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)

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Abstract:

Background and Aims:

Evidence suggests patients with inflammatory bowel disease (IBD) receiving TNF-antagonists have attenuated response to vaccination against COVID-19. We sought to determine the impact of IBD and various medications for treatment of IBD on antibody responses to vaccination against COVID-19.

Methods:

Patients with IBD (n=270) and healthy controls (HC, n=116) were recruited prospectively and quantitative antibody responses assessed following COVID-19 vaccination. The impact of IBD and medications for treatment of IBD on vaccine response rates was investigated.

Results:

100% of HC seroconverted post-complete vaccination with two vaccine doses. 2% of patients with IBD failed to seroconvert. Median anti-spike protein (SP) immunoglobulin (Ig)G levels post-complete vaccination in our IBD cohort was significantly lower than HC (2,613 AU/mL versus 6,871 AU/mL, p=<0.001). A diagnosis of IBD was independently associated with lower anti-SP IgG levels (β coefficient -0.2, p = 0.001). Use of mRNA vaccines was independently associated with higher anti-SP IgG levels (β coefficient 0.25, p = < 0.001). Patients with IBD receiving TNF-inhibitors had significantly lower anti-SP IgG levels (2,445AU/mL) than IBD patients not receiving TNF-inhibitors (3868AU/mL)(p = < 0.001). Patients with IBD not receiving TNF-inhibitors still showed attenuated responses compared to HC (3868AU/mL versus 8747AU/mL p = 0.001).
Conclusions:

Patients with IBD have attenuated serological responses to SARS-CoV-2 vaccination. Use of anti-TNF therapy negatively impacts anti-SP IgG levels further. Patients who do not seroconvert post-vaccination are a particularly vulnerable cohort. Impaired responses to vaccination in our study highlight the importance of booster vaccination programmes for patients with IBD.

Keywords: Inflammatory bowel disease, COVID-19 vaccination, anti-tumour necrosis antagonists
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Conflicts of interests: None to declare.

Data availability statement:

The data underlying this article will be shared on reasonable request to the corresponding author.
Introduction:

A novel coronavirus referred to as SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) was identified in late 2019 as the causative agent of a respiratory syndrome named Coronavirus-19 disease (COVID-19) and has subsequently resulted in a worldwide pandemic. Given the immense morbidity and mortality associated with COVID-19 infection, accelerated vaccine development programs and mass vaccination campaigns were conducted with the introduction of novel vaccines {1-4}. Induction of protective immunity against SARS-CoV-2 following vaccination is critical to mitigate against severe disease, morbidity and mortality.

There are evidence patients with inflammatory bowel disease (IBD) are susceptible to vaccine preventable infections, suggesting vaccines confer sub-optimal protection in this cohort {5-8}. Both the International Organisation for the Study of Inflammatory Bowel Disease (IOIBD) and the COVID-19 European Crohn’s and Colitis Organisation (ECCO) taskforce advise patients with IBD should be vaccinated against SARS-CoV-2 at the earliest opportunity possible and vaccination should not be deferred because a patient is receiving immune-modifying therapies {9,10}. The CLARITY study, the largest study to date looking at antibody responses to COVID-19 vaccination in patients with IBD found anti-spike protein (SP) immunoglobulin (Ig)G levels are lower following vaccination with a single-dose COVID-19 vaccine in patients with IBD treated with infliximab (IFX) compared with vedolizumab (VZ) {11}. A recent study from Israel found similar results with reduced antibody levels post vaccination with the mRNA vaccine BNT162b2 in patients with IBD treated with anti-TNF therapy compared to healthy controls and patients with IBD not receiving anti-TNF agents {12}.

To date data is limited on the impact of vaccine class on response rates in patients with IBD {11-13} or how different IBD therapies impact vaccine response after complete vaccination which we aimed to address in this study. We conducted a prospective multi-centre study supported by the INITIative collaborative research network (www.initiativeibd.ie) to assess immune response to both mRNA and
Viral-vector (VV) based COVID-19 vaccines in patients with IBD (biologic and non-biologic treated patients) compared to healthy controls (HC).

Methods and Materials:

Study design and participants:

VARIATION (VAriability in Response in IBD AgainsT SARS-COV-2 ImmunisatiON) is a multicentre, prospective, observational cohort study conducted to assess the impact of IBD itself and medications for treating IBD on SARS-CoV-2 acquisition and immune response to both mRNA and viral-vector based COVID-19 vaccines compared to HCs. IBD diagnosis was defined by accepted criteria. HC group included volunteers (healthcare professionals) without known gastrointestinal disease. Patients were recruited at the time of attendance at infusion units or clinical outpatients from 4 Irish hospitals between January 2021 and November 2021. Baseline bloods were analysed prior to participants receiving vaccination, before their second vaccine and post-complete vaccination. Vaccines currently available in Ireland during this study were two mRNA vaccines (mRNA-1273 (Moderna)) or BNT162b2 (PFIZER/BIONTECH)) or two viral-vector vaccines (ChAdOx1 (AstraZeneca) or Ad26.CoV2.S (Janssen) vaccine). The study was approved by the relevant hospital research ethics committees.

Study Procedure:

Eligible participants were evaluated at ≥1 of the following time points: (i) before first vaccination, (ii) prior to second vaccination, (iii) post complete vaccination. Variables recorded for participants included basic demographics (age, gender, co-morbidities and BMI) for all subjects. Data relating to IBD history including age at diagnosis, disease duration, IBD subtype, current medication at the time of vaccination, Faecal calprotectin (FCP), C-reactive protein (CRP) and albumin levels at the time of vaccination were collected.
Blood samples were collected using the serum clot activator-coated blood collection tubes (BD Diagnostics, Cat 367837). All samples were spun for 10 min at 2000 x g at room temperature. Serum was aliquoted and stored at -80°C until analysis.

**Outcomes:**

The primary endpoint was immune response to vaccine assessed by quantitative immunoassay to evaluate levels of IgG to SARS-CoV-2 spike protein following first vaccination dose and complete vaccination.

Secondary endpoints included prevalence of COVID-19 infection in our IBD cohort compared to our HCs by assessing semi-quantitative IgM in combination with IgG nucleocapsid (NC) antibody test (IgGNC) and the impact on levels of binding quantitative IgG SP, the impact of vaccine class, medications for treatment of IBD and IBD activity (FCP, CRP and albumin) on immune response to vaccination.

**Laboratory Methods:**

Laboratory analyses were conducted in a single laboratory (Core Laboratory in the Clinical Research Centre, University College Dublin, Ireland) using the SARS-CoV-2 IgG 75 chemiluminescent microparticle immunoassay (CMIA) (Abbott Laboratories, IL, USA) run on the Architect i2000SR platform (Abbott Diagnostics) and the Abbott Alinity ci platform (Abbott Diagnostics) and the Abbott SARS-CoV-2 IgM assay run on the Abbott Architect i2000SR platform. Three analyses were conducted: (i) Abbott Alinity semi-quantitative IgM (IgMSP) assay. A cut-off of 1.0 (index value) which indicates reactivity/positivity was applied. (ii) Quantitative IgG spike protein receptor binding domain (IgGSP RBD) serology, a quantitative measurement of anti-SP IgG that can be helpful to evaluate an individual’s humoral response to vaccines. 50 AU/mL and above in this test are considered as positive. (iii) Abbott Alinity IgG nucleocapsid (NC) antibody test (IgGNC) was measured.
to confirm whether participants had previously contracted COVID-19 infection. This is a semi-quantitative CMIA assay and the index values of 1.4 and above is considered as positive.

For the Abbott assays, 75µL of serum was used for the detection of IgG or IgM antibody to SARS-CoV-2 (25 µL+50 µL dead volume). Qualitative results and index values reported by the instrument were used for analysis. For the IgG assays, signal/cut-off (S/CO) ratio of ≥1.4 was interpreted as reactive and an S/CO ratio of <1.4 was interpreted as non-reactive. Calibration was performed and positive quality control (QC) S/CO 1.65–8.40 and negative quality control S/CO ≤ 0.78 were fulfilled prior to analyses of patient samples. For the IgM assay, a S/CO ratio of ≥1.0 was interpreted as reactive and an S/CO ratio of <1.0 was interpreted as non-reactive, with positive QC S/CO 1.39–6.67 and negative quality control S/CO ≤ 0.52 fulfilled prior to analyses of patient samples.

Statistics:

Statistical analyses were undertaken using SPSS version 26 (IBM). Participants with missing data were excluded from analysis. All tests were two tailed, and p values <0.05 were considered significant. Continuous data were reported as median and IQR, and discrete data as numbers and percentages, unless otherwise stated. Univariate analyses, using Mann-Whitney U tests, Kruskal-Wallis non-parametric test or one-way analysis of variance (ANOVA) were used to identify demographic and treatment related factors including medication type and vaccine class associated with anti-SP IgG levels. A two-tailed Spearman's correlation co-efficient was used to assess for differences in median anti-SP IgG levels in our IBD patients with FCP, CRP and albumin levels. We used a multivariable logistic regression model to identify factors independently associated with seropositivity including BMI, age at vaccination, gender and IBD subtype. Standardized β coefficients were obtained from linear regression.
Results:

Basic Demographics:

386 subjects were recruited. 270 participants had a confirmed diagnosis of IBD and 116 HCs. Basic demographics for our IBD cohort and HC cohort are summarised in Table 1. Median age [IQR] for HCs was 34.9 (28.3 – 49.6) years, compared to 40.6 (30.0 – 49.4) years in our IBD cohort (p=ns). 19% (n = 22) of HCs were male compared to 60% (n = 162) in our IBD cohort (p = < 0.01). Median BMI (IQR) for HCs was 24.7 (21.96 – 26.44) compared to 25.1 (22.28 – 28.37) in our IBD cohort (p=ns). The frequency of co-morbidities in each cohort was low with no significant difference seen between HCs and our IBD cohort (p =ns) and are summarised in Table 1.

Evidence of prior COVID-19 vaccination at baseline:

108 HCs and 147 IBD patients had serum samples for IgM, IgG nucleocapsid (NC) antibody and IgG SP antibody levels prior to vaccination to assess for prior exposure to SARS-CoV-2. We observed similar frequency of prior COVID-19 infection in the two groups; 6/147 (4.1%) patients were positive for IgM and/or IgG nucleocapsid (NC) antibody compared to 3/108 (3.8%) in our HCs.

Serological response to COVID-19 vaccination:

71 HCs (61.7%) received an mRNA vaccine and 44 (38.3%) received a VV vaccine. In our IBD cohort 215 patients (86.3%) were administered an mRNA vaccine and 34 (13.7%) a VV vaccine. Breakdown of vaccines administered is summarised in Table 1.

Median time from first vaccine administration to serologic assessment in our IBD cohort was 3.4 weeks (2.1 – 4.4) and in our control cohort was 3.0 weeks (2.8 – 12) (p= 0.06). Seroconversion post-vaccination was defined as anti-SP IgG levels of 50 AU/mL or above. After first vaccination 94 (79.5%)
patients in our IBD cohort seroconverted compared to 95 (96.9%) HCs (p = <0.001). Median anti-SP IgG levels post one vaccine dose in our IBD cohort was 266.0 AU/mL (63.6 – 919.2) compared to 837.8 AU/mL (348.0 – 1867.7) in HCs [p= <0.001] (Figure 1a).

92 HCs and 225 IBD patients had serum samples for anti-SARS-CoV-2 antibody levels after their second vaccine. Median time from second vaccine administration to serologic assessment in our IBD cohort was 6.8 weeks (4.4 – 10.0) and in HCs, 8.5 weeks (8.1 – 9.1) (p = 0.01). After second vaccination, 221 (98.2%) IBD patients and 92 (100%) HCs seroconverted (p = 0.33). Of the four IBD patients who did not seroconvert, 3 received an mRNA vaccine and one a VV vaccine. All four patients were receiving anti-TNF therapy. The median anti-SP IgG level post-complete vaccination in our IBD cohort was 2613.3 AU/mL (872.5 – 5851.3) compared to 6871.8 AU/mL (2182.4 – 11554.3) in our HCs (p=<0.001). Overall anti-SP IgG level post-complete vaccination in our IBD cohort were significantly lower than HCs (Table 1 & Figure 1a).

Both IBD patients and HCs who received the VV vaccine had significantly lower anti-SP IgG levels than those receiving mRNA vaccines (943.1 AU/mL versus 4448.4 AU/mL, p = <0.001) (Figure 1b). Anti-SP IgG levels for HCs and patients with IBD who received VV vaccine were similar; 784.1 AU/mL (336.1 – 2039.5) in IBD patients compared to 1160.7 AU/mL (565.0 – 2156.9) in our HCs post-complete vaccination (p = 0.93) (Figure 1b). However, a significant difference was observed in the magnitude of serologic response to mRNA vaccines in patients with IBD; median mRNA vaccine induced anti-SP IgG levels post-vaccination was 2931.95 AU/mL (1001.5 – 6636.5) in patients with IBD compared to 8747.6 AU/mL (5745.7 – 15488.3) in our control group (p = 0.001) (Figure 1b).

Nonetheless IBD patients who received a full course of mRNA vaccine had significantly higher median anti-SP IgG levels post-vaccination compared to those who received VV vaccines (p = < 0.001) (Figure 1b).
Factors impacting IgG SP antibody levels post-complete vaccination:

Using a model of stepwise backward linear regression, we examined the impact of gender, age at vaccination, BMI, vaccine type and a diagnosis of IBD on anti-SP IgG levels. We found receiving an mRNA vaccine positively impacted your final IgG result [β coefficient 0.25, p = < 0.001] whereas the presence of IBD itself negatively impacted final anti-SP IgG levels [β coefficient -0.2, p = 0.001]. Age at vaccination, gender or BMI had no impact on final anti-SP IgG levels (Supplementary Table 1a & 1b).

We next sought to evaluate whether the differences observed were related to underlying diagnosis of IBD or treatment. 270 patients with confirmed IBD were recruited. 113 (42.0%) patients had Ulcerative Colitis (UC), 150 (55.5%) Crohn’s Disease (CD) and 7 (2.6%) inflammatory bowel disease unclassified (IBD-U). Median disease duration was 9.5 years (4.9 – 16.9). Breakdown of patients’ medications at the time of vaccination, median FCP, CRP and albumin levels are summarised in Supplementary Table 2.

Following complete vaccination median anti-SP IgG levels in our cohort on anti-TNF therapy was significantly lower with median level of 2047.1 AU/mL (712.5 – 4980.7) compared to 3657.8 AU/mL (1477.3 – 13890.9) for those not receiving anti-TNF agents (p = <0.001). Median anti-SP IgG levels were further broken down according to IBD medication at time of vaccination and are summarised in Table 2. Patients were grouped into seven cohorts dependent on medications patients were receiving: (i) anti-TNF, (ii) anti-Integrins, (iii) anti-IL 12/23 (iv) immunomodulators (v) steroids (vi) tofacitinib (vii) 5-asa/no medications. Patients receiving anti-TNF therapy, anti-IL 12/23 therapy and JAK inhibitors had significantly lower median anti-SP IgG levels compared to patients receiving 5-ASA/no medications, immunomodulators or steroids (p = 0.007). Median anti-SP IgG levels and the proportion of patients with a limited antibody response (anti-SP IgG < 4000) dependent on patients’
medication are summarised in Table 2. In patients receiving anti-TNF therapy there was no difference seen in median anti-SP IgG levels post complete vaccination between those receiving monotherapy with anti-TNF therapy (n = 121) compared to those receiving combination therapy with anti-TNF therapy and immunomodulator (n = 23). Median anti-SP IgG levels for those on monotherapy was 2047.1 (722.7 – 4931.6) compared to 2458.7 (496.9 – 6539.9) (p = 0.72).

FCP levels demonstrated no clear relationship with anti-SP IgG levels after first or second vaccines (Spearmans correlation coefficient -0.98, p=0.46 post first vaccine, Spearmans correlation coefficient 0.69, p=0.412 post second vaccination). We found no evidence that IBD activity determined the magnitude of serologic response to vaccination.

Factors impacting anti-SP IgG levels post vaccination against COVID-19 in our IBD cohort:

Using a model of stepwise backward linear regression, we investigated the impact of gender, age at vaccination, vaccine type, IBD subtype, use of anti-TNF therapy, 5-ASAs, immunomodulators and vedolizumab on anti-SP IgG levels after receiving two vaccines. We found use of anti-TNF therapy at the time of vaccination (β coefficient -0.3, p = < 0.001) and older age at vaccination (β coefficient - 0.13, p = 0.05) negatively impacted final anti-SP IgG levels achieved. Gender, vaccine type, a diagnosis of UC, use of 5-ASAs, immunomodulators or vedolizumab had no impact on final anti-SP IgG levels (Table 3a and 3b).

Impact of anti-TNF therapy on vaccine response:

To determine if reduced antibody response observed in patients with IBD was secondary to IBD itself or anti-TNF therapy, we compared median anti-SP IgG levels between HCs, patients with IBD on anti-TNF therapy and patients with IBD not receiving anti-TNF therapy. We excluded participants receiving VV vaccines from this analysis given the low antibody titre observed in all study participants who received this class of vaccine. In total we included 65 HCs, 120 patients with IBD receiving anti-TNF therapy and 74 patients with IBD not receiving anti-TNF therapy. Median anti-SP
IgG levels in our HCs were 8747.6 AU/mL (5745.8 – 15488.3), compared to 3867.6 AU/mL (1758.7 – 14490.2) in our IBD patients not receiving anti-TNF therapy and 2444.6 AU/mL (771.0 – 5409.2) (p = < 0.001). Median anti-SP IgG levels in IBD patients not receiving anti-TNF therapy were significantly lower than HCs (p = 0.001) (Figure 2).

**Durability of vaccine response:**

58 patients with IBD (95% on TNF inhibitors) had an additional follow-up serology sample to allow assessment of the durability of the response after their initial post-vaccination IgG level. 45 (94.8%) were on anti-TNF therapy and 13 not on anti-TNF therapy (9 VDZ, 4 immunomodulators). Median time to second serum sample was 12.4 (9.7 – 16.4) weeks. There was a significant drop in IgG levels from 3952.85 AU/mL (1046.8 – 7094.7) at the first timepoint checked post-complete vaccination to 921.1 AU/mL (343.1 – 2102.7) on follow-up sampling at 12 weeks (p = <0.001) (Figure 3). Median anti-SP IgG levels were numerically lower in our cohort receiving anti-TNF therapy (794.8 AU/mL (308.7 – 1665.9)) compared to those not receiving anti-TNF therapy (3136.9 AU/mL (798.9 – 5298.90)) on final follow-up samples (p =0.28) (Figure 3).

**Participants with confirmed COVID-19 infection:**

5 HCs were found to have previous infection with COVID-19 and 7 patients with IBD, only 4 of which had follow-up antibody levels post vaccination analysed. HC participants with previous COVID-19 infection (n= 5) had significantly higher anti-SP IgG levels post complete vaccination (20,719.6 AU/mL (19981.0 – 35044.2)) compared to IBD patients (n=4) with prior infection (3,938.2 AU/mL (1261.3 – 14979.1)), HCs with no previous confirmed infection (n =87) (6117.4 AU/mL (2080.2 – 10887.7)) and patients with IBD with no previous confirmed infection (n= 222) (2637.8 AU/mL (870.5 -5819.9)) (p = < 0.001) (Table 4) suggesting that the additive effect of COVID-19 infection to vaccine generated immunity is reduced in patients with IBD.
Discussion:

In our study we have shown a diagnosis of IBD is an independent risk factor associated with reduced antibody response to vaccination against COVID-19. Patients with IBD have significantly lower anti spike protein IgG levels compared to participants without a diagnosis of IBD. Notably, patients receiving anti-TNF agents, anti-IL 12/23 and JAK inhibitors have the lowest level of vaccine induced immunity. Patients with IBD were found to have a significantly robust antibody response after vaccination with an mRNA vaccine compared to a VV vaccine. A small cohort of patients with IBD (2%) do not seroconvert following 2 doses of vaccine leaving them with limited protection against COVID-19 infection. Strategies such as passive immunisation or novel anti-viral therapies such as paxlovid or molnupiravir {14,15} may be beneficial in this vulnerable cohort. However further prospective research with the use of these therapies in this vulnerable cohort is needed.

Patients with IBD treated with anti-TNF agents are a vulnerable cohort and vaccination against opportunistic infections are advised {5, 16}. Our study is the first to compare anti-spike protein specific IgG levels in patients with IBD and HCs who received either mRNA or viral-vector vaccines post-complete vaccination. We found study participants (patients with IBD or HCs) who received mRNA vaccines had higher titres of anti-SP IgG than participants who received VV vaccines. On sub-analysis, patients with IBD who received an mRNA vaccine had significantly lower Ab levels than HCs. Given the robust evidence available that antibody levels drop significantly in the general population as early as 6-months post vaccination {17, 18} our patients with IBD are a vulnerable cohort and vaccination with an mRNA-based vaccine is superior. A significantly larger proportion of HC seroconverted after one vaccine compared to patients with IBD. These findings are in keeping with a large retrospective study demonstrating superior protective immunity against COVID-19 in patients with IBD after two vaccine doses {19}. 
We found a significant impairment in the humoral immune response post-vaccination against COVID-19 in patients with IBD, especially those receiving anti-TNF agents. This finding is in agreement with previous studies in patients with other immune mediated diseases or profoundly immunosuppressed patients [20 – 23]. Numerous agents especially anti-TNF agents can alter patients’ immune response to established vaccinations [6-8]. Recent studies found lower rates of anti-SARS-CoV-2 spike antibody levels in patients treated with anti-TNF therapy after a single-dose of COVID-19 vaccine compared to VDZ [11] and lower antibody levels after two doses of COVID-19 vaccines in patients receiving anti-TNF therapy [12]. TNF-α has a variety of important immunomodulatory actions including, maturation of antigen presenting cells, co-stimulation of antigen-activated T cells and driving B-cell immunoglobulin synthesis [24-26]. Therefore, neutralisation of TNF-α negatively impacts both innate and adaptive immunity and this may explain lower antibody titres following vaccination in this cohort. Our data raises concern for our patients with IBD especially those receiving anti-TNF therapy as reduced anti-SARS-CoV-2 antibody levels may ultimately increase susceptibility to COVID-19 infection. Although numbers were small, we noted patients receiving both anti-IL 12/23 therapy and JAK inhibitors also had significantly lower anti-SARS-CoV-2 spike antibody levels. Data on vaccine response rates in patients receiving anti-IL 12/23 therapy is limited. From current studies no unique antibody levels dependent on medication class are available [11, 12]. Similarly, data is limited in vaccine response rates dependent on use of JAK inhibitors. One study looking at patients treated with JAK inhibitors for rheumatoid arthritis or psoriatic arthritis found seroconversion rates of only 88% [27] however no large studies in the field of IBD are available to date. Further large prospective studies are needed in patients with IBD to look at the impact of anti-IL 12/23 and JAK inhibitors on vaccine response rates.

To date there is no well-defined threshold for anti-SP IgG antibody level that predicts resistance to COVID-19 infection, however, the level of anti-SP IgG correlates well with the titre of direct neutralising antibodies (NA) which mediate much of the protective effect of vaccination [28-30]. An
anti-SP IgG Ab level of > 4000 has been found to correlate to >95% probability of high NA titre {31}. The proportion of patients with a limited antibody reserve (anti-SP IgG < 4000) was significantly higher in patients on anti-TNF therapy, anti-IL 12/23 therapy and JAK inhibitors, indicating these patients are particularly vulnerable for re-infection.

Our study is the first to report a diagnosis of IBD negatively impacts anti-SP IgG titres post-vaccination against COVID-19. In agreement with previous work {11,12} we found immunosuppression with anti-TNF therapy negatively impacts antibody response. However, a diagnosis of IBD itself also negatively impacts anti-SP IgG levels with IBD patients not receiving anti-TNF therapy having significantly lower anti-SARS-CoV-2 spike antibody levels than HCs. Our data raises the question as to why patients with IBD mount a less robust response to vaccination? This is most likely multifactorial and may be impacted by immune-system dysfunction and/or dysbiosis.

There is a body of evidence highlighting immune-system dysfunction in patients with IBD {32, 33}. Defects in dendritic cells, macrophages and pathogen recognition receptors are seen in patients with IBD {34-36} and could impact systemic immunogenicity and aspects such as patient’s response to vaccination. It is also recognised that patient with IBD, even those in remission, have a high frequency of intestinal dysbiosis with reduced microbial diversity {37-40}. Recent publications suggest antibiotic therapy can reduce vaccine response. This may suggest this lack of microbial diversity may condition altered immune responses to vaccination as seen in our patients with IBD {41, 42}. Both these topic should be the subject of future research to help understand mechanism of altered vaccine response rates in IBD patients.

Immunity against SARS-CoV-2 wanes in all age groups within 6-months of receipt of the second vaccine dose {43, 44}. In our study a subset of patients had two sets of bloods post-compete vaccination. We found a significant drop in Ab levels in this cohort with median Ab levels dropping to 921 AU/ml compared to 3953 AU/ml over a 12-week period from complete vaccination. Lin et al reported similar results with immunity to vaccination against COVID-19 waning over time in patients
with IBD treated with anti-TNF drugs \cite{45}. Given this waning Ab response the British Society of Gastroenterology advise a third dose of a SARS-CoV-2 vaccine should be offered no earlier than 8-weeks after the second dose to all patients with IBD receiving any immunosuppressive treatment \cite{46}. Our results would support this approach and raise the question as to whether antibody response rates to the COVID-19 vaccine should be serially monitored in patients with IBD.

SARS-CoV-2 infection risk in patients with IBD is comparable to the general population \cite{16}. Reassuringly from samples taken prior to vaccination in our study rates of COVID-19 infection between HCs and our IBD cohort were similar (3.8\% versus 4.1\%). Of our patients with IBD who contracted COVID-19 all had mild symptoms and none required hospital admission. Finally, we found HCs who were previously infected with SARS-CoV2 had significantly higher Ab levels compared either to previously infected patients with IBD or uninfected HCs. The attenuated antibody response observed in patients with IBD who were exposed to the virus and subsequently received 2 doses of vaccine reinforces the advice that there should be no delay in vaccinating patients with IBD with previous infection with COVID-19 once symptoms have resolved.

Limitations of our study include difference in gender ratio between IBD and HC groups and the relatively young age of participants (however this does reflect typical IBD population). Endoscopic data at the time of recruitment for patients with IBD was limited, therefore determining whether disease activity impacts Ab response was not possible. Number of patients on individual medication types including JAK inhibitors, anti-IL 12/23 inhibitors and patients on combination therapy were limited. Numbers of participants with a second set of follow-up antibody levels were small and we had no HC samples to compare them to. Numbers of patients and HCs with COVID-19 infection were small on sub-analysis.

In conclusion, our study highlights that although seroconversion rates in patients with IBD vaccinated against COVID-19 are high, a small cohort of patients remain vulnerable to infection.
Patients with IBD independent of medication use are a vulnerable cohort of patients with reduced antibody levels post-vaccination against COVID-19. Patients treated with anti-TNF agents are even a more vulnerable cohort with significantly lower antibody levels than those not receiving these agents. Further larger prospective studies are required to investigate the impact of JAK inhibitors, anti-IL 12/23 inhibitors on vaccine response rates but our limited data would indicate these patients are also a vulnerable cohort with lower antibody levels. Patients with IBD should receive mRNA vaccines instead of VV vaccines to boost antibody response. Given the significant drop in antibody levels as early as 12-weeks post-vaccination in patients with IBD booster vaccination programmes are essential for patients with IBD especially patients immunosuppressed. Further studies are needed to determine if serology testing should be considered in immunosuppressed IBD patients to detect suboptimal vaccine responses or if altering patients scheduling of their biologic therapy at the time of vaccination could improve immune response.
Contributors: JD researched the data, performed part of the statistical analysis, contributed to laboratory analysis and wrote the manuscript; NOM, RS and PG researched the data and contributed to laboratory analysis; MT and RS were responsible for laboratory analysis of serum samples; JS, GC, EMD, MB, GH and COM recruited patients from various centres to take part in this study and reviewed the manuscript. HM recruited patients for the study, performed part of the statistical analysis and reviewed the manuscript. EJR, DD and PD contributed to the discussion and reviewed the manuscript. GD researched the data, recruited participants, contributed to and reviewed the manuscript. All authors participated in final approval of the version to be published.
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Table 1: Demographic characteristics of study participants and median IgG SP antibody levels

|                          | IBD Cohort (n = 270) | Control Cohort (n = 116) | P value |
|--------------------------|----------------------|--------------------------|---------|
| Median age [IQR]         | 40.6 (30.0 – 49.4)   | 34.8 (28.3–49.6)         | 0.1a    |
| Male [%]                 | 162 (60.0%)          | 22 (9.0%)                | <0.01b  |
| BMI [IQR]                | 25.0 (22.3 – 28.4)   | 24.3 (22.0 – 26.4)       | 0.08a   |
| Co-morbidities (Total)   | 15 (5.5%)            | 6 (3.5%)                 | 0.56    |
| HTN                      | 7                    | 4                        |         |
| Diabetes mellitus        | 1                    | 2                        |         |
| PSC                      | 5                    | 0                        |         |
| IHD                      | 2                    | 0                        |         |
| mRNA vaccine             | 217 (80.4%)          | 71 (61.2%)                |         |
| **BNT162b2**             | 207 (76.7%)          | 71 (61.2%)                |         |
| **mRNA-1273**            | 10 (3.7%)            | 0 (0%)                   |         |
| VV vaccine               | 33 (12.2%)           | 44 (37.9%)                |         |
| **ChAdOx1**              | 29 (10.7%)           | 44 (37.9%)                |         |
| **Ad26.Cov2.S**          | 4 (1.5%)             | 0 (0%)                   |         |
| Serological evidence of COVID-19 infection prior to vaccination | 6 (4.1%) | 3 (3.8%) | 0.42b |
| Seroconversion post 1st vaccine | 94(79.5%) | 95(96.9%) | < 0.001b |
| Median Anti-SP IgG levels post 1st vaccine (AU/ml) | 288.1 ((n = 118) 64.4 – 957.9) | 837.8 ((n = 98) 344.9 – 1868.0) | < 0.001a |
| Seroconversion post 2nd vaccine | 221 [98.2%] | 92[100%] | 0.33b |
| Median IgG levels post 2nd vaccine (AU/MI) | 2581.3 ((n = 228) 873.5 – 5948.3) | 6871.8 ((n = 98) 2182.4 – 11554.3) | < 0.001a |
| mRNA vaccine             | 2931.9 (1010.0 – 6534.5) | 8747.6 (5754.8 – 15488.3) | < 0.001a |
| VV vaccine               | 814.0 (336.1 – 2039.5) | 1160.7 (565.0 – 2156.9)  | 0.33a   |

*a Mann–Whitney U test  
*b Chi square test
Table 2: Median anti-SP IgG levels dependent of medication at time of vaccination

| Medication (n = 213)                | Anti-SP IgG (AU/ml)       | P value * | Limited Antibody Reserve (Anti-SP Ab < 4000) % (n) |
|-------------------------------------|--------------------------|-----------|-----------------------------------------------|
| 5-ASA/No medications (n= 31)       | 4107 (1882 – 15463)      |           | 48.4 (15)                                     |
| Anti-TNF therapy (n=145)            | 2153 (717 – 5022)        |           | 69.7 (101)                                    |
| Anti-integrins (n= 14)              | 4916 (2125 – 11198)      | 0.007     | 42.9 (6)                                      |
| Anti-IL 12/23 (n = 8)               | 1988 (711 – 13270)       |           | 75 (6)                                        |
| Immunomodulators (n= 16)            | 3337 (1034 – 18974)      |           | 56.3 (9)                                      |
| Steroids (n= 9)                     | 3760 (1300 – 14223)      |           | 55.6 (5)                                      |
| JAK Inhibitors (n= 4)               | 1960 (517 – 28542)       |           | 75 (3)                                        |

*Kruskal-Wallis test
Table 3: Factors impacting anti-SP antibody levels in IBD cohort. (A model of backward linear regression was undertaken to determine the impact of the above factors on IgG SP antibody levels in patients with IBD. A p value < 0.05 was significant and factors were removed in a stepwise approach).

| N = 228 | β co-efficient | T   | p Value |
|---------|----------------|------|---------|
| Age at vaccination | -0.11 | -1.6 | 0.02 |
| Male gender | 0.03 | 0.4 | 0.70 |
| mRNA vaccine | 0.10 | 1.5 | 0.15 |
| UC | -0.002 | -0.02 | 0.98 |
| Anti-TNF | -0.26 | -3.4 | 0.001 |
| Vedolizumab | 0.06 | 0.78 | 0.45 |
| 5-ASA | 0.003 | 0.04 | 0.97 |
| Immunomodulator | 0.06 | 0.88 | 0.38 |

Table 3a: All the factors included in the initial multivariate regression analysis

| N = 228 | β co-efficient | T   | p value |
|---------|----------------|------|---------|
| Age at vaccination | -0.13 | -1.9 | 0.05 |
| Anti-TNF | -0.3 | -4.4 | <0.001 |

Table 3b: Factors remaining in the final multivariate regression analysis.
Table 4: Impact of previous COVID-19 infection on anti-SP IgG levels

|                                | Median anti-SP IgG levels (AU/ml) post second vaccination | P Value * |
|--------------------------------|----------------------------------------------------------|-----------|
| IBD COVID-19 negative (n= 222) | 2,637.8 (870.5 -5819.9)                                   |           |
| IBD cohort previous infection (n = 4) | 3,938.2 (1261.3 – 14979.1)                                 | <0.001    |
| HC COVID-19 negative (n= 87)     | 6,117.4 (2080.2 – 10887.7)                                 |           |
| Control Cohort previous infection (n= 5) | 20,719.6 (19981.0 – 35044.2)                               |           |

*Kruskal-Wallis test
Figure 1 Differences in median IgG levels across three time points

Values measured by ELISA are presented as a logarithmic scale prior to vaccination represented as Time 0, following one vaccination (Time 1) and two doses of vaccination (Time 2) against COVID-19. Boxplots represent median anti IgG SP levels and error bars represent interquartile range.

(A) The difference in anti-SP IgG levels between IBD patients and healthy controls across three time points. Statistical analysis was carried out using independent-samples Mann-Whitney U test. (B) The difference in anti-SP IgG levels between IBD patients receiving mRNA vaccines and VV vaccines and healthy controls (HC) receiving mRNA vaccines and VV vaccines post two vaccines against COVID-19. Patients with IBD have significantly reduced levels of IgG spike protein Ab levels post one and two vaccines against COVID-19 vaccine. Statistical analysis was carried out using independent-samples Mann-Whitney U test and independent-samples Kruskal–Wallis test.
Figure 2: Differences in median anti-SP Levels dependent on medication for treatment of IBD.

Values measured by ELISA are presented as a linear scale (y axis represented as y = y/100) of IgG SP antibody levels post vaccination against COVID-19. Differences in anti-SP IgG levels post two vaccines of an mRNA vaccine in IBD patients on anti-TNF therapy, not receiving anti-TNF therapy and healthy controls. Statistical analysis was carried out using independent-samples Mann Whitney U test between each group.
Figure 3: Change in median IgG levels over vaccination period in patients with IBD.

In this graph anti-SP IgG antibody levels are represented on the Y axis with error bars representing the interquartile range and time points at serum analysis represented on the x axis. Time 0 represents anti-SP IgG levels prior to vaccination, Time 1 represents anti-SP IgG levels after first vaccine, Time 2 represents first set of anti-SP IgG levels after complete vaccination against COVID-19 at a median of 6 weeks post vaccination and Time 3 represents second anti-SP IgG levels post complete vaccination at a median time of 12 weeks post complete vaccination. Statistical analysis was carried out using related-samples Friedmans 2-way ANOVA by ranks test for trends in anti SP IgG levels over the four timepoint. A Mann Whitney U test was used to compare median anti-SP IgG levels between our anti-TNF and non-anti-TNF cohort at timepoint 3.