Viral infections and complications in inflammatory bowel disease

Rogier L. Goetgebuer

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Viral Infections and Complications in Inflammatory Bowel Disease

Virale infecties en complicaties bij inflammatoire darmziekten

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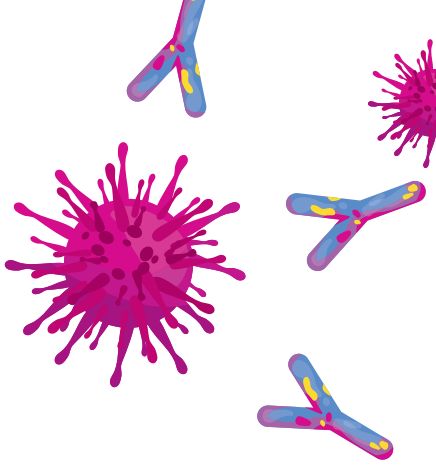

CHAPTER 1

General introduction and outline of the thesis

General introduction

Inflammatory bowel disease and infections

Inflammatory bowel disease (IBD), encompassing both Crohn's disease (CD) and ulcerative colitis (UC), is an immune-mediated inflammatory disease (IMID) with a relapsing and remitting character affecting the gastro-intestinal tract.(1, 2) The current prevalence rate is exceeding 0,3% in Western countries in Europe and North America and continues to rise with an estimated prevalence of 1.0% in 2030.(3, 4) As for other IMID's, immunomodulators (thiopurines and methotrexate) and the biological agent anti-tumor necrosis factor α (anti-TNF) have become widespread available in the past decades and are generally accepted as long-term treatment options to maintain disease remission in moderate to severe IBD.(5, 6)

Thiopurines are antimetabolites that have been used for over 50 years and have the capacity to impact on T-cell activity, macrophages and the barrier function of the intestinal mucosa.(7) Methotrexate is a folate antagonist that inhibits cellular proliferation of immune cells by preventing pyrimidine and purine synthesis required for DNA and RNA synthesis.(8) Anti-TNF agents have brought a major breakthrough in the treatment of IMIDs after their introduction in the late 1990s. They are monoclonal antibodies to the pro-inflammatory cytokine TNF-α, a key pathological cytokine in IBD.(9)

A recent Dutch population-based cohort study demonstrated that exposure to immunomodulators in patients with CD increased from 30% between 1991-1998 to 70% between 2006-2011. Similarly, exposure to biologicals increased from 3% to 41% in these era's, respectively.(10) A major drawback of this increasing trend of exposure to immunosuppressants is the increased risk for serious infections (SIs) and opportunistic infections (OIs), some of which are vaccine-preventable. An SI is an infection that causes hospitalization or results in permanent organ damage or death. An OI can be defined as an infection that is generally non-pathogenic but can become a serious disease as a result of an impaired immune system due to a predisposing disease or its treatment.(12) Because of the lack of a clear definition of OI, however, there is a lot of heterogeneity in reporting this adverse event in randomized controlled trials and cohort studies.(15)

In 2021, the European Crohn's and Colitis Organization (ECCO) published an updated version of the guideline on prevention, diagnosis and management of infections in patients with IBD, including viral, mycobacterial, bacterial, fungal and parasitic infections and vaccination strategies.(12) Next to general risk

factors such as older age, malnutrition, obesity and other comorbidities, IBD patients are mostly at risk because of their immunocompromised state due their medication use and active disease.(12) Thiopurines and anti-TNF agents are both associated with an increased risk of infections, especially when used in combination.(14, 16) Especially during the outbreak of severe acute respiratory syndrome coronavirus-2 (SARS-Cov-2) in 2020,(11) the safety of patients with IBD and their vaccination responses have been areas of concern.

In the past decade, new biologic agents and small molecules have become available for the treatment of IBD, such as vedolizumab, ustekinumab, tofacinitb, upadacitinib and filgotinib. Vedolizumab, approved for both CD and UC, is a gut-selective monoclonal antibody to $\alpha_4\alpha_7$ integrin that prevents leukocyte infiltration in the gastrointestinal mucosa and has been regarded as having a favourable safety profile, likely due to its selective mode of action.(12, 13) Ustekinumab is a monoclonal antibody directed against pro-inflammatory interleukins 12 and 23 and is also approved for both CD and UC. Tofacitinib, upadacitinib en filgotinib are janus kinase inhibitors that are approved for the treatment of UC. Although there are no data available directly comparing the safety between agents, lower rates of SIs and OIs are suggested in some of these newer agents as compared to anti-TNF.(12, 14, 15)

Despite several studies on SIs and OIs in patients with IBD, it remains a challenge for gastroenterologists to weigh the risk of disease related complications requiring aggressive immunosuppressive treatment against treatment-related complications such as infections, especially when more and more treatment options become available.

Viral infections

Forty percent of OIs are caused by viruses and risk factors are young age and use of thiopurines.(17, 18) It is mainly herpesviruses that cause these infections as they are ubiquitously and latently present in humans, and can be reactivated in an immunocompromised host.(17) Other viruses, like HPV and viruses that cause hepatitis, can also stay chronically present in humans and cause more severe outcomes in the immunosuppressed host as compared to an immunocompetent carrier. Table 1 provides an overview of the serious and opportunistic viral infections and complications that are studied in this thesis with their prevalence rates, complications, risks and risk factors. *Epstein Barr virus* is a very common virus with a seroprevalence of up to 100% in adults. For IBD patients specifically, both EBV-related infectious and malignant complications have been described such as hemophagytic lymphohistiocytosis (HLH), lymphoproliferative diseases

and gastric carcinoma.(18, 20). After a primary infection, T-cells are responsible for a lifelong 'immunosurveillance' of EBV – by controlling proliferation of latently infected B-cells. Decreased T-cell activity might lead to a loss of control of this proliferation and result in reactivation of the virus and ultimately to malignant transformation.(21-23) *Cytomegalovirus (CMV)* is another herpesvirus and similar to EBV, a primary infection is followed by a latent infection in myeloid progenitor cells and peripheral blood monocytes.(24) The seroprevalence of CMV in adults ranges from 40 to 100% worldwide. In healthy individuals, a CMV infection is often asymptomatic or results in a self-limiting mononucleosis-like disease.(24) In immunocompromised patients, primary infection or reactivation of the virus can result in systemic CMV disease, leading to pneumonitis, retinitis, encephalitis and in IBD patients in particular, CMV associated colitis.(25)

Cervical cancer is the fourth most common type of cancer in women worldwide and virtually all cancers result from a persistent infection with high risk types of the *human papillomavirus* (HPV) through 3 stages of cervical intraepithelial neoplasia (CIN).(26-28) In immunocompromised women, induction of carcinogenesis and progression through preneoplastic stages after HPV-infection might be accelerated due to impaired detection of oncogenic signals.(28)

Table 1. Overview of viral infections and complications studied in this thesis, adapted from ECCO

	Seroprevalence in IBD	Complication	Risks	Risk factors	Diagnosis	Therapy
CMV	40-100%	CMV colitis	10% to 30% in steroid-refractory acute colitis	Steroids	CMV IgG/IgM, DNA in blood, CMV inclusion bodies, IHC, PCR on biopsies	Taper steroids, antiviral therapy in steroid refractory disease
EBV	~95-100% in adults	Lymphoma	Incidence rate 0.9 per 1000 patientyears	Thiopurines, male sex, older age	EBV IgG/IgM, EBV DNA, IHC on biopsies	Stop immunosuppressants, referral to hemato-oncologist
HEV	1-17 %	Acute or chronic hepatitis E infection	Unknown	Unknown	HEV IgG/IgM, PCR RNA blood	Stop immunosuppressants, ribavirin in persisting viremia (> 3 months)
HPV	-up to 80% lifetime risk	Cervical cancer	1% of all HPV infections	Smoking Young age, immunomodulators, steroids	Cervical smear, HPV PCR	Referral to gynaecologist

Hepatitis E virus (HEV) infection in humans leads to silent seroconversion in the vast majority of individuals, however, a minority develops mild symptomatic hepatitis, mainly in middle-aged or elderly males with underlying liver disease. In recent years chronic courses of HEV infection have been described in immunosuppressed individuals.(29) Development of chronic hepatitis E may be due to delayed antibody production and prolonged HEV-viremia in an immunocompromised individual. It is largely unknown whether IBD patients are at increased risk of a worse outcome of this infection.

Vaccinations in IBD

Many of the more common infections that patients with IBD are at increased risk for, such as influenza and pneumococcus, are preventable with vaccinations.(30, 31) It has been shown that vaccination against influenza reduces risk of infection in immunocompromised patients.(32) European and American guidelines recommend that all IBD patients should receive non-live vaccines at diagnosis and during follow-up.(12, 33) Although self-reported vaccination uptake rates differ among IBD study populations, varying from 23-80%, most of the studies on this subject report low values. (34-38) Reasons for low vaccination uptake rates are concerns about efficacy, safety and side effects and lack of awareness among patients.(35-37) Annual vaccination reviews and use of immunosuppressive medication are associated with better vaccination uptake.(35) Also, actively providing education and information on vaccination behaviour has been shown to improve compliance to vaccination recommendations.(34, 38)

Since many patients will receive non-live vaccinations when they are already on immunosuppressants, physicians should be aware that vaccination responses may be blunted. Use of anti-TNF and immunomodulators, especially when used combined, is associated with a lower serological response to influenza, hepatitis B, pneumococcus and hepatitis A vaccination.(39-46) For newer biologicals and small molecules, it remains largely unknown if they affect vaccination responses as well. Table 2 provides an overview of known vaccination responses to vaccines that were studied in this thesis at the time of starting this work.

The COVID-19 (Corona Virus Disease 2019) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) became a major health crisis in the beginning of 2020.(47, 48) Rapidly, several vaccines including new vaccination strategies with mRNA platforms were developed that target the spike protein of SARS-CoV-2, the protein that binds to the ACE-2 receptor for viral entry. Although these vaccines had proven safety and efficacy in human

clinical trials,(49-52) immunocompromised patients were not included in these trials and so immunogenicity of this vaccination in the IBD population remained largely unknown.

Table 2. Overview of vaccinations studied in this thesis and therapies associated with reduced response adapted from ECCO

	Dosing, schedule and remarks	Type of vaccine	Vaccination	Therapies associated with a reduced vaccine response
Influenza	Annual vaccination recommended for all patients on immunosuppressive therapy, according to national guidelines	Non-live	Inactivated non-live trivalent	Thiopurines, anti-TNF, combination therapy of anti-TNF and thiopurines, steroids
SARS-Cov-2	Schedule and dosage according to national guidelines	Non-live	mRNA based	Unknown

Aims and outline of the thesis

In this thesis we aimed to further explore the risks of specific, partly opportunistic, viral infections in IBD patients, identify risk factors and investigate the response to vaccination in patients on immunosuppressive therapies. The thesis is divided into four sections.

The first part of this thesis focuses on two common herpesvirus infections that can lead to OIs in the immunocompromised patient. **Chapter 2** describes a case series of different complications related to EBV infection in patients with IBD, including haemophagocytic lymphohistiocytosis (HLH) and an EBV-positive mucocutaneous ulcer (EBV-MCU). Also, we described a case of a patient with severe acute colitis and a reactivation of an EBV infection in the intestinal mucosa. This phenomenon is by far more often described with CMV, however, the clinical significance of these reactivations remains a matter of debate. In **Chapter 3,** we investigated the attitude and practice variation of diagnostic and management strategies for CMV colitis in IBD patients among Dutch gastroenterologists.

In part two of this thesis the risk of CIN and cervical cancer in women with IBD is studied. HPV infection is virtually always responsible for this (pre)malignant disease. Previous studies show conflicting results on the risk of cervical neoplasia in IBD women and therefore recommendations for intensified screening are hard to implement and many patients remain unvaccinated. In **Chapter 4,** we

assessed the risk of cervical neoplasia in a large cohort of women in the Dutch IBD Biobank (Parelsnoer) cohort compared to a general population cohort from the nationwide pathology registry (PALGA). We identified risk factors for high-grade neoplasia and studied adherence of IBD women to the national cervical cancer screening programme. Immunosuppressive drugs are theoretically a risk factor for persistent HPV infection and cervical neoplasia. In **Chapter 5**, we therefore explored in more detail the risk of exposure to immunomodulators and biologicals specifically by comparing the women exposed to these medications to women that were not exposed in our cohort.

In part three and **Chapter 6**, we describe a patient with hepatitis E during vedolizumab therapy for Crohn's disease. HEV is a feco-orally transmitted viral infection and as vedolizumab is associated with an increased risk of gastrointestinal infections, we presented the reassuring outcome of this rarely described infection in patients with IBD in this case.

In the fourth and last part of this thesis, the immune response to vaccination in IBD patients treated with anti-TNF and other biologic agents is studied. Previous studies showed that vaccine response can be blunted by the effects of anti-TNF and other immunosuppressive agents. Immune protection by vaccination is mediated through a complex interplay of humoral and cellular responses and based on these previous studies, it is likely that TNF is involved in this process. For most new drugs, however, effects on vaccine response are largely unknown. In **Chapter 7**, we describe the results of a prospective cohort study comparing the vaccine response in CD patients using the anti-TNF agent adalimumab with CD patients using ustekinumab and healthy controls.

The protocol of this study was the basis of the study described in **Chapter 8** that was set up in the height of the pandemic. In this study, we investigated the immune response to the mRNA SARS-Cov-2 vaccination in a large cohort of patients with immune-mediated inflammatory diseases of which most were IBD patients treated with several (combinations of) immunosuppressive drugs.

Finally, in **Chapter 9,** we conclude with the main findings of this thesis in a summary and general discussion.

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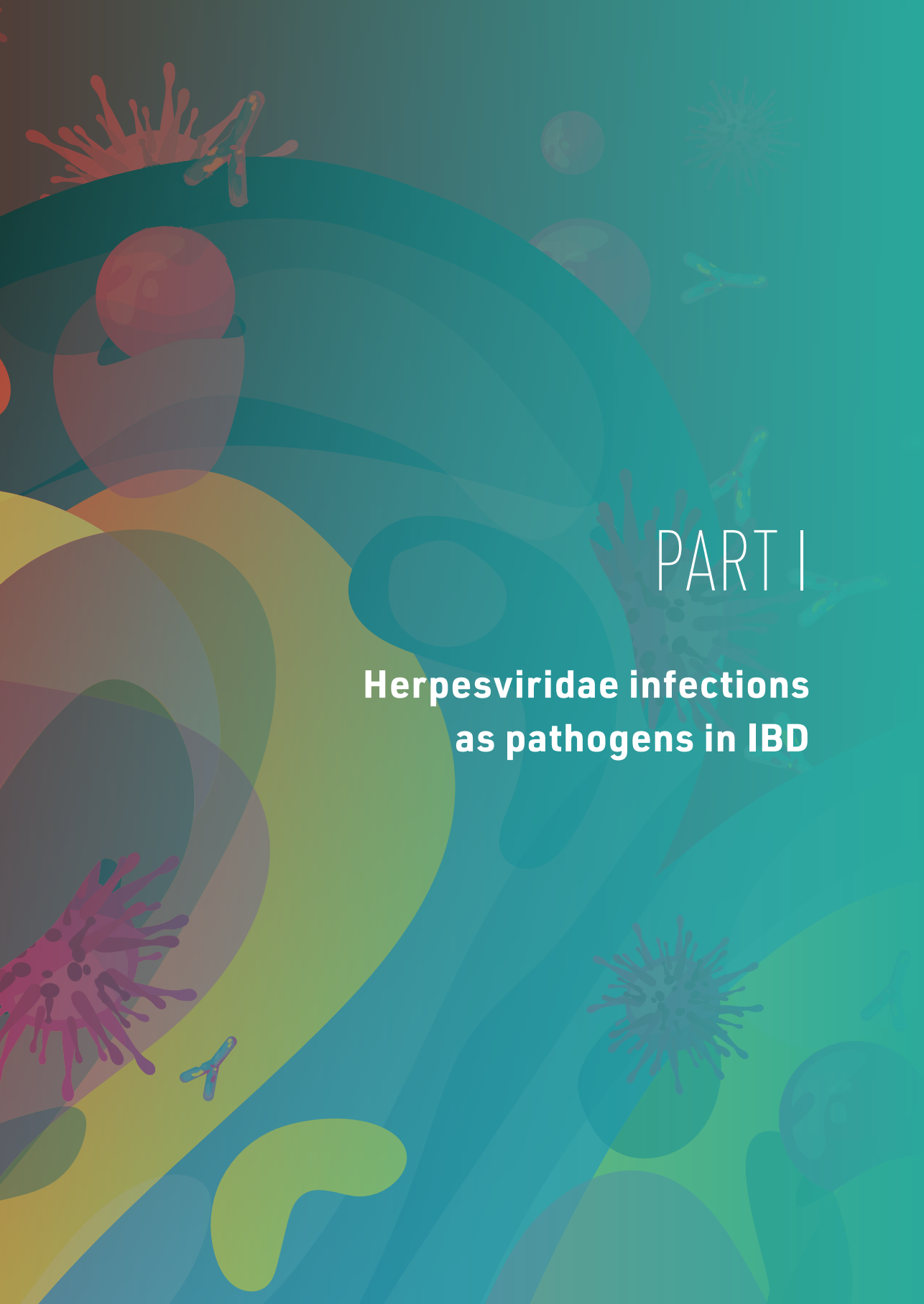
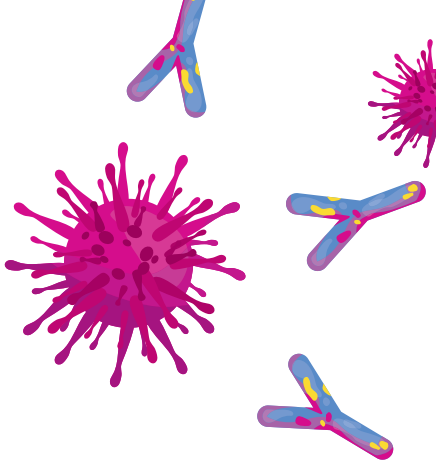


PART I

Herpesviridae infections as pathogens in IBD



CHAPTER 2

Clinical and endoscopic complications of Epstein-Barr virus in Inflammatory Bowel Disease: an illustrative case series

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Abstract

Epstein-Barr virus (EBV) is a proposed trigger in the etiopathogenesis of Inflammatory Bowel Disease (IBD) and is associated with lymphoproliferative diseases. Nevertheless, testing EBV DNA in intestinal mucosa and screening for EBV infection before initiation of drug therapy are not routinely performed. In this short communication, we describe the disease course of three IBD patients with EBV infection, varying from EBV reactivation during disease flare up to a trigger of EBV-mucocutaneous ulcer (EBV-MCU) and haemophagocytic lymphohistiocytosis (HLH).

Introduction

Epstein-Barr virus (EBV) has been proposed as a trigger in the complex multifactorial etiopathogenesis of inflammatory bowel disease (IBD) [1], as well as an aggravating agent during flares and for perpetuation of the inflammatory process.[2] In addition, EBV-associated lymphoproliferative disease in IBD is a feared complication, mostly attributed to immunosuppressive agents.[3] Nevertheless, indications for testing the presence of EBV in intestinal mucosa of IBD patients are unclear and serologic EBV screening before initiation of drug therapy is not routinely performed. To increase awareness of its relevance, we describe the disease course of three IBD patients with an EBV infection.

Case 1

A 29-year-old male patient with ulcerative proctitis, Montreal classification E1S2, in complete remission for four years, presented with a four week history of bloody diarrhoea, low-grade fever and weight loss. Physical examination was unremarkable. Blood results showed: CRP (C-reactive protein) 109 (<10) mg/L, haemoglobin (Hb) 6.6 (8.6-10.5) mmol/L and white blood cell count (WBC) 12.1 (3.5-10) x 10^9/L. Renal function and liver tests were normal. Sigmoidoscopy revealed a diffuse, erythematous, thickened mucosa with erosions and fibrin. Scattered throughout the mucosa there were numerous typical small cavities, measuring 2 to 5 mm; *Figure 1A*. Pathology examination demonstrated chronic active inflammation with ulceration, cryptitis, crypt abscesses and several EBV-encoded RNA (EBER)-positive lymphocytes (non-blasts); *Figure 1B*. Polymerase chain reaction (PCR) on biopsy specimens tested positive for EBV, and negative for HSV1 and -2 and CMV. Serology was positive for immunoglobulin G (IgG) EBV viral capsid antigen (VCA), Epstein-Barr nucleid acid (EBNA) and early antigen (EA) antibodies and negative for immunoglobulin M (IgM) EBV VCA. A severe exacerbation of ulcerative colitis complicated by reactivation of EBV was diagnosed.

Patient started induction therapy with infliximab and azathioprine and showed a rapid clinical improvement. No complications of EBV were seen and patient remained in long-term clinical, biochemical and endoscopic remission.

Case 2

A 34-year-old male patient with ulcerative colitis, Montreal classification E2S2, with long-term clinical remission with mesalamine and 6-mercaptopurine (6-MP) presented with abdominal pain, night sweats hematochezia and severe anal pain. Sigmoidoscopy revealed a deep ulcer measuring 15x20 mm in the

descending colon, several smaller ulcers in the sigmoid colon and a large circumferential rectal ulcer measuring 10 cm, localized above the anal ring; *Figure 1C*. Colon biopsies showed a large area of chronic active, necrotic ulceration with a polymorphous infiltrate of lymphocytes, medium and large sized immunoblasts. The immunoblasts were especially EBV encoded RNA (EBER) immunohistochemistry positive; *Figure 1D*. Clonality was proven with immunoglobulin gene rearrangement analysis. Blood tests showed: CRP 43 (<10) mg/L, Hb 7.4 (8.6-10.5) mmol/L, WBC 4.6 (3.5-10) x 10^9/L and lactate dehydrogenase (LDH) 153 (<248) U/L. Renal and liver function were unremarkable. PCR for EBV-DNA on the biopsies was positive.

As EBV-related mucocutaneous ulcer (EBV-MCU) and diffuse large B-cell lymphoma were considered, 6-MP was stopped, patient was referred to the haematologist and a positron emission tomography (PET) scan was performed. The PET scan showed only local FDG-avidity in the descending colon and rectum with two enlarged perirectal lymph nodes. EBV-MCU was the most likely diagnosis. After 4 weeks the ulcer slightly decreased in size, but colonic biopsies showed persistent necrotic ulceration with EBV-positive immunoblasts. Treatment with four infusions rituximab was prescribed anda rapid clinical recovery occurred. During one year follow-up gradual improvement of the ulcer was seen, without signs of lymphoma in the colon biopsies. Patient remained in clinical remission with mesalamine monotherapy.

Case 3

A 17-year-old female patient, presented with a two weeks history of fever, night sweats and painless cervical lymphadenopathy. She had been treated with azathioprine for one year for Crohn's disease (CD), Montreal classification A1L1B1. EBV status was unknown prior to presentation. At physical examination an enlarged submandibular lymph node was noted. Blood tests showed CRP 45 (<10) mg/L, Hb 5,6 (7.5-9.5) mmol/L, WBC 1.6 (3.5-10) x 10^9/L (lymphocytes 24.2% neutrophils 1.17 (1.5-7.5) x 10^9/L), bilirubin 109 (0-16) umol/L, ALAT 193 (<34) U/L, ferritin 1798 (10-140) ug/L, fibrinogen 3.5 (1.5-3.6) g/L, triglycerides 1.58 (0.4-1.6) mmol/L and soluble IL-2 receptor 67200 (0-2500) pg/mL. A primary EBV-infection was concluded after measuring high levels of EBV IgM antibodies and EBV viral load of 112.000 IU/mL using PCR.Computed tomography (CT) scan showed diffuse lymphadenopathy and hepatosplenomegaly. Lymph node aspiration showed architectural distortion with small T-cell and B-cell lymphocytes, some of which EBER positive, compatible with a primary EBV-infection. Bone marrow aspirate revealed increased numbers of macrophages and haemophagocytosis, after which haemophagocytic lymphohistiocytosis

(HLH) was diagnosed. Treatment with azathioprine was stopped and oral dexamethasone and acyclovir were started. Shortly thereafter, a rapid fall of the EBV viral load was measured. Ferritin rose to a maximum of 4978 ug/L and total bilirubin and ALAT to a maximum of 201 umol/L and 768 U/L, respectively. Acyclovir was discontinued after 10 days, dexamethasone was tapered in 30 days.

Three months later, EBV viral load had decreased to a minimal value and hepatosplenomegaly had disappeared. Without immunosuppressive medication, she remained in long-term remission.

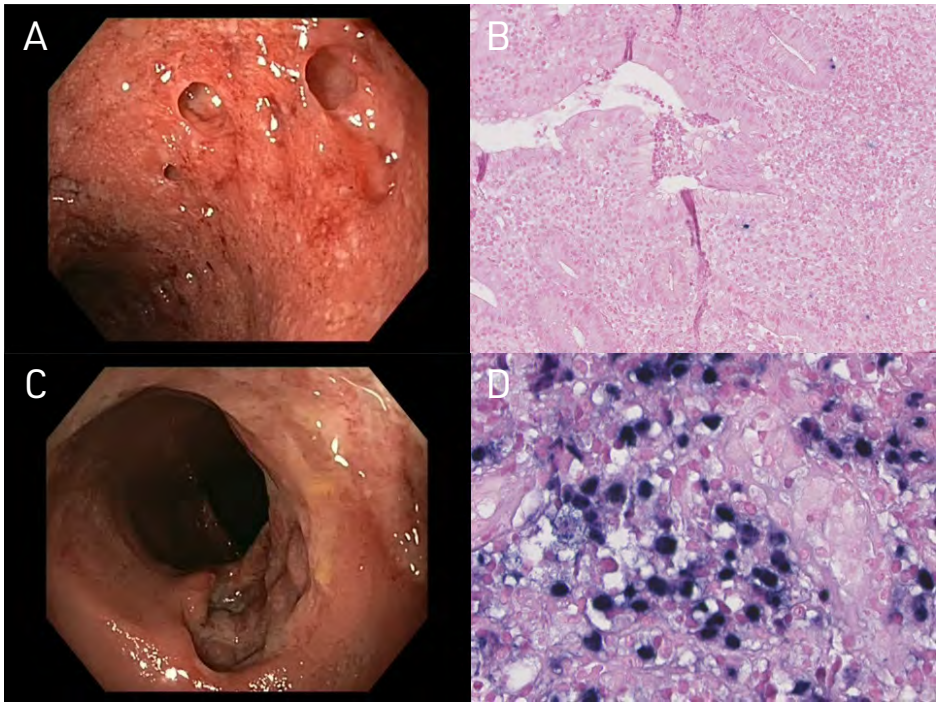

Figure 1. (A) punched-out ulcers as endoscopic findings of EBV-associated colitis in active ulcerative colitis , associated with reactivation of EBV infection; **(B)** diffuse severe chronic and active inflammation, cryptitis and crypts abscesses. Few EBER-positive lymphocytes in the lamina propria in the colon-epithelium (EBER x 200); **(C)** deep punched out ulcer of 15x20 mm in descending colon; **(D)** chronic active ulcerative inflammation with EBV-positive immunoblasts (EBER, x 200)

Discussion

These cases illustrate the spectrum of clinical and endoscopic complications of EBV infection in IBD patients. Several studies have reported on the presence of EBV in the intestinal mucosa of IBD patients with active inflammation and observed prevalences are as high as 64% using PCR assays of the EBV genome in inflamed colonic mucosa.[4,1] However, it remains unclear whether the virus is involved in the pathogenesis or is an innocent bystander. The first case of this series is in line with previous observations that presence of EBV in inflamed colonic mucosa and increased proliferation are associated with severe mucosal inflammation.[5] Active inflammation with intramucosal expansion of EBV-infected B-lymphocytes might cause local impairment of viral immunity and subsequently self-perpetuation of the disease process.[2,5] Mucosal immunity may be impaired because of the IBD itself, or may result from immunosuppressive medication. The immunomodulatory effects of EBV could delay the resolution of the IBD associated inflammation, thus contributing to disease progression.

The colonic mucosal cavities seen in in case 1 are similar to the punched-out ulcers that have been described in CMV associated colitis.[6] To our knowledge, this is the first case in which this type of endoscopic findings is attributed to an associated solitary EBV infection. In CMV-associated colitis antiviral treatment has the potential to shorten duration of severe exacerbations.[7] Although our patient responded rapidly to immunosuppressive therapy only, antiviral treatment may be valuable in patients with refractory disease showing signs of EBV-related disease.[8]

EBV-positive mucocutaneous ulcer (EBV-MCU), as described in case 2, is a rare B-cell lymphoproliferative disorder that can affect the oropharynx, gastrointestinal tract and skin.[9] Main risk factors for the development of EBV-MCU are immunosuppression and age-related immunosenescence.[9] Principle treatment consists of cessation of immunosuppressive medication, however in some patients more intensive therapy is necessary.[9] Lymphoproliferative disorders occur more often in IBD, particularly in patients on thiopurines and are frequently associated with EBV-infection.[3] Thiopurines may be responsible for decreased immunosurveillance of EBV-infected B-cells. Since the absolute lymphoma risk is still very low, it remains unclear whether this association justifies restrictive use of thiopurines for IBD.[3]

The European Crohn's and Colitis organisation guideline recommends to consider screening for EBV infection before initiation of thiopurines.[7] Consideration implies this is not routinely performed in clinical practice. Case 3 illustrates a rare complication of an EBV-infection in a young IBD patient treated with azathioprine. HLH is a potentially fatal lymphoproliferative disorder in which macrophages are overstimulated resulting in phagocytosis of all bone marrow derived cells.[10] Although a primary EBV-infection is considered the main initiator of this severe complication, other infections have been identified as triggers too.[11] Screening for EBV before start of thiopurines can identify high-risk individuals and lead to more restrictive use of thiopurines or more intensive surveillance. However, since the vast majority of adults will be seropositive and thiopurines remain a valuable option also in a negative serostatus, screening strategies are an issue of debate.[12]

In conclusion, EBV infection is associated with a variety of clinical manifestations in IBD patients, which is illustrated albeit not restricted to the described cases in this manuscript. Awareness of EBV-infection in IBD patients should be increased, and biopsies should be assessed for the presence of EBV in patients with specific endoscopic findings of mucosal cavities, ulcerative tumours or large ulcerative punched-out lesions. In addition, screening for EBV infection prior to initiation of immunosuppressive medication may be useful to create alertness for EBV-related complications during follow-up, and to carefully weigh the risks and benefits of the immunosuppressive treatment, especially in children and adolescents.

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CHAPTER 3

The diagnosis and management of CMV colitis in IBD patients shows high practice variation: a national survey among gastroenterologists

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Abstract

Background and aims: Clinical guidelines on cytomegalovirus (CMV) colitis in inflammatory bowel disease (IBD) are hampered by the low quality of evidence.In this study, we aim to explore the attitude and management of CMV colitis in IBD among gastroenterologists.

Methods: A web-based survey was distributed to adult and pediatric gastroenterologists and trainees in academic and general hospitals in the Netherlands. The survey comprised data collection on respondents' demographics, attitude towards the importance of CMV infection in IBD on a visual analogue scale (from 0 to 100), and diagnostic and therapeutic strategies.

Results: A total of 73/131 invited respondents from 32 hospitals completed the survey (response rate 56%). The importance of CMV infection was scored at median 74/100. Respondents indicated CMV testing as appropriate in the clinical setting of steroid-refractory colitis (69% of respondents), hospitalized patients with active colitis (64%), immunomodulator or biological refractory colitis (55%) and active colitis irrespective of medication use (14%). CMV diagnostics include histology of colonic biopsies (88% of respondents), tissue CMV PCR (43%), serum CMV PCR (60%), CMV serology (25%) and fecal CMV PCR (4%). 82% of respondents starts antiviral therapy after a positive CMV test on colonic biopsies (histology or PCR).

Conclusions: Most Dutch gastroenterologists acknowledge the importance of CMV colitis in IBD. Strategies vary greatly with regard to the indication for testing and diagnostic method, as well as indication for start of antiviral therapy. These findings underline the need for pragmatic clinical studies on different management strategies, in order to reduce practice variation and improve quality of care.

Introduction

Cytomegalovirus (CMV) infection is associated with severe flares of inflammatory bowel disease (IBD). Especially in the challenging clinical settings of acute severe colitis and therapy refractory colitis, a diagnosis of CMV colitis should be considered. A higher prevalence of CMV infections and CMV disease has been reported in ulcerative colitis as compared to Crohn's disease.(1) CMV infection is associated with resistance to immunosuppressive treatment, disease duration and severity, risk of colectomy (in both pediatric and adults patients) and therefore increased costs. (2-4) In addition, the start of antiviral therapy after diagnosis is associated with an improved outcome in adult patients. (5) The prevalence of CMV infection and CMV disease in IBD is unclear, mostly due to the variety of definitions and the variety of (use of) diagnostic tests.(1) In addition, exposure to corticosteroids or thiopurines, but not anti-TNF agents, has been associated with an increased risk of CMV reactivation in IBD patients. (6) In pediatric patients also, steroid resistance is associated with CMV infection. (4) Despite these observations, the clinical significance of CMV associated colitis in IBD remains a matter of debate. Hypotheses range from CMV as a non-pathogenic innocent bystander to CMV as an important disease-mediating factor perpetuating the disease process. (7-9) The viral load is proposed to differentiate both situations but it remains unclear which diagnostic strategy is most accurate. A high viral load in PCR testing on blood (>2000 copies DNA/ml) or mucosal biopsies (>250 copies DNA/mg) and high inclusions in the mucosal biopsies (>5 positive cells) are associated with steroid resistance and effectiveness of antiviral therapy. (8)

As a consequence of above-mentioned uncertainties, many recommendations regarding CMV colitis in current international guidelines are based on low to moderate evidence levels. In addition, the recommendations on the indication and methods of diagnostic testing are different. Although practical algorithms for diagnosis and treatment of CMV infection in IBD have been published in addition to the international guidelines (7, 8), clinicians are often puzzled by the conflicting recommendations. The objective of this study was to assess the diagnostic and management strategies for CMV colitis in IBD patients among Dutch adult and pediatric gastroenterologists.

Materials and methods

Study design

This is a national survey study among an expert group of adult and pediatric gastroenterologists with IBD focus in the Netherlands. The survey was sent to the members of the Initiative on Crohn's and Colitis, the Dutch network of adult gastroenterologists with an IBD differentiation. The survey was also sent to the network of Dutch pediatric gastroenterologists and gastroenterology trainees with IBD differentiation as determined by the supervisor from each academic hospital. It was conducted between 8 April 2019 and 9 June 2019. A targeted email reminder was sent to non-responders. The online survey tool Limesurvey was used to conduct the survey.

Survey design

We created a survey containing 15 open and closed questions (Supplementary Appendix). The survey contained the following 5 topics: a) demographic data on the respondents including sex, age, clinical role, size and type of hospital, years of work experience, b) the general attitude towards CMV, which was tested on a visual analogue scale, ranging from 'innocent bystander' (0) to 'aggravating factor which requires treatment' (100), c) clinical settings to test for CMV d) diagnostic CMV tests, e) indication for and therapy of CMV infection. Respondents were questioned on their preferred diagnostic and therapeutic approach and were allowed to provide open answers if their preferred approach was not captured by the survey.

Study definitions

Work experience in the field of (pediatric) gastroenterology was split up in two groups: five years or less and more than five years of experience. Consensus was defined as agreement by at least 75% of the respondents.

Statistical analysis

We only included complete surveys in the statistical analysis. The data was analyzed using IBM SPSS Statistics version 25. Continuous variables were reported as median (IQR). Categorical variables were summarized as frequency and percentages. Analyses were performed using descriptive statistics.

Independent Sample T-test was used to compare continuous variables between subgroups. Chi-square tests or Fisher Exact tests were used to compare categorical data. Statistical significance was defined as $p \leq 0.05$ and all tests were two-sided.

Ethical considerations

No approval of research ethics committees was required to accomplish the goals of this study because the survey did not involve patient material and consent for using data was assumed by participating in the survey.

Results

Respondents profile

A total of 73 /131 invited medical professionals completed the survey; response rate was 56% (Table 1). Of the respondents, 38/73 answers were obtained in the first round and 35/73 after a targeted reminder. The median age of the respondents was 42 years (interquartile range 36-49). These specialists are affiliated in 32 different hospitals, and represent 45% from the total of 69 Dutch hospitals. The response rate was lower in non-academic doctors vs academic doctors (non-academic 48.7 % vs academic 65.5%, p = 0.056). There was no difference in response rate between adult and pediatric gastroenterologists (adult 55.9%, pediatric 55.0%, p = 0.943). Non-respondents were older than respondents (mean age 47 vs. 43 years, p = 0.017). Table 1 shows the demographics of the respondents.

General attitude towards CMV infection in IBD

With regard to the general attitude towards CMV infection in IBD, the respondents scored a median of 74 (IQR 63-80) on a visual analogue scale ranging from 0 to 100 (Figure 1). No significant differences were found when comparing medians between subgroups, academic versus non-academic (p = 0.561), shorter versus longer work experience (p = 0.215) and adult versus pediatric gastroenterologists (p = 0.422).

Table 1. Details of the respondents

Demographics	*n* = 73 (%)
Sex	
Male	37 (51%)
Female	36 (49%)
Clinician	
Gastroenterologist	49 (67%)
Gastroenterology trainee	13 (18%)
Pediatric gastroenterologist	11 (15%)
Type of hospital	
Academic	36 (49%)
Non-academic	37 (51%)
Work experience within field of IBD	
<5 years	25 (34%)
>5 years	48 (66%)

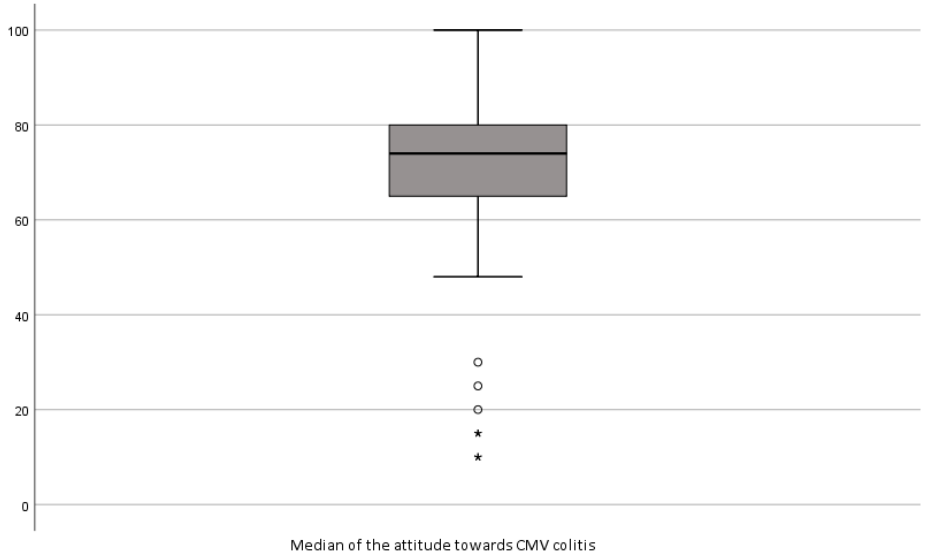

Figure 1. General attitude towards the association of CMV infection in patients with IBD on a visual analogue scale, ranging from 'innocent bystander' (0) to 'aggravating factor which requires treatment' (100). ○: mild outliers more than 1.5 times outside the IQR; ★: strong outliers more than 3 times outside the IQR.

Diagnosis

Respondents indicated testing for CMV appropriate in the clinical settings of severe exacerbations (64.4%), corticosteroid refractory exacerbations (68.5%), and immunomodulatory or biological refractory exacerbations (54.8%) (Table 2). Again, no significant differences were found between subgroups (Supplementary Table 1).

The diagnostic test options to detect CMV consisted of CMV serology, PCR testing on blood, feces or colonic biopsies and histopathology on colonic biopsies (haematoxylin and eosin (H&E) staining and/or immunohistochemistry (IHC)) (Table 3). Significantly more respondents from academic hospitals reported the use of PCR on colonic biopsies (18/36 versus 9/37, p = 0.030). Adult gastroenterologists perform significantly more PCR tests on blood in comparison with pediatric gastroenterologists (41/62 versus 3/11, p = 0.021; Supplementary Table 1).

According to the respondents, endoscopic findings suggestive for CMV colitis were large punched-out ulcers (65.8%), multiple small ulcers (43.8%), small erosions (16.4%) and inflammatory polyps (4.1%). Fourteen respondents (19.2%) mentioned that no specific endoscopic findings are suggestive for CMV colitis.

Table 2. Survey results: indication for diagnostic testing

	Total (n =73)
When do you perform diagnostic tests to detect CMV?	
Not or rarely	2 (3%)
Every exacerbation	10 (14%)
Severe exacerbations with the need for hospitalization	47 (64%)
Corticosteroid refractory exacerbations	50 (69%)
Immunomodulatory/biological refractory exacerbations	40 (55%)
Exacerbation with the need to perform a colonoscopy	3 (4%)
Other[a]	4 (6%)

Other indications included: refractory disease with a unknown cause (n = 1), every exacerbation on immunosuppressant therapy (n = 1), presence of endoscopic ulcers (n = 1), and CMV positive biopsies (n = 1)

Table 3. Survey results: diagnostic tests for CMV colitis

	Total (n =73)
What kind of diagnostic tests do you perform to detect CMV colitis?	
Virology	
CMV serology	18 (25%)
PCR CMV in blood	44 (60%)
PCR CMV in feces	3 (4%)
PCR CMV in colonic biopsies (n = 62)[a]	27 (44%)
If yes to PCR CMV in colonic biopsies, using (n =27)	
Quantitative result	15 (56%)
Qualitative result	8 (30%)
Unknown to the respondent	4 (15%)
Histopathology	
Colonic biopsies on CMV, reviewed by a pathologist	64 (88%)
If yes to colonic biopsies, reviewed by a pathologist, using (n = 64)	
H&E staining only	5 (8%)
H&E staining and IHC qualitative	25 (39%)
H&E staining and IHC quantitative	17 (27%)
Unknown to the respondent	17 (27%)
Other[b]	1 (1%)

a: 11 respondents provided inconsistent information on the questions regarding PCR testing in colonic biopsies and were not included in this table. b: performs colonic biopsies and if these are positive than they also perform a PCR test to determine the viral load (n = 1)

Treatment

Indications to consider treatment with antiviral therapy according to the respondents were positive PCR testing on blood, colonic biopsies or feces (respectively 43.8%, 24.7% and 1.4% of the respondents) or a positive histopathologic result (74.0%). Eighty-two percent of the respondents consider treatment based on positive colonic biopsies (histopathology or PCR) (Table 4). Adult gastroenterologists (32/62 versus 0/11, p = 0.002) consider treatment more often after a positive PCR on blood compared to pediatric gastroenterologists (Supplementary Table 1).

Thirty-seven respondents (54%) only consider treatment if the test reaches a certain threshold and 22 respondents (30%) only consider treatment based on a positive qualitative result. Thirty respondents (41%) perform two different tests. Of those, 23 respondents only need one of two tests to be positive before starting treatment, and seven respondents start treatment if both tests are positive. Only three respondents (4%) perform three tests and start treatment if two out of three tests are positive.

In case of CMV colitis, 82% of the respondents treat patients with ganciclovir intravenous or valganciclovir oral based on the clinical condition of the patient and 15% always start treatment with ganciclovir intravenous and they switch to valganciclovir oral depending on the clinical condition of the patient. Another respondent treats a patient orally or intravenously based on the viral load. No significant differences were seen between the subgroups.

Table 4. Survey results: indications to start treatment

	Total (*n* = 73)
When do you start treatment?	
Positive CMV PCR on blood	32 (44%)
Positive CMV PCR on colonic biopsies	18 (25%)
Positive CMV PCR feces	1 (1%)
Positive IHC and/or H&E staining on colonic biopsies	54 (74%)
Other[a]	6 (8%)

a: other indication to start treatment included: after consulting the IBD team (n = 1), no response to maximal medical treatment (n = 1), in case of proven systemic disease (n = 1), positive test on colonic biopsies, but PCR or histopathology not specified (n = 2), in case of a positive histopathologic result, after determining viral load (n = 1)

Discussion

Diagnostic and therapeutic strategies for CMV colitis in IBD patients remain a challenge. In this study, we observed a varying attitude among Dutch adult and pediatric gastroenterologists regarding the importance of CMV colitis in IBD patients. In addition, there is a lack of consensus regarding indications for testing, diagnostic and therapeutic strategies. This high practice variation raises concern for under- and overuse of diagnostic testing and therapy for CMV-associated colitis in IBD and may be associated with an adversely affected disease course and/or higher costs.(3)

With regards to indications, a majority of respondents in our study use diagnostic testing for CMV in case of severe exacerbations requiring hospitalization and in patients with steroid and immunomodulator or biological therapy refractory disease. This approach is consistent with literature and is recommended in most international guidelines.(10-13) Nonetheless, agreement on the specific indications for CMV diagnostics is still below 75% in this study, and testing is performed less often than recommended in most guidelines. This practice variation may be caused by the dissimilar indications for testing that are recommended in available guidelines. These vary from testing CMV serology at baseline in every patient (ECCO), to moderate to severe, in particular steroid-refractory, active colitis (BSG), immunosuppressive treatment refractory colitis (ECCO), acute severe ulcerative colitis (ACG and ESPGHAN) and biologic resistant active Crohn's disease (ACG).(9, 10, 12-14)

In the recently updated version (2021) of the ECCO guideline on opportunistic infections, screening for CMV is recommended in all IBD patients at baseline and especially before starting immunosuppressive therapy.(10) In this study (in which the survey was sent out before publication of the updated version of the ECCO guideline), 65% of the respondents never or rarely test CMV serology at baseline in newly diagnosed patients. This strategy is not consistent with the ECCO recommendation. However a study on 699 patients showed that latent CMV infection does not influence long-term disease outcome in IBD and testing for CMV at baseline was therefore not recommended.(15)

The diagnostic method on endoscopic detection and assessment of colonic biopsies varies greatly among respondents. In this study, 65% of the respondents finds punched-out ulcers more likely to be associated with CMV, whereas up to 19% of respondents finds no specific findings suggestive for CMV colitis.

According to literature, the endoscopic images compatible with CMV colitis are various. An increased awareness may be triggered by findings of irregular ulcerations or wide mucosal defects and longitudinal ulcerations.(16, 17)

Most respondents (88%) use histopathology on colonic biopsies as a diagnostic method and acknowledge that H&E staining only is insufficient. IHC on colonic biopsies is considered the golden standard for diagnosing CMV colitis (18). Although several respondents choose this test, many respondents still do not know how their pathologist assesses the presence of CMV on biopsies and most respondents do not use a cut-off value as a diagnostic tool. PCR testing on colonic biopsies is performed by only 37% of respondents. However, a high viral load of PCR in tissue is associated with steroid resistance and response to antiviral therapy and it is suggested that less number of biopsies are needed for a diagnosis as compared to IHC.(19, 20) Current guidelines do not specify a treatment cut-off value for the different recommended diagnostic tests, although patients with higher loads in both PCR and IHC on biopsies benefit more from antiviral therapy than patients with lower values.(7, 8) Several cut-off values have been proposed, but we show in this study that clinicians are often unaware of these treatment strategies and therefore do not use them in clinical practice.

More than 60% of the respondents, 66% of adult and 27% of pediatric gastroenterologists, perform a CMV PCR test on blood as a diagnostic test for CMV colitis. Although this test has a high specificity (87-100%) for CMV colitis, the sensitivity of this test is low (44-60%) when using H&E and/or IHC and PCR testing on biopsies as the reference standard even with a low cut-off level 250 copies/mL.(21, 22) Based on these studies, the ECCO guideline recommends to not rely on a blood-based test solely for a diagnosis of CMV colitis. Since most respondents choose only one diagnostic test for a diagnosis of CMV colitis, it is important for clinicians to acknowledge the low sensitivity of a blood PCR CMV test even with a low cut-off level.(8)The limited use of a blood test by pediatric gastroenterologist is likely caused by a consensus paper in which 19 pediatric IBD experts agree that detection of CMV in blood is not clinically meaningful in a child with acute severe ulcerative colitis.(23)

Only a few respondents use PCR testing on feces in patients with a suspicion of CMV colitis, despite several studies showing high diagnostic value of this test using IHC and H&E staining on biopsies as a reference.(24, 25) More studies are needed to add feces PCR to the arsenal of diagnostic tests for CMV colitis in IBD, but it could be a promising non-invasive alternative to other invasive test options.

Although this survey provides a good nationwide representation of clinical practice from IBD specialists working in various academic and non-academic hospitals, a few limitations need to be addressed. Potentially, non-responders to the survey, which were older and more often non-academic, find the subject of CMV colitis in IBD patients less relevant and therefore did not participate in this survey. This may have led to a non-response bias, resulting in a higher mean attitude towards CMV and respondents being more likely to choose diagnostic testing and treatment. However, a response rate of 56% is consistent with the mean response rate in survey studies by physicians and acceptable when taking non-response bias into account. (26) Another possible limitation is that there may be a discrepancy between the answers from the respondents in the survey and real practice, due to a lack of knowledge amongst gastroenterologists on the practice of the pathologists and microbiologists in their hospitals.

Conclusion

In conclusion, we show that variability in diagnosis and indication for treatment of CMV colitis is high. This lack of consensus raises concern on practice variation, which could lead to under- and over treatment of a potential severe complication in (pediatric) IBD patients. More strategic studies are needed to provide a more detailed recommendation for the diagnosis and treatment of CMV colitis in IBD patients in future guidelines, and may preferably include a combination of diagnostic tests including cut-off values to start therapy.

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Appendix: Questionnaire

Diagnosis and management of CMV infection in Inflammatory Bowel Disease in daily practice

Part A: General questions

Question 1 (open question): In which hospital do you work?

Question 2 (open question): What is your age?

Question 3 (multiple choice): What is your gender?

Question 4 (open question): How many years of experience in gastroenterology do you have?

Question 5 (open question): How many IBD patients are in the practice of your hospital?

Question 6 (multiple choice, only 1 answer possible): What is your position and level of training?

a. Gastroenterology trainee
b. Internal medicine specialist
c. Gastroenterologist
d. Gastroenterology pediatrician

Part B: Attitude

Question 7: What is your attitude towards CMV infection in IBD patients with active colitis? Visual analogue scale, ranging from 'innocent bystander' (0) to 'aggravating factor which requires treatment' (100)

Part C: When do you perform diagnostic tests to detect CMV?

Question 8 (multiple choice, only one answer possible): Do you perform a serologic test to detect CMV in new IBD patients?

a. No, rarely
b. Always
c. Only in patients with severe active disease
d. Other

Question 9 (multiple choice, multiple answers could be given): When do you use diagnostics tests to detect CMV-colitis in patients with active IBD?

a. Not/rarely
b. Every exacerbation
c. Severe exacerbations with the need for hospitalization
d. Corticosteroid refractory exacerbations
e. Immunomodulatory/biological refractory exacerbations
f. Other

Question 10 (multiple choice, multiple answers could be given): Which endoscopic findings do you find suggestive for CMV colitis?

a. No specific findings
b. Small erosions
c. Multiple small ulcers (0.1-1 cm)
d. Larger punched-out ulcers (>1 cm)
e. Inflammation polyps
f. Other

Part D: What kind of diagnostic tests do you perform to detect CMV colitis

Question 11 (multiple choice, multiple answers could be given): Which diagnostics tests do you perform in patients with active IBD and with a suspicion of CMV-colitis?

a. CMV serology
b. PCR on blood
c. Colonic biopsies reviewed by a pathologist (H&E staining or immunohistochemistry)
d. PCR on colonic biopsies (virology)
e. PCR on faeces
f. Other

Question 12 (multiple choice, only one answer possible): How does the pathologist review the colonic biopsies in the hospital?

a. Only H&E staining
b. H&E staining and qualitative immunohistochemistry
c. H&E staining and quantitative immunohistochemistry
d. I don't know

Question 13 (multiple choice, only one answer possible): Do you use a PCR to detect CMV on colonic biopsies?

a. No/rarely
b. Yes, a qualitative result
c. Yes, a quantitative result
d. Other

Part E: Treatment

Question 14 (multiple choice, multiple answers could be given): When do you start treatment?

a. Positive CMV PCR on blood
b. Positive colonic biopsies after a review by a pathologist
c. Positive CMV PCR on colonic biopsies
d. Positive CMV PCR on faeces
e. Other

Sub-question 14a (multiple choice, only one answer possible): Is a positive test result enough or do you only start treatment with enough viral load/positive amount of cells? This question was asked for all the options given in question 14.

a. A positive result is enough
b. Only starting treatment with enough viral load/positive amount of cells

Sub-question 14b (multiple choice, only one answer possible): Do both tests need to be positive or is one test enough? This questions was asked if the respondent chose more than 1 answer in question 14.

a. Both option need to be positive
b. Only [option 1] needs to be positive
c. Only [option 2] needs to be positive
d. One test needs to be positive

Sub-question 14c (multiple choice, only one answer possible): Is one positive result enough, or do you need two or three positive result in order to start treatment? This questions was asked if the respondent chose more than 2 answer in question 14.

a. All three need to be positive
b. Two need to be positive
c. One needs to be positive

Sub-question 14d (multiple choice, only one answer possible): How many tests need to be positive? This questions was asked if the respondent chose all four answers in question 14.

a. One positive test
b. Two positive tests
c. Three positive tests
d. All four tests need to be positive

Question 15 (multiple choice, only one answer possible): How do you treat CMV colitis in patients with active IBD?

a. Not/rarely
b. Depending on the clinical condition: ganciclovir intravenous or valganciclovir oral
c. Always ganciclovir intravenous first, with a switch to oral valganciclovir depending on the clinical condition
d. Other

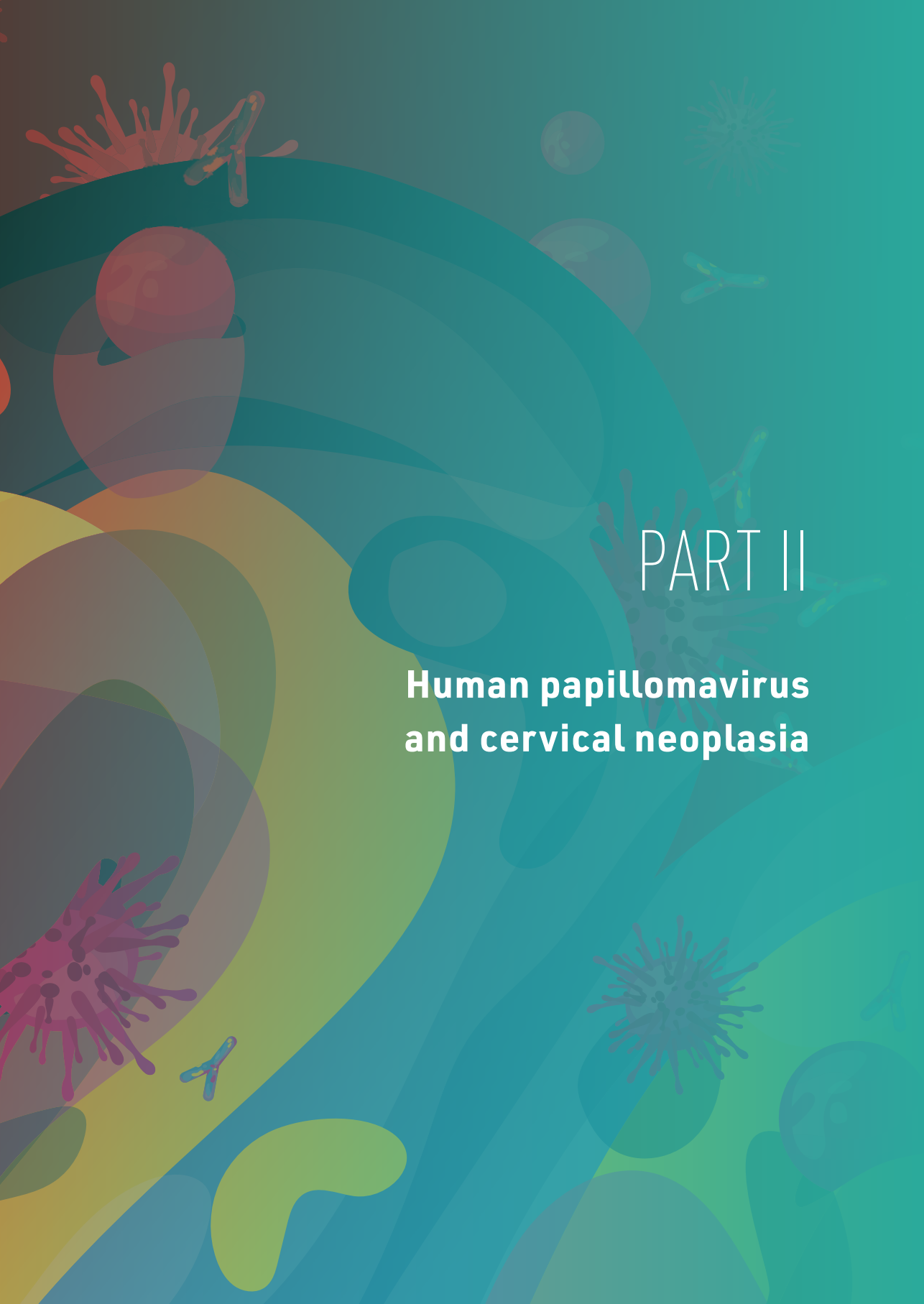
Supplementary Table 1. Survey results after categorizing respondents by demographics

Subgroups	Academic (n = 36)	Non- academic (n = 37)	p- value	Work experience <5 years(n = 25)	Work experience >5 years (n = 48)	p- value	Gastro-enterologists and trainees(n = 62)	Paediatricians (n = 11)	p- value
When do you perform diagnostic tests to detect CMV?									
Not/rarely	1 (3%)	1 (3%)	1	0	2 (4%)	0.543	2 (3%)	0	1
Every exacerbation	5 (14%)	5 (14%)	1	4 (16%)	6 (13%)	0.727	9 (15%)	1 (9%)	1
Severe exacerbations with the need for hospitalization	25 (70%)	22 (60%)	0.465	15 (60%)	32 (67%)	0.613	42 (68%)	5 (46 %)	0.183
Corticosteroid refractory exacerbations	24 (67%)	26 (70%)	0.804	19 (76%)	31 (65%)	0.428	42 (68%)	8 (73%)	1
Immunomodulatory/ biological refractory exacerbations	17 (47%)	23 (62%)	0.243	16 (64%)	24 (50%)	0.324	34 (55%)	6 (46%)	
Exacerbation with the need to perform a colonoscopy	2 (6%)	1 (3%)	0.615	1 (4%)	2 (4%)	1	1 (2%)	2 (18%)	0.057
Which endoscopic findings do you find suggestive for CMV colitis?									
No specific findings	8 (22%)	6 (16%)	0.564	2 (8%)	12 (25.0%)	0.118	**8 (13%)**	**6 (55%)**	**0.005**
Small erosions	6 (17%)	6 (16%)	1.00	**1 (4%)**	**11 (23%)**	**0.048**	10 (16%)	2 (18%)	1
Multiple small ulcers	15 (42%)	17 (46%)	0.815	14 (56%)	18 (38%)	0.145	29 (47%)	3 (27%)	0.328
Larger punched-out ulcers	24 (67%)	24 (65%)	1.00	19 (76%)	29 (60%)	0.206	**45 (73%)**	**3 (27%)**	**0.006**
Inflammation polyps	2 (6%)	1 (3%)	0.615	1 (4%)	2 (4%)	1.00	1 (2%)	2 (18%)	0.057

Supplementary Table 1. Continued

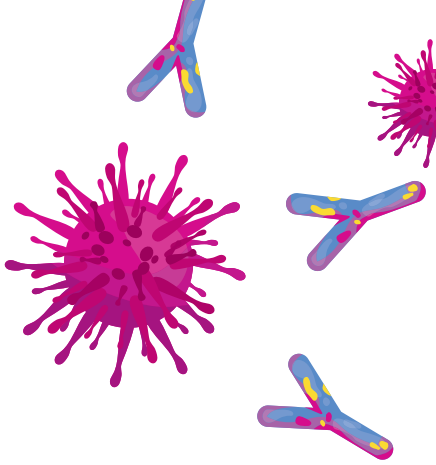
Subgroups	Academic (n = 36)	Non- academic (n = 37)	p- value	Work experience <5 years (n = 25)	Work experience >5 years (n = 48)	p- value	Gastro-enterologists and trainees (n = 62)	Paediatricians (n = 11)	p- value
Which diagnostics tests do you perform in patients with active IBD and with a suspicion of CMV-colitis?									
CMV serology	8 (22%)	10 (27%)	0.787	4 (16%)	14 (29%)	0.263	14 (23%)	4 (36%)	0.447
PCR CMV blood	22 (61%)	22 (60%)	1.00	13 (52%)	31 (65%)	0.324	**41 (66%)**	**3 (27%)**	**0.021**
PCR CMV colonic biopsies	**18 (50%)**	**9 (24%)**	**0.030**	11 (44%)	20 (42%)	1.00	25 (40%)	6 (55%)	0.511
PCR CMV feces	1 (3%)	2 (5%)	1.00	0	3 (6%)	0.547	2 (3%)	1 (9%)	0.392
Colonic biopsies reviewed by a pathologist	29 (81%)	35 (95%)	0.085	24 (96%)	40 (83%)	0.152	54 (87%)	10 (91%)	1
When do you start treatment?									
Positive CMV PCR blood	17 (47%)	15 (41%)	0.640	10 (40%)	22 (46%)	0.804	**32 (52%)**	**0**	**0.002**
Positive CMV PCR colonic biopsies	12 (33%)	6 (16%)	0.109	4 (16%)	14 (29%)	0.263	14 (23%)	4 (36%)	0.447
Positive CMV PCR feces	1 (3%)	0	0.493	0	1 (2%)	1.00	0	1 (9%)	0.151
Positive colonic biopsies reviewed by a pathologist	24 (67%)	30 (81%)	0.190	19 (76%)	35 (73%)	1.00	44 (71%)	10 (91%)	0.268

Significant differences are presented in bold. Chi-square tests or Fisher Exact tests were used depending on numbers. Statistical significance was defined as $p \leq 0.05$ and all tests were two-sided.



PART II

Human papillomavirus and cervical neoplasia



CHAPTER 4

Increased risk of high-grade cervical neoplasia in women with inflammatory bowel disease: a case-controlled cohort study

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Abstract

Background and aims: Women with inflammatory bowel disease (IBD) may be at higher risk for cervical intraepithelial neoplasia (CIN). However, data are conflicting. The aim of this study is to assess the risk of high-grade dysplasia and cancer (CIN2+) in IBD women and identify risk factors.

Methods: Clinical data from adult IBD women in a multicentre Dutch IBD prospective cohort (PSI) from 2007 onwards were linked to cervical cytology and histology records from the Dutch nationwide cytology and pathology database (PALGA) from 2000 to 2016. Patients were frequency matched 1:4 to a general population cohort. Standardized detection rates (SDR) were calculated for CIN2+. Longitudinal data were assessed to calculate CIN2+ risk during follow-up using incidence rate ratios (IRR) and risk factors were identified in multivariable analysis.

Results: Cervical records were available from 2,098 IBD women (77%) and 8,379 in the matched cohort; median follow-up 13 years. CIN2+ detection rate was higher in the IBD cohort than in the matched cohort (SDR 1.27, 95%CI 1.05-1.52). Women with IBD had an increased risk of CIN2+ (IRR 1.66, 95%CI 1.21-2.25), and persistent or recurrent CIN during follow-up (OR 1.89, 95%CI 1.06-3.38). Risk factors for CIN2+ in IBD women were smoking and disease location (ileocolonic (L3) or upper-GI (L4)). CIN2+ risk was not associated with exposure to immunosuppressants.

Conclusion: Women with IBD are at increased risk for CIN2+ lesions. These results underline the importance of HPV vaccination and adherence to cervical cancer screening guidelines in IBD women, regardless of exposure to immunosuppressants.

Introduction

IBD is a chronic inflammatory disease characterized by an exaggerated and self-sustained immune response in the gut and extra-intestinal tissues. Over the past decades, immunomodulators and biological agents have become available widely for the treatment of Crohn's disease (CD) and ulcerative colitis (UC).[1,2] Due to their chronic inflammatory state and frequent use of immunosuppressive medication, patients with IBD are generally considered as at risk of immunocompromise.

Cervical cancer is the fourth most common type of cancer in women worldwide and virtually all cancers result from a persistent infection with high-risk types of the human papillomavirus (hrHPV). The development of cancer from a persistent hrHPV-infection follows a stepwise progression via two stages of squamous intraepithelial lesions (low and high SIL) equivalent to the histologic diagnosis of cervical intraepithelial neoplasia (CIN) 1 and CIN 2/3, respectively.[3-5] In immunocompromised women, impaired detection of oncogenic signals or decreased immunosurveillance might accelerate the progression of CIN to invasive cancer.[6] The risk of cervical neoplasia and cancer in women with IBD has been studied previously, however, results are conflicting. Some studies reported an increased incidence of cervical abnormalities,[7-11] whereas others did not find a significantly higher incidence amongst women with IBD.[12-15] These studies use different outcomes; solely cervical cytology results, or cervical dysplasia or cancer risk and both population-based and single centre IBD cohorts were studied. In addition, most of these cohorts lack details on longitudinal follow up and detailed information on screening behaviour, urbanization, education level and IBD disease characteristics such as Montreal classification. The current European Crohn's and colitis organization (ECCO) guideline recommends an intensified screening approach in immunocompromised IBD women[16] and American guidelines recommend intensified screening only in IBD women using immunosuppressive medication.[17,18] However, these recommendations are based on low level of evidence.[18]

The aim of this study was to assess the detection rate and risk of CIN and cervical cancer in women with IBD as compared to the general Dutch female population and to assess the influence of IBD disease characteristics and exposure to immunosuppressive medication. A secondary aim of this study was to assess screening behaviour and adherence to the cervical cancer screening program for women with IBD.

Materials and methods

Data collection

A multicentre cohort study was performed within the Dutch nationwide IBD biobank registry named Parelsnoer Institute (PSI). PSI started in 2007 as a collaborative project of the eight University Medical Centres in the Netherlands, and comprises clinical data that are collected with a standardized information model and biomaterial.[19] The following data from all women in PSI were collected: year of birth, IBD-type, age at time of diagnosis, Montreal classification[20]; for CD location (L) and behaviour (B); for UC extension (E), smoking status, education level and exposure to immunosuppressive medication (immunomodulators and biologicals). Clinical data from all female IBD patients in the PSI cohort were linked to data on cervical cytology and histology in the Dutch nationwide network and registry of histology and cytopathology (PALGA).[21] In PALGA, individuals are identified by a code comprised from birth date and the first eight letters of the surname. This code was used to link the PSI and PALGA databases. All cervical records between January 2000 and December 2016 were retrieved from the PALGA database, including indication for cytological assessment, i.e. within the national screening program or by other indications. Each woman with IBD from the PSI cohort was randomly frequency matched by age and year of first available cervical record in PALGA to four women from the general population. To correct for the higher prevalence of cervical lesions in women living in urbanized areas,[22] the four-digit postal code from each woman was used to identify women living in low (<100,000 inhabitants) and high (>100,000 inhabitants) level urbanization areas. After matching, women without cytological or histological result (i.e. hrHPV test only) within the study period were excluded (Supplementary Figure 1).

Definitions and follow-up according to population cervical cancer screening

CIN and cervical cancer were coded according to the systemized nomenclature of medicine (SNOMED).[23] CIN1 was defined as mild dysplasia, CIN2 as moderate dysplasia, CIN3 as severe dysplasia or carcinoma in situ, and cervical cancer as invasive cervical squamous cell carcinoma or non-clear cell adenocarcinoma. CIN2+ was defined as the combination of CIN2, CIN3 and cervical cancer. Since only histological diagnoses were included as an endpoint in this study, the historical CIN classification was used instead of the two-tiered Bethesda classification for cytological screening.[24]

The number of screening episodes in a 5-year period was calculated as a proxy of screening behaviour. A screening episode started with a primary test and if abnormal or inconclusive, this primary test was followed by a secondary test. An episode ended after 4 years following the primary test when no (adequate) follow-up test had been performed, or when follow-up had been completed according to the Dutch cervical cancer screening program.[25] Thus, by definition, post-diagnostic follow-up smears were attributed to the same episode as the diagnosed lesion. Screening behaviour was measured for each woman by dividing the number of screening episodes by the number of 5-year follow-up periods (1: 0-5 years, 2: 5-10 years, 3: 10-15 years, 4: > 15 years) during follow-up.

Statistical analysis

Standardized detection ratios

The primary outcome was CIN2+ detection rate, defined as the percentage of episodes resulting in a histological diagnosis of CIN2+. Standardized detection ratios (SDRs) were calculated by correcting the observed detection rates from the IBD cohort by the expected detection rates based on 5-year age categories, 5-year time periods and urbanization level. The expected detection rates were the calculated detection rates in the matched cohort. A two-tailed P-value <0.05 was considered statistically significant and 95% confidence intervals (CI) were calculated assuming a Poisson distribution.

Incidence rate ratios during follow-up

Follow-up for each woman started on the first available cervical record in the PALGA database (index date) and ended at 31st December 2016. Women were censored after the occurrence of the highest grade of cervical neoplasia during follow-up or end of follow-up. Incidence rates (IR) per 100,000 person-years were calculated for both the IBD cohort and the matched cohort and incidence rate ratios (IRR) were computed. A sensitivity analysis was performed after exclusion of women with cervical neoplasia at the first screen within the study period. Kaplan Meier survival analyses were performed for the risk of CIN1 and CIN2+ diagnoses and statistical differences were calculated with a log-rank test. The effect of age on CIN2+ detection was visualised using attained age as time metric on the x-axis in a secondary analysis. Attained age was defined as the age at diagnosis of first occurrence of the highest CIN diagnosis during follow-up or age at end of follow-up. Cox proportional hazards regression analysis was performed to calculate hazard ratios (HRs) in order to quantify the effect of IBD on the risk of CIN2+ in the IBD cohort, adjusting for urbanization and screening behaviour.

Persistent or recurrent CIN lesions

Patients with persistent or recurrent CIN or CIN2+ lesions were identified by detection of two histologically confirmed CIN or CIN2+ lesions respectively, with a time interval of at least 18 months, since the majority of transient and productive hrHPV infections and low grade abnormal smears regress spontaneously within this time frame.[5] Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated.

Risk factors

Univariable and multivariable logistic regression models were performed to identify risk factors for CIN2+ within the IBD cohort. Smoking was divided in current smoking and never or former smoking if patients withdrew within 6 months before inclusion in PSI. High education level was defined as having a college or university degree. Exposure to immunosuppressive medication was defined as at least one data entry of an immunomodulator (thiopurines, methotrexate) or a biologic agent (anti-TNFα, vedolizumab, ustekinumab) in PSI. Exposure was further subdivided in less or more than one year exposure. Risk factors with a significance level of <0.20 in univariable analyses were taken into account in the multivariable analysis.

Coverage for cervical testing

All women living in the Netherlands receive an invitation to participate in the national cervical cancer screening program every five years between ages 30 and 60 years.[26] Adherence to the national cervical cancer screening program was defined as the proportion of women with at least one primary cytology test performed within the program. Five year coverage rate for cervical smear testing was defined as the percentage of women within the screening age group that had at least one cervical test in the five years before the reference date, either within the organized screening program or outside of the programme (i.e. by indication). For 5-year coverage rates, periods of five consecutive years were analyzed. For example: the coverage rate of 2016 is based on tests performed in the 2012-2016 period for women born between 1952 and 1986. Our results were compared with data from the nationwide monitoring of the National cervical cancer screening program in 2016 (for the year 2010) and 2017 (for years 2011-2016)[25] These coverage rates are calculated using the number of total women in the Dutch population aged 30 to 64 years adjusted for the risk of hysterectomy as denominator from Statistics Netherlands (CBS) and a proxy of the number of screens available in each 5-year period from PALGA as numerator for each

year.[25] These data were compared with the coverage rates in the IBD cohort for significant differences using two-tailed chi-squared tests and P-value <0.05 was considered statistically significant.

Ethical Approval

All patients in the PSI-IBD dataset provided written informed consent. The scientific boards of the Dutch IBD biobank and PALGA approved the study. The ethics committees of all eight participating UMCs granted permission to link study objects from the PSI cohort to their own cervical records collected in PALGA under strict privacy procedures. Consent by women for the use of their data stored in PALGA is implicit according to the Dutch Ethical Code of reuse of data and PALGA's own privacy policy.

Results

Study population

A total of 2,098 IBD women (median age at inclusion 42 years) were included. The matched cohort comprised 8,392 women. Median follow-up was 13 years in both cohorts (range 0-16 years). The IBD cohort comprised 1,382 (66%) patients with CD and 716 (34%) patients with UC, IBD-unclassified (IBD-U) or IBD-indeterminate (IBD-I). Within the IBD cohort, 554 (26.4%) women were smokers and 461 (34.6%) had a high education level. A total of 1,030 (49%) patients were exposed to immunomodulators and 707 (34%) to biologic agents (Table 1). CD patients were more often smokers (33.8% vs 15.0%, P: < 0.001) and were more often exposed to immunosuppresssants (immunomodulators 53.0 % vs 41.7%, biologicals 42.2% vs 16.9%, P: <0.001) than UC patients (Supplementary Table 1). The vast majority of patients exposed to biologics had been exposed to anti-TNFα agents. Seven patients (1%) had been only exposed to other biologics (vedolizumab, ustekinumab). Number of screening episodes in a 5 year period was significantly higher in the IBD cohort than in the matched cohort, 30% in the IBD cohort had more than one screening episode in a 5 year period, compared to 20.9% in the matched cohort (P: <0.001) (Table 1).

Table 1. Patient demographics from PSI for IBD women and screening behaviour for IBD and matched women

		IBD women N (%)
Total number of women		**2,098**
Diagnosis	CD	1,382 (66)
	UC, IBD-U or IBD-I	716 (34)
Age at IBD diagnosis	<25 years	**772 (37)**
	≥25 years	**1,321 (63)**
	N/A	**5 (0)**
Smoking status[a]	Never/>6 months	1,466 (70)
	Current/<6 months	554 (26)
	N/A	78 (4)
Education level[b]	Low	1,352 (64)
	High	700 (33)
	N/A	46 (2)
Medication exposure[c]		
Immunomodulator	No	1068 (51)
	< 1 year	237 (11)
	> 1 year	793 (38)
Biologicals	No	1391 (66)
	< 1 year	227 (11)
	> 1 year	480 (23)
Crohn's disease		
Montreal L	L1	256 (19)
	L2	277 (20)
	L3	530 (38)
	L4 or L1-3 + L4	155 (11)
	N/A	164 (12)
Montreal B	B1	495 (36)
	B2	191 (14)
	B3	192 (14)
	B1-3 + p	347 (25)
	N/A	157 (11)
Ulcerative colitis		
Montreal E	E1	56 (8)
	E2	238 (33)
	E3	346 (48)
	N/A	76 (11)

Table 1. Continued

		IBD women N (%)	Matched women N (%)	P value
Total number of women		**2,098**	**8,379**	
Total number of screening episodes		**6,654**	**23,344**	
Number of screening episodes per woman in a 5 year period				
	1	1,451 (69)	6,595 (79)	<0.001
	>1	567 (27)	1,646 (20)	
	>2	80 (4)	138 (1)	
Urbanization level	**>100,000**	632 (30)	2516 (30)	0.931
	<100,000	1466 (70)	5863 (70)	

[a]Smoking was defined as current smoker or former smokers who quitted within 6 months prior to inclusion in PSI. [b]High education level was defined as having a college or university degree. [c]Exposure to medication use was defined as at least one data entry of an immunomodulator (thiopurines, methotrexate) or a biological (anti-TNFα, vedolizumab) in the database. Abbreviations: IBD: inflammatory bowel disease, PSI: Parelsnoer Institute, N: number; IQR interquartile range, CD: Crohn's disease, UC: ulcerative colitis, IBD-U: IBD-unclassified, IBD-I: IBD-indeterminate, N/A: not available, L: location, B: behaviour, E: extent.

Standardized detection rates

Over the whole study period, significantly more CIN2+ lesions were detected in the IBD cohort compared to the matched cohort (SDR 1.27, 95% CI 1.05-1.52). This difference was mainly due to more CIN2+ lesions in the 35 to 39 years of age group (Table 2). No differences were observed in detection rates of CIN1 lesions (SDR 0.95, 95% CI 0.68-1.37) CIN3 lesions (SDR 1.21, 95% CI 0.94-1.55) or cervical cancer (SDR 0.30, 95% CI 0.03-1.08) (Table 2, Supplementary Table 2). Significant more CIN2+ lesions were detected in the 2006-2010 time period. Urbanization was not a strong influencing factor for detecting CIN2+ (Table 2).

Incidence rate of CIN2+ during longitudinal follow-up

The risk of progression of a normal smear towards CIN2+ was higher in IBD women than in women from the matched cohort. After exclusion of women with an abnormal smear at first available cytopathology record, during the total of 24,159 person years, 109 IBD women were diagnosed with CIN2+, versus 320 matched women during 97,163 person years. The risk of developing a CIN2+ lesion was significantly higher in the IBD cohort; incidence rate ratio (IRR) for CIN2+ for IBD women was 1.66 (95% CI 1.21-2.25) compared to the matched cohort. This was due to an increased risk of CIN2 (IRR 1.83, 95% CI 1.15-2.91) and CIN3 (IRR 1.56, 95% CI 1.01-2.41), not cervical cancer (IRR 1.14, 95% CI 0.16-5.13). No difference was observed in women developing CIN1 as highest grade of cervical neoplasia (IRR 0.95, 95% CI 0.57-1.60) (Table 3, Figure 1A-B). The cumulative incidence for CIN2+

as highest grade of cervical neoplasia during follow-up increased with age (Figure 1C). Including women with prevalent lesions at the first available cytopathology record resulted in a lower IRRs but still a significantly higher CIN2+ risk for IBD women (IRR 1.37, 95%CI 1.10-1.70) (Supplementary Table 3, Supplementary Figure 2A-C). After correcting for screening behaviour and urbanization in a Cox proportional hazards model, CIN2+ risk in IBD women was also increased (HR 1.46, 95% CI 1.07-2.00) (Table 4).

Table 2. Standardized detection ratios of cervical intraepithelial lesions and cervical cancer for IBD women by age, time period and urbanization, follow-up period 2000-2016 as compared to matched cohort

		CIN1[a]				CIN2+[a]			
	No. prim. tests[b]	Obs[b]	Exp[b]	SDR[b]	95% CI[c]	Obs[b]	Exp[b]	SDR[b]	95% CI[c]
Overall detection rate[b]	6,654	35	35.6	0.98	0.68-1.37	**118**	**93.2**	**1.27**	**1.05-1.52**
Screening age									
<29	348	7	7.3	0.96	0.38-1.98	12	16.7	0.72	0.37-1.26
29-34	1,457	11	6.4	1.72	0.86-3.08	40	35.0	1.14	0.82-1.56
35-39	1,068	3	7.1	0.42	0.09-1.24	23	12.8	**1.80**	**1.14-2.70**
40-44	1,136	9	6.0	1.50	0.68-2.85	17	10.2	1.67	0.97-2.67
45-49	1,060	2	4.4	0.45	0.05-1.64	14	8.5	1.65	0.90-2.76
50-54	706	0	2.0			6	4.2	1.42	0.52-3.11
55-59	594	3	1.7	1.77	0.36-5.16	5	2.4	2.08	0.68-4.86
≥60	285	0	0			1	0.9	1.11	0.03-6.19
Total	6,654	35	35.0	1.00	0.70-1.39	118	90.7	**1.30**	**1.08-1.56**
Time period									
2000-2005	2,157	5	9.3	0.54	0.17-1.26	31	25.5	1.22	0.83-1.73
2006-2010	2,006	15	11.1	1.35	0.76-2.23	38	25.7	**1.48**	**1.05-2.03**
2011-2016	2,491	15	15.4	0.97	0.54-1.61	49	37.1	1.32	0.98-1.75
Total	6,654	35	35.8	0.98	0.68-1.36	118	89.5	**1.26**	**1.04-1.51**
Urbanization									
High level	1,962	9	13.3	0.68	0.31-1.29	43	33.4	1.29	0.93-1.73
Low level	4,692	26	22.4	1.16	0.76-1.70	75	61.0	1.23	0.97-1.54
Total	6,654	35	35.8	0.98	0.68-1.36	118	94.4	**1.25**	**1.04-1.50**

[a]CIN: Cervical intraepithelial neoplasia; CIN1: mild dysplasia; CIN2: moderate dysplasia; CIN3: severe dysplasia or carcinoma in situ; cervical cancer: invasive cervical squamous cell carcinoma and non-clear cell adenocarcinoma; CIN2+: CIN2 or higher grade of neoplasiabNo. of prim tests: number of primary screening tests; Detection rate is the percentage of episodes starting with a primary cytology or histology screen test resulting in a histological diagnosis of CIN or cervical cancer. Obs.: detection rate in the IBD cohort. Exp.: detection rate in the age and year of screening matched cohort. SDR Standardized detection ratio: defined as observed detection rate in IBD cohort compared to the expected detection rate. c95% CI: 95% confidence interval based on a Poisson distribution. Bold numbers: statistically different.

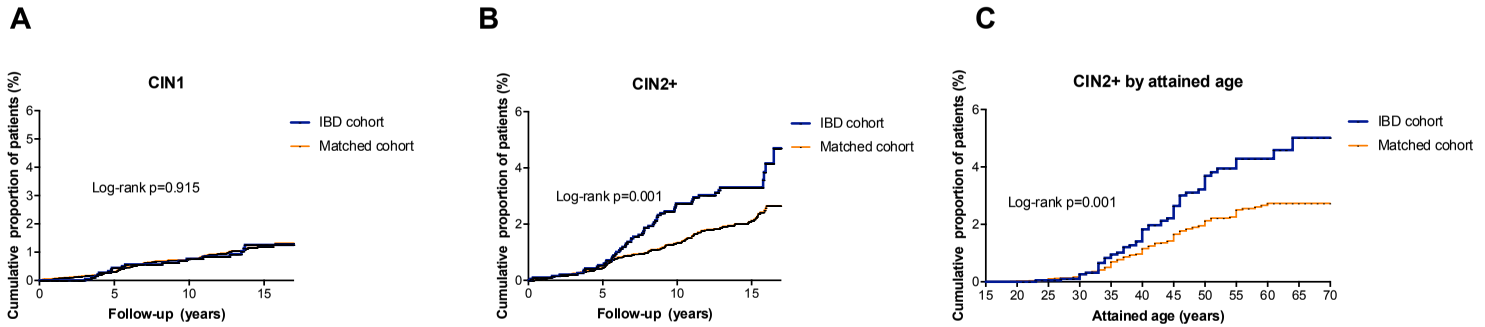

Figure 1 A-C. Kaplan Meier estimates for CIN1 and CIN2+ lesions as worst diagnosis for the IBD cohort and matched cohort by years of follow-up and attained age excluding women with a primary abnormal screen.
A: Proportion of women with CIN1 as highest grade of dysplasia during follow-up. B: Proportion of women with CIN2+ as highest grade of dysplasia during follow-up. C: Proportion of women with CIN2+ as highest grade of dysplasia by attained age. *Attained age is defined as the age at diagnosis of CIN2+ or age at end of follow-up.*

Abbreviations: CIN = cervical intraepithelial neoplasia. CIN2+ = CIN2, CIN3 or cervical cancer. IBD = inflammatory bowel disease.

Table 3. Observed number of CIN and cervical cancer cases, person-years, incidence rates per 1,000 person-years and incidence rate ratios for women with IBD compared to matched women from general population excluding women with an abnormal primary screen

	Person-years	Obs-No	IR (95% CI)	IRR (95% CI)
CIN1				
IBD women	23,726	18	0.76 (0.45-1.20)	0.95 (0.57-1.60)
Matched women	92,956	74	0.80 (0.63-1.01)	
CIN2				
IBD women	23,235	26	1.12 (0.73-1.64)	**1.83 (1.15-2.91)**
Matched women	93,167	57	0.61 (0.46-0.79)	
CIN3				
IBD women	23,228	28	1.21 (0.80-1.74)	**1.56 (1.01-2.41)**
Matched women	93,030	72	0.77 (0.61-0.97)	
Cervical cancer				
IBD women	23,383	2	0.09 (0.01-0.28)	1.14 (0.16-5.13)
Matched women	93,381	7	0.07 (0.03-0.15)	
CIN2+				
IBD women	23,070	56	2.43 (1.83-3.15)	**1.66 (1.21-2.25)**
Matched women	92,726	136	1.47 (1.23-1.74)	

Abbreviations: CI: confidence interval; CIN, cervical intraepithelial neoplasia; CIN2+, CIN2, 3 or cervical cancer; IBD: inflammatory bowel disease. No. number; IR incidence rate; IRR incidence rate ratio.

Table 4. Univariable and multivariable Hazard ratios for different risk factors for CIN2+ over time in the study population excluding women with a primary abnormal screen

	CIN2+			
	Univariable		Multivariable	
	HR	95%CI	HR	95%CI
Case				
No IBD	1.00	Ref	1.00	Ref
IBD	1.66	1.21-2.26	1.46	1.07-2.00
Urbanization				
Low level	1.00	Ref	1.00	Ref
High level	1.08	0.79-1.47	1.11	0.81-1.51
Screening episodes in a 5 year period				
1 episode	1.00	Ref	1.00	Ref
1-2 episodes	1.74	1.27-2.38	1.68	1.23-2.30
>2 episodes	5.84	3.55-9.60	5.39	3.26-8.92

Abbreviations: CIN: Cervical intraepithelial neoplasia. CIN2+: CIN2, CIN 3 or cervical cancer; IBD: inflammatory bowel disease. HR: Hazard ratio, CI: confidence interval.

Persistent or recurrent CIN lesions

In the IBD cohort an increased risk of persistent or recurrent CIN lesions was observed. A total of 17 (0.8%) IBD women had persistent CIN lesions during follow-up, compared to 36 (0.4%) in the matched cohort (OR 1.89, 95% CI 1.06-3.38, p 0.028). A total of 11 (0.5%) IBD women had persistent CIN2+ lesions during follow-up, compared to 15 (0.2%) in the matched cohort (OR 2.94, 95% CI 1.08-6.1, p 0.004).

Risk factors for CIN2+ in the IBD cohort

In multivariable analysis, CIN2+ risk was associated with ileocolonic (L3) and/or upper-GI (L4) location in women with CD (adjusted OR 1.84, 95% CI 1.05-3.24), smoking (adjusted OR 3.20, 95% CI 1.90-5.40) and more than one or two screening episodes within a five-year period (adjusted OR 2.00, 95% CI 1.16-3.44 and 5.02, 95% CI 1.89-13.35) respectively). Exposure to immunomodulators or biologic agents was not associated with CIN2+ risk (Table 5).

Table 5. Univariable and multivariable Odds ratios for different risk factors for a CIN2+ diagnosis in 2000-2016 for women with IBD

IBD cohort	CIN2+		CIN2+	
	Univariable		Multivariable	
	OR	95%CI	OR	95%CI
Screening episodes in a 5 year period				
1 episode	1.00	Ref	1.00	Ref
>1 episode	**1.64**	**1.08-2.48**	**2.00**	**1.16-3.44**
>2 episodes	**3.26**	**1.60-6.62**	**5.02**	**1.89-13.35**
Urbanization				
Low level	1.00	Ref	1.00	
High level	**1.43**	**0.96-2.13**	1.41	0.81-2.44
Disease type				
UC	1.00	Ref	1.00	1.00
CD	**1.39**	**0.90-2.13**	0.96	0.61-1.53
Age at diagnosis				
≥25 years	1.00	Ref	1.00	Ref
<25 years	**1.60**	**1.09-2.36**	1.54	0.91-2.59
CD behaviour				
B1	1.00	Ref		
B2, B3 or all p	0.79	0.49-1.26		
CD location				
L1 or L2	1.00	Ref	1.00	Ref
L3 or all L4	**1.92**	**1.14-3.24**	**1.84**	**1.05-3.24**
UC extent				
E1 or E2	1.00	Ref		
E3	0.67	0.31-1.45		
Education level				
Low	1.00	Ref	1.00	Ref
High	**0.77**	**0.51-1.15**	0.63	0.37-1.09
Smoking status				
No	1.00	Ref	1.00	Ref
Yes	**2.59**	**1.74-3.86**	**3.20**	**1.90-5.40**
Exposure to immunomodulators				
No	1.00	Ref	1.00	Ref
< 1 year	**0.42**	**0.18-0.99**	0.37	0.13-1.09
> 1 year	0.89	0.59-1.33	0.91	0.54-1.55
Exposure to biologicals				
No	1.00	Ref		
< 1 year	0.72	0.36-1.47		
> 1 year	0.96	0.61-1.54		

CIN: Cervical intraepithelial neoplasia. CIN2+: CIN2 or higher grade of neoplasia; IBD: inflammatory bowel disease. OR: Odds ratio, CI: confidence interval; CD: Crohn's disease; B: behaviour, L: location; UC: ulcerative colitis, E: extent

Coverage for cervical testing

IBD women participated significantly less often in the national cervical cancer screening program than women from the general population in 2010 and from 2012 to 2016 (Table 6). Cervical screening outside the national program was significantly more often performed in the IBD cohort than in the general population from 2011 to 2016 (Table 6). In 2012, the five-year coverage rate for total cervical screen testing was significantly higher in the IBD cohort than in the general population (82.7% vs. 77.3%, p <0.001), but declined to lower rates after that year.) The observed decline is most importantly explained by a decline in the number of IBD patients tested by indication (outside the national screening program), which declined from 16.8% in the period from 2008 to 2012 to 9.7% from 2012 to 2016. In addition, the adherence rate of IBD patients to the screening program declined slightly over the years from 2010 to 2016 (66.6% to 64.5%), a trend similar to the general population (69.6% to 67.4%).

Table 6. Five year coverage rate of cervical smear testing from 2010 to 2016 in percentages for IBD women compared to women from general population*

	Total cervical screen testing			National cervical cancer screening program			Screens on indication (outside screening program)[a]		
	IBD	General population*	**p-value**[b]	**IBD**	General population*	**p-value**[b]	**IBD**	General population*	**p-value**[b]
2010	76.7%	79,0%	**0.015**	66.6%	69,6%	**0.005**	10.1%	9,4%	0.312
2011	77.5%	77,8%	0.747	66.7%	68,4%	0.118	10.8%	9,4%	**0.042**
2012	82.7%	77,3%	**<0.001**	65.9%	67,9%	**0.056**	16.8%	9,4%	**<0.001**
2013	75.2%	77,2%	**0.043**	63.8%	67,9%	**<0.001**	10.4%	9,2%	**<0.001**
2014	76.7%	76,7%	0.965	65.4%	67,7%	**0.029**	11.3%	8,9%	**<0.001**
2015	74.8%	76,3%	0.122	64.3%	67,7%	**0.001**	10.5%	8,6%	**0.002**
2016	74.2%	75,9%	0.496	64.5%	67,4%	**0.005**	9.7%	8,4%	**0.035**

Abbreviations: IBD: inflammatory bowel disease. aOpportunistic, indicative or secondary tests only. bChi-squared tests were used to test for significant differences and two-tailed P-value <0.05 was considered statistically significant. *The coverage rates in Monitors 2016 (for year 2010) and 2017 (for years 2011 to 2016) are calculated using a denominator that is calculated with the following data: All women aged 30 to 64 years in the Dutch population, as reported by CBS on 1 January of each year. The year corresponds with the year at the end of the 5-year coverage period. The population is adjusted per five-year age group for the risk of hysterectomy.

Discussion

Results from our case-controlled cohort study show a higher detection rate of CIN2+ lesions in IBD women than in matched women from the general population. According to current guidelines, these lesions require treatment in most cases.[27] The difference in CIN2+ detection rate was highest in IBD women between the age of 35 and 39 years. The detection rate of cervical cancer was not significantly different between the two groups, probably due to the sample size. Even after correcting for their screening behaviour, IBD women were still at increased risk of CIN2 and CIN3 lesions during follow-up. Also, after excluding all women with prevalent CIN lesions at the first screen, the risk for CIN2+ remained increased. Risk factors associated with CIN2+ in IBD women were smoking and ileocolonic (L3) and/or upper-GI (L4) location. Exposure to immunosuppressive medication was not identified as a risk factor.

Our study supports previous observations that IBD women are at increased risk of high-grade CIN.[7-11] In addition to previous data, we have shown that during longitudinal follow-up, women with IBD show a higher rate of progression from normal smears to CIN2+ and more often have persistent or recurrent CIN lesions than women in the general population. A higher rate of persistence of an hrHPV infection might explain for both findings. Transient and productive HrHPV infections and cytological low-grade abnormal smears, histologically mostly classified as CIN1, are highly prevalent and known to clear or regress spontaneously in many patients, especially in young women.[5,27] However, as opposed to transient or productive hrHPV infections, it is persistent or transforming infections that are essential in carcinogenesis.[5,28,29]

In our IBD cohort, ileocolonic (L3) or upper-GI (L4) location in women with Crohn's disease and smoking were risk factors for CIN2+ in multivariable analysis, whereas exposure to immunosuppressants was not associated with CIN2+. Onset of IBD before the age of 25 was a risk factor in univariable analysis only. Although younger age at IBD onset has already been identified as a risk factor[9], increased risk by disease location in Crohn's disease is a novel finding. Both young age at IBD onset and L3 and/or L4 disease location may be associated with a severe disease expression which might increase risk for CIN lesions since chronic systemic inflammation can impair innate and adaptive cellular immune responses and may therefore result in a decreased clearance of hrHPV.[30] Studies on immunosuppressive medication as a risk factor for CIN and cervical cancer in IBD patients display discordant results. Some studies have previously found a significant association,[8-11,15,31] while others have not.[7,13,14] In our study exposure

to immunomodulators and biologics was solely studied as: no exposure, less than 1 year or more than 1 year. It would have been interesting to study the relation between timing of exposure to immunosuppressive medication and occurrence of CIN. Unfortunately, data on immunosuppressive medication was heterogeneously collected and data collected for the scope of this study did not allow to look into this in more detail. Further studies are needed to scrutinize the exact role for immunosuppressive medication in cervical neoplasia risk, split on duration of exposure, age of start, combination therapy and use of corticosteroids. Smoking was strongly associated with CIN2+ in our IBD cohort. This is consistent with previous findings, both in the general population[32,33] and amongst women with IBD.[8,14] In our IBD cohort the risk of CIN2+ in active smokers was higher than the estimated 2-fold risk of CIN2+ in ever smokers in the general population,[33-35] suggesting a combined effect of IBD and exposure to cigarette smoke.

IBD women had a higher screening frequency than women from the general population, as shown by the number of screening episodes within a five-year period. This might be explained by the fact that IBD women are referred to a gynecologist more often or are more aware of the increased risk and request intensified screening. This more frequent screening behaviour could easily have influenced the incidence rate of CIN2+ in our study population. Undeniably, an increased number of cervical smears per individual increases the chance of detecting abnormalities. However, the hazard ratio for acquiring CIN2+ was still higher in the IBD cohort than in the matched cohort after correcting for this important confounder in multivariable analysis.

This is one of the few studies reporting on screening behaviour and adherence to a national cervical cancer screening program amongst IBD patients.[13,14,36,37] Current ECCO guidelines advice to improve the rate of adherence in IBD women, based on a study by Long et al showing a suboptimal rate of cervical smear testing in IBD patients.[16,36] Our study underlines this advice, especially since we observed a decline in screening rate over the past years, due to less frequent testing both within and outside of the national screening program.

Prevention of cervical neoplasia requires two important interventions. First, vaccination for HPV in all females up to 26 years of age, preferably before sexual activity, is recommended for all women as primary prevention strategy.[16] Normal immunogenic response to HPV vaccination has been reported in patients on immunosuppressive medication.[38] HPV vaccination was only introduced in the Netherlands in 2008 for girls turning 13 years. Since this vaccinated

population has not reached the screening age of 30 years during the study period, reported associations are in all probability unaffected by this vaccination program. Data regarding efficacy in terms of decreasing incidence of cervical dysplasia in immunocompromised individuals are expected in the following years. Given the burden of other HPV-related (penile, oral and anal) cancers in men, vaccination in young males is also highly worth considering.[39,40] Next to that, secondary prevention by means of screening for premalignant cervical lesions within in a national cervical cancer screening program is advised. ECCO recommends for IBD women to follow European guidelines on cervical cancer screening for the general population [16,41] and intensified screening approach for immunocompromised women. American guidelines also suggest intensified screening for IBD women using immunosuppressive medication, but not for all women with IBD.[17,18] This risk stratification is not fully substantiated by our data. A decision on an intensified screening program in IBD women requires careful consideration of burden to patients, costs and benefits. Based on available evidence, we recommend to encourage all IBD women to adhere to national cervical cancer screening programs and increased awareness among physicians is warranted.

Despite the novel longitudinal data presented in this multicentre cohort study, a few limitations of this study warrant consideration. Since our IBD cohort comprises only patients from tertiary referral centres, reflecting a population with more severe disease[42], results of this study might not be completely generalizable to all IBD patients. Also, we did not have data on several other possible confounders such as sexual behaviour and oral contraceptive use.[43] It has been shown that a higher proportion of women with inflammatory bowel disease have sexual dysfunction compared to matched controls.[44] Since sexual activity is a strong risk factor for CIN[32] it might be hypothesized that the association with IBD is even stronger. Unfortunately, we were not able to draw conclusions on hrHPV status, since these data were only collected limitedly. Also, there was not enough power to identify risk factors for persistent or recurrent lesions, in particular exposure to immunosuppressive medication. Furthermore, we were not able to collect data from PALGA before the year 2000. Some women might have had a history of CIN before the index date of our follow-up period which may have put them at higher risk of a subsequent lesion. Lastly, a group of women in the IBD cohort might have had a CIN2+ diagnosis before their IBD diagnosis. We did not exclude these women, based on the fact that IBD is a chronic disease that often starts years before the actual date of diagnosis.

Moreover, since higher rates of cervical neoplasia were detected even to up to 10 years before IBD diagnosis[9], we believe including these women in the cohort was justified.

In conclusion, this study demonstrates that IBD is a risk factor for high-grade cervical neoplasia, especially in women who smoke and have a severe CD phenotype. Close surveillance of low-grade lesions and treatment of high-grade CIN is warranted given that persistent lesions were more prevalent in women with IBD, possibly reflecting a decreased clearance of hrHPV. Vaccination for HPV and adherence to cervical cancer screening programs should be strongly encouraged in all IBD women, regardless of immunosuppressant use.

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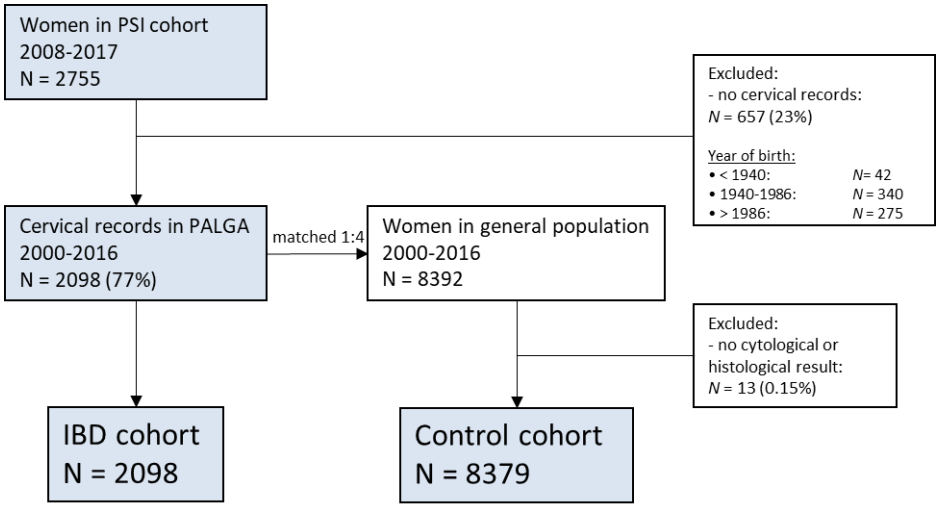
Supplementary figures and tables

Supplementary figure 1. Flowchart of study population



Abbreviations: PSI: Parelsnoer Institute; N: number; PALGA: Dutch nationwide network and registry of histology and cytopathology; IBD: Inflammatory bowel disease;

Supplementary Table 1. Patient demographics of women with Crohn's disease and ulcerative colitis

		CDN (%)	UC, IBD-U/IN (%)	P value
Total number of women		**1382 (65.9)**	**716 (34.1)**	
Age at IBD diagnosis	<25 years	586 (42.4)	186 (26.1)	<0.001^
N/A for 5 (0.2%)	≥25 years	795 (57.6)	526 (73.9)	
Montreal L	L1	256 (21.0)		
N/A for 164 (11.9%)	L2	277 (22.7)		
	L3	530 (43.5)		
	L4	6 (0.5)		
	L1+L4	28 (2.3)		
	L2+L4	30 (2.5)		
	L3+L4	91 (7.5)		
Montreal B	B1	495 (40.4)		
N/A for 157 (11.4%)	B2	191 (15.6)		
	B3	192 (15.7)		
	B1p	123 (10.0)		
	B2p	60 (4.8)		
	B3p	164 (13.4)		
Montreal E	E1		56 (8.8)	
N/A for 76 (10.6%)	E2		238 (37.2)	
	E3		346 (54.1)	
Smoking status	Never	882 (66.2)	584 (85.0)	<0.001^
N/A for 78 (3.7%)	Current/<6 months	451 (33.8)	103 (15.0)	
Education level				
N/A for 46 (2.2%)	Low	911 (65.4)	472 (66.4)	0.777^
	High	461 (34.6)	239 (33.6)	
Medication exposure				
N/A for 33 (1.6%)				
Immunomodulator	No	650 (47.0)	418 (58.4)	
	<1 year	170 (12.3)	67 (9.4)	
	>1 year	562 (40.7)	231 (32.3)	<0.001^
Biological	No	796 (57.6)	595 (83.1)	
	<1 year	174 (12.6)	53 (7.4)	
	>1 year	412 (29.8)	68 (9.5)	<0.001^
Number of screening episodes in a 5 year period				
N/A for 38 (1.8%)	1	956 (69.2)	495 (69.1)	0.413^
	2	375 (27.2)	191 (26.7)	
	>2	50 (3.6)	30 (4.2)	

Abbreviations: IBD: inflammatory bowel disease, PSI: Parelsnoer Institute, N: number; yrs: years; IQR interquartile range, CD: Crohn's disease, UC: ulcerative colitis, IBD-U: IBD-unclassified, IBD-I: IBD-indeterminate, N/A: not applicable, L: location, B: behavior, E: extent, 5-ASA, 5-aminosalicylic acid * Mann-Whitney U test ^ Chi square test ** independent samples T-test

Supplementary Table 2. Standardized detection ratios of CIN2, CIN3 and cervical cancer for IBD women by age, time period and urbanization, follow-up period 2000-2016 as compared to matched cohort

		CIN2[a]				CIN3[a]				Cervical cancer[a]			
	No. prim. tests[b]	Obs[b]	Exp[b]	SDR[b]	95% CI[c]	Obs[b]	Exp[b]	SDR[b]	95% CI[c]	Obs [b]	Exp[b]	SDR[b]	95% CI[c]
Overall detection rate[b]	**6,654**	52	34	**1.53**	**1.14-2.01**	64	52.7	1.21	0.94-1.55	2	6.7	0.30	0.03-1.08
Screening age													
<29	348	7	8.3	0.84	0.34-1.74	5	8.0	0.63	0.20-1.46	0	0.3		
29-34	1,457	15	11.7	1.28	0.71-2.12	25	21.7	1.15	0.75-1.70	0	1.5		
35-39	1,068	11	4.2	**2.62**	**1.31-4.69**	11	7.9	1.39	0.69-2.49	1	1.1	0.91	0.01-5.06
40-44	1,136	10	4.3	**2.33**	**1.11-4.28**	7	5.7	1.23	0.49-2.53	0	0		
45-49	1,060	5	2.4	2.08	0.67-4.86	8	4.2	1.91	0.82-3.75	1	1.1	0.91	0.01-5.06
50-54	706	1	1.5	0.67	0.02-3.72	5	2.1	1.91	0.51-4.88	0	0.7		
55-59	594	3	0.9	3.33	0.67-9.74	2	1.2	1.67	0.20-6.02	0	0.6		
≥60	285	0	0			1	0.6	1.67	0.02-9.27	0	0.3		
Total	6,654	52	33	**1.57**	**1.17-2.05**	64	51.4	1.25	0.96-1.59	2	5.5	0.36	0.04-1.31
Time period													
2000-2005	2,157	12	7.9	1.52	0.78-2.65	19	17.3	1.10	0.66-1.72	0	2.2		
2006-2010	2,006	19	8.7	**2.18**	**1.31-3.41**	19	16.0	1.19	0.71-1.85	0	2.0		
2011-2016	2,491	21	17.7	1.19	0.73-1.81	26	17.4	1.49	0.98-2.19	2	2.5	0.80	0.10-2.89
Total	6,654	52	34.3	**1.51**	**1.13-1.98**	64	50.7	1.26	0.97-1.61	2	6.6	0.30	0.03-1.10
Urbanization													
High level	1,962	17	15.4	1.10	0.64-1.77	24	17.2	1.40	0.89-2.08	2	0.6		
Low level	4,692	35	23.0	**1.52**	**1.06-2.12**	40	31.7	1.26	0.90-1.72	0	4.7		
Total	6,654	52	38.4	**1.35**	**1.01-1.78**	64	48.9	**1.31**	**1.01-1.67**	2	5.3	0.38	0.04-1.37

[a] CIN: Cervical intraepithelial neoplasia; CIN1: mild dysplasia; CIN2: moderate dysplasia; CIN3: severe dysplasia or carcinoma in situ; cervical cancer: invasive cervical squamous cell carcinoma and non-clear cell adenocarcinoma; CIN2+: CIN2 or worse [b] No. of prim tests: number of primary screening tests; Detection rate is the percentage of episodes starting with a primary cytology or histology screen test resulting in a histological diagnosis of CIN or cervical cancer. Obs: detection rate in the IBD cohort. Exp: detection rate in the age and year of screening matched cohort. SDR Standardized detection ratio: defined as observed detection rate in IBD cohort compared to the expected detection rate. [c] 95% CI: 95% confidence interval based on a Poisson distribution. Bold numbers: statistically different.

Supplementary Table 3. Observed number of CIN and cervical cancer cases, person-years, incidence rates per 1,000 person-years and incidence rate ratios for women with IBD compared to matched women from general population in total study population

	Person-years	Obs-No	IR (95% CI)	IRR (95% CI)
CIN1				
IBD women	24,737	31	1.25 (0.85-1.80)	1.09 (0.73-1.61)
Matched women	98,730	114	1.16 (0.95-1.39)	
CIN2				
IBD women	24,624	46	1.87 (1.37-2.49)	**1.58 (1.12-2.22)**
Matched women	98,821	117	1.18 (0.98-1.42)	
CIN3				
IBD women	24,482	61	2.49 (1.91-3.20)	**1.34 (1.00-1.78)**
Matched women	98,083	183	1.86 (1.60-2.16)	
Cervical cancer				
IBD women	24,936	2	0.08 (0.01-0.27)	0.40 (0.06-1.47)
Matched women	99,406	20	0.20 (0.13-0.31)	
CIN2+				
IBD women	24,159	109	4.51 (3.71-5.44)	**1.37 (1.10-1.70)**
Matched women	97,163	320	3.29 (2.94-3.68)	

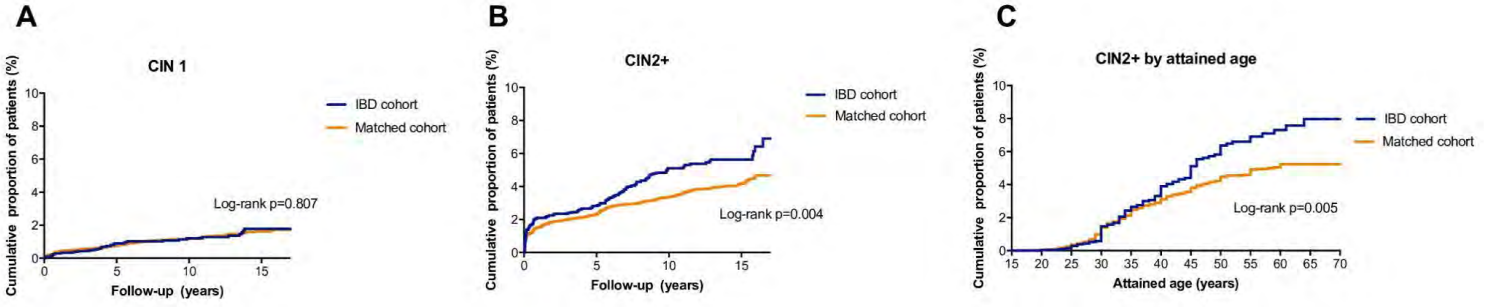
Abbreviations: CI: confidence interval; CIN, cervical intraepithelial neoplasia; CIN2+, CIN2, 3 or cervical cancer; IBD: inflammatory bowel disease. No. number; IR incidence rate; IRR incidence rate ratio

Supplementary Table 4. Univariable and multivariable Hazard ratios for different risk factors for CIN2+ over time in total study population

	CIN2+			
	Univariable		Multivariable	
	HR	95%CI	HR	95%CI
Case				
No IBD	1.00	Reference	1.00	Reference
IBD	1.37	1.10-1.70	1.28	1.03-1.60
Urbanization				
Low level	1.00	Reference	1.00	Reference
High level	1.31	1.07-1.60	1.33	1.09-1.62
Screening episodes in a 5 year period				
1 episode	1.00	Reference	1.00	Reference
1-2 episodes	1.31	1.05-1.63	1.28	1.03-1.60
>2 episodes	3.42	2.31-5.07	3.31	2.22-4.92

Abbreviations: CIN: Cervical intraepithelial neoplasia. CIN2+: CIN2, CIN 3 or cervical cancer; IBD: inflammatory bowel disease. HR: Hazard ratio, CI: confidence interval

Supplementary Figure 2A-C. Kaplan Meier estimates for a CIN1 or CIN2+ lesion as worst diagnosis for IBD cohort and matched cohort by years of follow-up attained age in total study population.

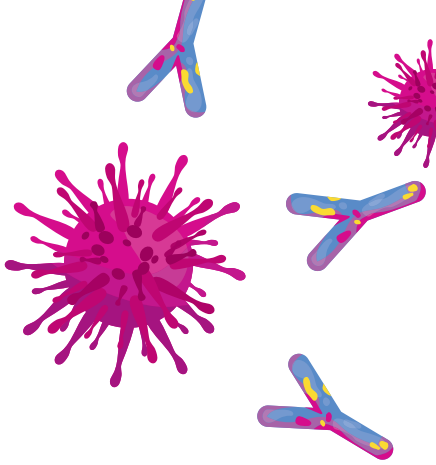


A: Proportion of women with CIN1 as highest grade of dysplasia during follow-up. B: Proportion of women with CIN2+ as highest grade of dysplasia during follow-up. C: Proportion of women with CIN2+ as highest grade of dysplasia by attained age. Attained age is defined as the age at diagnosis of CIN2+ or age at end of follow-up.

Abbreviations: CIN = cervical intraepithelial neoplasia. CIN2+ = CIN2, CIN3 or cervical cancer; IBD = inflammatory bowel disease; OR: Odds ratio. Attained age = age at diagnosis of CIN2+ or age at end of follow-up.

PART III

Hepatitis E in IBD



CHAPTER 6

Hepatitis E infection with a benign course during vedolizumab treatment for Crohn's disease: a case report

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Abstract

We present a 49 year old female patient with Crohn's disease (CD) in remission on vedolizumab therapy who experienced a symptomatic, though benign, course of acute hepatitis E. Routine blood tests showed substantial elevation of liver enzymes and polymerase chain reaction (PCR) testing confirmed hepatitis E virus (HEV) infection. Vedolizumab therapy was paused, liver enzymes improved three weeks after infection and normalized after six months. The patient recovered completely from mild symptoms. This case shows that hepatitis E is a potential cause of acute hepatitis during vedolizumab therapy, and in this case the infection has run a benign course.

Introduction

Vedolizumab is a gut-selective antibody to a4b7 integrin and has been proven effective in the treatment of CD.[1] By its gut specific mode of action, preventing leukocyte infiltration into the gastrointestinal mucosa, vedolizumab may theoretically be associated with an increased risk of gastrointestinal infections. A lower immunogenicity to the oral cholera vaccination was seen in patients on vedolizumab suggesting that inhibition of the $\alpha_4\beta_7$–MAdCAM-1 interaction might decrease immune priming in the gut and therefore lead to a decreased immune response.[2] Since hepatitis E virus (HEV) is transmitted through the oral-fecal route, inflammatory bowel disease (IBD) patients treated with vedolizumab, might therefore be at risk of HEV infection. Long-term safety data of up to 10 years have shown a reassuring safety profile without increased risk of serious or opportunistic infections.[3]

However, current knowledge on the occurrence and subsequent risk or consequences of HEV infection in IBD patients is limited to a few case reports[4-6] and a seroprevalence study, which showed a lower seroprevalence to HEV in IBD patients as compared to healthy blood donors.[7] Due to the paucity of literature, the clinical course of acute HEV infections in IBD is unclear, especially with regard to specific IBD medication. In addition, it is yet unknown whether data published on the risk of chronic HEV infections with potential progression to cirrhosis and liver failure in other immunocompromised patients may be applicable to IBD patients.[8] This report describes the benign clinical course of acute HEV infection in a CD patient receiving vedolizumab.

Case report

A 49 year old Caucasian female was diagnosed with CD in 2009 (Montreal classification A2 L3 B1) and had been in durable clinical remission after the start of vedolizumab for 3 years. A few hours after a scheduled vedolizumab infusion, she mentioned onset of severe muscle cramps and backache radiating to her feet. These symptoms were partially relieved by use of acetaminophen and completely resolved in three days. She did not have nausea, abdominal pain, diarrhea or fever. On the day of infusion, routine blood testing showed markedly elevated liver enzymes (AST 659 U/L, ALT 1293 U/L, GGT 541 U/L, ALP 261 U/L, bilirubin 7 umol/L) and mildly elevated inflammatory markers (WBC 10.4 x 10^9/L, CRP 8.4 mg/L) (Table 1). These results were only known after vedolizumab had already been administered. In addition to a stable dose of vedolizumab (300 mg every 8 weeks), she used over-the-counter vitamin supplements and estradiol. She did not take any new drugs or dietary supplements and denied

alcohol use. The serum antinuclear antibody titre was 1:320, anti-smooth muscle antibody and anti-mitochondrial antibodies were negative. Abdominal sonography was unremarkable with regard to liver parenchyma, vasculature, and biliary tree. Serological evaluation of viral hepatitis B and C, Epstein Barr virus, cytomegalovirus and human immunodeficiency virus was negative. She had been vaccinated against hepatitis A. Hepatitis E IgM and IgG were positive, and viral load of hepatitis E RNA was 4.55 x 10^5 IU/mL. Genotype testing revealed type 3i. A diagnosis of acute hepatitis E infection was made. The liver enzymes gradually improved over the subsequent weeks and returned to normal within six months. We intended to pause vedolizumab until complete clearing of the virus. One month after presentation we noticed a decrease in viral load and 42 days after presentation, viral load was undetectable. Fecal excretion of HEV was negative eight weeks after initial diagnosis. Vedolizumab therapy was restarted thereafter, and the standard 8 weeks schedule was delayed only by a few days. The cause of infection in our patient is likely consumption of pork liver a few weeks before laboratory assessment. Patient has remained in clinical and biochemical CD remission after restart of vedolizumab.

Discussion

This case illustrates a benign course of HEV infection in an immunocompromised CD patient. The clinical course was self-limiting and HEV was cleared from the body spontaneously in several weeks, despite being exposed to vedolizumab during her infection. This is the first case report on acute HEV infection during vedolizumab therapy. Although the long-term safety data on systemic complications with use of vedolizumab are reassuring, an increased risk of gastrointestinal infections is still plausible given the gut-selective mechanism of the drug.[2, 3] Since hepatitis E is a feco-oral transmitted viral infection, vedolizumab therapy might theoretically have put our patient at increased risk. Only three other case reports of HEV infection in IBD have been published.[4-6] The first one describes a patient with an acute hepatitis E that was fortuitously detected several months after starting infliximab for ulcerative colitis (UC). This patient experienced an asymptomatic course of hepatitis E infection and spontaneous clearance of the virus despite the use of immunosuppressive agents.[4] The second case describes a chronic HEV infection in a pregnant woman using azathioprine and infliximab for UC. Infection persisted during pregnancy after discontinuation of the drugs and was only cleared shortly after delivery.[5] The third case describes a male CD patient on 6-mercaptopurine, prednisolone and adalimumab therapy with a chronic HEV infection successfully treated with lowering the dose of immunosuppressants and a 24-week course of

ribavirin.[6] It remains largely unknown whether the use of immunosuppressive medication in IBD should be considered as a risk factor for acquiring an HEV infection.

In recent years chronic HEV infections have been described mostly in solid organ transplant recipients.[8] Chronic hepatitis E infection is defined by detection of HEV RNA in plasma or stool during a period longer than three months. Development of chronic hepatitis E may be due to delayed antibody production and prolonged HEV-viremia, possibly due to a decreased immune priming. Since chronically infected patients may develop a rapidly progressive liver disease[9], it seems prudent to closely monitor HEV infection in IBD patients. Chronic hepatitis E can be effectively treated with reduction of dose of immunosuppression or with three months of ribavirin monotherapy.[10]

In our patient, a self-limiting course was observed without the need of any intervention, despite the drug being administered during active HEV infection. This case report supports the evaluation of HEV in cases of hepatitis during vedolizumab therapy. Although this report shows a benign course of HEV infection, physicians should be vigilant for the possibility of a chronic infection in immunocompromised patients, and close monitoring of acute infections seems warranted.

IRB and informed consent

The patient has read through the final version of the manuscript and given us written consent for publication. This research was not subject to the Dutch Medical Research Involving Human Subject Act (WMO) as approved by the medical ethical committee of the Erasmus Medical Center (file number MEC-2021-0534).

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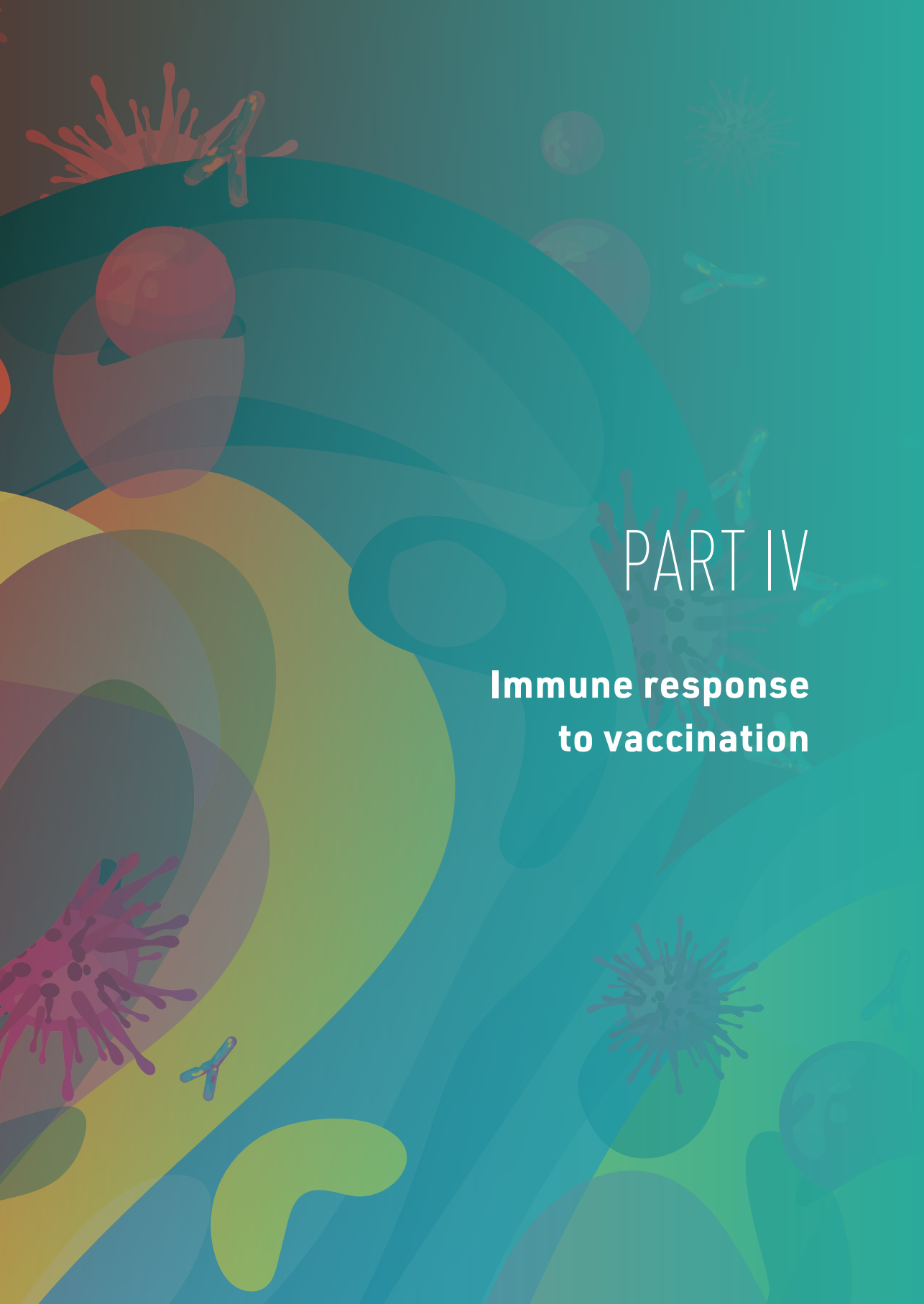
Table 1. Laboratory findings in our patient

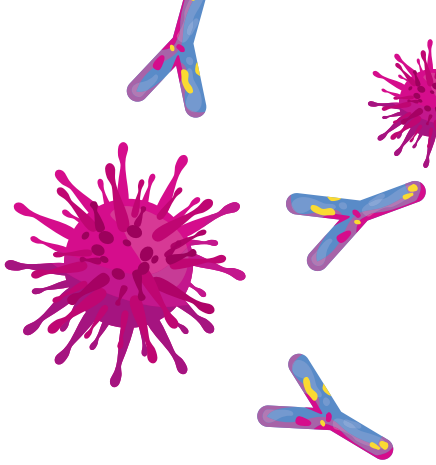
	Reference value	Unit			Vedolizumab infusion					Vedolizumab infusion
			06-06-2019	20-08-2019	21-04-2020	24-04-2020	15-05-2020	05-06-2020	17-06-2020	03-07-2020
Hemoglobin	7.5-9.5	mmol/L			7.8	8.5				
Platelets	150-370	10^9/L			277	313				
WBC	3.5-10.0	10^9/L			10.4	10.3				
PT-INR						0.9				
Albumin	35-50	g/L				44				
CRP	< 10	mg/L			8.4	5.1				
AST	< 31	U/L		17	659	197	21	18		
ALAT	< 34	U/L		8	1293	697	13	9		
LDH	< 247	U/L		172	334	224	221	170		
GGT	< 38	U/L			541	576	109	48		
AP	< 98	U/L		53	261	283	78	58		
Total bilirubin	< 17	mcmol/L			7	8	6	7		
IgG anti-HEV			N			P				
IgM anti-HEV			N			P				
HEV RNA blood		IU/mL				4,55 E5/P	4.32 E2/P	< 100/N		
HEV RNA feces		IU/mL							NEG	

Abbreviations: WBC, White blood cell count; PT-INR, Prothrombin time-International ratio; CRP, C-reactive protein; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; LDH, Lactate dehydrogenase; GGT, Gamma-glutamyltranspeptidase; AP, Alkaline phosphatase; HEV, hepatitis E virus; P, positive; N, negative; RNA, ribonucleic acid



PART IV

Immune response to vaccination



CHAPTER 7

High Immunogenicity to Influenza Vaccination in Crohn's Disease Patients Treated with Ustekinumab

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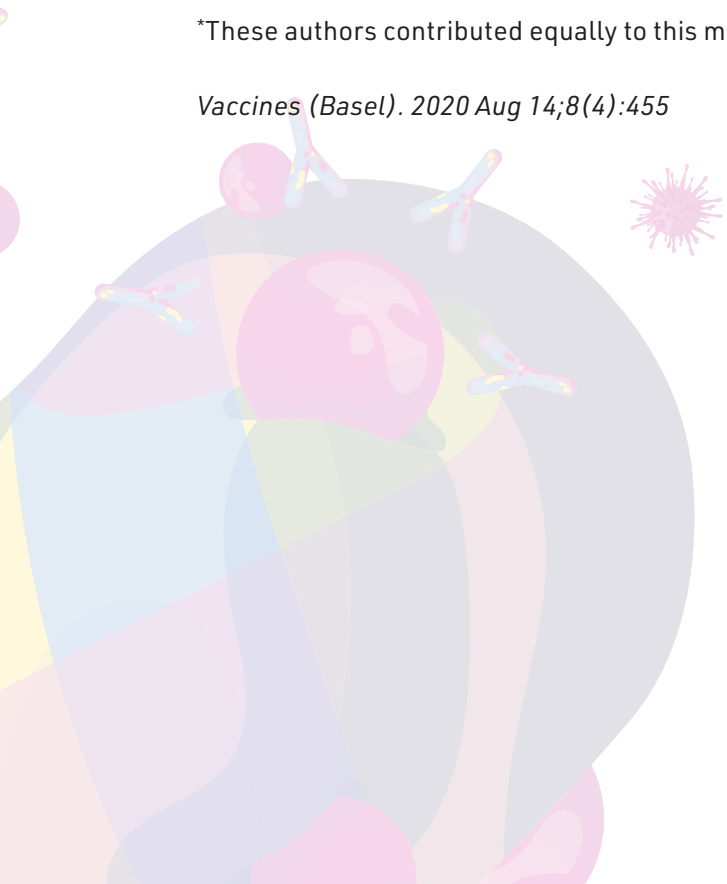

Abstract

Influenza vaccination can be less effective in patients treated with immunosuppressive therapy. However, little is known about the effects of ustekinumab, an anti-IL-12/23 agent used to treat Crohn's disease (CD), on vaccination response. In this prospective study, we assessed immune responses to seasonal influenza vaccination in CD patients treated with ustekinumab, compared to CD patients treated with anti-TNFα therapy (adalimumab) and healthy controls. Humoral responses were assessed with hemagglutinin inhibition (HI) assays. Influenza-specific total $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ T-cell responses were measured with flow cytometry. Fifteen patients treated with ustekinumab, 12 with adalimumab and 20 healthy controls were vaccinated for seasonal influenza in September 2018. Seroprotection rates against all vaccine strains in the ustekinumab group were high and comparable to healthy controls. Seroconversion rates were comparable, and for A/H3N2 highest in the ustekinumab group. HI titres were significantly higher in the ustekinumab group and healthy controls than in the adalimumab group for the B/Victoria strain. Post-vaccination T-cell responses in the ustekinumab group were similar to healthy controls. One month post-vaccination proliferation of $CD3^+CD8^+$ T-cells was highest in the ustekinumab group. In conclusion, ustekinumab does not impair immune responses to inactivated influenza vaccination. Therefore, CD patients treated with ustekinumab can be effectively vaccinated for seasonal influenza.

Introduction

Patients with inflammatory bowel disease (IBD) are frequently treated with immunomodulatory or immunosuppressive medication. Due to these therapies and the underlying inflammatory disease, they are at risk of more severe complications of infectious diseases [1]. Influenza causes significant morbidity and mortality in the general population [2] and the incidence of severe influenza is even higher in IBD patients, as demonstrated by higher rates of hospitalization (5.4% in IBD patients vs. 1.9% in healthy controls) [3]. Vaccination against influenza reduces the risk of infection in immunocompromised patients [4]. However, influenza vaccination may be less effective in patients treated with immunosuppressive therapies [5, 6, 7] and immunological mechanisms of the impaired vaccination response in IBD patients are often poorly understood [8].

Over the past decades, immunomodulatory and biologic therapies for the treatment of Crohn's disease (CD) and ulcerative colitis (UC) have become widely available. Adalimumab is a frequently prescribed anti-TNFα agent that is administered subcutaneously and has proven efficacy for CD since 2006 [9]. Use of anti-TNFα agents and immunomodulators, especially when used combined, is associated with a lower serological response to influenza vaccination in both children and adults with IBD [5, 6, 7, 10, 11, 12, 13]. This is explained by the involvement of TNFα in B-cell and T-cell interactions to achieve adequate antibody production [14, 15]. Ustekinumab, a human monoclonal antibody directed against the p40 subunit of interleukin (IL)-12 and IL-23 that normally binds to the interleukin-12 receptor β1 (IL-12Rβ1) of Th1 and Th17 cells, has more recently been approved as a treatment option for moderate-to-severe CD [9] and UC [16]. Although ustekinumab is effective and the safety profile reassuring [17, 18], infections remain feared complications and preventive measures including annual influenza vaccination is currently advised by the European Crohn's and Colitis Organisation (ECCO) guidelines [19]. Yet, little is known about the effects of ustekinumab on the immune responses to vaccinations.

Ustekinumab selectively inhibits IL-12 and IL-23 and thereby mainly Th1 and Th17 cell development [20]. However, IL-12Rβ1–mediated signaling via STAT3 and probably also STAT4, affected by ustekinumab treatment, plays a role in the generation of T follicular helper (T_{FH}) cells [21]. As T_{FH} cells are important for the B-T cell interaction to generate high-affinity antibodies, humoral responses may be compromised [22]. In this study, we aim to investigate the humoral and

cellular immune response after the inactivated 2018-2019 trivalent influenza vaccination (TIV) in adults with CD treated with ustekinumab (UST) compared to those treated with adalimumab (ADA) and healthy controls (HC).

Materials and Methods

Study Design and Population

We performed a prospective study on a selected cohort from a vaccination biobank in the Erasmus Medical Centre. All adult CD patients treated with either ustekinumab or adalimumab who wished to receive the seasonal influenza vaccination in September 2018 were asked to participate in the biobank study and were included following written informed consent. Healthcare workers who were offered the influenza vaccination for their occupation were selected from the biobank after age and sex matching to the CD patients, and included as healthy controls.

Data Collection and Analysis

At baseline, informed consent forms were signed and medical history was collected from participants and electronic patient files. Medical IBD history was classified using Montreal classification.[23] We collected medication use including dose at moment of vaccination. Ustekinumab was routinely injected in a dose of 90 mg every eight weeks or 12 weeks and adalimumab in a dose of 40 mg once every two weeks, defined as standard dose. More frequent injections were classified as escalated dose. Blood sampling was performed prior to the administration of the TIV. The 2018/2019 inactivated TIV (Influvac®; Abbott biologicals®) contained 15 microgram of HA antigen of each of the following influenza virus strains: A/Michigan/45/2015 (H1N1)pdm09-like virus; A/Singapore/INFIMH-16-0019/2016 (H3N2)-like virus; and B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage), and was administered intramuscularly in the deltoid. Patients were followed-up at one (T1), three (T3) and nine months (T9) post-vaccination. During each patient visit blood samples were collected in a BD Vacutainer® Serum Separating Tubes II Advance and a BD Vacutainer® Cell Preparation Tube. Within 24 hours after collection, serum samples were centrifuged and stored at -20 °C until further use. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient Ficoll separation and thereafter washed with phosphate buffer saline (PBS). Subsequently, PBMCs were counted and frozen in mononuclear cell medium with 10% dimethyl sulfoxide (DMSO) at a minimum of 2×10^6 mononuclear cells per ampule. These samples were stored overnight in Nalgene® Mr. Frosty™ Freezing Containers (Thermo Scientific) at -80°C and transferred to liquid nitrogen thereafter.

Laboratory Assessments

Hemagglutination Inhibition Assay

To assess antibody responses against the influenza virus vaccine strains, a hemagglutinin inhibition (HI) assay was performed simultaneously on all available serum samples, using a standard protocol [24, 25]. Briefly, sera were pre-treated with Vibrio cholerae neuramidase (dilution of 1:5 of an in-house produced cholera filtrate), by incubation overnight at 37 °C and heat-inactivation for one hour at 56 °C. Nonspecific agglutination in sera was eliminated, if present, by incubating 15 parts of the serum-cholera filtrate mixture with one part 100% turkey erythrocytes for one hour at 4 °C. Due to the pre-treatment steps, a starting serum dilution of 1:10 was used for all experiments. Three hemagglutinin antigens, each representing a strain of virus contained in the vaccine, were added and twofold serial dilutions were made up to 1:20,480. The highest dilution of antiserum that was still able to block agglutination between test influenza viruses and 1% turkey erythrocytes was considered the HI titre.

T-cell Proliferation Assay Using Flow Cytometry

Six doses of 2018/2019 inactivated TIV vaccine were dialysed (3 ml) with a slide-a-lyzer (Thermo Scientific) for contaminant removal to avoid interference in the T-cell proliferation assay. The amount of purified membrane glycoprotein subunit was analysed with a bicinchoninic acid (BCA) assay (Thermo Scientific™, Pierce™) and compared to undialysed vaccine content. If there was no difference in the amount of protein between dialysed and undialysed vaccine, we assumed no membrane protein was lost.PBMCs were thawed at 37^{0}C and washed twice with IMDM (Gibco Invitrogen, USA), supplemented with 2 mM L-glutamine, 100 U/ml penicillin (Lonza BioWhittaker, Switzerland) and 100 µg/ml streptomycin (Lonza BioWhittaker, Switzerland) (PSG) and 10% heat-inactivated fetal bovine serum (HI-FBS; Sigma-Aldrich, USA), further referred to as I10F. Subsequently, PBMCs were incubated with 50 U/ml Benzonase (Merck Millipore, USA) in I10F for 30 minutes at 37 °C, washed once and cultured over night at a density of 1–3 × 10^5 cells/well in RPMI-1640 supplemented with HI-FBS and PSG, further referred to as R10F. The next day, cells were washed once with PBS and labelled with 600 nM CFSE (in PBS) for 5 minutes at 37 °C. Afterwards, PBMCs were washed with R10F, plated at a density of approximately 1.5 × 10^5 cells per well in R10F and cultured for five days. Per donor and time point three wells were left unstimulated, while three wells were stimulated with 100 ng/well of the dialysed purified membrane glycoprotein subunit preparations of the 2018/2019 TIV [26]. Concanavalin A (ConA) was used as a positive control at a concentration of 5 µg/ml. Five days after stimulation PBMCs were stained for CD3, CD4 and CD8. Briefly, cells were washed once with PBS containing 2mM EDTA and 0.05% BSA (FACS

buffer) and then stained for 15 minutes at 4^0C in FACS buffer with the following monoclonal antibody-fluorochrome conjugates: CD3/APC Cy7 (1:50 dilution, BD Pharmingen), CD4/V450 (1:50 dilution, BD Horizon) and CD8/PE-Cy7 (1:25 dilution, eBioscience). After staining, cells were washed twice with FACS buffer and flow cytometry was performed with a BD FACSLyric™ flowcytometer (BD Bioscience, USA).

Outcomes and Parameters

Functional antibody responses were assessed with the HI assay. The assay was performed in duplo and geometric mean titres were calculated. For calculation purposes, HI titres <10 were adjusted to 1. From these results, the following outcomes were calculated: (1) seroprotection rate: the percentage of participants per study group with an antibody titre above 40, which is considered the best surrogate correlate of protection [27]; (2) seroconversion rate: the percentage of participants in the study group that had at least a fourfold increase of the post-vaccination antibody concentration when compared to the pre-vaccination antibody concentration; (3) geometric mean titres (GMT) per time point per study group. We corrected for high pre-vaccination antibody titres, using a log10 transformation of GMTs and a linear regression formula described by Beyer and colleagues [28], which results in a 'reset' of pre-vaccination antibody titres to zero. Data were back log transformed to show interpretable results.

Cellular responses were assessed by the proliferation of influenza specific $CD3^+$, $CD4^+$ and $CD8^+$ T-cells. Stimulation indexes (SI) were calculated by dividing the percentage of proliferated cells in stimulated samples by the percentage of proliferated cells in unstimulated samples per donor, time point and T-cell subset (total CD3, CD4 or CD8).

Data Analysis

FACS data was analysed with FlowJo version 10.6.1. Gating strategies used for analysis are shown in Figure S1. We set the mean background of proliferation in unstimulated samples to 1.5 and applied the same gating strategy to stimulated samples. SPSS version 24 was used for data analysis. A Shapiro-Wilk test was used to assess normality of distributions. Comparison of parametric continuous variables between the three groups was done using one-way ANOVA and of non-parametric continuous variables using Kruskal-Wallis tests. Comparison of non-parametric continuous variables between two groups was done using Mann-Whitney U tests. Fisher's exact tests were used for comparing differences in categorical variables. To prevent finding significances due to multiple testing, we

only performed testing between two groups when the comparison between three groups showed a p-value of <0.10. A p-value <0.05 was considered significant. Outliers were detected with the Tukey's box-plot method which defines outliers as being outside the interquartile interval (Q1 – 1.5·IQR, Q3 + 1.5·IQR). Missing data were excluded per variable. GraphPad Prism version 5.0 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com) was used to create figures.

Ethical Considerations

all subjects gave their informed consent for inclusion before they participated in the study. This study has been exempted from medical ethical approval requirements by the Medical Ethical Research Committee of the Erasmus Medical Center on November 13, 2017, due to the biobank format of the Vaccination Cohort study (COVA study, MEC-2014-398). The study has been conducted according to the principles of the declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013).

Results

Forty-seven subjects were enrolled in this study between September 2018 and November 2018. We studied the 2018-2019 TIV vaccine response of 47 individuals in three different study groups: 15 CD patients using ustekinumab, 12 CD patients using adalimumab, and 20 healthy controls with influenza vaccination history. Demographic baseline characteristics were comparable between the three groups and described in Table 1. The average median age of the total study population was 39 years (IQR 29-50) and 57 percent was female. Median duration of use of adalimumab was 32 months, and 19 months for ustekinumab (p = 0.022). In the ustekinumab group, one patient was injected every seven weeks and one patient every six weeks. In the adalimumab group two patients were injected weekly, two patients every 10 days and one every four weeks. Three patients in the ustekinumab group additionally used an immunomodulator (thiopurines or methotrexate) compared to two patients in the adalimumab group. Montreal classification, use of co-medication and influenza vaccination history did not differ significantly between the three groups.

Table 1. Baseline characteristics.

	UST	ADA	HC	Difference between groups
	n **= 15**	***n*** **= 12**	***n*** **= 20**	**Sig. (p-value)**
Gender				
Female, *n* (%)	11 (73.3)	5 (41.7)	11 (55.0)	0.260†
Pregnant, *n* (%)	1 (9.1)	2 (40.0)	0 (0)	0.079†
Age				
Median, years (IQR)	36 (26-56)	45 (28-59)	36 (29-49)	0.688‡
Country of birth				
Netherlands, *n* (%)	13 (86.7)	11 (91.7)	19 (95.0)	0.808†
BMI				
Mean, kg/m^2 (SD)	24.5 (4.6)	25.3 (5.2)	24.0 (4.3)	0.723§
Lifestyle				
Smoker, *n* (%)	5 (33.3)	1 (8.3)	3 (15.0)	0.201†
Alcohol, *n* (%)	9 (60.0)	7 (58.3)	19 (95.0)	**0.016**†
Duration of CD				
Median, years (IQR)	15 (9-25)	14 (8-35)	NA	0.845¶
Disease Location				
Ileal (L1)	3 (20.0)	2 (16.7)		
Colonic (L2)	1 (6.7)	1 (8.3)		
Ileocolonic (L3)	8 (53.3)	7 (58.3)		
L3 + upper gastrointestinal (L3+4)	3 (20.0)	2 (16.7)	NA	1.000†
Disease Behaviour				
Non-stricturing, non-penetrating (B1)	4 (26.7)	4 (36.4)		
Stricturing (B2)	7 (46.7)	6 (54.5)		
Penetrating (B3)	4 (26.7)	1 (9.1)	NA	0.666†
Perianal disease (p)	4 (26.7)	3 (25.0)	NA	1.000†
Duration medication,				
Median, months (IQR)	13 (5-19)	32 (15-82)	NA	**0.022**¶
Dose medication				
Standard dose	13 (86.7)	7 (66.7)		
Escalated dose	2 (13.3)	4 (33.3)	NA	**0.357**†
Immunosuppressive co-medication* *n* (%)				
None	9 (60.0)	7 (58.3)		
Low dose corticosteroids	2 (13.3)	3 (25.0)		
High dose corticosteroids	1 (6.7)	0 (0.0)		
Methotrexate	2 (13.3)	0 (0.0)	NA	0.643†
Thiopurines	1 (6.7)	2 (16.7)		
Influenza vaccine history, *n* (%)				
never before	3 (20.0)	3 (25.0)	6 (30.0)	
once before (2017)	0 (0.0)	2 (16.7)	2 (10.0)	
twice before (2016, 2017)	1 (6.7)	0 (0.0)	1 (5.0)	
thrice before (2015–2017)	0 (0.0)	1 (8.3)	1 (5.0)	
more than thrice before	5 (33.3)	5 (41.7)	4 (20.0)	0.537†
at least once, but not 2017	6 (40.0)	1 (8.3)	6 (30.0)	

Percentages within study groups. T-tests were used to calculate differences between continuous variables, chi-square tests were used for categorical variables. UST = ustekinumab group, ADA = adalimumab group, HC = healthy controls. CD = Crohn's Disease, NA = not applicable. * used while vaccinated or during the 3 months before. Low-dose corticosteroids = prednisone <10mg/day or budesonide (<9mg/day). High-dose corticosteroids = prednisone ≥10mg/day (at least 14 consecutive days or 700 mg total). † Fisher's exact test, ‡ Kruskal-Wallis test, § one-way ANOVA, ¶ Mann-Whitney U test.

Humoral Immune Response

Seroprotection Rates

Pre-vaccination seroprotection rates for all three strains were not significantly different between the groups (Table 2). Seroprotection rates to the H3N2 strain one month post-vaccination were 100 percent in all three groups, and remained 100 percent three months post-vaccination in healthy controls and the ustekinumab group. In the adalimumab group, seroprotection rates were lower three months post-vaccination compared to the other two groups, reaching borderline significance (81.8%, p = 0.056). Seroprotection rates to the H1N1 strain were higher than 90.0 percent one month post-vaccination and at least 78.6 percent three months post-vaccination for the three study groups and did not differ significantly (Table 2). Pre- and post-vaccination titres were lowest to the B/Victoria strain, especially in the adalimumab group (T1 and T3: 63.6%), however there was no significant difference between study groups.

Table 2. Seroprotection rates per study group (% HI-titers ≥1:40).

		UST	ADA	HC	Overall	UST vs. HC	UST vs. ADA	ADA vs. HC
		%	*%*	*%*	*p-value*	*p-value*	*p-value*	*p-value*
A/H3N2	T0	71.4	75.0	90.0	0.328			
	T1	100	100	100	1			
	T3	100	81.8	100	0.056	1	0.183	0.118
A/H1N1pdm09	T0	57.1	58.3	55.0	0.982			
	T1	91.7	90.0	100	0.379			
	T3	78.6	90.9	90	0.561			
B/Victoria	T0	42.9	33.3	60.0	0.311			
	T1	92.3	63.6	85.0	0.170			
	T3	92.9	63.6	75.0	0.202			

UST = ustekinumab group, ADA = adalimumab group, HC = healthy controls. Significances were calculated with Fisher's exact tests.

Seroconversion Rates

Seroconversion rates to the H3N2 strain were significantly different in the three groups at three months post-vaccination (T3: p = 0.014, Table 3) and borderline significant at one month post-vaccination (T1: p = 0.064). The ustekinumab group had higher seroconversion rates compared to the adalimumab group (T3: p = 0.015, Table 3) and the healthy controls (T1: p = 0.038, T3: p = 0.035, Table 3). Seroconversion rates to the other influenza vaccine strains in the three study groups were highest in the ustekinumab group and lowest in the adalimumab group, although this reached no significance.

Table 3. Seroconversion rates per study group (% ≥4-fold increase).

		UST	ADA	HC	Overall	UST vs. HC	UST vs. ADA	ADA vs. HC
		%	%	%	*p-value*	*p-value*	*p-value*	*p-value*
A/H3N2	T0-T1	69.2	27.3	30.0	0.064	0.038	0.100	1
	T0-T3	71.4	18.2	30.0	0.014	0.035	0.015	0.676
A/ H1N1pdm09	T0-T1	75.0	40.0	50.0	0.288			
	T0-T3	50.0	36.4	45.0	0.863			
B/Victoria	T0-T1	61.5	27.3	35.0	0.227			
	T0-T3	50.0	27.3	30.0	0.520			

UST = ustekinumab group, ADA = adalimumab group, HC = healthy controls. Significances were calculated with Fisher's exact test.

Antibody Titres

The post-vaccination antibody titres in the ustekinumab group were comparable to those of the healthy controls. In the adalimumab group, geometric mean titres (GMT) were lower compared to the other two groups for all influenza vaccine strains at both T1 and T3, except for the H1N1 strain three months post-vaccination (Table 4, Figure 1). This reached significance for the B/Victoria strain at both T1 and T3, when comparing the three groups (T1: $p = 0.031$ and T3: $p = 0.028$, Table 4, Figure 2,) and specifically the ustekinumab and adalimumab group ($p = 0.028$ and $p = 0.009$, Table 4) respectively.

As pre-vaccination titres in the ustekinumab group were significantly lower than in the healthy controls and the adalimumab group ($p = 0.013$), we studied antibody titres after correction for high pre-vaccination titres in the latter two (Table 4). Post-correction antibody titres at T3 for the H3N2 strain were significantly lower in the adalimumab group compared to healthy controls and the ustekinumab group ($p = 0.041$, Table 4). For the B/Victoria strain, post-correction antibody titres were significantly higher for both T1 and T3 in the ustekinumab group compared to the other two groups (T1: $p = 0.014$, T3: $p = 0.015$, Table 4).

Table 4. Geometric mean antibody titres (GMT) per study group per time point.

		UST	ADA	HC	Overall	UST vs. HC	UST vs. ADA	ADA vc HC
A/H3N2	*GMT*				*p-value*	*p-value*	*p-value*	*p-value*
	T0	26	59	163	0.013	0.008	0.252	0.586
	T1	437	215	474	0.171			
	T3	372	132	427	0.071			
	Post-correction GMT							
	T1	203	75	141	0.159			
	T3	132	35	85	0.041	0.396	0.025	0.036
A/H1N1pdm09	*GMT*							
	T0	15	18	26	0.856			
	T1	195	127	184	0.786			
	T3	80	101	120	0.905			
	Post-correction GMT							
	T1	107	60	91	0.261			
	T3	27	29	33	0.947			
B/Victoria	*GMT*							
	T0	12	9	17	0.337			
	T1	129	30	90	0.031	0.073	0.028	0.306
	T3	111	26	62	0.028	0.125	0.009	0.220
	Post-correction GMT							
	T1	53	13	31	0.014	0.043	0.005	0.197
	T3	42	10	21	0.015	0.036	0.006	0.227

UST = ustekinumab group, ADA = adalimumab group, HC = healthy controls. GMT = geometric mean antibody titre. Post-correction GMT = transformed post-vaccination GMTs corrected for high pre-vaccination titres. Significance between GMT and post-correction GMT values was calculated with a Kruskal Wallis test. If Kruskal-Wallis test showed a significant difference, differences between separate groups were calculated with Mann-Whitney U-tests.

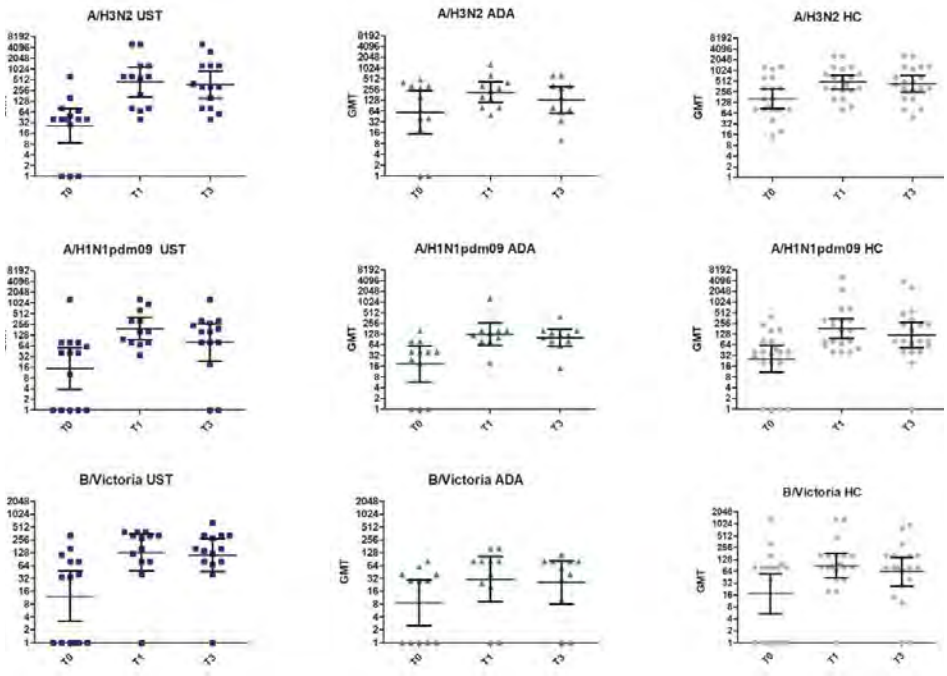

Figure 1. HI titres for each participant to influenza A/H3N2, A/H1N1pdm09 and B/Victoria vaccination per strain and study group. T0 = pre-vaccination, T1 = one month post-vaccination, T3 = three months post-vaccination, T9 = nine months post-vaccination. UST = ustekinumab group, ADA = adalimumab group, HC = healthy controls. Geometric mean titres (GMT) and 95% confidence intervals are shown.

Cellular Immune Response

T-cell proliferation was studied per group, per time point and per T-cell subset (example shown in Figure S2). In general, stimulation indexes showed a pattern of increased proliferation from baseline to T1 and T3 (with the exception of $CD3^+CD8^+$ response in healthy controls) and a decrease between T3 and T9 (with the exception of the $CD3^+$ and $CD3^+CD8^+$ response in the ustekinumab group) (Figure 3). In all three groups, baseline $CD3^+$ and $CD3^+CD4^+$ responses were low (mean SI <1.36). However, $CD3^+CD8^+$ baseline responses were relatively high (mean SI >1.49). When comparing time points and T-cell subsets, no significant differences were found between the three study groups. However, when we compared the groups one by one, we found a significant higher $CD3^+CD8^+$ response one month after vaccination for the ustekinumab group compared to healthy controls ($p = 0.025$).

Overall, 95% confidence intervals were large and a few donors from all groups showed exceptional high responses (Figure 3). When excluding these outliers, stimulation indexes were significant different between the three groups one month post-vaccination in the $CD3^{+}CD8^{+}$ subset (p = 0.031) in favour of the ustekinumab group (UST vs. HC, p = 0.009) (Figures S3 and S4).

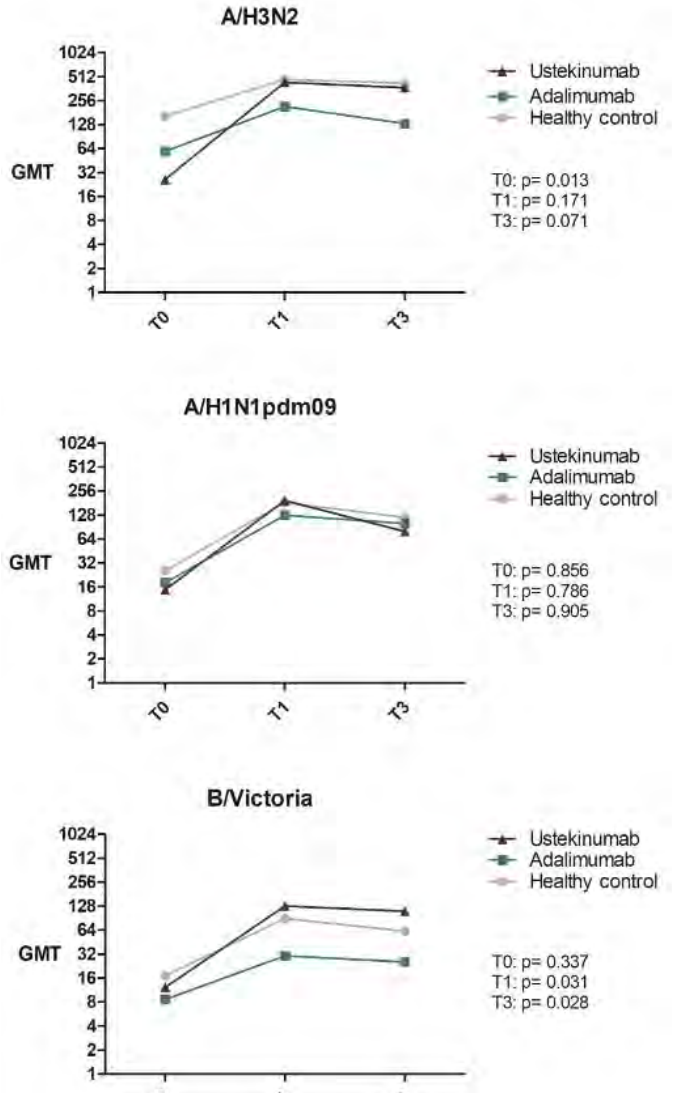

Figure 2. Dynamics of geometric mean HI titres (GMT) to influenza A/H3N2, A/H1N1pdm09 and B/Victoria vaccination according to study groups. Comparisons between groups were tested using Kruskal Wallis tests. A p-value <0.05 indicates statistical significance.

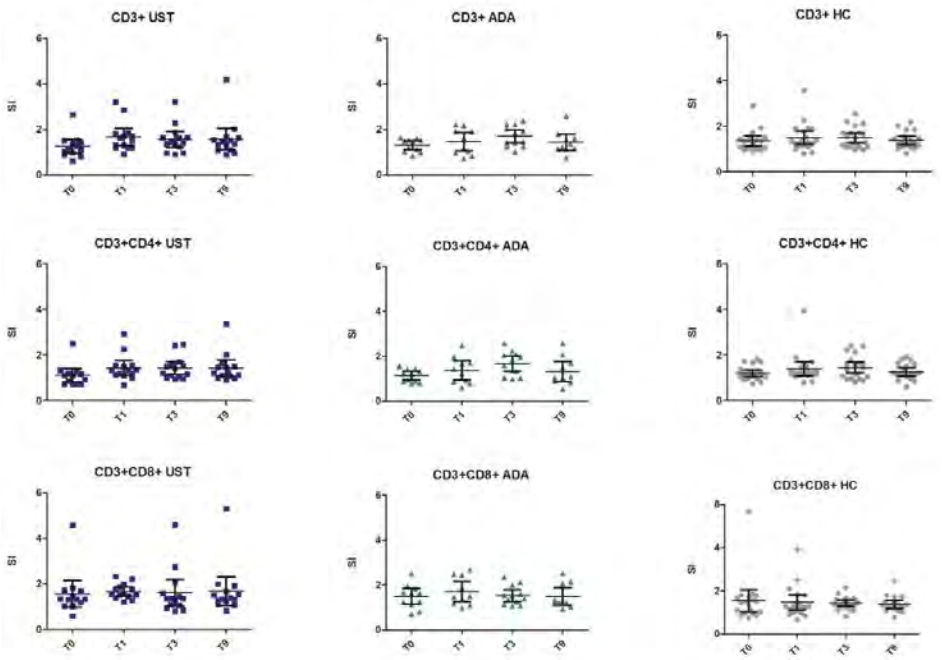

Figure 3. Stimulation indexes for each participant in the three study groups per T-cell subset. T0 = pre-vaccination, T1 = one month post-vaccination, T3 = three months post-vaccination, T9 = nine months post-vaccination. SI = stimulation index. UST = ustekinumab group, ADA = adalimumab group, HC = healthy controls. 95% confidence intervals are shown.

Correlation Between Humoral and Cellular Immune Response

To assess a possible relationship between the humoral and cellular immune responses, we calculated correlations between HI assay titres (GMTs for the three different vaccine strains) and the stimulation indexes (for the three different subsets of T-cell populations) (Figure S5). The highest Spearman correlation coefficient was found between the GMTs for the H1N1 strain and the SI for the $CD3^+$ T-cells ($R = 0.278$, $p = 0.002$).

Discussion

Influenza vaccination is recommended in IBD patients according to international guidelines, however, immunomodulatory or immunosuppressive treatment may impair vaccine responses. This prospective cohort study showed that B-cell as well as T-cell responses to inactivated TIV in patients with CD during ustekinumab treatment were maintained and not impaired compared to healthy

controls. Patients treated with ustekinumab had comparable seroprotection rates post-vaccination as healthy controls and better sustained seroprotection rates to the H3N2 strain than patients treated with adalimumab. Seroconversion rates were also higher in the ustekinumab group compared to healthy controls and the adalimumab group at three months post-vaccination for the H3N2 strain. After correction for high pre-vaccination titres using a linear regression formula described by Beyer et al [28] post-correction post-vaccination titres were significantly higher in the ustekinumab group compared to the adalimumab group and healthy controls for the B/Victoria strain. Cellular immune responses in the ustekinumab group were not impaired either. The $CD8^+$ T cell response one month post-vaccination was even significantly higher than in healthy controls.

To our knowledge, this is the first study that shows the immune response to vaccination in CD patients treated with ustekinumab. Our results are in line with a previous study in psoriasis patients treated with ustekinumab. This study showed no differences in the immune response to pneumococcal or tetanus toxoid vaccinations in patients treated with ustekinumab compared to controls [29]. In another study, higher antibody responses to hepatitis B virus vaccination were found in patients treated with ustekinumab compared to patients treated with infliximab or adalimumab [30]. Immune response to influenza vaccination in patients treated with ustekinumab have not been reported yet. Our results indicate that blocking IL-12 and IL-23 does not influence immune responses to vaccination as has been previously hypothesized [31]. T_{FH} cells could still be generated, as studies in IL-12Rβ1-deficient adults have shown that the level of T_{FH} cells was not reduced in the absence of IL-12Rβ1 [32]. Alternatively, if the generation of T_{FH} cells is impaired due to the effect of a lacking signal to IL-12Rβ1 on the STAT3 (and 4) pathway, extra follicular T helper cells might take over T_{FH} cells functions [21, 33].

Although measured against different influenza strains, the HI assay responses in our healthy controls were comparable to those in previous studies, or even higher [34, 35]. Higher GMTs can be explained by the influenza vaccination history in our study population Seroconversion rates might be lower than in non-immune populations due to high pre-vaccination titres. Although antibody titres only increase slightly after repeated annual influenza vaccination, they still prevent laboratory proven influenza infections [35]. Several previous studies have shown decreased immune responses to influenza vaccination in IBD patients using anti-TNFα agents [6, 7, 11, 12, 13]. This is in line with our results from the HI assay, but not reflected by our T-cell proliferation data.

We found no previous studies on cellular responses after influenza vaccination in adult IBD patients. In children with IBD it was shown, in line with our data, that lymphocyte proliferation in general and after stimulation with tetanus antigen and adenovirus antigen was not impaired by several immunosuppressive therapies [36]. For T-cell proliferation assays in liver transplant recipients who were vaccinated for seasonal influenza higher SI indexes in healthy controls and patients were reported compared to our data [26]. However, due to the use of a thymidine assay to measure the influenza-specific T-cell response at that time, the results might not be comparable to our flow cytometry results. A study investigating T-cell responses after influenza vaccination reported short-lived $CD4^+$ T cell responses when PBMCs were stimulated with live (attenuated) virus strains [35]. This is in contrast with our data showing that the T-cell response was still high (or even highest) three months post-vaccination.

In this era of new therapy targets and personalized treatment, immune response to vaccination might be an extra aspect influencing the choice of therapy, in addition to commonly weighed factors such as effectiveness, safety and costs. Combination therapy with anti-TNFα agents and an immunomodulatory agent is more effective for the treatment of CD than monotherapy, most likely due to both suppression of immunogenicity and an additive effects of the two drugs to reach disease remission [37]. However, this combined strategy is also associated with a higher risk of infections [38] and may have a negative impact on immune responses to vaccination [5, 11, 12]. Several ways to improve the influenza vaccination response during anti-TNFα therapy have been investigated. A booster vaccination failed to show better protection rates [7, 39] and timing relative to infliximab infusion neither showed to affect serological protection [13]. Recently, a study found that four times higher dose vaccination resulted in higher antibody responses to influenza vaccination compared to the standard dose, without leading to more adverse effects [40]. Yet, 'high dose' vaccination is currently only recommended by American guidelines for patients aged 65 years or older [41]. Current evidence does not support the use of immunomodulatory agents combined with ustekinumab [17]. Similar to our results in the ustekinumab group, a recent study showed that immune responses to influenza vaccination in patients treated with vedolizumab, a monoclonal antibody against the $\alpha_4\beta_7$ integrin, were not altered either [40]. Interestingly, the immune response to an enterally administered vaccine was impaired during treatment with vedolizumab, possibly reflecting the gut-selective action of this therapy [42].

The ECCO recommends routine influenza vaccination of patients on immunomodulators [19]. However, reported influenza vaccination uptake rates among IBD patients are low (28 to 61%) [1, 43, 44, 45], amongst others due to concerns about effectiveness and their unawareness of the recommendation [1, 43, 44]. With our results, we provide evidence for high immunogenicity of influenza vaccination in CD patients treated with ustekinumab. As vaccination check-ups and active vaccination recommendations by treating physicians or supportive nurses are associated with improved vaccination uptake [43, 45], we strongly support involved nurses and physicians to recommend annual influenza vaccination to their patients treated with ustekinumab. This advice is similar for CD patients treated with adalimumab, because even though anti-TNFα treatment is associated with a lower serological response, the $CD4^+$ and $CD8^+$ T-cell responses showed to be non-inferior in this study.

Few limitations need to be taken into account with the interpretation of our results. First, this study is hampered by a small sample size and the number of patients on combination therapy with an immunomodulatory agent was too small to do a subgroup analysis. Since lowest influenza vaccine responses in IBD patients are reported in patients using combination therapy with an anti-TNFα agent and an immunomodulatory agent [5, 11, 12], this would have been an interesting addition. However, since immunomodulatory use was equally distributed amongst the patient groups, this will not have affected their comparison. The heterogeneity in the dose of medication in both adalimumab and ustekinumab users at moment of vaccination might have influenced the results. Furthermore, in this study CD patients were treated with adalimumab. Although anti-TNFα agents are comparable in efficacy and side effects, vaccine responses may differ, and cannot be generalized to other anti-TNFα agents than adalimumab. There was a significant higher baseline GMT for the A/H3N2 strain in healthcare workers compared to the ustekinumab group. Since the influenza vaccination history was comparable between the three study groups, this might be explained by a higher exposure to influenza in healthcare workers [46]. By using a linear regression formula we were able to correct for this possible confounder. Also, since the composition of influenza vaccinations change annually it is hard to compare our results one by one with previous and future studies. While we broadly examined influenza-specific immune responses by studying both humoral and cellular responses, in-depth details remain to be elucidated. The HI assays showed that functional antibodies are present in ustekinumab-treated patients, but we cannot conclude anything about the isotypes of the antibody response. With the T-cell proliferation we showed comparable proliferation of influenza-specific $CD4^+$ and $CD8^+$ T cells in all study populations, but the effector

functions of $CD4^+$ and $CD8^+$ T cells are still unknown. Therefore, the performance of an intracellular cytokine staining would have been of additional value. Lastly, we compared immunological outcome measures between groups and could therefore not directly draw conclusions about morbidity due to influenza infections in these cohorts.

Conclusions

In this study, we demonstrated that CD patients treated with ustekinumab have adequate B- and T-cell responses to influenza vaccination. Therefore, our data support the plea for influenza vaccination in CD patients treated with ustekinumab to protect them from severe infections.

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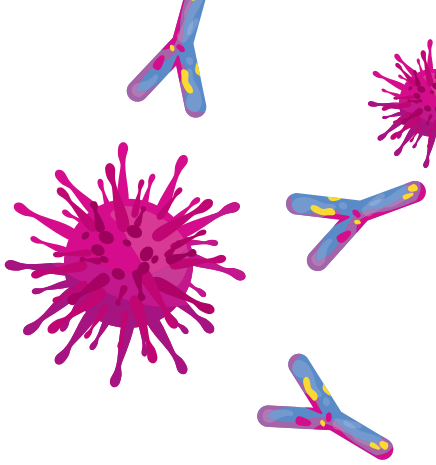
Supplementary materials

https://www.mdpi.com/2076-393X/8/3/455/s1



CHAPTER 8

Accelerated waning of immunity to SARS-CoV-2 mRNA vaccines in patients with immune mediated inflammatory diseases

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Abstract

Background: Limited information is available on the impact of immunosuppressants on COVID-19 vaccination in patients with immune-mediated inflammatory diseases (IMID).

Methods: This observational cohort study examined the immunogenicity of SARS-CoV-2 mRNA vaccines in adult patients with inflammatory bowel disease, rheumatoid arthritis, ankylosing spondylitis, or psoriatic disease, with or without maintenance immunosuppressive therapies. Antibody and T cell responses to SARS-COV-2, including neutralization against SARS-CoV-2 variants were determined before and after 1 and 2 vaccine doses.

Results: We prospectively followed 150 subjects, 26 healthy controls, 9 IMID patients on no treatment, 44 on anti-TNF, 16 on anti-TNF with methotrexate/azathioprine (MTX/AZA), 10 on anti-IL-23, 28 on anti-IL-12/23, 9 on anti-IL-17, and 8 on MTX/AZA. Antibody and T cell responses to SARS-CoV-2 were detected in all participants, increasing from dose 1 to dose 2 and declining 3 months later, with greater attrition in IMID patients compared to healthy controls. Antibody levels and neutralization efficacy against variants of concern were substantially lower in anti-TNF treated patients than in healthy controls and were undetectable against Omicron by 3 months after dose 2.

Conclusions: Our findings support the need for a third dose of mRNA vaccine and for continued monitoring of immunity in these patient groups.

Introduction

The COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remains a serious health crisis (1, 2). COVID-19 infections can vary from asymptomatic or mild through to severe disease, with lethal complications such as progressive pneumonia, acute respiratory distress syndrome and organ failure driven by hyperinflammation and a cytokine storm syndrome. Patients with immune-mediated inflammatory diseases (IMID), such as inflammatory bowel disease (IBD), psoriatic disease, rheumatoid arthritis (RA) and spondyloarthritis (SpA), are frequently treated with immunosuppressants and biologics and therefore may be at increased risk for COVID-19 (3, 4). Age and underlying comorbidities as well as the use of some immunosuppressants have been shown to be risk factors for developing COVID-19 among IMID patients (3, 5). Glucocorticoids and combination therapy of immunomodulators and biologics have been shown to increase the risk of severe outcomes of COVID-19 (4, 6).

Although many IMID patients mount adequate serological responses to vaccination after two doses of an mRNA vaccine, a proportion of IMID patients show reduced responses compared to healthy controls (7-14), as confirmed in recent meta-analyses (15, 16). Patients receiving glucocorticoids, methotrexate, mycophenolate, anti-TNF and B-cell depleting therapy may have attenuated serological responses to COVID-19 vaccines (7, 11, 13, 15, 17-24). Moreover, two recent studies showed that patients on anti-TNF therapy have greater waning of humoral immunity compared to healthy controls (13, 21).

Data regarding the cellular immune responses to vaccination are still relatively scarce and conflicting. Several studies have shown unimpaired T cell responses to SARS-CoV-2 vaccines in immunocompromised patients compared to healthy individuals (13, 25-28), though a follow-up study showed that a proportion of IMID patients on immunosuppression had reduced T cell responses to a second dose of vaccine (29). In another study, methotrexate limited $CD8^+$ T cell responses to vaccination in a cohort of IMID patients (30). To gain further insight into immunity to mRNA vaccines in IMID patients on different maintenance therapies, we investigated serological and T cell responses against SARS-CoV-2 before and after one or two doses of mRNA vaccine. The results show substantial variation in responses within different treatment groups. Notably, we observed decreased serological responses in anti-TNF treated patients, including decreased efficacy of neutralization of variants of concern, with no neutralizing capacity against the Omicron variant. T cell cytokine production, including IFN-γ, IL-2 and IL-4 increased from one to two doses of vaccine and correlated with

humoral responses. Importantly, both antibody and T cell responses in the IMID treatment groups showed greater waning by 3 months post second dose of mRNA vaccine compared to healthy controls. These data highlight the need for third doses of SARS-CoV-2 mRNA vaccines and for continued monitoring of responses in these patients.

Methods

Study design and participants

Patient recruitment: In this observational multicenter cohort study, we investigated the IMmune resPonse After COVID-19 vaccination during maintenance Therapy in immune-mediated inflammatory diseases (IMPACT). IMID patients being treated at Mount Sinai Hospital, University Health Network/ Toronto Western Hospital or Women's College Hospital in Toronto, Canada who were receiving BNT162b (Pfizer-BioNTech) and/or mRNA-1273 (Moderna) SARS-CoV-2 vaccines were recruited between January 8 and October 4, 2021. In Canada the vaccine schedule between dose 1 and 2 was increased from the standard 21 or 28 days to allow faster roll out of dose 1 and as a result, in our cohort there was a median of 60.5 days, IQR [45.5-72] between the 2 doses.

Inclusion criteria for this study were adult IMID patients being treated with anti-TNF therapies (infliximab, adalimumab, golimumab, etanercept or certolizumab pegol), anti-IL-17 therapy (ixekizumab, secukinumab), methotrexate (MTX) or azathioprine (AZA) monotherapy, combination therapy of MTX/AZA plus anti-TNF therapies, anti-IL-12/23 (ustekinumab) therapy, anti-IL-23 therapy (guselkumab, risankizumab) or no immunosuppressants. A group of healthy volunteers, without an IMID and without immunosuppression were also recruited as a control cohort. Excluded were individuals younger than 18 years, those who had a past SARS-CoV-2 infection, patients on vedolizumab or oral steroids and those receiving COVID-19 vaccines other than mRNA.

Sample and data collection: Patient information and medical history were collected at each visit. Participation was terminated when all the blood samples were collected or when a patient opted out. Clinical data included basic demographics (age, sex, weight, height), relevant past medical and surgical history, and medication use at inclusion. Questions about prior COVID-19 diagnosis or exposure, vaccination history and side effects, changes in medical history or medication and disease activity were collected at each study visit. Blood samples were drawn from the participants at up to 4 time points:

T1 = pre-vaccination, T2 = median 26 days after dose 1, T3 = median 16 days after dose 2 and T4 = median 106 days after dose 2. Peripheral blood samples were collected in BD Vacutainer® sodium heparin tubes for plasma antibody assessment and peripheral blood mononuclear cell (PBMC) separation. All samples were labelled with unique patient identifiers. Researchers were blinded to the identity and clinical details of the subjects. Plasma samples were stored at -80^{0}C. PBMCs were isolated by density centrifugation using Ficoll-Paque PLUS (GE Healthcare). PBMCs were cryopreserved in 10% DMSO in FBS (Wisent Bioproducts) and stored in liquid nitrogen at a minimum of 2x10^6 mononuclear cells per vial.

Automated ELISAs

Frozen plasma was thawed and treated with 1% final Triton X-100 for one hour. Samples were analyzed by automated ELISA for IgGs to the spike trimer (spike), the spike receptor binding domain (RBD), and the nucleocapsid (NP; all antigens and secondary antibodies are produced in mammalian cells and were provided by Dr. Yves Durocher at the National Research Council of Canada, NRC, Montréal, QC, Canada) as previously reported (31). Luminescence values for each sample/assay were normalized to synthetic standards profiled in a 4-fold dilution series on each plate (Human anti-nucleocapsid IgG, #A02039, clone HC2003, GenScript, Piscataway, NJ, USA and humanized anti-RBD/spike IgG: VHH72hFc1X7; NRC). The synthetic references, as well as a pool of positive samples from convalescent patients with high IgG level to all three antigens and negative controls (pre-COVID era samples, blank and IgG, 1 µg/ml; #I4506, Millipore-Sigma, Oakville, ON, Canada) were also added to each plate in a 4-fold dilutions series to enable quality controls across the plates and batches of samples. For each assay, $\log_{10}$ raw values and relative ratio of samples were compared to prior runs to confirm that the sample density distribution is within range; automated scripts, blinded to sample description and meta-data were used to extract relative ratios to the synthetic references. The assay was calibrated to the World Health Organization (WHO) reference (Code 20/136, National Institute for Biological Standards and Control, NIBSC, Potters Bar EN6 3QG, United Kingdom); a table of conversion of relative ratios for each assay to Binding International Units/ml (BAU/ml) is provided (Supplemental Table 4). Seropositivity was defined based on both receiver operating characteristic (ROC) analysis of negative (pre-COVID era) and positive (PCR confirmed COVID-19 cases) samples (<1% false positive rate threshold) and on deviation from the log means of the negative controls (≥ 3 standard deviations). In some of the figures, the median convalescent values for serum samples from 340 PCR confirmed COVID-19 cases 21–115 days after symptom onset(31) is displayed as a reference point. Since the assays saturate in

healthy controls after two doses of vaccine, all samples were processed both at the dilution used for determination of seroconversion, and a 1/16 further dilution for evaluation of the quantitative differences in antibody responses.

Spike-pseudotyped lentivirus neutralization assays

The lentivirus neutralization assay and the generation of spike pseudotyped lentivirus particles were performed as described previously (32). Briefly, the lentivirus particles were generated by co-transfection in HEK293TN cells (System Biosciences, Palo Alto, CA, USA, LV900A-1) of the Wuhan Hu-1 sequence with a D614G mutation (wild-type SARS-CoV-2), or the variants B.1.617.2 (Delta), B.1.351 (Beta), P.1 (Gamma), and B. 1.1.529 (Omicron constructs with packaging (psPAX2, Addgene, Watertown, MA, USA, #12260) and reporter (luciferase expressing pHAGE-CMV-Luc2-IRES-ZsGreen-W, provided by Drs. Jesse Bloom and Katharine Crawford, Fred Hutchison Cancer Research Center, Seattle, WA) constructs. Heat-inactivated (30 min at 56°C) plasma was serially diluted and incubated with the lentiviral particles (1h, 37°C) prior to addition to cells (HEK293T-ACE2/TMPRSS2, previously described (32) for 48h; luminescence signals were detected with the Bright-Glo Luciferase assay system (Promega, E2620) on an EnVision multimode plate reader (Perkin Elmer. GraphPad Prism 9 was used to calculate 50% neutralization titer (ID_{50}) using non-linear regression. The WHO International Standard (20/136) was evaluated in this assay, and a mean ID_{50} value of 5744 corresponded with 1000 IU/ml.

T cell cytokine secretion assay

Cellular immune responses to COVID-19 vaccination were determined by measuring the release of cytokines and cytotoxic molecules in cell culture supernatants following stimulation with peptide arrays using the LEGENDplex multiplex bead assay as previously described (41, 42). Briefly, $1x10^6$ PBMCs were seeded per well in 96-well round bottom plates with 1 µg/ml each of SARS-CoV-2 spike or Nucleoprotein (NP) peptide pools (JPT peptide technologies, GMBH, Berlin, Germany). PBMCs were cultured with anti-CD28 (clone 9.3, Bio X Cell) and anti-CD3 (clone OKT3, Bio X Cell) as a positive control, or with equimolar DMSO as a negative control. Samples with no response to positive control were not included in the analysis. After 48h incubation at 37°C, cell culture supernatants were collected and stored at -80°C. Release of cytokines and cytotoxic molecules (IL-2, 4, 17, IFN-γ, TNF, Granzyme A,B, Perforin, sFASL) in the supernatants were analyzed using LEGENDplex CD8/NK multiplex cytokine bead assay (BioLegend) as per manufacturer's instructions. Samples were acquired on the BD LSR Fortessa flow cytometer using BD FACSDiva software.

Data are reported as square root (sqrt) transformed values in pg/ml after subtracting background signal from wells containing PBMCs cultured with DMSO containing media alone, as indicated by "Δ".

Statistical analysis

T cell cytokine secretion data were analyzed using the LEGENDplex™ Data Analysis Software Suite, pandas data analysis library for Python and GraphPad Prism v9.3.1 (43). Antibody data were analyzed with R (version 4.1.1) using package ggplot2 and custom R scripts. GraphPad Prism v 9.2.0 was used to analyze the neutralization and antibody data. Models controlled for baseline (timepoint 1) T cell/antibody data and included an interaction term between time point and the variable of interest. All multivariate analyses were performed using R (version 4.1.1) and SAS 9.4. Longitudinal multivariate analysis on antibody data and T cell cytokine secretion was performed using linear mixed models and P 0.05 was considered statistically significant.

Study approval

This study was approved by the ethics boards of the University of Toronto (REB protocol #27673), Mount Sinai Hospital/Sinai Health System (MSH REB #21-0022-E), University Health Network-Toronto Western Hospital division (REB # 21-5096) and Women's College Hospital (REB approval 2021-0023-E). Written informed consent was obtained from all participants prior to participation.

Funding

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Results

Study population and design

Of 177 initially recruited subjects, 150 met the inclusion criteria for this study (see methods). PBMCs and plasma were collected for T cell and antibody responses at up to four time points, before and after vaccination with mRNA vaccines (Figure 1A). This cohort was vaccinated according to Canadian scheduling guidelines at the time, resulting in a median time between dose 1 and dose 2 of the mRNA vaccines of 60.5 days, IQR [45.5-72]. Baseline characteristics of

the study subjects are shown in Table 1. Of note, age, and BMI, but not vaccine interval, were significantly different between groups and multivariate analysis of the data took these differences into account (Supplemental Tables 1-3). The patients ultimately analyzed included 26 healthy controls, 9 IMID patients not on treatment, 44 IMID patients on anti-TNF, 16 on anti-TNF with MTX/AZA, 10 on anti-IL-23, 28 on anti-IL-12/23, 9 on anti-IL-17 and 8 on MTX/AZA.

Antibody responses are reduced in anti-TNF treated subjects and wane over time

Antibody responses were measured by automated ELISA (Enzyme Linked Immunosorbent Assay; (31) see methods). For the entire cohort, antibody responses increased from T1 to T2 to T3, and decreased by T4 (Figure 1B; see Supplemental Table 4 for conversion to World Health Organization standards). Responses to nucleocapsid (NP) were used to rule out exposure to SARS-CoV-2 (Supplemental Figure 1). After the first dose (T2), 97.6% and 80% of participants seroconverted for spike and RBD IgG, respectively, and the relative ratios were greater than the medians of the convalescents in 44.6% and 13% of the participants (Figure 1B, Supplemental Table 5). Seroconversion increased to 100% for spike and 99.2% for RBD soon after the second dose (T3) and the anti-spike and anti-RBD IgG levels were greater than the median levels of convalescent patients in 96.1% and 85.3% of participants, with a median relative ratio of 1.91 for spike and 1.55 for RBD (Figure 1B, Supplemental Table 5). Analysis of antibody responses by vaccine type at T3 showed that two doses of the mRNA-1273 vaccine elicits a stronger humoral response than BNT162b, with mixed mRNA vaccines inducing significantly higher levels of anti-spike and anti-RBD IgG than two doses of BNT162b (Supplemental Figure 2A). Although all data were included in the figures, as most of the cohort was vaccinated twice with BNT162b2, univariate statistical analysis between treatment groups was performed only on samples from the BNT162b/BNT162b participants. Among the BNT162b/BNT162 cohort, males had a slightly lower response to RBD than females, whereas antibody response differences by age were not significant (Supplemental Figure 2B, C).

Participants undergoing anti-TNF, and anti-TNF+MTX/AZA therapies had significantly lower levels of anti-RBD and anti-spike antibodies than those in the healthy control, IMID-untreated, and anti-IL-12/23 groups after the first dose of vaccine (Figure 1C, Supplemental Figure 1). Comparison between the groups after the second dose (T3) indicates that for the BNT162b/BNT162b group, participants taking anti-TNF had significantly lower levels of anti-spike IgG than those in the healthy control, untreated IMID patients, and anti-IL-12/23 groups

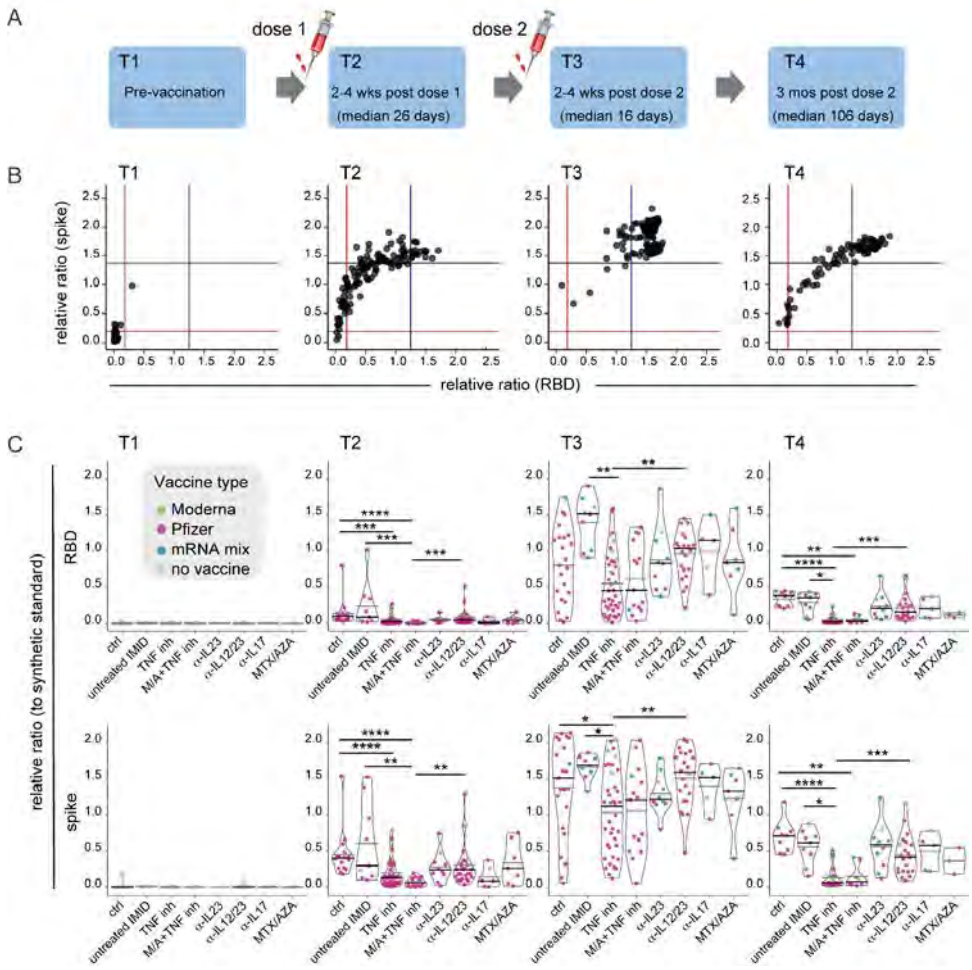

Figure 1. Antibody responses after 1 or 2 doses of mRNA vaccine. (A) Schematic diagram of the sampling schedule**. (B)** IgG response to vaccination across all participants at each time point (defined in Figure 1A). Anti-spike (y-axis) and anti-RBD (x-axis) IgG levels at indicated time points. The dark blue line is the median ratio in convalescent patients (340 samples collected 21 to 115 days post symptom onset). The red line is the seropositivity threshold, set to pass both a 1% false positive rate (FPR) and ≥3 standard deviations from the $\log_{10}$ means of the negative controls. See Supplemental Table S5 for percentage of samples that pass these thresholds. **(C)** IgG responses to vaccination in IMID patients. Violin plots show the relative ratios of RBD and spike at the indicated time points in IMID patients under mono- and combination therapy (0.0039 µl sample used, see Supplemental Figure 1 for the second dilution and Supplemental Table 4 for conversion to BAU/ml). T1, n=111; T2 n= 130, T3, n=130, T4, n= 87. The dot colors indicate the type of vaccine, Pfizer refers to BNT162b; Moderna to mRNA-1273; mRNA mix = first dose BNT162b, second dose mRNA-1273. MTX = methotrexate, AZA = azathioprine. Black and gray lines indicate median and mean ratio values for each violin, respectively. Plots are faceted based on the groups/treatments. Comparisons were made by Dunn's multiple comparisons test based only on the BNT162b/BNT162b group. **p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001.*

(Figure 1C; anti-RBD was significant against the untreated IMID but not the healthy controls). Multivariate analysis of treatment groups controlling for age and sex confirmed the deficits in anti-RBD and anti-spike in the anti-TNF group after the second dose, whether the entire cohort or only the BNT162b/BNT162b participants were evaluated (Supplemental Figure 3, Supplemental Table 1).

Anti-RBD and -spike antibody levels decreased by T4 (median 106 days post-dose 2), with a more rapid decline in anti-RBD than anti-spike levels (Figure 1B). At that time point, only 67.8% and 50.5% of the participants show relative ratios greater than the medians of the convalescents for spike and RBD, respectively (Figure 1B, Supplemental Table 5). When data were analyzed by study group, we again observed that the anti-TNF and anti-TNF+MTX/AZA therapy groups were associated with a statistically significant drop in anti-RBD and anti-spike IgG levels compared to the healthy control, untreated IMID, and anti-IL-12/23 groups (Figure 1C, Supplemental Table 1). Multivariate analysis of treatment groups controlling for age and sex confirmed the deficits in anti-RBD and anti-spike in the anti-TNF and anti-TNF+MTX/AZA groups at T4, whether the entire cohort or only the BNT162b/BNT162b participants were evaluated (Supplemental Figure 3, Supplemental Table 1).

IMID patients undergoing anti-TNF therapy show significantly lower neutralization responses than other groups

To verify whether the observed deficits in binding antibody detected by ELISA were accompanied by alterations in neutralization potential, we performed spike-pseudotyped lentiviral neutralization assays with serum from T3 and T4 using spike protein from either wild-type strain, or the B.1.351 (Beta), P.1 (Gamma), B.1.617.2 (Delta), and/or B. 1.1.529 (Omicron) variants of concern (VOCs). Across all participants at T3, samples neutralized the wildtype strain more efficiently than any of the VOCs tested (Supplemental Figure 4A). Previous studies have shown that antibody binding to either spike or RBD generally correlates with neutralization activity (32). Consistently, Spearman's correlations (ρ = 0.59-0.67) were detected between anti-spike or anti-RBD, but not NP IgG levels and neutralization of either the wild-type lentivirus (Figure 2A) or each of the VOCs (Supplemental Figure 4B). Participants on anti-TNF and anti-TNF+MTX/AZA showed significantly lower neutralization response to the wild-type and all variants, as compared to controls or untreated IMID groups (Figure 2B), consistent with the ELISA data.

Table 1. Characteristics of study participants at baseline and vaccination interval between two doses of an mRNA vaccine

	control	untreated IMID	Anti-TNF agents	MTX/AZA + anti-TNF agents	anti-IL-23 agents	Anti-IL-12/23 agents	Anti-IL-17 agents	MTX/AZA	
	n=26	n=9	n=44	n=16	n=10	n=28	n=9	n=8	Sig. (p-value)
IMID*	N/A								
IBD		9	30	10	0	27	0	4	
Psoriasis		1	3	1	8	1	2	2	
PsA		0	7	3	2	1	7	2	
AS		0	8	3	0	0	1	0	
RA		1	1	0	0	0	1	1	
Age median years [IQR]	36 [26-46]	33 [27-41]	38 [30-51]	53 [44-59]	48 [45-61]	34 [28-47]	49 [46-61]	42 [31-55]	<0.001^
Sex male (%)	16 (62)	5 (56)	18 (41)	8 (50)	5 (50)	13 (46)	6 (67)	4 (50)	0.772~
BMI median kg/m2 [IQR]	25 [23-28]	26 [22-27]	22 [24-26]	26 [24-28]	27 [24-35]	22 [21-24]	32 [26-34]	26[25-33]	0.001^
Vaccine interval median days [IQR]	74 [35-84]	54 [31-64]	60 [45-69]	64 [50-72]	74 [35-84]	62 [49-69]	65 [52-75]	58 [21-97]	0.372^

*multiple IMIDs per patient possible ^Kruskal Wallis test. ~Fisher's exact test. **1 patient in this study group was also on methotrexate. IMID; immune mediated inflammatory disease, TNF; tumor necrosis factor alpha, MTX, methotrexate; AZA, thiopurine, IL; interleukin, n; number, N/A; not applicable, IQR; interquartile range, BMI; body mass index, sig; significance, IBD; inflammatory bowel disease, PsA; psoriatic arthritis, RA: rheumatoid arthritis, AS; ankylosing spondylarthritis, kg/m^2; kilogram per square meter

In accordance with the waning binding antibody levels detected across all samples at T4, median neutralization was reduced in comparison to T3 for both the wild-type and Delta lentiviruses that were profiled across both time points (Supplemental Figure 4, compare panels A and C). The participants on anti-TNF and anti-TNF+MTX/AZA again showed significantly lower neutralization response in comparison to the control groups against the wild-type and Delta lentiviruses (Figure 2C). Reduction in the neutralization ability in other treatment groups was also observed at T4, including the anti-IL12/IL23 group which showed significantly lower neutralization of both the wild-type and the Delta lentiviruses as compared to the control group (Figure 2C). Consistent with recent reports (33-37), Omicron spike-pseudotyped lentiviral particles were about an order of magnitude more difficult to neutralize than wild-type and Delta variants, across the T4 samples (Supplemental Figure 4C). Moreover, sera from anti-TNF, anti-TNF+MTX/AZA or MTX/AZA showed no detectable neutralization of Omicron in our assay, with all treatment groups showing significant defects in neutralization compared to the controls (Figure 2C). Overall, these data demonstrate weaker neutralization responses to mRNA vaccines at all the time points and for all VOCs tested for the participants on anti-TNF agents, and a mixed response for the other treatment groups, with an exacerbation of these deficits at T4 and against Omicron.

IMID patients show increased T responses to successive vaccine doses, with greater waning after dose 2

To assess memory T cell responses to SARS-CoV-2, PBMCs were stimulated with spike or NP peptide pools for 48hrs. A quantitative multiplex bead-based immunoassay was used to measure the levels of 9 secreted cytokines and cytotoxic molecules in the supernatants in response to spike peptide stimulation and results are reported after subtracting the values from negative control wells. The response to NP was used as an additional control to detect memory responses to previous virus exposure. NP-specific responses pre-vaccination were minimal, consistent with study subjects being SARS-CoV-2 naïve and suggesting minimal impact of cross-reactive T cells from previous coronavirus infections (Supplemental Figure 5). The cytokines IFN-γ, IL-2, IL-17A and IL-4 were increased over baseline (T1) after one or two doses of mRNA vaccine in all patient groups (T2 and T3), with the response predominantly of the Th1 phenotype as characterized by high levels of IFN-γ and IL-2 (Figure 3, Supplemental figure 6). Molecules associated with cytotoxicity such as granzyme (Gzm) A, GzmB, perforin and sFasL were also increased over baseline following one dose of vaccine and did not consistently increase with the second dose (Figure 4, Supplemental Figure 6). TNF was not detected over baseline (data not shown). Most study groups showed a wide range of responses to spike peptide

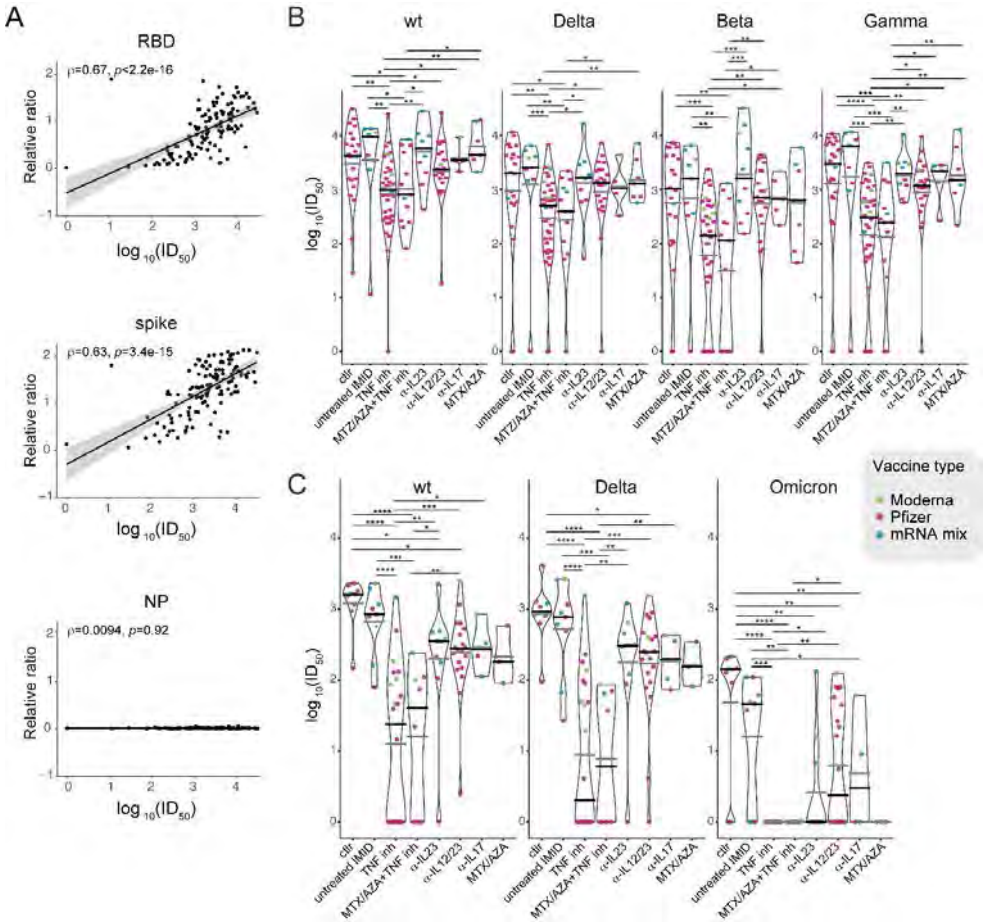

Figure 2. Variant neutralization after two doses of vaccine. **(A)** Spearman correlation at T3 between the indicated antibody levels determined by ELISA (y axis) and the neutralization of the wild-type spike lentivirus (x axis; see Supplemental Figure S4B for correlations with the VOCs). **(B, C)** Violin plots of $\log_{10}$ (ID_{50}) – the serum dilution that inhibits 50% of the lentivirus infection – values of samples at (**B**), T3 (2-4 weeks post dose 2), n=129 and (**C**), T4 (3 months post dose 2), n=86. Lentiviral particles used: wild-type (Wuhan Hu-1 sequence with a D614G mutation), B.1.617.2 (Delta), B.1.351 (Beta), P.1 (Gamma), and B. 1.1.529 (Omicron). The distribution is stratified by study groups/treatments. The dots colors indicate the type of vaccine. Black lines indicate the median and gray lines the mean ratio value for each violin. Comparisons were made by Dunn's multiple comparisons test for the entire cohort. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, ****$p \leq 0.0001$.

pools after first or second vaccine doses (Figures 3, 4, Supplemental Figure 6) When multivariate analysis was performed on the BNT162b/BNT162b group only, after controlling for age and sex we observed deficits in IFN-γ production in the untreated IMID, anti-TNF, MTZ/AZA, anti-12/23 and anti-IL-23 treatment groups relative to healthy controls at T2, which largely recovered by T3 (Supplemental Figure 7, Supplemental Table 1). However, by T4, IFN-γ and IL-2 responses were lower in most treatment groups as well as in untreated IMID patients relative to controls (Supplemental Figure 7, Supplemental Table 1). When results from all subjects were pooled, there was an increase in response from first to second dose for all 8 readouts (Figure 5A, B). By 3 months after dose 2 (T4), we saw an overall decrease in IL-2, IL-4, IL-17, sFasL and Granzyme A (Figure 5A, B). We also noted higher IL-4 responses following vaccination with mRNA-1273 compared to BNT162b or mixed doses (Supplemental Figure 8A). Although T cell responses overall were similar based on age or sex (Supplemental Figure 8B, C), multivariate analysis revealed lower IL-4 responses in the over 60 group (Supplemental Table 2).

Levels of secreted IL-2 were positively correlated with plasma IgG against RBD (r=0.50) and whole spike trimer (r=0.51). Similarly, there was a positive correlation between IL-4 and plasma IgG against RBD (r=0.58) and whole spike trimer (r=0.59), and between IFN-γ and RBD IgG (r=0.36) and whole spike trimer IgG (r=0.36) (all p values <0.0001) (Figure 6).

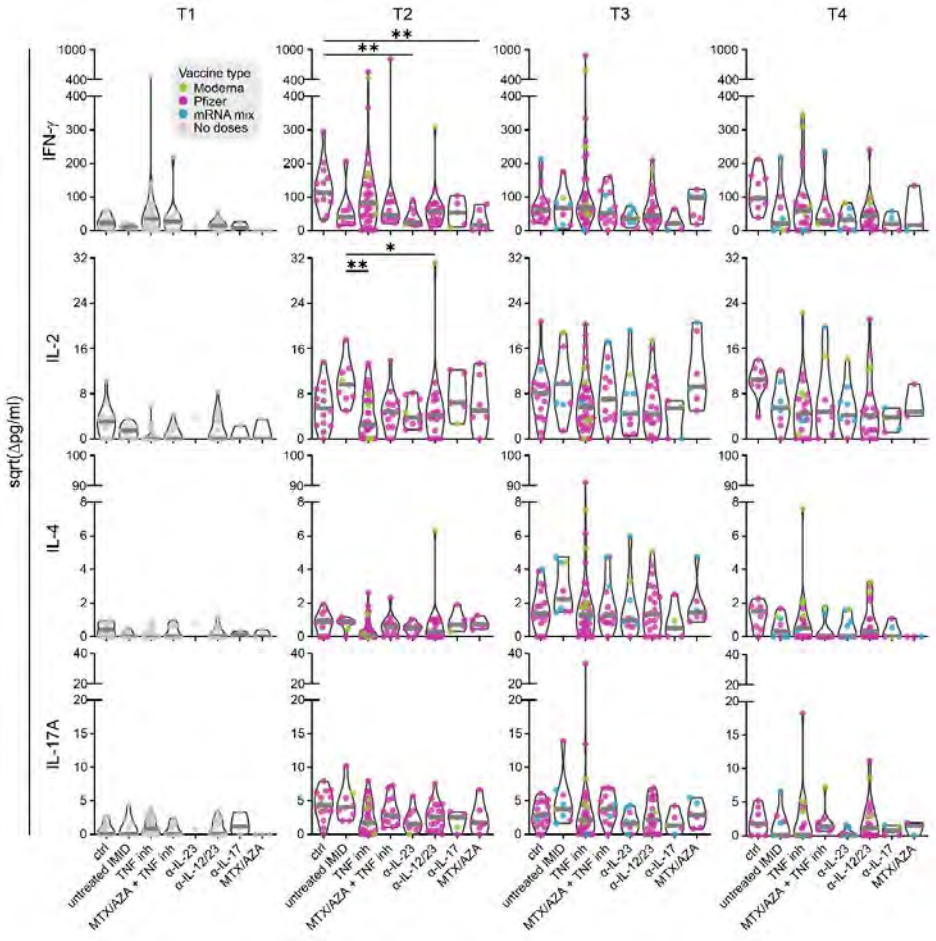

Figure 3. Cytokine responses in each group prior to vaccination and after first and second doses of mRNA vaccine. Cytokine release in cell culture supernatants was analyzed by multiplex bead array following 48h stimulation with SARS-CoV-2 spike peptide pools. Violin plots show IFN-γ, IL-2, IL-17A and IL-4 release at T1 (pre-vaccination); n=100, T2; n=114, T3; n=123, and T4; n= 85, with timepoints defined in Figure 1A. Colored dots represent the type of vaccine as indicated in the inset legend. The gray line indicates the median. Values are reported in pg/ml after subtracting background signal from wells containing PBMCs cultured with DMSO alone, as indicated by "Δ". Ctrl = Healthy controls, inh = inhibitor, MTX = methotrexate, AZA = thiopurines. Comparisons between groups in entire cohort were made by Dunn's multiple comparisons test after excluding outliers and subjects with an NP IgG response. *p≤0.05, **p≤0.01.

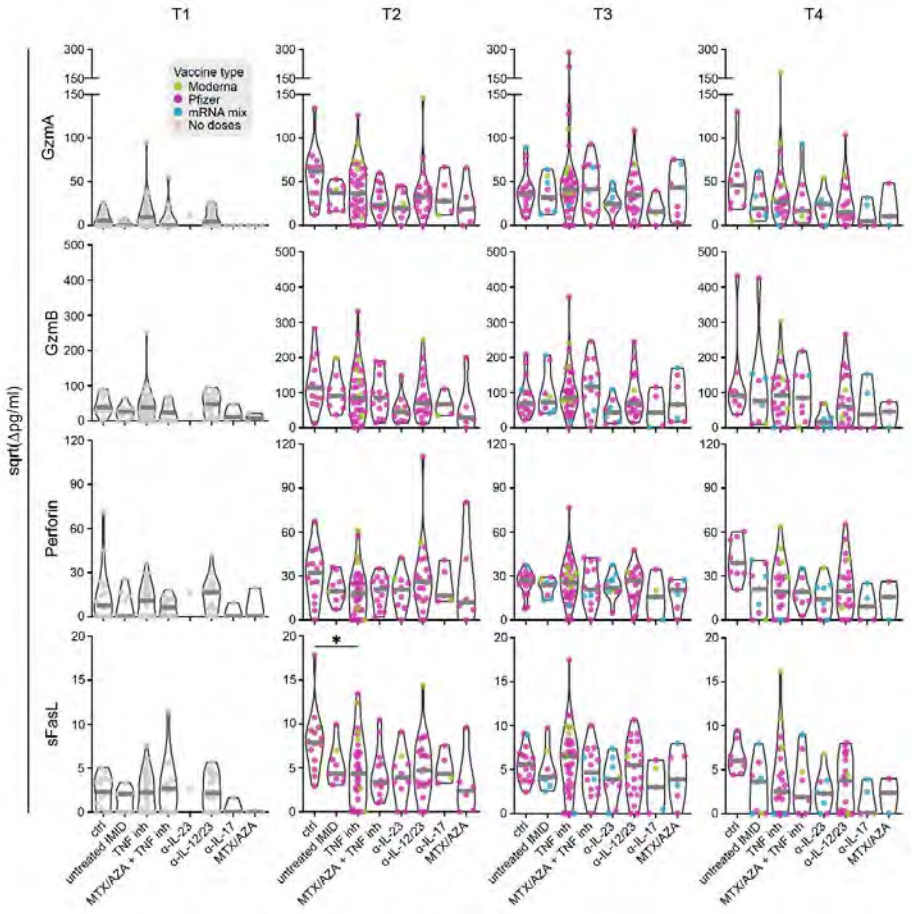

Figure 4. Cytotoxic responses in each group before or after first and second doses of mRNA vaccine. The release of cytotoxic molecules in cell culture supernatants was analyzed by multiplex bead array following 48h stimulation with SARS-CoV-2 spike peptide pools. Violin plots show release of Granzyme (Gzm) A, B, perforin or sFASL release at T1, n=100, T2; n=114, T3; n=123, and T4; n= 85 (with T1-T4 defined in Figure 1A). The dot colors indicate the type of vaccine as indicated in the inset legend. The gray line indicates the median. Values are reported in pg/ml after subtracting background signal from wells containing PBMCs cultured with DMSO alone, as indicated by "Δ". Ctrl = Healthy controls, inh = inhibitor, MTX = methotrexate, AZA = thiopurines. Comparisons on entire cohort were made by Dunn's multiple comparisons test after excluding outliers and subjects with an NP IgG response. *p≤0.05.

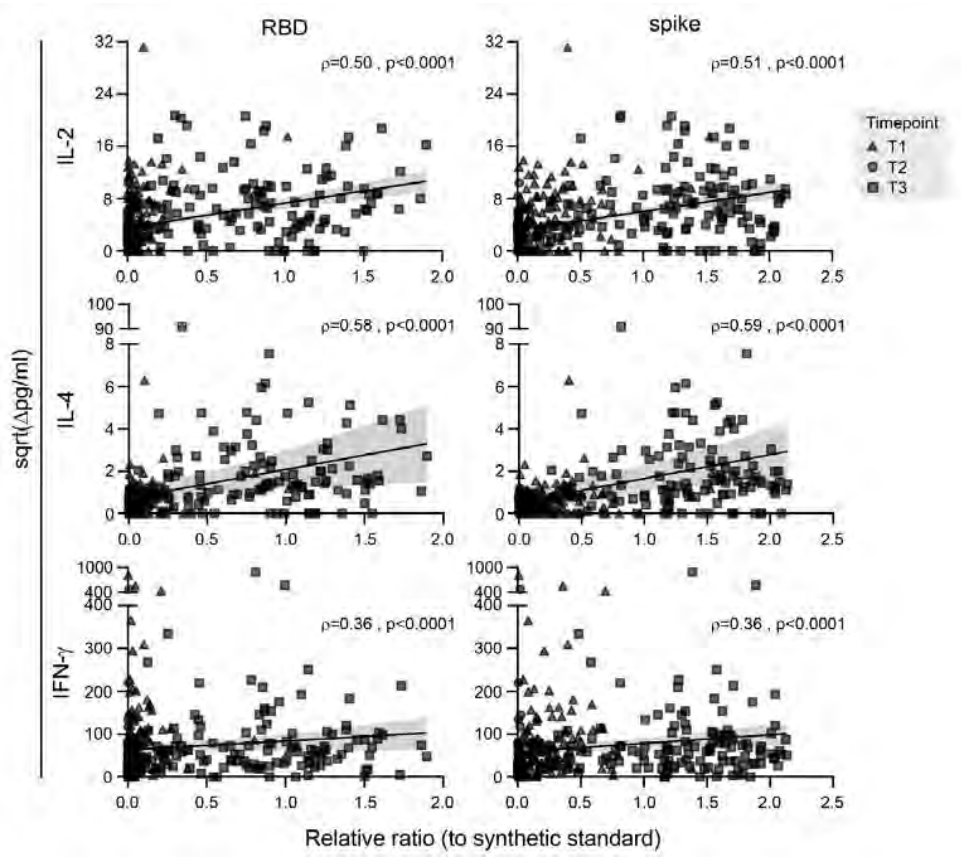

Figure 5. Cytokine and cytotoxic responses in all patient groups in response to spike peptide pools over time. The release of cytokines in cell culture supernatants were analyzed by multiplex bead array following 48h stimulation with SARSCoV-2 S peptide pools. (A) IL-2 and IL-4 responses across all participants at timepoints T1 -T4 as defined in Figure 1A. T1; n=100, T2; n=114, T3; n=123, and T4; n= 85. (B) Violin plots show release of cytokines and cytotoxic molecules in all study subjects pooled. The median is indicated by the gray line. Pairwise comparisons were made by mixed-effects ANOVA after excluding outliers and subjects with an NP IgG response. *p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001.

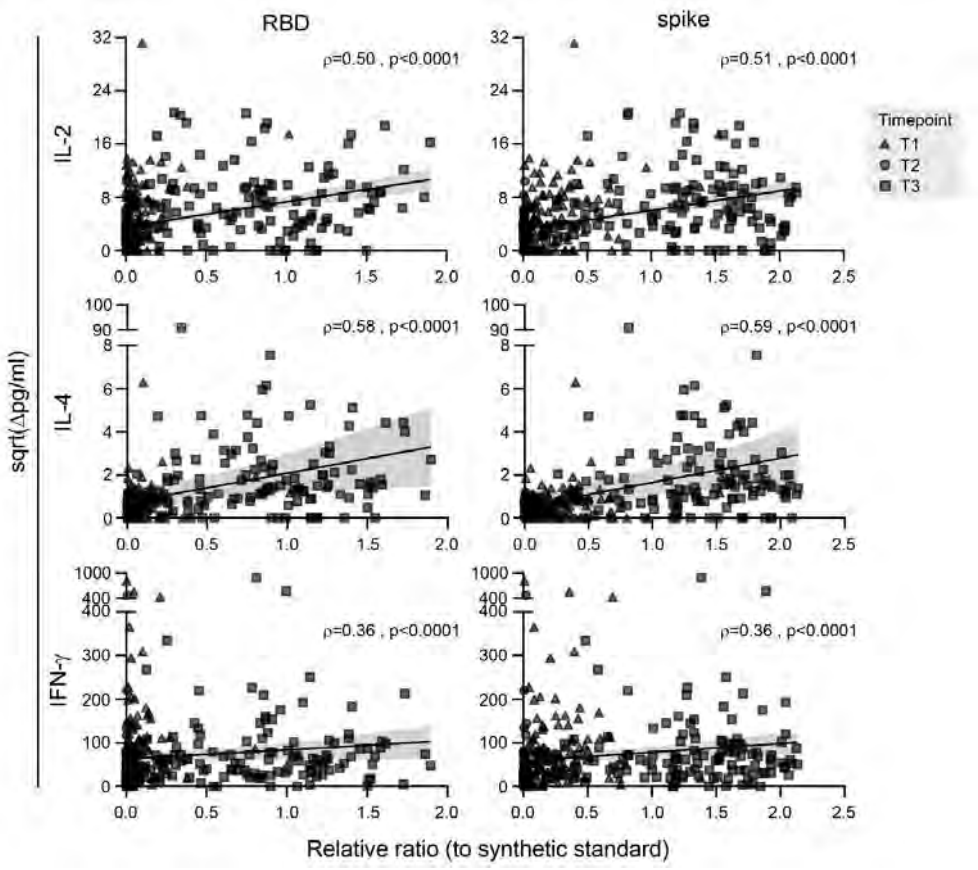

Figure 6. Correlation between IgG levels and T cell cytokine responses at all sampled time points. The solid black line is the linear regression, and the gray shading indicates the 95% confidence interval. p-values and Spearman's rho coefficients are indicated in each graph, n=337.

Discussion

Here we studied a cohort of patients with inflammatory bowel disease, psoriatic disease, ankylosing spondylitis or rheumatoid arthritis, treated with biologics (anti-TNF, anti-IL-12/23, anti-IL-23, anti-IL-17) or antimetabolites to assess their response to COVID-19 mRNA vaccines. There is limited information available on the degree of immunosuppression in this group, raising concern as to how their treatments could impact the response to the vaccines. Although there was considerable variability within groups, 100% of participants seroconverted for spike after 2 doses of vaccine. There was also a clear indication of higher responses to mRNA-1273 vaccine compared to BNT162b vaccine with respect to antibody levels and neutralization titers, as well as T cell IL-4 production. Of concern, antibody

levels and neutralization activity were lower in the anti-TNF treated study subjects even after two doses of vaccine and showed accelerated waning by 3 months post dose 2, with neutralization of the Omicron spike-pseudotyped lentivirus undetectable in this group at that time point We also note that the responses of other treatment groups are markedly reduced against Omicron at T4, compared to healthy controls. Our data are consistent with recent data suggesting reduced vaccine efficacy against Omicron infection in immunocompromised patients (14). The observed waning of antibody responses to mRNA vaccines in anti-TNF patients agree with two other recently published studies (13, 21). We also observed that IMID patients overall showed more substantial waning of both antibody and T cell responses compared to healthy controls.

The vaccine dose interval used in our study was a median of 60.5 days rather than the standard 21 or 28 days. This was due to the policy in place in Canada at the time, to maximize first doses when vaccines were in limited supply. Subsequent analysis has shown that an interval between vaccine dose 1 and vaccine dose 2 of greater than 8 weeks resulted in enhanced humoral and T cell immunity in healthy subjects (38), therefore our longer vaccine interval could impact the results relative to studies which used the standard interval. A limitation of our study is that several of our study groups, particularly those on anti-IL-17, anti-IL-23 or on MTX/AZA as well as the untreated IMID patients, were under powered, making it difficult to draw conclusions about these specific groups, although they do contribute to the overall analysis of the IMID patients as a group. Additional limitations are that we grouped patients together by drug class, regardless of disease or the specific drug product, which may contribute heterogeneity to our results. However, we were underpowered to investigate these differences further.

T cell responses, including IL-4, IL-2 and IFN-γ production, showed a significant correlation with RBD and spike -specific antibody responses. There was substantial induction of T cell cytokines and release of cytotoxic molecules following spike peptide pool stimulation of PBMCs collected following one dose of vaccine and this increased further for all readouts after two doses. Multivariate analysis of the data showed that several groups had decreased IFN-γ after dose 1 of vaccine, but these deficits were largely corrected following the second vaccine dose. When data were pooled for all subjects, it was apparent that cytokine responses including IL-4 and IL-17 were dependent on 2 doses of vaccine. IL-4 is an important mediator of B cell proliferation, which in turn impacts antibody levels and B cell memory (39). This lower IL-4 response after 1 dose as compared to 2 doses of vaccine highlights the need for second doses to maximize B cell responses. Of note, a recent report showed that atopic dermatitis as well as

asthma patients treated with either IL-4 or IL-5 receptor antagonists had reduced antibody responses following two doses of mRNA vaccines compared to healthy controls, consistent with the importance of T cell IL-4 in the antibody response to SARS-CoV-2 mRNA vaccines (40).

Taken together, our study shows generally robust T cell responses in most patient groups treated with immunosuppressants or biologics after 2 doses of mRNA vaccine, improving with a second dose but with significantly more attenuation in IMID patients than healthy controls by 3 months after the second dose. We observed substantial deficits in antibody responses even after two doses of vaccine in the anti-TNF treated patients, with more substantial waning immunity by three months after dose two and a complete inability to neutralize the Omicron variant. These findings highlight the need for a third vaccine dose, particularly in patients undergoing treatment with anti-TNF agents. As there is limited information available about the duration of immune memory induced by mRNA vaccines, it will also be important to follow these responses for longer time periods and to evaluate the impact of additional vaccine doses in this cohort as well as the possible contribution of natural infection to persistence of immune response.

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Supplementary material

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9220925/bin/jciinsight-7-159721-s227.pdf

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9220925/bin/jciinsight-7-159721-s229.xlsx

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9220925/bin/jciinsight-7-159721-s230.xlsx

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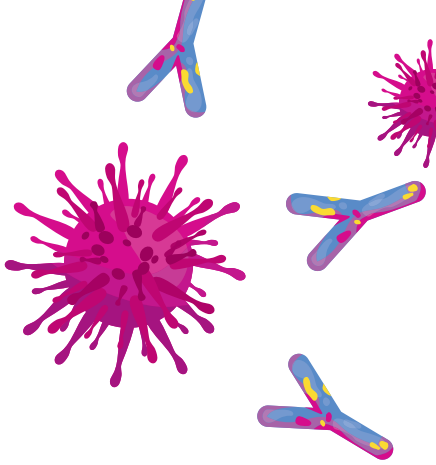
PART V

Discussion



CHAPTER 9

Summary, general discussion and future perspectives

Summary

This thesis aimed to clarify the impact of several viral infections, their complications and vaccination responses in patients with inflammatory bowel disease (IBD). The first part focuses on two herpes viruses, Epstein-Barr virus (EBV) and cytomegalovirus (CMV), that can cause severe opportunistic infections, mostly as a rare complication of the use of immunosuppressants. In the second part we studied the risk of the premalignant condition cervical intraepithelial neoplasia (CIN) and cervical cancer caused by human papillomavirus (HPV) in women with IBD and we aimed to identify risk factors, in particular by studying the exposure to immunosuppressants in detail. The third part describes an uncommon case of a hepatitis E virus (HEV) infection in a patient using the gut-selective biologic agent vedolizumab. In the last part of this thesis, we focused on vaccination responses to influenza vaccination and severe acute respiratory coronavirus 2 (SARS-Cov-2) vaccination in immunocompromised patients with IBD and other immune-mediated inflammatory diseases (IMID). In this chapter, a summary with a general discussion and perspectives on future research opportunities is provided.

General discussion

Part I: Herpesviridae infections in IBD

Most opportunistic infections (OIs) in IBD patients are caused by viruses, mainly the herpesviruses CMV, EBV and other herpesviruses.(1) The use of thiopurines is the most important risk factor for these viral infections.(1) OIs can be defined as infections that generally have limited or no capacity to result in severe disease in immunocompetent individuals, but can become problematic in patients that are immunocompromised.(2, 3) Most herpesviruses are acquired early in life, often asymptomatic, and remain latent lifelong in immunocompetent people. Because of decreased immunosurveillance by specific T-cells, either caused by medication or an altered immunity due to the inflammatory disease itself, proliferation of infected cells causes reactivation of the latent virus.

In **Chapter 2**, we described three IBD patients with an opportunistic EBV-infection with different disease manifestations. Two patients, both treated with a thiopurine for IBD, presented with severe haematological complications caused by EBV. In one case we diagnosed an EBV-related mucocutaneous ulcer (EBV-MCU) in the rectum of a 34-year-old male. This rare lymphoproliferative disease is driven by reactivation of a latent EBV infection in the intestinal mucosa and is

caused by age-related immunosenescence or immunosuppressive medication. It may recover completely after cessation of the immunosuppressive medication, although some cases require more intensive therapy.(4) In the other case, we describe a diagnosis of haemophagocytic lymphohistiocytosis (HLH) after a primary EBV infection a 17-year-old female. In this rare condition, which can be fatal, a severe inflammatory response is seen by overactivation of the mononuclear phagocyte system.(5)

In **Chapter 3,** we focused on the attitude towards CMV colitis amongst Dutch (pediatric) gastroenterologists and identified their diagnostic and therapeutic strategies using a web-based survey. We concluded that most Dutch gastroenterologists acknowledge the importance of CMV colitis in IBD. The general attitude towards a CMV infection in IBD was scored a median of 74/100 on a visual analogue scale in the direction of being a disease aggravating factor requiring treatment as opposed to being an innocent bystander. However, agreement on the indications for diagnostic testing was below 75% and we suggest that this may be caused by dissimilar indications for testing that are recommended in the guidelines.(3, 6-8) In case of acute severe colitis, especially if patients are immunocompromised by the use of steroids or other immunosuppressants, a concomitant CMV infection should always be considered. (3, 6-8). There is a high variety of definitions used for CMV colitis and different (use) of diagnostic tests and strategies might complicate a consistent practice for clinicians.(9) Indeed, we showed a high variability in diagnosis and indications of CMV colitis amongst physicians. Most strikingly, many gastroenterologists do not know how their pathologist assesses the presence of CMV on biopsies and most of them do not use a cut-off value to guide treatment. Although not incorporated yet in current guidelines, recent treatment algorithms do suggest using cut-off values for the different diagnostic tests as higher loads of CMV benefit more from antiviral therapy than lower values.(10, 11) Our data raise concern about this variation leading to under- or overtreatment of a potentially severe complication in IBD patients.

Part II Human papillomavirus and cervical neoplasia

Infection with a high-risk human papillomavirus (hrHPV) precedes almost exclusively all cervical cancers. Due to similar mechanisms causing opportunistic herpes virus infections described in part I, immunocompromised women with IBD might theoretically be at increased risk for a persistent hrHPV infection and subsequently cervical cancer.(12) Persistent hrHPV infections can, usually after decades, also lead to vaginal and vulvar cancers in women, penile cancers in men, and anal and oropharyngeal cancers in both men and women.(13) Also,

the risk of genital warts is increased in patients with IBD.(14) The lifetime risk of acquiring an HPV-infection is approximately 80-90% and the majority of these infections are cleared within 2 years. However, about 20% of HPV infections persist and approximately 1% of all infection with an oncogenic HPV subtype in women will eventually undergo malignant transformation into cervical cancer.(15) So far, conflicting evidence has hampered recommendations for women with IBD regarding intensified cervical cancer screening.(16-24) In **Chapter 4,** we studied the risk of cervical neoplasia in women from the Dutch IBD Parelsnoer cohort (PSI) using the nationwide pathology registry (PALGA). We compared the IBD cohort with a cohort from the general population matched in a 1:4 ratio by age and first available year of screening. We showed that women in the IBD cohort had a higher detection rate of high-grade CIN and cervical cancer (CIN2+) compared with women from the matched cohort (standardized detection rate (SDR) 1.27, 95% CI 1.05-1.52), mainly due to a higher detection rate of CIN2 lesions in women aged 35 to 44. The detection rates of CIN1 and CIN3 lesions and cervical cancer individually, were not significantly different between the two cohorts. Since only nine cervical cancers were identified in the entire cohort, we concluded that our sample size was too small to assess the cervical cancer risk. We also showed that women in the IBD cohort more often had persistent or recurrent lesions (0.8%) compared with women in the matched cohort (0.4%). By excluding women with prevalent lesions at baseline, we showed that women in the IBD cohort had a higher rate of progression to CIN2+ as compared to women in the matched cohort. Both factors can be explained by a higher rate of HPV persistence, which is the factor responsible for carcinogenesis.(25)

By having access to detailed data from the PSI database we identified that young age of IBD onset and ileocolonic and/or upper GI disease location in Crohn's disease increased the risk for CIN2+ lesions. Both characteristics may be associated with a more severe IBD phenotype. Smoking was another independent risk factor for CIN2+ lesions in our cohort as has been clearly been identified before, both in the general population (26, 27) and in women with IBD.(17, 23) Theoretically, and as shown by several previous studies, use of immunosuppressive medication increases the risk for CIN2+.(17-20, 24, 28) A current international guideline on cervical cancer screening therefore only recommends an intensified screening approach for IBD women if they are using immunosuppressants.(29) Surprisingly, in this study, we were not able to identify that exposure to thiopurines or biologicals, studied as never, less than 1 year or more than 1 year exposure, was a risk factor for CIN2+. In **Chapter 5**, we studied in more detail the risk of cumulative exposure to immunosuppressive medication in a selection of the PSI cohort with exact known start and stop dates.

In this substudy we showed that for each year of exposure to immunomodulators (thiopurines and methotrexate) the risk of CIN2+ did increase with a hazard ratio of 1.16 (95% CI 1.08-1.25) per treatment year. Cumulative exposure to biologics was not associated with CIN2+. These findings underline previous observations that long-term use of immunomodulators increases the risk for CIN2+,(18, 30) and could suggest a need for intensified screening in this population. This decision requires a careful consideration of burden, costs and benefits as more intensified screening might also increase the detection of transient infections and potentially lead to overtreatment of low-grade CIN lesions.

In **Chapter 4,** we studied coverage rates of cervical smear testing in the IBD cohort and compared these rates with data from the nationwide monitoring of the cervical cancer screening programme. We showed that there was an overall decline in participation in the screening programme, both in women with IBD as in the general population. Also, we found a remarkable decline in coverage rates for IBD women outside of the screening programme. These findings should alert clinicians to stress the importance of cervical smear testing to their patients. The most recent ECCO guideline recommends a cervical smear at IBD diagnosis for all female patients.(3) Based on our data, patients should be encouraged to participate in a national cervical cancer screening programme.

Part III Hepatitis E in Vedolizumab

As literature on HEV infection in IBD patients is limited to several case reports(31-33) and seroprevalence studies(34-36), the impact of this potentially chronic and severe disease in the IBD population is largely unknown. In **Chapter 6**, we describe the outcome of an HEV infection in a 47-year-old patient with Crohn's disease treated with vedolizumab. This case shows that a feco-orally transmitted viral infection has run a benign, self-limiting course despite vedolizumab therapy. Although vedolizumab might theoretically, by its gut-selective mechanism of action, increase the risk of gastrointestinal infections,(37) numerous studies have shown a low risk of serious infections(38-41) and a comparable or even favourable safety profile as compared to anti-TNF.(42, 43) The risk of clostridium difficile or other gastrointestinal infections might be slightly increased, however this risk continues to be evaluated.(39)

Although the HEV infection in this patient ran a self-limiting course, physicians should be vigilant for the possibility of prolonged viremia and chronic hepatitis E in immunocompromised individuals. In case of elevated liver enzymes, hepatitis E should be considered, and in case of a positive HEV RNA test, close monitoring of enzymes and viral load is warranted.

Part IV Vaccinations

Vaccination is one of the most important preventive measures for patients with an immune-mediated inflammatory disease (IMID) like IBD to lower the risk of infectious complications.(44) Next to adhering to routine vaccination programmes including SARS-Cov-2, ECCO specifically recommends vaccination against influenza, pneumococcus, herpes zoster, hepatitis A and B and HPV for patients with IBD.(3) Unfortunately, the immune response to vaccinations may be impaired by the use of immunosuppressants.(3, 45) Immunogenicity of vaccines in the IMID population has mostly been studied in patients using immunomodulators and anti-TNF.(45)

In **Chapter 7**, we studied the immunogenicity of the inactivated trivalent influenza vaccine (TIV) in CD patients using ustekinumab as compared to CD patients using adalimumab and healthy controls. We showed that both humoral and cellular immune responses to TIV were maintained in the ustekinumab group and not impaired as compared to healthy controls. This study suggests that blocking IL-12 and IL-23 does not impact the formation of antibodies and this is in line with a previous study that showed no difference in immune response to pneumococcus and tetanus vaccines in patients with psoriasis treated with ustekinumab compared to healthy controls.(46, 47) We saw significant lower titres for some of the influenza strains in the adalimumab group as compared to healthy controls. However, seroprotection rates were overall sufficient and not significantly different between the study groups. This is line with another Dutch study in rheumatology patients showing sufficient seroprotection rates despite lower titres in anti-TNF treated patients.(48) Numerous other studies have shown lower humoral responses to influenza vaccination in IBD patients treated with anti-TNF, especially when used in combination with an immunomodulator.(49-52) Unfortunately, our study was underpowered to study the effects of combination therapy on the immune response. It remains largely unknown what the clinical consequences are of this reduced humoral response in anti-TNF treated patients. Both serological and cellular parameters can serve as predictors of immune response and correlates of protection. There is increasing evidence suggesting their usefulness in evaluation of vaccine efficiency. As T-cell responses were robust in our study and seroprotection rates were sufficient, patients treated with anti-TNF most likely still benefit from vaccinations and should be strongly encouraged to get vaccinated.

Coronavirus disease 2019 (COVID-19) infections caused by SARS-Cov-2 range from asymptomatic or mild, to severe disease with lethal complications such as progressive pneumonia, acute respiratory distress syndrome and organ

failure driven by a cytokine storm syndrome. Because of their altered immunity and increased risk of serious and opportunistic infections, the management of immunocompromised patients with IBD and other IMIDs and vaccination strategies became a great area of concern when the pandemic started. **Chapter 8** describes the results of a multi-centre prospective cohort study looking at humoral and cellular responses to SARS-Cov-2 vaccination in a large cohort of IMID patients on various biologicals or immunomodulators and healthy controls. We demonstrated that all participants showed seroconversion after two doses of vaccine, with substantial variation in response within the different treatment groups. Of note, higher antibody levels and neutralizing capacity were seen in participants that received the mRNA-1273 (Moderna) vaccine compared with the BNT162b (BionTech/Pfizer) vaccine. We showed that anti-TNF treated patients had lower antibody levels and neutralizing capacity three months after two doses compared with other study groups. Notably, neutralization of the Omicron variant (the main variant of concern at time of publication of this study) was undetectable in the anti-TNF treated patients, and significantly lower in the other IMID patients compared with healthy controls. Significantly lower neutralizing capacity to Omicron was also shown in immunocompromised patients in another study.(53) Although in this study, a third dose did seem to protect well against hospitalization, titres in most patients using immunosuppressants did not increase after a third dose in a recent Dutch study.(54)

In our study, T-cell responses were generally robust in most patient groups and correlated well with the humoral responses. There was however, also significantly more attenuation of T-cell cytokine production in IMID patients as compared to healthy controls three months after the second dose. These results highlighted the need for third doses of SARS-Cov-2 mRNA vaccines and continued monitoring in immunocompromised patients.

Future perspectives

EBV-driven lymphoproliferative diseases are very rare complications in IBD patients and are associated with exposure to thiopurines. Screening for EBV could be a tool to lower their risk and is currently recommended by the ECCO. However, as most EBV-lymphomas are caused by reactivations, and not all lymphomas are EBV-driven, future studies are needed to investigate its added value and cost-effectiveness. As higher levels of EBV DNA in blood have been shown to be predictive for post-transplant related lymphoproliferative diseases (PTLD) in post-transplant patients, studies on EBV load in blood, preferably combined with

EBV load in intestinal mucosa of patients with IBD, could assess if this would be a useful biomarker in this population as well. As more selective and safer treatment options become available, thiopurines may be less frequently used in the near future and this may decrease the risk of EBV related complications. Future studies comparing efficacy and safety between medication classes in long-term follow-up and cost-effectiveness analyses are needed to help further guide decision-making. Both CMV and EBV can be found in the intestinal mucosa of IBD patients with active disease and the clinical significance of this remains a matter of debate. Especially for EBV, future studies are needed to assess if the virus is involved in the disease process and is a perpetuating factor of inflammation requiring antiviral therapy, or resolves upon anti-inflammatory treatment. As for CMV, cut-off values in viral load might predict the risk of response to antiviral therapy and colectomy. Future prospective studies including patients with colitis, especially in the clinical scenario of acute severe colitis with a high risk of colectomy, should incorporate viral detection on biopsies to better understand the presence of CMV and EBV and its consequences. As diagnosis and management of CMV colitis is highly variable among gastroenterologists, strategic studies are needed to provide a more detailed recommendation for this infection in IBD patients in future guidelines, and should preferably include investigating a combination of diagnostic tests including cut-off values to guide therapy.

The risk of high-grade cervical neoplastic lesions is increased in women with IBD and risk factors are smoking, ileocolonic and upper gastrointestinal disease location in women with CD. The risk increases with each year of exposure to immunomodulators. An intensified cervical cancer screening programme might detect lesions timely, however future studies are needed to identify if a certain approach improves morbidity from CIN lesions and cervical cancer and is cost-effective. ECCO recommends a cervical smear for each woman at IBD diagnosis, but this approach has not been specifically studied and may increase overtreatment in women that are not at increased risk of complications. Since the start of the HPV vaccination programme, prevalence of genital warts and cervical precancers has significantly declined in the general population, even in unvaccinated persons.(13) This may outweigh the benefits of an intensified screening programme in the following years. The effect of vaccination on the incidence of cervical neoplasia in immunocompromised individuals is expected to be known soon. Given the burden of other HPV-related (vaginal, vulvar, penile, oral and anal) cancers in both women and men, vaccination in young males is recommended and catch-up vaccinations in adult patients at risk should likely also be considered best practice. The newest HPV vaccine covers nine virus-like particles of HPV instead of the original bivalent and quadrivalent vaccines.

As vaccination response to the HPV vaccine has only been studied once in IBD patients, more vaccine response studies are needed including to this newest vaccine. Also, studies should be performed on cervical neoplasia risk when using new therapies.

Hepatitis E has rarely been studied in IBD patients and diagnostic tools such as antibodies might be impacted by the use of immunosuppressive drugs. More seroprevalence studies are needed to assess the clinical relevance of this infection in the IBD population and the risks of chronic hepatitis E in the immunocompromised patient. HEV has been proposed as a trigger for the development of autoimmune hepatitis (AIH) after measuring higher antibody titers in these patients in two studies.(55, 56) Studying the role of HEV as a trigger for IBD or as a factor contributing to mucosal inflammation might be an interesting experiment.

Although influenza vaccination reduces the risk of infection in immunocompromised patients, little is known about the effects of newer medications on immune response to vaccination. Before the COVID-19 pandemic, studies on vaccine response in immunocompromised IBD and other IMID patients were largely performed in patients using immunomodulators and anti-TNF, and studies on cellular immune responses were limited. Evaluation of vaccine efficacy is usually done by measuring immunogenicity (antibody levels); yet, even in healthy individuals, it remains unclear whether measurement of antibody levels is the optimal correlate of protection.(57) Future studies on immunogenicity of vaccines in IBD patients should include patients treated with new therapies and should look at both humoral and cellular immune responses. Also, studies on morbidity due to influenza infections, effects on quality of life and work productivity and impact of vaccination on these outcomes are scarce or absent. These areas also offer important areas for future research.

During the pandemic, numerous national and international cohort studies on immunogenicity of SARS-Cov-2 vaccination in different patient populations, such as immunocompromised patient with IBD and other IMIDs, were set up – which was right after the newly-developed vaccines were rolled out globally. In the short term, follow-up data are expected on the longevity of antibodies and neutralizing capacity against new variants after three and four doses of vaccination. IBD patients treated with anti-TNF are at risk for lower titres and neutralizing capacity against new variants. However, although immunogenicity to vaccines is blunted in this patient group, studies on clinical outcomes of COVID-19 in IBD patients are reassuring – especially in those treated with anti-

TNF.(58-60) Anti-TNF may even have a protective role in developing the cytokine storm associated with severe COVID-19.(61) Although third and fourth doses for IBD patients are highly recommended, next to studies on immunogenicity, more studies are needed on the clinical benefits of vaccinations such as the risk of breakthrough infections and severe disease. Also, use of antiviral therapy and monoclonal antibodies against SARS-Cov-2 might be indicated for those with a weakened immune response, and studies on the use of these agents are needed.

In conclusion, the world of IBD therapy is rapidly evolving with many new therapeutic options becoming available in the coming years. Even in the five years that it took to complete this thesis, new therapeutic agents such as JAK-inhibitors, S1P modulators and selective anti IL-23 antibodies have become available – or will soon be. This rapidly evolving world, requires a continuous monitoring of safety signals that may not have been apparent in Phase III trials but may still create concern during long-term follow-up. Often, combination therapy with two new (biologic) agents is considered, but lack of safety data limits the combined use of these drugs in clinical practice. Real-world evidence studies using long-term follow-up data comparing efficacy and safety of different (combinations of) agents, preferably from large international registries, will help in identifying those safety signals. These data would allow for a better risk stratification for each individual patient and help clinicians in guiding their patients in more personalized treatment strategies. With this thesis we aimed to contribute in understanding risks of specific viral infections and the immunogenicity of vaccinations in the IBD population. Incorporating results from this research in future guidelines will help clinicians to further optimize care for their patients.

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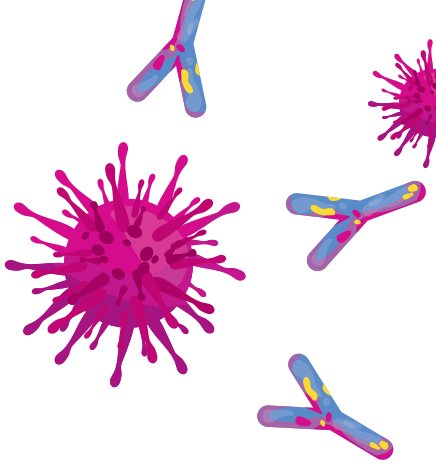
APPENDICES



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Inleiding

Dit proefschrift had tot doel de impact van enkele virale infecties, complicaties daarvan en de vaccinatierespons bij patiënten met inflammatoire darmziekten (IBD) te onderzoeken. Het eerste deel richt zich op twee herpesvirussen, het Epstein-Barr-virus (EBV) en het cytomegalovirus (CMV). Deze virussen kunnen ernstige opportunistische infecties veroorzaken, meestal een zeldzaam gevolg van het gebruik van immuunsuppressiva. In het tweede deel bestudeerden we het risico op de premaligne aandoening cervicale intra-epitheliale neoplasie (CIN) en baarmoederhalskanker welke worden veroorzaakt door het humaan papillomavirus (HPV), bij vrouwen met IBD. We onderzochten welke risicofactoren er zijn, met name door de blootstelling aan immuunsuppressiva in detail te bestuderen. Het derde deel beschrijft een zeldzame casus van een infectie met het hepatitis E-virus (HEV) bij een patiënt die het darm-selectieve medicijn vedolizumab gebruikt. In het laatste deel van dit proefschrift hebben we ons gericht op de immuunrespons op griepvaccinatie en vaccinatie tegen 'severe acute respiratory coronavirus 2' (SARS-Cov-2) bij immuungecompromitteerde patiënten met IBD en andere immuungemedieerde ontstekingsziekten (IMID). In dit hoofdstuk wordt een Nederlandstalige samenvatting gegeven van de algemene discussie en toekomstperspectieven.

Herpesvirus infecties bij inflammatoir darmlijden

De meeste opportunistische infecties (OI's) bij IBD-patiënten worden veroorzaakt door herpesvirussen, zoals CMV en EBV. Het gebruik van de medicatiegroep thiopurines is de belangrijkste risicofactor voor deze virale infecties. OI's kunnen worden gedefinieerd als infecties die over het algemeen niet in staat zijn om ernstige ziekte te veroorzaken bij immuuncompetente individuen, maar dat wel kunnen doen bij mensen met een verzwakt immuunsysteem. De meeste herpesvirussen maakt men op jonge leeftijd door, zijn vaak asymptomatisch, en blijven dan latent levenslang aanwezig. Door verminderde waakzaamheid van bepaalde T-lymfocyten, ten gevolge van medicatie of een veranderde immuniteit als gevolg van de ontstekingsziekte zelf, kan reactivering van het latente virus uit geïnfecteerde cellen optreden.

In **hoofdstuk 2** beschreven we drie patiënten met IBD die een EBV-infectie doormaakten met verschillende ziekteverschijnselen. Twee van hen, beiden behandeld met een thiopurine, maakten een ernstige hematologische complicatie veroorzaakt door EBV door. Bij de eerste patiënt, een 34-jarige man, werd een EBV-gerelateerde mucocutane zweer (EBV-MCU) in het rectum

gediagnosticeerd. Deze zeldzame lymfoproliferatieve ziekte wordt veroorzaakt door reactivering van een latente EBV-infectie in het darmslijmvlies en wordt veroorzaakt door veroudering van cellen of gebruik van immuunsuppressiva. Het beeld kan volledig herstellen na staken van de immuunsuppressiva, hoewel in sommige gevallen een intensievere therapie nodig is. De tweede casus beschrijft een 17-jarige patiënte met de ziekte van Crohn met een hemofagocytische lymfohistiocytose (HLH) na een primaire EBV-infectie. Bij deze zeldzame aandoening leidt een overactivatie van het mononucleaire fagocytensysteem tot een zeer ernstige ontstekingsreactie die fataal kan zijn.

In **hoofdstuk 3** onderzochten we de mening van Nederlandse (kinder-) MDL-artsen over het ziektebeeld CMV-colitis bij IBD. We vroegen naar hun diagnostische en therapeutische strategieën door middel van een online enquête. We concludeerden dat de meeste Nederlandse MDL-artsen het belang van CMV-colitis bij IBD onderkennen. Een CMV-infectie wordt vaker gezien als een factor die de onderliggende IBD verergert en behandeling vereist dan als een 'innocent bystander'. Er was echter minder dan 75% overeenstemming over de indicaties voor diagnostisch onderzoek. Mogelijk wordt dit veroorzaakt door de verschillende indicaties die worden aanbevolen in de richtlijnen. Ook wordt er een grote verscheidenheid aan definities voor CMV-colitis gebruikt. Verschillen in (gebruik van) diagnostische testen en teststrategieën zou een consistente handelwijze voor clinici kunnen bemoeilijken. We toonden inderdaad een grote variabiliteit aan onder artsen in diagnostiek naar en behandeling van CMV-colitis. Opvallend was dat veel MDL-artsen niet weten hoe hun patholoog de aanwezigheid van CMV op biopsieën beoordeelt. De meesten gebruiken geen afkapwaarde om de behandeling te sturen. Hoewel afkapwaardes nog niet zijn opgenomen in de huidige richtlijnen, suggereren recent gepubliceerde behandelingsalgoritmen wel degelijk dat het gebruik van afkapwaarden zinvol is. Patiënten met een hogere CMV-load hebben namelijk meer baat bij antivirale therapie dan patiënten met een lage CMV load. Onze uitkomsten zijn verontrustend omdat deze praktijkvariatie tot onder- of overbehandeling van een mogelijk ernstige complicatie bij IBD-patiënten zou kunnen leiden.

Humaan papillomavirus en cervicale neoplasie

Baarmoederhalskanker wordt vrijwel altijd voorafgegaan door een infectie met een hoog risico humaan papillomavirus (hrHPV). Op een vergelijkbare manier zoals herpesvirussen opportunistische infecties kunnen veroorzaken en zoals beschreven in deel I, zouden immuungecompromitteerde vrouwen met IBD in theorie een verhoogd risico kunnen lopen op een persisterende

infectie en vervolgens baarmoederhalskanker. Persisterende hrHPV-infecties kunnen, meestal na tientallen jaren, ook leiden tot vaginale en vulvaire kanker, peniskanker bij mannen en anale en mond- en keelkanker bij zowel mannen als vrouwen. Ook is het risico op genitale wratten verhoogd bij patiënten met IBD. Het lifetime risico op het krijgen van een HPV-infectie is ongeveer 80-90% en de meeste van deze infecties verdwijnen binnen 2 jaar. Ongeveer 20% van de cervicale HPV-infecties persisteert en ongeveer 1% van alle HPV-infecties met een oncogeen subtype zal uiteindelijk ontaarden in baarmoederhalskanker. Tegenstrijdige onderzoeksresultaten maken het lastig om aanbevelingen te doen met betrekking tot (geïntensiveerde) baarmoederhalskankerscreening voor vrouwen met IBD. In **hoofdstuk 4** bestudeerden we het risico op cervicale neoplasie bij alle vrouwen in het Nederlandse IBD Parelsnoer cohort (PSI) met behulp van de landelijke pathologieregistratie (PALGA). Het IBD cohort werd vergeleken met een cohort uit de algemene bevolking, gematcht naar leeftijd en jaar van de eerst beschikbare testuitslag in een 1 op 4 verhouding. We toonden aan dat er in het IBD-cohort een hoger detectiepercentage was van hooggradige CIN en baarmoederhalskanker (CIN2+) in vergelijking met het gematchte cohort (standardized detection rate (SDR) 1,27, 95% CI 1,05-1,52), voornamelijk vanwege een hoger detectiepercentage van CIN2-laesies bij vrouwen van 35 tot 44 jaar. De detectiepercentages van CIN1- en CIN3-laesies en baarmoederhalskanker afzonderlijk waren niet significant verschillend tussen de twee cohorten. Aangezien slechts negen patiënten met baarmoederhalskanker werden geïdentificeerd in het gehele cohort, concludeerden we dat onze onderzoekspopulatie te klein was om het risico op baarmoederhalskanker te kunnen beoordelen. We toonden ook aan dat vrouwen in het IBD-cohort vaker persisterende of terugkerende afwijkingen hadden (0,8%) in vergelijking met vrouwen in het gematchte cohort (0,4%). Door vrouwen bij wie het eerst beschikbare cervixonderzoek reeds afwijkend was uit te sluiten in een andere analyse, toonden we aan dat vrouwen in het IBD-cohort sneller CIN2+ ontwikkelden vergeleken met vrouwen uit het gematchte cohort. Beide factoren kunnen worden verklaard door meer persisterende HPV-infecties, wat verantwoordelijk is voor de carcinogenese.

Met behulp van gedetailleerde klinische gegevens uit de Parelsnoer database, konden we aantonen dat IBD-specifieke ziektekenmerken, zoals jonge leeftijd bij ontstaan van IBD en ileocolonische en/of ziektelocatie in de bovenste tractus digestivus bij de ziekte van Crohn, het risico op CIN2+ laesies verhogen. Roken was ook een onafhankelijke risicofactor voor CIN2+ laesies in ons cohort. Dit werd eerder reeds in andere studies aangetoond, zowel in de algemene populatie als in de IBD populatie. Theoretisch gezien, en zoals blijkt uit verschillende

eerdere onderzoeken, verhoogt het gebruik van immuunsuppressiva het risico op CIN2+. Een huidige internationale richtlijn voor baarmoederhalskankerscreening adviseert daarom alleen een geïntensiveerde screening voor vrouwen met IBD die immuunsuppressiva gebruiken. Verrassend genoeg konden we in deze studie niet vaststellen dat blootstelling aan thiopurines of biologicals een risicofactor voor CIN2+ was, waarbij blootstelling alleen werd bestudeerd als nooit, minder dan 1 jaar of meer dan 1 jaar blootstelling. In **hoofdstuk 5** bestudeerden we het risico op cumulatieve blootstelling aan immuunsuppressiva in een selectie van het Parelsnoer cohort waarvan de exacte start- en stopdata bekend waren. In deze substudie lieten we zien dat blootstelling aan immuunmodulatoren (thiopurines en methotrexaat) het risico op CIN2+ vergroot met een hazard ratio van 1,16 (95% CI 1,08-1,25) per behandelingsjaar. Cumulatieve blootstelling aan biologicals was niet geassocieerd met CIN2+. Deze bevindingen bevestigen eerdere observaties dat langdurig gebruik van immuunmodulatoren het risico op CIN2+ verhoogt en zouden aanleiding kunnen geven tot intensievere screening van deze groep patiënten. Deze beslissing vereist een zorgvuldige afweging van lasten, kosten en baten, aangezien intensievere screening ook de detectie van voorbijgaande infecties zou kunnen verhogen en daarmee zou kunnen leiden tot overbehandeling van laaggradige CIN-laesies.

In **hoofdstuk 4** hebben we de dekkingsgraad van het laten nemen van uitstrijkjes in het IBD-cohort bestudeerd en vergeleken met gegevens uit de landelijke monitoring van het bevolkingsonderzoek naar baarmoederhalskanker. We lieten zien dat deelname aan het bevolkingsonderzoek in het algemeen afnam, zowel bij vrouwen met IBD als bij de algemene bevolking. Ook vonden we een opmerkelijke daling van de dekkingsgraad bij vrouwen met IBD buiten het bevolkingsonderzoek om. Deze bevindingen maken duidelijk dat er meer aandacht moet zijn onder artsen voor het benadrukken van het belang van het laten afnemen uitstrijkjes. De meest recente ECCO-richtlijn adviseert om bij elke vrouw bij het vaststellen van IBD een uitstrijkje te adviseren. Op basis van onze gegevens moeten patiënten ten minste worden aangemoedigd om deel te nemen aan een nationaal screeningsprogramma voor baarmoederhalskanker.

Hepatitis E in Vedolizumab

Aangezien de literatuur over hepatitis E bij IBD-patiënten beperkt is tot enkele casusbeschrijvingen en studies naar de seroprevalentie van HEV, is de impact van deze, potentieel chronische en ernstige, ziekte in de IBD-populatie grotendeels onbekend. In **hoofdstuk 6** beschrijven we het beloop van een HEV-infectie bij een 47-jarige patiënte met de ziekte van Crohn die werd behandeld met vedolizumab. Deze casus laat zien dat een feco-oraal overdraagbare virale infectie ondanks

behandeling met vedolizumab een gunstig beloop heeft gehad. Hoewel vedolizumab in theorie, vanwege een darm-selectief werkingsmechanisme, het risico op gastro-intestinale infecties zou kunnen verhogen, wordt tot nu toe een laag risico op ernstige infecties en een vergelijkbaar of zelfs gunstig veiligheidsprofiel in vergelijking met anti-TNF beschreven. Het risico op gastro-intestinale infecties zoals een clostridium difficile infectie is mogelijk licht verhoogd, maar meer onderzoek is nodig om dit te bewijzen. Alhoewel de HEV-infectie in deze casus een gunstig beloop had, dienen artsen waakzaam te zijn voor de mogelijkheid van langdurige viremie en chronische hepatitis E bij immuungecompromitteerde patiënten. Als verhoogde leverenzymen worden gemeten bij een patiënt met IBD moet een hepatitis E worden overwogen en bij een positieve HEV RNA-test is frequente controle van de leverenzymen en de viral load aangewezen.

Vaccinaties

Vaccinatie is een van de belangrijkste preventieve maatregelen voor patiënten met een immuungemedieerde ontstekingsziekte (IMID) zoals IBD om het risico op infectieuze complicaties te verlagen. Naast het volgen van rijksvaccinatieprogramma's beveelt ECCO specifiek vaccinaties aan tegen influenza, pneumokokken, herpes zoster, hepatitis A en B, HPV en SARS-Cov-2. Helaas kan het gebruik van immuunsuppressiva de immuunrespons op vaccinaties negatief beïnvloeden. De immunogeniciteit van vaccins bij patienten met IMID's, is voornamelijk bestudeerd bij patiënten die immunomodulatoren en anti-TNF gebruiken.

In **hoofdstuk 7** bestudeerden we de immunogeniciteit van het geïnactiveerde trivalente griepvaccin (TIV) bij CD-patiënten die ustekinumab gebruikten in vergelijking met CD-patiënten die adalimumab gebruikten en gezonde controles. We toonden aan dat zowel de humorale als de cellulaire immuunrespons op TIV behouden bleven in de ustekinumab-groep en niet verminderd was in vergelijking met gezonde controles. Deze studie suggereert dat het blokkeren van IL-12 en IL-23 geen invloed heeft op de vorming van antilichamen en dit komt overeen met een eerdere studie die geen verschil aantoonde in de immuunrespons op pneumokokken- en tetanusvaccins bij met ustekinumab behandelde psoriasispatiënten in vergelijking met gezonde controles. We zagen wel significant lagere titers voor sommige influenzastammen in de adalimumab-groep in vergelijking met gezonde controles. De seroprotectiepercentages waren echter over het algemeen voldoende en verschilden niet significant tussen de onderzoeksgroepen. Dit komt overeen met een andere Nederlandse studie bij reumatologiepatiënten die voldoende hoge seroprotectie liet zien ondanks

lagere titers bij met anti-TNF behandelde patiënten. Tal van andere studies toonden een lagere humorale respons op griepvaccinatie aan bij IBD-patiënten die werden behandeld met anti-TNF, vooral wanneer gebruikt in combinatie met een immuunmodulator. Helaas had ons onderzoek onvoldoende power om de effecten van combinatietherapie op de immuunrespons te bestuderen. Het blijft grotendeels onbekend wat de klinische gevolgen zijn van deze verminderde humorale respons bij met anti-TNF behandelde patiënten. Zowel serologische als cellulaire parameters kunnen dienen als voorspellers van de immuunrespons en de beschermingsgraad. Er is steeds meer bewijs dat suggereert dat het meten van de cellulaire immuunrespons nuttig is bij de evaluatie van de efficiëntie van vaccins. Aangezien zowel de T-celrespons als de seroprotectiepercentages goed waren in onze studie, hebben patiënten die met anti-TNF worden behandeld zeer waarschijnlijk wel baat bij vaccinaties en adviseren we om ze sterk aan te moedigen om zich te laten vaccineren.

COVID-19-infecties, veroorzaakt door SARS-Cov-2, variëren van asymptomatisch of mild, tot ernstige ziekte met dodelijke complicaties zoals een ernstige pneumonie, het acute respiratory distress syndrome en multi-orgaanfalen veroorzaakt door een cytokinestormsyndroom. Vanwege hun veranderde immuniteit en een verhoogd risico op ernstige en opportunistische infecties, werd de behandeling van immuuncompromitteerde patiënten met IBD en andere IMID's bij de uitbraak van de pandemie een grote bron van zorg. **Hoofdstuk 8** beschrijft de resultaten van een multicenter prospectieve cohortstudie waarin gekeken is naar humorale en cellulaire respons op SARS-Cov-2-vaccinatie in een groot cohort van IMID-patiënten die behandeld werden met biologicals of immuunmodulatoren en gezonde controles. We toonden aan dat alle deelnemers seroconversie vertoonden na twee doses, met substantiële variatie in de respons binnen de verschillende behandelingsgroepen. Er werden meer antilichamen en een beter neutraliserend vermogen waargenomen bij deelnemers die het mRNA-1273 (Moderna) -vaccin kregen in vergelijking met het BNT162b (BionTech/Pfizer) -vaccin. We toonden aan dat met anti-TNF behandelde patiënten drie maanden na twee doses minder antilichamen en minder neutraliserend vermogen hadden dan patiënten in de andere onderzoeksgroepen. Met name neutralisatie van de Omicron-variant, die op het moment van publicatie van deze studie net de belangrijkste variant was, was niet detecteerbaar bij de met anti-TNF behandelde patiënten en significant lager bij de andere IMID-patiënten in vergelijking met gezonde controles. Het neutralisatievermogen van de Omicron-variant was in een ander onderzoek ook significant lager bij immuungecompromitteerde patiënten.

Hoewel in dit onderzoek een derde dosis zeer goed leek te beschermen tegen ziekenhuisopname, namen de titers niet toe na een derde dosis bij patienten die immuunsuppressiva gebruikten in een recente Nederlandse studie.

In onze studie was de T-celrespons over het algemeen robuust in de meeste patiëntengroepen en correleerde deze goed met de humorale respons. Er was echter significant minder productie van T-cel cytokinen bij IMID-patiënten dan bij gezonde controles drie maanden na de tweede dosis. Deze resultaten benadrukten de noodzaak van derde doses SARS-Cov-2-mRNA-vaccins en monitoring bij immuungecompromitteerde patiënten.

Toekomstperspectieven

EBV-gerelateerde lymfoproliferatieve ziekten zijn zeer zeldzame complicaties bij IBD-patiënten en zijn geassocieerd met blootstelling aan thiopurines. Screening op EBV zou een hulpmiddel kunnen zijn om hun risico op lymfomen te verlagen en wordt momenteel aanbevolen door de ECCO. Aangezien de meeste EBV-lymfomen echter worden veroorzaakt door reactivaties in plaats van primaire infecties en niet alle lymfomen EBV-gerelateerd zijn, moet de toegevoegde waarde en kosteneffectiviteit van EBV screening beter worden onderzocht. Het is aangetoond dat een hogere viral load van EBV-DNA in het bloed voorspellend is voor het ontstaan van post-transplantatiegerelateerde lymfoproliferatieve ziekten (PTLD) bij transplantatiepatiënten. Het moet nog beter worden uitgezocht of EBV-DNA in het het bloed ook een bruikbare biomarker in de IBD populatie zou kunnen zijn. Aangezien steeds meer selectieve en veiligere behandelingsopties beschikbaar komen, zullen thiopurines in de nabije toekomst wellicht minder vaak worden voorgeschreven en zal het risico op EBV-gerelateerde complicaties hierdoor mogelijk afnemen. Er zijn meer studies nodig die de werkzaamheid en veiligheid tussen medicatiegroepen op de lange termijn vergelijken, alsmede kosteneffectiviteitsanalyses.

Zowel CMV als EBV kunnen frequent worden aangetoond in het darmslijmvlies van IBD-patiënten met actieve ziekte en de klinische betekenis ervan blijft een punt van discussie. Vooral voor EBV is er meer onderzoek nodig om aan te tonen of het virus in de mucosa betrokken is bij het ziekteproces, de ziekte onderhoudt en antivirale therapie vereist, of vanzelf verdwijnt na ontstekingsremmende behandeling. Wat CMV betreft, zouden afkapwaarden in de viral load het risico op succes van antivirale therapie en colectomie kunnen voorspellen. In toekomstige prospectieve studies bij patiënten met colitis, vooral in het klinische scenario van acute ernstige colitis met een hoog risico op colectomie, zouden darmbiopten met CMV en EBV diagnostiek moeten meenemen om de aanwezigheid van deze

virussen en de gevolgen daarvan beter te begrijpen. Aangezien diagnostiek naar en behandeling van CMV-colitis zeer variabel zijn onder MDL-artsen, zijn strategische studies nodig om in toekomstige richtlijnen een meer gedetailleerde aanbeveling voor deze infectie bij IBD-patiënten te geven, en bij voorkeur een combinatie van diagnostische tests inclusief afkapwaarden onderzoeken om de therapie beter te sturen.

Het risico op hooggradige CIN (CIN2+) is verhoogd bij vrouwen met IBD en risicofactoren zijn roken, ziektelocatie in het ileum en colon en de bovenste tractus digestivus bij vrouwen met CD. Het risico verhoogt met elk jaar blootstelling aan immuunmodulatoren. Een geïntensiveerd screeningsprogramma naar baarmoederhalskanker zou afwijkingen tijdig kunnen opsporen, maar toekomstige studies zijn nodig om vast te stellen of een dergelijke aanpak de morbiditeit van CIN-laesies en baarmoederhalskanker verbetert en kosteneffectief is. ECCO beveelt een uitstrijkje aan voor elke vrouw bij het vaststellen van IBD, maar deze benadering is niet specifiek onderzocht en kan leiden tot overbehandeling bij vrouwen die geen verhoogd risico op complicaties lopen. Sinds de start van het HPV-vaccinatieprogramma is het risico op cervicale neoplasie en genitale wratten bij vrouwen in de algemene bevolking drastisch verminderd, zelfs bij niet-gevaccineerde personen. Het zou de komende jaren kunnen blijken dat het eventuele voordeel van een geïntensiveerd screeningprogramma hier niet tegen opweegt. Ook zal binnenkort het effect van vaccinatie op de incidentie van cervicale neoplasie bij immuungecompromitteerde personen kunnen worden verwacht. Vanwege de gevolgen van HPV-gerelateerde (vaginale, vulvaire, penis-, orale en anale) kankers bij zowel vrouwen als mannen, is ook vaccinatie bij jonge mannen aanbevolen en zouden inhaalvaccinaties bij oudere hoog-risico patiënten ook zinvol kunnen zijn. Het nieuwste HPV-vaccin bevat negen virus-like HPV-deeltjes in plaats van de originele bivalente en quadrivalente vaccins. Aangezien de vaccinatierespons op het HPV-vaccin slechts één keer is onderzocht bij IBD-patiënten, zijn meer onderzoeken naar vaccinatierespons nodig, waaronder naar het nieuwste vaccin. Verder moet het risico op cervicale neoplasie bij het gebruik van nieuwe medicatie worden onderzocht.

Hepatitis E is zelden onderzocht bij IBD-patiënten en diagnostische hulpmiddelen zoals antistofmetingen kunnen worden beïnvloed door het gebruik van immuunsuppressiva. Er zijn meer seroprevalentiestudies nodig om de klinische relevantie van deze infectie in de IBD-populatie en de risico's van chronische hepatitis E bij de immuungecompromitteerde patiënt te beoordelen. Het is gesuggereerd dat HEV een uitlokkende factor zou kunnen zijn voor

de ontwikkeling van auto-immuunhepatitis (AIH) na het meten van hogere antilichaamtiters bij deze patiënten in twee studies. Onderzoek naar de rol van HEV als uitlokkende factor voor IBD of als bijdragende factor voor mucosale inflammatie zou een interessant experiment zijn.

Hoewel griepvaccinatie het risico op infectie bij immuungecompromitteerde patiënten vermindert, is er weinig bekend over de effecten van nieuwere medicijnen op de immuunrespons op vaccinatie. Vóór de COVID-19-pandemie waren onderzoeken naar de vaccinrespons bij immuungecompromitteerde IBD- en andere IMID-patiënten grotendeels beperkt tot patiënten die immuunmodulatoren en anti-TNF gebruikten. De cellulaire immuunrespons werd slechts beperkt onderzocht. De evaluatie van de effectiviteit van vaccinaties wordt doorgaans gebaseerd op de immunogeniciteit c.q. hoogte van antilichamen, maar zelfs bij gezonde personen blijft het onduidelijk hoe goed antistoffen de mate van bescherming weergeven. Toekomstige studies naar immunogeniciteit van vaccins bij IBD-patiënten moeten patiënten includeren die worden behandeld met nieuwe therapieën en zouden zowel naar humorale als cellulaire immuunrespons moeten kijken. Studies naar de morbiditeit van influenza, effecten op de kwaliteit van leven en arbeidsproductiviteit en de impact van vaccinatie op deze uitkomsten zijn schaars of afwezig. Dit zouden zinvolle onderwerpen voor toekomstig onderzoek kunnen zijn.

Ten tijde van de pandemie werden wereldwijd verschillende nationale en internationale cohortstudies opgezet naar de immunogeniciteit van SARS-Cov-2-vaccinatie bij verschillende patiëntpopulaties zoals immuungecompromitteerde patiënten met IBD- en andere IMIDs – vlak nadat de vaccins beschikbaar werden. Er worden op korte termijn data verwacht over de levensduur van antilichamen en het neutraliserende vermogen tegen nieuwe varianten na drie en vier vaccinaties. IBD-patiënten die worden behandeld met anti-TNF hebben een risico op lagere antistoftiters en verminderd neutraliserend vermogen tegen nieuwe varianten. Echter, hoewel de immunogeniciteit van het vaccin bij deze groep patiënten verminderd is, zijn studies naar de klinische uitkomsten van COVID-19 bij IBD-patiënten geruststellend, vooral tijdens behandeling met anti-TNF. Anti-TNF kan zelfs een beschermende rol spelen bij het ontwikkelen van de cytokinestorm die gepaard gaat met ernstige COVID-19. Hoewel de derde en vierde dosis voor IBD-patiënten sterk worden aanbevolen, zijn er naast studies naar immunogeniciteit meer studies nodig naar de klinische voordelen van vaccinaties, zoals het risico op doorbraakinfecties en ernstige ziekte. Ook kan het gebruik van antivirale

therapie en monoklonale antilichamen tegen SARS-Cov-2 geïndiceerd zijn voor mensen met een verminderde immuunrespons en meer onderzoek naar het gebruik van deze middelen is nodig.

Conclusie

De wereld van IBD-therapie evolueert snel en de komende jaren worden er veel nieuwe therapeutische opties beschikbaar. Zelfs in de laatste vijf jaren die nodig waren om dit proefschrift te voltooien, zijn er nieuwe therapeutische middelen zoals JAK-remmers, S1P-modulatoren en selectieve anti-IL-23-antilichamen op de markt gekomen of zullen binnenkort beschikbaar zijn. Deze snel evoluerende wereld vereist een continue monitoring van veiligheidssignalen die misschien niet duidelijk waren in fase III-onderzoeken, maar die toch zorgen kunnen baren tijdens de follow-up op de lange termijn. Vaak wordt combinatietherapie met twee nieuwe (biologische) middelen overwogen, maar gebrek aan veiligheidsgegevens maakt het gecombineerde gebruik van deze geneesmiddelen in de klinische praktijk lastig. 'Real-world evidence' studies met lange termijn follow-up data die de werkzaamheid en veiligheid van verschillende (combinaties van) middelen vergelijken, bij voorkeur uit grote internationale registers, zullen helpen bij het identificeren van die veiligheidssignalen. Deze gegevens zouden een betere risicostratificatie voor elke individuele patiënt mogelijk maken en clinici helpen bij het begeleiden van hun patiënten in meer gepersonaliseerde behandelstrategieën. Met dit proefschrift wilden we bijdragen aan het begrijpen van de risico's van specifieke virale infecties en de immunogeniciteit van vaccinaties in de IBD-populatie. Het opnemen van resultaten uit dit onderzoek in toekomstige richtlijnen zal clinici helpen om de zorg voor hun patiënten verder te optimaliseren.

List of abbreviations

5-ASA	5-aminosalicylic acid
95%CI	95% confidence interval
ACE	Angiotensine-converting-enzyme
ALT	Alanine aminotransferase
AMA	Anti-mitochondrial antibodies
ANA	Antinuclear antibody
AP	Alkaline phosphatase
ASMA	Anti-smooth muscle antibody
AST	Aspartate aminotransferase
BMI	Body mass index
CD	Crohn's disease
CI	Confidence interval
CIN	Cervical intraepithelial neoplasia
CMV	Cytomegalovirus
COVID-19	Corona Virus Disease 2019
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr virus
EBV-MCU	EBV-positive mucocutaneuousulcer
ECCO	European Crohn's and Colitis Organization
GGT	Gamma-glutamyltranspeptidase
HEV	Hepatitis E virus
HLH	hemophagytic lymphohistiocytosis
hrHPV	high-risk human papillomavirus
HR	Hazard ratio
HPV	human papillomavirus
IBD	Inflammatory bowel disease
IBD-I	Inflammatory bowel disease indeterminate
IBD-U	Inflammatory bowel disease unclassified
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IHC	Immunohistochemistry
IL	interleukin
IMID	Immune-mediated inflammatory disease
IQR	Interquartile range
IR	incidence rate
IRR	incidence rate ratio
JAK	janus kinase

LDH	Lactate dehydrogenase
mRNA	messenger RNA
N	number, negative
OI	opportunistic infection
OR	Odds ratio
P	positive
PALGA	Pathologisch Anatomisch Landelijk Geautomatiseerd Archief
PCR	Polymerase chain reaction
PSI	Parelsnoer Instituut
PT-INR	Prothrombin time-International ratio
PTLD	Post-transplant related lymphoproliferative diseases
RNA	Ribonucleic acid
S1P	Sphingosine-1-phosphate
SARS-Cov-2	severe acute respiratory syndrome coronavirus-2
SI	serious infection
SIL	squamous intraepithelial lesion
SD	standard deviation
SDR	standardized detection ratio
TNF	tumor necrosis factor α
UC	Ulcerative colitis
WBC	White blood cell count

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Other publications

Mahmoud R, Savelkoul EHJ, Mares W, **Goetgebuer RL,** Witteman BJM, de Koning DB, van Tuyl SAC, Minderhoud I, Lutgens MWMD, Akol-Simsek D, van Schaik FDM, Fidder HH, Jansen JM, van Boeckel PGA, Mahmmod N, Horjus-Talabur Horje CS, Römkens TEH, Colombel JF, Hoentjen F, Jharap B, Oldenburg B; Dutch Initiative on Crohn's and Colitis (ICC) and the AWARE study group.
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*Shared first authorship

PhD Portfolio

Name PhD student: Rogier Goetgebuer
PhD period: April 2018 – December 2022
Erasmus MC department Gastroenterology and Hepatology
Promotor: prof. dr. C.J. van der Woude
Co-promotores: Dr. A.C. de Vries

Courses and workshops

	Year	Workload	ECTS
Scientific Integrity Course, Erasmus MC, Rotterdam	2022	**8 hours**	**0,3**
eBROK course, NFU	2022	**42 hours**	**1,5**
Journal clubs Mount Sinai Hospital, Toronto, Canada	2020-2021	**15 hours**	**0,6**
Journal clubs Erasmus MC, Rotterdam	2018-2020	**30 hours**	**1,2**
Journal clubs Amsterdam UMC, Amsterdam	2021-2022	**15 hours**	**0,6**
Advanced Immunology – *Erasmus Postgraduate School of Molecular Medicine*	2019	**84 hours**	**3,0**
Biomedical English Writing Course - *Erasmus Postgraduate School of Molecular Medicine*		**12 hours**	**0,4**
Endnote Workshop, Erasmus MC, Rotterdam	2018	**8 hours**	**0,3**
Systematic Literature Retrieval in Pubmed workshop, Erasmus MC, Rotterdam	2018	**8 hours**	**0,3**
Systematic Literature Retrieval in other databases workshop, Erasmus MC, Rotterdam	2018	**8 hours**	**0,3**
Basic Introduction of SPSS – *Erasmus Postgraduate School of Molecular Medicine*	2018	**28 hours**	**1,0**
Total			**9,5**

Oral presentations

	Year	Workload	ECTS
CMV in acute colitis; innocent bystander or pathogen? Delta IBD day conference meeting, Leuven, Belgium	2022	**14**	**0,5**
High seroconversion rate to trivalent influenza vaccine during ustekinumab treatment in Crohn's disease: results from a prospective cohort study. 27th United European Gastroenterology Week, Barcelona, Spain	2019	**28**	**1**
High seroconversion rate to trivalent influenza vaccine during ustekinumab treatment in Crohn's disease: results from a prospective cohort study. Nederlandse Vereniging voor Gastroenterologie najaarscongres, Veldhoven, The Netherlands	2019	**14**	**0,5**
Prevalence of cervical dysplasia in women with inflammatory bowel disease: data from the Parelsnoer Institute (PSI) and PALGA database (PAP-IBD study). Nederlandse Vereniging voor Gastroenterologie voorjaarscongres, Veldhoven, The Netherlands	2019	**14**	**0,5**
Severe viral complications in IBD: is pre-emptive EBV and CMV screening useful? Delta IBD day conference meeting, Noordwijk,The Netherlands	2019	**14**	**0,5**
Total			**3,0**

Poster presentations

	Year	Workload	ECTS
Accelerated waning of immunity to SARS-Cov-2 mRNA vaccines in patients with immune mediated inflammatory diseases **Digestive Disease Week, San Diego**	2022	**14**	**0,5**
Women with IBD are at risk for CIN 2+ lesions. Results from the PAP-IBD study: Prevalence and Persistence of Cervical Intraepithelial Neoplasia (CIN) in women with Inflammatory Bowel Disease **Digestive Disease Week, San Diego**	2019	**14**	**0,5**
Women with IBD are at risk for CIN 2+ lesions. Results from the PAP-IBD study: Prevalence and Persistence of Cervical Intraepithelial Neoplasia (CIN) in women with Inflammatory Bowel Disease **14th Congress of ECCO, Copenhagen**	2019	**14**	**0,5**
Total			**1,5**

Attended (inter)national conferences

	Year	Workload	ECTS
Digestive Disease Week – *San Diego, United States of America*	2022	**28 hours**	**1,0**
17th congress of European Crohn's and Colitis Organization – *Vienna/Online*	2022	**28 hours**	**1,0**
Half-yearly spring meeting of the Dutch association of Gastroenterology, Veldhoven,The Netherlands	2022	**16 hours**	**0,6**
Half-yearly spring meeting of the Dutch association of Gastroenterology, Veldhoven,The Netherlands	2021	**16 hours**	**0,6**
27th United European Gastroenterology Week, *Barcelona, Spain*	2019	**28 hours**	**1,0**
Digestive Disease Week – *San Diego, United States of America*	2019	**28 hours**	**1,0**
14th congress of European Crohn's and Colitis Organization – *Copenhagen, Denemarken*	2019	**28 hours**	**1,0**
Half-yearly fall meeting of the Dutch association of Gastroenterology, Veldhoven,The Netherlands	2019	**16 hours**	**0,6**
Half-yearly spring meeting of the Dutch association of Gastroenterology, Veldhoven,The Netherlands	2019	**16 hours**	**0,6**
Half-yearly fall meeting of the Dutch association of Gastroenterology, Veldhoven, The Netherlands	2018	**16 hours**	**0,6**
Total			**8,0**

Attended seminars

	Year	Workload	ECTS
IBD Research Day Amsterdam UMC Fall	2022	4 hours	0,2
IBD Research Day Amsterdam UMC Spring	2021	4 hours	0,2
IBD Today and Tomorrow Amsterdam	2021	16 hours	0,6
Regionale IBD avond: Beterketen	2021	**4 hours**	**0,2**
Immunologie Kennis Netwerk: zorg op maat door multidisciplinaire samenwerking	2020	**4 hours**	**0,2**
Nationale ICC dag: de kunst van leven met IBD	2019	**6 hours**	**0,2**
IBD onderwijsdag AIOS MDL	2019	**14 hours**	**0,5**
Regionale IBD avond Beterketen	2019	**4 hours**	**0,2**
Nationale ICC dag: IBD binnen en buiten de lijnen	2018	**6 hours**	**0,2**
Total			**2,5**

Educational activities and lecturing

	Year	Workload	ECTS
Lecture 'IBD and vaccination' Curriculumonderwijs AIOS MDL, Amsterdam UMC,The Netherlands	2022	**14 hours**	**0,5**
Tillotts webinar CMV a clinical challenge	2022	**14 hours**	**0,5**
Tillots presentation Viral infections in IBD	2022	**14 hours**	**0,5**
Vaccinations in IBD: do they work? IBD rounds Mount Sinai Hospital, Toronto, Canada	2021	**14 hours**	**0,5**
Supervising research project, master student Medicine, Erasmus University Rotterdam,The Netherlands	2020-2022	**80 hours**	**2,8**
Lecture Post-UEGW symposium Erasmus MC	2020	**14 hours**	**0,5**
Supervising research project, international graduate student, Erasmus MC, Rotterdam	2019	**40 hours**	**1,4**
Supervising research project, medical student, Erasmus MC, Rotterdam	2019	**40 hours**	**1,4**
Lecture 'abdominal pain, inflammatory bowel disease first year medicine students	2018-2020	**12 hours**	**0,4**
Total			**7,5**

Memberships

Nederlandse Vereniging van Maag-darm-leverartsen (NVMDL), Nederlandse Vereniging voor Gastroenterologie (NVGE), European Crohn's and Colitis Organization (ECCO)

	Year
Faculty member Young Gastro Event *Janssen Immunology*. Involved in organizing Medical Education in IBD for Dutch and Belgian young gastroenterologists and residents	2019-2020
Member national IBD-committee *Dutch Gastroenterology Society (NVMDL)*. Involved in development of advanced IBD residency for Dutch gastroenterology residents	2017-2019

Dankwoord

De afgelopen 5 jaar heb ik de kans gekregen om dit werk neer te zetten. Ik had nooit gedacht dat een promotieonderzoek zo'n belangrijke fase van m'n leven in beslag zou nemen. Naast het kennis maken met de medische wetenschap, heb ik leren samenwerken, successen vieren, tegenslagen verwerken en dingen in perspectief plaatsen. Het meest waardevolle is dat ik mijn horizon heb kunnen verbreden en veel verschillende mensen heb leren kennen, waarvan velen ook hebben bijgedragen aan dit werk. Graag wil ik iedereen die heeft bijgedragen aan mijn promotie van harte bedanken.

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Dear **professor Silverberg**, dear **Mark**, thank you very much for accepting me to the advanced IBD fellowship. Unfortunately, the timing for the start of the fellowship was challenging. I really appreciated the effort you've put in to helping me making me feel at home. During my team at Sinai you taught me a lot about medical treatment in IBD and it was really valuable to notice the cultural differences in our countries. I was very lucky that you allowed me to start working on IMPACT and we managed to make it a success in only a short period of time. Although we eventually mostly collaborated virtually, I do feel we were able to build up a good professional connection and I cherish my time in Toronto very much.

Daarnaast wil de leden van de beoordelingscommissie: **prof. dr. Samsom, prof. dr. Rijnders** en **prof. dr. Dijkstra,** van harte bedanken voor de tijd die jullie in de beoordeling van dit manuscript hebben gestoken en de bemoedigende woorden. Ook wil ik de overige leden van de promotiecommissie, **dr. Herma Fidder, dr. Nanne de Boer, dr. Johan van Limbergen** en **prof. dr. Joao Sabino** bedanken dat jullie als opponent wilde deelnemen in de commissie. Notably, I would like to thank **prof. Mark Silverberg** for traveling all the way from Canada to be an opponent in the committee.

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About the author

Rogier Goetgebuer was born on June 18, 1987 in Rotterdam,The Netherlands. He attended secondary school at Erasmiaans Gymnasium in Rotterdam and graduated cum laude in 2005. In September 2005 he started studying medicine at Utrecht University. After a research semester and his final rotations at the department of gastroenterology and hepatology in University Medical Center Utrecht, the seed for his interest in gastroenterology was planted. After finishing his medical degree in 2012 he started working as medical doctor and resident at the department of Internal Medicine at Diakonessenhuis hospital in Utrecht. In 2015 he started his training in gastroenterology at Meander Medical Center in Amersfoort. With a growing interest in inflammatory bowel disease he applied for a PhD trajectory at the Erasmus Medical Center in Rotterdam. He finished his gastroenterology specialty training in 2020. After working several months as advanced IBD fellow at Mount Sinai Hospital in Toronto, Canada, he is now working as IBD staff member in Amsterdam University Medical Center in Amsterdam.