine doses (1.07 [0.97–1.17]). Importantly, there was no interaction between

study group and time between booster vaccination and measurement of antibody levels nor between study group and positive anti-SARS-CoV-2 nucleocapsid IgG seral status. A multivariable linear regression model additionally adjusting for steroid use indicated no impact on antibody concentrations (Figure S3). When assessing potential bivariate correlations between IBD activity and antibody concentrations, a weak correlation was found for calprotectin levels ≤ 2 months before booster vaccination, but no correlation was found for calprotectin levels ≤ 2 months after booster vaccination or patient-reported outcome (PRO2) (Figure S4). However, multivariable modelling indicated no strong effect of faecal calprotectin levels before vaccination, but strong effects of anti-TNF treatment and time since booster vaccination on the serological response (Table S2).

3.3 | Serum antibody concentrations decline over time following SARS-CoV-2 booster vaccination and remain reduced in anti-TNF-treated patients

To investigate whether anti-TNF therapy impacts antibody kinetics after SARS-CoV-2 booster vaccination, we visualised the rolling geometric means of anti-spike IgG concentrations in IBD patients treated with anti-TNF or non-anti-TNF biologics (Figure 4A). While mean antibody concentrations were consistently reduced in the anti-TNF group during the investigated time period, antibody decay starting approximately 40 days after vaccination could be observed in both groups. Importantly, antibody concentrations sustainably stayed above the seroconversion threshold. When stratifying antibody levels by time intervals, the geometric mean of anti-spike IgG concentrations was reduced at 10–16 weeks compared to 2–9 weeks after vaccination in anti-TNF- ($p < 0.001$) and non-anti-TNF-treated ($p = 0.019$) IBD patients, respectively (Figure 4B).

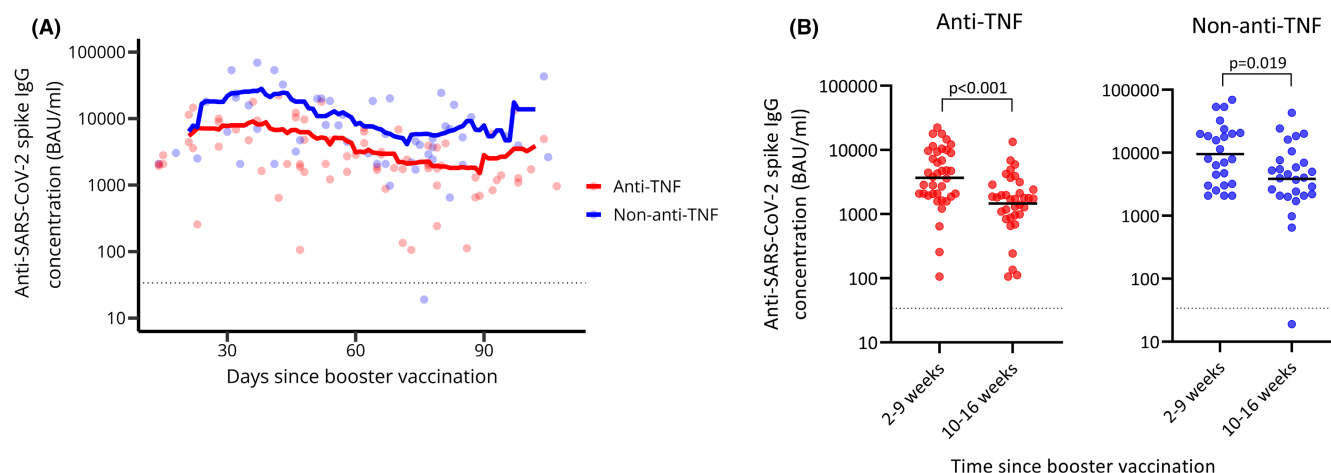


FIGURE 4 Anti-spike IgG concentration following SARS-CoV-2 booster vaccination over time. For peer review (A) depicted are rolling geometric means which are calculated by including the values from 7 days on each side of the indicated day (15-day window), stratified by treatment. Individual values are depicted in transparent colours. (B) Geometric means of anti-spike IgG concentrations, stratified by indicated time intervals post-SARS-CoV-2 booster vaccination. Statistical analyses are based on Wilcoxon rank-sum tests with holm correction. Dotted lines represent the threshold for seroconversion (33.8 BAU/ml), respectively.

3.4 | High concentrations of anti-spike antibodies did not protect from breakthrough infections during the omicron wave

Despite high concentrations of anti-spike IgG concentrations in our healthy control group, 29 out of 54 healthy subjects (53.7%) experienced breakthrough infections during the observed time period (Figure 5A). When comparing anti-spike IgG concentrations of subjects with or without breakthrough infection, no difference in geometric mean of anti-spike IgG concentrations was found ($p = 0.931$), suggesting that high antibody levels cannot protect from breakthrough infection during the SARS-CoV-2 B.1.1.529 (omicron) wave (Figure 5B).

3.5 | SARS-CoV-2 reactive T cell immunity is reduced in anti-TNF treated IBD patients following a third vaccination

Since T cell immunity plays a central role in the protection against SARS-CoV-2, we further quantified T cell-mediated release of IFN- γ upon stimulation of whole blood with SARS-CoV-2 antigens.¹⁸ Strikingly, a higher proportion of anti-TNF-treated IBD patients failed to mount an adequate T cell response after booster vaccination compared to healthy controls (15/73 [20.5%] vs 2/100 [2.0%]; $p = 0.00031$; Figure 6). The proportion of T cell immunity-negative individuals was numerically also higher in non-anti-TNF-treated IBD patients than in healthy controls, but in this case without being significant (5/52 [9.6%] vs 2/100 [2%]; $p = 0.093$). Similarly, no significant difference could be observed between anti-TNF-treated and non-anti-TNF-treated IBD patients (15/73 [20.5%] vs 5/52 [9.6%]; $p = 0.14$). Notably, IBD patients with adequate T cell immunity reported higher clinical IBD activity than patients lacking robust T cell

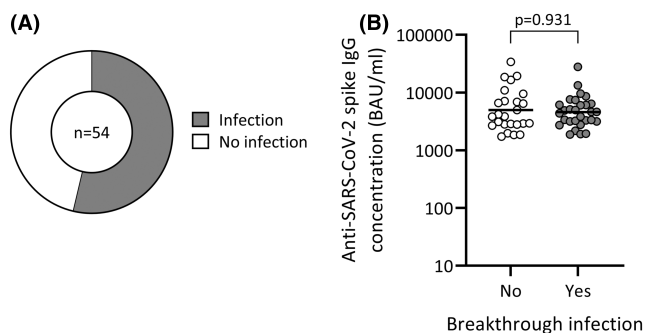


FIGURE 5 Breakthrough infections during the omicron wave are common and not prevented by robust serologic immune response. (A) Number of breakthrough infections experienced by healthy subjects following third dose SARS-CoV-2 vaccination. (B) Geometric means of anti-spike IgG concentrations in healthy subjects with or without breakthrough infection. Statistical analysis is based on Wilcoxon rank sum. Observed median time periods were 213 and 217 days since booster vaccination for participants with and without infection, respectively. Time periods correlated with the first B.1.1.529 (omicron) wave in 2022

responses (Figure S5A). However, disease activity was similar in IBD patients under anti-TNF and non-anti-TNF treatment (Figure S5B).

3.6 | T cell immunity is uncoupled from humoral immune response in IBD patients following a third SARS-CoV-2 vaccination

When comparing SARS-CoV-2 spike antibody concentrations in T cell immunity positive and negative individuals from our study population, antibody concentrations differed only marginally, suggesting no correlation between humoral and cellular immune responses (geometric means 4318.2 BAU/ml vs 3635.9 BAU/ml; $p = 0.88$; Figure 7A). Furthermore, uncoupling of antibody and T cell responses was visualised by ordering antibody concentrations by magnitude while highlighting T cell negative individuals per study group (Figure 7B). When anti-spike IgG concentrations were categorised into quartiles, similar proportions of anti-TNF-treated patients with inadequate T cell response were seen in all quartiles (1st (highest) quartile: 6/56 [10.7%]; 2nd quartile: 7/56 [12.5%]; 3rd quartile: 3/56 [5.4%]; 4th (lowest) quartile: 6/56 [10.7%]; $p = 0.61$ based on chi-squared test).

4 | DISCUSSION

Immunocompromised patients face a greater risk of infectious diseases, and immunogenicity towards vaccines is reduced by several immunosuppressive drugs.^{19,20} Therefore, vaccination campaigns worldwide recommend a SARS-CoV-2 booster vaccine for immunosuppressed patients. Booster vaccines were shown to be safe in IBD patients and several studies analysed booster vaccine-elicited immune responses in different subgroups of immunocompromised

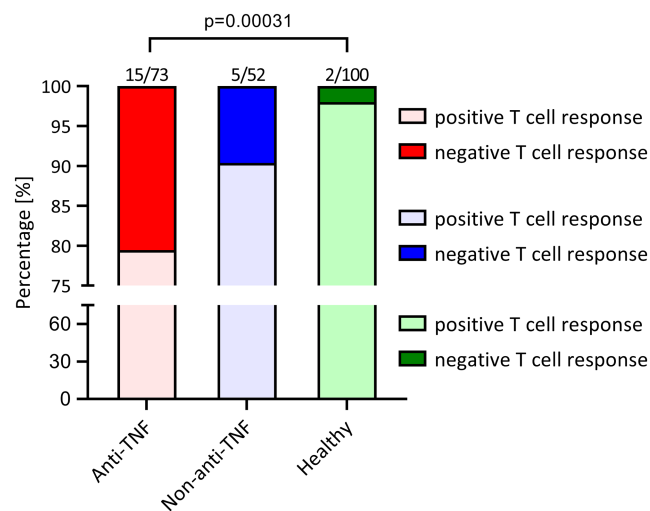


FIGURE 6 T cell immunity following SARS-CoV-2 booster vaccination is impaired by anti-TNF treatment in IBD patients. Proportion of participants who failed to develop an adequate T cell response following booster vaccination, stratified by study group. Numbers above bars indicate the number of participants without adequate T cell immunity. Statistical analysis comparing anti-TNF-treated IBD patients with healthy controls is based on Holm-corrected Fisher's exact test ($p = 0.00031$).

patients.²¹⁻²⁷ However, to our knowledge, this is the first study to prospectively characterise the humoral and functional cellular immune responses following a third dose with SARS-CoV-2 mRNA vaccines in IBD patients receiving immunosuppressive treatment and healthy controls.

First, we demonstrated that anti-TNF treatment attenuates the anti-spike IgG concentration following third dose vaccination, compared to treatment with non-anti-TNF biologics and healthy controls, respectively (Figure 2A,B). These observations are in line with findings from CLARITY-IBD, VIP, PREVENT-COVID, HERCULES and Wagner et al., while the STOP COVID-19 in IBD study could not find reduced antibody concentrations in anti-TNF-treated IBD patients when comparing them to IBD patients without immunosuppressive treatment.^{24-26,28-30} Importantly, it must be noted that the reduced antibody responses found in this study, might at least partially be influenced by already reduced antibody concentrations in anti-TNF-treated patients after two vaccine doses as shown before.^{10-12,31-37}

Second, multivariable linear regression modelling revealed that anti-TNF treatment is the strongest predictor of impaired humoral immunity after third dose vaccination (Figure 3). Similar to previous reports, reduced anti-spike antibody concentrations were further associated with time between booster and antibody measurement and vaccination with BNT162b2 (compared to mRNA-1273), whereas prior infection resulted in higher antibody concentrations.^{25,28,30,34} Given the correlation between COVID-19 severity and infection-elicited antibody responses, it will be interesting to see if the latter effect also establishes after infection with SARS-CoV-2 omicron variants which cause less severe disease.^{38,39} In contradiction to the CLARITY-IBD and VIP studies, but in line with the STOP COVID-19

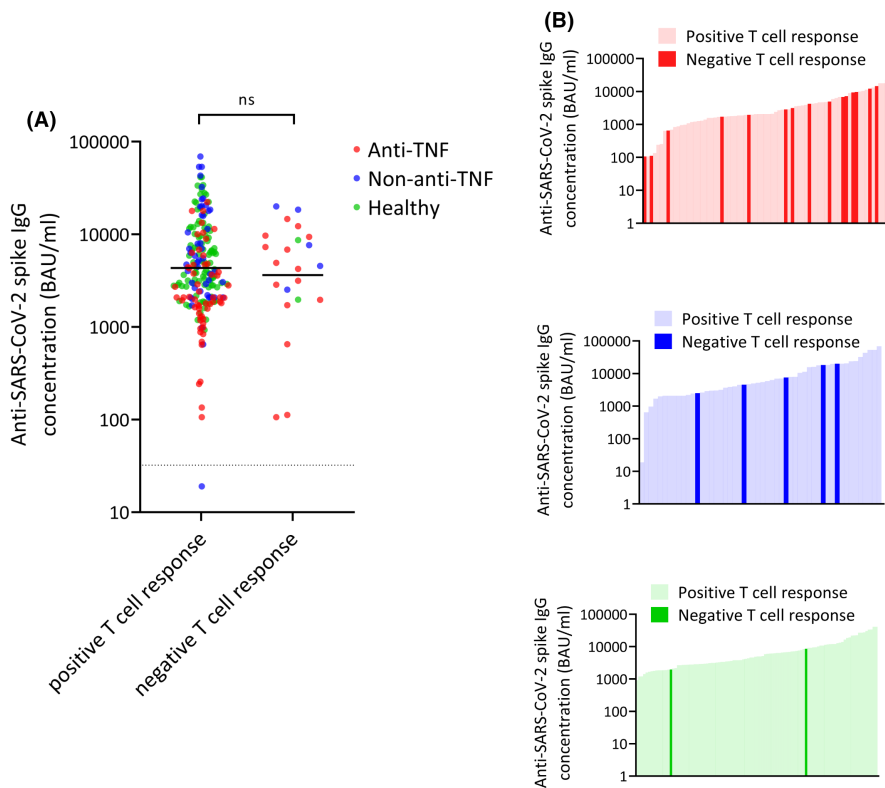


FIGURE 7 Cellular immunity following SARS-CoV-2 booster vaccination is uncoupled from humoral immune responses. (A) Anti-SARS-CoV-2 spike IgG concentrations of participants with and without an adequate T cell response. Colours correspond to the respective study group as indicated. Statistical analysis is based on a Wilcoxon rank sum test with continuity correction. (B) Each bar represents the anti-SARS-CoV2 spike IgG concentration measured in one study participant. Antibody concentrations are ordered by magnitude from lowest to highest. Red, blue and green colours correspond to the study groups anti-TNF, non-anti-TNF and healthy, respectively. Dark colours indicate participants who failed to develop an adequate T-cell response. Inadequate T cell responses were seen in all quartiles of anti-TNF-treated IBD patients.

in IBD study, age did not impact antibody concentrations in our participants.^{25,26,30} Moreover, as the first study to investigate this, we could not find an impact of disease activity on antibody concentrations following booster vaccination. Of note, our study included 26 patients who used steroids at the time of third dose vaccination. However, a multivariable linear regression model covarying steroid use indicated no impact on antibody concentrations (Figure S3), which is in line with the CLARITY-IBD but in contrast to the STOP COVID-19 in IBD study.^{25,30} While combination therapy with immunomodulators and anti-TNF agents was previously shown to attenuate antibody responses after SARS-CoV-2 vaccination, we try to limit the number of patients receiving long-term combination therapy in our IBD centres due to an increased risk for developing lymphoma or severe COVID-19.^{37,40–42} Given the low number of patients with combined immunosuppressive therapy in this study, we did not covariate for this potential confounder in our model. Future studies will be required to investigate if third dose SARS-CoV-2 vaccine responses in IBD patients are also affected by other potential confounders such as vitamin D deficiency which has been hypothesised to influence severity of COVID-19.^{43,44}

Third, we found that antibody concentrations decline similarly over time in anti-TNF and non-anti-TNF-treated individuals, respectively (Figure 4A,B). In both groups, the rolling mean anti-spike IgG concentration clearly stayed above the threshold for seroconversion, indicating that a substantial immune response is provided at least up to 16 weeks after third dose SARS-CoV-2 vaccination. However, the constant antibody decay in both groups plus the consistent reduction of antibody concentrations in anti-TNF-treated patients potentially indicate that antibody concentrations might fall

below the seroconversion threshold earlier in patients under anti-TNF treatment. These findings point towards a benefit of a fourth vaccine dose in anti-TNF patients in order to guarantee optimal protection. Rolling mean antibody concentrations were not visualised for healthy participants due to the lack of variation in the time intervals since booster vaccination. Antibody decay in IBD patients following a third vaccine dose was reported previously, but unlike a previous report, we did not see an increased decay in anti-TNF-treated patients.^{25,30} Long-term longitudinal sampling will be required to determine the exact rate of antibody-decay in IBD patients under immunosuppressive treatment and to identify the best timing for fourth dose administration.

Fourth, we uncovered that high concentrations of anti-spike IgG concentrations did not protect from SARS-CoV-2 breakthrough infections during times of high B.1.1.529 (omicron) prevalence (Figure 5A,B), which is in line with previous reports in IBD patients and healthy controls.^{25,45,46} The current data suggests that neutralising antibodies are most important for preventing SARS-CoV-2 infection but both antibody and CD8⁺ T cell responses are crucial for preventing severe disease.⁴⁷ Given that B.1.1.529 (omicron) escapes most neutralising antibodies, the risk of infection following vaccination remains high during the omicron wave, which is reflected by our analysis of breakthrough infections. However, vaccines remain our most powerful tool to prevent severe COVID-19.

Lastly, we demonstrated that a higher proportion of IBD patients under anti-TNF treatment had inadequate SARS-CoV-2-directed T cell immunity after third dose vaccination, when compared to healthy controls (Figure 6). Importantly, time

intervals between booster vaccination and assessment of T cell immunity were similar in T cell negative and positive individuals (Figure S6; $p = 0.398$), indicating that attenuated T cell responses are a result of anti-TNF treatment rather than differences in time since booster vaccination. Since T cell positive IBD patients reported higher disease activity than T cell negative patients, we cannot rule out the possibility that disease activity contributes to the development of adequate T cell immunity after booster vaccination. However, since similar disease activity was reported by anti-TNF- and non-anti-TNF-treated IBD patients, we assume that our findings are not biased by this potential effect. Robust T cell immunity plays a pivotal role in protection against SARS-CoV-2 omicron subvariants or when neutralising antibody concentrations are waning or low.^{47–49} Furthermore, CD8+ T cells are required for protection against severe COVID-19 in immunocompromised patients.⁵⁰ Therefore, our results indicate that our finding of impaired T cell responses might add to the potentially elevated risk of severe COVID-19 (based on reduced concentration of SARS-CoV-2-reactive antibodies) in anti-TNF-treated patients. Contrary to our findings, previous reports from the CORALE-IBD and CLARITY-IBD studies showed that T cell responses to SARS-CoV-2 vaccination are augmented in anti-TNF-treated IBD patients after two vaccine doses, and not impaired after three vaccine doses.^{26,51} Notably, these studies only focused on quantification of SARS-CoV-2-reactive T cells or clonal depth rather than qualitative measures of immunity such as IFN- γ secretion. Recently, another study reported that after two doses, a higher proportion of IBD patients lacked adequate SARS-CoV-2-directed IFN- γ secretion, when compared to healthy controls.⁵² Importantly, this is the first study to demonstrate impaired functional T cell immunity in anti-TNF-treated IBD patients after three doses of SARS-CoV-2 mRNA vaccines. Interestingly, no correlation between humoral and T cell immunity was detected in this study (Figure 7A,B). While the number of SARS-CoV-2-reactive T cells was shown to be uncoupled from antibody concentrations by the CLARITY study, this is the first study to demonstrate uncoupling of antibody concentrations and IFN- γ secretion upon challenge with SARS-CoV-2 antigens.^{26,34} Since robust CD8+ T cell responses can protect against SARS-CoV-2 even when antibody concentrations are low, this highlights the importance of assessing both immune mechanisms in order to accurately identify at-risk patients.

We acknowledge the lack of seral virus neutralisation analyses as a limitation of this study. To account for this limitation we used a serological assay that employs a trimeric spike protein with improved detection of a broad repertoire of IgG neutralising antibodies and has been validated for neutralising IgG estimation by the manufacturer.^{53,54} Future studies should focus on investigating how the presented findings affect neutralisation of current SARS-CoV-2 variants that can escape vaccine-elicited immune responses such as the omicron subvariants BA.2 and BA.5.⁵⁵ Moreover, our study design is limited by the assignment of participants to groups based on the respective action mode of IBD treatment – similarly to the RECOVER

trial—rather than specific biologic agents and the lack of longitudinal sampling.¹¹ Notably, no previous study could find differences in antibody levels between patients treated with infliximab vs other anti-TNF antagonists, or between patients treated with vedolizumab vs ustekinumab.⁴²

In conclusion, our results provide important insights into the immunogenicity following a third dose of BNT162b2 or mRNA-1273 in IBD patients and highlight that patients treated with anti-TNF antagonists experience impaired immunogenicity on both a humoral and cellular level. Our findings have important and direct implications for global health policy makers and will contribute to establishing evidence-based guidelines for future vaccine doses. In the face of constantly evolving SARS-CoV-2 variants and the increasing threat of vaccine fatigue, it is more important than ever to identify at-risk patient groups.⁵⁶ While robust humoral immune responses cannot protect against breakthrough infections with the SARS-CoV-2 B.1.1.529 (omicron) variant, our data indicate that a fourth vaccine dose may still be beneficial for anti-TNF-treated IBD patients in order to guarantee adequate long-term protection against severe COVID-19.

AUTHOR CONTRIBUTIONS

Simon Woelfel: Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (supporting); investigation (equal); supervision (equal); validation (equal); visualization (lead); writing – original draft (lead); writing – review and editing (lead). **Joel Dütschler:** Conceptualization (equal); funding acquisition (lead); investigation (lead); methodology (equal); project administration (equal); supervision (equal); writing – review and editing (supporting). **Marius König:** Investigation (equal); writing – review and editing (supporting). **Nicole Graf:** Conceptualization (supporting); data curation (lead); formal analysis (lead); software (lead); validation (equal); visualization (equal); writing – original draft (supporting); writing – review and editing (supporting). **Vasileios Oikonomou:** Investigation (equal); writing – review and editing (supporting). **Claudia Krieger:** Investigation (equal); writing – review and editing (supporting). **Samuel Truniger:** Investigation (equal); writing – review and editing (supporting). **Annett Franke:** Investigation (equal); writing – review and editing (supporting). **Annika Eckhold:** Data curation (equal); investigation (equal); project administration (lead); writing – review and editing (supporting). **Kristina Forsch:** Data curation (supporting); project administration (supporting); writing – review and editing (supporting). **Jacqueline Wyss:** Investigation (equal); writing – review and editing (supporting). **Niklas Krupka:** Investigation (equal); writing – review and editing (supporting). **Werner Albrich:** Conceptualization (supporting); writing – review and editing (supporting). **Nicola Fabian Frei:** Investigation (equal); writing – review and editing (supporting). **Nora Geissler:** Investigation (equal); writing – review and editing (supporting). **Peter Schaub:** Investigation (equal); writing – review and editing (supporting). **Matthias Friedrich:** Funding acquisition (equal); writing – review and editing (equal). **Benjamin Misselwitz:**

Conceptualization (supporting); investigation (equal); supervision (equal); writing – review and editing (supporting). **Wolfgang Korte:** Conceptualization (supporting); funding acquisition (equal); methodology (lead); project administration (supporting); supervision (supporting); writing – review and editing (supporting). **Justus Johannus Bürgi:** Methodology (lead); project administration (equal); resources (equal); writing – review and editing (supporting). **Stephan Brand:** Conceptualization (equal); funding acquisition (lead); supervision (lead); validation (equal); writing – original draft (supporting); writing – review and editing (lead).

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DATA AVAILABILITY STATEMENT

Access to the study protocol of this project including the statistical analysis plan can be provided by the study investigators upon request. De-identified participant data collected in the context of this study, will be made available after publication and will remain accessible for 5 years. Before data access is granted, the individual investigator has to apply for data access by outlining the specifics of the proposed data use. This application has to be evaluated and accepted by an independent review committee. Additional data analyses have to be in line with the aims of the approved study proposal. Inquiries for data use and access to the study protocol are to be directed to stephan.brand@kssg.ch. Before data can be accessed, investigators have to agree to specific terms of data use by signing a data access agreement.

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section.

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