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Immunogenicity of SARS-CoV-2 primary vaccination and boosters in patients with immune-mediated inflammatory diseases: Impact of immunosuppressive therapy

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ABSTRACT

Introduction: Patients with immune-mediated inflammatory diseases (IMID) are at increased risk of severe COVID-19 infection. Their immunosuppressive treatment may lead to attenuated vaccine responses. In this study, we assessed the impact of specific immunosuppressive treatments on the immunogenicity of primary SARS-CoV-2 vaccination as well as booster vaccinations in IMID patients.

Material and methods: We included participants with IMID from the Danish ENFORCE cohort with baseline sampling prior to SARS-CoV-2 vaccination. Participants were followed for two years, with scheduled visits prior to vaccine dose 2, day 90, 180, 365 and 730 as well as before and after each booster dose. At all visits, seroconversion rates and quantitative SARS-CoV-2 Spike IgG antibody levels were assessed. Vaccine hyporesponsiveness, defined as $<2\log_{10}$ increase in SARS-CoV-2 Spike IgG levels from baseline, was evaluated at day 90 and again after the first booster.

Results: We included 282 patients with IMID and 482 immunocompetent controls. At day 90, patients with IMID treated with anti-CD20 antibodies or fingolimod exhibited markedly reduced seroconversion rates (27 % and 60 %, respectively, vs 100 % for controls), significantly lower antibody levels (2251 AU/mL [95 % CI: 888–5703] and 1743 AU/mL [95 % CI:784–3873] vs 186,308 AU/mL [95 % CI, 171366–202,552]) and higher odds of vaccine hyporesponsiveness (odds ratio (OR) = 67.5 [95 % CI, 25.4–179.7] and 82.5 [95 % CI, 29.6–196.3]). This impaired response persisted throughout the follow-up period, and anti-CD20 antibodies and fingolimod treated patients never reached antibody titers comparable to day 90 titers in controls, despite repeated booster vaccinations.

Conclusion: Anti-CD20 antibody treatment and fingolimod severely impair humoral vaccine responses in IMID patients. In contrast, IMID patients treated with Methotrexate, TNF-alpha inhibitors, or other immunosuppres-

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sants mounted efficient vaccine responses. These findings support that tailored vaccine schedules with early and frequent boosters are crucial to protect this high-risk population.

Clinical Trial registration: EudraCT no, 2020–006003-42, NVK no. 1–10–72-337-20.

1. Introduction

In 2020, the COVID-19 pandemic spread rapidly across the globe. Over the following 5 years, more than 750 million confirmed cases and 7 million deaths have been reported [1,2]. Immunocompromised patients are particularly at risk of severe infections, with high rates of hospitalization and mortality [3–5]. Patients with immune-mediated inflammatory diseases (IMIDs), such as rheumatological diseases, multiple sclerosis (MS) and inflammatory bowel diseases (IBD) are considered part of this high-risk group [6]. The impaired immune system in patients with IMID is primarily attributable to immunomodulatory therapies rather than the underlying disease per se. Thus, patients with IMID do not generally have an increased risk of severe COVID-19. However, certain immunosuppressive treatments are strongly associated with increased COVID-19 morbidity [7–9].

Depending on the specific IMID diagnosis and disease severity, patients receive a range of treatment regimens, including conventional disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate (MTX) and azathioprine, monoclonal antibodies (mAbs) such as anti-CD20 antibodies and tumor necrosis factor (TNF)-α-inhibitors, sphingosine-1-phosphate receptor modulators such as fingolimod, synthetic targeted JAK-STAT inhibitors or glucocorticoids. These therapies act on distinct components of the immune system, exerting various effects on vaccine-induced immune responses. Anti-CD20 antibodies, such as rituximab, deplete most B-cells, including naïve, memory and activated B-cells [10]. Methotrexate and TNFα-inhibitors prevent B-cell proliferation and maturation [11,12], while fingolimod prevents T- and B- lymphocytes from exiting the lymph nodes and entering the bloodstream, leading to peripheral lymphocytopenia [13]. Despite differences in mode-of-action, these immunomodulatory therapies all have the potential to inhibit B-cell proliferation and differentiation leading to impaired antibody responses.

Since the rollout of the first SARS-CoV-2 vaccines in December 2020 [14], several studies have investigated vaccine responses in patients with IMID and the impact of immunosuppressive therapy on effectiveness and immunogenicity [15,16]. In particular, anti-CD20 antibodies [17,18] and fingolimod [19,20] have been associated with markedly lower vaccine responses, but varying degrees of diminished antibody production have also been observed in patients treated with TNF-α inhibitors [21,22], methotrexate [23] and glucocorticoids [24]. However, most studies have focused on one or a few different immunosuppressive regimens and limited time points. In this study, we aimed to assess the longitudinal SARS-CoV-2 vaccine-induced immune response in patients with IMID across multiple treatment classes, following both the primary vaccination series and subsequent booster doses.

2. Materials and methods

This is a sub-study within the Danish National Cohort Study of Effectiveness and Safety of SARS-CoV-2 Vaccines (ENFORCE). ENFORCE is a non-randomized, open-label phase IV study, with seven study sites across all Danish regions. Danish residents were eligible for participation if they were > 18 years of age and had received an offer of vaccination through the Danish SARS-CoV-2 program (clinicaltrails.gov, identifier: NCT04760132). Participants were enrolled prior to their first vaccination dose, during the enrollment period from February to August 2021. All participants received written and oral information about the study and provided written consent. ENFORCE has previously been described in detail by Stærke et al. [25].

The ENFORCE study protocol was approved by the Danish Medicines Agency (Eudra CT number:2020–006003-42) and the National Committee on Health Research Ethics (no. 1–10–72-337-20).

2.1. Study population and data collection

In this study, we included ENFORCE participants diagnosed with IMID, such as rheumatological diseases, MS and IBD. Using each participant's unique civil registration number (CPR), diagnoses were obtained from the Danish National Patient Register (LPR). A control group, matched on age and gender, was included from the ENFORCE cohort.

Medical treatment for the IMIDs was grouped as follows; anti-CD20 antibodies, MTX, TNF-α inhibitors, azathioprine, glucocorticoids, fingolimod, salazopyrin/mesalazine, other treatments and no treatment. Anti-CD20 treatment was recorded if the most recent dose had been administered within the previous 12 months, while all other medications were reported if the participants were actively treated at the time of enrollment.

At enrollment, we collected baseline data, including age and sex. From the Danish Vaccine Registry (DDV) we retrieved information on vaccination dates and types (BNT162b2, mRNA-1273, and ChAdOx1). Information on specific diagnoses, and immunosuppressive treatments were obtained from electronic medical records (NordEPJ, MidtEPJ, EPJ SYD, and Sundhedsplatformen).

Follow-up visits were scheduled before the second vaccine dose (on day 21 or 28, depending on the vaccine type) and at approximately day 90 (±14 days), day 180 (±14 days), day 365 (±14 days), and day 730 (±14 days). Additionally, all participants were invited to attend visits both before and around 28 days after each booster vaccination.

Participants were excluded from analysis if they were SARS-CoV-2 receptor binding domain (RBD) IgG positive at enrollment, lacked baseline antibody results, were lost to follow-up before visit 3 or withdrew consent at any point during the study.

2.2. Serology measurements and cellular immune response

Serum and plasma samples were collected at each study visit. Total serum SARS-CoV-2 RBD antibody levels were measured to assess seroconversion, and quantification of SARS-CoV-2 Spike, RBD and nucleocapsid IgG was performed. Neutralizing antibodies were quantified by measuring their ability to block the binding between SARS-CoV-2 Spike and the human ACE2 receptor. Approximately 10 % of all participants were enrolled in a sub-study where peripheral blood mononuclear cells (PBMCs) were isolated after each visit. These PBMCs were used to quantify the percentages of SARS-CoV-2 Spike-specific CD4+ and CD8+ T cells, by using an activation induced marker (AIM) assay. This assay detects expressions of CD69, OX40 (CD134), and 41BB (CD137). We defined SARS-CoV-2-specific T cells as those co-expressing two or more of these AIMs. Details on the laboratory work have been described previously [25–27].

Hyporesponsiveness was evaluated at day 90 and four weeks after the first booster. All participants were categorized into two groups, based on the change in Spike IgG levels from baseline: high responders (≥ 2 log10 fold change) and low responders (<2 log10 fold change) [28]. This threshold was selected as it robustly identified a suboptimal response, as evidenced by its low prevalence (3.2 %) in immunocompetent controls.

2.3. Infections

SARS-CoV-2 nucleocapsid measurements were used to assess the

proportion of participants infected during the study period. Infection was defined as a nucleocapsid IgG > 3000 AU/mL and a two-fold increase compared to baseline values. Furthermore, information on positive SARS-CoV-2 PCR samples was collected from the Danish National Microbiology Database (MiBa, Statens Serum Institut, Copenhagen, Denmark).

2.4. Statistical analyses

Baseline demographics for categorical variables are reported as percentages. Continuous variables are summarized as medians with interquartile ranges (IQR). Serological data are presented as Geometric Mean Titers (GMT) with 95 % confidence intervals (CI) in tables and line plots and visualized using boxplots showing the median, lower and upper quartiles, and whiskers extending to the lower adjacent value (25th percentile $-1.5 \times$ IQR) and upper adjacent value (75th percentile $+1.5 \times$ IQR) on a $\log_{10}$ scale. Outliers are not displayed separately. Cellular immune responses are reported as percentages. Differences between treatment groups are evaluated using the Kruskal–Wallis test followed by Dunn's test for pairwise comparisons, with Bonferroni correction applied to adjust for multiple testing. Risk factors for hyporesponsiveness are assessed at day 90 and after the first booster dose using binary logistic regression, based on variables selected a priori, including multiple treatments, age at enrollment, sex and vaccine brand. Hyporesponsiveness is presented in a forest plot and a table with odds ratios (OR) compared to controls. All statistical analyses were conducted in STATA 19 (StataCorp LLC, College Station, TX, USA). Odds ratios were visualized using R.

3. Results

We included 282 patients with IMID in this study: 101 with rheumatological disease, 77 with MS, 92 with IBD and 12 suffering from >1 of these diseases. Additionally, 482 immunocompetent controls matched on age (+/− one year) and sex were included. Immunosuppressive treatments were prescribed to 221 (78.4 %) of the 282 patients with IMID. Their median age at enrollment was 55 years (IQR 45–66) and almost two-thirds were female (n = 180, 63.8 %). The majority received BNT162b2 as their first vaccine dose (n = 234, 83.0 %) (Table 1).

3.1. Serological response

At day 90 after starting the primary vaccination series, seroconversion rates differed significantly between treatment groups. Only 27 % of patients treated with anti-CD20 antibodies, 60 % of fingolimod-treated patients and 83 % of patients who received glucocorticoids were seropositive, compared to >90 % among all other treatment groups and 100 % of untreated IMID patients and healthy controls. After the first booster, the seroconversion rate was 45 % and 77 % among patients treated with anti-CD20 or fingolimod, respectively, increasing to 67 % and 91 % after the second booster (Fig. 1, supplementary table 2).

Following the primary vaccination, SARS-CoV-2 Spike IgG levels increased across all patient groups, although the magnitude of the response varied considerably. There was a significant difference in GMT SARS-CoV-2 levels between patients receiving fingolimod (1743 AU/mL [95 % CI:784–3873], antiCD20 antibodies (2251 AU/mL [95 % CI: 888–5703]), glucocorticoids (48,034 AU/mL [95 % CI: 15660–147,337]), TNF-α inhibitors (74,096 AU/mL [95 % CI: 51660–106,276]), MTX (84,069 AU/mL [95 % CI, 54549–129,564]), and healthy controls (186,308 AU/mL [95 % CI, 171366–202,552]). After the first booster, treatment with anti-CD20 antibodies, TNF-α inhibitors, glucocorticoids and fingolimod was consistently associated with reduced antibody levels. Even after the second booster (i.e. 4th vaccination), participants receiving anti-CD20 antibodies or fingolimod had lower antibody levels than the day 90 titers after the primary 2-dose vaccination observed in healthy controls (Fig. 2, Supplementary Fig. 2). Antibody levels from all visits are shown in supplementary tables 3 and 4.

3.2. Neutralizing antibodies

Neutralizing antibody levels were reduced in patients treated with TNF-α inhibitors compared to controls after the initial vaccination series (3.5 AU/mL [95 %CI: 2.5–4.9] vs 5.7 AU/mL [95 % CI: 5.2–6.3]). In patients treated with anti-CD20 antibodies (1.7 AU/mL, [95 % CI: 0.7–4.0), fingolimod (0.8 AU/mL [95 % CI: 0.0–48.5) and in patients receiving no treatment (4.5 AU/mL [95 % CI: 3.3–6.2]), we also observed a tendency towards reduced neutralizing antibody levels. However, after Bonferroni correction, there were no significant differences compared to controls. Most treatment groups reached high levels of neutralizing antibodies after the first booster, while patients receiving fingolimod and anti-CD20 antibodies first reached levels comparable to the day 90 levels measured in healthy controls after the first and second booster, respectively (Supplementary Fig. 3, Supplementary Table 5 and 6).

3.3. Hyporesponsiveness

Serological hyporesponsiveness was observed in 69.2 % (n = 18/26) of participants treated with anti-CD20 antibodies and in 73.3 % (n = 11/15) of patients receiving fingolimod after the initial two vaccine doses. In contrast, only 6.1–13.3 % of participants across all other treatment groups, 1.7 % (n = 1/60) of patients receiving no immunosuppressive treatment and 3.2 % (N = 15/465) of controls were categorized as low responders (Table 2). The OR of being a low responder was 82.5 [95 % CI: 23.5–289.3] in patients treated with fingolimod, 67.5 [95 %CI: 25.4–179.7] in patients receiving anti-CD20 antibodies and 3.3 [95 % CI: 1.05–10.6] in patients treated with TNF-α inhibitors compared to controls (Fig. 3, Supplementary Table 7). When adjusting for multiple treatments there was no longer a significant difference between patients receiving TNF-α inhibitors and controls (OR = 2.6 [95 % CI: 0.8–8.8]) (Supplementary Table 7).

After the first booster, 68.2 % (n = 15/22) of patients treated with anti-CD20 antibodies remained low responders, while hyporesponsiveness had decreased in patients receiving fingolimod (30.8 %, n = 4/13) (Table 2). The unadjusted OR for being a low responder was significant in all groups except participants treated with TNF-α inhibitors and salazopyrin/mesalazine. Adjusted ORs (aOR) are shown in Supplementary Table 7.

After the second booster, 100 % of participants were high responders, except among those treated with anti-CD20 and fingolimod, where 40 % (n = 6/15) and 18.2 % (n = 2/11), respectively, remained low responders (Table 2). It was not possible to calculate OR after the second vaccine booster because there were too few low responders.

3.4. Cellular immunity responses

Due to few IMID patients participating in the cellular immunity substudy, analysis between different treatment groups was not possible. Instead, we pooled all treatment groups into one group. There was no significant difference between the percentage of SARS-CoV-2 specific CD4+ or CD8+ T-cells in IMID patients treated with immunosuppressive treatment and controls at any time point. Prior to the first booster and at day 365, we found a significant difference between untreated IMID patients and controls, but there was no significant difference at day 90 or after the first booster. Percentages per visit are shown in Supplementary Table 8.

3.5. Infections

Throughout the study period, a significant increase in nucleocapsid

Table 1
Baseline demographics and characteristics.

	Total	Anti-CD20 antibodies	Methotrexate	TNF-alpha inhibitors	Azathioprine	Glucocorticoids	Fingolimod	Salazopyrin/ Mesalazine	Other treatment	No treatment	Controls
Participants, n (%)	282	27 (9.6)	44 (15.6)	45 (16.0)	16 (5.7)	18 (6.4)	16 (5.7)	34 (12.1)	50 (17.7)	61 (21.6)	482
Diagnosis, n (%)											
Rheumatological disease	101 (35.8)	9 (33.3)	44 (100.0)	22 (48.9)	0 (0.0)	8 (44.4)	0 (0.0)	6 (17.6)	11 (22.0)	18 (29.5)	NA
Multiple sclerosis	77 (27.3)	18 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	15 (93.8)	0 (0.0)	28 (56.0)	16 (26.2)	NA
Inflammatory bowel disease	92 (32.6)	0 (0.0)	0 (0.0)	20 (44.4)	15 (93.8)	8 (44.4)	0 (0.0)	26 (76.5)	8 (16.0)	26 (42.6)	NA
>1 autoimmune disease	12 (4.3)	0 (0.0)	0 (0.0)	3 (6.7)	1 (6.3)	2 (11.1)	1 (6.3)	2 (5.9)	3 (6.0)	1 (1.6)	NA
Age at enrollment, median (IQR)	55 (45–66)	48 (38–58)	69 (58–76.5)	49 (40–64)	51 (35.5–54.5)	59.5 (40–68)	48 (46–52.5)	55.5 (49–69)	51.5 (43–60)	61 (53–69)	56 (48–68)
Age group, n (%)											
18–24 years	2 (0.7)	0 (0.0)	0 (0.0)	1 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.00)	0 (0.0)	4 (0.83)
25–39 years	39 (13.8)	7 (25.9)	1 (2.3)	10 (22.2)	5 (31.3)	3 (16.7)	1 (6.3)	5 (14.7)	9 (18.00)	3 (4.92)	52 (10.79)
40–64 years	167 (59.2)	17 (63.0)	17 (38.6)	25 (55.6)	10 (62.5)	9 (50.0)	15 (93.8)	19 (55.9)	34 (68.00)	36 (59.02)	285 (59.13)
65–79 years	62 (22.0)	3 (11.1)	21 (47.7)	6 (13.3)	1 (6.3)	6 (33.3)	0 (0.0)	9 (26.5)	6 (12.00)	17 (27.87)	120 (24.90)
>80 years	12 (4.3)	0 (0.0)	5 (11.4)	3 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)	0 (0.0)	5 (8.20)	21 (4.36)
Gender, n (%)											
Male	102 (36.2)	9 (33.3)	11 (25.0)	19 (42.2)	10 (62.5)	7 (38.9)	3 (18.8)	16 (47.1)	15 (30.0)	21 (34.4)	176 (36.5)
Female	180 (63.8)	18 (66.7)	33 (75.0)	26 (57.8)	6 (37.5)	11 (61.1)	13 (81.2)	18 (52.9)	35 (70.0)	40 (65.6)	306 (63.5)
Vaccine name (1st vaccination), n (%)											
ChAdOx1	3 (1.6)	1 (3.7)	0 (0.0)	1 (2.2)	1 (6.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (1.2)
mRNA-1273	45 (16.0)	1 (3.7)	13 (29.5)	6 (13.3)	0 (0.0)	1 (5.6)	0 (0.0)	8 (23.5)	5 (10.0)	13 (21.3)	83 (17.2)
BNT162b2	234 (83.0)	25 (92.6)	31 (70.5)	38 (84.4)	15 (93.8)	17 (94.4)	16 (100.0)	26 (76.5)	45 (90.0)	48 (78.7)	393 (81.5)

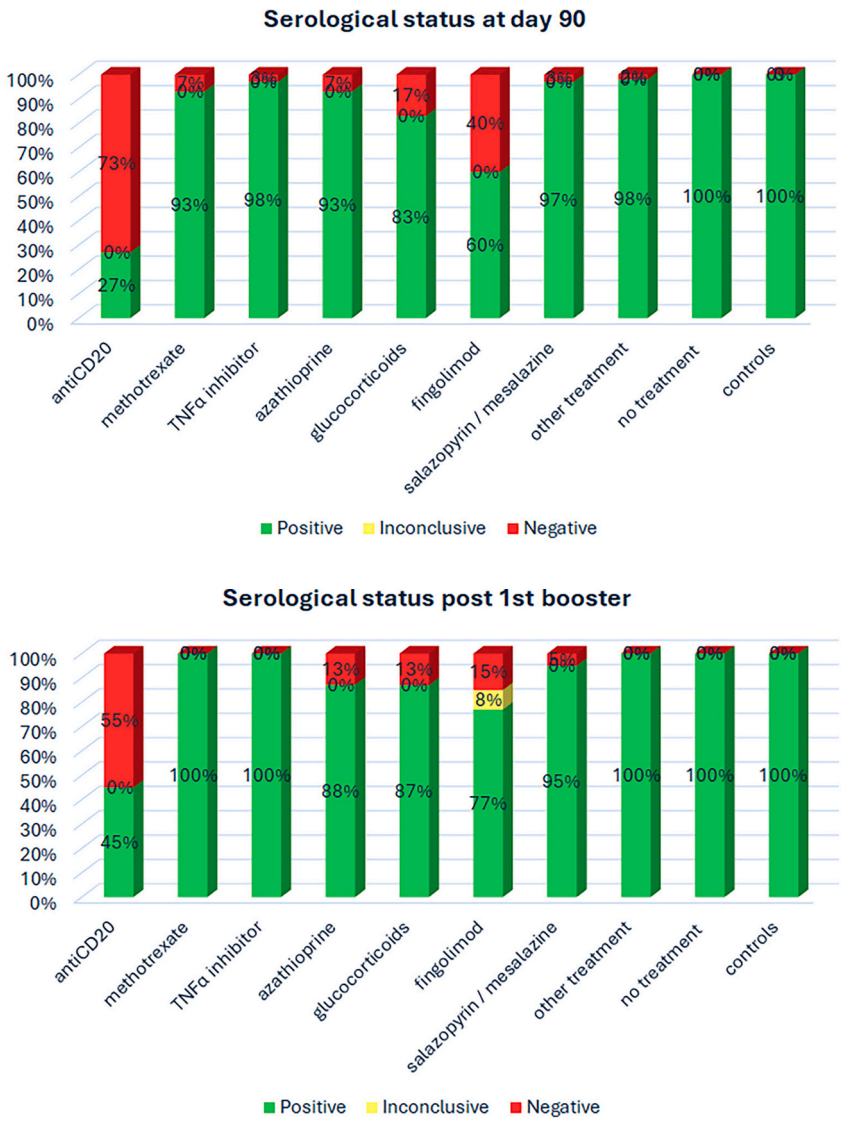

Fig. 1. Serological status per treatment and visit.
Serological status is based on total SARS-CoV-2 RBD IgG at A) day 90 and B) 28 days after the first booster vaccine dose, measured using an ELISA-based assay. Data is stratified by treatment group. The ratio was calculated as optical density OD value divided by the average OD of the negative controls plus 0.16. Results are presented as positive (ratio > 1.1), inconclusive (ratio 0.9–1.1) or negative (ratio < 0.9).

IgG (N-IgG+) was observed in 196 participants (69.5 %), while 173 (61.3 %) tested positive for SARS-CoV-2 in a throat swab PCR test (PCR+). In total, 228 participants with IMID contracted SARS-CoV-2. N-IgG+ PCR- was observed in 55 participants (19.5 %). N-IgG- PCR+ was comparable across all treatment groups, except in patients treated with anti-CD20 antibodies, where 66.7 % ($n = 18/27$) had a positive PCR test, but only 37.0 % ($n = 10/27$) exhibited a significant N-antibody increase. Overall, 32 (18.5 %) of participants who tested positive with a throat swab did not seroconvert in the nucleocapsid IgG analysis (Table 3). Of these, 11 (34.4 %) were considered vaccine-low responders. The OR of nucleocapsid IgG seroconversion, independent of PCR results, during the study was 0.45 [95 % CI: 0.23–0.88] among vaccine-low responders compared to vaccine-high responders at day 90.

4. Discussion

In this study, we present data on seroconversion, Spike-specific IgG levels, neutralizing antibodies, hyporesponsiveness, and T cell responses following primary SARS-COV-2 vaccination and booster doses in patients with IMID. Our results confirm that anti-CD20 antibody and fingolimod treatment strongly attenuated serological responses among patients with IMID, characterized by reduced seroconversion rates, lower antibody levels and persistent hyporesponsiveness [15,17–19]. Notably, while each booster dose elicited a measurable response in these patients, antibody levels remained consistently below the day 90 titers observed in healthy controls, even after two booster doses. This aligns with previous studies, showing continuously diminished responses [29–33]. Importantly, although the immune responses remained attenuated, we observed increases in seroconversion rates and antibody levels following each booster. These findings highlight the critical role of timely and repeated booster vaccinations in ensuring adequate protection for these vulnerable patients, particularly those receiving anti-CD20 antibodies, who are at increased risk of developing severe COVID-19 [9].

Previous studies on vaccine response among patients with IMID on immunosuppressive treatment with MTX, TNF-α inhibitors, and glucocorticoids, also identified a decreased humoral response [22,24,34,35]. In our study, we observed lower GMT levels of SARS-CoV-2 IgG in these groups, although no significant differences in odds for hyporesponsiveness were found. The appropriateness of the threshold used to

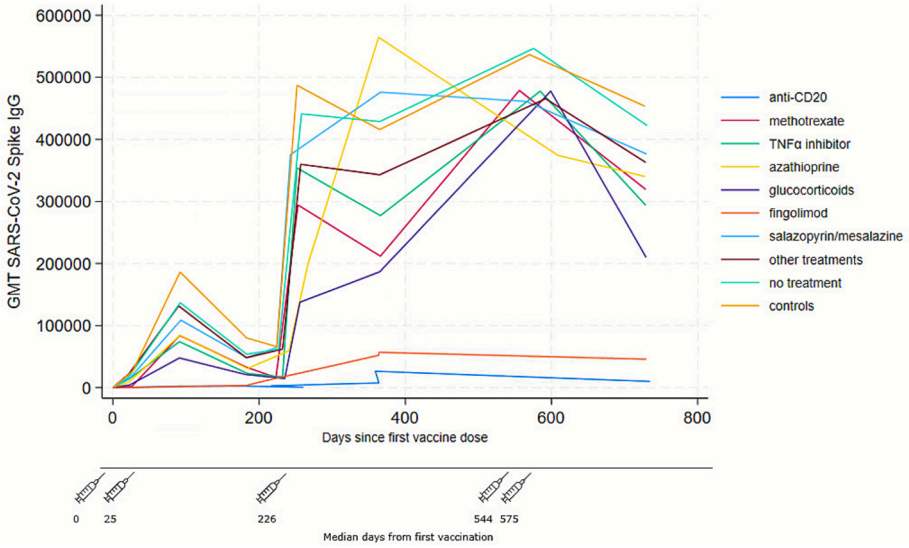

Fig. 2. SARS-CoV-2 Spike IgG per treatment and visit.
SARS-CoV-2 Spike IgG levels were assessed using a multi-antigen serology assay (Meso Scale), stratified by treatment groups and shown for each visit. Data is presented as Geometric mean total spike IgG (AU/mL) Statistical comparisons were performed using the Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction. * indicates statistically significant differences ($p < 0.05$ after Bonferroni correction for multiple comparisons).

Table 2
Percentage of high and low responders by treatment at day 90 and after the first and second booster.

Treatment, n (%)	Day 90		Post 1st booster		Post 2nd booster	
	High responder	Low responder	High responder	Low responder	High responder	Low responder
AntiCD20	8 (30.8)	18 (69.2)	7 (31.8)	15 (68.2)	9 (60.0)	6 (40.0)
Methotrexate	40 (90.9)	4 (9.1)	30 (93.8)	2 (6.3)	29 (100.0)	0 (0.0)
TNF-alpha inhibitor	36 (90.0)	4 (10.0)	33 (97.1)	1 (2.9)	24 (100.0)	0 (0.0)
Azathioprine	13 (86.7)	2 (13.3)	7 (87.5)	1 (12.5)	6 (100.0)	0 (0.0)
Glucocorticoids	16 (88.9)	2 (11.1)	14 (87.5)	2 (12.5)	10 (100.0)	0 (0.0)
Fingolimod	4 (26.7)	11 (73.3)	9 (69.2)	4 (30.8)	9 (81.8)	2 (18.2)
Salazopyrin/mesalazine	31 (93.9)	2 (6.1)	22 (95.7)	1 (4.4)	14 (100.0)	0 (0.0)
Other treatment	45 (91.8)	4 (8.2)	33 (94.3)	2 (5.7)	23 (100.0)	0 (0.0)
No treatment	59 (98.3)	1 (1.7)	49 (100.0)	0 (0.0)	32 (100.0)	0 (0.0)
Controls	450 (96.8)	15 (3.2)	332 (99.7)	1 (0.3)	225 (100.0)	0 (0.0)

Note: Responsiveness status is based on the increase in SARS-CoV-2 Spike IgG from baseline to day 90. Low responder is defined as ≤ 2 $\log_{10}$-fold increase, high responder as >2 $\log_{10}$-fold increase.

define vaccine hyporesponsiveness may be subject to scrutiny. Had all healthy controls exhibited high responses at day 90, it could be argued that the threshold was set too low; conversely, a high proportion of low responders among controls would suggest an overly stringent definition. Given that only 3 % of healthy controls were classified as hyporesponders, we consider the chosen threshold and the resulting calculations to be appropriate and well-calibrated. Even if the total number of IMID patients included in this study is high, each treatment group includes relatively few participants. This reduces the power of our analyses. Hence, it is possible that other treatments besides anti-CD20 and fingolimod could impact vaccine-responsiveness given studies with larger sample sizes. Nevertheless, it is evident that fingolimod and anti-CD20 antibody treatments pose the highest risk for hyporesponsiveness in IMID patients.

According to SARS-CoV-2 test results and quantification of nucleocapsid IgG, 80.9 % of all participants were infected with SARS-CoV-2 during the study period. This aligns with national data from a study conducted in Danish blood donors, estimating that two-thirds of the Danish population contracted COVID-19 between November 2021 and March 2022 [36]. In Denmark, a throat swab to detect SARS-CoV-2 by PCR was encouraged and possible without a referral during the first years of the pandemic [37]. Over time, antigen tests were increasingly used, and after March 2023, PCR tests were no longer available without a referral, further encouraging the use of antigen tests [38]. Most antigen test results were not recorded and thus, the true number of infected patients was probably higher than reported here. This, combined with asymptomatic infections, may explain why 55 of the participants in our study presented with a significant nucleocapsid IgG without a positive PCR test. We also identified 32 participants with a positive SARS-CoV-2 PCR test but no significant increase in nucleocapsid IgG. One explanation to this discrepancy may be the time interval between infection and sampling. If a participant tested positive by PCR several months before the subsequent study visit, antibody decay may result in a falsely low nucleocapsid IgG titer, potentially masking an initial significant increase. Interestingly, we found a marked difference between the nucleocapsid and PCR results among patients treated with anti-CD20 antibodies [39]. While self-isolation among high-risk groups may partly explain the lower infection rates observed early in the pandemic, it is unlikely to account for the consistently low nucleocapsid IgG titers throughout the study, particularly in patients with confirmed PCR-positive SARS-CoV-2 infection. Instead, we propose that treatment-induced B-cell depletion, and the resulting inability to respond to and produce antibodies against new antigens, is the primary explanation among these patients [10,40]. This interpretation is supported by our finding that low responders were less likely to be classified as having had an infection based on the nucleocapsid assay, suggesting that the observed pattern reflects not only true absence of infection, but also an inability to produce IgG antibodies. Previous studies from the ENFORCE

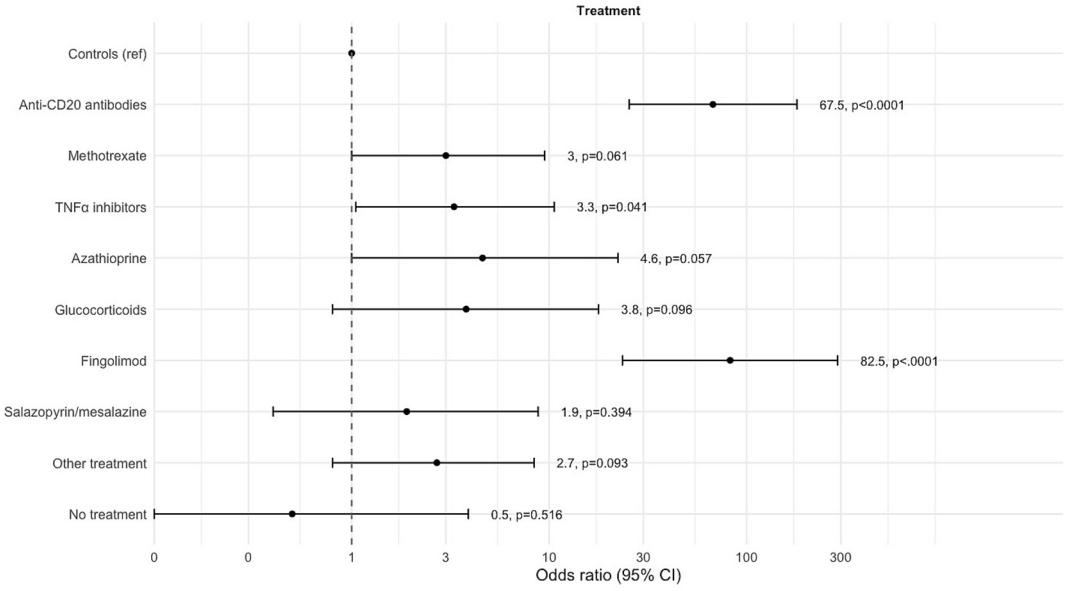

Fig. 3. Odds ratio for hyporesponsiveness at day 90.
Hyporesponsiveness was defined as a ≤ 2 $\log_{10}$ fold increase in SARS-CoV-2 Spike IgG levels from baseline to day 90. Multivariable logistic regression was performed to calculate odds ratios (ORs). ORs are presented with 95 % confidence intervals (CIs) and *p*-values in a forest plot stratitified by treatment. An OR > 1 indicate increased odds of hyporesponsiveness, while an OR < 1 indicates decreased odds. Statistical significance is defined as a 95 % CI not crossing 1 and a p-value <0.05.

Table 3
SARS-CoV-2 infected participants throughout the study by PCR and anti-nucleocapsid IgG.

Treatment, n (%)	Total	N-IgG+	PCR+	N-IgG+ PCR+	N-IgG- PCR+	N-IgG+ PCR-
Total*	228 (80.9)	196 (69.5)	186 (66.0)	141 (50.0)	32 (11.3)	55 (19.5)
antiCD20	20 (74.1)	10 (37.0)	18 (66.7)	8 (29.6)	10 (37.0)	2 (7.4)
methotrexate	31 (70.5)	31 (70.5)	19 (43.2)	19 (43.2)	0 (0.0)	12 (27.3)
TNF-alpha inhibitors	37 (82.2)	34 (75.6)	26 (57.8)	23 (51.1)	3 (6.7)	11 (24.4)
azathioprine	14 (87.5)	13 (81.3)	11 (68.8)	10 (62.5)	1 (6.3)	3 (18.8)
glucocorticoid	14 (77.8)	13 (72.2)	12 (66.7)	11 (61.1)	1 (5.6)	2 (11.1)
fingolimod	12 (75.0)	10 (62.5)	12 (75.0)	10 (62.5)	2 (12.5)	0 (0.0)
salazopyrin/ mesalazine	27 (79.4)	21 (61.8)	23 (67.6)	17 (50.0)	6 (17.6)	4 (11.8)
other treatment	44 (88.0)	39 (78.0)	32 (64.0)	27 (54.0)	5 (10.0)	12 (24.0)
no treatment	49 (80.3)	43 (70.5)	33 (54.1)	27 (44.3)	6 (9.8)	16 (26.2)
controls	NA	355 (73.7)	NA	NA	NA	NA

Note: Infection was defined as A) a positive SARS-COV-2 PCR (PCR+) and/or B) a nucleocapsid IgG $>$ 3000 AU/mL and a two-fold increase compared to baseline values (N-IgG+).

cohort demonstrated a 96.9 % nucleocapsid seroconversion rate among PCR-positive participants, further strengthening the interpretation that immunosuppression may be associated with a diminished nucleocapsid antibody response [41]. Consequently, nucleocapsid IgG is an unreliable marker of prior SARS-CoV-2 infection in patients receiving B-cell depleting therapies and cannot be used to accurately estimate infection rates in this population.

One strength of this study is the large amount of immunological data collected. Instead of limiting our analysis to a single outcome, we assessed both serological responses, neutralizing antibodies and T-cell responses. By performing these analyses at multiple time points, both at fixed intervals and before and after each booster, we were able to evaluate the immunogenicity induced from both the primary and booster vaccination and track antibody dynamics over time. However, our serological quantification analysis has an upper detection limit, resulting in a plateau at approximately 5.5 log10, as illustrated in Supplementary Fig. 2. This does not reflect a biological ceiling in the participants' ability to produce antibodies, but rather a limitation of the assay due to insufficient dilution, which restricts the detection range [42]. Importantly, we do not believe this affects our hyporesponsiveness analysis, as neither anti-CD20 antibody- nor fingolimod-treated patients reached SARS-CoV-2 GMT levels near this threshold.

It should also be noted that some treatment groups, particularly in the sub-study assessing T cell immunity, are small. Data on cellular immunity was only available for a subgroup of study participants and therefore, the observed differences should be interpreted with some caution. Although a larger sample size would strengthen the conclusions, the observed trend – with a majority of IMID patients, including those treated with immunosuppressive treatment, mounting a significant T-cell response, is consistent with previous studies [31,43–45]. Furthermore, we did not adjust for specific diagnosis, which could introduce some bias. However, any confounding is likely to be limited, as these conditions do not generally impair the immune system but rather cause immune dysregulation. This is supported by the fact that only one person in the "no treatment" group was categorized as a low responder. The attenuated vaccine responses in these groups are therefore primarily driven by the immunosuppressive therapy.

Lastly, we only recorded the immunosuppressive treatments the patients were actively treated with at the time of enrollment, or within the past year in the case of anti-CD20 antibodies. We do not have information on subsequent changes of medication during the 2-year follow-up period. This may introduce bias, particularly in the later phase of the study.

In conclusion, treatment with anti-CD20 antibodies or fingolimod was consistently associated with impaired humoral responses to SARS-CoV-2 vaccines, despite the administration of multiple booster doses. In contrast, although other immunomodulatory therapies such as MTX, TNF-α inhibitors, glucocorticoids and azathioprine may also affect the

humoral immune response negatively, the SARS-CoV-2 vaccine-induced immunity was not significantly impaired compared to controls, and booster vaccination elicited robust antibody production in these patients. This provides reassurance for a large proportion of patients with IMID, for whom standard booster strategies appear effective. However, a subset of patients remains at risk of inadequate response, underscoring the importance of early and frequent boosters in this population, and emphasizing the need for tailored vaccination strategies, particularly for individuals receiving B-cell-depleting or sphingosine-1-phosphate receptor-modulating therapies. These strategies could include more frequent booster doses, serological monitoring to guide vaccination timing, or the use of pre-exposure prophylactic antibodies. Our findings are further important for the development of future guidelines for passive immunotherapy with monoclonal antibodies against SARS-CoV-2 in these specific subgroups.

Author contribution

OSS, LJØ, JL, NBS, HN, ISJ, LW, TB and MT conceptualized the design of ENFORCE. This sub-study was conceptualized by CSL, FDL, OSS and LJØ. HN, ISJ, LW, TB, JB, FDL, VK, SOL, LDR and LSK did the clinical visits and collected the data. AKJ, LLD and MT performed the laboratory analysis. FDL performed the data curation, statistical analysis and visualization. FDL, CSL, OSS and JL contributed to the interpretation of the results. FDL drafted the manuscript. All authors critically revised and approved the manuscript. All authors attest they meet the ICMJE criteria for authorship.

CRediT authorship contribution statement

Fredrikke Dam Larsen: Writing – original draft, Visualization, Supervision, Investigation, Formal analysis, Data curation, Conceptualization. **Anna Karina Juhl:** Writing – review & editing, Investigation, Data curation. **Lisa Loksø Dietz:** Writing – review & editing, Investigation, Data curation. **Henrik Nielsen:** Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization. **Nina Breinholt Stærke:** Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization. **Isik Somuncu Johansen:** Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization. **Lothar Wiese:** Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization. **Thomas Benfield:** Writing – review & editing, Supervision, Project administration, Investigation, Conceptualization. **Jacob Bodilsen:** Writing – review & editing, Supervision, Investigation. **Vibeke Klastrup:** Writing – review & editing, Investigation. **Susan Olaf Lindvig:** Writing – review & editing, Investigation. **Line Dahlerup Rasmussen:** Writing – review & editing, Investigation. **Lene Surland Knudsen:** Writing – review & editing, Investigation. **Martin Tolstrup:** Writing – review & editing, Supervision, Methodology, Data curation. **Lars Jørgen Østergaard:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Jens Lundgren:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization. **Ole Schmeltz Søgaard:** Writing – review & editing, Project administration, Methodology, Conceptualization. **Carsten Schade Larsen:** Writing – review & editing, Supervision, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to improve the language and readability for parts of the manuscript, as well as coding assistance. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2025.128155.

Data availability

Data will be made available on request.

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