Reproduction and Inflammatory Bowel Disease

finding the balance

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Reproduction in Patients with Inflammatory Bowel Disease: finding the balance

Zwangerschap bij patiënten met Inflammatoire Darmziekten: op zoek naar de balans

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Chapter 1:

General introduction & outline of the thesis

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GENERAL INTRODUCTION

Inflammatory Bowel Disease (IBD) represents a lifelong relapsing inflammatory condition of the gastrointestinal tract. It comprises of Crohn's disease (CD) and ulcerative colitis (UC); CD is characterized by transmural inflammation which may occur anywhere along the gastrointestinal tract; from the oral cavity until the anus, and UC is characterized by continuous inflammation of the colon[1, 2]. In the minority of cases the term IBD Unclassified (IBDU) is used when inflammation is restricted to the colon without specific features of CD or UC. The disease arises from an interaction between genetic and environmental factors, however, the exact etiology remains unknown and as a result curative medical therapy is not yet available. IBD is predominantly seen in developed countries, and the incidence increases exponentially in the Western world whereas in the 21 century the estimated prevalence in Europe is approximately 0.3%[3]. It is striking that the majority of patients are diagnosed during reproductive years; approximately 50% are diagnosed before the age of 35[4]. Overall, IBD is associated with increasing prevalence owing to the lack of cure, low mortality, and young age of onset. As most patients are diagnosed during reproductive years and incidence is increasing, fertility and pregnancy are important topics for gastroenterologists and other physicians treating patients with IBD.

FERTILITY

Women with IBD have less children compared with the general population, which is thought to be a result of poor knowledge and incorrect believes regarding IBD and pregnancy[5, 6]. Fertility in women and men is not influenced by the presences of IBD itself. However, active IBD has been associated with subfertility in both women and men. Subfertility may be a result of inflammation of the colon and surrounding ovaries and fallopian tubes in the case of women and may possibly be due to depression, decreased libido and malnutrition in both women and men. In women with UC, surgical resection of the colon and with Ileal Pouch Anal Anastomosis (IPAA) is associated with a threefold increased risk of subfertility[7, 8]. This is possibly the result of tubal obstruction and increased risk of hydrosalpinx following pelvic surgery. Subfertility rates are lower after laparoscopic intervention compared with laparotomy, underlining the theory that post-surgical adhesion formation leads to subfertility. If IPAA surgery influences male fertility has not been studied.

IBD medication does not influence fertility in women. In men, sulfasalazine reversible decreases both sperm count and motility in a dose dependent fashion[9, 10]. In case of a reproduction wish, male patients using sulfasalazine should be advised to switch to another 5-ASA. Methotrexate causes oligospermia and is contraindicated for women and men wishing to conceive because of the teratogenic effect[11, 12]. It is advised to discontinue methotrexate 4-6 months before conception for both women and men[13]. Steroids may cause decreased

sperm concentration and motility, however, there seems to be no association between decreased fertility and corticosteroid use[14, 15]. Thiopurines are not associated with male fertility and adverse pregnancy outcomes, it is therefore not recommended to discontinue thiopurines at the time of conception[16-18]. Studies regarding the influence of anti-TNF-α on male fertility are scarce and conflicting. Reduced sperm motility has been described in male IBD patients using infliximab[19], however, a study in male patients with spondyloarthropathies showed improved sperm quality after receiving anti-TNF-α treatment[20]. There is no evidence that paternal anti-TNF-α use for IBD at the time of conception is associated with adverse pregnancy outcomes such as congenital abnormalities, preterm birth and children born small for congenital age[21]. In most studies infliximab was used, the effect of adalimumab on male fertility remains barely studied.

IBD AND PREGNANCY

Women with IBD more often experience adverse pregnancy outcomes compared with the general population[22]. In particular, active disease at the time of conception and pregnancy is associated with a higher rate of spontaneous abortions, preterm delivery, low birth weight and thrombo-embolic events[23, 24]. On the contrary, most pregnancies in women with quiescent disease are uncomplicated. This underlines the importance of maintaining disease remission before and during pregnancy. Remission may be maintained by continuing IBD treatment, as disease activity is more harmful than most types of medication[25]. In the case conception occurs at a time of disease remission, the risk of a relapse during pregnancy is similar to the relapse risk in non-pregnant patients over the course of 9 months, which is approximately 30%[26, 27]. If conception occurs at a time of active disease women have an increased risk of persistent activity during the entire pregnancy. Pre-conceptional counselling is therefore of utmost importance as it improves drug adherence and subsequently reduces disease relapse during pregnancy[28]. During pre-conceptional counselling the following topic should be discussed; current medication and if indicated advise to switch to another IBD drug if current treatment is contraindicated, the importance of disease remission, folic acid intake, life style such as smoking and alcohol intake, mode of delivery and breastfeeding.

Auto-immune diseases may be altered during pregnancy as immunological adaptions are necessary for intrauterine implantation and maintenance of the semi-allogeneic fetus. Other auto-immune diseases such as rheumatoid arthritis, have a positive influence on disease course during pregnancy[29]. However, the effect of pregnancy on the disease course of IBD remains elusive. In addition, women with UC relapse more often during pregnancy than women with CD, irrespective of IBD medication and periconceptional disease activity[30]. The reason for the difference in relapse risk between UC and CD during pregnancy has yet to be elucidated.

ENDOSCOPY DURING PREGNANCY

Clinical scores, such as body weight and abdominal complaints and laboratory work up, such as hematocrit are of limited value to assess disease activity during pregnancy as most women experience abdominal complaints, become anemic and experience weight change during pregnancy. A lower endoscopy is considered relatively safe during pregnancy and may be warranted to assess disease activity[31], although studies exist showing an association between endoscopy during pregnancy and adverse pregnancy outcomes[32]. Overall, the safety of endoscopic interventions during pregnancy remains a topic of debate.

IBD TREATMENT DURING PREGNANCY

As IBD often occurs in patients in childbearing age, inevitably some women will require treatment during pregnancy. During pre-conceptional counselling, medication should be reviewed and if necessary high-risk drugs should be switched to low-risk drugs before contraception cessation.

5-ASA are considered to be of low risk during pregnancy. However, formulations containing dibutyl phthalate coating were associated with male urogenital tract malformations in animal studies and is possibly associated with precocious puberty and should preferably be avoided[33, 34]. As sulfasalazine decreases folic acid levels, women are advised to use 2mg folic acid per day when contemplating pregnancy.

Corticosteroids may be needed during pregnancy for induction of disease remission. The use of corticosteroids during pregnancy is associated with an increased risk of gestational hypertension and diabetes, for which regular follow-up of a gynecologist is indicated. An increased risk of orofacial clefts has been reported in women using corticosteroids in the first trimester[35], although a more recent large nationwide cohort study showed no association between maternal corticosteroid use during pregnancy and orofacial clefts[36]. Furthermore, corticosteroid use in the third trimester may lead to neonatal adrenal suppression. The preferred corticosteroid during pregnancy is prednisone because of the limited placental transmission[37], and corticosteroids more prone to cross the placenta should be avoided, such as hydrocortisone and dexamethasone. Overall, the use of corticosteroid during pregnancy seems of low risk and is indicated in the case of a disease flare during pregnancy.

Methotrexate is teratogenic and should be discontinued 4-6 months prior to contraception cessation[38]. Fertile women starting or using methotrexate should be actively counselled and a treatment switch is necessary in women who express a current or future pregnancy wish.

Thiopurines, such as azathioprine and mercaptopurine are both unable to cross the placenta however the active end metabolites 6-thioguaninenucleotides, which are associated with therapeutic efficacy, cross the placenta. Studies assessing pregnancy outcomes in case of maternal thiopurine use are conflicting; studies exist that describe no association between thiopurine use and adverse outcomes[39-41], however, other studies describe an increased risk of adverse outcomes whilst using thiopurine during pregnancy[42, 43]. All studies were retrospective of nature, therefore confounding factors were not adjusted for, such as disease activity, folic acid intact and obstetrical complications. Risks of disease activity probably outweigh risks of thiopurine use during pregnancy, however, prospective studies on this topic, adjusting for confounding factors, are lacking.

Anti-TNF-α treatment is capable of crossing the placenta. The transmission over the placenta increases exponentially, starting in the second trimester, resulting in higher levels in the newborn than mother at term[44, 45]. Anti-TNF-α treatment during pregnancy is not associated with adverse pregnancy outcomes such as spontaneous abortions, congenital abnormalities, preterm birth and low birth weight[46, 47]. However, anti-TNF-α has been detected in children after birth until 9 month of age for adalimumab and until 12 months of age for infliximab [48]. Therefore, concerns have been raised about the immune development of the child, subsequently the infections risk and the response to vaccinations. Current pregnancy guidelines advise to stop anti-TNF-α treatment around gestational week 24-26 to limit fetal exposure if patients are in sustained remission[38]. A previous study has shown that anti-TNF-α cessation is of low risk for the mother in the case of sustained remission[49]. However, if women are not in remission from 6 months prior to conception until 20 weeks of gestation anti-TNF-α should be continued the entire pregnancy to minimize the relapse risk. These guidelines make no distinction between the different types of anti-TNF-α, however, recent studies show higher infliximab levels and slower clearance in children compared with adalimumab. This underlines the different pharmacokinetics of the anti-TNF-α types and indicates that future guidelines probably should be adjusted for each anti-TNF-α type individually.

LONG-TERM HEALTH OUTCOMES OF CHILDREN EXPOSED TO IMMUNE SUPPRESSIVE TREATMENT IN UTERO

Long-term health outcomes of children born to women with IBD, especially children exposed to immune suppressive therapy in utero, are relatively unexplored and need to be elucidated. Current studies assessing infection risk in children exposed to anti-TNF-α and/or thiopurine are conflicting. An increased infection risk has been reported in children exposed to the combination of anti-TNF-α and thiopurine compared with children exposed to anti-TNF-α

monotherapy[48]. Other studies however failed to find a correlation between exposure to immune suppressive treatment in utero and infection risk[49, 50]. Overall, health outcomes seem comparable to the non-IBD population[49]. Follow-up in most studies are until 1 year, therefore long-term implications of in utero exposure to immune suppressive therapy is unknown.

Non-live vaccines seem safe in patients using anti-TNF-α[51] and children exposed to anti-TNF-α in utero[52]. However, live vaccines are contraindicated in immune compromised individuals. As detectable anti-TNF-α levels are found in children until 12 months, live vaccines should be avoided until anti-TNF-α is cleared. A recent study showed no effect of anti-TNF-α exposure in utero on response rate to vaccines, furthermore, the administrations of a live vaccine was not associated with adverse reactions[53]. However, in the absence of robust evidence, guidelines remain to advise that live vaccines should be postponed until drug clearance is confirmed.

AIMS AND OUTLINE OF THIS THESIS

The aim of this thesis was to assess several clinical topics on IBD and reproduction.

PART 1. PRE-PREGNANCY

For IBD patients with a reproduction wish, pre-conceptional counselling is of utmost importance to assess medical treatment and strive for sustained disease remission. Studies regarding the influence of anti-TNF-α on male fertility are scarce and conflicting and mostly describe the influence of infliximab. In **Chapter 2** we describe the effect of adalimumab use for IBD on male fertility. In addition, health outcomes of children with paternal adalimumab exposure during conception are shown. Incorrect beliefs and insufficient knowledge regarding IBD and pregnancy still remains among patients resulting in uncontrolled drug cessation and may subsequently lead to adverse pregnancy outcomes. In **Chapter 3** the importance of pre-conceptional counselling is emphasized in an editorial.

PART 2. PREGNANCY

Most IBD patients require medical treatment to remain in remission and inevitable some women will need to maintain treatment during pregnancy. Nowadays, thiopurines and anti-TNF-α form the corner stone of IBD treatment and may be used as monotherapy or in combination. In **Chapter 4** we describe the effect of maternal thiopurine use during pregnancy on pregnancy outcomes and health outcomes of in utero exposed children. In **Chapter 5** the effect of anti-TNF-α treatment on pregnancy outcomes and health outcomes of children is emphasized. Also differences between anti-TNF-α type are described. During pregnancy, interventions may be needed to assess disease activity. We commented on a study assessing the risk of an endoscopy during pregnancy in **Chapter 6**.

PART 3. POST-PREGNANCY

Long-term health outcomes of children born to mothers with IBD are relatively unexplored. In **Chapter 7** we describe health outcomes of children, who were exposed to different types of immunosuppressive therapy in utero, until 5 years of age. Previous studies have shown that anti-TNF-α exposed children may continue to have detectable anti-TNF-α levels until 1 year of age which may have implications on vaccination response. In **Chapter 8** the efficacy of hepatitis B vaccination in anti-TNF-α exposed children is described. Finally, in **Chapter 9** the main finding and conclusions of our studies will be summarized and discussed.

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Part 1.
Pre-pregnancy

Chapter 2:

Semen quality and birth outcomes: the effect of adalimumab use on reproduction in males with Inflammatory Bowel Disease

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Submitted

ABSTRACT

Objectives: Adalimumab represents an increasingly prescribed anti-TNF-α agent for the treatment of Inflammatory Bowel Disease (IBD), however, there is no data available on the impact of adalimumab use on semen quality. Furthermore, data regarding the influence of paternal adalimumab use during conception on birth outcomes are scarce. Our primary aim was therefore to assess the impact of adalimumab on semen quality and our secondary aim was to assess the influence of paternal adalimumab use on birth outcomes.

Methods: Male IBD patients naïve to adalimumab planning to start adalimumab, were prospectively recruited at our IBD outpatient clinic between October 2009 and April 2011. A semen analysis was performed before the start of adalimumab treatment, and subsequently after 3 months and 6 months of treatment. In addition, male IBD patients who conceived while using adalimumab were retrospectively recruited at our outpatient clinic between July 2015 and July 2016 and birth outcomes such as, birth weight, gestational age and congenital abnormalities were obtained.

Results: Semen analyses was performed in 7 patients. All patients had active disease at inclusion. Six patients responded to treatment. Overall, adalimumab treatment did not have a deleterious influence on semen quality. In addition, we identified 17 children who were conceived while the father was using adalimumab. One child was born small for gestational age, no other adverse birth outcomes were reported.

Conclusions: This small sample size study suggests that adalimumab has no deleterious influence on spermatogenesis or birth outcomes of children fathered by IBD patients using adalimumab.

INTRODUCTION

Inflammatory Bowel Disease (IBD) typically affects patients in their reproductive years and as a result, reproduction represents a frequently encountered issue in clinical practice.[1-3] Fertility and pregnancy is extensively studied in women with IBD, however, the fertility of male IBD patients is also an important topic in clinical practice and the studies focussing on this subject are scarce.

With the introduction of anti-TNF-α agents, the field of IBD therapeutics has undergone a dynamic evolution. TNF-α is a cytokine with pro-inflammatory effects that plays an important role in the pathogenesis of IBD.[4-6] Multiple anti-TNF-α agents, including infliximab (IFX), adalimumab (ADA) and certolizumab pegol (CZP) have proven to be effective in the treatment of IBD.[7-12] TNF-α is among others, produced by germ cells, and it is present in physiologically low levels in seminal plasma.[13] During inflammatory state TNF-α levels increase, which has a negative effect on spermatogenesis and sperm motility.[14, 15] An in vitro study showed that semen quality declines after incubation with TNF- α in a dose- and time-dependent manner.[16] Treatment with anti-TNF-α agents could therefore be beneficial by reducing the harmful high levels of TNF-α in case of inflammation.

Human studies regarding anti-TNF-α treatment and semen parameters are scarce and conflicting. A study including 10 male IBD patients showed a possible negative effect of IFX on sperm motility.[17] On the other hand, studies conducted in male patients with other inflammatory conditions, such as spondyloarthritis and ankylosing spondylitis, showed that sperm quality decreased during disease activity, but after treatment with anti-TNF-α, sperm quality was comparable to the sperm of healthy controls.[18-20]

Thus, there are indications that TNF-α may play an important role in spermatogenesis and that its proper functioning may be influenced by the systemic use of an anti-TNF-α drug. IFX is the most frequently studied, however, although ADA is increasingly prescribed for IBD, there are no data available on ADA use and the effect on semen quality. The primary aim of this study was therefore to assess the impact of ADA treatment on semen parameters. Our secondary aim was to analyse pregnancy outcomes of children who were conceived while the father was using ADA.

MATERIALS AND METHODS

To assess the impact of ADA on semen quality, male IBD patients planning to start treatment with ADA were prospectively enrolled between October 2009 and April 2011, at our IBD outpatient clinic at the Erasmus University Medical Center, a tertiary hospital. Patients with previous documented fertility problems were excluded. Once enrolled in the study, the following information was collected from the treating physician: diagnosis, comorbidity, disease behavior and disease location according to the Montreal classification, age, disease duration in years, Body Mass Index (BMI), prior IBD surgery, reason for ADA treatment, concomitant treatment and smoking status. The ADA induction regimen was applied as follows: 160 mg at week 1, 80 mg at week 3 and subsequently a standard dose of 40 mg every other week. Disease activity was assessed during every visit and relapse was defined as: a Harvey Bradshaw Index (HBI) of ≥ 5 and/or C- reactive protein (CRP) ≥ 9.0 mg/mL and/or presence of inflammation seen during endoscopy. A semen analysis was performed before the start of ADA treatment and subsequently after 3 months and 6 months of treatment. Because spermatogenesis takes up till 3 months, all samples taken during ADA treatment reflect spermatogenesis at the time of systemic anti-TNF-α drug use. Before handing in a semen sample, patients were asked to refrain from ejaculation for 3 - 5 days and to hand in the semen sample within one hour after ejaculation. During visits, patients were asked about recent illness, particularly febrile illness. The following semen parameters were assessed: semen volume, sperm concentration, progressive motility, pH and the presence of leukocytes. All analyses were performed and references were used according to the current WHO manual.[21] In addition, total motile sperm count (TMSC) was assessed, which is an indicator for the severity of male factor infertility.[22, 23] The TMSC is obtained by multiplying the sperm concentration by the volume of the ejaculate and the proportion of A (fast forward progressive) and B (slow progressive) motile sperms divided by 100%.[23, 24]

Multiple variables influence semen quality such as BMI, smoking, age, concomitant medication use and disease activity. In an additional analysis, we therefore compared semen quality before ADA treatment and during ADA treatment in the 6 patients that responded to treatment. Thus, patients served as their own control. If patients handed in 2 semen samples during ADA treatment, the averages of the 2 samples was used.

For our secondary aim, all male IBD patients were identified at the outpatient clinic between July 2015 and July 2016 who had previously conceived while using ADA. Birth outcomes of these pregnancies were obtained from the patients and/or the mothers. The following birth outcomes were collected: birth weight, gestational age at birth, preterm birth, small for gestational age (SGA) and the presence of congenital abnormalities. Only in case an adverse birth outcome was reported, data regarding paternal smoking, maternal smoking, medical

history of mother, medication use of mother and obstetric complications were documented.
Definitions

Standard ADA dose is 40mg subcutaneously every other week. Preterm birth is defined as a delivery before 37 weeks of gestation. SGA is a weight below the 2 SD for gestational age according to the Dutch reference curve.[25]

Statistical analysis

All analyses were performed using IBM SPSS statistics version 23.0. Categorical data are shown as absolute numbers with percentages and were compared using Fisher's exact tests. Descriptive statistics of continuous variables are displayed as medians with interquartile ranges (IQR) and were compared using Mann Whitney U Tests. Paired data was analysed using the Wilcoxon signed rank test. All tests were performed two tailed and tested at a significance level of 0.05.

Ethical Considerations

Medical ethical committee approved this study and all patients gave informed consent before inclusion. This study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

RESULTS

Semen analyses

Seven male IBD patients were included in the study for semen analyses. Characteristics per individual are shown in **Table 1**.

Table 1. Baseline characteristics

	Diagnosis and comorbidity	Disease behavior (Montreal)	Disease location (Montreal)	Age	Disease duration (years)	BMI	Prior IBD Surgery	Reason adalimumab treatment	Concomitant treatment	Smoking
Patient 1	CD, PSC	B1	L2	25	9	22,6	No	Luminal Crohn's disease	Ursodeoxycholic acid Entocort (mnth3-mnth6)	No
Patient 2	CD	B1	L3	27	11	18,5	No	Luminal Crohn's disease	Hydrocortison Azathioprine	No
Patient 3	CD, Morbus Hashimoto	B1	L2	42	1	34,9	No	Luminal Crohn's disease and extra intestinal manifestation	Prednison (first 3 mnths) Azathioprine Thyrax Augmentin (month 5)	No
Patient 4	CD	B1	L3	30	1	22,4	No	Luminal Crohn's disease	Entocort (first 3 months)	No
Patient 5	CD	B1	L3	34	6	23,4	No	Luminal Crohn's disease	Prednison (first month)	Yes
Patient 6	CD	B1,p	L2	25	2	26,0	Perianal fistula	Active perianal fistula	Ciproxin (first 3 months)	No
Patient 7	CD, Hemophilia A	B1	L1	31	1	22,0	No	Luminal Crohn's disease	None	No

IBD inflammatory Bowel Disease; CD Crohn's disease; PSC Primary Sclerosing Cholangitis; BMI Body Mass Index; EIM extra intestinal manifestation

The average age was 30 years (range 25-42) and all patients were diagnosed with Crohn`s disease (CD). At baseline, all patients had active CD; 6 patients had active luminal disease and 1 patient had an active perianal fistula. None of the patient underwent abdominal surgery for their IBD prior to inclusion. In terms of response to medical treatment; all patients responded to treatment except patient 2. Patient 2 was a primary non-responder and stopped ADA treatment after 3 months, however, a semen sample was obtained after 3 months before treatment cessation. In addition, patient 5 needed a dose escalation after 5 months to a weekly dose of 40 mg because of an incomplete response. Patients 4, 5 and 7 were in remission at month 3 and patients 1, 3, 4, 6 and 7 were in remission at month 6. An overview of disease activity is shown in **Table 2**.

Table 2. Disease activity				
	Clinical activity (HBI≥5)	CRP (mg/mL)	Endoscopy	Additional clinical information
Patient 1 (responder)				Fever after 5 months of ADA treatment; focus was not found
Baseline	Yes	30	Moderate- severe colitis (pancolitis)	
3 months	Yes	7	Moderate- severe colitis (pancolitis)	
6 months	No	6	N/A (endoscopy not performed)	
Patient 2 (primary non-responder)				Stopped ADA treatment after 3 months
Baseline	Yes	19	Mild activity terminal ileum and rectum	
3 months	Yes	19	N/A (endoscopy not performed)	
6 months	Yes	10	Severe colitis right hemi colon	
Patient 3 (responder)				Pneumonia, adequately treated with amoxicillin after 4 month of ADA treatment
Baseline	Yes	3	N/A (endoscopy not performed)	
3 months	Yes	13	N/A (endoscopy not performed)	
6 months	No	6	Mild colitis in sigmoid, mucosal healing observed	
Patient 4 (responder)				
Baseline	Yes	1	Moderate-severe colitis right hemicolon and ileum	
3 months	No	1	N/A (endoscopy not performed)	
6 months	No	1	N/A (endoscopy not performed)	
Patient 5 (responder)				Clinical and laboratory relapse after 5 months, remission after dose intensification
Baseline	Yes	12	Mild-moderate colitis (pancolitis)	
3 months	No	3	N/A (endoscopy not performed)	
6 months	Yes	13	Mild-moderate colitis (pancolitis)	
Patient 6 (responder)				Perianal abscess after 3 months of treatment.
Baseline	Yes	1	Perinal fistula, mild proctitis	
3 months	Yes	1	N/A (endoscopy not performed)	Fistula closed after 6 months
6 months	No	1	N/A (endoscopy not performed)	
Patient 7 (responder)				
Baseline	Yes	18	Disease activity jejunum	
3 months	No	2	N/A (endoscopy not performed)	
6 months	No	4	No disease activity small bowel	

HBI Harvey Bradshaw Index; CRP C-reactive protein; reference CRP: < 9.0 mg/mL

A baseline sample was obtained from all 7 patients; 6 patients handed in a sample after 3 months of treatment and 4 patients handed in a sample after 6 months of treatment. All semen samples were analysed within one hour after ejaculation. Reference values for semen parameters according to the WHO manual are stated in **Table 3**.

Table 3. Semen composition before and during ADA treatment (n=6)			
	Before start ADA	During ADA treatment	P value
Median semen volume (ml) (IQR) *Reference lower limit: 1.5*	2.2 (1.6-3.4)	2.1 (1.3-3.4)	0.69
Sperm concentration (10^6/ml) (IQR) *Reference lower limit: 15*	16 (11-29)	33 (24-51)	0.05
Progressive motility in % (IQR) *Reference lower limit: 32*	35 (22-49)	32 (24-46)	0.92
pH (IQR) *Reference lower limit: 7.2*	8.0 (7.7-8.0)	7.7(7.6-7.7)	0.08
Presence of leucocytes (%) *Reference: 0*	0 (0.0)	0 (0.0)	1.00
TMSC (IQR) *Reference > 20 × 10^6 spermatozoa*	14 (4-41)	20 (8-71)	0.25

ADA adalimumab, IQR interquartile range; TMSC total motile sperm count

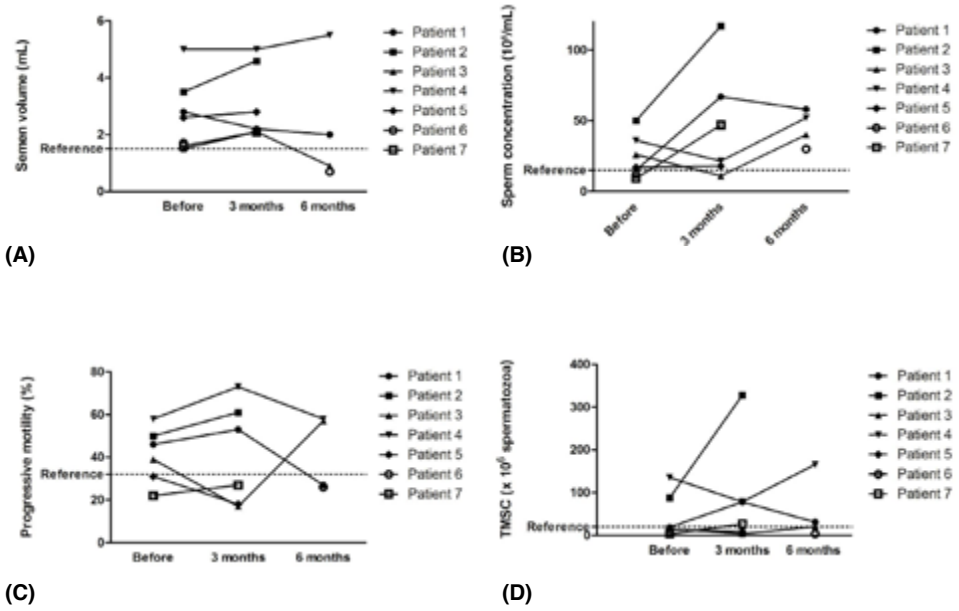

Figure 1. Semen parameters before and during adalimumab treatment **(A)** semen volume (mL) before and during adalimumab treatment **(B)** Sperm concentration (106/mL) before and during adalimumab treatment **(C)** Progressive motility (%) before and during adalimumab treatment **(D)** TMSC (x 106 spermatozoa) before and during adalimumab treatment

Semen volume was normal in all patients at baseline and at month 3. However, after 6 months patient 3 and patient 6 had a decreased semen volume (**Figure 1A**).

Sperm concentration was low at baseline in patient 6 and patient 7 (**Figure 1B**). The subsequent analyses showed normal sperm concentration for both these patients. Patient 3 had a normal sperm concentration at baseline, a low concentration at month 3, which normalized again at month 6. This patient suffered from a pneumonia during the study, which was diagnosed after 4 months of ADA treatment and was treated successfully.

Progressive motility was below the reference rate at baseline in patients 5, 6 and 7 (**Figure 1C**). After treatment initiating, results remained abnormal for all 3 patients, but the progressive motility rate improved in patients 6 and 7. Patients 1 and 3 both had an episode of febrile illness during the study period; pneumonia and fever without a focus, both had a low progressive motility rate at the time of illness, which was normal during other measurements. TMSC was below threshold in patients 1, 3, 5, 6, and 7 at baseline (**Figure 1D**). TMSC of the 2 patients with a normal value at baseline remained normal during ADA treatment. During ADA treatment, TMSC normalized in patients 1 and 7 but remained low in patients 3, 5 and 6. Semen quality before and during ADA treatment of the 6 responders were compared and

shown in **Table 3**. Patients 1, 3 and 4 handed in 2 sample during ADA treatment, therefore, the average of these samples were used. Overall, semen volume, sperm concentration, progressive motility, TMSC, pH and the presence of leucocytes did not differ significantly before and during ADA treatment.

Outcomes of pregnancies conceived under adalimumab treatment

We retrospectively identified 17 children who were conceived by 12 male IBD patients who were using ADA at time of conception. Birth outcomes of these children are shown separately in **Table 4**. There were 11 fathers diagnosed with CD and 1 father with ulcerative colitis. Three fathers also participated in the first part of the study were we assessed semen quality before and during ADA treatment. All children were born between 2008 and 2014. None of the children had a congenital abnormality. All children had a normal birth weight and gestational age at birth, none of the children were born preterm, however, one child was born small for gestational age (SGA). The father of this child smoked at the time of conception, however, mother was healthy, did not smoke nor consume alcohol during pregnancy, did not use medication and had an uncomplicated pregnancy. The reason why this child was born SGA remains unknown.

Table 4. Pregnancy outcomes of children that were conceived while the father used adalimumab (n=17)	
Median birth weight in grams (IQR)	3107 (2964-3760)
Median gestational age in weeks (IQR)	39.5 (38.2-40.5)
Preterm delivery (<37.0 weeks)	None
Low birth weight (<2500 grams)	None
Small for gestational age (%)	1 (5.9%)
Congenital abnormalities	None

DISCUSSION

This small sample size study suggests that ADA has no deleterious impact on semen quality. In addition, we found no association between paternal ADA use during conception and adverse birth outcomes.

In our study, we observed no significant difference in semen volume, progressive motility, pH, the presence of leukocytes and TMSC before and during ADA treatment. Sperm concentration was higher during ADA treatment than before the start of ADA, reaching a borderline statistical difference (p=0.05), indicating a positive effect of ADA treatment on sperm concentration. If this is an effect of systemic anti-TNF-α on spermatogenesis or a result of adequate IBD treatment could not be determined. The fact that semen quality did not change delirious during ADA treatment suggests that the interference of ADA with spermatogenesis may be

minimal if not non-existing. However, these results must be interpreted with caution, keeping in mind that DNA damage does not necessarily result in an altered microscopic appearance of sperm cells.

Our findings are consistent with previous studies, demonstrating that the use of anti-TNF-α does not influence sperm parameters.[20, 26] The study by Mahadevan et al, [17] however, showed a possible negative effect of IFX on sperm morphology and sperm motility. It should be mentioned that cut-off values to differentiate between normal an abnormal semen parameters are under constant debate. The WHO manual, containing references that classify semen parameters as normal or abnormal, has been revised in 2010 and now contains lower cut-off values because the previous classification poorly predicted pregnancy chance.[27] Also the relevance of the new cut-off values in predicting pregnancy change remains questionable, as shown in a recent study,[22] indicating that semen parameters, especially sperm morphology, should be interpreted with caution.

Sperm morphology was not assessed in our study as the ability of sperm morphology to predict likelihood of pregnancy remains controversial. Previous studies demonstrated a negative association between number of abnormal sperm morphology and likelihood of pregnancy.[28-30] However, outcomes of studies assessing the predictability of sperm morphology on outcomes in couples undergoing intrauterine insemination (IUI) and in vitro fertilization (IVF) vary widely.[31-36] In addition, two recent studies found no relation between sperm morphology and likelihood of conception.[37, 38]

A systematic review from Tavernier et al. found that fertility was decreased in men with CD.[39] If this is a result of voluntary childlessness or involuntary infertility due to the underlying disease remains unclear. A small study showed a decreased semen quality in men suffering from CD without using IBD treatment, which was possibly a result of the underlying disease activity but, because of the small sample size, statistical significance could not be achieved.[40] A significant correlation between disease activity and sperm quality was, however, demonstrated in men with spondyloarthritis.[20] This indicates there is a relation between active inflammatory disease and decreased male fertility; as a result, adequate treatment of the underlying disease may have a beneficial effect on fertility. It should be noted that, in our study, patients had disease activity before the start of ADA treatment and remission was achieved in almost all patients at the end of the follow-up period, which may have influenced semen parameters.

We found no association between paternal ADA use while children were conceived and adverse birth outcomes, which is consistent with previous studies.[41-44] A recent population

based study assessed birth outcomes of 372 children fathered by men that were treated with anti-TNF-α within 3 months prior to conception.[42] No association was found between paternal anti-TNF-α use and congenital abnormalities, preterm birth and SGA. Also after adjusting for maternal and paternal age, maternal smoking, BMI and parity, results were reassuring.
To make sure that the conception occurs with semen that is produced without any influence of a drug, one must take into account the biological half time of that particular drug and the 60 to 80 days that is needed for spermatogenesis. Depending on the drug, it can therefore take up to 4-6 months after drug cessation to produce sperm without drug influence. In the period after drug cessation IBD patients have a considerable risk of a disease relapse, but in case of anti-TNF-α treatment there are additional risks such as an allergic reactions and loss of response after drug re-initiation. Thus, it is important to realize that in male IBD patients the reproductive plans influence the therapeutic strategy.

This small case-series obviously has several limitations. Due to the small sample size and biological variation of semen quality over time, conclusions cannot be drawn. We aimed to correct for factors influencing semen quality such as, obesity, smoking, increased age and medication use,[45, 46] by comparing semen quality of each individual before and during ADA treatment, thus each patient served as their own control. However, because of the small sample size it remains difficult to correct for confounding factors. In addition, birth outcomes were obtained from patient reporting and have not be confirmed by assessing medical charts. However, this is de first study assessing semen quality in male IBD patients using adalimumab. As no information is available on ADA use and the effect on male fertility in IBD patients, this study may aid in the decision making in clinical practice. In addition, this study may create a paradigm for future studies on this topic as larger prospective studies are needed.

In conclusion, this small sample size study suggests that ADA has no significant influence on sperm parameters and health outcomes of children fathered by IBD patients using ADA. Because of our small sample size these results should not be generalized. Thorough identification and prospective reporting and follow-up of all pregnancies with indirect exposure to ADA are crucial steps towards better evaluation of the safety of ADA use by the future fathers.

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Chapter 3:

Preconceptional Counselling of IBD Patients

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Inflammatory bowel disease [IBD] is often diagnosed during the reproductive years.[1] Therefore managing patients necessitates discussing wishes regarding future children in order to improve the patient's knowledge and to avoid misbeliefs and inappropriate concerns regarding the safety of IBD medications. As active disease during conception and pregnancy is related to adverse pregnancy outcomes, the mainstay of treating these patients is maintaining disease remission and subsequently most often maintaining IBD drugs.[2] Although it is known that in utero exposure to most IBD drugs is of low risk for the child, drugs such as antitumour necrosis factor alpha [anti-TNF-α], vedolizumab and thiopurines do cross the placenta, and the long-term effect of these drugs are yet unknown. This uncertainty makes counselling parents-to-be challenging. In this issue of the journal, it is clearly demonstrated that IBD patients have incorrect beliefs and insufficient knowledge regarding IBD treatment during pregnancy and lactation. First, Ellul et al. report the perspectives of 348 women with IBD on fertility, pregnancy, and lactation.[3] Patients were included from nine IBD centers, were aged between 16 and 50 years, and nearly half of these women gave birth to at least one child. The majority [> 60%] had serious concerns about the effect of IBD on pregnancy and/or believed that IBD medications cause fetal harm. Although most women would consult their physicians about continuing drugs during pregnancy, 15% would stop all IBD medication anyway. In addition, 27.2% of patients were unsure if breastfeeding was safe while using IBD drugs. In this study it was nicely demonstrated that if patients received adequate counselling by health care professionals, this was related to higher numbers of pregnancies and a decreased number of patients considering voluntary childlessness. Second, Gallinger et al. assessed IBD medication adherence in 204 women between 16 and 50 years of age, among whom 101 patients reported a current or previous pregnancy.[4] Almost half of these women stopped their prescribed IBD medication because of concerns regarding the safety of IBD drugs. In this study group, 19.8% even stopped medication without consulting a physician. These studies demonstrate that misbeliefs affect drug adherence, which in turn may increase the risk of a disease relapse during pregnancy, with subsequently an increased risk of adverse pregnancy outcomes. Therefore, the above-mentioned studies emphasize the importance of adequate preconceptional counselling. Recently it was shown that this approach was effective in optimising adherence and, in this study, a reduction in disease relapse was seen during pregnancy compared with outcomes among women who did not received adequate counselling.[5] Several pregnancy guidelines, including the European Crohn's and Colitis Organisation [ECCO] pregnancy guideline, provide practical advice with regard to preconceptional counselling.[2,6] This advice includes aspects of managing IBD patients not only during pregnancy but also in the pre-pregnancy period. An often-discussed drug in relation to pregnancy is anti-TNF-α. In this issue, the risks of anti-TNF-α treatment during pregnancy are reported by Shihab et al. in a meta-analysis of six studies.[7] A total of 1242 pregnancies in women with IBD, of whom 482 were exposed to an anti-TNF-α drug, were included for analysis. Exposure to anti-TNF-α treatment during pregnancy was not associated with an increased risk

of congenital abnormalities, preterm birth, or low birthweight. In addition, when compared with the general population, anti-TNF-α treatment during pregnancy was not associated with an increased risk of congenital abnormalities. These results are reassuring regarding the risk of congenital abnormalities and short-term health outcomes in children that were exposed to anti-TNF-α in utero, and adds to the current advice to continue anti-TNF-α drugs in women contemplating pregnancy and during pregnancy. However, the effects on long-term health outcomes such as growth, infectious diseases, the effectiveness of vaccinations and the effect on the immature immune system remain to be elucidated. Therefore, according to pregnancy guidelines, anti-TNF-α may be stopped around week 24 of pregnancy in a patient who is in sustained remission to limit fetal exposure. It has been shown that this strategy is of low risk for the mother and at the same time minimises fetal drug exposure.[8] In conclusion, controlling health risks of IBD mothers-to-be and their children requires finding the right balance between maintaining disease remission and at the same time minimising fetal drug exposure. Based on the studies in this issue of JCC, discussing wishes for a future child should be included in the management of all fertile IBD patients. This gives the opportunity to timely counsel IBD patients and to correct existing misbeliefs regarding the effects of IBD drugs on the health outcomes of their children. This proactive strategy will improve adherence to therapy and will increase the chance of a favourable pregnancy outcome.

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Part 2. Pregnancy

Chapter 4.1:

Use of Thiopurines During Conception and Pregnancy Is Not Associated With Adverse Pregnancy Outcomes or Health of Infants at One Year in a Prospective Study

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ABSTRACT

BACKGROUND & AIMS

Most data on the safety of thiopurine therapy for inflammatory bowel disease (IBD) during pregnancy come from retrospective studies, which makes it difficult to adjust for confounding factors. We performed a prospective cohort study to determine whether thiopurine use affects pregnancy outcomes or health outcomes of children.

METHODS

We performed a prospective study of all women who visited the IBD preconception outpatient clinic at our tertiary health center in The Netherlands from December 2008 through May 2016. Patients were counseled before pregnancy and seen bimonthly during pregnancy. We collected and analyzed data on medication use, as well as lifestyle and clinical factors, during conception and pregnancy. Pregnancy outcomes (live birth, spontaneous abortion, elective abortion, and stillbirth), birth outcomes (gestational age, birth weight, and congenital abnormalities), and health outcomes of infants 1 year after birth were compared between women who did and did not use a thiopurine during conception and pregnancy. In addition, health outcomes of infants 1 year after birth were compared with infants born to mothers without IBD from the same geographic region.

RESULTS

Our study comprised 309 women with confirmed IBD (216 with Crohn's disease, 85 with ulcerative colitis, and 8 with IBD unclassified). During the study period, 311 pregnancies of 232 women resulted in a live birth; a thiopurine was used during 108 pregnancies (35%). After correction for diagnosis, fertility treatment, and disease activity, there was no association between thiopurine use and spontaneous abortions. Birth outcomes were similar between women who did and did not use a thiopurine. Among infants 1 year of age, there were no differences in median growth, number of infections, allergies, adverse reactions to vaccinations, or chronic diseases between those born to women who did and did not use a thiopurine or between women with and without IBD.

CONCLUSIONS

In this prospective cohort study, we found no association between maternal thiopurine use during pregnancy and increased spontaneous abortions, adverse birth outcomes, or adverse health outcomes of infants 1 year after birth.

INTRODUCTION

Inflammatory bowel disease (IBD) is typically diagnosed during reproductive years[1]; therefore, women with IBD will often become pregnant after diagnosis. Maintaining disease remission during conception and pregnancy is of utmost importance because active disease is related to adverse pregnancy outcomes such as spontaneous abortion, preterm birth, and low birth weight.[2–6] For this reason, it is advised to maintain most IBD drugs throughout pregnancy including azathioprine (AZA) and mercaptopurine (MP). It has previously been shown that the pharmacologic active end metabolites 6-thioguanine nucleotides (6-TGN), which are associated with therapeutic efficacy but also with the development of myelotoxicity,[7–9] can cross the human placenta.[10,11] In addition, mild hematologic abnormalities such as anemia, leukopenia, and thrombocytopenia have been observed in infants at birth.[11,12] Some previous clinical studies demonstrated that there is no association between maternal thiopurine use and adverse pregnancy outcomes.[13–15] Others did find an association but concluded that this was probably caused by confounding factors.[16–18] Because these studies are all retrospective in nature, it was not possible to determine the influence of confounding factors such as disease activity, smoking, folic acid intake, and obstetric complications. To our best knowledge, only 1 prospective study has been published showing normal Appearance, Pulse, Grimace, Activity, Respiration scores and no major congenital abnormalities in 31 thiopurine-exposed infants; however, 60% had mild anemia at birth.[11] In addition, besides 1 study showing normal global medical and psychosocial health development in 30 thiopurine-exposed children,[19] clinical studies regarding long-term health outcomes are lacking. Overall, large prospective studies assessing the effect of thiopurine use on pregnancy outcomes and health outcomes, including adjustments for important confounding factors, are lacking. The aim of this study was therefore to assess the influence of maternal thiopurine use on pregnancy outcomes (live birth, spontaneous abortion, elective abortion, and stillbirth), birth outcomes (gestational age, birth weight, and congenital abnormalities), and clinical health outcomes of thiopurine-exposed infants 1 year after birth in a large prospective IBD cohort.

METHODS

Study Design

To determine whether thiopurine use during pregnancy negatively affects health outcomes of infants, we analyzed data from our ongoing single-center prospective cohort study at the Erasmus University Medical Center (Rotterdam, The Netherlands), a tertiary health center. All women with confirmed IBD who visited the IBD preconception outpatient clinic between December 2008 and May 2016 were enrolled. At our IBD outpatient clinic, patients are counseled according to the current IBD pregnancy guidelines[20,21] and seen bimonthly during pregnancy by an experienced IBD physician. Before pregnancy, patients are counseled on

IBD medication use, lifestyle habits such as smoking and alcohol use, folic acid intake, and the importance of disease remission 6 months before conception to minimize the risk of a relapse during pregnancy.6 In addition, patients were asked about their obstetrical history such as previous pregnancies, polycystic ovarian syndrome (PCOS), and if patients received fertility treatment. During pregnancy, data regarding medication adherence and confounding variables such as smoking, alcohol use, folic acid intake, disease activity, and obstetric complications were collected. The following obstetrical complications were documented: hyperemesis gravidarum, cholestasis of pregnancy, in utero growth restriction, gestational hypertension, preeclampsia and Hemolysis Elevated Liver enzymes and Low Platelets (HELLP). Paternal factors that could influence pregnancy outcomes such as age, smoking, daily alcohol use, body mass index (BMI) >30 kg/m2, and medication use were also documented. Disease activity was assessed by the treating gastroenterologist, which was based on clinical symptoms (Harvey-Bradshaw Index [HBI] or Simplified Clinical Colitis Activity Index [SCCAI]), blood analysis, and fecal calprotectin, and if necessary an endoscopy was performed.

Outcomes

Pregnancy outcomes were defined as live birth, spontaneous abortion, elective abortion, stillbirth, or current pregnancy. If pregnancy outcomes could not be retraced, patients were excluded from analysis. Birth outcomes such as gestational age, birth weight, mode of delivery, and congenital abnormalities were noted during the first visit after delivery. One-year health outcomes were obtained through telephonic questionnaire with mothers and/or from the general practitioner after consent of both parents. The 1-year outcomes included growth, infections for which antibiotic treatment were needed, hospitalization because of an infection, allergies, adverse reactions to vaccinations, and the presence of eczema.

Study Group

Patients using a thiopurine during conception were assigned to the study group. According to IBD pregnancy guidelines, all pregnant patients using a thiopurine are advised to continue treatment. However, since a publication in 2014 showing a major effect of pregnancy on thiopurine metabolism,[11] 6-methylmercaptopurine (6-MMP) and 6-TGN levels are measured during pregnancy, and thiopurine dose is adjusted accordingly. Thiopurine S-methyltransferase (TPMT) genotype testing is only performed in thiopurine-naive patients before treatment initiation at our hospital.

Pregnancy outcomes, birth outcomes, and 1-year health outcomes of women using a thiopurine were compared with the control group, consisting of women with IBD who were not using a thiopurine during pregnancy. In addition, the 1-year health outcomes were compared with non-IBD controls consisting of 459 children from the same geographic region.[22]

Sample Size

The primary outcomes that were used for the sample size calculation were spontaneous abortion, small for gestational age (SGA), and infection risk of infants up to 1 year. Because we used multiple outcomes, a sample size calculation could not be performed for all documented variables; however, the number of patients was sufficient to identify differences of 15%–20% between study and control group for the primary outcomes.

On the basis of a previous study, we expected a risk of a pregnancy ending in a spontaneous abortions of approximately 20%.[23] In case of an increase of 20% in the study group, at a significance level of .05 and a power of 80%, 82 patients per arm were needed.

The rate of children born SGA was 10% in a large prospective healthy birth cohort from the same geographic region as our study.[22] In case of an increase of 15% in the study group, at a significance level of .05 and a power of 80%, 100 patients per arm were needed.

On the basis of a previous Dutch study, the risk of an infection requiring treatment with an antibiotic was expected to be 43%.[24] In case of an increase of 20% in the study group, at a significance level of .05 and a power of 80%, 97 patients per arm were needed.

Definitions

Abnormal growth was defined as growth for age and gender deviating >2 standard deviations (SDs) from the mean Dutch growth chart. Low birth weight was a weight <2500 g at birth. Preterm birth was defined as a delivery before 37 weeks of gestation. SGA was a weight below the 2 SDs for gestational age according to the Dutch reference curve.[25] The presence of disease activity was assessed by the treating physician and based on the combination of clinical symptoms (HBI >5 or SCCAI >2), C-reactive protein >9.0 mg/L, and fecal calprotectin >200 mg/g, and when strongly indicated, an endoscopy was performed.

Statistical Analysis

All analyses were performed by using IBM SPSS statistics (version 21.0; Chicago, IL). Descriptive statistics of continuous data are displayed as medians with interquartile ranges (IQRs) or means with SDs and compared by using Student t tests or Mann-Whitney U tests. Categorical data are shown as absolute numbers with percentages and compared by using c2 or Fisher exact tests. The tests were performed two-tailed and tested at a significance level of .05. Univariate analysis and multivariate analysis with 95% confidence interval (CI) for the risk of adverse outcomes in the study group were executed with logistic regression. All possible factors associated with adverse pregnancy outcomes (maternal age, fertility treatment, disease activity, diagnosis, thiopurine use, biological use, combination of a thiopurine and a biological, pre-pregnancy BMI, preconception counseling, abdominal IBD surgery, surgery for perianal fistula and/or abscesses, smoking, alcohol use, and the following paternal factors: age, smoking, daily alcohol use, BMI >30 kg/m2, and medication use) and adverse 1-year health outcomes (maternal age, thiopurine use, biological use, combination of a thiopurine

and a biological, smoking, pre-pregnancy BMI, mode of delivery, preterm birth, low birth weight, and breastfeeding) were first analyzed in a univariate binary logistic regression analysis to determine the independent correlation. Subsequently, all univariate variables with a P value .05 and variables chosen by the clinician's rationale (ie, maternal age) were included in the model. After backward elimination, the final multivariate logistic regression model was determined. Interactions were tested between the variables in the multivariate model and were included in the final model when significant (P < .01). A Bonferroni correction was applied in case of multiple comparison testing.

Ethical Consideration

This study was approved by the local ethics committee of the Erasmus Medical Center (Rotterdam, The Netherlands). Legal guardians of the children signed informed consent before data were collected from the general practitioner.

RESULTS

The cohort consisted of 309 women with confirmed IBD who had expressed 1 or more pregnancy wishes between December 2008 and June 2016 at our IBD outpatient preconception clinic. The maternal baseline characteristics at inclusion are displayed separately (**Table 1**), which are the characteristics that did not change during the study period. At the time of analysis, 464 pregnancy wishes were documented that had resulted in 413 pregnancies, of which 311 (75.3%) resulted in a live birth, 78 (18.9%) in a spontaneous abortion, 2 (0.5%) in an elective abortion, 1 (0.2%) in a stillbirth, and 21 women (5.1%) were still pregnant at the time of analysis. Of the remaining cases, 22 women were not yet pregnant, 11 women did not have a reproduction wish anymore, and 18 women were lost to follow-up (**Figure 1**).

Table 1. Maternal baseline characteristics (n=309)

Diagnosis (%)	
Crohn's disease	216 (69.9)
Ulcerative colitis	85 (27.5)
IBD unclassified	8 (2.6)
Disease location CD (Montreal) (%)	
L1 Ileal	54 (25.1)
L2 Colonic	43 (20.0)
L3 Ileocolonic	118 (54.9)
Disease behavior CD (Montreal) (%)	
B1 Non stricturing non penetrating	116 (54.5)
B2 Structuring	14 (6.6)
B3 Penetrating	49 (23.0)
B2+ B3 Stricturing and penetrating	34 (16.0)
P Perianal fistulizing disease (%)	56 (18.5)
Disease extent UC and IBDU (Montreal) (%)	
E1 Proctitis	9 (10.0)
E2 Left-sided colitis	39 (43.3)
E3 Pancolitis	42 (46.7)
Extra intestinal manifestations (%)	51 (17.6)
IBD surgery (%)	
Partial or complete bowel resection	68 (22.7)
Surgery for perianal fistula and/or abscess	31 (10.7)
Education level (%)	
Low	12 (4.5)
Secondary	137 (51.9)
High	115 (43.6)

Relapse during pregnancy

A relapse occurred during 100 pregnancies (32%) that had ended in a live birth. Relapse rate was similar in study group (n = 31, 29%) and control group (n = 69, 35%) (P = .30). The types

of disease activity were as follows: luminal activity (n = 88), active perianal fistula (n = 8), extraintestinal manifestations (n = 2), and a combination of an active fistula and extraintestinal manifestations (n = 2). Disease activity was ongoing from conception in 32 pregnancies; others were new during pregnancy. Active perianal fistula and extraintestinal manifestations were diagnosed during clinical physical examination. Luminal disease activity was identified during an endoscopy (n = 41), abdominal ultrasound (n = 6), magnetic resonance imaging scan (n = 2), clinical symptom in combination with fecal calprotectin >200 mg/g (n = 22), and in some cases solely on the basis of clinical symptoms (n = 17). These clinical symptoms were described by patients as active luminal IBD. In all cases, the treating physician increased the current IBD drug dose or started a new IBD drug.

Figure 1. Flow chart

Spontaneous abortions

For the analyses regarding spontaneous abortion, all pregnancies were included that had ended in a live birth, that had passed the 24th week of gestation, or that had ended in a spontaneous abortion. A total of 410 pregnancies were identified, of which 149 pregnancies (36%) were assigned to the study group. Overall, pregnancies of women using a thiopurine ended in a spontaneous abortion more often (24%, n = 35) than pregnancies of women not using a thiopurine (16%, n = 43), with a trend toward statistical significance in the univariate analyses (crude odds ratio [cOR], 1.63; 95% CI,
0.99–2.69) (**Table 2**). A multivariate regression analysis was performed, with spontaneous abortions included as the dependent variable. After correction for IBD type, fertility treatment, and disease activity, which were the variables that remained in the final multivariate model after backward elimination, thiopurine was not associated with spontaneous abortions (adjusted odds ratio [aOR], 1.26; 95% CI, 0.73–2.20). Variables that did have a statistically significant or borderline significant association with spontaneous abortion were fertility treatment (aOR, 2.15; 95% CI, 1.07–4.34), the presence of Crohn's disease (CD) (aOR, 2.33; 95% CI, 1.20–4.49), and disease activity (aOR, 1.81; 95% CI, 0.95–3.44).

Table 2. Pregnancy outcomes and birth outcomes: univariate and multivariate logistic regression analysis						
All pregnancies > 24 weeks of gestation	**Study group (n=146)**	**Control group (n=263)**	***P* value**	**Crude OR (95% CI)**	**Adjusted OR (95% CI)**	***P* value**
Spontaneous abortions (%)	35 (24.0)	43 (16.3)	.06	1.63 (0.99-2.69)	1.15 (0.66-2.01)a	.62
Live births	**Study group (n=108)**	**Control group (n=203)**				
Median birthweight in grams (IQR)	3360 (3018-3630)	3326 (2898-3640)	.74	-	-	-
Small for gestational age (%)	5 (4.8)	12 (6.2)	.80	0.77 (0.26-2.25)	0.59 (0.18-1.96)b	.39
Median gestational age in weeks (IQR)	38.6 (37.4-40.0)	39.0 (38.0-40.0)	.39	-	-	-
Low birth weight (%)	10 (9.4)	21 (10.4)	.84	0.89 (0.40-1.97)	0.66 (0.28-1.57)b	.34
Preterm birth (%)	12 (11.4)	21 (10.4)	.85	1.11 (0.52-2.36)	0.90 (0.40-2.0)b	.81
Major congenital abnormalities (%)	4 (3.9)	4 (2.1)	.46	-	-	-

[a.] Adjusted for disease activity during conception, fertility treatment and diagnosis

[b.] Adjusted for smoking

Live births

From the 311 live births, 108 infants (35%) were exposed to a thiopurine during pregnancy. Maternal characteristics per child are separately displayed (**Table 3**). Of these infants, 95 (88%) were exposed to AZA, 12 (11%) to MP, and 1 (1%) to thioguanine (TG). Most women (n = 96, 89%) used thiopurine during the entire pregnancy; however, 8 women (7%) stopped thiopurine treatment in the first trimester, and 4 women (4%) stopped in the third trimester. There were various reasons for stopping thiopurine such as elevated 6-MMP level (n = 2), side effects (n = 1), advice from physician (n = 1), anemia (n = 1), abnormal liver enzymes (n = 1), and because of fear of harming their unborn child (n = 6). There were no women who started a thiopurine during pregnancy.

No differences regarding birth outcomes were observed between the study group and the control group (**Table 2**). After correction for smoking in the multivariate logistic regression analysis, there was no association between thiopurine use and the outcomes SGA, low birth weight, and preterm birth. Smoking was the only independent variable with significant association with all 3 related outcomes, SGA (cOR, 4.15; 95% CI, 1.22–14.12), low birth weight (cOR, 3.04; 95% CI, 1.12–8.30), and preterm birth (cOR, 2.62; 95% CI, 1.15–6.00).

In total, 49 pregnancies (12%) were achieved after fertility treatment. Notably, the presence of PCOS was more often reported in women who had undergone fertility treatment (n = 16, 33%) than in other women (n =13, 4%) (P = .0001).

The presence of paternal factors (ie, age, smoking, daily alcohol use, BMI >30 kg/m2, and medication use) were not associated with adverse pregnancy outcomes.

Thiopurine dose during pregnancy

Median thiopurine dose at inclusion was as follows: 1.95 mg/kg AZA (IQR, 1.65–2.22), 0.98 mg/kg MP (IQR, 0.60–1.29), and 1 mother used 0.29 mg/kg TG. Dose optimization was based

Table 3. Maternal characteristics per live birth (n=311)			
	Study group (n=108)	**Control group (n=203)**	***P* value**
Median maternal age in years (IQR)	30.3 (28.2-32.9)	30.4 (27.7-33.3)	.80
Diagnosis (%)			
Crohn's disease	84 (77.8)	135 (66.5)	.05
Ulcerative colitis	18 (16.7)	64 (31.5)	<.01
IBD unclassified	6 (5.6)	4 (2.0)	.10
Disease location CD (Montreal) (%)			
L1 Ileal	15 (17.9)	35 (26.3)	.19
L2 Colonic	15 (17.9)	36 (27.1)	.14
L3 Ileocolonic	54 (64.3)	62 (46.6)	.01
Disease behavior CD (Montreal) (%)			
B1 *Non structuring non penetrating*	41 (48.8)	76 (57.1)	.26
B2 Stricturing	4 (4.8)	6 (4.5)	1.00
B3 Penetrating	27 (32.1)	28 (21.1)	.08
B2+ B3 Stricturing and penetrating	12 (14.3)	23 (17.3)	.71
P Perianal fistulizing disease	30 (28.0)	33 (16.4)	.02
Disease extent UC and IBDU (Montreal) (%)			
E1 *Proctitis*	2 (8.3)	9 (13.2)	.72
E2 Left-sided colitis	12 (50.0)	30 (44.1)	.64
E3 Pancolitis	10 (41.7)	29 (42.6)	1.00
Median disease duration in years (IQR)	7.8 (4.2-11.9)	8.0 (4.4-11.5)	.87
Extra intestinal manifestations (%)	18 (17.3)	37 (19.0)	.76
IBD surgery (%) prior to pregnancy			
Partial or complete bowel resection	17 (15.7)	45 (22.4)	.18
Surgery for perianal fistula and/or abscesses	13 (12.5)	21 (10.7)	.70
Median pre-pregnancy BMI (IQR)	23.8 (21.3-26.3)	22.8 (20.9-25.6)	.22
Concomitant medication (%)			
5-ASA	21 (19.4)	55 (27.1)	.66
Oral steroids	11 (10.2)	31 (15.3)	.23
Suppositories/enemas with steroids	12 (11.1)	27 (13.3)	.72
Anti-TNF-α	35 (32.4)	98 (48.3)	<.01
stopped (<25 weeks of gestation)	18 (54.5)	55 (57.9)	.84
Fertility treatment (%)	9 (8.5)	24 (12.1)	.44
PCOS (%)	4 (3.7)	9 (4.4)	1.00
Folic acid use (%)	80 (76.9)	151 (79.1)	.66
Smoking < 3 months prior to pregnancy (%)	21 (20.2)	32 (16.3)	.43
Smoking during pregnancy (%)	12 (11.7)	15 (7.7)	.29
Disease activity during pregnancy (%)	31 (28.7)	69 (34.7)	.31
Disease activity treated successfully before delivery (%)	22 (73.3)	40 (63.5)	0.50

5-ASA 5-aminosalicylate; Anti-TNF-α anti-tumor necrosis factor; CD Crohn's disease; UC ulcerative colitis; IBD Inflammatory Bowel Disease; IBDU Inflammatory Bowel Disease Unclassified; IQR interquartile range; BMI Body Mass Index; PCOS polycystic ovarian syndrome

on 6-MMP/6-TGN measurements. TPMT genotype was performed in 22 patients; 21 patients had a normal phenotype, and 1 patient had a heterozygote phenotype, for which the dose was optimised before treatment initiation. During pregnancy, an increased 6-MMP level was measured in 11 patients, for which the treating physician lowered the thiopurine dose. All 11 patients were using AZA. Of these patients, 3 experienced disease activity during pregnancy; 1 patient had ongoing activity from conception, and 2 patients had a new relapse after lowering their treatment dose. The 6-TGN measurement was not performed before pregnancy in the patient with ongoing disease. The 6-TGN level decreased in the 2 patients with a new relapse after lowering the AZA dose; however, it remained within normal range. All relapses were treated adequately before delivery, and all 11 pregnancies resulted in the birth of healthy

infants who were born at term with a normal birth weight for their gestational age. Median AZA dose for the 11 patients who lowered their dose during pregnancy was 1.92 mg/kg (IQR, 1.70–2.22) before dose adjustment and 1.11 mg/kg (IQR, 0.87–1.54) after dose adjustment.

Sub analyses: spontaneous abortions and birth outcomes

Women in the control group more often used anti– tumor necrosis factor (TNF) (n = 120, 46%) during pregnancy than women in the study group (n = 50, 34%) (P = .02). In addition, steroids were more often used in the control group (n = 37, 14%) than in the study group (n = 15, 10%) (P = .28). Because these IBD drugs could influence pregnancy outcomes, we performed a sub analysis where we excluded all pregnancies in which the mother used anti-TNF-α and/ or steroids.

In the sub analysis regarding spontaneous abortions, 208 pregnancies were included, of which 44 (21%) had ended in a spontaneous abortion. Spontaneous abortion rate in the study group was similar to the control group (cOR, 0.93; 95% CI, 0.47–1.83). In the sub analyses regarding birth outcomes, there was also no association between thiopurine exposure and adverse pregnancy outcomes (**Supplementary Table 1**).

Congenital abnormalities

There were 8 infants (2.6%) born with a major congenital malformation according to the EUROCAT definitions.[26] Mothers of 4 children used a thiopurine during pregnancy, 3 AZA and 1 MP. The major congenital abnormalities included polydactyly (n = 3), cleft lip and/ or palate (n = 3), ventricular septum defect (VSD) (n = 1), and hypospadias (n = 1) (**Table 4**).

Table 4. Major congenital abnormalities according to the EUROCAT definitions (n=8)

Type	Medication during conception and 1th trimester	Folic acid use	Maternal age at conception
Polydactyly	Azathioprine	No	21
Polydactyly	Methotrexate during conception, infliximab	No	18
Polydactyly	Azathioprine	No	28
Cleft palate	Prednison, probiotics	No	25
Cleft palate	Entocort	Yes	28
Cleft palate	Azathioprine, Infliximab, Omeprazol, Thyrax	Yes	33
VSD	Infliximab	No	29
Hypospadia	Mercaptopurine, 5-ASA	Yes	30

5-ASA 5-aminosalicylate; VSD ventricular septum defect

One-year health outcomes

Information regarding 1-year health outcomes was from the study group died shortly after birth because of available from 224 infants (72%) (**Table 5**); other infants did not yet reach the age of 1, we were unable to contact mother/general practitioner, and unfortunately, a child from the study group died shortly after birth because of extreme prematurity after his

Table 5. One-year health outcomes of infants: univariate and multivariate logistic regression analysis						
	Study group (n=83)	Control group (n=141)	*P* value	Crude OR (95%CI)	Adjusted OR (95%CI)	*P* value
Growth deficiency (%)	2 (2.4)	4 (2.8)	1.00	-	-	-
Infection (yes/no) (%)	27 (33.8)	44 (31.7)	.77	1.03 (0.58-1.85)	1.02 (0.57-1.83)a	1.00
Number of infections (%)						
0	53 (66.2)	89 (64.0)	.77	1.10 (0.62-1.97)	1.12 (0.63-1.99)a	.71
1-2	24 (30.0)	41 (29.5)	1.00	1.02 (0.56-1.87)	1.01(0.55-1.85)a	.97
≥ 3	3 (3.8)	9 (6.5)	.54	0.56 (0.15-2.14)	-	.40
Hospitalization because of an infection (%)	8 (9.9)	10 (7.2)	.61	1.28 (0.49-3.31)	1.30 (0.50-3.28)a	.60
Allergies (%)	10 (12.0)	10 (7.1)	.20	1.83 (0.73-4.61)	1.72 (0.67-4.38)b	.25
Adverse reaction(s) to vaccination(s) (%)	0 (0.0)	1 (0.7)	1.00	-	-	-
Eczema (%)	13 (18.3)	22 (18.3)	1.00	1.00 (0.47-2.13)	0.98 (0.45-2.13)c	.95

a Adjusted for preterm birth

b Adjusted for smoking during pregnancy

c Adjusted for mode of delivery

mother developed HELPP syndrome during pregnancy. Overall, there were no differences regarding 1-year health outcomes between groups.

Growth

There were 2 thiopurine-exposed children (2%) with a primary growth deficiency and 4 children (3%) in the control group with a growth deficiency (3 primary growth deficiencies and 1 secondary growth deficiency). There was no association between thiopurine exposure in utero and growth deficiencies.

Infections

Information regarding infections was retrieved for 219 infants. In total, 77 infants received 109 oral antibiotic courses for an infection. There were 142 infants (65%) without antibiotic use, 55 (25%) received 1 treatment, 10 (5%) received 2 treatments, and 12 children (5%) received 3 or more treatments. The types of infections requiring antibiotic treatment were acute otitis media (n = 51), upper respiratory infections (n = 28), other ear nose throat infections (n = 9), urinary tract infection (n = 3), respiratory syncytial virus (RS virus) (n = 2), secondary infected eczema (n = 1), secondary infected mycosis (n = 1), paronychia (n = 1), tonsillitis (n = 1), infection in toe (n = 1), impetigo (n = 1), and furuncles (n = 1), and the reason for 9 oral antibiotic treatments were unknown. In the univariate analysis and after adjustment in the multivariate regression analysis for preterm birth, which is a well-known risk factor for infections,[27–29] there was no association between thiopurine use and infection risk.

There were 19 hospital admissions because of a serious infection. Most infants were admitted because of an RS virus infection (n = 6); other reasons for hospital admission were severe otitis media infection (n = 4), upper respiratory infection (n = 4), fever (n = 2), urinary tract infection (n = 1), scarlet fever (n = 1), and furuncles (n = 1). In the univariate analysis and after

adjustment for preterm birth in the multivariate regression analysis, there was no association between hospital admission and in utero exposure to thiopurine.

Adverse reactions to vaccinations

One adverse reaction had been reported. A child from the control group had been admitted to hospital because of a high fever shortly after a vaccination; however, treatment was not needed.

Allergies

There were 20 infants with an allergy reported by their parents, of which 10 children were exposed to a thiopurine during pregnancy. A cow milk allergy was most often reported, namely in 12 infants, and was confirmed in 6 cases by the general practitioner. In 3 cases, parents reported an allergic reaction after eating a meal; however, the specific type of food to which the infants reacted was unknown. Other allergies that were reported by parents were allergies to an antibiotic (n = 2), hay fever (n = 2), and sun cream (n = 1). Thiopurine exposure in utero was not associated with allergies in both the univariate analysis and after adjustment for smoking in the multivariate analysis. In the univariate analyses, smoking was the only independent variable that was associated with allergies (odds ratio [OR], 3.27; 95% CI, 1.07–10.04).

Eczema

Thiopurine exposure was not associated with eczema in both the univariate analysis and after adjustment for cesarean section in the multivariate analysis. In the univariate analyses, a cesarean section was the only independent variable that was associated with eczema (OR, 3.31; 95% CI, 1.55–7.06).

Sub analyses: one-year health outcomes

In the sub analyses for 1-year health outcomes in which we excluded all infants who had been exposed in utero to anti-TNF-α and/or steroids, thiopurine exposure was also not associated with adverse 1-year health outcomes (Supplementary Table 2).

Non–inflammatory bowel disease controls: we found no statistical significant differences between the study group and non-IBD controls regarding the following 1-year health outcomes: growth abnormalities (P = 1.00), number of infants requiring 1 or more antibiotic treatments (P = .21), allergies (P = .18), and the presence of eczema (P = 1.00). Adverse reactions to vaccination were not documented for the non-IBD controls.

DISCUSSION

In this prospective cohort study, we found no association between maternal thiopurine use for IBD and adverse pregnancy outcomes, including spontaneous abortions, and adverse birth outcomes. In addition, we found no association between thiopurine exposure in utero and adverse health outcomes of infants up to 1 year of age.

To our best knowledge, this is the largest prospective follow-up study assessing the effect of thiopurine use for IBD during pregnancy. In contrast to previous retrospective and population-based studies, we were able to adjust for confounding factors influencing pregnancy outcomes and health outcomes.

The overall rate of spontaneous abortions in our IBD cohort was 19.0%. This is higher than the 8.6% demonstrated in a previous retrospective multicenter study in women with IBD.[13] Studies of the overall population showed that between 12% and 15% of all clinically recognized pregnancies result in a spontaneous abortion.[30,31] However, when including biochemical pregnancies, defined as pregnancy losses shortly after conception that could be confused with a normal period, the prevalence is approximately 20%.[23] This is similar to our IBD cohort, which mainly consists of women who planned their pregnancy carefully and often discovered their pregnancy at an early stage.

We found no association between thiopurine use and spontaneous abortions; however, fertility treatment and CD were both associated with spontaneous abortions in our cohort. Women who had undergone fertility treatment were more often diagnosed with PCOS, which is associated with spontaneous abortions.[32–34] In addition, the association between CD and spontaneous abortions is in line with previous studies.[35–37]

Paternal preconception risk factors for adverse pregnancy outcomes have been scarcely investigated. [38,39] In our cohort we did not find an association between adverse pregnancy outcomes and paternal factors.

In our IBD cohort, 8 infants (2.6%) were born with major congenital malformations, which is consistent with the overall European population.[40] This indicates that children born to women with IBD, with or without thiopurine therapy, have no increased risk of a major congenital abnormality.

The absolute number of infections in thiopurine-exposed children for which antibiotics were prescribed is comparable to the 0–4 years age group of the Dutch general practice.[24] In addition, when comparing the number of infections in thiopurine-exposed children with a

non-IBD cohort from the same geographic region, no difference was observed.[22] There were also no differences in growth, allergies, and eczema observed between thiopurine-exposed infants and the non-IBD cohort.[22]

It should be noted that this cohort received counseling and strict follow-up during pregnancy, which has a beneficial effect on pregnancy outcomes.[41] This should be taken into account when comparing results with other studies. In addition, disease activity could not be assessed in a standard manner because some clinical scores and laboratory work-up are of limited value during pregnancy. Most pregnant women will experience abdominal complaints during pregnancy, independent of their underlying IBD, and clinical scores such as hematocrit and body weight are of little use because many pregnant women will become anemic and will gain weight over time. An endoscopy is only performed when strongly indicated and will be postponed until after the delivery if possible. Furthermore, this study lacks a non-IBD control group; therefore, outcomes were compared with outcomes of a non-IBD pregnancy cohort from the same geographic region. Thus, controls were part of a different follow-up protocol, which may have an effect on the reported outcomes.
Our study, however, has an important advantage over previous studies, namely this is a single-center prospective study and all patients were treated in a specialized IBD pregnancy center according to the current pregnancy guidelines.[20,21] Because of the prospective nature of the study and stringent per protocol follow-up, we were able to firmly assess the influence of confounding factors on the outcomes.

CONCLUSION

In this prospective cohort study, we found no association between maternal thiopurine use during pregnancy and spontaneous abortions, adverse birth outcomes, or adverse health outcomes of infants 1 year after birth.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1. Sub-analysis pregnancy outcomes (anti-TNF-α and steroid use excluded): univariate logistic regression analysis				
All pregnancies > 24 weeks of gestation	**Study group (n=88)**	**Control group (n=120)**	***P* value**	**Crude OR (95% CI)**
Spontaneous abortions (%)	18 (20.5)	26 (21.7)	.83	0.93 (0.47-1.83)
Live births	**Study group (n=65)**	**Control group (n=88)**		
Median birthweight in grams (IQR)	3366 (3028-3620)	3290 (2840-3644)	.39	-
Small for gestational age (%)	5 (6.0)	5 (7.7)	.68	1.32 (0.37-4.76)
Median gestational age in weeks (IQR)	39.0 (37.5-40.0)	39.1 (38.0-40.0)	.70	-
Low birth weight	6 (9.2)	10 (11.5)	.65	0.78 (0.27-2.28)
Preterm birth (%)	6 (9.2)	11 (12.6)	.51	0.70 (0.25-2.01)
Major congenital abnormalities (%)	3 (4.6)	0 (0.0)	.08	-

IQR interquartile range

Supplementary Table 2. Sub-analysis (anti-TNF-α and steroid use excluded): univariate and multivariate logistic regression analysis of the 1-year health outcomes						
	Study group (n=65)	**Control group (n=52)**	***P* value****	**Crude OR (95%CI)**	**Adjusted OR (95%CI)**	***P* value**
Growth deficiency (%)	2 (3.8)	1 (1.5)	.58	-	-	-
Antibiotic use for infection (%)	18 (36.0)	20 (30.8)	.69	1.27 (0.58-2.77)	1.29 (0.59-2.84)a	.52
Hospitalization because of infection (%)	4 (8.0)	4 (6.2)	.73	-	-	-
Allergies (%)	7 (13.7)	2 (3.0)	.04	-	-	-
Adverse reaction(s) to vaccination(s) (%)	0 (0.0)	1 (1.5)*	1.00	-	-	-
Chronic disease (%)	0 (0.0)	0 (0.0)	1.00	-	-	-
Eczema (%)	10 (16.9)	9 (19.6)	.80	1.19 (0.44-3.23)	1.09 (0.40-3.02)b	.73

* Fever and torticollis after vaccination, hospital admission, no intervention

** Significance level ≤ 0.007 (Bonferroni correction)

a Adjusted for preterm birth

b Adjusted for mode of delivery

Chapter 4.2:

Commentary on "Pregnancy outcomes in women with inflammatory bowel disease following exposure to thiopurines and antitumor necrosis factor drugs: A systematic review with meta-analysis"

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With great interest, we read the systematic review and meta-analysis by Mozaffari et al.[1] who underline that active inflammatory bowel disease (IBD) during pregnancy is related to adverse pregnancy outcomes.[2–5] We agree with the authors that studies assessing the safety of IBD drugs during pregnancy to maintain disease remission such as thiopurines and antitumor necrosis factor alpha are conflicting.

The authors concluded that the presence of congenital abnormalities was higher in thiopurine-exposed children than in children who were not exposed to an IBD drug in utero. This conclusion is based on the results of two studies: a population-based study by Norgard et al.[6] and a partial prospective and partial retrospective study by Coelho et al.[7] Only the study by Norgard et al. demonstrated a high rate of congenital abnormalities (n = 4; 15.4%) in 26 thiopurine-exposed children. In this study, women suffering from Crohn's disease with an active prescription for azathioprine or mercaptopurine during the time of conception until the end of the third trimester were retrospectively evaluated. However, due to the nature of this study, medication adherence could not be assessed. In addition, no data on folic acid intake were obtained, which is of importance when describing congenital abnormalities. Also, specific details regarding the described congenital abnormalities (i.e. minor or major congenital abnormalities) are lacking.

Other studies exist showing an association between thiopurine use and its adverse pregnancy outcomes, including congenital abnormalities.[8–10] However, due to the retrospective nature of these studies, it was not possible to determine the influence of confounding factors such as disease activity, smoking, folic acid intake, and obstetrical complications. Therefore, the authors stated that they could not rule out the possibility that the association was caused by confounding factors. Overall, most clinical studies, including one small prospective study,[11] show that there is no association between maternal thiopurine use and adverse pregnancy outcomes, including congenital abnormalities.7,[11–13] Therefore, we feel that the study by Mozaffari et al. should be interpreted with caution as the evidence that thiopurine use during pregnancy leads to a higher risk of congenital abnormalities is frail.

More prospective studies assessing the effect of thiopurine use on pregnancy outcomes, including adjustments for important confounding factors, are needed. However, based on the current available data, we advise to continue thiopurine treatment throughout pregnancy, as disease activity during pregnancy likely outweighs possible risks related to thiopurine use, including congenital abnormalities.

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Chapter 5:

Anti-TNF-α levels in cord blood at birth are associated with anti-TNF-α type

5

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ABSTRACT

Background and Aims

Pregnancy guidelines for women with Inflammatory Bowel Disease (IBD) provide recommendations regarding anti-TNF-α cessation during pregnancy, in order to limit fetal exposure. Although infliximab (IFX) leads to higher anti-TNF-α concentrations in cord blood than adalimumab (ADA), recommendations are similar. We aimed to demonstrate the effect of anti-TNF-α cessation during pregnancy on fetal exposure, for IFX and ADA separately.

Methods

We conducted a prospective single center cohort study. Women with IBD, using IFX or ADA, were followed-up during pregnancy. In case of sustained disease remission, anti-TNF-α was stopped in the third trimester. At birth, anti-TNF-α concentration was measured in cord blood. A linear regression model was developed to demonstrate anti-TNF-α concentration in cord blood at birth. In addition, outcomes such as disease activity, pregnancy outcomes and 1-year health outcomes of infants were collected.

Results

We included 131 pregnancies that resulted in a live birth (73 IFX, 58 ADA). At birth, 94 cord blood samples were obtained (52 IFX, 42 ADA), showing significantly higher levels of IFX than ADA ($p<0.0001$). Anti-TNF-α type and stop week were used in the linear regression model. During the third trimester, IFX transportation over the placenta increases exponentially, however, ADA transportation is limited and increases in a linear fashion. Overall, health outcomes were comparable.

Conclusions

Our linear regression model shows that ADA may be continued longer during pregnancy as transportation over the placenta is lower than IFX. This may reduce relapse risk of the mother without increasing fetal anti-TNF-α exposure.

INTRODUCTION

Active inflammatory bowel disease (IBD) during conception and pregnancy has been associated with adverse pregnancy outcomes such as spontaneous abortion, intrauterine growth restriction, preterm birth and low birth weight.[1-4] Current pregnancy guidelines therefore advise to conceive at time of disease remission.[5, 6] In order to maintain disease remission, anti-TNF-α IgG1 monoclonal antibodies such as infliximab (IFX) and adalimumab (ADA) are increasingly being used. These drugs, however, are actively transported over the placenta to the fetus.[7, 8] The transportation of IgG1 antibodies over the placenta increases exponentially during the third trimester, resulting in higher fetal levels than maternal levels at term.[9-11]

Previous studies among women suffering from IBD suggested that the use of IFX and ADA during pregnancy is not related to adverse pregnancy outcomes nor to adverse health outcomes of children until 1 year of age.[12-17] However, a case series reported severe neutropenia at birth in 4 IFX-exposed children[18] and also a case of a fatal disseminated Bacille Calmette-Guerin (BCG) infection of an IFX-exposed child has been reported.[19] In addition, an increased infection risk was seen in children exposed in utero to a combination of an immunomodulator and either IFX or ADA,[20, 21] and also long-term health outcomes of anti-TNF-α exposed infants are scarcely studied. In this context, the safety of exposure to high levels of anti-TNF-α is difficult to take for granted and should preferably be avoided.
Current pregnancy guidelines provide recommendations on how to balance between limiting drug exposure in utero to avoid health risks for the fetus and maintaining maternal disease remission. If women are not in sustained disease remission, it is advised to continue anti-TNF-α treatment throughout the entire pregnancy as active IBD increases the risk of adverse pregnancy outcomes. However, in case women are in sustained disease remission, anti-TNF-α treatment cessation may be considered in the third trimester to minimise fetal drug exposure. Anti-TNF-α cessation in the third trimester seems feasible in this latter group as it does not lead to an increased relapse risk.[17]

These current pregnancy guidelines, however, do not differentiate between IFX and ADA, although different pharmacokinetics are expected. A recent study, comparing anti-TNF-α levels in cord blood and the time of infant drug clearance between IFX-exposed infants and ADA-exposed infants, found significantly higher anti-TNF-α levels in cord blood and a longer time to anti-TNF-α clearance in IFX-exposed infants than in ADA-exposed infants.[20] This suggests that it may be possible to extend ADA treatment longer during the third trimester of pregnancy than IFX treatment, without increasing anti-TNF-α levels in the newborn.
The primary aim of our study was to demonstrate the effect of gestational anti-TNF-α cessation on fetal drug exposure in a linear regression model for IFX and ADA separately.

The secondary aims were to assess disease course during pregnancy, birth outcomes, adverse events after anti-TNF-α re-initiation and infants health outcomes at 1 year for IFX and ADA separately.

MATERIAL AND METHODS

Study design

All IBD women treated with either IFX or ADA who visited the preconception outpatient clinic at the Erasmus University Medical Center Rotterdam, a tertiary health center, from December 2008 through June 2016 were prospectively enrolled. If patients did not attend follow-up visits at our clinic and/or if the pregnancy outcomes could not be retraced, they were excluded from analyses. This cohort consisted partly of women from a previously published cohort[17] and partly of additional follow-up information. During visits at our IBD outpatient clinic, patients were counselled before pregnancy and seen bi-monthly during pregnancy by an experienced IBD physician. In case of disease activity, women were seen every second week at the outpatient clinic. Patients were counselled on medication use, folic acid intake, life style habits (e.g. smoking, alcohol use) and the importance of achieving and maintaining disease remission before conception and during pregnancy. Data regarding disease activity, medication adherence, smoking, alcohol use, folic acid intake and obstetric complications were collected during each visit. Disease activity was assessed based on clinical symptoms (Harvey Bradshaw Index (HBI) or Simplified Clinical Colitis Activity Index (SCCAI), blood analysis, fecal calprotectin (FCP) measurement and an endoscopy was performed when necessary. In case of disease relapse at any time from 6 months before conception until gestational week 20, anti-TNF-α treatment would be continued during the entire pregnancy. In case of sustained disease remission from 6 months before conception until gestational week 20, the option of discontinuing anti-TNF-α treatment in the third trimester was discussed in a multidisciplinary team, including the gynecologist and the patient. All patients were additionally informed by an experienced IBD nurse regarding anti-TNF-α measurements in cord blood and peripheral maternal blood at birth. During this consult, patients received written instructions on how to obtain the blood samples at birth, which they handed over to their gynecologist. During delivery, blood samples were collected by the gynecologist and sent directly to the laboratory of Gastroenterology at the Erasmus MC.

Birth outcomes such as, gestational age at birth, birth weight and the presence of congenital abnormalities were noted during the first visit after delivery. After 1 year, health outcomes from infants were obtained through telephonic questionnaire with mothers and/or by obtaining medical information from the general practitioner after consent of both parents. The 1-year outcomes included growth, infections for which systemic antibiotic treatment were needed, hospitalization for an infection, allergies, chronic diseases, adverse reactions to vaccinations and the presence of eczema.

Outcome measurements

Anti-TNF-α serum level measurements were performed since 2010 at our clinic. These measurements were done by ELISA from peripheral blood, as described in previous papers.[9, 22]

Definitions

Abnormal growth is defined as a growth or height for age and gender deviating > 2 standard deviation (SD) from the mean Dutch growth chart. Preterm birth is defined as a delivery before 37 weeks of gestation. Small for gestational age (SGA) is a weight below the 2 SD for gestational age according to the Dutch reference curve.[23] The presence of disease activity was assessed by the treating physician and based on the combination of clinical symptoms (HBI > 5 or SCCAI > 2), C-reactive protein (CRP) > 9.0 mg/l, FCP measurement > 200 µg/g and when strongly indicated, an endoscopy was performed. The standard dose of anti-TNF-α treatment is 5mg/kg intravenously every 8 weeks for IFX and 40mg subcutaneously every 2 weeks for ADA.

Study size

The primary aim was to assess the difference in anti-TNF-α cord blood concentrations between IFX and ADA and subsequently use the anti-TNF-α cord blood concentrations as dependent variable in a linear regression model. The null hypothesis assumed no difference in anti-TNF-α cord blood concentration between IFX-users and ADA-users. We used the median anti-TNF-α level in cord blood and range from a previous study[20] and estimated the mean and standard deviation according to the method devised by Hozo et al.[24] We expected the mean anti-TNF-α concentration of ADA-users to be lower than the mean anti-TNF-α concentration of IFX-users, therefore, a one-side test with alpha of 2.5% was used. To detect a lower mean anti-TNF-α concentration in cord blood of 3.5 µg/mL in ADA-users, with a power of 90%, the estimated sample size was 31 per arm.

Statistical considerations

All analyzes were performed using IBM SPSS statistics (version 21.0 Chicago III, USA). Descriptive statistics of continuous data are displayed as medians with interquartile ranges (IQR) or means with standard deviations (SD), and compared using Students T-tests or Mann Whitney U tests. Categorical data are shown as absolute numbers with percentages, and compared using Fisher's exact tests. The tests were performed two tailed unless stated differently, and tested at a significance level of 0.05. Univariate and multivariate analysis are shown with a 95% confidence interval (CI). Anti-TNF-α cord blood data was normalized by log transformation. Simple linear regression analyses were performed to determine the association between variables and anti-TNF-α concentration in cord blood at birth. All possible predictors (i.e. maternal age, diagnosis, pre-pregnancy body mass index (BMI), pre-pregnancy weight, anti-TNF-α stop week, smoking, type anti-TNF-α, thiopurine use, steroid

use, gestational age at birth, birth weight, bowel resection prior to pregnancy, anti-TNF-α dose and disease relapse during pregnancy) were considered. Subsequently, only variables with a p-value<0.20 in the univariate analysis and variables based on clinician's rationale (i.e. disease activity during pregnancy, anti-TNF-α dose and concomitant thiopurine use) were considered for the multiple linear regression model. These variables were carefully weight to create to most favorable model. In the final multivariate model, interactions were tested between the independent variables and were included when significant (p<0.01).

Ethical statement

This study was approved by the local ethics committee of the Erasmus University Medical Center (Rotterdam, The Netherlands). Legal guardians of the children signed informed consent before data was collected from the general practitioner.

RESULTS

A total of 416 pregnancies were recorded at our outpatient clinic; during 170 (41%) pregnancies mothers were treated with anti-TNF-α. Of these pregnancies, 136 (80%) resulted in a live birth, 28 (16%) resulted in spontaneous abortion, 5 (3%) were pregnant at the time of analyses and 1 (1%) pregnancy was terminated by an elective abortion.

Live births

We included 136 live births from 102 mothers (82 (80%) with Crohn's disease (CD), 19 (19%) with ulcerative colitis (UC) and 1 (1%) with IBD unclassified). During conception and/or pregnancy, 76 (56%) infants were exposed to IFX and 60 (44%) were exposed to ADA in utero. There were 5 women who stopped anti-TNF-α treatment before the second trimester (3 IFX, 2 ADA), which was advised by a physician other than a gastroenterologist. These women were excluded from further analyses. Baseline characteristics per pregnancy are shown in **Table 1**. In our cohort, women with CD more often used ADA and women with UC more often used IFX. In addition, IFX was more often prescribed in case of an extensive CD and in combination with a thiopurine. There were no other differences in the baseline characteristics between IFX-users and ADA-users.

Maternal outcomes

Maternal outcomes are displayed in **Table 2**. There were no differences regarding weight gain during pregnancy, anti-TNF-α stop week, disease relapse during pregnancy, and successful treatment of the relapse between IFX- users and ADA-users. In addition, we observed no differences in adverse events after restarting anti-TNF-α post-partum such as, allergic reactions, loss of response or disease relapse within 3 months post-partum between these groups.

Table 1. Baseline characteristics per pregnancy (n=131)			
	IFX (n=73)	ADA (n=58)	*P* value
Median maternal age during conception (IQR)	30 (27-33)	30 (28-33)	0.59
Education level (%)			
High	27 (43)	18 (35)	0.44
Middle	34 (55)	33 (65)	0.34
Low	1 (2)	0 (0)	1.00
Diagnosis (%)			
Crohn's disease	54 (74)	51 (88)	0.05
Ulcerative colitis	18 (25)	6 (10)	0.04
IBD unclassified	1 (1)	1 (2)	1.00
Disease location CD (Montreal) (%)			
L1 Ileal	10 (19)	15 (30)	0.25
L2 Colonic	8 (15)	15 (30)	0.10
L3 Ileocolonic	35 (66)	20 (40)	0.01
Disease behaviour CD (Montreal) (%)			
B1 Non structuring non penetrating	44 (61)	28 (51)	0.28
B2 Stricturing	2 (3)	4 (7)	0.40
B3 Penetrating	14 (19)	14 (26)	0.52
B2+ B3 Stricturing and penetrating	12 (17)	9 (16)	1.00
P Perianal fistulizing disease (%)	20 (28)	19 (33)	0.57
Disease extent UC / IBDU (Montreal) (%)			
E1 Proctitis	0 (0)	0 (0)	1.00
E2 Left-sided colitis	4 (21)	4 (57)	0.15
E3 Pancolitis	15 (79)	3 (43)	0.15
Disease duration in years (IQR)	7 (3-11)	7 (5-11)	0.58
Duration of anti-TNF-α treatment in months (IQR)	24 (8-40)	23 (8-38)	0.74
Anti-TNF-α dose (%)			
Standard dose	50 (69)	47 (81)	0.11
Increased dose	3 (4)	0 (0)	0.13
Increased frequency	19 (26)	11 (19)	0.30
Increased dose and frequency	1 (1)	0 (0)	1.00
Co-medication			
Mesalazine	5 (7)	5 (9)	0.75
Steroids (systemic)	9 (12)	10 (17)	0.46
Thiopurine	29 (40)	5 (9)	0.0001
IBD surgery (%)			
Abdominal surgery	14 (19)	14 (25)	0.52
Perianal surgery	13 (19)	12 (22)	0.82
EIM prior to pregnancy (%)	15 (22)	14 (26)	0.68
Parity (%)			
Nulliparous	47 (65)	30 (54)	0.21
Multiparous	24 (34)	26 (46)	0.20
Median pre-pregnancy BMI (IQR)	25 (22-27)	23 (21-27)	0.25
Disease relapse in the preceding year (%)	20 (29)	22 (47)	0.08
Folic acid intake (%)	55 (83)	45 (83)	1.00
Smoking (%)	5 (7)	6 (11)	0.53
Fertility treatment (%)	3 (4)	5 (9)	0.47

IBD Inflammatory Bowel Disease; IFX Infliximab; ADA Adalimumab; CD Crohn's disease; UC Ulcerative Colitis; IBDU IBD Unclassified; EMI extra-intestinal manifestation; BMI Body Mass Index.

Disease relapse

Overall, 42 (32%) women had a disease relapse in the year before conception and 38 (30%) women experienced disease activity during pregnancy of which, 15 (40%) were ongoing from

Table 2. Maternal and pregnancy outcomes (n=131)			
	IFX (n=73)	**ADA (n=58)**	***P* value**
Median weight gain in kilograms during pregnancy (IQR)	12 (10-18)	12 (10-15)	0.90
Anti-TNF-α stopped during pregnancy < week 25 (%)	37 (52)	33 (57)	0.60
Median anti-TNF-α stop week during pregnancy (IQR)	23 (21-32)	23 (22-37)	0.19
Total disease activity during pregnancy (%)	19 (26)	19 (35)	0.33
Activity ongoing from conception (%)	7 (10)	8 (15)	0.42
New relapse during pregnancy (%)	12 (16)	11 (20)	0.65
Successful relapse treatment before delivery (%)	14 (78)	11 (58)	0.30
Median anti-TNF-α restart week postpartum (IQR)	3 (1-4)	2 (0-3.5)	0.20
Allergic reaction at restart anti-TNF-α (%)	2 (3)*	1 (2)**	1.00
Loss of response after restart anti-TNF-α (%)	1 (1)***	1 (2)****	1.00
Disease relapse within 3 months postpartum (%)	7 (12)	8 (19)	0.40
Median birthweight in kilograms (IQR)	3.3 (3.0-3.7)	3.4 (3.1-3.6)	0.50
Low birth weight (%)	6 (9)	4 (7)	1.00
Median gestational age (IQR)	39 (38-40)	39 (38-40)	0.75
Preterm birth (%)	7 (10)	2 (3)	0.18
Small for Gestational Age (SGA) (%)	3 (4)	1 (2)	0.63
Major congenital abnormalities (%)	3 (4.7)*****	0 (0)	0.25
Breastfeeding > 2 weeks (%)	18 (30)	16 (33)	0.68
Mode of delivery (%) Vaginal delivery Caesarean section	 32 (48) 35 (52)	 34 (61) 22 (39)	 0.21 0.21

*Infusion reaction, both treated with hydrocortisone and clemastine; ** Itch after ADA administration, treated with antihistamine; *** Decreased effect IFX after restart, switched to ADA after 5 months; ****Decreased effect ADA, switched to vedolizumab after 5 months; ***** VSD (n=1), cleft palate (n=1) and polydactyly (n=1).

IQR interquartile range.

conception and 23 (60%) were new relapses. The types of disease activity were as follows: 33 (87%) women had luminal disease activity, 4 (10%) women had an active perianal fistula and 1 (3%) woman had an active extra-intestinal manifestation (EIM). Active perianal fistula and EIM were diagnosed during clinical physical examination. Luminal disease activity was diagnosed by endoscopy (n=12, 37%), abdominal ultrasound (n=1, 3%) and in other cases, activity was diagnosed based on clinical symptoms and laboratory work-up (CRP and/or FCP measurement) (n=20, 60%). Disease activity was treated as follows; in 6 cases, anti-TNF-α was re-started or never discontinued because of disease activity, 11 women were treated with corticosteroids, 2 women were treated with antibiotics and 1 women by means of nasogastric feeding. In other cases, the delivery was advanced or activity was not treated as requested by patient. Treatment was successful in 25 (68%) cases before delivery.

As advised by current pregnancy guidelines, women discontinued anti-TNF-α treatment before the third trimester in case of sustained disease remission. Overall, there were 37 (52%) women using IFX and 33 (57%) women using ADA who discontinued treatment between gestational week 12 and 25 (p=0.60). In case women stopped treatment between gestational week 12 and 25, the rate of new relapses during pregnancy was 4 (11%) for women using IFX and 3 (10%) for women using ADA, which was not statistically significant (p=1.00). In case women continued anti-TNF-α after gestational week 25, the rate of new relapses during pregnancy was 8 (24%) for women using IFX and 8 (33%) for women using ADA, however, this difference was also not statistically significant (p=0.55).

Pregnancy outcomes

No differences were observed in terms of birth weight, gestational age at birth, the presence of congenital abnormalities and mode of delivery between IFX-exposed infants and ADA-exposed infants **(Table 2)**. Three children were born with a congenital abnormality. Two of the 3 mothers did not use folic acid at time of conception. In addition, the mother of the child born with a polydactyly used methotrexate at the time of conception. She stopped the methotrexate and started folic acid after she discovered her pregnancy. The other mothers did not use teratogenic medication.

Drug concentration in cord blood and maternal blood

At birth, cord blood samples in which anti-TNF-α concentration was measured were obtained from 94 mothers; 52 used IFX and 42 used ADA during pregnancy. Results are shown in **Table 3**. Anti-TNF-α levels in cord blood and the ratio cord blood level / maternal level were

Table 3. Outcomes of mother-newborn pairs with anti-TNF-α measurement in cord blood (n=94)

	IFX (n=52)	ADA (n=42)	*P* value
Median anti-TNF-α concentration in cord blood (μg/mL) (IQR)	4.9 (1.9-14.7)	1.1 (0.4-37.0)	**0.0001**
Median anti-TNF-α level in maternal blood at birth (μg/mL) (IQR)	1.7 (0.4-6.9)	0.6 (0.3-3.6)	0.05
Median ratio of cord blood level / maternal level (IQR)	2.63 (1.67-4.03)	1.36 (1.00-2.08)	**0.0001**
Median anti-TNF-α stop week (IQR)	25 (21-32)	23 (22-37)	0.56
Anti-TNF-α stopped during pregnancy before gestational week 25 (%)	26 (50)	26 (62)	0.30
Disease relapse during pregnancy (%)	15 (29)	14 (36)	0.50
Median duration of anti-TNF-α treatment in months before pregnancy (IQR)	24 (8-40)	22 (8-38)	0.73
Standard dose anti-TNF-α (%)	35 (67)	33 (79)	0.25

IQR interquartile range; IFX infliximab; ADA adalimumab

both higher in IFX-users than ADA-users. In addition, anti-TNF-α levels in maternal blood were higher in IFX-users than ADA-users (p=0.05). There were no other differences between women using IFX and women using ADA regarding anti-TNF-α stop week during pregnancy, duration of anti-TNF-α treatment before conception, relapse rate and number of women using the standard dose.

Table 4. Simple linear regression analyses: assessing association between variables and anti-TNF-α cord blood concentration

Independent variables	Standardized β coefficients	*P* value
Maternal age	0.031	0.71
Diagnosis	-0.078	0.41
Pre-pregnancy BMI	-0.174	0.35
Pre-pregnancy weight	-0.004	0.98
Anti-TNF-α stop week	0.738	**0.0001**
Smoking	-0.071	0.39
Type anti-TNF-α	-0.510	**0.0001**
Thiopurine	-0.020	0.81
Steroids	0.027	0.77
Gestational age	-0.113	0.27
Birth weight	0.053	0.56
Prior bowel resection	0.012	0.89
Standard anti-TNF-α dose	0.003	0.97
Relapse during pregnancy	-0.118	0.24
Mode of delivery	0.05	0.58

BMI body mass index

Linear regression model

The simple linear regression analyses, assessing the association between variables and anti-TNF-α concentration in cord blood at birth, are shown in **Table 4**. Only the variables anti-TNF-α stop week and type anti-TNF-α were strongly associated with anti-TNF-α concentration in cord blood. There was no significant correlation between these two variables. No other variables improved the model. Therefore, in the final multiple linear regression model, anti-TNF-α stop week and type anti-TNF-α were used as independent variables and anti-TNF-α cord blood concentration at birth as dependent variable **(Figure 1)**. This model shows that IFX transportation over the placenta increases exponentially during the third trimester of pregnancy. However, ADA transportation over the placenta is significantly lower than IFX and increases in a linear fashion.

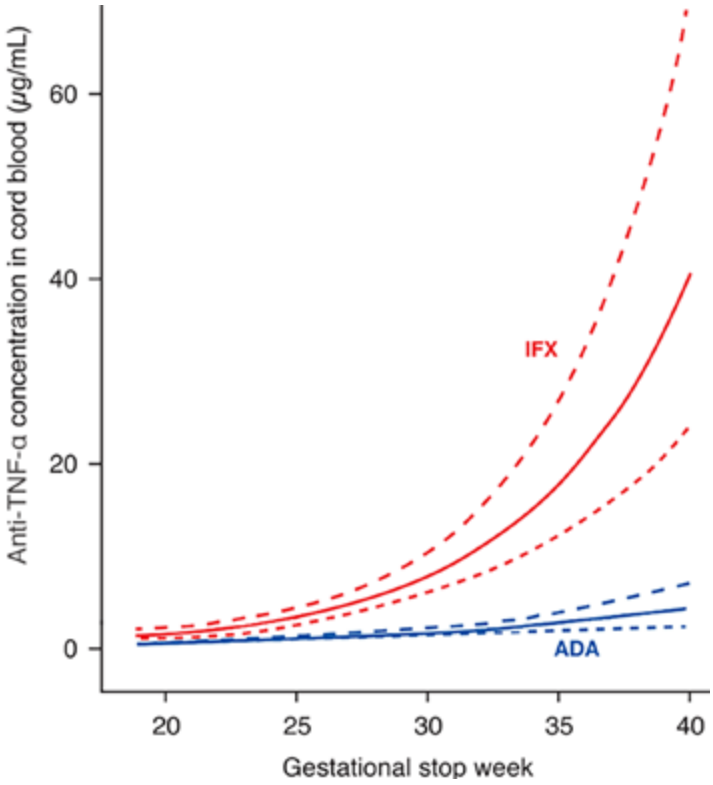

Figure 1. Multiple linear regression model predicting IFX and ADA cord blood concentration at birth based on gestational stop week. The means are represented by the continuous lines and the 95% confidence intervals are represented by the dotted lines.

One- year health outcomes of infants exposed to anti-TNF-α in utero

Out of all live births, health outcomes until 1 year of age were obtained from 93 infants (71%). These results are shown separately **(Table 5)**. Information was not obtained if infants did not yet reach the age of 1 or if we were unable to contact mother for a telephonic questionnaire. There were no differences regarding growth, infection rate, hospitalization because of an infection, allergies, chronic diseases, adverse reactions to vaccinations and the presence of eczema between IFX-exposed infants and ADA-exposed infants.

Table 5. Health outcomes of infants at 1 year (n=93)

	IFX (n=50)	ADA (n=43)	*P* value
Growth (%)			
Normal	49 (98)	42 (98)	1.00
Abnormal	1 (2)*	1 (2)**	1.00
Number of infections treated with antibiotics (%)			
0	35 (76)	31 (71)	0.82
1-2	10 (22)	10 (23)	0.48
3	1 (2)	3 (7)	0.35
Hospitalization because of infection (%)	5 (10)	3 (7)	0.72
Allergies (%)	3 (7)	5 (11)	0.48
Chronic diseases (%)	0 (0)	1 (2)***	1.00
Adverse reaction to vaccination (%)	0 (0)	0 (0)	1.00
Eczema (%)	12 (26)	6 (14)	0.20

*Secondary growth failure; **Primary growth failure; ***Cystic fibrosis

Growth

There were 2 infants (2%) with an abnormal growth (1 IFX, 1 ADA). One infant, diagnosed with cystic fibrosis (CF), had a primary growth failure. The second infant had a secondary growth failure, however, the cause was still unknown at the time of analyses.

Infections

In total, 25 infants (27%) received one or more antibiotic treatments in the first year of life, of whom 12 (48%) were exposed to IFX. None of the infants received more than 3 antibiotic treatments. Anti-TNF-α type was not associated with the number of antibiotic-treated infections. A total of 33 oral antibiotic treatments were prescribed for the following types of infections: acute otitis media (n=16), upper respiratory infections (n=11), urinary tract infection (n=2), infected toe (n=1), paronychia (n=1), impetigo (n=1) and furuncles (n=1). Information regarding anti-TNF-α cord blood level and infection rate was collected from 55 mother-child pairs. The correlation between cord blood level at birth and infection rate is demonstrated

in **Figure 2**. Median cord blood level of children who did not receive antibiotics for an infection (n=38) was 2.0 µg/mL (IQR 1.0-7.8), children who had 1 antibiotic-treated infection (n=13) was 3.1 µg/mL (IQR 0.4-10.6) and children who received 2 or more antibiotic treatment for an infection (n=4) was 3.9 µg/mL (IQR 0.1-18.7). There was no statistically significant difference regarding anti-TNF-α cord blood level between children without antibiotic-treated infections, children with 1 antibiotic-treated infection and children with 2 or more antibiotic-treated infections.

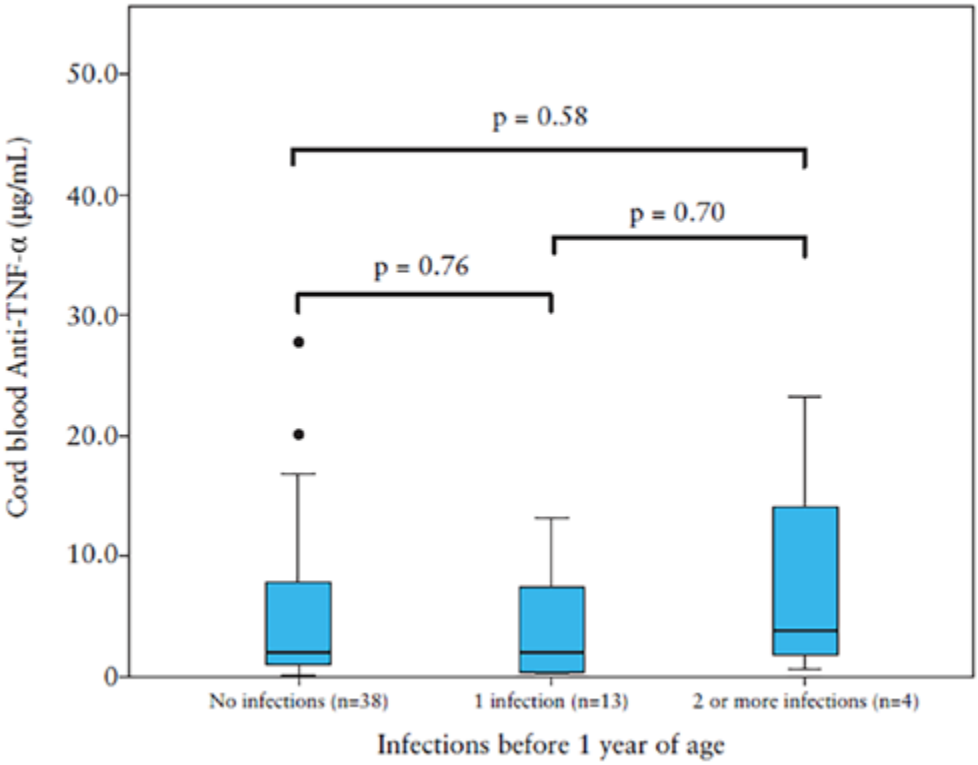

Figure 2. Boxplot showing the association between anti-TNF-α cord blood level and infections rate within 1 year. One extreme outlier in the 1 infection group is not shown. One child-mother couple had an anti-TNF-α cord blood level of 56.0. Mother used 10mg/kg IFX every 7 weeks. This pregnancy was complicated by preeclampsia with preterm birth after 35.5 gestational weeks. Last infusion was given 36 days before delivery.

The rate of children who received at least one antibiotic treatment was similar for infants that were exposed to combination treatment of anti-TNF-α and an immunomodulator (n=6, 26%) and infants exposed to anti-TNF-α monotherapy (n=17, 30%) (P=1.00).

There were 8 infants (9%) admitted to hospital because of a severe infection. The indications for hospital admission were as follows: respiratory infections (n=4), respiratory syncytial virus (RS-virus) (n=2), furuncles (n=1) and staphylococcal infection of the skin (n=1).

There were 4 children exposed to IFX (50%). Median cord blood level at birth of children admitted to hospital was 3.5 µg/mL (IQR 0.3-8.9) and the median cord blood level at birth of children not admitted to hospital was 2.0 µg/mL (IQR 0.9-7.8). There was no statistically significant difference regarding anti-TNF-α cord blood level between children admitted to hospital and children not admitted to hospital because of an infection in the first year of life (p=0.87).

There was no difference in hospital admission rate between anti-TNF-α types. Furthermore, there was no difference in hospital admissions because of a severe infection between infants exposed to combination therapy (n=2, 8%) and infants exposed to anti-TNF-α monotherapy (n=6, 9%) (P=1.00).

Allergies

Eight infants (9%) suffered from an allergy of which, 3 (38%) were exposed to IFX and 5 (62%) to ADA. The following types of allergies were reported: cow milk allergy (n=4), antibiotics (n=2), allergic rhinitis (n=1) and a sun allergy that resolved completely shortly after diagnosis (n=1). There was no correlation between anti-TNF-α type and allergies.

Adverse reaction to vaccinations

Live vaccinations were avoided in case anti-TNF-α levels were above 3 µg/mL in the offspring. In The Netherlands, the first live vaccination is administered at the age of 14 months. None of the infants had anti-TNF-α levels above 3 µg/mL at 14 months of age, moreover none of the infants had anti-TNF-α levels exceeding 1 µg/mL at 6 months of age. Furthermore, none of the infants in our cohort had an adverse reaction to a vaccination.

DISCUSSION

We developed a linear regression model to demonstrate the effect of anti-TNF-α cessation during pregnancy on fetal exposure, for IFX and ADA separately. Anti-TNF-α concentrations in cord blood and maternal blood were higher in the IFX group than the ADA group. Our linear regression shows that IFX transportation over the placenta increases exponentially during the third trimester of pregnancy. However, ADA transportation over the placenta is lower and increases in a linear fashion. Therefore, ADA may be continued longer during pregnancy than IFX without leading to higher anti-TNF-α concentrations in the newborn. In cases of disease activity in women with a current or future pregnancy and in pregnant women who need to step-up to anti-TNF-α therapy, ADA may therefore be preferred over IFX. Furthermore, we did not observe significant differences in disease course, maternal outcomes and birth outcomes between women using IFX and women using ADA during pregnancy.

Continuing ADA longer during pregnancy may avoid low maternal ADA levels and subsequently an increased relapse risk in mothers. In addition, a shorter anti-TNF-α drug holiday may reduce the risk of an adverse event after drug re-initiation such as, an allergic reaction and loss of response. We did not observe significant differences between the anti-TNF-α types regarding relapse rate and adverse events after drug re-initiation. However, our study may be underpowered and therefore, further evaluation in larger prospective studies is needed.
Anti-TNF-α concentrations in cord blood varied widely between individuals, which is similar to non-pregnant patients.[22, 25] This variation is possibly due to several factors influencing pharmacokinetics of anti-TNF-α such as, anti-drug antibody formation, concomitant immunosuppressive therapy, serum CRP concentration, BMI and serum albumin concentration.[22, 26, 27] In addition, physiological changes during pregnancy may also alter pharmacokinetics of anti-TNF-α. The latter was demonstrated in a recent study showing a significant increase of the maternal anti-TNF-α level during pregnancy in women using IFX with a fixed dosing schedule, irrespective of albumin level and BMI.[28] The underlying mechanism of this finding remains unknown and needs further evaluation.

Because of the relative small sample size, this study may be underpowered to demonstrate an association between potential predictor variables and anti-TNF-α concentration in cord blood. However, despite this small sample size, we did find a very strong association between anti-TNF-α concentration in cord blood and the variables anti-TNF-α type and stop week. These robust findings indicate that these variables are of utmost importance when predicting anti-TNF-α concentration in cord blood and underlines the large pharmacokinetic differences between IFX and ADA.

One-year health outcomes such as, growth, infection rate, hospitalization because of an infection, allergies, chronic diseases, adverse reactions to vaccinations and the presence of eczema were similar between IFX-exposed and ADA-exposed infants. One or more infections were documented in 31% of infants, which is in line with a non-controlled follow-up study assessing the health outcomes of 25 anti-TNF-α exposed children, of whom 32% were treated for an infection in the first year of life.[29] The infection rate in our cohort is also in line with the overall Dutch population.[30] However, infection rate was higher in infants from non-IBD controls from the same geographic region, namely 40%.[17] It should be noted that infection data are biased as parents of infants with a cord blood concentration of 3 μg/mL or higher were advised not to bring their child to day care, to be extra cautious of infectious sources, and to postpone all live vaccinations before the repeated anti-TNF-α measurement was below 3 μg/mL. Therefore, the actual infection risk in the first year in anti-TNF-α exposed infants may be higher than shown in our study. The cut-off level of 3μg/mL is however arbitrary and was initially based on adult studies showing an association between IFX level > 3μg/mL and response to treatment.[31] Subsequent studies showed that higher trough levels may be needed for response in the case of ADA treatment. However, we conservatively continued to use the lower cut-off value based on the pharmacokinetics of IFX in order to minimise health risks for the newborns.

The top 3 indications for which antibiotics are prescribed in the general Dutch population are respiratory tract infections (45%), ear infections (20%), and urinary tract infections (10%)[30] which resembled the indications in our study population; ear infections (48%), upper respiratory infections (33%) and urinary tract infections (6%).

It should be noted that this cohort received stringent counselling and follow-up before and during pregnancy. This strict follow-up has a beneficial effect on pregnancy outcomes and should be taken in to account when comparing results with other studies.[32] In addition, disease activity during pregnancy could not be assessed in a standard manner. During pregnancy, some clinical scores and laboratory work-up are of limited value. Pregnant women often experience abdominal complaints, regardless of their underlying IBD and clinical scores such as, hematocrit and body weight to assess disease activity are of little value; as pregnant women often become anemic and most women gain weight during pregnancy. An endoscopy, to assess disease activity, was only performed in case there was a strong indication. Furthermore, cord blood samples were not obtained from all pregnancies because anti-TNF-α measurements were performed only since 2010 at our clinic and there were sample missing because of logistic problems, however, missing samples were missing at random. In addition, we did not find a correlation between anti-TNF-α cord blood levels and the impact on the developing immune system of the child. However, this study may be

underpowered to demonstrate a correlation and therefore further prospective studies on this topic are needed.

In conclusion, ADA may be continued longer during pregnancy than IFX because of the lower placental transmission. Continuing ADA longer during pregnancy may reduce relapse risk of the mother without increasing fetal anti-TNF-α exposure.

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Chapter 5.2:

IBD: Exposure to anti-TNF-α agents in utero: controlling health risks

5

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A recent study reports on drug clearance in newborn children after in utero exposure to anti-TNF-α antibodies, infliximab and adalimumab. As women with IBD are increasingly exposed to these drugs due to changing treatment paradigms and earlier diagnosis, this commentary explores these clinically important results.

Active Crohn's disease and ulcerative colitis during conception and pregnancy have been associated with an increased risk of adverse pregnancy outcomes, the most consistently described are preterm delivery (before 37 weeks of gestation), low birthweight (<2500gram) and small for gestational age[2]. Thus, conception is preferable at a time of disease remission, and maintenance treatment should be continued throughout the pregnancy to control disease activity. Anti-TNF-α antibodies, such as infliximab and adalimumab, are known to actively cross the placenta, and umbilical cord blood levels might exceed maternal levels[3]. Now, Julsgaard et al.[1] present the results of a study investigating drug clearance in 80 newborn children following in utero exposure to infliximab and adalimumab. This is a very relevant clinical study as the anti-TNF-α drug clearance of in utero exposed children has not been extensively describes.

In the latest study, pregnant females with IBD receiving treatment with the anti-TNF-α agents infliximab (n=44) and adalimumab (n=36) were recruited from 14 tertiary hospitals in Denmark, Australia and New Zealand. Anti-TNF-α concentration was measured in umbilical cord blood and in peripheral maternal blood during delivery; if detected in umbilical cord blood, anti-TNF-α levels in the newborn child were followed-up every 3 months until undetectable. Notably, umbilical cord blood levels of anti-TNF-α agents from adalimumab-exposed children were lower than those from infliximab-exposed children1. Nine months post-delivery, none of the adalimumab-exposed children had detectable anti-TNF-α levels, but five (11%) of the infliximab-exposed children did, and one child had a detectable level at 1 year of age. Health and development of the children was assessed by questioning mothers 1 year after delivery. Anti-TNF-α levels in umbilical cords did not correlate with an increased infection risk in infants; however, children exposed to a combined treatment of an anti-TNF-α agent and an thiopurine had an infection risk twice that of children exposed to anti-TNF-α monotherapy. No other health risks were described and infant development was normal in all but one.

The results of this study necessitate an in-depth discussion on the management of IBD during pregnancy: how can we limit in utero drug exposure to avoid health risks for the child and at the same time maintain disease remission in the mother? To achieve this difficult balance, several pregnancy guidelines already include recommendations that are helpful for day-to-day clinical practice[2,4]. These guidelines recommend that all female patients with IBD who want to become pregnant should receive personalized counselling. This counselling includes an assessment of medications, discontinuing those that are teratogenic, as well

as an assessment of disease activity. In cases of disease activity in females contemplating pregnancy, and subsequently in pregnant females who need to step up to anti-TNF-α therapy, adalimumab might be preferred over infliximab because of the lower umbilical cord blood level at birth[4] and the faster drug clearance in children as shown by Julgaard et al[1]. However, we strongly advise not switching from infliximab to adalimumab in cases of stable disease remission in pregnant patients, or in females contemplating pregnancy, because of the risk of a disease flare.

During counselling, it is advised to strive for remission for at least 6 months before contemplating pregnancy. These strict remission rules influence the health of the offspring because it increases the likelihood of switching to monotherapy and/or stopping anti-TNF-α treatment around week 22–24 of gestation[2,4]. These modifications will minimise in utero drug exposure and is not associated with an increased health risk for the mother—stopping anti-TNF-α therapy in pregnant females in sustained remission is not related with a relapse, allergic reactions upon reinitiating, or secondary loss of response[6]. In the study by Julsgaard et al.[1], 31% of females were not in sustained remission at the time of conception and a large proportion continuing treatment after 30 weeks of gestation and were using a combination of an anti-TNF-α and an thiopurine. Children exposed to combination treatments had a higher infection risk than children exposed to monotherapies. This finding emphasizes the importance of disease remission prior to conception. However, to date no clear data indicate the optimal time to switch to monotherapy in the overall IBD population, including patients who wish to become pregnant. Thus, this decision should be individualized, based on relapse risk and personal preference, and made by a multidisciplinary team. Preferably, a timely switch to monotherapy before contemplating pregnancy is advisable in order to monitor the maintenance of disease remission. If patients are able to switch to monotherapy, discontinuation of thiopurines is preferable because of the stronger evidence of anti-TNF-α therapeutics in maintaining remission[4]. In addition, evidence suggests that maternal thiopurine metabolism is altered during pregnancy and that newborn children from mothers receiving thiopurines have an increased risk of anaemia at birth, although non-exposed children were not included in this study[7].

Of utmost interest is the finding of Julsgaard et al. that the half-life of infliximab and adalimumab in newborn children was increased compared to adult non-pregnant patients. The underlying mechanism for this finding remains obscure and needs further evaluation. However, one hypothesis is that the immature immune system of the infant and the absence of inflammation results in increased levels of circulating anti-TNF-α drugs. Further research is required to identify the effects of anti-TNF-α drugs on the immature immune system. Additionally, research on the efficacy and safety of vaccinations in newborn children with prolonged anti-TNF-α levels is warranted as this is barely understood. Concerns were raised

after the death of a 4.5 month old infliximab-exposed boy following a disseminated Bacille Calmette-Guerin vaccination. However, little is known about the health risks of postponing vaccinations in children that were exposed to anti-TNF-α in utero. Therefore, with regards to the prolonged anti-TNF-α half-life, we propose that the decision of postponing vaccinations is made on a case-by-case evaluation, which would include continuing measurement of anti-TNF-α levels in newborn children until undetectable. As a result, children with high anti-TNF-α levels will not be exposed to live vaccinations with possible health risks and children with undetectable levels will not be exposed to unnecessary health risks caused by postponing vaccinations.

As breastfeeding did not affect anti-TNF-α clearance, this study is also reassuring with regards to the health risks of breastfeeding in exposed children, although it is unclear whether anti-TNF-α therapy was continued or commenced after delivery in the case of anti-TNF-α cessation in the third trimester of pregnancy[1]. The safety of breastfeeding is further supported by previous studies that reported low anti-TNF-α concentrations in breastmilk[8-10].
In conclusion, controlling health risks requires a careful balance between maintaining disease remission in mothers with IBD and minimising foetal drug exposure. Adequate counselling prior to conception leads to optimisation of disease control before and during pregnancy, with the possibility of lowering and even stopping IBD drugs. Consequently, fetal drug exposure is reduced, which leads to lower health risks in the newborn child.

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Chapter 6:

Endoscopy for Inflammatory Bowel Disease During Pregnancy: Only When There Is a Strong Indication

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With great interest we have read the study by Ludvigsson et al,[1] published in the October issue of Gastroenterology, who performed a population-based study to examine the risks of endoscopy during pregnancy. This nationwide Swedish study showed that exposure to any endoscopy during pregnancy, independent of trimester and indication, was related to preterm birth and children born small for gestational age. However, when restricting data to women without a gastrointestinal disease, risk estimates decreased, and when limiting controls to women with endoscopy <1 year before or after pregnancy, endoscopy was not associated with any adverse pregnancy outcome.

We agree with the authors that studies regarding the safety of endoscopy during pregnancy are limited[2,3]; therefore, this first large study adds to the current literature. However, we feel that the risks and benefits of endoscopy during pregnancy in women suffering from inflammatory bowel disease (IBD) were not enough emphasized. First, owing to the study design, the indication for endoscopy during pregnancy could not be determined. Overall, there are numerous indications for endoscopy, each with its own disease identity, etiology, and treatment,[4] that may be unrelated to an underlying disease. The risks and benefits of endoscopy during pregnancy and effect on pregnancy outcomes are obviously highly dependent on the indication. Second, accurate adjustment for disease activity is lacking. Previous studies have shown that disease activity, but not the presence of quiescent IBD during pregnancy, is associated with adverse pregnancy outcomes such as preterm birth and low birth weight.[5] Therefore, it is of utmost importance to accurately assess disease activity for women with IBD because it has a major influence on pregnancy outcome. An attempt to adjust for disease activity was done by limiting the control group to women with endoscopy <1 year before or after pregnancy; however, in the case of IBD, a plausible reason for endoscopy in the year before pregnancy is to confirm disease remission before stopping all contraceptive methods. Thus, it remains unclear to what extent the variables—endoscopy, the presence of IBD, and disease activity—influence pregnancy outcome. In addition, no data regarding follow-up before and during pregnancy were reported for women with IBD, which is of interest because preconception care and strict follow-up positively influences pregnancy outcome.[6] Also, part of the data is collected before the introduction of anti-tumor necrosis factor treatment for IBD; therefore, this study only partly represents the current era of novel IBD treatment.

In conclusion, this study adds to the current literature regarding the safety of endoscopy during pregnancy; however, the applicability of this study seems limited for women suffering from IBD.

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Part 3.
Post-pregnancy

Chapter 7:

Long-term health outcomes of 1000 children born to mothers with Inflammatory Bowel Disease in the anti-TNF-α era

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Submitted

ABSTRACT

Background & Aims

The aim of this study was to describe the long-term health outcomes of children born to mothers with Inflammatory Bowel Disease (IBD) and to assess the impact of maternal IBD medication use on these outcomes.

Methods

We performed a multicenter retrospective study in The Netherlands. Women with IBD who gave birth between 1999 and 2016 were enrolled from 20 participating hospitals. Information regarding disease characteristics, medication use, lifestyle, pregnancy outcomes and long-term health outcomes of children were retrieved from mothers. After consent of both parents, outcomes until 5 years were also collected from general practitioners (GPs). Our primary aim was to assess infection rate and our secondary aims were to assess adverse reactions to vaccinations, growth, auto-immune diseases and malignancies.

Results

We included 1000 children born to 605 IBD mothers (369 (61%) Crohn's Disease (CD), 217 (36%) ulcerative colitis (UC), 19 (3%) IBD unclassified (IBDU). In total, 198 children (20%) had intrauterine exposure to anti-TNF-α (60 with concomitant thiopurine), 241 (24%) to thiopurine monotherapy. The 561 children (56%) not exposed to any of the two treatments were part of to the control group.

The number of antibiotic-treated infections and hospital admissions due to infections were not associated with maternal IBD medication. In addition, adverse reactions to vaccinations, growth failure, auto-immune diseases and malignancies were also not associated with in utero exposure to IBD medication. All outcomes correspond with the general age-adjusted population.

Conclusion

In our study, we found no association between maternal immunosuppressive IBD medication use during pregnancy and adverse long-term health outcomes of children until 5 years of age.

INTRODUCTION

Inflammatory bowel diseases (IBD) represent chronic diseases that may be maintained in remission by different types of immunosuppressive medication. The disease is incurable and the majority of patients needs life-long treatment with these drugs. IBD typically affects patients in their reproductive years and, inevitably, a part of these women will require treatment during pregnancy. Anti-TNF-α and immunomodulators, as monotherapy or in combination, are used increasingly to maintain disease remission[1, 2] and both types of drugs cross the human placenta. Anti-TNF-α compounds, such as infliximab (IFX) and adalimumab (ADA), are IgG1 monoclonal antibodies that are actively transported over the placenta, particularly during the final weeks of gestation,[3-6] the pharmacological active end metabolites 6- thioguaninenucleotides (6-TGN) of thiopurines cross the human placenta as well.[7, 8] Women using anti-TNF-α and/or thiopurine during pregnancy do not have a higher risk of adverse pregnancy outcomes, such as preterm delivery, children with low birth weight or congenital abnormalities[9-15], but the impact of in utero exposure on the development of the child's immune system, growth and risk of auto-immune diseases and malignancies later in life remains relatively unexplored.

A recent study showed changes in the immune system of 7 infants exposed to anti-TNF-α during pregnancy who had detectable levels of anti-TNF-α until 6 months of age[16]. Exposed infants had a more immature B- and T- helper phenotype and a decreased response after mycobacterial challenge compared to non-exposed controls, although no serious infections were reported during a follow-up period of 18 months. Data of clinical studies regarding infection risk in infants exposed to anti-TNF-α and/or thiopurine show conflicting results. An almost 3-fold increased infection risk was found in a recent prospective study of infants exposed in utero to the combination of anti-TNF-α and thiopurine maintenance therapy compared with infants exposed to anti-TNF-α monotherapy.[17] Also preliminary data from the United States national PIANO registry indicated a higher rate of infections in infants exposed in utero to combination treatment in 2012[18]. However, a more recent report from the same registry demonstrated similar infection rates for infants exposed to anti-TNF-α monotherapy and infants exposed to combination therapy in utero[19]. A prospective study, conducted by our study group, also did not find an increased infection risk in infants exposed to anti-TNF-α monotherapy or in combination with a thiopurine after one year of follow-up[20]. In addition, a recent retrospective study did not find an increased risk of hospital admission because of an infection in children exposed to anti-TNF-α monotherapy or combination therapy in utero[21]. Overall, the follow-up time in these previous mentioned studies were mostly until one year and studies often report only one health outcome, mostly infections. Data on long-term implications on overall children's development after fetal exposure to therapeutic anti-TNF-α and/or thiopurine remains therefore essential. Abnormalities regarding immune

development, growth failure, auto-immune diseases and malignancies are often discovered after the age of one year and therefore longer follow-up studies assessing these outcomes are needed.

The primary aim of our study was to assess the effect of exposure to IBD drugs in utero on the infection rate until 5 years of age. The secondary aims were to assess the effect of in utero exposure to IBD drug on adverse reactions to vaccinations, growth development and the risk of auto-immune diseases and malignancies in the first 5 years of life.

METHODS

Study design and outcomes

We conducted a multicentre retrospective study in The Netherlands. All Dutch hospitals with a gastroenterology department were asked to participate in this study. In each participating hospital, women diagnosed with IBD and with a known pregnancy resulting in a live birth were identified and invited per letter. All women diagnosed with IBD who gave birth between 1999 and 2016 were asked to respond. After consent, the following information was retrieved during a telephone interview: IBD disease characteristics, education level, medication use during pregnancy, life style habits during pregnancy (i.e. smoking, folic acid intake), IBD surgery prior to pregnancy, mode of delivery, breastfeeding and the following obstetrical complications: hyperemesis gravidarum, cholestasis of pregnancy, in utero growth restriction, gestational hypertension or preeclampsia and hemolysis, elevated liver enzymes, low platelet count (HELLP). In addition, the following birth outcomes were documented: birth weight, gestational age at birth and the presence of congenital abnormalities and the following long-term health outcomes until 5 years of age: number of infections requiring systemic antibiotic treatment, severe infections necessitating hospitalization, day care attendance, growth failure necessitating referral to a paediatrician, vaccination rate, adverse reactions to vaccinations, auto-immune diseases and malignancies. If mothers did not correctly record pivotal information, such as medication use during pregnancy, patients and their offspring were excluded from the study.

After receiving informed consent from both parents, information regarding children's long-term health outcomes was also collected from their general practitioners (GPs). In The Netherlands, all residents are registered in a general practice that provides primary care. The GP is the gatekeeper to hospital- and specialist care and specialists report back to GPs. As a result, GPs have at their disposal the most accurate and complete information regarding health outcomes of patients. We therefore used information provided by the GPs regarding long-term health outcomes of children. In case information from the GP was missing we

used maternally reported outcomes. However, for each long-term health outcome we first compared GPs and mothers reported outcomes and in case there was a significant difference, only information provided by GPs was used. Health outcomes of children were collected until 5 years of age.

Study population

The study population consisted of mothers diagnosed with IBD and their offspring born between 1999 and 2016. We assessed disease characteristics, pregnancy complications, birth outcomes and long-term health outcomes of children. To assess whether maternal IBD medication use influences pregnancy and long-term health outcomes of children, women using anti-TNF-α monotherapy, thiopurine monotherapy or the combination of anti-TNF-α with thiopurine were analyzed separately and compared with controls consisting of women with IBD not using these IBD treatments.

Statistical analyses

All analyses were performed using IBM SPSS statistics (version 21.0 Chicago III, USA). Descriptive statistics of continuous data are displayed as medians with interquartile ranges (IQR) or means with standard deviations (SD) if they are approximately normally distributed. Normally distributed data was compared using Students t-tests and non-normally distributed data was compared using the nonparametric Mann Whitney U test in the case of two independent samples or the Kruskal-Wallis test in the case of > 2 dependent samples. Categorical data are shown as absolute numbers with percentages and compared using Fisher's exact tests. The tests were performed two tailed and tested at a significance level of 0.05, unless stated differently. To analyze the risk of the adverse outcomes; major congenital abnormalities, preterm birth and small for gestational age (SGA) univariable as well as multivariable logistic regression was performed. Candidate predictors of all three outcomes were anti-TNF-α and/or thiopurine use, systemic corticosteroids, IBD type, maternal age and smoking during pregnancy. For preterm birth and SGA disease activity during pregnancy, obstetric complication and endoscopy during pregnancy were also considered. Subsequently, variables with a p-value of ≤ 0.10 and variables chosen by the clinician's rationale were included in a multivariable logistic model. Interaction terms were tested between these variables and included in the final model if significant ($p<0.01$).

Because the follow-up was shorter for children of mothers using anti-TNF-α and/or thiopurine than for children in the control group, outcomes were compared per year. Kaplan-Meier survival curves were plotted to depict the proportion of children admitted to hospital admission because of a severe infection over time. Hospital admission was compared between maternal treatment group using the log-rank test and Breslow test. A Cox regression model was used

to adjust for preterm birth and maternal systemic corticosteroid use during pregnancy for each treatment group separately.

A Bonferroni correction was applied to correct for multiple comparisons between outcomes of the three treatment groups and control group. For these analyzes, a statistical significant difference was defined as a p-value <0.017.

Sample size

The primary outcome that was used for the sample size calculation was the number of infections per year. Based on a previous Dutch study, the rate of infections requiring antibiotic treatment is 43% per year for children between 0-4 years of age.[22] We expected the number of infections to be higher in children who were exposed to anti-TNF-α; therefore, a one-side test was used. In case of an increased infection risk of 15% in children that were exposed to anti-TNF-α, at a significance level of 0.05 and a power of 90%: 189 children per arm were needed.

Definitions

Growth failure is defined as abnormal growth necessitating referral to a paediatrician. Preterm birth is defined as a delivery before 37 weeks of gestation. SGA is a weight below 2 SD for gestational age according to the Dutch reference curve.[23] The periconceptional period is defined as the period 8 weeks before conception until 2 weeks after conception. The EUROCAT guideline was used to classify congenital abnormalities.[24] Severe infection is defined as an infection for which hospital admission was necessary.

Ethical consideration

This study was approved by the ethics committee of the Erasmus Medical Center (Rotterdam, The Netherlands) and all participating hospitals. Legal guardians of the children signed informed consent before data was collected from the general practitioner.

RESULTS

In total, 20 hospitals participated in this study; 7 university hospitals and 13 general hospitals. We have sent 1913 invitation letters to women diagnosed with IBD. In our invitation letter we asked women to respond if they fitted the inclusion criteria and if they were willing to participate. In addition, we asked all eligible women from the Dutch Crohn's and Colitis Association (CCUVN) to participate in the study. **Figure 1** shows a flow chart of the inclusion in our study.

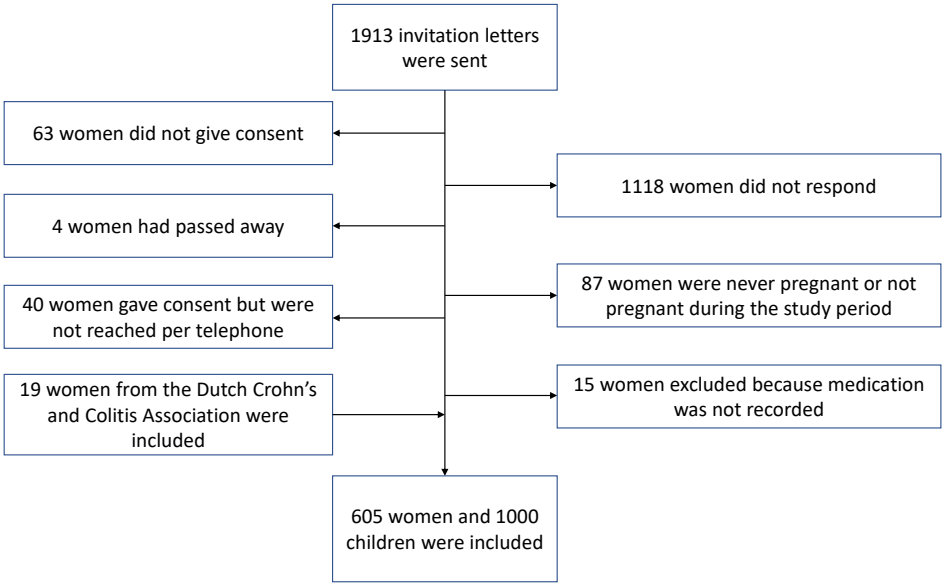


Figure 1. Flow chart of inclusions.

In total, we included 1000 children born to 605 mothers (369 (61%) Crohn's disease (CD), 217 (36%) ulcerative colitis (UC) and 19 (3%) IBD unclassified (IBDU)). Seven twin-pregnancies were included in the study. Overall, 198 (20%) of children were exposed to anti-TNF-α in utero (118 IFX, 80 ADA). There were 138 (14%) children exposed to anti-TNF-α monotherapy, 241 (24%) children exposed to thiopurine monotherapy and 60 (6%) children exposed to the combination of anti-TNF-α and thiopurine. One patient used methotrexate in combination with anti-TNF-α but stopped the methotrexate at pregnancy week 6. All other mothers discontinued methotrexate before pregnancy. In the control group, there were 232 children not exposed to anti-TNF-α or thiopurine; 249 were exposed to 5-ASA and 80 to systemic corticosteroids. Eleven women stopped anti-TNF-α in the first trimester, 87 stopped anti-TNF-α in the second trimester and 100 women used anti-TNF-α in the third trimester. Overall, 301 (30%) children were exposed to a thiopurine. Baseline characteristics are shown in

Table 1. Baseline characteristics treatment group (N=1000)							
	Controls (n=561)	**Anti-TNF-α mono-therapy (n=138)**	**P-value**	**Thiopurine mono-therapy (n=241)**	**P-value**	**Anti-TNF-α & thiopurine (n=60)**	**P-value**
Median maternal age at birth (IQR)	32 (29-35)	30 (28-33)	**0.0001**	32 (29-35)	0.41	31 (30-34)	0.45
Education level (%)							
High	265 (50)	57 (47)	0.48	112 (50)	0.94	32 (56)	0.49
Secondary	227 (43)	56 (46)	0.61	95 (42)	0.87	23 (40)	0.78
Low	34 (7)	9 (7)	0.69	18 (8)	0.44	2 (4)	0.56
Diagnosis (%)							
Crohn's disease	288 (51)	115 (83)	**0.0001**	171 (71)	**0.0001**	43 (72)	**0.003**
Ulcerative colitis	249 (45)	22 (16)	**0.0001**	63 (26)	**0.0001**	15 (25)	**0.004**
IBD unclassified	24 (4)	1 (1)	0.04	7 (3)	0.43	2 (3)	1.00
Disease location CD (Montreal) (%)							
L1 Ileal	35 (12)	15 (14)	0.74	15 (9)	0.28	7 (18)	0.32
L2 Colonic	71 (25)	26 (24)	0.90	40 (24)	0.82	5 (13)	0.11
L3 Ileocolonic	178 (63)	68 (62)	1.00	114 (67)	0.31	27 (69)	0.48
Disease behaviour CD (Montreal) (%)							
B1 Non stricturing non penetrating	134 (51)	61 (54)	0.50	73 (44)	0.24	16 (38)	0.18
B2 Stricturing	51 (19)	9 (8)	**0.006**	30 (18)	0.90	3 (7)	0.08
B3 Penetrating	48 (18)	21 (19)	0.89	41 (25)	0.11	15 (36)	**0.01**
B2+ B3 Stricturing and penetrating	33 (12)	21 (19)	0.11	21 (13)	1.00	8 (19)	0.23
P Perianal fistulizing disease (%)	68 (24)	39 (34)	0.05	40 (24)	0.91	23 (56)	**0.0001**
Disease extent UC / IBDU (Montreal) (%)							
E1 Proctitis	90 (38)	2 (11)	0.02	18 (29)	0.24	3 (22)	0.26
E2 Left-sided colitis	54 (23)	5 (26)	0.78	19 (31)	0.25	2 (14)	0.74
E3 Pancolitis	92 (39)	12 (63)	0.05	25 (40)	0.88	9 (64)	0.09
Disease duration in years (IQR)	8 (5-13)	9 (4-12)	0.66	7 (5-12)	0.13	8 (5-12)	0.66
Concomitant IBD medication use (%)							
Systemic steroid (alone or in combination with 5-ASA)	80 (14)	24 (18)	0.35	46 (19)	0.09	6 (11)	0.44
5-ASA	248 (44)	4 (3)	**0.0001**	56 (23)	**0.0001**	3 (5)	**0.0001**
No IBD medication	233 (42)	-	-	-	-	-	-
IBD abdominal surgery prior to pregnancy (%)	142 (25)	38 (28)	0.59	59 (24)	0.66	10 (17)	0.20
Nulliparous (%)	272 (49)	78 (57)	0.09	134 (56)	0.08	33 (56)	0.34
Folic acid intake (%)	504 (94)	122 (98)	0.07	210 (91)	0.12	49 (93)	0.55

All outcomes of children exposed to IBD therapy were compared with the controls. The Bonferroni correction was applied to adjust for multiple testing. A statistical significant difference was defined as a p value < 0.017.

IQR interquartile range; CD Crohn's disease; UC ulcerative colitis; IBDU IBD unclassified

Table 1. The children exposed to anti-TNF-α monotherapy, thiopurine monotherapy or a combination of both anti-TNF-α and thiopurines were compared with the controls. Women using anti-TNF-α monotherapy during pregnancy were younger (30 years (IQR 28-33)) than women not using anti-TNF-α and/or thiopurine (32 years (IQR 29-35) (p=0.0001). Women diagnosed with CD more often used anti-TNF-α and/or thiopurine than women diagnosed with UC. Women using anti-TNF-α monotherapy less often had stricturing CD (n=9, 8%) than women not using these drugs (n=51, 19%) (p=0.006). Within the combination treatment group more women had penetrating disease (n=15, 36%) than women not using anti-TNF-α and/or thiopurine (n=48, 18%) (p=0.01), and women in the combination treatment group more often had perianal fistulizing disease (n=23, 56%) than women not using anti-TNF-α and/or thiopurine (n=68, 24%) (p=0.0001).

Maternal outcomes

Maternal outcomes per pregnancy are displayed in **Table 2**. During pregnancy, there was no difference between study groups and women not using anti-TNF-α and/or thiopurine regarding relapse risk, endoscopy during pregnancy, obstetric complications, systemic corticosteroid use and smoking during the entire pregnancy. Approximately 50% of women discontinued anti-TNF-α before the third trimester in both the anti-TNF-α monotherapy group and the combination group. Women not using anti-TNF-α and/or thiopurine more often breastfed (n=314, 57%) than women using anti-TNF-α monotherapy (n=39, 29%) (p=0.0001), women using thiopurine monotherapy (n=51, 21%) (p=0.001) and women using combination treatment (n=11, 19%) (p=0.001).

Table 2. Maternal outcomes (N=1000)

	Controls (n=561)	Anti-TNF-α monotherapy (n=138)	P-value	Thiopurine monotherapy (n=241)	P-value	Anti-TNF-α & thiopurine (n=60)	P-value
Disease activity during pregnancy (%)	144 (26)	31 (23)	0.45	43 (18)	0.02	12 (20)	0.43
Endoscopy during pregnancy (sigmoidoscopy / colonoscopy) (%)	39 (7)	13 (10)	0.36	17 (7)	1.00	10 (17)	0.02
Anti-TNF-α cessation in the third trimester (%)	-	67 (49)	-	-	-	31 (52)	-
Obstetric complications during pregnancy (%)	58 (10)	15 (11)	0.75	32 (13)	0.22	12 (20)	0.03
Systemic corticosteroid use (%)	80 (14)	24 (17)	0.35	44 (18)	0.17	6 (10)	0.44
Smoking during entire pregnancy (%)	25 (5)	12 (9)	0.05	14 (6)	0.59	3 (5)	0.74
Breastfeeding > 4 weeks (%)	314 (57)	39 (29)	**0.0001**	51 (21)	**0.0001**	11 (19)	**0.0001**

All outcomes of children exposed to IBD therapy were compared with the controls. The Bonferroni correction was applied to adjust for multiple testing. A statistical significant difference was defined as a p value < 0.017.

Obstetric complications

All obstetric complications are displayed in **Table 3**. Overall, gestational hypertension / preeclampsia (n=55, 6%) and gestational diabetes (n=23, 2%) were the most frequent reported obstetrical complication during pregnancy. Other reported obstetrical complications were intrahepatic cholestasis of pregnancy (ICP) (n=16, 2%), HELLP (n=11, 1%), hyperemesis gravidarum (n=7, 0.7%) and in utero growth restriction (n=5 (0.5%)). When comparing study groups with women not using anti-TNF-α and/or thiopurine, we found a higher rate of ICP in women using thiopurine monotherapy (n=9, 4%) and in women using a thiopurine in combination with anti-TNF-α (n=3, 5%), than in women without anti-TNF-α and/or thiopurine use (n=2, 0.4%), p=0.002 and p=0.01 respectively. There was no difference regarding other obstetric complications between the study group and women not using anti-TNF-α and/or thiopurine.

Table 3. Types of obstetric complications (n=1000)

	Controls (n=561)	Anti-TNF-α mono-therapy (n=138)	P-value	Thiopurine monotherapy (n=241)	P-value	Anti-TNF-α & thiopu-rine (n=60)	P-value
Hyperemesis Gravidarum	4 (0.7)	0 (0)	1.00	3 (1)	0.43	0 (0)	1.00
Cholestasis of Pregnancy	3 (0.5)	1 (0.7)	0.59	9 (4)	**0.002**	3 (5)	**0.01**
In Utero Growth Restriction	2 (0.4)	1 (0.7)	0.48	2 (0.8)	0.59	0 (0)	1.00
Hypertension / preeclampsia	30 (5)	6 (4)	0.83	11 (5)	0.73	7 (12)	0.08
HELLP	5 (1)	1 (1)	1.00	3 (1)	0.70	2 (3)	0.14
Gestational Diabetes	13 (2)	6 (4)	0.24	4 (2)	0.79	0 (0)	0.63

All outcomes of the study groups were compared with the controls. The Bonferroni correction was applied to adjust for multiple testing. A statistical significant difference was defined as a p value < 0.017.

HELLP hemolysis, elevated liver enzymes, low platelet count

Birth outcomes

Birth outcomes are displayed in **Table 4**. There were no differences between the study groups and women not using anti-TNF-α and/or thiopurine regarding birth weight, gestational age at birth, SGA, preterm birth, the presence of congenital abnormalities and mode of delivery. Women using anti-TNF-α monotherapy more often underwent caesarean section (n=59, 43%) compared to women not using anti-TNF-α and/or thiopurine (n=140, 25%) (p=0.001). We found no other differences regarding birth outcomes between women using anti-TNF-α and/or thiopurine during pregnancy and women not using these drugs.

Major congenital abnormalities were seen in 26 (2.6%) children. Logistic regression analysis showed no association between major congenital abnormalities and maternal anti-TNF-α use, thiopurine use, combination treatment of anti-TNF-α and thiopurine, systemic corticosteroid use, IBD type (CD or UC/IBDU), maternal age or smoking during pregnancy **(Supplementary Table 1)**.

Table 4. Birth outcomes (n=1000)							
	Controls (n=561)	Anti-TNF-α monotherapy (n=138)	P-value	Thiopurine monotherapy (n=241)	P-value	Anti-TNF-α & thiopurine (n=60)	P-value
Birth weight in kg (IQR)	3.3 (3.0-3.7)	3.3 (3.0-3.7)	0.70	3.3 (3.0-3.6)	0.94	3.2 (2.8-3.5)	0.08
Gestational age in weeks (IQR)	39 (38-40)	39 (38-40)	0.57	39 (38-40)	0.09	39 (37-40)	0.04
Small for gestational age (%)	22 (4)	4 (3)	0.80	7 (3)	0.54	1 (2)	0.72
Preterm birth (%)	59 (11)	14 (10)	1.00	35 (15)	0.12	10 (17)	0.19
Major congenital abnormalities* (%)	10 (2)	6 (4)	0.10	7 (3)	0.30	3 (5)	0.12
Caesarean section (%)	140 (25)	59 (43)	**0.0001**	74 (31)	0.08	20 (35)	0.16

All outcomes of the study groups were compared with the controls. The Bonferroni correction was applied to adjust for multiple testing. A statistical significant difference was defined as a p value < 0.017.

IQR interquartile range

*According to the EURCAT guideline

In total 118 (12%) children were born preterm. In a univariable logistic regression analysis we found an association between preterm birth and the variables; disease activity during pregnancy (p=0.02), systemic corticosteroid use (p=0.0001) and obstetrical complications (p=0.0001) **(Supplementary Table 2)**. In our multivariable analyses the variables systemic corticosteroid use and obstetrical complications remained, showing a strong correlation between these variable and preterm birth.

A total of 35 (0.4%) children were born SGA. In a multivariable logistic regression analysis, SGA was associated with maternal disease activity during pregnancy (p=0.07) and smoking during pregnancy (p=0.02). However, there was no association between SGA and maternal IBD medication, IBD type, maternal age, obstetric complications and endoscopy during pregnancy **(Supplementary Table 3)**.

Sub analysis for children born to mothers with ICP

There were 16 children born to mothers with ICP, of whom 12 (75%) used a thiopurine during pregnancy. None of the children born to mothers with ICP had a congenital abnormality and none were born SGA. Women with ICP gave birth preterm more often (n=8, 50%) than women without ICP (n=109, 11%) (p=0.0001) and women with ICP had a caesarean section (n=9, 56%) more often than women without ICP (n=283, 29%) (p= 0.03).

Long-term health outcomes of children

Complete information regarding long-term health outcomes, including number of antibiotic-treated infections per year, of 645 (65%) children was retrieved from the GPs. Median follow-up time was 60 (IQR 36-60) months. Compared to children not exposed to anti-TNF-α and/or thiopurine, follow-up was shorter for children exposed to anti-TNF-α monotherapy (24 (IQR 12-48) months) (p=0.0001), children exposed to thiopurine monotherapy (60 (IQR 36-

60) months) (p=0.0001) and children exposed to combination treatment of anti-TNF-α and thiopurine (36 (IQR 12-60) months) (p=0.0001).

Antibiotic-treated infections

Because the number of infections reported by mothers is highly susceptible to recall bias, we compared infection rate reported by the GP and the mother in cases for which both was available. Information regarding infections was available from both GP and mother for 359 children. The infection rate for these 359 children reported by the GPs (median 0.20 infections per year (IQR 0.00-0.60)) was slightly higher than the infection rate reported by mothers (median 0.20 infections per year (IQR 0.00-0.40) (p= 0.001). Because of the significant difference in reporting between mothers and GPs, only GPs information regarding antibiotic treated infections per follow-up year was used (n=645).

The proportion of children receiving one or more antibiotic treatments was the highest in the first year (n=241, 37.4%) and the second year (n=222, 37.5%), and decreased in the following

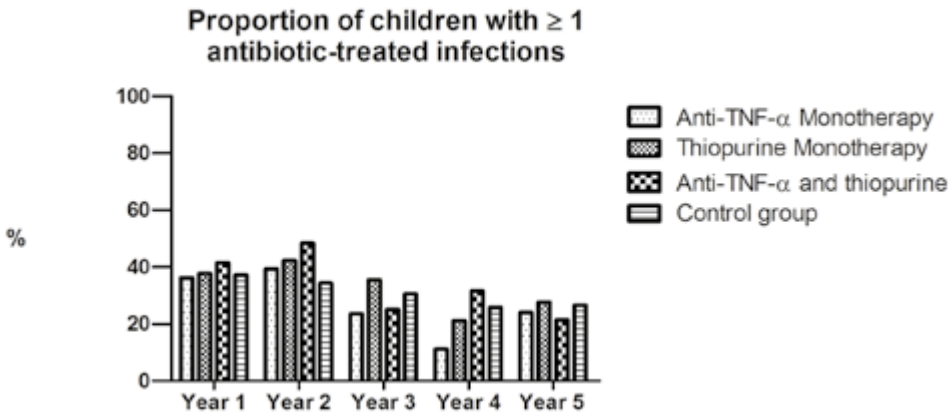


Figure 2. Proportion of children who received 1 or more antibiotic treatment because of an infection for each follow-up year per treatment group.

years; third year (n=163, 30.7%), fourth year (n=110, 23.8%) and fifth year (n=107, 26.5%).

Median number of antibiotic-treated infections per person per year of the 645 included children was 0.33 (IQR 0.00-0.67). There were 1209 infections treated with antibiotics by the GP during the follow-up of 2724 person years, resulting in 444 antibiotic courses per 1000 person-years. The following infections were reported: 502 (42%) ear nose and throat (ENT) related infections, 441 (37%) respiratory tract infections, 61 (5%) skin infections, 60 (5%) urinary tract infections, 28 (2%) children were treated for a fever not specified, 8 (1%) parasitic infections, 7 (1%) gastroenteritis and in 95 (7%) cases the exact reason for the antibiotic prescription was unknown.

To determine whether maternal IBD treatment was associated with antibiotic-treated infections, we assessed the number of antibiotic-treated infections of children exposed to anti-TNF-α monotherapy, thiopurine monotherapy, combination therapy with anti-TNF-α and thiopurine and children not exposed to any of the two drugs for each year (Figure 2). Subsequently, we compared the outcomes of each study group with children not exposed to any of the two drugs for each year. Overall, for each follow-up year, we found no differences between each study group and children not exposed to anti-TNF-α and/or thiopurine regarding number of antibiotic-treated infections. In addition, we found no differences between children of mothers who received anti-TNF-α in the third trimester compared to children of mothers who stopped anti-TNF-α treatment before the third trimester in each year.

Overall, most children (85%) attended day care. Median age at which the child first attended daycare was 8 (IQR 3-14) months. There was no association between maternal IBD treatment with regards to day care attendance and age during first daycare attendance.
During the study period, pneumococcal vaccination for infants was introduced in The Netherlands (April 2006) resulting in a reduction of invasive pneumococcal disease by 35% in Dutch children < 2 years of age[25]. In our cohort, 747 (75%) children were born after April 2006, of whom 99% received all vaccinations according to the Dutch national vaccination program, including pneumococcal vaccination. In the control group 351 (63%) children received pneumococcal vaccination, which is significantly lower than the number of children that received the pneumococcal vaccination in the anti-TNF-α monotherapy group (n=138, 100%) (p=0.0001), thiopurine monotherapy group (n=198, 82%) (p=0.0001) and combination group (n=60, 100%) (p=0.0001). Therefore, a sub-analysis was performed for children born after April 2006. In these sub-analyses, the median number of antibiotic-treated infections per person per year reported by GPs was 0.33 (IQR 0.00-0.73). There were 848 infections treated with antibiotics by the GP during the follow-up of 1864 person years: 455 antibiotic courses per 1000 person-years. The number of antibiotic courses per 1000 person-years was similar for children born after April 2006 compared to the total study group (p=0.79).

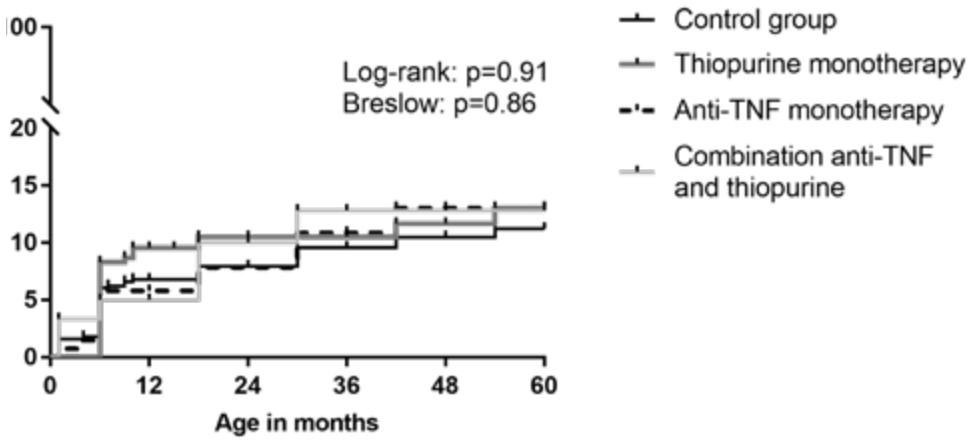
Hospital admission because of an infection

Information regarding hospital admission because of an infection was retrieved from mothers and/or GPs in all 1000 children. There was no significant difference regarding the reported number of hospital admissions. Mothers reported a hospital admission rate of 8.1% and GPs reported a hospital admission rate of 10.1% (p=0.16). Therefore, we used information from mothers in case information from the GP was missing (n=1000).

In total, 107 (11%) children were admitted to hospital because of an infection of whom 6 were admitted twice. Total follow-up of children in our study was 3944 person years, resulting

in 29 infections per 1000 person years necessitating hospital admission. Median age during hospital admission was 6 (IQR 6-18) months. Most hospital admissions occurred in the first year of life (77 cases; 69%).

The reasons for hospital admission were the following: acute respiratory tract infection (n=35, 31%), viral infections (n=27, 24%) (respiratory syncytial virus (n=12), Epstein Barr virus (n=1), human metapneumovirus (n=1), Rota virus (n=1) or Noro virus (n=3), viral infection unclassified (n=9)), infection unspecified (n=14, 12%), sepsis (n=9, 8%), gastroenteritis (n=7, 6%), urinary tract infection (n=5, 4%), pyelonephritis (n=5, 4%), bacterial skin infection (n=3, 3%), appendicitis (n=1, 1%), meningitis (n=1, 1%), herpes stomatitis with dehydration (n=1, 1%), tonsillitis (n=1, 1%), mastoiditis (n=1, 1%), multiple infections during chemotherapy (n=1, 1%), acute otitis media with dehydration (n=1, 1%) and mesenteric lymphadenitis (n=1, 1%).
To determine whether maternal IBD treatment was associated with hospital admission because of an infection a Kaplan-Meier survival curve was plotted. **Figure 3** shows the proportion of children who were admitted to hospital because of an infection over time per IBD treatment group. No significant difference was found between the different IBD treatment groups: 59 (11%) in the group not exposed to anti-TNF-α and/or thiopurine, 29



No. at risk

Control group	**561**	**522**	**474**	**436**	**398**	**36**
Thiopurine monotherapy	**241**	**218**	**184**	**173**	**152**	**128**
Anti-TNF monotherapy	**138**	**130**	**90**	**59**	**40**	**24**
Combination anti-TNF and thiopurine	**60**	**57**	**36**	**31**	**25**	**18**

Figure 3. Kaplan-Meier estimates of time to hospital admission per treatment group.

(12%) in the thiopurine monotherapy group, 13 (9%) in the anti-TNF-α monotherapy group and 6 (10%) in the combination anti-TNF-α and thiopurine-group were admitted to hospital (Log-Rank: p=0.90). Consistent results were obtained by a Cox regression model, adjusting for preterm birth and maternal systemic corticosteroid use during pregnancy: hazard ratio were 1.00 for anti-TNF-α monotherapy (95% CI 0.55-1.77), 1.13 for thiopurine monotherapy (95% CI 0.74-1.73) and 1.06 for combination treatment of anti-TNF-α and thiopurine (95% CI 0.46-2.42).

As stated earlier, children exposed to IBD medication more often received pneumococcal vaccination, which may influence infection rate and subsequently hospital admission. Therefore, Kaplan-Meier survival curves were additionally plotted only for children born after April 2006, representing the current vaccination policy in the Netherlands. In this subgroup, no significant difference was found between the different IBD treatment groups: 34 (10%) in the group not exposed to anti-TNF-α and/or thiopurine, 25 (13%) in the thiopurine monotherapy group, 13 (9%) in the anti-TNF-α monotherapy group and 6 (10%) in the combination anti-TNF-α and thiopurine-group were admitted to hospital (Log-Rank: p=0.76).

Adverse reaction to vaccination

There were 100 children who were exposed to anti-TNF-α in the third trimester of pregnancy. Hospital admissions due to an infection occurred more often in children from mothers who continued anti-TNF-α treatment in the third trimester (n=14, 14%) than in children from mothers who only received anti-TNF-α in the first and/or second trimester (n=5, 5%). However, when comparing children born to women who stopped anti-TNF-α before the third trimester with children born to women who continued in the third trimester, a statistical difference was not reached (hazard ratio 2.60, 95% CI 0.94-7.24) (p=0.07).

There was no discrepancy regarding reported adverse reactions to vaccination between mothers and GPs. In total, 7 adverse reactions to vaccinations were reported; 4 children were admitted to hospital shortly after vaccination because of a high fever, 2 children received antibiotic treatment because of an infection shortly after vaccination and 1 child received anti-histamine treatment because of a severe erythema at the injection site. No severe life threatening reactions were reported. The mothers of these children used the following IBD treatment during pregnancy: thiopurine monotherapy (n=2), anti-TNF-α and thiopurine (n=1), no anti-TNF-α or thiopurine (n=4).

Growth

There was no discrepancy regarding reported growth failure between mothers and GPs. In total, there were 16 (1.6%) children with growth failure. All children with growth failure were

referred to a pediatrician for further analysis and treatment. Mothers used the following IBD treatment during pregnancy: anti-TNF-α monotherapy (n=4), thiopurine monotherapy (n=3), no anti-TNF-α or thiopurine (n=9).

Auto-immune diseases and malignancies

There was no discrepancy regarding reported auto-immune diseases and malignancies between mothers and GPs. There was 1 (0.1%) child diagnosed with an auto-immune disease before the age of 5 years: this was a boy diagnosed with Diabetes Mellitus type 1 at the age of 4 years. He was born to a mother with UC whom did not use IBD medication during pregnancy nor experienced disease activity during pregnancy.

There were 2 (0.2%) children diagnosed with a malignancy before the age of 5 years. A girl, born to a mother with UC who used azathioprine and corticosteroids during the entire pregnancy, was diagnosed at the age of 3 years with a rhabdomyosarcoma of her left orbita. In addition, a girl born to a mother with UC who used corticosteroids during the entire pregnancy was diagnosed with leukemia at the age of 2 years.

Sub analysis for children born to mothers with ICP

For each follow-up year, infection rate was similar for children born to women with ICP and children born to women without ICP. The proportion of one or more infections treated with antibiotics in children born to mothers with ICP is as follows and was compared using Fishers exact tests to the corresponding proportion of children born to women without ICP: year 1 (n=5, 42%) (p=0.77), year 2 (n=5, 45%) (p=0.76), year 3 (=3, 30%) (p=1.00), year 4 (n=3, 43%) (p=0.37), year 5 (n=3, 50%) (p=0.19).

None of the children born to mothers with ICP were admitted to hospital because of a severe infection. In addition, none of the children had an adverse reaction to a vaccination, were diagnosed with growth failure or were diagnosed with an auto-immune disease or malignancy during follow-up.

Sensitivity analyses

Because systemic corticosteroid use may influence outcomes, sensitivity analyses were performed by excluding all women who used systemic corticosteroids during pregnancy. In total, there were 154 (15%) women who used systemic corticosteroids during pregnancy. The rate of systemic corticosteroid used was similar in all treatment groups compared with controls **(Table 2)**. Maternal outcomes are shown in the Supplementary **Table 4**. Other

than a lower rate of disease activity during pregnancy in the thiopurine monotherapy group compared with controls, excluding women using systemic corticosteroids did not change maternal outcomes. Birth outcomes are displayed in the **Supplementary Table 5**. Birth outcomes in the sensitivity analyses were similar to the total group. Regarding long-term outcomes, antibiotic-treated infections per person per year was not statistically significant different between the study groups and the control group. In addition, excluding women who used systemic corticosteroid did not influence hospital admission rate, adverse reactions to vaccines, growth auto-immune diseases and malignancies.

DISCUSSION

In this large multicenter retrospective study, we did not find an association between maternal anti-TNF-α, thiopurine or combination treatment for IBD during pregnancy and adverse long-term health outcomes of children until 5 years of age.

We found an increased risk of ICP in women using a thiopurine during pregnancy. This association has not been described previously. The pathogenesis of ICP is multifactorial, possibly including hormonal, environmental, genetic and dietary influences[26, 27]. Azathioprine and 6-MP are both associated with liver enzyme abnormalities[28, 29]. Cholestasis as a side effect generally occurs a few weeks until 3 months after starting treatment or after dose escalation[30]. Pregnancy has an important effect on maternal thiopurine metabolism leading to decreased 6-TGN and increased 6-MMP concentrations[8]. This finding therefore underlines the importance of introducing thiopurine drug monitoring during pregnancy, as drug dose could be decreased to avoid maternal exposure to high levels of 6-MMP. However, it should be noted that maternal thiopurine use did not influence long-term health outcomes of exposed children. In addition, the small sample size should be taken into account when interpreting this outcome. More studies are needed to confirm this association.

In our study, 26 infants (2.6%) were born with a major congenital abnormality, which is consistent with data reported from the overall European population[31]. Anti-TNF-α and/or thiopurine use was not associated with the presence of major congenital abnormalities. Preterm birth was associated with obstetric complications and corticosteroid use, however, in the univariable analyses we also found an association with disease activity. The association between preterm birth and corticosteroid use may possibly be due to the fact that women used corticosteroids because of disease activity. The outcome SGA was associated with disease activity and smoking during pregnancy, which is consistent with previous studies[32, 33]. IBD medication was not associated with SGA.

In general practice, the incidence of acute infections in children necessitating antibiotics is high[34]. In our study population, 444 antibiotic courses per 1000 person-years were prescribed. The number of antibiotic-treated infections in our study is in line with the results of a national survey of the Dutch general practice where 472 antibiotic courses per 1000 person-years were reported in children of 0-4 years[22]. We found that the highest number of antibiotic-treated infections was reported before the age of 2 years and decreased significantly with age. This trend coincides with the general Dutch population[35, 36]. The most frequent indication for antibiotics in our study were ENT and respiratory tract infections, which is also comparable with children of the same age in the general Dutch population[35, 37, 38].

In contrast to acute infections, the incidence of serious infections are rare and account for approximately 1% of all infections[39]. In our study, there were 29 infections per 1000 person years necessitating hospital admission. The number of serious infections in our cohort is slightly higher than the number reported in a Belgian population study. In this study 21 serious infections per 1000 person years were seen[39]. However, it should be noted that the definition of a serious infection differed between these studies. In contrast to the latter study, we also included viral infections requiring hospital admission instead of only bacterial infections. Bearing this in mind, the risk of a severe infection in the IBD population does not seem to be increased.

In our study we found a trend towards more severe infections in children necessitating hospital admission who were born to women who continuing anti-TNF-α in the third trimester compared with children born to women who discontinuing anti-TNF-α before the third trimester. Parental counseling may have diminished the risk of infections as parents were possible more cautious of infectious sources. Therefore, this difference may actually be larger than reported in our study, however, these data were not recorded in this study. On the contrary, physicians may admit a child to hospital more easily out of precaution because the mother used anti-TNF-α in the third trimester. Further long-term follow-up studies, taking the above-mentioned confounding factors into account, are needed to assess whether anti-TNF-α exposure in the third trimester is associated with a higher risk of severe infections.

Antibiotic prescription in children is influenced by seasonal differences, with a peak incidence in the winter[40]. We addressed the seasonal influence on prescription rate by including information per completed follow-up year. Therefore, our outcomes were not influenced by seasonal differences.

No life threatening allergic reactions to vaccines occurred in our study group. Life threatening allergic reactions to vaccinations, however, occur very rarely, approximately 1 in 1.000.000 doses,[41] and therefore our study group is too small to draw firm conclusion. The reactions reported in our study were mild and may have occurred coincidental to vaccination. Children born to mothers with IBD, with or without IBD medication exposure, do not seem to have an increased risk of adverse reactions to vaccines.

Growth failure occurs approximately in 2.3% of all children in The Netherlands[42]. A Dutch cohort study reported growth failure in 15 out of 459 (1.8%) children in a non-IBD control group[20]. The rate of growth failure in our study is comparable to the general Dutch population.

We found no association between auto-immune diseases or malignancies in offspring of mothers with IBD, with or without IBD medication use during pregnancy. The incidence of a malignancy in the age group 0-5 years in The Netherlands according to the most recent estimates from 2017 of The Netherlands Cancer Registry is 199 per 100.000 person a year[43]. The incidence of a malignancy in our IBD study group (0.2%) is therefore the same as the overall Dutch population (0.2%).

Although our study is a large nationwide multicenter study, it was limited by its retrospective design. To address this limitation, we collected information regarding long-term health outcomes from GPs. In the Netherlands GPs are the gatekeepers to hospital- and specialist care and as a result, patients will need a referral from their GP when consulting a medical specialist. In addition, it is standard policy in The Netherlands that physicians report back to GPs. As a result, GPs possess the most comprehensive and accurate information about patients' health. Information from 645 children was collected from their GPs. Others were missing because parents did not give consent or because the GP was not willing to share information. Information regarding maternal health and medication use was collected during telephonic interview and is therefore subject to recall bias. In order to minimize this bias we excluded all women that were unsure about medication use during pregnancy. In addition, our study was subject to response bias as only 32% of the invited women participated in the study. It is possible that most invited women did not fit the inclusion criteria and therefore did not respond to our invitation, however, this was not assessed. Furthermore, women with adverse pregnancy outcomes may be more prone to participate, influencing reported outcomes. In addition, our study lacks a non-IBD control group. For each health outcome a literature search was conducted to compared our study group with the general population, however, the children in the general population were part of a different study protocol, which may have affected reported outcomes. Finally, It should be noted that all our analyses assume measurements of different children to be independent, however, this may not be the case for children born by the same mother.

Our study does have advantages over previous published studies. This is the largest long-term study to assess implications of maternal IBD medication during pregnancy on children's health outcomes. Multiple outcomes, presenting overall health of children born to mothers with IBD, were assessed. Exposure to IBD medication in utero may not only influence infection risk but also other health outcomes such as growth, response to vaccinations, malignancies and auto-immune diseases. In addition, the type of infections per follow-up year were retrieved from the GP. In that way, infection rate per age group and type of infection per year could

be compared with overall Dutch population. The type of infections necessitating hospital admission were also retrieved. Hospital admission for other indications could therefore be easily excluded. Finally, this is a national study and as a result GPs and pediatricians work according to the same protocols. Differences in antibiotic prescribing and hospital admission are therefore not expected between different regions.

CONCLUSION

In our multicenter retrospective study assessing long-term health outcomes of 1000 children born to mothers with IBD, we found an association between thiopurine use during pregnancy and intrahepatic cholestasis of pregnancy, without affecting birth outcomes and long-term health outcomes of children. Overall, we found no association between maternal medication use for IBD during pregnancy and antibiotic-treated infections, infections needing hospital admission, adverse reactions to vaccination, growth failure, auto-immune diseases and malignancies in children until 5 years of age.

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SUPPLEMENTARY TABLES

Supplementary Table 1. Variables associated with major congenital abnormalities (n=26)						
	Univariate analyses			Multivariate analyses		
	OR	95% CI	P-value	OR	95% CI	P-value
Anti-TNF-*α*	2.20	0.96-5.00	0.06	2.04	0.89-4.66	0.09
Thiopurine	1.47	0.66-3.27	0.35			
Anti-TNF-*α* and thiopurine	2.10	0.61-7.19	0.24			
Systemic corticosteroids	1.67	0.66-4.23	0.28			
IBD type	0.67	0.31-1.46	0.31			
Maternal age	0.92	0.85-1.00	0.04	0.92	0.85-1.00	0.06
Smoking during pregnancy	0.69	0.09-5.18	0.72			

Correlation disease activity and smoking during pregnancy: Pearson correlation -0.10, p=0.001.

Anti-TNF-α * maternal age was added to the model, however, did not contribute significantly, p=0.85.

Supplementary Table 2. Variables associated with preterm birth (n=118)						
	Univariate analyses			Multivariate analyses		
	OR	95% CI	P-value	OR	95% CI	P-value
Anti-TNF-α	1.05	0.65-1.69	0.85			
Thiopurine	1.53	1.02-2.28	0.04			
Anti-TNF-α and thiopurine	1.55	0.76-3.15	0.22			
Systemic corticosteroids	2.87	1.86-4.45	0.0001	2.74	1.63-4.60	0.0001
Disease activity	1.70	1.11-2.58	0.02	1.07	0.65-1.77	0.79
IBD type	0.88	0.62-1.26	0.49			
Maternal age	0.96	0.92-1.01	0.08			
Smoking during pregnancy	0.97	0.41-2.32	0.94			
OBGYN complication	1.36	1.22-1.51	0.0001	4.23	2.65-6.75	0.0001
Endoscopy during pregnancy	1.72	0.93-3.18	0.08			

Correlation disease activity and systemic corticosteroids: Pearson correlation 0.42, p=0.0001. The variable disease activity * systemic corticosteroids was added to the model, however, did not contribute significantly: p=0.48.

Correlation disease activity and thiopurine: Pearson correlation -0.73, p=0.02. The variable disease activity * thiopurine was added to the model, however, also did not contribute significantly: p=0.29.

Supplementary Table 3. Variables associated with SGA (n=35)						
	Univariate analyses			Multivariate analyses		
	OR	95% CI	P-value	OR	95% CI	P-value
Anti-TNF-α	0.71	0.27-1.87	0.49			
Thiopurine	0.73	0.33-1.64	0.45			
Anti-TNF-α and thiopurine	0.48	0.06-3.56	0.47			
Systemic corticosteroids	0.76	0.26-2.18	0.61			
Disease activity	1.92	0.93-3.96	0.08	2.00	0.95-4.16	0.07
IBD type	1.34	0.75-2.41	0.32			
Maternal age	0.95	0.88-1.03	0.22			
Smoking during pregnancy	3.44	1.27-9.33	0.02	3.42	1.26-9.31	0.02
OBGYN complication	1.05	0.84-1.32	0.67			
Endoscopy during pregnancy	1.57	0.54-4.58	0.41			

Correlation disease activity and smoking during pregnancy: Pearson correlation 0.04, p=0.89.

Supplementary Table 4. Sensitivity analyses (excl. systemic corticosteroids): Maternal outcomes (N=846)							
	Controls (n=481)	**Anti-TNF-α monotherapy (n=114)**	**P-value**	**Thiopurine monotherapy (n=197)**	**P-value**	**Anti-TNF-α & thiopurine (n=54)**	**P-value**
Disease activity during pregnancy (%)	92 (19)	14 (12)	0.10	18 (9)	**0.001**	7 (13)	0.35
Endoscopy during pregnancy (sigmoidoscopy / colonoscopy) (%)	25 (5)	5 (5)	1.00	10 (5)	1.00	5 (9)	0.22
Anti-TNF cessation in the third trimester (%)	-	59 (52)	-	-	-	29 (54)	-
Obstetric complications during pregnancy (%)	46 (10)	11 (10)	1.00	26 (13)	0.17	10 (19)	0.06
Smoking during entire pregnancy (%)	23 (5)	9 (8)	0.17	13 (7)	0.35	3 (6)	0.73
Breastfeeding > 4 weeks (%)	284 (60)	30 (27)	**0.0001**	45 (23)	**0.0001**	11 (21)	**0.0001**

All outcomes of children exposed to IBD therapy were compared with the controls. The Bonferroni correction was applied to adjust for multiple testing. A statistical significant difference was defined as a p value < 0.02.

Supplementary Table 5. Sensitivity analyses (excl. systemic corticosteroids): Birth outcomes (n=846)							
	Controls (n=481)	**Anti-TNF-α monotherapy (n=114)**	**P-value**	**Thiopurine monotherapy (n=197)**	**P-value**	**Anti-TNF-α & thiopurine (n=54)**	**P-value**
Birth weight in kg (IQR)	3.4 (3.0-3.7)	3.4 (3.0-3.8)	0.88	3.4 (3.1-3.7)	0.69	3.2 (2.8-3.6)	0.33
Gestational age in weeks (IQR)	39 (38-40)	39 (38-40)	0.29	39 (38-40)	0.03	39 (37-40)	0.10
Small for gestational age (%)	20 (4)	4 (4)	1.00	4 (2)	0.25	1 (2)	0.71
Preterm birth (%)	38 (8)	10 (9)	0.71	25 (13)	0.06	8 (15)	0.12
Major congenital abnormalities* (%)	7 (2)	5 (4)	0.06	5 (3)	0.35	3 (6)	0.07
Nulliparous (%)	231 (48)	68 (60)	0.02	111 (56)	0.05	30 (57)	0.25
Caesarean section (%)	117 (24)	52 (46)	**0.0001**	58 (30)	0.18	20 (39)	0.04

All outcomes of the study groups were compared with the controls. The Bonferroni correction was applied to adjust for multiple testing. A statistical significant difference was defined as a p value < 0.02.

IQR interquartile range

*According to the EURCAT guideline

Chapter 8:

Hepatitis B vaccination effective in children exposed to anti-TNF alpha in utero

8

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ABSTRACT

Introduction

Neonates exposed to TNF-α inhibitors in utero are born with detectable drug levels which can still be detected throughout the first year of life. Since 2011, the hepatitis B virus (HBV) vaccine is routinely administered to all newborns in the Netherlands. Adults treated with anti-TNF-α have been reported to respond inadequately to the HBV vaccine. The aim of this study was to compare anti-HBs levels in anti-TNF-α exposed children with non- exposed children following routine Dutch HBV vaccination.

Methods

We performed a cross-sectional, controlled cohort study from 2014-2017 in a single, tertiary referral center. Pregnant women treated with anti-TNF-α for Inflammatory Bowel Disease (IBD) and their subsequent children were recruited from the IBD preconception outpatient clinic. Pregnant women not treated with anti-TNF-α for IBD and their subsequent children were eligible as controls. Adherence to the Dutch National Vaccination Programme was mandatory for participation in this study. A venous blood sample was obtained one month after final HBV vaccination. Anti-HBs levels were measured by ELISA.

Results

Anti-HBs levels at 12 months did not differ between the anti-TNF-α exposed (n=15) and the control group (n=12) (>1000 IU/L vs >1000 IU/L, p=0.59). All children were successfully immunised against HBV, defined as anti-HBs>10 IU/L. Median anti-TNF-α levels determined in cord blood at birth were 9.0 µg/mL (IQR: 3.0-15.0 µg/mL) for IFX and 0.4. µg/mL (IQR: 0.3-0.6 µg/mL) for ADA. There were no differences in general birth and health outcomes.

Conclusion

Children born with detectable anti-TNF-α levels can be effectively vaccinated against HBV.

INTRODUCTION

Anti-TNF-α agents such as adalimumab (ADA) and infliximab (IFX) are commonly prescribed treatments for Inflammatory Bowel Disease (IBD) by successfully achieving and maintaining disease remission.[1] IBD is a chronic, relapsing and remitting disease, which typically affects people in their childbearing years.[2] Active disease during pregnancy has been associated with adverse pregnancy outcomes,[3-14] however more recent publications show acceptable pregnancy outcomes by means of adequate disease control in terms of stringent IBD treatment and follow-up throughout pregnancy.[15-17] Anti-TNF-α agents have been accepted as generally low risk drugs during pregnancy as they are not associated with an increased risk of congenital abnormalities, preterm birth, low birth weight or miscarriages.[18, 19] Nonetheless, anti-TNF-α actively crosses the placenta in the second and especially the third trimester of pregnancy, resulting in detectable anti-TNF-α levels in the neonate which can still be detectable throughout the first year of life. [20-25] The effects of anti-TNF-α on the developing immune system of the child has been scarcely investigated.

Hepatitis B virus (HBV) is a major global health issue associated with significant morbidity and mortality.[26] Prevention is possible through vaccination and since 2011, the hepatitis B virus vaccine is routinely administered to all infants in The Netherlands. Interestingly, several reports suggest that the HBV vaccine in IBD patients treated with anti-TNF-α agents fails to yield protective anti-HBs levels after a primary vaccination regimen.[27-32] Although the efficacy of several vaccines have been investigated in anti-TNF-α exposed children,[33, 34] the efficacy of the HBV vaccine has not been investigated in infants born to mothers treated with anti-TNF-α during pregnancy. The aim of this study is therefore to assess response to the HBV vaccine in children born to IBD mothers treated with anti-TNF-α during pregnancy in terms of anti-HBs levels after the administration of the final dose of the HBV vaccine.

MATERIALS AND METHODS

Study design

We performed a cross-sectional, controlled cohort study to determine HBV immunity in children who were exposed to anti-TNF-α in utero. Pregnant women with IBD were informed about the study from May 2014 through December 2016 at our IBD preconception outpatient clinic (POC) of the Erasmus University Medical Centre Rotterdam, a tertiary health care centre. At the IBD outpatient clinic, women with IBD were counselled before pregnancy and seen every second month during pregnancy by an experienced IBD physician. During visits, women were counselled on medication use, life style habits (e.g. smoking, alcohol use), folic acid intake, and the importance of disease remission before and during pregnancy. Disease activity was assessed by the treating physician and treated according to current IBD

pregnancy guidelines. At birth, an umbilical cord blood sample was drawn. In this sample anti-TNF-α levels were measured.

In The Netherlands, the HBV vaccine (Engerix-B Junior, rDNA vaccine) is routinely administered to infants in the first year of life at 6 weeks, 3, 4 and 11 months. To determine HBV immunity, a venous blood sample was obtained from the infant at the age of 12 months, one month after the final HBV vaccination, to assess anti-HBs level. Anti-HBs levels were measured by a commercially available enzyme-linked immune sorbent assay (ELISA). Written informed consent from both parents was obtained before the blood sample was collected. Children with an anti-HBs level of ≥ 10 IU/L were considered immune. In case of insufficient anti-HBs levels (< 10 IU/L) a HBV booster vaccine was given and anti-HBs measurement was repeated after 3 months. If the anti-HBs level exceeded 1000 IU/L, anti-HBs level was displayed as > 1000 IU/L.

In addition, birth outcomes such as birth weight, gestational age and the presence of congenital abnormalities were obtained from mothers during the first visit after delivery. One year after birth, health outcomes were obtained from the general practitioner (GP) with informed consent of both parents. The following health outcomes were collected: growth, number of infections treated with antibiotics, hospital admissions because of infection, chronic diseases, allergies, adverse reactions to a vaccination and the presence of eczema. If data from the GP was unavailable, health outcomes as reported by the mother were used.
Exclusion criteria

All women with IBD who visited our outpatient clinic during pregnancy were asked to participate in this study, however, the following exclusion criteria were applied: mothers infected with HBV, hepatitis C virus (HCV) or human immunodeficiency virus (HIV), children with other immunocompromising conditions and children not vaccinated according to the Dutch National Vaccination Programme.

Anti-TNF-α exposed group

The anti-TNF-α exposed group consisted of children born to mothers with IBD treated with anti-TNF-α during part of the pregnancy or the entire pregnancy. Women in the study group were treated with anti-TNF-α during pregnancy, at least until the end of the second trimester. In case of sustained disease remission from 6 month before conception until gestational week 22, the option of discontinuing anti-TNF-α treatment was discussed in a multidisciplinary team. In all other cases, anti-TNF-α treatment was continued throughout the entire pregnancy.
The control group consisted of children born the mothers with IBD not treated with anti-TNF-α, however, any other IBD medication was permitted.

Definitions

Abnormal growth is defined as growth or height for age and gender deviating > 2 standard deviations (SD) from the mean Dutch growth chart. Preterm birth is defined as delivery before 37 weeks of gestation. Small for gestational age (SGA) is a weight below 2 SD for gestational age according to the Dutch reference chart.[35] The presence of disease activity was assessed by the treating physician and based on the combination of clinical symptoms (HBI > 5 or SCCAI > 2), C-reactive protein (CRP) > 9.0 mg/l, FCP measurement > 200 µg/g and when strongly indicated, an endoscopy was performed.

Sample size

In healthy children and adults, HBV immunisation rates after vaccination are high and considered to be around 95%.[36, 37] In adult IBD patients treated with anti-TNF-α, the immunisation rates after primary vaccination regimen are reported to be approximately 50-60%.[29, 31, 32] At a one-sided significance level of 0.05 and a power of 80%, this would result in an anti-TNF-α exposed group of 12 children and a control group of the same size.

Statistical considerations

All analyses were performed using IBM SPSS statistics (version 21.0 Chicago III, USA). Descriptive statistics of continuous data are displayed as medians with interquartile ranges (IQR) and compared using Mann Whitney U tests. Categorical data are shown as absolute numbers with percentages, and compared using Chi-square or Fisher's exact tests. The tests were performed two tailed unless stated differently, and tested at a significance level of 0.05.

Ethical statement

This study was approved by the local ethics committee of the Erasmus Medical Centre (Rotterdam, The Netherlands). Legal guardians of the child signed informed consent before a venous blood sample was obtained from the child and again before data was collected from the GP.

RESULTS

In total 192 pregnant women (86 treated with anti-TNF-α) were invited for participation in this study. Thirty mother-child pairs were included in the study (15,6%). The anti-HBs measurement was not performed in 3 children; 2 samples were lost because of logistic problems and 1 sample could not be analysed because too little blood was drawn. Therefore, these children were excluded from further analyses. Overall, there were 15 children assigned to the anti-TNF-α exposed and 12 children to the control group. Baseline characteristics are shown in **Table 1**.

Anti-TNF alpha exposed group

In the anti-TNF-α exposed group, 8 (53%) children were exposed to IFX and 7 (47%) children were exposed to ADA. The median anti-TNF-α stop week was gestational week 25 (IQR 22-29) in the IFX group and gestational week 23 (IQR 22-24) in the ADA group (p=0.34). Anti-TNF-α cord blood measurements were obtained from 10 children; 6 IFX-exposed children and 4 ADA-exposed children. Median anti-TNF-α concentration in cord blood was 9.0 µg/mL (IQR 3.0-15.0) in IFX-exposed children and 0.4 µg/mL (IQR 0.3-0.6) in ADA-exposed children, which was a statistically significant difference (p=0.01). However, all children in the anti-TNF-α exposed group responded adequately to the vaccine and were considered immune for HBV. There was no difference between IFX-exposed children and ADA-exposed children.

In addition, we separately analysed the 5 mothers who received anti-TNF-α treatment in the third trimester of pregnancy, of whom 4 cord blood measurements were obtained at birth. All 5 mothers used IFX during pregnancy. The median anti-TNF-α stop week in this group was gestational week 29 (IQR 27-40) and the median anti-TNF-α concentration in cord blood was 12 µg/mL (IQR 8-16). Overall, all children that were exposed to anti-TNF-α in the third trimester of pregnancy responded adequately to the vaccine and were considered immune for HBV. None of the anti-TNF-α exposed children required the HBV booster vaccine.

Control group

The control group consisted of 12 children. Maternal IBD medication use is shown in **Table 1**, which was, other than anti-TNF-α, similar to the anti-TNF-α exposed group. All children in the control group responded adequately to the vaccine and were considered immune for HBV. None of these children required the HBV booster vaccine.

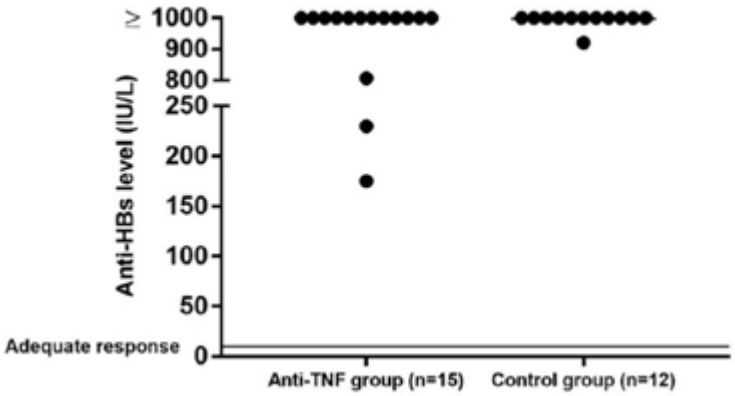

Figure 1. Anti-HBs levels (IU/L) for infants in anti-TNF-α exposed group and the control group.

Table 1. Baseline characteristics (N=27)			
	Study group (n=15)	**Control group (n=12)**	**P-value**
Median maternal age during conception (IQR)	31 (29-33)	30 (27-35)	1.00
Diagnosis (%)			
Crohn's disease	12 (80)	8 (66)	0.66
Ulcerative colitis	3 (20)	2 (17)	1.00
IBD unclassified	0 (0)	2 (17)	0.20
Disease location CD (Montreal) (%)			
L1 Ileal	3 (20)	1 (13)	0.61
L2 Colonic	1 (9)	1 (13)	1.00
L3 Ileocolonic	8 (73)	6 (74)	1.00
Disease behaviour CD (Montreal) (%)			
B1 Non structuring non penetrating	7 (47)	7 (88)	0.34
B2 Stricturing	1 (8)	0 (0)	1.00
B3 Penetrating	3 (25)	1 (12)	0.61
B2+ B3 Stricturing and penetrating	1 (8)	0 (0)	1.00
P Perianal fistulizing disease (%)	4 (29)	1 (8)	0.33
Disease extent UC / IBDU (Montreal) (%)			
E1 Proctitis	0(0)	2 (50)	0.47
E2 Left-sided colitis	0(0)	2 (50)	0.47
E3 Pancolitis	3 (100)	0 (0)	0.23
Disease duration in years (IQR)	10 (7-12)	7 (4-9)	0.38
Anti-TNF-α type (%)			
IFX	8 (53)	-	-
ADA	7 (47)	-	-
Co-medication			
Mesalazine	2 (13)	5 (42)	0.19
Steroids (systemic)	3 (20)	2 (17)	1.00
Thiopurine	4 (27)	4 (33)	1.00
IBD surgery (%)			
Abdominal surgery	4 (27)	2 (17)	0.66
Perianal surgery	3 (23)	0 (0)	0.22
Nulliparous (%)	6 (50)	4 (36)	0.68
Disease relapse during (%)	2 (13)	2 (17)	1.00
Smoking during pregnancy (%)	1 (7)	1 (9)	1.00
Folic acid intake (%)	15 (100)	12 (100)	1.00
Mode of delivery (%)			
Vaginal	8 (57)	8(67)	0.70
Caesarean section	6 (43)	4 (33)	0.70

IQR interquartile range; CD Crohn's disease; UC ulcerative colitis; IBDU IBD unclassified; BMI Body Mass Index.

Birth outcomes

Birth outcomes are shown in **Table 2**. There were no differences between anti-TNF-α exposed group and control group regarding birth weight, low birth weight, gestational age, preterm birth, small for gestational age and the presence of congenital abnormalities. Mothers in the control group more often breastfed than mothers in the anti-TNF-α exposed group.

Table 2. Birth outcomes (N=27)			
	Study group (n=15)	**Control group (n=12)**	**P-value**
Birth weight in grams (IQR)	3495 (3285-3835)	3478 (3215-3866)	0.91
Low birth weight (%)	0 (0)	0 (0)	1.00
Gestational age in weeks (IQR)	39.4 (38.5-41.0)	38.5 (37.6-40.0)	0.08
Preterm birth (%)	0 (0)	1 (8)	0.44
Small for gestational age (%)	0 (0)	0 (0)	1.00
Major congenital abnormalities (%)	0 (0)	0 (0)	1.00
Breastfeeding more than 2 weeks (%)	2 (14)	8 (67)	**0.01**

IQR Interquartile range

One-year health outcomes

We obtained one-year health outcomes from 25 children (15 from the ant-TNF-α exposed, and 10 from the control group) as shown in **Table 3**. Health outcomes were provided by the GP in 14 cases, in other cases information from the parents was used. Overall, we found no differences in growth and health outcomes of children at one year of age between anti-TNF-α exposed children and controls.

Table 3. One-year health outcomes (N=27)			
	Study group (n=15)	**Control group (n=12)**	**P-value**
Growth deficiency (%)	0 (0)	0 (0)	1.00
Chronic disease (%)	0 (0)	0 (0)	1.00
Nr. of infections treated with systemic antibiotics (%)			
0	11 (73)	9 (90)	0.61
1	3 (20)	1 (10)	0.63
2 or more	1 (7)	0 (0)	1.00
Hospitalization because of an infection (%)	0 (0)	0 (0)	1.00
Allergies (%)	0 (0)	0 (0)	1.00
Adverse reactions to vaccination (%)	0 (0)	0 (0)	1.00
Eczema (%)	4 (40)	1 (11)	0.30

DISCUSSION

This study assessed anti-HBs levels after routine HBV vaccination in children exposed to anti-TNF-α in utero. Given the high morbidity and mortality of the sequelae of HBV infection,[26] we believe it is highly important to assess the efficacy of the vaccination regimen in children exposed to anti-TNF-α in utero. This study suggests that HBV infection in these children can be effectively prevented, and that we do not observe the large difference in immunisation rates as reported in the studies conducted in adult IBD patients. The data we present are reassuring, but caution is still warranted in its interpretation. First of all, the sample size was calculated based on an expected, large difference in immunisation rate between anti-TNF-α exposed and unexposed children. The power calculation was based on data from literature in an adult IBD population whom were vaccinated against HBV while treated with anti-TNF-α.[29, 31, 32] The difference in HBV vaccination response between IBD patients treated with anti-TNF-α and healthy individuals is quite impressive. Naturally, this study is unable to detect smaller differences in immunisation rate between the two groups.

The overall participation rate in this study was very low (15,6%) Even though this was not one of the parameters measured; a large proportion of parents enrolled in this study expressed that participation was associated with a high burden in terms of ethical concerns of invasive diagnostics in their healthy child. Especially the parents of children who were not exposed to anti-TNF-α in utero expressed fear of unnecessary exposing their healthy child to a painful and possibly traumatising venous puncture. In our view, these ethical concerns should weigh heavy in designing future similar studies. Importantly, these concerns led to the decision to limit this study to the investigation of the HBV vaccination response, as additional vaccination response measurements would require a larger blood sample and potentially burden the child and parents even more.

A previous study suggested a dose-dependent response to the HBV vaccine in IBD patients, showing a double three dose vaccine-series to be more effective than the single three dose vaccine series.[28] In the present study, all children were vaccinated with a four-dose vaccine series yielding protective anti-HBs levels. The vaccination regimen, however, varies from country to country, for example in the United States the three-dose regimen is used. Therefore these study results might not be applicable to every country. This study was unable to investigate the efficacy of a three dose vaccine series in anti-TNF-α exposed children.
Anti-HBs levels have been shown to decline in time.[38, 39] In an uncontrolled study performed in paediatric IBD patients treated with IFX, 50% of patients did not have protective antibodies against HBV. Insufficient anti-HBs levels were associated with older age and the intensity of the IFX dose regimen. Anti-HBs levels in the present study were measured four weeks after the final vaccination dose. Follow up of anti-HBs levels in the children in the present

study would be interesting, as the rate of decline of anti-HBs levels in this specific population remains unknown.

Furthermore, it is important to realise that IBD mothers treated with anti-TNF-α during pregnancy discontinued their anti-TNF-α treatment at the end of the second trimester if the disease was in sustained remission.[23] The goal of this policy is to limit anti-TNF-α levels in the neonate. Therefore, this cohort of anti-TNF-α exposed children may have overall lower drug levels than children born to mothers who continued anti-TNF-α throughout the entire pregnancy. It may be possible that children with higher anti-TNF-α levels at birth have a less adequate vaccine response, although we did not observe this effect in this small cohort.
In conclusion, the present study shows children exposed to anti-TNF-α in utero can be effectively vaccinated against the hepatitis B virus.

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Chapter 9:

Summary and general discussion

SUMMARY & GENERAL DISCUSSION

This thesis aimed to provide more insight into important clinical topics regarding IBD and reproduction. Finding the balance between IBD treatment in fertile patients to maintain disease remission and at the same time consider fetal safety remains challenging. In the first part of this thesis we describe anti-TNF-α treatment for male IBD patients wishing to procreate and the importance of preconceptional counselling. The second part focusses on anti-TNF-α and thiopurine use and gastrointestinal endoscopy during pregnancy. In the third part we describe long-term health outcomes of children born to mothers with IBD and the efficacy of hepatitis B vaccination in children with detectable anti-TNF-α levels at birth. Finally, the general discussion and future perspective for research will be described.

PART 1. PRE-PREGNANCY

The field of IBD therapy has undergone a dynamic evolution with the introduction of anti-TNF-α therapy. TNF-α is a proinflammatory cytokine that is associated with the pathogenesis of IBD and anti-TNF-α treatment has proven to be effective in the treatment of IBD[1-4]. TNF-α is also present in seminal plasma in physiologically low levels, and those levels increase during inflammatory state with negative effect on spermatogenesis and sperm motility[5, 6]. Data regarding the use of anti-TNF-α agents in male IBD patient who wish to procreate are scarce. A study describing infliximab use in 10 male IBD patients wishing to reproduce showed a possible negative effect of infliximab on sperm motility[7]. However, studies of male patients using anti-TNF-α because of other inflammatory diseases found no negative effects of anti-TNF-α on sperm quality[8, 9]. After the introduction of infliximab as the first anti-TNF-α drug for IBD, nowadays different types of anti-TNF-α are used for the treatment of IBD. Currently adalimumab is often used, however, studies assessing adalimumab use by male IBD patients on semen quality are entirely lacking. In **Chapter 2** we aimed to describe the effect of adalimumab use in male IBD patients on semen parameters and pregnancy outcomes of children who were conceived while the father was using adalimumab. This study consists partly of a prospective part assessing the effect of adalimumab on semen quality and partly of a retrospective clinical part assessing the effect of paternal adalimumab use on child's health outcomes, such as birth weight, gestational age and the presence of congenital abnormalities. The result of this study showed no association between adalimumab use and adverse effects on sperm quality or adverse health outcomes of children.

Health risks of IBD mothers-to-be and their children are an import clinical topic. IBD patients still tend to have incorrect believes regarding IBD treatment and pregnancy[10-12]. As a result, patients consider voluntary childlessness more often than women without IBD. In the case

of pregnancy and lactation, women tend to stop medication for their IBD as it is often believed that medication causes harm to their offspring. In **Chapter 3** the importance of preconceptional counselling is described in an editorial. Stopping maintenance drugs during pregnancy increases the risk of disease relapse which leads to an increased risk of adverse pregnancy outcomes such as, preterm birth and low birth weight. Most IBD drugs, however, are not associated with adverse pregnancy outcomes and should therefore be continued during pregnancy to maintain disease remission. During preconceptional counselling medication should be assessed and adjusted accordingly in order to find the right balance between disease remission and minimising fetal drug exposure. This approach is effective in reducing disease relapse during pregnancy by optimizing adherence to medication. Timely counselling of women with IBD in their reproductive years should have positive effects on health outcomes of their children and physicians should therefore adapt a proactive strategy to improve medication adherence during pregnancy which subsequently will increase the chance of favorable pregnancy outcomes.

PART 2. PREGNANCY

During pregnancy, IBD medication may be needed to maintain disease remission. Currently, thiopurine and anti-TNF-α, as monotherapy or as combination therapy, form the corner stone of IBD treatment. In **Chapter 4.1** we described a prospective cohort study assessing the effect of thiopurine use during pregnancy. Previous studies assessing this effect were all retrospective studies and outcomes were conflicting[13-18]. Adverse outcomes were possibly due to confounding factor, however, because of the retrospective nature of these studies it was not possible to correct for these factors. In the study of this chapter, we assessed the effect of thiopurine use during pregnancy in a prospective manner in which we were able to adjust for confounding factors such as; disease activity, smoking, folic acid intake, and obstetric complications. In this study we prospectively assessed 410 women during pregnancy of whom 149 used a thiopurine. We found that, after adjusting for confounding factors, thiopurine did not increase the risk of a spontaneous abortion nor adverse pregnancy outcomes. In addition, no increased risk of adverse one-year health outcomes were found in thiopurine-exposed infants. Pregnancy does seem to effect 6-methylmercaptopurine (6-MMP) metabolism as we found increased 6-MMP levels during pregnancy in women using stable dosing of thiopurine, however, without leading to adverse events in mothers or fetus. This is in line with a previous published study demonstrating increased 6-MMP levels and decreased 6-thioguanine nucleotide (6-TGN) levels during pregnancy in women on stable dose therapy[19]. These alterations may be avoided by therapeutic drug monitoring although the implication of this altered thiopurine metabolism during pregnancy remains to be elucidated. **Chapter 4.2** briefly comments on a meta-analysis investigating the effect of thiopurine use

during pregnancy in women with IBD[20]. This study concluded that thiopurine use during pregnancy leads to an increased risk of congenital abnormalities. This conclusion is based on one small retrospective study[21], showing congenital abnormalities in 4 out of 26 thiopurine-exposed children, without adjusting for confounding factors such as folic acid intake. This outcome should therefore be interpreted with caution. More recent studies, including the prospective study described in this thesis, found no association between thiopurine use and congenital abnormalities. Therefore, based on the available literature, women with IBD should be advised to continue thiopurine during pregnancy as disease activity likely outweighs the possible risks of thiopurine use.

Different types of anti-TNF-α drugs are currently used to induce and maintain disease remission. Most often studied during pregnancy are infliximab and adalimumab. Both drugs are IgG1 monoclonal antibodies that are actively transported over the placenta, mainly during the third trimester resulting in higher drug levels in the newborn than mother[22-24]. A previous study assessing drug levels in cord blood at birth of IBD women demonstrated significant higher infliximab levels than adalimumab levels and found longer time to drug clearance in infliximab-exposed infants than adalimumab-exposed infants[25]. This suggest that the placental transmission of different anti-TNF-α may vary. Current guidelines provide recommendation regarding anti-TNF-α cessation during pregnancy in order to minimise fetal exposure[26]. Women may discontinue anti-TNF-α in the third trimester in case of sustained disease remission, as this does not lead to a higher risk of disease relapse[27]. These recommendation however do not differentiate between the different types of anti-TNF-α. In **Chapter 5.1** we illustrate the effect of anti-TNF-α use during pregnancy on fetal exposure for infliximab and adalimumab separately. The study described in this chapter illustrates that placental transmission of infliximab increases exponentially leading to high fetal concentrations at term, and that placental transportation of adalimumab increases more gradual in a linear fashion. Relapse rate and one-year health outcomes of exposed-infants were comparable between the two drugs. Overall, this study shows that adalimumab may be continued longer during pregnancy than infliximab without leading to high drug levels in the newborns. In **Chapter 5.2** comments were made on a study investigating the concentration of infliximab and adalimumab in cord blood at birth and the clearance rate of these drugs in newborns who were exposed in utero[25]. Adalimumab seems to be the preferable anti-TNF-α drug during pregnancy as it may be continued longer during pregnancy without leading to high anti-TNF-α concentrations in offspring and drug clearance in newborns is faster. The importance of disease remission before and during pregnancy however should be emphasized. Therefore, it is not advisable to switch from infliximab to adalimumab during stable disease or in women contemplating pregnancy because of the risk of a disease relapse. During preconceptional counselling, IBD medication should be assessed carefully to find the optimal balance between maintaining disease remission and at the same time minimise fetal drug exposure.

Women with IBD are at increased risk of needing a gastrointestinal endoscopy during pregnancy. In **Chapter 6** we briefly comment on a population-based cohort study concluding that endoscopy during pregnancy is associated with increased risk of preterm birth and SGA[28]. However, essential confounding factors such as disease activity, which is known to be associated with preterm birth and SGA, was not taken into account. The implications of this study therefore seems limited for women suffering from IBD. In the case of IBD, lower endoscopies are more likely to be needed than higher endoscopies. Previous studies assessing the safety of lower gastrointestinal endoscopy in women with IBD show that lower endoscopy during pregnancy seems to be of low risk for mother and child[29-31]. However, studies assessing the risks of lower endoscopy during pregnancy in women with IBD are limited. A lower endoscopy during pregnancy should therefore only be performed when there is a strong indication.

PART 3. POST-PREGNANCY

During pregnancy, different types of IBD medication may be needed to induce and maintain disease remission. Most IBD drug are not associated with adverse pregnancy outcomes such as low birth weight, preterm birth and congenital abnormalities[32-34]. However, the implications on long-term health outcomes of exposed children are relatively unexplored. In **Chapter 7** we investigated the long-term health of 1000 children born to mothers with IBD and assessed the effect of different types of drugs. We assessed the association of anti-TNF-α monotherapy, thiopurine monotherapy and the combination of anti-TNF-α and thiopurine with the following outcomes until 5 years of age: infections needing antibiotic treatment, infections needing hospital admission, adverse reactions to vaccinations, growth, auto-immune diseases and malignancies. We found no association between any of the types of IBD drugs and adverse long-term health outcomes. Because corticosteroids may influence outcomes, a sensitivity analysis was performed by excluding all women using corticosteroids. Also the sensitivity analysis showed no association between IBD medication and adverse long-term outcomes. We did however find an association between thiopurine use and intrahepatic cholestasis of pregnancy (ICP), without affecting pregnancy outcomes or long-term health outcomes. It should however be noted that the sample size is small and more studies will be needed to confirm this association. Overall, this study is reassuring regarding the use of IBD medication during pregnancy indicating that anti-TNF-α and thiopurines have no or little influence on long-term health development of in utero exposed children.

Studies in adults and children have shown insufficient response to vaccinations during anti-TNF-α treatment[35, 36]. Infants exposed to anti-TNF-α in utero are born with detectable anti-TNF-α levels, which is cleared in the following months depending anti-TNF-α type and the time of last drug administration. In **Chapter 8** we investigated the response to the hepatitis

B vaccine (HBV), which is administered in the first year of life, in infants who were exposed to anti-TNF-α in utero and had detectable anti-TNF-α levels at birth. In this controlled cohort study we found that children born with detectable anti-TNF-α levels respond adequately to the vaccination against HBV.

GENERAL DISCUSSION & FUTURE PERSPECTIVES

As described in this thesis, an uncomplicated pregnancy course is feasible for patients suffering from IBD. However, complications may occur due to disease activity during conception and pregnancy underlining the importance of disease remission before and during pregnancy. We show in this thesis that anti-TNF-α and thiopurine as maintenance treatment are of low risk during pregnancy for mother and their offspring. Patients knowledge regarding reproductive related issues remains poor which subsequently leads to negative view on IBD treatment during pregnancy. It is understandable that patients wishing to reproduce are reluctant of using systemic drugs as it may affect pregnancy outcome. However, disease activity is associated with adverse pregnancy outcomes and disease remission is the most important factor in a successful pregnancy. Therefore, studies assessing the safety of IBD drugs during pregnancy are of utmost importance as these studies help to reassure patients wishing to reproduce.

Therapeutic drug monitoring (TDM) is used in patients with IBD and involves measurements of active drug metabolites and anti-bodies, which can be used to optimise therapeutics. This strategy is based on the assumption that drug exposure is related to outcome and that there is an inter-individual variability in how patients metabolize the drug. TDM is not used during pregnancy. As demonstrated in this thesis we found increased 6-MMP levels during pregnancy in women on stable dose thiopurine. Alteration of 6-MMP and 6-TGN levels during pregnancy was also previously observed in a prospective study demonstrating a major effect of pregnancy on thiopurine metabolism[19]. Aberrant 6-MMP and 6-TGN levels during pregnancy may be avoided by monitoring maternal thiopurine metabolites during pregnancy and adjust dose accordingly. However, during pregnancy, increased 6-MMP levels in mothers have not been related to hepatotoxicity or myelotoxicity and increased 6-TGN levels do not seem to be associated with teratogenicity. This raises the question what the effect is of these alterations during pregnancy on maternal and fetal health. In addition, a recent study demonstrated that infliximab levels also rises significantly during pregnancy in women on a stable dose regime however adalimumab levels remain stable[37]. Measuring maternal anti-TNF-α during pregnancy may aid in the decision to stop anti-TNF-α in the third trimester if women are in sustained remission or to make dose adjustments. However, the implication of TDM during pregnancy on relapse risk remains unknown. Larger prospective studies will be needed do

give more insight into the altered thiopurine and anti-TNF-α metabolism during pregnancy. Furthermore, the role of TDM during pregnancy need to be evaluated.
Investigating the effect of IBD drugs on pregnancy outcomes remains challenging as data is mostly provided by post-hoc analyses, cohort studies and retrospective studies. Because it involved pregnant women, drug safety is never studied in randomized controlled trials (RCTs) as this would be considered unethical. This makes it even more challenging than for non-pregnant patient to provide evidence regarding drug safety. As a physician it is important to realize these challenges. In case of pregnancy, large follow-up cohort will provide the best possible information regarding drug safety and health outcomes of mothers and their children.

IBD therapy is evolving and new medical treatments are rapidly introduced. As discussed in the latter paragraph, drug safety is not studied in women during pregnancy or men willing to conceive in RCTs. The safety of these new treatments, however, will need to be studied before widely introducing them in patients with an active pregnancy wish or during pregnancy. Little is known about the safety of newer IBD drugs such as, vedolizumab, ustekinumab and golimumab which should be taken into account when starting an anti-TNF-α in patients with a current or future pregnancy wish. If possible, it is advisable to start an anti-TNF-α that has been studied during pregnancy such as adalimumab until future studies provide more insight into the safety of the newer IBD drugs.

Anti-TNF-α are IgG1 antibodies that are known to be actively transported over the placenta during pregnancy. However, there seems to be a difference regarding the amount of placental transmission between different anti-TNF-α drugs as shown in this thesis. Little is known about the exact mechanism of placental transportation of anti-TNF-α drugs and which placental receptors are involved in this transmission. Future studies may provide more insight into this mechanism and may aid in the development of new drugs preventing anti-TNF-α transmission over the placenta.

Overall, this thesis adds to the current data regarding reproduction in patients with IBD and shows favorable outcomes for mothers and children in case disease remission is maintained. This underlines the importance of finding the balance between maintaining disease remission in mothers with IBD drugs and at the same time minimising fetal drug exposure.

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Chapter 10:

Nederlandse samenvatting
List of co-authors
PhD portfolio
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Curriculum Vitae

NEDERLANDSE SAMENVATTING

Inflammatoire darm ziekten (IBD) zoals colitis ulcerosa en de ziekte van Crohn zijn chronische ontstekingsziekten van het maagdarmkanaal. Deze ontstekingsziekten zijn ongeneselijk en patiënten hebben vaak levenslang medicatie nodig om de ziekte rustig te houden. Omdat deze ziekten vaak op jonge leeftijd optreedt (50% van de patiënten zijn jonger dan 35 jaar als de diagnose gesteld wordt) is het onvermijdelijk dat patiënten ook tijdens conceptie en zwangerschap behandeling nodig hebben. In dit proefschrift worden vraagstukken rondom IBD en zwangerschap belicht. In het eerste deel beschrijven we de veiligheid van anti-TNF-α behandeling voor IBD bij mannen rondom conceptie en beschrijven we het belang van goede voorlichting voor een beoogde zwangerschap. In het tweede deel beschrijven we de veiligheid van het gebruik van de medicijnen thiopurine en anti-TNF-α tijdens de zwangerschap en de veiligheid van het ondergaan van een kijkonderzoek van de dikke darm tijdens de zwangerschap. In deel drie worden de lange termijn gezondheidsuitkomsten van de kinderen van moeders met IBD beschreven evenals de werkzaamheid van het hepatitis B vaccine in kinderen die intra-uterien zijn blootgesteld aan anti-TNF-α. Tot slot worden suggesties voor toekomst onderzoek gepresenteerd.

DEEL 1. VOOR DE ZWANGERSCHAP

Met de introductie van anti-TNF-α is er veel veranderd op het gebied van de behandeling van IBD. TNF-α is een pro-inflammatoir cytokine dat geassocieerd is met de pathogenese van IBD en meerdere studies hebben laten zien dat anti-TNF-α effectief is bij de behandeling van IBD. TNF-α is echter ook in lage hoeveelheden aanwezig in semen. Bij inflammatie stijgt de hoeveelheid TNF-α met negatieve gevolgen voor de sperma kwaliteit. De gevolgen van anti-TNF-α gebruik op sperma kwaliteit is echter weinig onderzocht. Er bestaat een studie die laat zien dat infliximab gebruik door mannen met IBD een mogelijk ongunstig effect heeft op de bewegelijkheid van sperma. Maar onderzoeken die zijn uitgevoerd bij mannen met andere auto-immuun ziekten lieten geen ongunstig effect zien van anti-TNF-α op de sperma kwaliteit. Na de introductie van infliximab, de eerste anti-TNF-α die op de markt kwam voor de behandeling van IBD, zijn er inmiddels meerdere type anti-TNF-α beschikbaar zoals adalimumab, welke vandaag de dag vaak wordt gebruikt als behandeling van IBD. Het effect van adalimumab op sperma kwaliteit is nooit onderzocht. In **hoofdstuk 2** wordt beschreven wat het effect is van adalimumab gebruik op sperma kwaliteit en de geboorte uitkomsten van kinderen van vaders die adalimumab gebruikte ten tijde van conceptie. Het eerste deel is een prospectief deel waar sperma kwaliteit wordt beschreven van mannen die adalimumab gebruiken en het tweede deel is een retrospectief deel welke de geboorte uitkomsten zoals geboortegewicht, zwangerschapsduur en aangeboren afwijkingen, beschrijft van de kinderen

van vaders die adalimumab gebruikte ten tijde van conceptie. Er werd in deze studie geen ongunstige effecten van adalimumab gebruik op sperma kwaliteit of geboorte uitkomsten gezien.

De gezondheidsrisico's van vrouwen met IBD die zwanger willen worden of zwanger zijn, zijn belangrijke klinische vraagstukken. Patiënten met IBD blijken tot op heden nog veel onjuiste opvatting te hebben betreffende IBD medicatie en zwangerschap. Het resultaat is dat patiënten met IBD er vaker voor kiezen om geen kinderen de krijgen vergeleken met mensen zonder IBD. Daarnaast stoppen patiënten vaak medicatie tijdens zwangerschap of borstvoeding omdat ze in de veronderstelling zijn dat medicijngebruik schadelijk is voor het (ongeboren) kind. In **hoofdstuk 3** wordt het belang van goede voorlichting aan patiënten met IBD die een zwangerschapswens hebben beschreven. Het stoppen van IBD medicatie tijdens de zwangerschap vergroot de kans op een opvlamming van de ziekte en als gevolg daarvan hebben deze patiënten een grotere risico op ongunstige zwangerschapsuitkomsten, zoals vroeggeboorten en een laag geboortegewicht. De meeste IBD medicijnen zijn echter niet geassocieerd met schadelijke effecten tijdens de zwangerschap en het wordt daarom aangeraden om deze medicijnen tijdens de zwangerschap te continueren om een opvlamming van de ziekte te voorkomen. Tijdens de voorlichting is het van belang dat de huidige medicatie wordt beoordeelt en zo nodig wordt aangepast. Dit om ervoor te zorgen dat moeders in remissie blijven tijdens de zwangerschap en tegelijkertijd dat medicatie blootstelling aan het ongeboren kind zo laag mogelijk is. Hierin is ook een belangrijke rol weggelegd voor de behandeld arts, namelijk om tijdig een eventuele toekomstige zwangerschapswens te bespreken met IBD vrouwen om complicaties te voorkomen.

DEEL 2. TIJDENS DE ZWANGERSCHAP

Tijdens de zwangerschap is het vaak nodig om de onderhoudsmedicatie te continueren om de ziekte in remissie te houden. Vandaag de dag vormen thiopurines en anti-TNF-α, als monotherapie of in combinatie, de hoeksteen van de behandeling van IBD. In **hoofdstuk 4.1** beschrijven we een prospectieve studie naar thiopurine gebruik voor IBD tijdens de zwangerschap. Alle voorgaande onderzoeken waren retrospectief van aard en uitkomsten tegenstrijdig. Het is mogelijk dat andere factoren meespeelden waardoor vrouwen die thiopurine gebruikte vaker ongunstige zwangerschapsuitkomsten hadden dan vrouwen zonder thiopurine, echter omdat deze studies retrospectief van aard waren was het onmogelijk om te corrigeren voor beïnvloedende factoren. In dit hoofdstuk wordt het effect van thiopurine gebruik tijdens de zwangerschap op een prospectieve manier onderzocht waardoor het mogelijk was om te corrigeren voor factoren die invloed hebben op de uitkomsten, zoals ziekte activiteit, roken, foliumzuur gebruik en gynaecologische complicaties tijdens de zwangerschap. Er werden in totaal 410 vrouwen geïncludeerd waarvan 149

thiopurine gebruikten. Na correctie voor de bovenstaande factoren, vonden we dat thiopurine geen verhoogd risico geeft op miskramen of andere ongunstige zwangerschapsuitkomsten zoals aangeboren afwijkingen, vroeggeboorte en laag geboortegewicht. Daarnaast vonden we geen verband tussen thiopurine gebruik tijdens de zwangerschap en ongunstige gezondheidsuitkomsten van kinderen tot 1 jaar.

Thiopurines worden na inname via een complex metabolisme door diverse enzymen omgezet in onder andere 6-thioguaninenucleotides (6TGN), waaraan de farmacologische werking van thiopurine grotendeels wordt toegeschreven. Tevens wordt bij deze omzetting het metaboliet 6-methylmercaptopurine (6-MMP) gevormd welke is geassocieerd met levertoxiciteit. Bij de behandeling met thiopurines wordt er naar gestreefd om zowel de 6-TGN als de 6-MMP waarden binnen referentiewaarden te houden om het werkingsmechanisme zo optimaal mogelijk en het risico op bijwerkingen zo laag mogelijk te houden. Tijdens de zwangerschap vonden wij dat de 6-MMP spiegel drastisch steeg zonder dat de dosering van het medicijn werd aangepast. Deze stijging had echter geen negatieve gevolgen voor de gezondheid van moeder of kind. Een stijging van 6-MMP en daling van 6-TGN werd al eerder beschreven in een prospectief onderzoek van 30 patiënten die thiopurine gebruikte tijdens de zwangerschap zonder dat dit heeft geleid tot ongunstige uitkomsten van moeder en kind. Doses aanpassingen tijdens de zwangerschap zouden deze hoge 6-MMP spiegels mogelijk kunnen voorkomen echter wat de relatie is met de gezondheidsuitkomsten van moeder en kind is niet onderzocht. In **hoofdstuk 4.2** becommentariëren we een meta-analyse die concludeert dat thiopurine gebruik tijdens de zwangerschap een verhoogt risico geeft op aangeboren afwijkingen van het kind. Deze conclusie is echter gebaseerd op een kleine retrospectieve studie die laat zien dat 4 van de 26 kinderen die intra-uterien zijn blootgesteld aan thiopurine een aangeboren afwijkingen hadden, zonder te corrigeren voor factoren die invloed hebben op deze uitkomst zoals het gebruik van foliumzuur. Meer recente studies, waaronder de studie beschreven in dit proefschrift, laten geen verband zien tussen aangeboren afwijkingen en thiopurine gebruik tijdens de zwangerschap. Wij adviseren daarom om thiopurine door te gebruiken tijdens de zwangerschap omdat de risico's van een opvlamming zwaarder wegen dan de risico's van thiopurine gebruik.

Er zijn meerdere type anti-TNF-α beschikbaar voor de behandeling van IBD. Infliximab en adalimumab zijn het meest onderzocht tijdens de zwangerschap. Dit zijn beide immunoglobulines van het type IgG1 welke actief over de placenta worden getransporteerd naar het ongeboren kind voor passieve immunisatie. Eerdere studies lieten zien dat infliximab in veel hogere waarden aanwezig is bij het kind dan adalimumab en daarnaast blijft infliximab langer meetbaar in het kind dan adalimumab. Dit suggereert dat de transmissie van deze medicijnen over de placenta verschilt. Huidige richtlijnen adviseren om anti-TNF-α gedurende de gehele zwangerschap te blijven gebruiken indien een patiënte niet in remissie is of een

opvlamming heeft gehad in de 6 maanden voor conceptie. Indien patiënten tijdens de zwangerschap en gedurende de 6 maanden voor de zwangerschap in remissie zijn, dan is het mogelijk om anti-TNF-α behandeling te staken in het derde trimester. In het geval van langdurige remissie leidt dit namelijk niet tot een verhoogd risico op een opvlamming en tegelijkertijd wordt te blootstelling van het kind geminimaliseerd. De richtlijnen houden echter geen rekening met de verschillen tussen anti-TNF-α typen ondanks dat er aanwijzingen zijn dat er verschillen zijn in transmissie over de placenta. In **hoofdstuk 5.1** illustreren we het effect van anti-TNF-α gebruik tijdens de zwangerschap voor infliximab en adalimumab apart. De transmissie van infliximab is significant hoger en neemt exponentieel toe gedurende de zwangerschap, de transmissie van adalimumab is veel lager en neemt gestaag toe. Gezondheidsuitkomsten van moeder en kind zijn voor infliximab en adalimumab vergelijkbaar. Deze studie laat zien dat adalimumab veel langer tijdens de zwangerschap gecontinueerd kan worden zonder dat dit leidt tot hoge spiegels bij het ongeboren kind. Richtlijnen zullen in de toekomst het advies ten aanzien van het stoppen van anti-TNF-α tijdens de zwangerschap moeten aanpassen aan het type anti-TNF-α. In **hoofdstuk 5.2** becommentariëren we een studie die de concentratie van infliximab en adalimumab in pasgeborenen, die intra-uterien werden blootgesteld aan deze medicatie, onderzocht. Deze studie laat zien dat adalimumab eerder wordt geklaard door het kind dan infliximab, derhalve heeft adalimumab de voorkeur over infliximab tijdens de zwangerschap. Het is echter essentieel om het belang van ziekte remissie voorop te stellen. Het is daarom niet verstandig om tijdens de zwangerschap te switchen van infliximab naar adalimumab vanwege het risico op een opvlamming van de ziekte. Dit risico onderstreept weer het belang van tijdige voorlichting aan vrouwen met IBD die een (toekomstige) zwangerschapswens hebben.

Vrouwen met IBD hebben een hoger risico dat ze een kijkonderzoek van de dikke darm moeten ondergaan tijdens de zwangerschap dan vrouwen zonder IBD. In **hoofdstuk 6** geven we commentaar op een studie die concludeert dat een kijkonderzoek van de dikke darm tijdens de zwangerschap een vergroot risico geeft op vroeggeboorte en een laag geboortegewicht. Er is in deze studie echter onvoldoende gecorrigeerd voor beïnvloedende factoren, zoals ziekte activiteit. Zoals eerder beschreven geeft actieve IBD namelijk ook een vergroot risico op vroeg geboorte en een laag geboortegewicht. Andere studies naar de risico's van een kijkonderzoek tijdens de zwangerschap laten geen vergroot risico zien op ongunstige uitkomsten. Omdat onderzoeken betreffende een kijkonderzoek tijdens de zwangerschap tegenstrijdig zijn en omdat een kijkonderzoek op zichzelf risico's met zich meebrengt, adviseren we om een kijkonderzoek enkel uit te voeren als daar een harde indicatie voor is.

DEEL 3. NA DE ZWANGERSCHAP

IBD medicijnen zijn soms nodig tijdens de zwangerschap om de ziekte rustig te houden. De meeste van deze medicijnen hebben geen effect op de zwangerschapsuitkomsten zoals geboortegewicht, vroeggeboorte en aangeboren afwijkingen. Wat het effect is van medicijngebruik tijdens de zwangerschap op de lange termijn gezondheid van blootgestelde kinderen is weinig onderzocht. In **hoofdstuk 7** beschrijven we de lange termijn gezondheidsuitkomsten van 1000 kinderen van moeders met IBD. We hebben gekeken naar het effect van anti-TNF-α, thiopurine en de combinatie van anti-TNF-α en thiopurine op de volgende gezondheidsuitkomsten in kinderen tot 5 jaar: infecties waarvoor antibiotische behandeling of ziekenhuisopname noodzakelijk was; allergische reacties op vaccinaties; groei; auto-immuun ziekten en maligniteiten. We vonden geen relatie tussen het gebruik van deze medicijnen en ongunstige lange termijn gezondheidsuitkomsten van het kind. Omdat corticosteroïden ook regelmatig worden gebruikt om een opvlamming van IBD te behandelen en het gebruik hiervan invloed kan hebben op de gezondheidsuitkomsten van het kind werden aanvullend aparte analyses gedaan waar alle patiënten met corticosteroïd gebruik werden geëxcludeerd. Ook in deze analyses vonden we geen relatie tussen het gebruik van anti-TNF-α, thiopurine of de combinatie en ongunstige lange termijn gezondheidsuitkomsten van het kind. We vonden echter wel een verband tussen thiopurine gebruik en zwangerschapscholestase. Deze uitkomst is echter gebaseerd op zeer kleine aantallen, derhalve zullen grotere studies deze uitkomst moeten bevestigen.

Het gebruik van anti-TNF-α kan de werkzaamheid van een vaccinatie beïnvloeden. Eerdere studies lieten zien dat IBD patiënten die anti-TNF-α gebruiken onvoldoende reageerden op de hepatitis B vaccinatie en dus onvoldoende beschermd waren. In **hoofdstuk 8** beschrijven we de werkzaamheid van het hepatitis B vaccine, welke in het eerste levelsjaar wordt gegeven, in kinderen die intra-uterien werden blootgesteld aan anti-TNF-α en ook meetbare anti-TNF-α spiegels hadden bij geboorte. Deze studie laat zien dat kinderen van moeders die tijdens de zwangerschap anti-TNF-α gebruikte een normale reactie laten zien op het hepatitis B vaccine en voldoende beschermd zijn tegen hepatitis B na vaccinatie.

AANBEVELINGEN VOOR TOEKOMSTIG ONDERZOEK

Zoals beschreven in dit proefschrift, is een ongecompliceerde zwangerschap voor vrouwen met IBD goed mogelijk. Echter is het wel belangrijk dat de ziekte rustig blijft omdat ziekte activiteit een vergroot risico geeft op ongunstige zwangerschapsuitkomsten. In dit proefschrift laten we zien dat de meest gebruikte medicijnen voor IBD; thiopurine en anti-TNF-α, gecontinueerd kunnen worden en dat het gebruik niet leidt tot ongunstige korte

termijn en lange termijn uitkomsten van moeder en kind. Deze uitkomsten dragen bij aan de kennis omtrent IBD en zwangerschap en kunnen gebruikt worden bij de voorlichting van IBD patiënten met een (toekomstige) zwangerschapswens.

Voor zowel thiopurine als anti-TNF-α is het mogelijk om de dosering aan te passen aan de hand van de spiegels in het bloed van de patiënt. Op deze manier blijven de spiegels binnen bepaalde referentiewaarden. Dit wordt echter niet gedaan tijdens de zwangerschap. Zoals beschreven in dit proefschrift blijken de waarden van 6-MMP en 6-TGN drastisch te veranderen bij moeders tijdens de zwangerschap ondanks dat ze dezelfde dosering blijven gebruiken. Ook heeft een recente studie laten zien dat infliximab spiegels stijgen tijdens de zwangerschap zonder de medicijn dosering werd aangepast. Deze veranderingen lijken geen effect te hebben op de gezondheid van moeders en het kind, echter grotere studies moeten dit nog bevestigen. Toekomstige studies zullen daarnaast moeten uitwijzen of medicijn aanpassingen op basis van deze spiegels tijdens de zwangerschap veilig is.
Onderzoeken naar medicijnen tijdens de zwangerschap blijft een uitdaging. Grote studies naar de veiligheid van nieuwe medicijnen worden nooit uitgevoerd bij zwangere patiënten uit ethische overwegingen. De data die beschikbaar is komt meestal van post-hoc analyses, retrospectieve studies en cohort studies. Het is belangrijk om bewust te zijn van de beperkingen van deze studies.

De behandelingsmogelijkheden voor IBD zijn constant in ontwikkeling en er komen steeds meer nieuwe medicijnen op de markt. Zoals besproken in de voorgaande alinea wordt de veiligheid van deze nieuwe middelen nooit direct onderzocht bij zwangere patiënten. Voorbeelden van nieuwere medicijnen voor de behandeling van IBD zijn vedolizumab, ustekinumab en golimumab. Toekomstige studies zullen zich moeten richten op de veiligheid van deze middelen tijdens de zwangerschap.

Anti-TNF-α zijn immunoglobulines die actief over de placenta heen worden getransporteerd naar het ongeboren kind toe. Het exacte mechanisme van deze transportatie is echter onbekend. Toekomstige studies moeten meer inzicht geven in dit mechanisme. Indien dit mechanisme kan worden opgehelderd, is het misschien ook mogelijk om medicijnen te ontwikkelen die aangrijpen op deze transportatie waardoor vrouwen medicijnen kunnen blijven gebruiken tijdens de zwangerschap zonder dat deze medicijnen naar het ongeboren kind worden getransporteerd.

Concluderend laat dit proefschrift zien dat conceptie en zwangerschap goed mogelijk is voor patiënten met IBD. Er dient te worden gestreefd naar een goede balans tussen ziekte remissie in moeders en tegelijkertijd lage blootstelling van medicatie aan het ongeboren kind.