On reproduction and other gender and sex-related issues in inflammatory bowel disease

(Inflammatory bowel disease `du deuxième sexe`)

Zuzana Zelinkova
On Reproduction and
Other Gender and Sex-related Issues
in Inflammatory Bowel Disease

Voortplanting en andere geslachtsgerelateerde aspecten
in inflammatoire darmziekten

Thesis
to obtain the degree of Doctor from the
Erasmus University Rotterdam
by command of the
rector magnificus
Prof.dr. H.G. Schmidt

and in accordance with the decision of the Doctorate Board
The public defence shall be held on
Wednesday 6 June 2012 at 09.30 hrs

Zuzana Zelinkova
born in Bratislava, Slovakia

ISBN: 978-80-970908-9-0
Cover: Jan Brázda (1917 - 2011) - Náklonnost, 1951
Lay-out: Adrián Struňák
Printed by SINEAL

The work described in this thesis was conducted at the Department of Gastroenterology and Hepatology at the Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands.

Financial support for the publication of this thesis was kindly provided by Abbott Immunology, Dr. Falk Pharma Benelux B.V., Ferring B.V. and Merck Sharp&Doehme Nederland B.V.

Copyright © 2012 Zuzana Zelinkova, Rotterdam, The Netherlands. All rights reserved. No part of this book may be reproduced or transmitted in any form or by any means, without prior written admission of the author.
PROMOTIECOMMISSIE

Promoters:
Prof.dr. E.J. Kuipers
Prof.dr. M.P. Peppelenbosch

Overige leden:
Prof.dr. H.L.A. Janssen
Prof.dr. J.D. Laman
Dr. W.J. de Jonge

Copromotor:
Dr. C.J. van der Woude
## CONTENTS

**Chapter 1**  
Research and Care of Inflammatory Bowel Disease – Time for a Gender-Specific Approach?  
Part I – Gender  

**Chapter 2**  
Differences in the Health-Related Quality of Life between Men and Women with Inflammatory Bowel Disease  
Part II – Sex  

**Chapter 3**  
Maternal Imprinting and Female Predominance in Familial Crohn’s Disease  

**Chapter 4**  
Adverse Drug Reactions to Anti-Tumor Necrosis Factor Agents Differ between Male and Female Inflammatory Bowel Disease Patients  
Part III – Reproduction  

**Chapter 5**  
Reproductive Wish Represents an Important Factor Influencing Therapeutic Strategy in Inflammatory Bowel Diseases  

**Chapter 6**  
Azathioprine Treatment during Lactation  

**Chapter 7**  
High Intra-Uterine Exposure to Infliximab Following Maternal Anti-Tnf Treatment During Pregnancy  

**Chapter 8**  
Effect of Adalimumab on Semen Quality in Inflammatory Bowel Disease Patients  

**Chapter 9**  
Evaluation of the Discontinuation of Infliximab during Pregnancy in Inflammatory Bowel Disease Patients  

**Chapter 10**  
Pregnancy and AntiTNF Use: Safety is not yet Assured  

**Chapter 11**  
Summary. Nederlandse Samenvatting. General Discussion  

**Dankwoord**  

**Curriculum Vitae**  

**List of Publications**
Chapter 1

Research and Care of Inflammatory Bowel Disease –
Time for a Gender-Specific Approach?
INTRODUCTION

No one would argue that men and women differ. The differences between the sexes are obvious and affect every aspect of life. The biological grounds for these differences are determined by the complementary yet distinct roles of the two sexes in the process of procreation. The success of human reproduction is related to the very efficient selection process for the proper genetic material that will, after 40 weeks of intense physical investment by the female, result in the birth of a healthy progenitor. This ultimate goal of procreation determines the basic differences between male and female biology that presumably functions at the level of every cell.

In general, each individual’s physiognomy, character, susceptibility to specific diseases and life expectancy results from an interaction between genes and the environment. At the cellular level, basic differences between the female XX and male XY genotypes leads to a great divergence in protein structure that influences a variety of biochemical processes. With such divergence at the cellular level, substantial differences are expected at all levels of various physiological and patho-physiological processes in males compared with females. This, in turn, would be reflected in the sexually dimorphic presentation and phenotype of different diseases, and in drug pharmacokinetics and pharmacodynamics as well as subjective perceptions of the burden of disease.

Yet, most medical interventions not related to reproductive organs are conducted in the same manner in both men and women. Medical textbooks only include a ‘unisex’ description of disease presentation, men and women are unequally represented during drug development and testing, and pharmaco-economic studies using quality of life assessments are still subject to important gender biases. Undoubtedly, every physician creates and uses his/her own gender-specific way of working on a daily basis, but without proper evidence-based grounds for such an approach. A rather isolated example is in cardiology, where the first report of increased mortality in women with coronary heart disease compared with men prompted a thorough analysis of the problem, and resulted in the creation of gender-specific guidelines. These observations pointing out the importance of gender/sex in health and disease have prompted further mechanistic studies on the cellular and molecular basis of the observed phenomena, but in most cases the sexual dimorphic features of the disease are yet to be elucidated. Once the main question, “to what extent and how does sex play a role in the pathogenesis of a particular disease?” is answered, the development of tailored therapies for men and women can start.

On the other hand, sex differences contribute to variations in biology and can be used as a tool for efficiently researching various physiological processes. Thus, introducing sex systematically as one of the variables in experimental design would be valuable, not only for understanding the role of sex in a particular biological process, but sex-related differences can also help to elucidate the details of the process itself.

The biological differences between women and men may greatly impact physiological and pathophysiological conditions. A main goal of medicine in the twenty-first century, providing truly personalized care for each patient, should start with the creation of respective female and male concepts of health and disease. To achieve this, each specialty needs to inventory its own gender-specific issues that can serve as the basis for further investigation into the contributions of gender/sex to disease pathogenesis and its impact on choosing appropriate therapeutic strategies.

GENDER AND SEX IN HEALTH AND DISEASE

General considerations and terminology

During the past two decades, the important influence of gender/sex on the type and presentation of some diseases has been put on the research agendas of several health organizations. Systematic research in this field showed that sex-related differences are present at virtually every level of functioning of the human body. At the level of clinical research, these differences are present in the sexual dimorphic prevalence, phenotypes, prognosis, and therapy success of a broad range of diseases. Additionally, the influence of gender on health-related quality of life (HRQOL) and the bias this influence may cause in the cost-effectiveness analysis of various therapeutic strategies is being recognized. These observations pointing out the importance of gender/sex in health and disease have prompted further mechanistic studies on the cellular and molecular basis of the observed phenomena, but in most cases the sexual dimorphic features of the disease are yet to be elucidated. Once the main question, “to what extent and how does sex play a role in the pathogenesis of a particular disease?” is answered, the development of tailored therapies for men and women can start.

On the other hand, sex differences contribute to variations in biology and can be used as a tool for efficiently researching various physiological processes. Thus, introducing sex systematically as one of the variables in experimental design would be valuable, not only for understanding the role of sex in a particular biological process, but sex-related differences can also help to elucidate the details of the process itself.

The non-recognition or underestimation of the biological contributions of gender/sex to human health is currently subject to active discussion. This discussion resulted in suggestions of several regulatory mechanisms that should ensure that gender/sex is taken into account at the initiation of experimental or trial designs. The use of unified terminology is one of the prerequisites for this process; especially, the terms sex and gender should be used consistently. According to the WHO definition, gender refers to “the socially constructed roles, behaviours, activities, and attributes that a given society considers appropriate for men and women”, whereas sex refers to the biological and physiological characteristics that define men and women. In line with these definitions, the Committee on Understanding the Biology of Sex and Gender Differences recommends using the term sex to classify males or females according to the reproductive organs and functions that derive from the chromosomal complement, and to use the term gender to refer to a person’s self-representation as male or female, or how that person is responded to by social institutions on the basis of the individual’s gender presentation.
“If health care systems are to respond adequately to problems caused by gender inequality, it is not enough to simply ‘add in’ a gender component late in a given project’s development. Research, interventions, health system reforms, health education, health outreach, and health policies and programmes must consider gender from the beginning.

Gender is thus not something that can be consigned to ‘watchdogs’ in a single office, since no one office can possibly involve itself in all phases of an organization’s activities. All health professionals must have knowledge and awareness of the ways in which gender affects health, so that they may address gender issues wherever appropriate thus rendering their work more effective” http://www.who.int/gender/gender_mainstreaming/en/index.html

ROLE OF GENDER/SEX IN INFLAMMATORY BOWEL DISEASE

INFLAMMATORY BOWEL DISEASE

Inflammatory bowel disease (IBD) is a chronic relapsing inflammatory condition that affects the gastrointestinal tract. The disease typically manifests itself in the second and third decade of life and persists throughout the individual’s lifetime. It comprises two conditions; Crohn’s disease (CD) and ulcerative colitis (UC). The two conditions differ in the localisation and extent of inflammation at the mucosal level. The transmural inflammation typical of CD is localised at any part of the gastrointestinal tract, whereas superficial inflammation is confined exclusively to the large bowel in UC. However, physicians in clinical practice encounter many disorders that at presentation and/or through the evolution of the disease cannot be definitely allocated to one these categories, and are then designated as unclassified colitis. Thus, the term IBD refers to a large spectrum of inflammatory conditions affecting the gastrointestinal tract.

The aetiology of IBD is unknown, but the evidence gathered supports the hypothesis that the inflammation of the intestinal mucosa results from an ill-directed immune response to commensal bacteria in a genetically-susceptible host. This feature of a problematic coexistence with autologous intestinal flora takes the aetiological viewpoint that IBD is a unique auto-immune condition in which the normal distinction between pathogens and commensals is disturbed. Additionally, up to half of IBD patients also have the extra-intestinal manifestations of sterile inflammation of joints, skin, uvea, and liver. These findings complement the view of IBD as an autoimmune disorder with an aberrant immune response that is systemic rather than confined to the mucosal surface.

The presentation of the disease ensues from the inflammation of the gut with typical symptoms of diarrhoea, rectal blood loss, abdominal pain, and weight loss. The lack of a thorough understanding of the aetio-pathogenesis of the disease in clinical practice is translated into the lack of curative treatment. Current therapy for IBD consists mainly of immune suppressive medication with all its potential short- and long-term toxicities. Furthermore, this therapy has a limited impact on the disease course, probably because it comes late in the pathogenetic process, leaving a significant proportion of patients progressing to structural or penetrating complications of the disease.

The epidemiologic reports on IBD vary depending on the population studied; but, generally, its incidence seems to be rising. Considering this increasing incidence with the current estimate of 2.2 million IBD patients in Europe, the chronic character of this incurable disease, as well as increasing health care costs, IBD represents an important clinical and public health issue.

GENDER

The course of IBD is characterized by flares and remissions, and no existing therapeutic approach can cure this disease. Improvement in the health-related quality of life (HRQOL) represents a primary goal of therapeutic strategies. In general, women report lower HRQOL, regardless of the questionnaire used or the underlying condition, and lower HRQOL in women has also consistently been reported in IBD. Female IBD patients have significantly reduced HRQOL compared with male patients and female gender is the strongest predictor of low HRQOL in CD.

This sexual-dimorphic perception of disease burden can be both gender-related and sex-determined. With regards to gender, the disease itself as well as medications to treat it cause issues typically weighted differentially by women and men. Ostomy, surgical scars, and scarring of the perineum resulting from perianal involvement in CD, the use of corticosteroids that are associated weight gain, striae, and Cushingoid habitus, are typical body image issues that can be perceived more intensely as a problem by women than men. Moreover, as discussed further in this chapter (Part Reproduction), IBD strongly interferes with reproduction, be it through its potential impact on fertility or because of issues related to the effect of the medication to treat IBD on the foetus, the latter typically being a female issue. These intuitive considerations have indeed been documented in a report of female IBD patients, who had greater concerns than men with regards to feelings about their bodies, attractiveness, feeling alone, and having children. However, reports dealing with gender roles in the determination of the HRQOL are scarce, and a detailed analysis of this phenomenon is still lacking.

The possibility of determining the sexual-dimorphic perception of HRQOL according to biological differences between the two sexes has not been given much attention in the scientific
literature. Yet, sex-related biological differences in several physiological processes can modulate gastrointestinal symptoms of active IBD as described previously. Some of these are in direct relation to IBD, such as the observation of worsening bowel symptoms during the premenstrual and menstrual phase of the menstrual cycle in patients with IBD and irritable bowel syndrome, respectively. Furthermore, considerable research has been devoted to studies of sex-differences in the perception of pain in general, and consistent findings show females are more sensitive than males to noxious stimuli. Although no such study has been performed specifically in an IBD patient population, in the context of these generally observed differences it seems plausible that the increased sensitivity of females to noxious stimuli, including visceral one, can be translated into a lower quality of life in female IBD patients compared with males. Finally, as discussed later, disease presentation, its natural course, and the responses to therapy differ between men and women with IBD, and these respective differences can contribute to the sex-specific perception of the impact the disease has on everyday life. Thus, women with IBD report consistently lower HRQOL than men. There are indications that this is owed to the gender-specific perception of disease complications, but biological differences between the two sexes are likely to play a role as well. A thorough analysis of the mechanism underlying this phenomenon is indispensable in order to develop gender/sex-specific interventions with the ultimate goal of improving the quality of life of IBD patients.

SEX

Because of the intestinal mucosal immune system’s aberrant response against autologous luminal flora, IBD belongs to the so-called immune-mediated or autoimmune diseases. The female predominance in autoimmune disorders has long been recognized, but the mechanism of this dimorphism is still unknown. Most studies dealing with the sexual dimorphism of immune responses and autoimmune features focus on ‘classic’ immune-mediated inflammatory diseases, i.e. systemic lupus erythematosus, multiple sclerosis, and rheumatoid arthritis. So far, no studies have been performed on this subject in IBD, although this chronic immune-mediated inflammatory condition affecting the gastrointestinal tract features clear sexual dimorphism with regards to female predominance in adults, disease phenotype with a higher prevalence of extra-intestinal manifestations in females, and disease behaviour, with a more complicated disease course and higher disease activity in females. Additionally, although not yet studied in depth, there are indications that females are more prone to experience immunogenicity of anti-TNF monoclonal antibodies, which may underlie the role of sex in immune reactions with an important impact on disease management.

Among the proposed mechanisms of the female-specific increased susceptibility to autoimmunity, two main pathways are implicated. Firstly, the influence of sex hormones on both innate and adaptive immune mechanisms have been studied and led to important insights into the modulation of the innate and adaptive immune responses through sex hormones.

However, this modulation cannot explain the whole spectrum of female autoimmune features, and probably only represents one part of the implicated pathologic mechanism. Secondly, our increasing understanding of the role of the X-chromosome in the sexual dimorphism of immune responses has turned the focus towards the mechanisms that would directly (i.e. not through the effect of sex hormones) involve X-chromosome anomalies in autoimmunity. Interestingly, one of the susceptibility loci for IBD was located on the X-chromosome, although this has not been shown in the genome-wide scan, possibly because the methodological specificities of gonosomal genetics were not taken into account. Furthermore, the strongest risk factor for IBD is a positive family history, which is subject to female imprinting, supporting the hypothesis of X-chromosome anomalies being involved in the pathogenesis of IBD.

Thus, there are indications that sex plays an important role in the aetio-pathogenesis of IBD. The underlying mechanism by which sex contributes to the specific disease presentation, prognosis, and therapy success remains to be unravelled. However, the extent of the problem must first be determined through well-designed epidemiological and observational studies in order to generate a hypothesis on the mechanisms of sex-mediated IBD aetio-pathogenesis.

REPRODUCTION

The importance of reproductive issues for chronically ill patients has long been underestimated and left out of both research and clinical practice agendas. That this essential need has been ignored in a life-long disease has led to the current situation in which most of the drugs used by chronically ill patients have insufficient data regarding the safety of their use during periconception and/or during pregnancy. Owing to the obvious ethical considerations, no drug enters the market approved for use by mothers- and fathers-to-be. The results of animal studies on teratogenicity generally have limited predictive value for the human situation. Human data on the safety of a particular drug in this setting are gathered with much difficulty through different registries, and are per definition obtained in a retrospective study design prone to selection and recall bias. Additionally, most of these studies are underpowered and suffer from the lack of a proper control population. The disease itself can have a great impact on all aspects of reproduction, resulting in a situation where neither healthy controls nor patients with the same disease but different phenotypes, which do not necessitate the use of a particular drug, can serve as a proper control. Thus, the patient with a reproductive wish who depends on the medication and the physician giving the pre-conceptional counselling are both left with a very limited body of evidence to enable them to make an informed decision.

IBD represents a typical example of this complicated situation. The peak incidence of the first presentation of IBD is in the third decade of life, and a quarter of the patients conceive for the first time after their diagnosis. Therefore, family planning for these young, chronically ill
patients often interferes with different therapeutic interventions, be it drugs or surgery, that are necessary to control the disease. Reproductive issues are of key concern to IBD patients. In this respect, it is important to note that IBD patients remain voluntarily childless more frequently than non-IBD controls. IBD patients refrain from having children because of concerns about the adverse reproductive outcome, fear of side-effects of the medication on the child, and medical advice given by physicians. This is not surprising considering the lack of prospective and controlled cohort studies which determines that the actual management of IBD patients with regard to fertility and pregnancy issues is based on expert opinion and a few case-control trials.

Reproduction in IBD patients is influenced by two overlapping factors; the disease itself, and the therapeutic interventions. The impact of the disease has been studied previously. The results of these studies are conflicting, ranging from an important impact of the disease on male and female fertility to (sub)normal fertility findings in this patient population. These discrepancies were thought to be because of the historical differences between the studied cohorts, with recent studies reflecting the use of novel therapeutics that would result in better disease control and unimpaired fertility. Moreover, keeping the disease in remission has been shown to be essential, not only for the success of conception, but also to ensure a favourable pregnancy outcome. Thus, IBD does not preclude male and female IBD patients having children, provided the disease is in remission, which again underscores the importance of the question of the safety of the medications necessary to control the disease in this setting.

In the past years, new therapeutic agents, i.e. monoclonal antibodies directed against human tumour-necrosis alpha (anti-TNF), were used to treat IBD. To date, two distinct anti-TNF agents have shown efficacy in the treatment of IBD: infliximab (IFX) and adalimumab (ADA). Because of the human specificities of these biologicals, it is not possible to do animal studies to evaluate their peri-conceptional safety or use during pregnancy. Human data on the use of IFX and ADA during pregnancy are limited to short-term observations, where IFX and ADA use during pregnancy seems to be safe for the child. However, concerns about placental transfer and an ensuing neonatal exposure to these agents post partum have triggered an active discussion about the proper management of IBD patients using anti-TNF agents during pregnancy. However, constructive discussion cannot take place in the absence of good quality clinical data and without careful interpretation of the immune processes of both the mother and child.

With regards to the safety of anti-TNF agents for fathers-to-be, data are limited to case series that analysed the effects of these agents on semen quality. The study by Mahadevan et al. in 10 patients using IFX showed a decreased sperm motility; however, in a study by Villiger in patients with ankylosing spondylarthritis using IFX, ADA, or etanercept compared with healthy controls, these defects in sperm quality were shown to also occur frequently in the general population. Thus, data on the effect of anti-TNF agents on semen quality are inconclusive. Furthermore, the translation of data on sperm motility and concentration into its quality with regards to DNA damage is only an approximation, and there is a great need for follow-up of children conceived by fathers who used anti-TNF agents. Favourable pregnancy outcomes with indirect exposure to IFX are reported, but no data are available on the use of adalimumab by future fathers.

In conclusion, choosing therapeutic strategies for IBD patients with reproductive wishes is difficult and guided by a compromise between disease activity and potential side-effects of the therapy on the embryo/foetus/newborn. Therefore, good quality data with prospective assessment of the outcomes of pregnancies and long-term patient follow-up together with an understanding of pharmacokinetics of particular drugs in this setting are of extreme importance to develop responsible therapeutic protocols for use during peri-conception and pregnancy.

**SUMMARY, AIM, AND OUTLINE OF THIS THESIS**

In conclusion, there is ample reason to assume that gender- and sex-specific issues, especially those related to procreation, are of great importance in both basic research and clinical care in IBD. There is sexual dimorphism in the perception of the disease, its incidence, presentation, phenotype, natural course, and in treatment success. These issues should be taken into account to optimise patient care. Importantly, however, hard data are lacking, hampering meaningful discussions on this subject. The current work was undertaken to fill this void.

In this thesis, the aim was to provide the grounds for a personalized approach to female and male IBD patients. More specifically, determining whether gender is really an important issue in IBD is explored by comparing the perception of quality of life in female patients versus male patients. The results show that female patients are significantly less capable of meeting life expectations compared with male patients, establishing that gender is an issue in IBD. These results described in part I of this thesis give ground for further mechanistic studies of the underlying sex-specific basis of gender-determined perceptions of IBD.

The sex-specific dimorphism of the disease aetio-pathogenesis and immune responses is delineated in part II of this thesis. In this part, we will establish that in familial Crohn’s disease the pathology manifests itself predominantly in females and involves epigenetic rather than classical genetic factors. Subsequently, we will show that once the disease manifests itself, the success of immune suppressive pharmacological intervention is sexually dimorphic: women are at higher risk of developing allergic complications to certain types of medication. The epigenetic factors leading to a female predominance in the familial presentation of Crohn’s disease may also result in more severe phenotypes, contributing to the
gender-specific differences in the perception of quality of life of IBD patients. In addition, more medication side effects will have a similar effect. Thus in toto, part I and part II of this thesis establish that gender is an issue in IBD and provides at least some insight into the sex-specific factors contributing to this effect.

Whenever gender is discussed, the issue of reproduction cannot be ignored. This is even more true in IBD, which typically involves young adults who might be expected to want to procreate. Thus, in part III of this thesis, this subject is explored and evidence is provided that reproductive wishes are indeed an issue in IBD patients and that this is an important factor guiding treatment strategies (Chapter 5). Subsequently, we will further investigate how use of immune suppressive medication by the mother influences the health of the child, both in utero (Chapter 7) and through lactation (Chapter 6). The effect of discontinuing biological therapies during pregnancy is investigated in Chapter 9. Finally, the effect on conception is investigated from the male perspective (Chapter 8); however, no significant effects were found, emphasizing the female-specific nature of the gender-related problems in IBD. Chapter 10 contains a comprehensive discussion of all issues related to anti-TNF use during pregnancy.

Summarizing, this thesis shows that a gender problem exists in IBD, that rational sex-dependent mechanistic explanations may be present, and describes the dilemma associated with pregnancy, especially for the mother, where the safety of continuing anti-TNF therapy is not yet assured. From the current investigation, it can be concluded that the time has come for a gender-specific approach in investigations of IBD and its clinical management.

REFERENCES


Part I - Gender
Differences in Health-Related Quality of Life between Men and Women with Inflammatory Bowel Disease

Zuzana Zelinkova, Lauran Vogelaar, Judith Baars, Tineke Markus, Ernst J Kuipers, Jan JV van Busschbach, C Janneke van der Woude

Submitted
ABSTRACT

Introduction
Clinical practice shows that gender-related issues in inflammatory bowel disease (IBD) are of great importance. Previous reports showed that female gender is an independent predictor of decreased quality of life. However, no data are available on the gender-specific perception of the respective factors influencing the disease-related quality of life.

Aims & methods
The aim of the study was to assess gender-related differences in the perception of respective factors influencing the quality of life in IBD patients. Between December 2006 and January 2007, a patient empowerment study has been performed as a patients-initiated study in cooperation with Dutch Patients’ Crohn’s and Colitis association (CCUVN). All patients willing to participate completed an online questionnaire consisting of multiple-choice questions on the CCUVN website. Differences between men and women in the perception of particular quality of life influencing factors were analysed statistically by chi-square test.

Results
In total 1067 (Crohn’s disease/ulcerative colitis, 617/450; 703 females/364 males, 66%/34%) replied. Significantly more females (F) than males (M) found that the disease limited them in everyday activities (53% F vs. 45% M, p=0.014). Only 9% of participating female patients vs. 19% of males almost never experienced disease-related limitations.

Significantly more females than males encountered serious limitations in professional life such as work (42% F vs. 33% M, p=0.015) and study (36% F vs. 19% M, p<0.001). Furthermore, the disease was limiting more females than males in family life (31% F vs. 21% M, p=0.002), social life (35% F vs. 23% M, p<0.001) and sport (44% F vs. 34% M, p=0.004). No differences in the impact of disease on these activities were observed with regards to the type of the disease.

Finally, as a result of the disease, significantly less female than male patients (44% and 55%, respectively; p=0.002) found that they were able to meet their daily expectations with regard to work or study, household, family and social activities.

Conclusion
Female inflammatory bowel disease patients experience more frequently the negative impact of the disease on the quality of life. Furthermore, there are important gender-related differences in the perception of particular factors influencing the quality of life.
PATIENTS AND METHODS

Between December 2006 and January 2007, a patient empowerment study has been performed as a patients-initiated study governed by Dutch Patients’ Crohn’s and Colitis association (CCUVN). The questionnaire was elaborated by a committee of patients. The part concerning quality of life consisted of two sets of questions dealing qualitatively (yes/no questions) and quantitatively (multiple choice questions with four categories) with the same quality of life areas. The quality of life areas/items were work and study, family, parenthood and hobbies/social activities. The quantitative questions included four degrees of experienced limitations – no limitations, hardly any limitations, serious limitations and extreme limitations. In order to analyse the gender-related differences in the perceptions of the limitations in different areas of life, the first two and the latter two degrees were pooled together.

The other parts of questionnaire concerned the individually reported compliance of patients (published previously by Baars et al Aliment Pharmacol Ther 2009) and the relationship with doctors.

The questionnaire was made public online on the CCUVN website and all patients willing to participate completed the survey online. The differences in reported quality of life restrictions between female and male patients were analysed by a chi-square test. This specific subanalysis has been performed with the agreement of the CCUVN.

RESULTS

In total 1067 (Crohn’s disease/ulcerative colitis, 617/450; 703 females/364 males, 66%/34%) replied. The baseline characteristics of the patient population are shown in Table 1.

First, the overall gender-related differences in the quality of life perception were analysed. Significantly more females (F) than males (M) found that the disease limited them in everyday activities in general (53% F vs. 45% M, p=0.014). Only 9% of participating female patients vs. 19% of males almost never experienced disease-related limitations.

Second, gender-related differences in the specific areas of the quality of life were assessed (Table 2). The question ‘If you think about everything you would like to do in your life, are there any things that you would like to do but the disease is limiting your ability to do it?’ was answered in a qualitative way per each item proposed in the questionnaire.

In general, significantly less female than male patients (44% and 55%, respectively; p=0.002) found that they were able to meet their expectations in everyday life as a result of the disease.
### Table 2. Percentages of male and female patients not able to satisfy their life expectations overall and with regard to specific quality of life areas. The question was formulated in the questionnaire as follows: ‘If you think about everything you would like to do in your life, are there any things that you would like to do but the disease is limiting your ability to do it?’

<table>
<thead>
<tr>
<th>Category</th>
<th>% of Female Patients</th>
<th>% of Male Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;No limitations&quot;</td>
<td>44*</td>
<td>55*</td>
</tr>
<tr>
<td>Travelling</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td>&quot;Outdoor&quot; activities</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Work</td>
<td>17*</td>
<td>10*</td>
</tr>
<tr>
<td>Shopping</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Parenthood</td>
<td>6*</td>
<td>0*</td>
</tr>
<tr>
<td>Social life</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Study</td>
<td>6*</td>
<td>1*</td>
</tr>
<tr>
<td>Going out</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Sport</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Energy for family/partner</td>
<td>4*</td>
<td>1*</td>
</tr>
<tr>
<td>Partnership</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Energy to continue</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Emigration</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hobby’s</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Household</td>
<td>1*</td>
<td>0*</td>
</tr>
<tr>
<td>Doing nice thinks</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Drink and eat</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Spontaneous activities</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Not be dependent on the toilets</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Read</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

*p<0.05

### Figure 1. Gender-stratified differences in the experienced limitations in professional life.
Significantly more females compared to males encountered serious limitations in professional life such as work (42% F vs. 33% M, p=0.015; Fig 1A) and study (36% F vs. 19% M, p<0.001; Fig 1B). Grey bar – no/hardly limited, black bar – very/extremely limited

![Figure 1A](image1a.png)  
![Figure 1B](image1b.png)

### Figure 2. Gender-stratified differences in the experienced limitations in personal life.
Significantly more females than males were limited by the disease in family life (31% F vs. 21% M, p=0.002; Fig 2A) and activities with partner (30% F vs. 21% M, p=0.007; Fig 2B). Grey bar – no/hardly limited, black bar – very/extremely limited

![Figure 2A](image2a.png)  
![Figure 2B](image2b.png)
Figure 3. Gender-stratified differences in the experienced limitations in hobbies. As a result of the disease, more females than males experienced limitations in sport (44% F vs. 34% M, p=0.004; Fig 3A), going out (37% F vs. 25% M, p=0.0001; Fig 3B) and going on holidays (39% F vs. 30% M, p=0.004; Fig 3C). Grey bar – no/hardly limited, black bar – very/extremely limited.

DISCUSSION

In this large patient-empowerment study, we found several gender-related differences in the perception of the respective domains that determine health-related quality in IBD patients. More women with IBD experience negative impact of the disease on their quality of life compared with men. Female-specific life domains in which they perceived more limitations were professional life, private life and hobbies. We found no differences between male and female patients with regard to the perception of physical restrictions.

In general, women report lower HRQOL regardless of the questionnaire used or underlying condition which is also seen in IBD patients18,19. Our observations are thus in line with this previously reported phenomenon in various areas of clinical medicine. The decreased HRQOL in women may result from gender-specific psychology in which the burden of the disease would be perceived more intensely by women. On the other hand, biological differences underlying a more complicated disease course in female IBD patients may contribute to the low HRQOL in women14-16. Considering the higher rates of disease-related symptoms in women than in men, as well as worse HRQOL perception in other diseases, our findings might be a logical consequence of both, psychological differences in the perception of disease burden as well as sex-specific disease biology17-19.

Despite the more complicated disease course of female patients, females did not experience more physical restrictions in our study but they did report more limitations in doing sports, going out and going on holidays. This can be explained by different level of health utility between males and females but can also originate from gender-specific social expectations.

Our observation of gender-related differences in the perception of various domains determining the health-related quality of life has important consequences for understanding of the measurements of quality of life in IBD patients. To date, the measurement of the respective quality of life domains is lacking. The classical health-related quality of life questionnaires which deal preferentially with perception of physical restrictions do not measure the domains investigated in our study. Once validated in a prospective manner, these observations would give ground for the development of a new gender-specific questionnaire.

Since the study was internet-based and the participants were CCUVN members, the possibility of selection bias cannot be excluded. Neither was it possible to correct the results for the disease activity. However, the large size of the study population allows us to assume that the homogeneity of active or severe disease distribution as well as psychological factors between genders has been conserved.

This patient-empowerment study shows patients’ perspectives without any direct influence by the researcher. New insight gained in this study on the gender-specific domains influencing HRQOL can help to generate efficacious tools for the fine-tuning of the treatment and monitoring of the IBD patients. More specifically, female patients might benefit more from the active attention paid by their caregivers for their daily limitations, including specific copying trainings. More research is needed in order to develop and validate gender-specific tools for the assessment of the health-related quality of life.

Differences in Health-Related Quality of Life between Men and Women with Inflammatory Bowel Disease
REFERENCES


Part II - Sex
Chapter 3

Maternal Imprinting and Female Predominance in Familial Crohn’s Disease

Zuzana Zelinkova, Pieter Stokkers, Klaas van der Linde, Ernst J Kuipers, Maikel P Peppelenbosch, C Janneke van der Woude

Accepted for publication in J Crohn’s Colitis
http://dx.doi.org/10.1016/j.crohns.2012.01.002
ABSTRACT

Background & Aim

Although the genetic risk factors for familial and sporadic inflammatory bowel disease (IBD) seem identical, the relative risk for contracting IBD in the familial setting is larger as that seen in the population at large, suggesting an important role of epi- and/or paragenetic factors in familial IBD. Epidemiological data indicate a female predominance in IBD, but how this relates to familial IBD has not been assessed.

Methods

Familial IBD patients (N=608) were compared with 415 sporadic IBD patients with regards to the patterns of sex and disease type distribution. The imprinting pattern in 87 families in which both a parent and a child had IBD was tested using Galton binominal statistics.

Results

The percentage of females in familial IBD population was significantly higher (61%; female/male ratio 1.5) compared with sporadic IBD (54%; female/male ratio 1.2; p=0.011). The analysis of offspring sex distribution pattern revealed significantly higher female to female transmission compared with female to male transmission rate (36 vs. 18, respectively; p=0.02). A significantly higher number of mother to child transmissions (55 vs. 32 of father to child transmissions) was observed (p=0.018). The female imprinting was specifically related to Crohn’s disease (31 vs. 14 mother vs. father to child transmissions, respectively; p=0.016).

Conclusion

We propose that a female sex-specific epigenetic inheritance pattern for Crohn's disease is a major contributing factor in the family-specific risk in Crohn’s disease. Sex-specific manifestation of familial Crohn's disease can partly explain the epidemiologically observed increased relative risk for females for contracting IBD.

INTRODUCTION

Among the major achievements in the field of genetically complex diseases has been the elucidation of the genetic risk factors contributing to the propensity for Crohn's disease and ulcerative colitis, recurrent gastrointestinal inflammatory disorders, with a still poorly understood etiology, that together constitute inflammatory bowel disease (IBD). The familial clustering of disease is highly suggestive of genetic factors playing role in the disease etiology and indeed especially in Crohn’s disease (CD) but also in ulcerative colitis (UC) a plethora of risk genes has been identified from large genome-wide association that seem to underlie the genetic basis of IBD. Nevertheless, although familial clustering of disease was an important reason to embark on GWAS of IBD to identify its genetic determinants, the frequency and nature of the risk alleles subsequently discovered is more or less similar in both familial and sporadic IBD. Thus the familial clustering of IBD seems only marginally dependent on genomic determinants, and by inference epigenetic and/or paragenetic factors must be important to explain the orders of magnitude in increased relative risk for contracting the disease in familial IBD versus sporadic IBD. The mechanistic basis of these factors remains obscure at best and identifying and understanding these, remains one of large outstanding questions in IBD research.

In the past years, it has become increasingly clear that gender is an important factor in both the clinical presentation of IBD and its subsequent response to therapy. There is a male predominance in pediatric population1 whilst an overall female predominance seen in IBD population start with the adolescent and adult onset of the disease2. Other sex-related biological differences are reflected in the disease phenotype, with female predominance in CD and more male patients in UC population and a sex-specific distribution of the different types of extra-intestinal manifestations3. The strength of the female predominance differs significantly depending on the cohort and can vary from equal sex distribution to female/male ratio of 2.5:14-12. Furthermore, some reports suggest a differential disease course, with a more complicated disease course in female patients13,14. Importantly, how these differences are related to familial disease versus sporadic disease dichotomy in IBD has not been investigated, but if detected could reveal important clues as to pathogenesis of familial IBD.

Some of the differences observed between male and female IBD fits well with a classical genetic interpretation, as recent reports have provided evidence for sex-specific risk variants for IBD. The R30Q DLG5 variant has been shown to confer differential risk for CD dependent on the sex in several independent populations15-17. Recently, a variant of IL-23 receptor, L310P, has been shown to protect from with ulcerative colitis in female but not in male population18. Furthermore, the two functional SNPs in the promoter region of IL-10 were found to be associated with ulcerative colitis only in female patients19. Thus some genetic variants seem only to contribute to IBD pathogenesis in the context of one particular gender. Although the effects detected are certainly too small to explain the female preponderance in epidemiology of IBD,
the detection of such sex-specific variants in especially IBD families indicates that a sex-specific manifestation of the disease may be important in familial IBD and call for further research to prove this notion.

The indications on one hand that gender may play a role as a modifier of the genetic predisposition for IBD, whereas the need for further data as to the nature of familial IBD prompted us to study the sex-specific inheritance pattern in the familial IBD patients’ population. First, we assessed the sex distribution pattern in IBD patients’ population with familial IBD compared with the baseline IBD population. Subsequently, the sex-related transmission pattern in the families with affected parent and child was studied. We detected a female sex-specific epigenetic inheritance pattern for CD that is a major contributing factor in the family-specific risk in IBD. Furthermore, this sex-specific manifestation of familial Crohn’s disease can largely explain the epidemiologically observed increased relative risk for females of contracting IBD observed in the population at large.

PATIENTS AND METHODS

Patients

IBD patients with family history of IBD were identified in the IBD research databases in two academic medical centres (AMC, Amsterdam; Erasmus MC, Rotterdam, The Netherlands). These research databases comprised all IBD patients under the follow-up at the outpatient clinic. Family history of IBD was considered as positive if first and/or second degree relatives of patients were affected by IBD. Family relationship, sex and the type of the disease (CD, UC) were retrieved from the databases and the sex distribution in the population of familial IBD was compared to the control IBD population. For the analysis of imprinting, families were selected where both parent and child were affected. Patients and their relatives participated in previously published inheritance studies20-22. Local medical ethical committee approval was obtained for these studies. Since the parent and child were affected. Patients and their relatives participated in previously published studies20-22. Local medical ethical committee approval was obtained for these studies. Since the parent and child were affected were identified. In total 92 families with parent–child pairs were found, of which in 5 families both parents were affected and these 5 families were excluded from further analysis. From 87 families analyzed, in 55 cases the mother was affected vs. 32 families with affected father (p=0.018), (Table 2, Fig. 1). In the subgroup analysis with stratification according to the disease which would make them not representative for the whole population. Thus specifically including thus the possibility that these patients represent a specific subgroup with a late onset disease which would make them not representative for the whole population. Thus specifically including thus the possibility that these patients represent a specific subgroup with a late onset-disease which would make them not representative for the whole population. Thus specifically including thus the possibility that these patients represent a specific subgroup with a late onset-

Statistical analysis

To compare the respective proportions of males and females in familial population with baseline population a chi-square test was used. Galton statistics using the two-tailed binomial distribution test with test proportion of 0.5 was used to analyze the imprinting pattern in the subset of the families in which both, parent and child had IBD.

RESULTS

Female preponderance in the risk for contracting IBD is strong in familial IBD and slight in the sporadic IBD manifestation

The control population comprised 415 IBD patients; 278 CD, 128 UC and 9 patients with unclassified IBD. There was a slight female predominance in the baseline population with female/male ratio 1.2. The control population was comprised of 608 IBD patients (363 patients with CD, 233 patients with UC and 12 patients with unclassified IBD) from 289 families with at least two family members affected with IBD. A higher percentage of CD patients was found in the control population compared to familial IBD population (respective 67% and 60%), this difference did not reach the statistical significance (p=0.05). The percentage of females in familial population was substantially and significantly higher compared with the control population, 61% (female/male ratio 1.5) vs. 54% (female/male ratio 1.2), p=0.028 (Table 1).

The definition of sporadic IBD is strongly dependent on the reliability of the family history. Once the possibility of documentation error is excluded by contacting the patients directly, still other factors can lead to wrong allocation of a patient as having the sporadic disease and include him/her erroneously in the control group. The most common of these factors are young age and/or short duration of the disease. Both these factors are placing the patient in the period of life where they or do not have children yet or not enough time has elapsed for other relatives to develop the disease. The inheritance studies using a control population with sporadic IBD disease are likely to suffer from this bias. We therefore performed a subanalysis of the differences of sex distribution between the familial IBD population and an arbitrary chosen subpopulation of the control population in which only patients older than 50 years were included. This subpopulation of 140 patients showed an underrepresentation of females, with female/male ratio of 0.9 which differed significantly from the sex distribution in familial IBD population with female/male ratio of 1.5 (p=0.006). To mean duration of the disease in this subpopulation of patients older than 50 years was significantly longer compared with the patients with sporadic disease younger than 50 years (19±5 SEM 1 vs. 11±0.5 years, p<0.001), excluding thus the possibility that these patients represent a specific subgroup with a late onset-disease which would make them not representative for the whole population. Thus specifically familial IBD is strongly skewed towards a female presentation of the disease and sporadic IBD much less so.

The female-specific manifestation of familial IBD is restricted to CD

Subsequently the sex-specific inheritance pattern was analyzed. The families where both, parent and child were affected were identified. In total 92 families with parent–child pairs were found, of which in 5 families both parents were affected and these 5 families were excluded from further analysis. From 87 families analyzed, in 55 cases the mother was affected vs. 32 families with affected father (p=0.018), (Table 2, Fig. 1). In the subgroup analysis with stratification according to the dis-
ease type (Table 2), this maternal imprinting was only present in the families where parent was affected with CD, with 31 families with affected mother vs. 14 families with affected father (p=0.016).

**Female specific transmission in familial CD**

An important question is whether the female sex specific manifestation of CD is only paragenetic (e.g. familial CD only occurs in presence female hormones), or also epigenetic (e.g. female specific transmission of risk via imprinted alleles). In the former case (paragenetic factors), the female bias in risk would be maintained over the generations even if the father transmits the disease. In case of the latter (e.g. an epigenetic factor imprinted through maternally-transmitted alleles), transmission of the disease via a father would not be associated with increased risk. For this analysis of the sex distribution among the affected offspring, two families with multiple affected offspring were excluded; the remaining 85 families were analyzed further. In 54 families with an affected mother, 36 of the affected offspring were females and 18 males (p=0.02), (Fig. 1). There was no sex-specific pattern of disease transmission in the subgroup of the families where the disease was transmitted from the father. In the subanalysis stratified according the disease type, this mother-to-daughter transmission of the disease was found only in the families where parent was affected with Crohn’s disease (Table 3). Thus a female specific imprinting in the inheritance of CD explains to large extent the female predominance in IBD and is a major contributor to the strongly increased risk in familial IBD when compared to sporadic IBD.

Table 1. Sex distribution in familial and baseline IBD patients’ population

<table>
<thead>
<tr>
<th></th>
<th>Familial IBD population</th>
<th>Baseline IBD population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nr of patients</td>
<td>608</td>
<td>415</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>363 (59.7%)</td>
<td>278 (59.6%)</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>233 (38.3%)</td>
<td>128 (37.4%)</td>
</tr>
<tr>
<td>Unclassified colitis</td>
<td>12 (2%)</td>
<td>9 (2%)</td>
</tr>
<tr>
<td>Female/Male (ratio)</td>
<td>369/239 (1.5)</td>
<td>223/192 (1.2)*</td>
</tr>
</tbody>
</table>

*p=0.028, chi square test

Table 2. Sex-specific inheritance

<table>
<thead>
<tr>
<th></th>
<th>Nr. of families</th>
<th>Mother to child</th>
<th>Father to child</th>
<th>Binomial test p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>87**</td>
<td>55</td>
<td>32</td>
<td>0.018</td>
</tr>
<tr>
<td>CD parent</td>
<td>45</td>
<td>31</td>
<td>14</td>
<td>0.016</td>
</tr>
<tr>
<td>UC parent</td>
<td>39</td>
<td>21</td>
<td>18</td>
<td>0.749</td>
</tr>
</tbody>
</table>

*binomial, two-tailed test p-value, test proportion 0.5
**three parents with unclassified colitis

Table 3. Sex distribution among affected offsprings

<table>
<thead>
<tr>
<th></th>
<th>Mother to child transmission</th>
<th>Father to child transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Nr of Families</td>
<td>54</td>
<td>31</td>
</tr>
<tr>
<td>- Female offspring</td>
<td>36</td>
<td>13</td>
</tr>
<tr>
<td>- Male offspring</td>
<td>18</td>
<td>18</td>
</tr>
</tbody>
</table>

*p=0.02*  *p=0.473*

<table>
<thead>
<tr>
<th>CD parent</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Female offspring</td>
<td>30</td>
<td>13</td>
</tr>
<tr>
<td>- Male offspring</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

*p=0.005*  *p=0.581*

<table>
<thead>
<tr>
<th>UC parent</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>- Female offspring</td>
<td>22</td>
<td>17</td>
</tr>
<tr>
<td>- Male offspring</td>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>

*p=0.832*  *p=1*

*binomial, two-tailed test p-value, test proportion 0.5

**Figure 1.** Sex-specific inheritance and the sex-distribution pattern among the offspring. In the families with parent and child affected, significantly higher transmission rate from the mother was observed (p=0.018*), (Figure 1A). Significantly higher percentage of affected female offspring was found in the families with maternal transmission whereas no sex-specific distribution pattern among the offsprings has been detected in the families with paternal transmission (Figure 1B; black bars – female offspring, grey bars – male offspring). *Galton binomial, two-tailed test, test proportion 0.5
DISCUSSION

In this study, we found that the female predominance characteristic for adult IBD population is a feature of familial IBD. In this familial IBD population with female predominance, we describe a specific pattern of inheritance in the families with parent and child affected with Crohn’s disease with significantly higher disease transmission rate from mothers. With respect to the sex of the offspring, this transmission is characterized by prevailing female sex among affected children.

Our findings are in line with previously reported female predominance in familial IBD and the phenomenon of increased risk of CD in offspring of mothers with CD in a non-Jewish familial IBD population. This sex-specific modification of genetic predisposition for IBD suggests the existence of genetic factors that are specifically translated into diseased phenotype in females. One of the possible genetic factors involved might be genomic imprinting, an epigenetic form of gene regulation in which only one member of the specific gene pair is expressed depending on whether it is inherited from the father or the mother. Genomic imprinting has been increasingly studied in the last years and has been suggested as etiological factor for several disorders with sex-specific distribution. It is tempting to speculate that this factor might play a role in the female imprinting observed in IBD familial population.

The possible genetic factors are the transmission of genetic variants present in mitochondrial DNA and X-chromosome. Although, in case of disease transmission through variants in mitochondrial DNA an equal distribution of the disease among both sexes of the offspring would be expected, this factor cannot fully be excluded if also paragenetic factors contribute. To address this possibility, multi-generation families would have to be analysed in order to detect the expected stop of the disease transmission through male intermediates. Thus, we cannot exclude the possibility of the transmission through genetic variants located in mitochondrial DNA and mitochondrial DNA analysis has not been studied yet in this context in IBD populations. Concerning the transmission through variant(s) located on X-chromosome, a susceptibility locus on X-chromosome has been found in a linkage analysis study with basic population of familial IBD but the genome-wide meta-analysis did not find susceptibility loci on this chromosome.

Although apparent absence of male transmission in familial CD fits ill with an important role for paragenetic factors in the risk of contracting familial IBD, there is still a slight bias towards female disease in our non-familial CD population. Although undetected familial cases will certainly contribute to this basis, in context of epidemiological data showing a male predominance in pediatric population with the onset of the disease prior to puberty one can speculate about the contributive role of female sex hormones as risk factor for females with inherited predisposition to develop IBD. Both, endogenous female sex hormones which cyclic changes starting around puberty, as well as exogenous female sex hormones through oral contraceptives (OCs) might play a role. Discrimination between these two factors based on epidemiological data is difficult as the onset of endogenous hormonal activity in Western society is often related to start of sexual life and with it associated use of the OCs. Interestingly, OCs have been proposed as a risk factor for the development of CD, although this risk seems to be modest with results of the meta-analysis by Godet et al. showing the pooled relative risk after adjusting for smoking of 1.44 (CI 1.12 – 1.86). Nevertheless, the contribution of these effects towards the increased risk for females for contracting IBD in patient population in toto, seems minor in comparison to the risk conferred to female specific transmission in especially familial CD.

In conclusion, we observed female predominance as a feature characteristic for familial inflammatory bowel disease. In CD, the female predominance may be related to the imprinting of the disease predisposition with a specific female to female transmission pattern. This transmission pattern suggests existence of female-specific modifier of genetic predisposition for the disease. The epigenetic female transmission of CD is a major contributing factor to the familial presentation of disease and explains to a large extent the epidemiologically-detected female bias in the risk for contracting IBD.
REFERENCES


Chapter 4

Adverse Drug Reactions to Anti-Tumor Necrosis Factor Agents Differ between Male and Female Inflammatory Bowel Disease Patients

Zuzana Zelinkova, Evelien Bultman, Lauran Vogelaar, Cheima Bouziane, Ernst J Kuipers, C Janneke van der Woude

Submitted
ABSTRACT

Background
Limited data are available on the sex-specific adverse drug reactions (ADR) to immunosuppressive medication. In this study we analyzed sex differences in the incidence of ADR to the immunosuppressive medication in inflammatory bowel disease (IBD) patients.

Methods
The electronic medical records of IBD patients were reviewed, the reported ADR to immune suppressive drugs used for IBD were noted and the sex differences in ADR were analyzed.

Results
In total, 843 IBD patients were included. No differences between males and females were observed with regard to specific ADR to thiopurines, methotrexate and cyclosporine but significantly more females (54 patients, 39% of all female users of anti-TNF) than males (23 patients, 23% of all male users of anti-TNF) experienced ADR to one or more anti-TNF agents (p=0.011, OR 2.2, 95% CI 1.2-3.8). The most frequent ADR were allergic reactions. As a result of ADR, 36 patients (15% of all patients using anti-TNF) stopped the treatment, with significantly higher stopping rate among females (27 females, 19% vs. 9 males, 9%, p=0.024).

Conclusion
Treatment with anti-TNF antibodies is accompanied by sex dimorphic profile of adverse drug reactions with female patients being more at risk for allergic reactions and subsequent discontinuation of the treatment.

INTRODUCTION

The existence of a sex dimorphic profile of adverse drug reactions (ADR) has been increasingly recognized in recent years. Several studies on various therapeutics pointed to differences between sexes in the incidence as well as character of ADR. In general, females seem to be more at risk for ADR to various medication, 70% of drug users with ADR in a large cohort of 2367 patients being women. In addition to this generally increased risk of ADR, female patients also differ from males in terms of types of ADR to a range of medication such as antiarrhythmics, antipsychotics, anti-retroviral drugs, and analgesics.

A limited number of small size studies performed in the field of auto-immune diseases and transplant medicine suggested existence of a sexual dimorphic profile of ADR to immunosuppressive medication, but this has not been studied in depth. Male sex has been reported as a risk factor for nodular regenerative hyperplasia in inflammatory bowel disease (IBD) patients treated with azathioprine. In rheumatoid arthritis patients’ population, males have been shown to be more at risk than females for methotrexate-associated interstitial pneumonia and to bacterial pneumonia complicating treatment with infliximab. For females, a higher incidence of azathioprine-related alopecia has been reported in transplant recipients.

In a pediatric population of Crohn’s disease (CD) patients, female sex was one of the risk factors for infusion reactions to anti-tumor necrosis factor (anti-TNF) treatment and in an adult population of ankylosing spondylitis patients, females were more at risk of discontinuation of anti-TNF agents.

The sexual dimorphism of the immune responses is a generally accepted concept that has been studied predominantly in the context of the female predominance in autoimmune disorders with the most important factor determining this dimorphism being the immunomodulatory properties of sex hormones. Considering these differences between the two sexes in basic immune reactions, further modulation of immune response by the immunosuppressive medication might have sex-specific consequences, including the quantitative and qualitative differences in ADR to these agents.

The limitations resulting from ADR for further therapeutic strategy in patients with chronic inflammatory conditions such as inflammatory bowel diseases (IBD) are important. The ADR occurring in up to 20% of IBD patients using immune suppressive and anti-TNF agents represent an important factor leading to the modulation or discontinuation of effective treatment. The thorough understanding of the underlying mechanism of ADR to these drugs, including the sex-related differences would help optimizing the therapy in these patients.

Therefore, in the present study we aimed to specifically determine the difference between male and female IBD patients in the occurrence and type of ADR to commonly used
immunosuppressive agents, including ‘classical’ immunosuppression, i.e. thiopurines and methotrexate, as well as anti-TNF agents. In this large retrospective study, we found a sex dimorphic profile of ADR to anti-TNF agents with females being at increased risk for allergic reactions compared with males limiting thus the long-term use of anti-TNF in substantial proportion of female IBD patients.

PATIENTS AND METHODS

Patients

IBD patients attending the outpatient clinic of the Department of Gastroenterology and Hepatology of the Erasmus MC were identified through the electronic diagnosis registration system. The medical records were reviewed with emphasis on details of drug treatment. Reported ADR to immunosuppressive and anti-TNF agents used for IBD were noted. Patients for whom the required information on drug use and potential side effects was not available in the electronic medical record and patients with only one registered contact and no further follow-up at the outpatient clinic were excluded.

Definition of ADR

All ADRs designated as such by the treating physician in the medical record were registered. The general definition of ADR used in clinical practice comprised the occurrence of the ADR in the temporal relationship with its disappearance upon discontinuation of the medication. In case of doubt about other concomitant factors contributing to the ADR a positive re-challenge was considered to be necessary for the event to be definitely categorized as ADR.

The ADR to immunosuppressive agents were divided into the following categories according to the type of symptom/event: gastro-intestinal, arthralgia and/or myalgia, cutaneous, infectious, malignancy, myelosuppression, hepatotoxicity, or pancreatitis. In case of anti-TNF agents, additional categories of allergic reactions, lupus-like syndrome, and injection-site reactions were used.

Gastro-intestinal ADR comprised abdominal pain recognized by the patient as different from the IBD-related pain, diarrhea, nausea, and vomiting. Cutaneous ADR were defined as any kind of reported skin abnormality that occurred in temporal relationship with the treatment and resolved after cessation of the medication. Remittent or opportunistic infections occurring during the immunosuppressive treatment were noted as infectious ADR. For malignancies, any malignancy that was revealed during the use of the treatment was categorized as ADR.

Myelosuppression was defined as leucopenia (leucocytes count<4.0x10^9/L), and/or anemia and/or thrombocytopenia (thrombocytes count<150x10^9/L). To categorize abnormal liver tests as hepatotoxic ADR, the increase of liver tests above 2 times upper normal value and absence of other causes, i.e. viral or autoimmune were required. Drug-induced pancreatitis was defined as a new abdominal pain and hyperamylasemia occurring during the treatment.

Any of the following symptoms occurring during or within one day after infusion alone or in combination were considered as allergic reactions: skin reactions, dyspnoe, chest pain, low blood pressure, angiooedema, fever and/or chills. Dyspnoe, skin abnormalities and arthralgia/myalgia occurring later than two days after infusion were categorized separately as potential delayed allergic reactions. Any motoric or sensoric loss, paresthesia and/or seizures were categorized as neurological ADRs. Lupus-like syndrome diagnosis was characterized as the combination of arthritis and/or flu-like symptoms or fever and presence of anti-nuclear and or anti-doublestrand DNA antibodies. Injection site reactions (applicable for adalimumab) were defined as pain or local skin reaction after injection.

Non-specific ADR which could not be categorized according to these criteria were analyzed together and are further referred as others.

Statistical analysis

The sex-related differences in categorical variable were analyzed statistically using two-sided chi-square testing, for continuous variables a two-sided independent t-test was used. P-values <0.05 were considered significant. The analysis was performed using SPSS PASW 17 software.

RESULTS

Demographic characteristics and the use of medication

In total, 843 patients were eligible for analysis, 386 males (46%), mean age 42 yrs (range 16-87) with a mean duration of the disease of 14 years (range 0-54); 578 patients with Crohn’s disease, 244 with ulcerative colitis (UC) and 21 with unclassified colitis. There were no differences between male and female patients with regard to age and disease duration; significantly more males suffered from ulcerative colitis (141 pts, 58% of all ulcerative colitis patients).

Seventy percent (586 pts) of patients used any kind of immunosuppressive agents during the disease course, the majority of the patients (546 pts, 65%) used thiopurines, 176 pts (21%) methotrexate, 46 pts (5%) cyclosporine and one patient tacrolimus. No differences between male and female patients were observed with regard to the frequency of use of immunosuppressive agents in general or of particular agent.
One third (240 pts, 28%) of patients were treated with anti-TNF, the majority of patients (227 pts, 27%) used infliximab, 96 (11%) adalimumab and five patients certolizumab. There were no sex-related differences in the use of anti-TNF agents (Table 1).

Adverse drug reactions to immune suppressive agents

In total 278 patients experienced ADR to immunosuppressive agents; of which 155 patients to thiopurines (28% of all thiopurine-treated patients), 44 to methotrexate (25%), 2 (4%) to cyclosporine, and 77 pts (32%) developed ADR to one or more anti-TNF agents.

Overall, there were no significant differences in the frequencies of the ADR to immune suppressive agents between males and females. In total, 27% of males (71 pts) experienced ADR to any kind of immune suppressive agent compared to 34% of females (108 pts; p=0.087), (Figure 1A).

Among thiopurines users, 26% of males (63 pts) and 31% of females (92 pts) suffered from ADR to thiopurines (p=0.183), (Figure 1B). The most frequent ADR to thiopurines were myelosuppression, hepatotoxicity (both in 33 pts, 6%) and gastro-intestinal ADR (23 pts, 4%), (Table 2). No differences between male and female patients were observed with regard to specific type of ADR to thiopurines.

In the group of patients treated with methotrexate, 27% (20 pts) of males and 23% (24 pts) of females experienced ADR (p=0.597), (Figure 1B). The most frequent ADR to methotrexate were hepatotoxicity and gastro-intestinal ADR (both in 10 pts, 6%), (Table 2).

Out of 46 pts treated with cyclosporine, two female patients experienced an ADR, one developed pseudo-membraneous colitis following treatment but she was also treated with systemic steroids. The second patient had a cutaneous reaction to cyclosporine.

Among 77 pts who developed ADR to one or more anti-TNF agents, significantly more females (54 pts, 39% of all anti-TNF treated women) than males (23 pts, 23% of all anti-TNF treated men) experienced ADR to an anti-TNF agent (p=0.011; OR 2.2, 95% CI 1.2-3.8), (Figure 2A). In the subanalysis of respective anti-TNF agents, significantly more females (51 pts, 38% of all IFX-treated women) than males (20 pts, 22% of all IFX-treated men) suffered from ADR to infliximab (p=0.009; OR 2.2, 95% CI 1.2-4.1). Relatively more females than males experienced ADR to adalimumab (16 females; 28% vs. 6 males; 15%), this difference was not significant though (p=0.216).

The most frequent ADR to both infliximab and adalimumab were allergic reactions (15% of all infliximab users and 7% of all patients treated with adalimumab) and for both agents a significantly higher rate of allergic reactions in females compared with males was observed (Table 3). There were no other sex-specific ADR observed to anti-TNF agents.

Of 77 patients experiencing ADR to an anti-TNF agent, 36 pts stopped the treatment (47%; overall discontinuation rate 15%), 27 pts (35%) switched to another anti-TNF agent, and 14 pts (18%) continued the treatment. As a result of ADR, 27 females (19% of all antiTNF-treated women) stopped the treatment compared with 9 males (9% of all antiTNF-treated men; p=0.024). Furthermore, significantly higher proportion of females (21 pts, 15% of all antiTNF-treated women) switched to another antiTNF agents compared with males (6 pts, 6% of all antiTNF-treated men; p=0.026) significantly more frequently than males.
Figure 1. Relative percentages of male and female patients experiencing adverse drug reactions to immunosuppressive agents in general (Figure 1A); to respective immunosuppressive agents (Figure 1B). Black bars – adverse drug reactions present, gray bars – no adverse drug reactions.

* two-sided chi-square test

Figure 2. Relative percentages of male and female patients experiencing adverse drug reactions to anti-TNF agents in general (Figure 2A) and stratified by respective anti-TNF agent (Figure 2B). Black bars – adverse drug reactions present, gray bars – no adverse drug reactions.

* two-sided chi-square test

Table 1. Demographic characteristics and the use of medication

<table>
<thead>
<tr>
<th></th>
<th>Males*</th>
<th>Females*</th>
<th>P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males/Females (% males)</td>
<td>386 (46%)</td>
<td>457</td>
<td></td>
</tr>
<tr>
<td>Age (mean, range)</td>
<td>43yrs (16-87)</td>
<td>42yrs (18-87)</td>
<td>p=0.138</td>
</tr>
<tr>
<td>Duration of the disease (mean, range)</td>
<td>14yrs (0-48)</td>
<td>14yrs (0-54)</td>
<td>p=0.168</td>
</tr>
<tr>
<td>CD/UC/Unclassified</td>
<td>233 (40%)/141 (58%)/12 (57%)</td>
<td>345/103/9</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Immunosuppressive agents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- thiopurines</td>
<td>265 (45%)</td>
<td>321</td>
<td>p=0.652</td>
</tr>
<tr>
<td>- methotrexate</td>
<td>247 (45%)</td>
<td>299</td>
<td>p=0.665</td>
</tr>
<tr>
<td>- cyclosporine</td>
<td>73 (42%)</td>
<td>103</td>
<td>p=0.203</td>
</tr>
<tr>
<td>- tacrolimus</td>
<td>26 (57%)</td>
<td>20</td>
<td>p=0.170</td>
</tr>
<tr>
<td>- Anti-TNF</td>
<td>73%</td>
<td>78%</td>
<td>p=0.097*</td>
</tr>
<tr>
<td>- infliximab</td>
<td>101 (42%)</td>
<td>139</td>
<td>p=0.193</td>
</tr>
<tr>
<td>- adalimumab</td>
<td>93 (41%)</td>
<td>134</td>
<td>p=0.102</td>
</tr>
<tr>
<td>- certolizumab</td>
<td>39 (41%)</td>
<td>60</td>
<td>p=0.328</td>
</tr>
<tr>
<td>- Others*</td>
<td>1 (20%)</td>
<td>4</td>
<td>p=0.401</td>
</tr>
</tbody>
</table>

* for each categorical variable number and percentage of males within the group is displayed
** p values for test of the sex-related differences; two-sided chi-square test for categorical variables and t-test for continuous variables

Table 2. Categories of adverse drug reactions to immunosuppressive agents, in general and stratified by sex

<table>
<thead>
<tr>
<th></th>
<th>Thiopurines N, % of all patients treated with thiopurines</th>
<th>Thiopurines N of males (%)**</th>
<th>Methotrexate N, % of all patients treated with methotrexate</th>
<th>Methotrexate N of males (%)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myelosupression</td>
<td>33 (6%)</td>
<td>12 (36%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Hepatotoxicity</td>
<td>33 (6%)</td>
<td>19 (58%)</td>
<td>10 (6%)</td>
<td>6 (60%)</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>10 (2%)</td>
<td>2 (20%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Gastro-intestinal side-effects</td>
<td>23 (4%)</td>
<td>8 (35%)</td>
<td>10 (6%)</td>
<td>6 (60%)</td>
</tr>
<tr>
<td>Arthralgia and/or myalgia</td>
<td>11 (2%)</td>
<td>5 (45%)</td>
<td>2 (1%)</td>
<td>1 (50%)</td>
</tr>
<tr>
<td>Cutaneous side-effects</td>
<td>10 (2%)</td>
<td>4 (40%)</td>
<td>3 (2%)</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>Infectious</td>
<td>6 (1%)</td>
<td>3 (50%)</td>
<td>2 (1%)</td>
<td>1 (50%)</td>
</tr>
<tr>
<td>Others*</td>
<td>23 (4%)</td>
<td>7 (30%)</td>
<td>13 (7%)</td>
<td>2 (15%)</td>
</tr>
<tr>
<td>Not specified</td>
<td>6 (1%)</td>
<td>3 (50%)</td>
<td>4 (2%)</td>
<td>1 (25%)</td>
</tr>
</tbody>
</table>

* headache, paresthesia, hair loss, fatigue, emotional instability, malaise; specific for methotrexate – injection site reaction
** no significant difference observed between males and females for none of the ADRs as tested with two-sided chi-square test
Table 3. Types of adverse drug reactions to respective anti-TNF agents, in general and stratified by sex

<table>
<thead>
<tr>
<th>Adverse Reaction</th>
<th>Infliximab N % of all patients treated with infliximab</th>
<th>Infliximab N of males (%)</th>
<th>P value*</th>
<th>Adalimumab N % of all patients treated with adalimumab</th>
<th>Adalimumab N of males (%)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic reaction</td>
<td>33 (15%)</td>
<td>7 (21%)</td>
<td>p=0.045</td>
<td>7 (7%)</td>
<td>0</td>
<td>p=0.039</td>
</tr>
<tr>
<td>Cutaneous SE</td>
<td>9 (4%)</td>
<td>5 (56%)</td>
<td>p=0.492</td>
<td>3 (3%)</td>
<td>3 (100%)</td>
<td>p=0.064</td>
</tr>
<tr>
<td>Neurological SE</td>
<td>4 (1.8%)</td>
<td>0</td>
<td>p=0.146</td>
<td>2 (2%)</td>
<td>0</td>
<td>p=0.512</td>
</tr>
<tr>
<td>Dyspnoe</td>
<td>4 (1.8%)</td>
<td>0</td>
<td>p=0.146</td>
<td>1 (1%)</td>
<td>0</td>
<td>p=1.00</td>
</tr>
<tr>
<td>Arthralgia and/or myalgia</td>
<td>11 (5%)</td>
<td>4 (45%)</td>
<td>p=1.00</td>
<td>3 (3%)</td>
<td>1 (33%)</td>
<td>p=1.00</td>
</tr>
<tr>
<td>Injection site reactions**</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
<td>3 (3%)</td>
<td>1 (33%)</td>
<td>p=1.00</td>
</tr>
<tr>
<td>Infectious SE</td>
<td>0</td>
<td>0</td>
<td></td>
<td>3 (3%)</td>
<td>1 (33%)</td>
<td>p=1.00</td>
</tr>
<tr>
<td>Malignancy</td>
<td>1 (0.4%)</td>
<td>1 (100%)</td>
<td>p=0.41</td>
<td>0</td>
<td>1 (33%)</td>
<td>p=1.00</td>
</tr>
<tr>
<td>Lupus-like</td>
<td>1 (0.4%)</td>
<td>0</td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Others***</td>
<td>8 (3.5%)</td>
<td>2 (25%)</td>
<td>p=0.476</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* two-sided chi-square test to compare the differences in the frequencies of specific adverse drug reaction between males and females
** in case of adalimumab
*** abdominal pain, hair loss, fatigue, malaise, emotional instability

DISCUSSION

In this large retrospective study, we studied sex differences in the frequency and type of adverse drug reactions to immune suppressive medication in inflammatory bowel disease patients. In contrast to thiopurines and methotrexate with similar rates of ADR in both sexes, we observed a sex dimorphic profile of adverse drug reactions to anti-TNF agents with higher frequency of ADR among female IBD patients compared with male patients. With regard to particular types of ADR, females experienced more often allergic reactions to monoclonal anti-TNF antibodies.

For each drug group with sex dimorphic ADR profile specific considerations for underlying mechanisms are applicable. The basic pharmacokinetic differences between the genders were at first considered to cause the predominance of ADR to some drug in females. Over the past years, it became evident that sex hormones, interacting with the particular drug’s metabolism and mechanisms of action, might also account for the sex differences in ADR to immune-modulating therapy. However, in the present study, only biological anti-TNF agents and no other immunosuppressive treatment showed a specific ADR sex dimorphic profile. In addition, suggesting a female-specific immunogenic potential related to biological therapy, ADR to monoclonal anti-TNF antibodies have been shown to be related to the development of antibodies against these agents. Anti-infliximab as well anti-adalimumab antibodies are a frequent problem in daily clinical practice.

ADR to monoclonal anti-TNF antibodies have been shown to be related to the development of antibodies against these agents. Anti-infliximab as well anti-adalimumab antibodies are...
found in the sera of patients with loss of response and/or adverse drug reactions to IFX or ADA\textsuperscript{19-23}, suggesting thus the humoral immune response as underlying mechanism of IFX- and ADA-related immunogenicity. Interestingly, some studies analyzing the sex differences in the humoral response to vaccinations showed higher antibody titers in females\textsuperscript{24, 25} and more local and systemic adverse reactions to influenza and rubella vaccines have been observed in women compared with men\textsuperscript{26-27}. The underlying mechanism of these sex differences in humoral immune response in general is not elucidated thus far, but taken all these observational data together, it is tempting to speculate that the use of monoclonal antibodies against TNF-alpha might result in higher anti-antiTNF antibodies formation rate in female patients which in turn would lead to the female-specific higher incidence of immune-mediated ADR.

This immunogenic potential of anti-TNF agents resulting in ADR has important consequences for the management of IBD patients using these drugs. In this study, up to 50% of patients experiencing ADR discontinued the treatment, with the overall discontinuation rate of all patients using anti-TNF agents being 15%. There are thus considerable potential limitations of long term use of these otherwise effective drugs. Understanding the underlying mechanisms, one of which might be female-specific immunogenicity would subsequently help to identify patients at risk and modify the therapeutic strategy accordingly.

To our knowledge, this is the first report studying the sex differences in ADR profile to immunosuppressive medication in a large cohort of patients with immune-mediated disease. The main limitation of our study is its retrospective design in which reporting bias of ADR is inevitable due to the lack of a standardized protocol that would ensure a meticulous screening of every treated patient in a prospective study design. This might particularly affect a study dealing with sex-related differences due to the psychosocial specificities of the two sexes in reporting ADRs. On the other hand, we analyzed sex-related ADRs profile to several immunosuppressive drugs in this large cohort. We found the sex dimorphism specifically applying only for anti-TNF agents and no other medication which would be the case if this reporting bias ensuing from retrospective design was substantially to modify the findings.

In conclusion, female inflammatory bowel disease patients are at increased risk of hypersensitivity reactions to anti-TNF agents compared with males. These adverse drug reactions have important clinical consequences as they lead to the discontinuation of the treatment in half of the patients experiencing these reactions. Further research, with a specific consideration of sex dimorphism of the immunogenic potential of anti-TNF agents is warranted in order to improve the clinical management of patients at risk for adverse drug reactions.

REFERENCES

Part III - Reproduction
Reproductive Wish Represents an Important Factor Influencing Therapeutic Strategy in Inflammatory Bowel Diseases

Zuzana Zelinkova, Peter BF Mensink, Jan Dees, Ernst J Kuipers, C Janneke van der Woude

Scand J Gastroenterol 2010;45:46-50
ABSTRACT

Background
Inflammatory bowel diseases (IBD) affect patients in reproductive age but little is known about the peri-conceptional use of medication for IBD.

Aim
To assess the type of medication used by IBD patients with reproductive wish and changes of the medication in the peri-conceptional period.

Methods
IBD patients with active conception plans and pregnant patients were prospectively recruited from the outpatient clinic of one academic medical center. IBD-related medication and changes of this medication for reasons of the conception wish or pregnancy were analyzed.

Results
In total 61 patients (51 females; 40 Crohn’s disease, 21 ulcerative colitis) were included. Thirteen (21%) patients used no medication, 44 (72%) used monotherapy and 4 (7%) patients used combination treatment. From patients on monotherapy, 11 (19%) patients used 5-aminosalicylates, 5 (9%) steroids, 11 (19%) thiopurines, 5 (9%) methotrexate and 11 (19%) used anti-TNF agents.

Thirty-seven patients (61%) consulted the physician prior to conception. One third of these patients required change of the medication due to the conception plans.

Conclusions
Two-thirds of patients with active reproductive wish use medication of uncertain peri-conceptional safety profile. The reproductive wish leads to a change in medication in one third of these patients, influencing thus substantially the therapeutic strategy.

INTRODUCTION

Inflammatory bowel diseases (IBD) typically affect patients in their reproductive years. It has been shown that reproductive issues are of key concern to IBD patients, especially women. In this respect, it is important to note that IBD patients remain voluntary childless more frequently than non-IBD controls. A recent study reported that IBD patients refrain from having children due to the concerns about the adverse reproductive outcome. Fear of side-effects of the medication on the child and medical advice given by physicians, were the most important reasons for voluntary childlessness in this study.

Previous studies primarily focused on the determination of factors influencing the pregnancy outcomes. Some IBD therapeutics have been shown to be related to adverse pregnancy outcomes, but these data are often retrospective and based on limited patient numbers or, as it is the case of biologicals, without long-term follow-up. The risk for complications during pregnancy seems to be primarily related to disease activity and not to the specific medication. The medical advice for IBD patients with active reproductive wish is therefore guided by a difficult compromise between the risks of active disease versus potential adverse effects of the therapy on the pregnancy outcome.

In the present study, we aimed to determine the actual clinical extent of this problem, i.e. how many patients with active reproductive plans need medication that is either contra-indicated or has an unclear safety profile for use during the peri-conceptional period. For this purpose, we prospectively assessed the medication used by the patients with active conception wish recruited from our outpatient clinic. In addition, we analyzed the medication changes resulting from the conception wish or pregnancy of IBD patients.

PATIENTS AND METHODS

Between April 2007 and April 2009, all consecutive IBD patients with conception plans or pregnancy ongoing during the study period were recruited from the IBD outpatient clinic of the Department of Gastroenterology and Hepatology, Erasmus Medical Center, Rotterdam, the Netherlands. The medication used in the peri-conceptional period and changes in the medication for reasons of the conception plans and pregnancy were noted at each outpatient clinic consultation. In case of doubts the patient and/or the physician were contacted personally to specify the reason for the change of medication.
RESULTS

General

In total 61 IBD patients with active reproductive wish were included. The basic demographic characteristics, the type and localization of inflammatory bowel disease are shown in Table 1. Of all included patients, 23 (38%) patients consulted the physician after the conception, 38 patients were included in the pre-conceptional period.

Medication use

Only 13 (23%) patients used no medication during conception. Forty-four (72%) patients were using monotherapy and 4 patients were on combination therapy (all on infliximab with either azathioprine (2 patients), prednisone (1 patient), or mesalazine (1 patient)). Of the patients on monotherapy, 11 (19%) were using 5-aminosalicylates, 11 (19%) thiopurines, 3 (5%) systemic steroids, 2 (4%) budesonide, 11 (19%) anti-TNF agents (4 infliximab and 7 adalimumab), 5 (9%) methotrexate and one patient was treated by a trial medication (Figure 1). The respective use of medication by female and male patients is shown in Table 2 and 3, together with the designated FDA categories.

Changes in the medication

For reasons of the pregnancy or active reproductive wish, 15 patients (25%) changed their medication. Eleven patients, 30% of all patients consulting the physician prior the conception, changed their medication because advised so by their gastroenterologists. The patients were advised to postpone (2 patients) or stop methotrexate (4 patients), switch methotrexate to anti-TNF agent (1 patient), stop budesonide (2 patients), and stop anti-TNF (1 patient); one patient was withdrawn from the trial because of the pregnancy. One patient was asked to temporary stop the treatment with 6-mercaptopurine by the fertility specialist prior the semen collection for in vitro fertilization. Three patients stopped their medication when they became pregnant, without consulting the physician (two patients were using azathioprine and one mesalazine). After having consulted the gastroenterologist, both patients who were previously using azathioprine restarted the treatment.

<table>
<thead>
<tr>
<th>Type of Medication</th>
<th>Nr of patients (%)</th>
<th>FDA category</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No medication</strong></td>
<td>13 (25%)</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Mono-therapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-aminosalicylates</td>
<td>9 (17%)</td>
<td>B</td>
</tr>
<tr>
<td>systemic steroids</td>
<td>2 (4%)</td>
<td>C</td>
</tr>
<tr>
<td>budesonide</td>
<td>2 (4%)</td>
<td>C</td>
</tr>
<tr>
<td>thiopurines</td>
<td>10 (20%)</td>
<td>D</td>
</tr>
<tr>
<td>methotrexate</td>
<td>3 (6%)</td>
<td>X</td>
</tr>
<tr>
<td>anti-TNF (infliximab/adalimumab)</td>
<td>9 (3/6)</td>
<td>B</td>
</tr>
<tr>
<td>study medication</td>
<td>1 (2%)</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Combination therapy</strong></td>
<td>2 (4%)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*one patient infliximab and azathioprine; one patient azathioprine and mesalazine
### Table 3. Medication of male IBD patients with active conception plans

<table>
<thead>
<tr>
<th>Type of Medication</th>
<th>Nr of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>No medication</td>
<td>0</td>
</tr>
<tr>
<td>Monotherapy</td>
<td>8</td>
</tr>
<tr>
<td>5-aminosalicylates</td>
<td>1</td>
</tr>
<tr>
<td>systemic steroids</td>
<td>1</td>
</tr>
<tr>
<td>budesonide</td>
<td>0</td>
</tr>
<tr>
<td>thiopurines</td>
<td>2</td>
</tr>
<tr>
<td>methotrexate</td>
<td>2</td>
</tr>
<tr>
<td>anti-TNF (infliximab/adalimumab)</td>
<td>2 (1/1)</td>
</tr>
<tr>
<td>Combination therapy*</td>
<td>2</td>
</tr>
</tbody>
</table>

*one patient infliximab and prednisone; one patient infliximab and azathioprine

**DISCUSSION**

In the present study, we report the actual medication use in inflammatory bowel disease patients with active reproductive wish. First, we found that the majority of the patients with active reproductive wish require medication for which limited information on the safety of the peri-conceptional use is available. Second, we found that in one third of these patients the medication was changed due to the active reproductive plans.

The first part of our observation helps to define the extent of the issue of medication use by IBD patients at fertile age. Only a minority of these patients did not require any medication (23%), or were using monotherapy with 5-aminosalicylates (19%), medication that has been shown to be safe in the peri-conceptional period. In total, nearly half of the patients were using anti-TNF agents or immunosuppressive agents. Anti-TNF agents seem to be relatively safe as also reflected by their FDA categorization B. However, one must keep in mind that only short term observations are available for these therapeutics so far. In addition, the safety of the use of anti-TNF medication during the last trimester is still questionable due to the transfer of anti-TNF through the placenta. The uncertainty about the safety of the use of anti-TNF during pregnancy is also reflected in the recently reported survey on anti-TNF use. In this French national survey, in one third of patients who became pregnant on anti-TNF therapy the therapy was stopped by the treating physician. Since a substantial proportion of the patients in our study (19%) were using anti-TNF agents, we believe that gastroenterologists should actively identify patients on anti-TNF treatment with conception plans in order to provide the right counseling prior conception.

One quarter of the IBD patients with active reproductive wish were using immunosuppressive agents, thiopurines or methotrexate. Thiopurines, although generally categorized as FDA category D have been shown to be safe at the dose used for IBD. The experience with thiopurines is longer than with anti-TNF agents, however, specific data on the long-term effects of the peri-conceptional and/or intrauterine exposure are not available. The original reports of congenital malformations resulting from intra-uterine exposure were not confirmed in IBD patients. Nevertheless, the relative contra-indication for the peri-conceptional use of thiopurines is still mentioned in general information provided by the manufacturer. This often leads to confusion in IBD patients as also demonstrated by our two patients who stopped thiopurines when they became pregnant. The second type of immunosuppressive medication used by our patients, methotrexate, is absolutely contra-indicated in the peri-conceptional period due to its teratogenicity and embryotoxicity. Based on this information on peri-conceptional safety of immunosuppressive agents, keeping in mind that important part of patients with active conception wish use this medication, we again would like to underline the necessity of pre-conceptional counseling for these patients.
In addition, few patients with active reproductive wish in our study were on combination therapy. The risks of combination therapy are rather difficult to estimate. Therefore, in our opinion, these patients necessitate a careful coaching, with extensive discussion of the potential risks with the mothers/fathers-to-be.

In the second part of our analysis we found that one third of the patients eventually required a change of medication for reasons of their conceptions plans. Reproductive wish represents thus an important factor influencing the therapeutic strategy in IBD patients. Nevertheless, in our study, one third of patients did not consult their reproductive plans with the physician prior to the conception. This represents an additional argument for the above advocated active approach of the gastroenterologists in identifying IBD patients with active conception wish. Our data clearly indicate the importance of the reproductive issues in the management of IBD patients. It is presumable that without proper coaching many patients with active reproductive wish would finally choose for voluntary childlessness out of fear for the side-effects of the medication on the child, as reported recently.

The results of the present study are potentially limited by reporting and selection biases. The first implies that the use of medication, in particular when potentially harmful, may act as incentive for patients to report their reproductive plans. However, the explicit question about the reproductive plans was asked by the treating physicians during the outpatient consultation which would lower the risk of underreporting. Concerning the selection bias, a typical referral center IBD population is characterized by complicated disease course and therefore also more frequent use of immunosuppressives which also applies for our group of patients.

Thus, reproductive wish represents an important factor influencing the therapeutic strategy in inflammatory bowel disease patients. This underlies the necessity of active approach with regards to the reproductive issues in these patients in everyday practice. Two-thirds of patients proceed to achieve pregnancy under the medication of unclear safety in this setting. Therefore, the information from post-marketing studies on the peri-conceptional safety of the novel medications used in IBD will be extremely valuable in the management of these patients.

REFERENCES

Azathioprine Treatment during Lactation

Zuzana Zelinkova, Inge P de Boer, Mirian J van Dijke, Ernst J Kuipers, C Janneke van der Woude

Published in part in Aliment Pharmacol Ther 2009; 30:90-1
Dear sir,

We read with interest the article by Christensen et al. demonstrating a low penetration of 6-mercaptopurine (6-MP) in maternal milk of eight inflammatory bowel disease (IBD) patients on azathioprine. Based on this reported low exposure of children to azathioprine (<1% of the maternal dose), breastfeeding during azathioprine therapy seems safe.

This study is of great importance as little is known about breastfeeding by IBD patients on azathioprine. However, considerable inter-individual variability in the absorption and metabolism of azathioprine makes it difficult to predict whether this very low dose in maternal milk would not eventually result in a clinically relevant cumulative dose in the individual child. Therefore, we propose to monitor the azathioprine metabolites levels in the breastfed child as studied earlier. We would like to demonstrate the feasibility of this approach in the case of a child born to a 31-year old mother with Crohn’s disease. The child was fed with maternal milk during 3 months, while the mother was treated with azathioprine, 100mg a day (1.4mg/kg). At day 8 of the breastfeeding, the peripheral blood levels of 6-methylmercaptopurine (6-MMP) and 6-thioguaninenucleotides (6-TGN) were assessed in the child, both appeared undetectable. At month 3, when the feeding with maternal milk was tapered to zero, the levels of 6-MMP and 6-TGN were again undetectable in the child whilst the mother had therapeutic levels (6-MMP 410 pmol/10^8 red blood cells, 6-TGN 470 pmol/10^8 red blood cells). During the six months of the follow-up, the child thrived and did not suffer from any infections.

Thus, breastfeeding by IBD patients on azathioprine is probably safe. However, until more experience is gained, we advocate the monitoring of azathioprine metabolites as a doable method to safeguard the minimal exposure of a breastfed child.

Best regards,

Zuzana Zelinkova, MD
Department of Gastroenterology&Hepatology
Erasmus MC, Rotterdam, The Netherlands

Inge P. de Boer, MD
Department of Pediatrics
Diakonessenhuis, Utrecht, The Netherlands

Mirian J. van Dijke, MD
General Practitioner, Utrecht, The Netherlands

Ernst J. Kuipers, MD
Department of Gastroenterology&Hepatology
Erasmus MC, Rotterdam, The Netherlands

C. Janneke van der Woude, MD
Department of Gastroenterology&Hepatology
Erasmus MC, Rotterdam, The Netherlands

ABSTRACT

Background
Lactation during the thiopurine therapy seems to be safe as thiopurines are excreted through breastmilk only in a very low concentration. However, it is unclear whether interindividual pharmacokinetic and pharmacodynamic differences cannot lead to the accumulation of the thiopurines metabolites with potential of toxic side-effects, namely myelosuppression in the newborn. Therefore, we proposed earlier the monitoring of the thiopurine metabolites in the child as a doable method to safeguard the safety of lactation during the treatment with thiopurines. Here, we report the results of the first five patients monitored according to this protocol.

Methods
Pregnant inflammatory bowel disease (IBD) patients using azathioprine were given the information about the monitoring of the lactation safety during the treatment with azathioprine. In case they wanted to breastfeed, the levels of 6-methylmercaptopurine (6-MMP) and 6-thioguaninenucleotides (6-TGN) were measured in the peripheral blood of the child during the second week of the breastfeeding and, when appropriate, at month 3.

Results
Five children born to mothers treated with azathioprine were breastfed and the levels of 6-MMP and 6-TGN were assessed during the second week of the full breastfeeding. None of the newborns had detectable levels at this point; in two children the levels of 6-TGN and 6-MMP were assessed again in the 3rd month of life and were again undetectable.

Conclusion
Lactation during the use of azathioprine does not lead to accumulation of the azathioprine metabolites in the newborn. However, until this approach is validated in a larger cohort, we advise to continue the monitoring of the thiopurines metabolites levels in the newborn.
INTRODUCTION

Thiopurines (azathioprine AZA and 6-mercaptopurine 6-MP) are used widely for the induction and maintenance of remission of inflammatory bowel disease (IBD). IBD affects typically young patients with family plans and therefore, the questions on the safety of the use of these drugs during pregnancy and lactation are frequently encountered in the clinical practice. The exposure of the foetus to low levels of the end-metabolites of thiopurines, 6-thioguaninenucleotides (6-TGN), documented recently5, 6 does not seem to lead to unfavourable pregnancy outcomes in terms of teratogenicity7. Thus, for the use of thiopurines during pregnancy of IBD patients the benefits of controlled disease are considered to outweigh the risks for the child and the continuation of this treatment during pregnancy is recommended8.

Once the child is born, the question arises of the safety of the use of these drugs during lactation. In contrast to the data on pregnancies, the effect these drugs may have on the child through lactation is much less studied. Thiopurines seem to have a very low penetration in the breast milk, as it has been demonstrated by the measuring of 6-MP levels in maternal milk of eight IBD patients on azathioprine1. Based on this report, breastfed children by mothers using thiopurines would be exposed to a very low dose of the thiopurine metabolites corresponding to less than 1% of the maternal dose1. This finding suggesting safety of the thiopurine use during lactation has been further supported by measurements of thiopurine metabolites in 4 children breastfed by mothers using azathioprine that showed absence of these metabolites in peripheral blood of the infants5. Thus, the use of thiopurine by breastfeeding women with IBD seems to be safe for the child.

Nevertheless, these reports are very limited in size. In addition, the dose of azathioprine used by mothers in the referred study on the thiopurines excretion in the breast milk1 was in 5 out of 8 patients included lower than the recommended dose for the use in IBD, i.e. 2-2.5mg/kg, which makes it difficult to apply these results for larger patient population. Furthermore, one must keep in mind that there is a considerable inter-individual variability in the absorption and metabolism of azathioprine2, 3. This variability makes it difficult to predict whether the documented very low dose of thiopurine metabolites in maternal milk would not eventually result in a clinically relevant cumulative dose in the individual child. Therefore, we proposed earlier the monitoring of the thiopurine metabolites in the child as a doable method to safeguard the safety of lactation during the treatment with thiopurines5. Here, we report the results of the first five patients monitored according to this protocol.

MATERIALS AND METHODS

Pregnant IBD patients using AZA were recruited from the outpatient IBD clinic specifically dedicated to pre-conceptional counselling of IBD patients. At the consultation, the current safety data on the breastfeeding during AZA therapy were explained to the patients. More specifically, they were given information that according to thus far published studies the use of AZA during lactation is probably safe but that the numbers of patients documented in this setting are limited. In case that wanted to breastfeed, they were offered the possibility of measuring the levels of 6-MMP and 6-TGN in the peripheral blood of the child during the second week of the breastfeeding. At the same time, maternal levels of 6-MMP and 6-TGN were assessed.

RESULTS

In total, 6 patients on azathioprine treatment wanted to breastfeed, one patient did not want to measure the 6-MMP and 6-TGN levels in her child; she was breastfeeding during the period of one year and the child thrived well. Remaining five patients consented to assess the 6-MMP and 6-TGN levels in their children, all of them had undetectable levels of these AZA metabolites. The 6-MMP and 6-TGN levels of 3 mothers and all five children are shown in Table 1.

<table>
<thead>
<tr>
<th>Case</th>
<th>Duration of breastfeeding*</th>
<th>Levels child**</th>
<th>Levels mother**</th>
<th>Dosis azathioprine mother</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7 days</td>
<td>undetectable</td>
<td>6-TGN 110</td>
<td>6-MMP 580</td>
</tr>
<tr>
<td>2</td>
<td>7 days</td>
<td>undetectable</td>
<td>Not assessed</td>
<td>200 mg/day</td>
</tr>
<tr>
<td>3</td>
<td>1 month</td>
<td>undetectable</td>
<td>Not assessed</td>
<td>150 mg/day</td>
</tr>
<tr>
<td>4</td>
<td>8 days 3 months</td>
<td>undetectable</td>
<td>6-TGN 470</td>
<td>6-MMP 410</td>
</tr>
<tr>
<td>5</td>
<td>8 days 3 months</td>
<td>undetectable</td>
<td>6-TGN 170</td>
<td>6-MMP 7150</td>
</tr>
</tbody>
</table>

* at the moment of the assessment of the 6-MMP and 6-TG levels in the child
** pmol/10^8 red blood cells
DISCUSSION

We report here the first results of the monitoring of thiopurines metabolites levels in five children breastfed by IBD patients treated with azathioprine. In none of the children, these levels were detectable which is in line with previous report using this monitoring method. So far, there were no cases of documented exposure of children to thiopurines in this setting. Furthermore, a recent report on the long term follow-up of 15 children breastfed for a median period of 6 months by mothers using AZA showed a normal development of these children during the follow-up of up to 4 years without an increased risk of infections. Thus, both pharmacokinetic studies and clinical follow-up suggest that the use of AZA during lactation is safe for the child.

However, in all these studies, the maternal dose of AZA was rather low, which corresponded with suboptimal therapeutic levels of 6-TGN in mothers in our study and low therapeutic levels in the report by Christensen. It has been documented that levels of 6-TGN vary greatly during pregnancy and this is also likely to be the case in the post partum period during which the maternal organism undergoes important changes in distribution volume and body weight. In addition, inter-individual differences in AZA metabolism in adults are well recognized which makes it difficult to use the results of these limited size studies for general recommendations. Furthermore, it has been shown that, compared with adults, neonates have higher activity of thiopurine S-methyltransferase (TPMT), an enzyme that plays crucial role in the metabolism of thiopurines. This illustrates clearly, why the predictions on the pharmacokinetics in the neonates cannot be made using the calculations derived from data obtained in adults. Thus, the variability of thiopurine pharmacokinetic on maternal side together with specificities of the thiopurine metabolism in the first months of life can both influence the final cumulative dose to which the child will be exposed.

Therefore, we believe, the available evidence does not allow general recommendations on the use of azathioprine during lactation without careful monitoring of the child, ideally by the means of measurements of thiopurine metabolites levels at two different time points during the first months of lactation.

REFERENCES

Chapter 7

High Intra-Uterine Exposure to Infliximab Following Maternal Anti-Tnf Treatment During Pregnancy

Zuzana Zelinkova, Colin de Haar, Lissy de Ridder, Marieke J Pierik, Ernst J Kuipers, Maikel P Peppelenbosch, C Janneke van der Woude

Aliment Pharmacol Ther 2011; 33:1053-8
ABSTRACT

Background
Typically, inflammatory bowel disease (IBD) patients are in their reproductive years, raising questions about safely using anti-tumor necrosis factor antibodies like infliximab (IFX) during pregnancy. IgG antibodies naturally cross the placenta, especially during the last trimester. To prevent fetal intra-uterine exposure, stopping IFX treatment at gestational week 30 is recommended. However, whether this limits intra-uterine and early post-natal IFX exposure is unestablished.

Aim
To determine the intra-uterine exposure to IFX following maternal treatment with IFX.

Methods
Four pregnant IBD patients intentionally continued IFX during pregnancy. IFX levels were assessed in newborns’ cord blood and the mothers’ peripheral blood at delivery. The children’s development during the first 3–6 months, infections, vaccine reactions and antibody responses to vaccinations against Haemophilus influenzae type b and Pneumococcus were assessed.

Results
The patients stopped IFX therapy at gestational week 21, 26, 26, and 30, respectively. In three infants, therapeutic IFX levels were present in cord blood at levels of 5.5–13.7 μg/mL and were two- to three-fold higher than in the peripheral blood of their mothers. During the 3–6-month follow-up, the children developed normally without signs of infections or allergic reactions, and had normal antibody titers after routine childhood vaccinations.

Conclusion
The use of IFX until gestational week 30 leads to fetal intra-uterine exposure to IFX at levels that exceed those in the mothers’ peripheral blood. Although no short-term complications were detected, the high IFX levels observed in newborns raise concerns about unknown effects of IFX on the developing immune system.

INTRODUCTION

Anti-tumor necrosis factor (anti-TNF) agents are frequently used powerful immunomodulatory agents available to clinicians to treat several autoimmune conditions including inflammatory bowel disease (IBD). Potential candidate patients for anti-TNF treatment are often young and in their reproductive years, which is also reflected in the observation that 20% of IBD patients with active reproductive wishes require treatment with anti-TNF agents. Therefore, the safety of anti-TNF use during conception and pregnancy has become a major concern for patients and their clinicians.

Of the various anti-TNF agents, infliximab (IFX), a chimeric monoclonal antibody against TNF, is the best characterised with respect to safety during human reproduction. Short-term data from two safety registries found no important teratogenicity issues. However, intra-uterine exposure to IFX resulting from placental antibody transfer was previously reported in children born to mothers treated with IFX during pregnancy. Like all IgG antibodies, IFX crosses the placenta beginning in the second trimester, probably reaching its maximal transport capacity during the third trimester. To limit this placental transfer stopping IFX treatment prior to gestational week 30 was proposed. Nevertheless, in view of the strong immunomodulatory properties of IFX and the lack of long-term outcome data, the safety of terminating IFX therapy at gestational week 30 is difficult to take for granted without data showing that this is sufficient to prevent transfer of significant amounts of anti-TNF to the unborn child.

Concern about the unpredictable outcomes of early exposure of the developing immune system to powerful TNF-alpha neutralization and the immunomodulatory potential of anti-TNF molecules is, at least on a theoretical basis, substantial. This concern, together with an absence of data in which the level of IFX transfer to the unborn child is actually assessed following termination of anti-TNF therapy during different gestational weeks, prompted us to determine the exposure of the newborn to IFX in this case series of four IBD patients intentionally treated with IFX until gestational week 30. We found that this approach is not sufficient to limit the placental transfer of IFX, resulting in IFX levels in the cord blood that exceed the therapeutic level for adults.

PATIENTS AND METHODS

Four patients (three with Crohn’s disease and one with ulcerative colitis, aged 19, 29, 29, and 31 years, respectively) were intentionally treated with IFX during pregnancy. All patients continued the remission-maintaining preconception dose regimen. In one patient, methotrexate was advised to be discontinued following her expressed wish to reproduce. Three patients were receiving IFX at a stable dose of 5 mg/kg every eight weeks, and one patient received 10 mg/kg
every eight weeks. Two patients had periconceptional IFX monotherapy, one patient was using azathioprine. The patient using methotrexate conceived within two months after stopping methotrexate and continuing IFX monotherapy. The other three patients had stable medication during the six months prior to conception. One patient stopped IFX at gestational week 21, two patients at week 26, and one patient at week 30. The respective gestational weeks at delivery are shown in Table 1. The decision of discontinuation of IFX was at the discretion of treating gastroenterologist who discussed all pros and cons of the treatment continuation with the patients at an early stage of their pregnancy. The timing of discontinuation was motivated by the current approach advising not to extend the use of IFX beyond 30 weeks of pregnancy. The exact date of the last infusion was based on the 8-weeks schedule with which patients entered the pregnancy so that the last infusion would be given prior or at the week 30 at the latest. At delivery, cord blood was collected from the newborns, and peripheral blood serum was obtained within two days after delivery from the patients.

All children received routine childhood vaccinations according to recommendations in the Netherlands, i.e. a combined vaccine for diphtheria, tetanus, pertussis, and polio; Haemophilus influenzae b; and pneumococcus at the ages of 2, 3, and 4 months. The response to bacterial vaccines was assessed at month 6 of life in two of the three children born with significant IFX levels.

The IFX serum levels were measured by ELISA, the assay has been adapted and validated in our laboratory based on the previously reported and validated assay4. High binding capacity 96-well ELISA plates (Nunc-ImmunoTM Plates) were coated overnight at 4°C with 50µl of 2µg/mL recombinant human TNF-alpha (Invitrogen) diluted in phosphate buffered saline (PBS). The plates were washed twice with PBS 0.05% Tween 20 (PBST). The remaining protein-binding sites were blocked during two hours at room temperature with 250µl of 3% non-fat dry milk (Elk Campina, Eindhoven, The Netherlands) diluted in PBST. The plates were washed five times with PBST and 100µl of serum, standard and control samples were added. For the negative control, a pooled human serum, obtained from random 40 different healthy individuals was used. To generate standard curve, serial concentrations of infliximab (Remicade®, Merck Sharp&Dohme) were used. The serum samples were serially diluted at concentrations of 1:1000 to 1:32000. All samples were diluted in PBST containing 1% bovine serum albumine (BSA). The samples were incubated during one hour at room temperature and subsequently washed four times with 0.05% PBST. The reaction was revealed using 100µl of 3,3',5,5'tetramethylbenzidine (ellobiscience) solution at room temperature and stopped with 100µl of 1M H2SO4. Reading was performed at two wave lengths; 450 and 620 nm using Biorad 680 plate reader. The samples were run in different concentrations as mentioned above and the final infliximab serum concentrations were interpolated from the standard curve. The results were rejected if difference between the calculated concentration of the serial dilution was higher than 20%, and the accepted measurements were considered as triplicates to calculate the mean.

The assay was tested and validated using serum samples from patients receiving IFX and those who were naïve to this drug. For the average inter-assay coefficient of variation calculation, 10 samples were run on different days and the standard deviation per patients sample was expressed as a percentage of the mean. The average inter-assay coefficient was 4%. The inter-assay coefficient was calculated using the serial dilutions of samples that were used instead of triplicates. For this calculation 3 dilutions of 10 samples were measured with the intra-assay variability coefficient of 4.4%.

Since the treatment protocol is considered a standard care, no approval of ethical committee has been solicited.

RESULTS

Pregnancy outcomes

Patients gave birth at gestational weeks 37, 36, 41, and 38, respectively (Table 1). Three children were healthy without congenital malformations. One baby girl, born to a 19 year old mother (patient Nr 3), experienced respiratory depression after birth owing to anesthetics used during delivery, and which resolved spontaneously. This child was also diagnosed with polydactyly of her left hand. Apart from infliximab, the mother had used methotrexate till two months prior to conception and had omitted to use the recommended folic acid supplementation during the first weeks of the pregnancy. Other risk factors for congenital malformations, such as alcohol consumption, smoking or consanguinity were not revealed.

During the follow-up of 4 to 11 months, all children developed normally, there were no signs of infections, and no abnormal reactions to vaccinations were observed. The antibody response to vaccinations against pneumococcus and Haemophilus influenzae b were assessed in two of the three children born with detectable IFX levels. Both children had protective levels of vaccine antibodies when checked at month 6.

IFX levels

Only one patient (patient 1) had undetectable levels of IFX in her peripheral blood at delivery and undetectable levels in her infant’s cord blood. This patient received her last IFX infusion at gestational week 21. In three other patients, therapeutic levels of IFX were found in the cord blood at levels of 13, 5.5 and 13.7 μg/mL, respectively. The levels in cord blood were higher than the levels measured in peripheral blood of mothers (p=0.032 with one-tailed paired t-test), which were 4.9, 2.4, and 5 μg/mL respectively.
**DISCUSSION**

In this case series, we show that the currently advocated approach of stopping IFX at gestational week 30 does not prevent the intra-uterine exposure of the fetus to significant levels of IFX. This finding is important in clinical practice because of the potential short-term as well as long-term complications of early exposure to anti-TNF.

Data from the safety registries\(^1\,^2\), as well as a recently published first prospective cohort on the outcomes of pregnancies exposed to infliximab\(^3\) did not reveal significant teratogenicity issues. In one out of the four patients presented in our study, polydactyly of the hand was diagnosed in a child born to a mother using IFX at conception and during the first two trimesters of the pregnancy. However, this patient also conceived only two months after stopping methotrexate. Digital malformations have been reported with the use of methotrexate but almost exclusively in combination with other birth defects\(^4\) which were not present in this child. To our knowledge, no isolated polydactyly has been reported in the case of the pre-natal exposure to infliximab. In addition, polydactyly is a very common birth defect in general population\(^5\). Thus, no clear association with the medication can be concluded, however, the role of the pre-conceptional use of methotrexate remains a subject of discussion in this patient.

Intra-uterine exposure to IFX of children born to mothers receiving IFX treatment was first suggested by Vasiliauskas et al. in 2006\(^6\). In this case report, the mother used IFX during the entire pregnancy, and clinically significant levels of IFX were detected in the child’s serum sample. The hypothesis of placental transfer of IFX was further supported by a study by Mahadevan et al.\(^7\) where authors reported clinically significant levels in the cord blood of the infant and serum samples taken directly postpartum from five children born to mothers treated with IFX. In this case series, the mean time between birth and last IFX infusion was 30 days. Kane et al.\(^8\) measured the levels of IFX in two children born to mothers treated with IFX until gestational week 32 and 24, respectively. In both children, IFX was undetectable; however, the samples were taken postpartum on days 15 and 57, respectively, which makes the assumption of a lack of intra-uterine and early postnatal exposure to IFX uncertain.

The kinetics of IgG antibodies in general are governed by the neonatal Fc receptor (FcRn)\(^9\). Antibodies are passively taken up by endothelial cells among other cell types and subsequently sequestered through binding to intracellularly expressed FcRn then shuttled back to the extracellular milieu. This recycling helps to increase the life time of the antibodies by protecting them from catabolism\(^10\). The same mechanism is used by maternal IgG antibodies that cross the placenta beginning in the second trimester\(^11\). IFX, being an IgG1 class antibody with a functional Fc, very probably crosses the placenta in the same way\(^12\) and is subject to the efficient FcRn-mediated protection from break down in the newborn. The biological half time of IFX in the newborn is also expected to be longer than in adults because of the high expression of

---

**Patient follow-up**

All four IBD patients remained in remission throughout their pregnancy and resumed IFX treatment within 4 weeks after delivery. The re-treatment with IFX did not result in allergic reactions and all patients remained in remission during the 4 to 11 months of follow-up after delivery.

**Table 1.** Pregnancy outcomes and medication during pregnancy

<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Infliximab dose</th>
<th>Co-medication</th>
<th>Infliximab stopped (gestational week)</th>
<th>Gestational week delivery</th>
<th>Birth weight (g)</th>
<th>Congenital malformations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5 mg/kg, every 8 weeks</td>
<td>None</td>
<td>21</td>
<td>37</td>
<td>2650</td>
<td>none</td>
</tr>
<tr>
<td>2</td>
<td>5 mg/kg, every 8 weeks</td>
<td>None</td>
<td>26</td>
<td>36</td>
<td>4030</td>
<td>none</td>
</tr>
<tr>
<td>3</td>
<td>5 mg/kg, every 8 weeks</td>
<td>None*</td>
<td>26</td>
<td>41</td>
<td>3030</td>
<td>Polydactyly left hand</td>
</tr>
<tr>
<td>4</td>
<td>10 mg/kg, every 8 week</td>
<td>Azathioprine 2 mg/kg</td>
<td>30</td>
<td>39</td>
<td>3185</td>
<td>none</td>
</tr>
</tbody>
</table>

*Methotrexate two months prior to conception

**Figure 1.** Infliximab levels in cord blood of newborns (black bars) and in the peripheral blood of the mothers (grey bars) at delivery. In the three patients with detectable IFX levels, the levels in newborn were higher than the levels at the peripheral blood of the mothers (one-sided paired t-test, p=0.032)
the FcRn during the first months of life. Indeed, the series of 5 children born with detectable IFX levels showed the persistence of IFX in the peripheral blood of the children for as long as 6 months. Thus, the placental transfer of IFX not only raises the issue of intra-uterine exposure but, most importantly, the concern about consequences for immune system functioning and development during the first weeks of life.

Children born with detectable levels of IFX do not seem to have an increased risk of infections in their first year of life and have normal responses to vaccinations with bacterial non-live vaccines. This was documented in the eight-patient series reported by Mahadevan et al. and is also supported by the findings in the current study. However, a fatal case of disseminated mycobacterial infection after BCG administered at month 3 to a child born to a mother treated with IFX during the entire pregnancy was recently reported. In this case, the likely mechanism of insufficient immune control of the live attenuated vaccine would be the neutralization of TNF-alpha by IFX, although no measurements of IFX levels in the child were performed. The specific infectious complications resulting from the use of anti-TNF agents are well known and, therefore, vaccinations with live antigens are also prohibited in patients on anti-TNF treatment. Because significant levels of IFX may persist for several months in children born to mothers treated with IFX, vaccinations with attenuated vaccines should be postponed until IFX levels are undetectable.

Early exposure of the immune system to powerful TNF-alpha blockade also raises concerns about the long-term consequences for the maturation of the immune system. In the context of the increasing number of reports of hepatosplenic T-cell lymphoma in young adolescents treated with combined immunosuppressive therapy containing, in most cases, anti-TNF, and keeping in mind the lack of long-term outcome data, we believe the actual approach should be to try to limit the intra-uterine and postnatal exposure of children to anti-TNF in general.

The decision to stop treatment with a drug that keeps the mother's underlying disease in remission during pregnancy is a difficult decision for the treating physician. A disease flare may represent a substantial risk not only for the patient, but also for the unborn child. On the other hand, in the absence of convincing long-term safety data of the perinatal exposure to IFX, the continuation of the treatment remains a subject of discussion. Simultaneously, the decision to stop IFX is difficult in the current absence of predictors for the disease course during pregnancy in this specific setting. In addition, in case of treatment with anti-TNF, allergic reactions during retreatment which had been postponed for more than 12 weeks represent another concern. In this limited series of patients, we did not observe any of these problems; all patients remained in remission after stopping IFX and no allergic reactions occurred during retreatment after delivery. Our observation is also supported by the results of the first prospective cohort of 35 IBD patients treated with IFX during first two trimesters of the pregnancy that did not show negative impact of this approach nor on the disease course neither on the pregnancy outcomes.

Interestingly, in three patients with detectable levels of IFX at delivery, the interval between the last IFX infusion was 10, 15, and 9 weeks, respectively. The elimination half-time of IFX for women being 18 days, one would expect no detectable levels in these patients at delivery. That these patients still had detectable levels of IFX may indicate changes in the pharmacokinetics of IFX during pregnancy that would lead to a longer biological half-life of IFX and, therefore, possibly may provide a safe way to stop treatment earlier in the pregnancy.

In conclusion, the use of IFX at the end of the second trimester of pregnancy leads to intra-uterine exposure of the fetus to IFX. This exposure does not seem to have a negative impact on the child in terms of increased infection rate and vaccination failure, but in specific situations, such as vaccinations with attenuated live antigens, the presence of IFX may have fatal consequences. Therefore, we propose that the levels of IFX be assessed in every child born to mothers treated with IFX during pregnancy, and to administer attenuated live vaccines only to children with undetectable IFX levels. Additionally, the long-term effects of this early exposure of the immune system to IFX are hard to predict. Therefore, for patients in whom the quiescent disease during pregnancy allows interruption of treatment, intra-uterine and postnatal exposure of newborns to IFX may probably be avoided by stopping IFX at the beginning of the second trimester. In order to determine the exact timing of the discontinuation of IFX during pregnancy, further studies on the pharmacokinetics in this setting need to be conducted.
REFERENCES


Chapter 8

Effect of Adalimumab on Semen Quality in Inflammatory Bowel Disease Patients

Zuzana Zelinkova, Cokkie van der Ent, Ernst J Kuipers, Gert R Dohle, C Janneke van der Woude
ABSTRACT

Background

Inflammatory bowel diseases (IBD) patients are typically in their reproductive years. Therefore, the frequently encountered clinical problem concerns the impact of the disease and therapy on the IBD patients’ fertility. Adalimumab (ADA) represents a recently introduced therapeutic effective in IBD. The wide-spread use of ADA, including IBD patients with active conception wish raises the question of its effect on the semen quality and outcomes of pregnancies conceived by males under ADA treatment.

Aim

First, the aim of this study was to assess the influence of ADA on the semen quality of IBD patients using ADA. Second, we analysed the outcomes of the pregnancies conceived under the use of ADA by male IBD patients.

Methods

Male IBD patients naïve to ADA and planning to start the treatment with ADA were included. The semen sample was obtained prior to starting the treatment, at month 3 and at month 6 of the treatment. The semen quality according the WHO criteria was assessed and the influence of ADA on the semen quality was evaluated intra-individually. In addition, the patients who became fathers during the treatment with ADA were identified at the outpatient clinic during their regular consultation and outcomes of these pregnancies were assessed.

Results

The effect of the treatment with ADA on the semen quality was assessed in seven IBD patients. No differences in the sperm concentration, percentage of cells with progressive motility and cells’ vitality were found between the baseline samples and samples obtained at month 3 and 6 during the treatment.

In addition, two patients conceived during the treatment with ADA. Both children were born à terme, without congenital malformations.

Conclusion

In this small sample size inflammatory bowel disease patients’ group, we did not observe any modifying effect of adalimumab on the semen quality.

INTRODUCTION

Inflammatory bowel disease (IBD) represents a chronic inflammatory disorder affecting gastrointestinal tract. The patients necessitate lifelong follow-up and intensive medical and/or surgical treatment. As the disease typically affects patients in their reproductive years, procreation represents a frequently encountered issue in the clinical practice. Reproduction of IBD patients is influenced by two overlapping factors, disease itself and the therapeutic interventions. In males, many factors interfere with the sperm quality and subsequent fertility, such as fever and/or presence of an inflammatory condition. Therefore, in order to enhance the chances for successful conception it is important to keep the disease under control.

Inherently, the issue of the safety of the use of a particular medication by the fathers-to-be becomes an important question in the clinical practice. In the past years, the field of IBD therapeutic has undergone a dynamic evolution with the introduction of new therapeutic agents, biologicals directed against human tumor-necrosis alpha (anti-TNF). To date, two distinct anti-TNF agents have shown their efficacy in the treatment of IBD, infliximab (IFX) and adalimumab (ADA). Thus far, very limited data are available on the safety of the use of anti-TNF agents by men with IBD who wish to conceive. Keeping in mind the limitations of small sample size, the periconceptual use of IFX by male patients is probably safe but no data are available on ADA use by future fathers.

Therefore, in the present study we aimed at filling this gap by first, studying the impact of the ADA treatment on the semen composition and second, to analyse the outcomes of pregnancies conceived under indirect exposure to ADA. We found no alterations of the sperm quality induced by the treatment and favourable outcomes of pregnancies identified retrospectively as indirectly (i.e. through male exposure in the peri-conceptional period) exposed to ADA.

PATIENTS AND METHODS

Between October 2009 and April 2011, male IBD patients planning to start treatment with ADA were recruited from the IBD outpatient clinic of the Erasmus MC. A semen sample was collected before the start of treatment, and subsequently at month 3 and month 6 of the treatment. At the initiation of treatment with ADA, induction regimen of 160 mg s.c. at week 1, 80 mg at week 3 was applied and subsequently patients continued the maintenance medication of 40 mg every other week. The quantitative and qualitative semen samples analysis was performed according to the WHO criteria.

Before each sampling the disease activity was assessed by CDAI and the co-medication was noted, together with the information on the smoking habits and alcohol use. The co-
medication had to remain stable during the 6-months follow-up. To assess whether the impact of the disease or and the treatment on the fertility is ensuing from a disturbed endocrine status, folliculi stimulating hormone, testosteron and inhibine-B levels were measured. Medical ethical committee approval was obtained for this part of the study and all patients were included after informed consent had been obtained.

In addition, all pregnancies conceived during ADA treatment were retrieved. The patients attending the IBD outpatient clinic treated with ADA were asked whether they conceived children during the treatment with ADA. The outcomes of these pregnancies – birth weight, gestational age at delivery and congenital malformations were obtained from the patients and/or their partners.

RESULTS

Effect of adalimumab on sperm composition

In total, 8 patients were included, all with Crohn’s disease, average age 32 years (range 25-46); with the indication for ADA as follows: 6 patients luminal disease, one patient fistulising disease and one patient with extra-intestinal manifestations of arthritis. In terms of response to medication, six patients responded to treatment, one stopped ADA after 3 months due to non-responsiveness and one patient needed dose escalation to weekly dose of 40 mg. One patient dropped out and no semen samples at month 3 and 6 could have been obtained from him. In two patients no samples at month 6 were available for analysis, one patient stopped the medication and one wished to stopped the participation at the study. In seven patients, at least one of the follow-up samples under ADA treatment could have been analysed. From these seven patients, three patients had baseline abnormalities of the sperm composition regarding sperm concentration and/or motility; in two patients these abnormalities remained present during the treatment.

Overall, no differences in semen volume, sperm concentration and motility (Figure 1), semen pH, agglutination and presence/absence of leucocytes induced by the treatment were noted. No abnormalities in the hormonal status were observed.

Outcomes of pregnancies conceived under adalimumab treatment

Two pregnancies with indirect exposure to ADA were identified. Both children were born à terme, at respective gestational weeks 40 and 38, with birth weight of 3820 grams and 2800 grams, and no congenital malformations.

DISCUSSION

In this small sample size Crohn’s disease patients’ group, we did not observe any modifying effect of adalimumab on the semen quality as assessed by analysis of the sperm composition and outcomes of two pregnancies conceived while fathers were using adalimumab.

The concerns about the use of anti-TNF agents in male IBD patients with conception wish are related to two areas; first, to the impact of anti-TNF agents on fertility and second, the outcome of the pregnancies conceived under indirect exposure to anti-TNF.

To study these questions, there are several methodological possibilities including animal studies, in vitro experiments, human studies assessing the effect of anti-TNF agents on semen quality in vivo and clinical trials assessing the outcomes of pregnancies with indirect exposure to ADA. It has been shown in vitro that TNF-α inhibits germ cell apoptosis and in animals studies TNF-α effect on seminiferous epithelium survival has been effectively blocked by infliximab. In a study with 10 male patients with different indications for infliximab treatment, decreased sperm motility but increased sperm concentration has been found after start of infliximab treatment. Thus, there are indications that TNF-α may play an important role in spermatogenesis and that its proper functioning might be influenced by the systemic use of an anti-TNF agent. However, the animal studies are difficult to be interpreted with regards to the experiments testing a humanized and thus species-specific antibody and the human data available thus far are still limited to few patients. It is therefore important to study these questions using a relevant methodological design in human.
Our study is the first study evaluating the effects of ADA treatment on sperm composition. The fact that we did not observe changes in semen composition after start of ADA treatment is reassuring and suggests that the interference of this particular agent with spermatogenesis would be minimal if not non-existing. However, these results must be interpreted with caution, keeping in mind that DNA damage does not necessarily need to be accompanied by changes in the microscopic appearance of sperm cells.

Therefore, a thorough identification, follow-up and subsequent reporting of all pregnancies with indirect exposure to ADA represents a crucial step towards final evaluation of the safety of ADA use by the future fathers. Thus far, no such cases have been documented and to our knowledge the two pregnancies we report here are the first indirectly exposed to ADA documented pregnancies. In line with the observation of lack of influence of ADA on the semen composition, both pregnancies we report here had favourable outcomes. However, the small sample size and retrospective character of identifying the cases call for caution in generalizing these observations. Nevertheless, these data helps to create the body of evidence serving the patients and their physician in the difficult decision-making once the reproductive issue comes up in a patient on an established therapy with ADA.

Once a male IBD patient who wishes to conceive is on an established treatment, the discontinuation of the treatment might have disastrous consequences for his disease course. In order to really insure the conception with semen produced without any influence of the drug, one must take into account not only the biological half time of a particular drug, but also the duration of the spermatogenesis that takes 60 to 80 days. Thus, depending on the drug kinetics, the time needed to produce ‘drug influence free’ sperm, can easily reach 4 to 6 months that patient needs to be off the drug. In this period, the patient carries important risk of relapse and in case of treatment with anti-TNF agents is also at increased risk of allergic reactions and loss of response once the treatment is resumed. Thus, it is important to realize, that also in males, the reproductive plans influence the therapeutic strategy in a substantial way.

In conclusion, our small sample size study suggests that there is not an important influence of adalimumab on the spermatogenesis. However, we do not feel that these small sample size data allow advocating of active peri-conceptional use of adalimumab by male IBD patients and extensive discussion should take place with the patient before he and his partner make their decision.

REFERENCES

Chapter 9

Evaluation of the Discontinuation of Infliximab during Pregnancy in Inflammatory Bowel Disease Patients

Zuzana Zelinkova, Cokkie van der Ent, Karlien F Bruin, Onno van Baalen, Hestia G Vermeulen, Herman J Smalbraak, Rob J Ouwendijk, Aad C Hoek, Sjoers D van der Werf, Ernst J Kuipers, C Janneke van der Woude and The Dutch Delta IBD group

Submitted
ABSTRACT

Objective
Discontinuation of infliximab (IFX) prior to the third trimester is recommended to limit early neonatal exposure to IFX. The aims of this study were first, to assess the course of inflammatory bowel disease during pregnancy after discontinuation of IFX and second, to evaluate the effect of this discontinuation on the neonatal exposure to IFX.

Design
Pregnant IBD patients using IFX were prospectively followed. In case of remission, IFX was discontinued prior to gestational week 30. Disease activity and complications of the resumption of the treatment were evaluated. IFX levels in the cord blood were assessed and correlated to the interval between last infusion and delivery.

Results
In total, 18 pregnancies in 17 IBD patients were followed; resulting in one spontaneous miscarriage at week 6 and 17 live births.

All twelve patients (71%) who discontinued the treatment prior gestational week 30 remained in remission; one patient experienced an allergic reaction to IFX at treatment resumption. Five patients (29%) were not in remission and received the last infusion between gestational weeks 30 and 34.

The cord blood was collected from 12 newborns. The mean cord blood IFX level in the patients who stopped IFX more than 10 weeks prior delivery was significantly lower than in the group with less than 10 weeks from the last infusion to delivery (2.8±SEM 1.1 µg/mL and 10±SEM 2.3 µg/mL, respectively, p=0.02).

Conclusion
In quiescent disease, early discontinuation of infliximab during pregnancy in inflammatory bowel disease is safe for patients and reduces neonatal exposure to infliximab.

INTRODUCTION

Inflammatory bowel disease (IBD) patients are typically in their reproductive age and procreation represents thus an important factor influencing the management of these patients1. A favourable pregnancy outcome is strongly related to a tight control of disease activity2 which brings up the issue of the safety of the use of the medication necessary to control the disease by the mothers-to-be.

In case of infliximab (IFX), a monoclonal chimeric anti-TNF antibody, short-term data from the safety registries and one retrospective study3-5 are available with regards to the safety of use during pregnancy. These registries did not reveal teratogenicity issues but the size of the populations included was limited and the reports were mostly retrospective. In addition, the exposure to IFX in majority of the patients in these studies was confined to the first trimester, while placental transfer of IFX starts from the second trimester6, leaving the newborn with IFX levels in the range of levels considered therapeutic in adults7-9. Therefore, the concerns about the safety of IFX use during pregnancy relate specifically to the use of this drug in the second and third trimester and there are very few data evaluating this specific clinical situation.

In order to limit neonatal exposure to IFX, it is currently recommended to discontinue treatment around gestational week 30. This specific timing of discontinuation has been set up rather arbitrary, as a compromise between the concerns about the child’s exposure and the risks of disease flare and allergic reactions at the treatment resumption carried by the mother. We have shown previously, that discontinuation of the treatment in the third trimester still results in significant exposure of the newborn to IFX9 and therefore, in order to limit the newborn’s exposure to IFX, treatment discontinuation in the second trimester should be considered. It is unclear whether this approach is safe for the mother and to which extent it reduces neonatal exposure to IFX. Therefore, the aim of this study was first, to assess the disease course during pregnancy after IFX discontinuation and second, to evaluate whether early discontinuation leads to the reduction of IFX levels in newborns.

MATERIAL AND METHODS

Patients and treatment protocol
Between April 2006 and April 2011, IBD patients with reproductive wish or already pregnant were recruited through specific outpatient clinic consultation. IBD patients were referred for this pre-conceptional consultation by their own gastroenterologists to the Erasmus MC, a tertiary referral center. The consultation was performed in a standardized manner according to ECCO guidelines10 by two physicians (ZZ and vdW). Patients were recruited from the region of South-West Holland by the physicians affiliated to the Dutch Delta IBD Study Group.
The patients with active reproductive wish or already pregnant using IFX were prospectively followed with regular bi-monthly outpatient clinic controls with clinical assessment of disease activity. In case of remission defined as complete absence of complaints recognized by patients as IBD-related and with pregnancy compatible weight gain (i.e. at least 6 to 10 kg weight gain as off beginning of the third trimester), IFX was discontinued prior to gestational week 30. Patients with unstable disease continued treatment throughout the third trimester. Disease activity and complications of the resumption of treatment were evaluated. For pregnancy outcomes, birth weight, gestational age at delivery and congenital malformations were noted.

As this approach, including assessment of infliximab levels in the newborn, represents standard care based on recommendations from different health-professional societies (AGA, ECCO), no specific approval by the medical ethical committee was solicited. Pregnancy outcomes and cord blood levels of infliximab of three of the patients included in this cohort have been reported previously.

**Infliximab levels in the newborns’ cord blood**

The cord blood was collected at delivery and IFX levels were assessed by ELISA in serum from cord blood as described earlier. Briefly, assay plates were coated with TNF-α and blocked with milk protein to prevent non-specific binding of IFX to the assay plates. Next, the plates were incubated with serum from peripheral blood or cord blood. To determine the absolute levels, a standard curve of IFX concentrations was used. After washing the serum samples, the bound IFX was detected using an antibody-peroxidase conjugate directed against the Fc-part of IgG1, followed by an enzymatic colour reaction. The assay was tested and validated using serum samples from patients receiving IFX and those who were naïve to these drugs.

**Statistical analysis**

For analysis of the effect of the timing of IFX discontinuation on perinatal IFX levels, patients were divided into two groups. The group of late discontinuation comprised patients with the time from the last infusion to delivery 10 weeks and less. The IFX levels in this group were compared to the group of early discontinuation with more than 10 weeks from the last infusion to delivery by t-test. For the correlation of gestational week of IFX discontinuation with IFX levels in the newborns a nonparametric Spearman’s correlation test was used.

**RESULTS**

In total 105 female patients received (pre)conceptional counselling. Of these patients, 17 patients (16%) used IFX during pregnancy, 16 patients were on established treatment, one patient started the treatment with IFX due to steroid-resistant disease during pregnancy. Basic demographic characteristics are shown in Table 1.

**Disease activity during pregnancy and IFX discontinuation**

In total, 18 pregnancies in 17 patients (mean age 29 years, range 18 to 37; 12 with Crohn’s disease and 5 with ulcerative colitis) were followed. Twelve patients (71%) were in remission and discontinued treatment between gestational weeks 18 and 27 (average week 23), Figure 1. After IFX discontinuation, all 12 patients remained in remission during pregnancy. Five patients (29%) were not fully in remission, three of them had complaints of diarrhoea and occasional rectal blood loss in the last two weeks prior infliximab infusion. Two patients experienced an acute flare of the disease, both in the first trimester and were treated with prednisolone, one patient successfully. In the second patient with steroid-resistant disease, IFX treatment was initiated in the 4th month of pregnancy with good response. All these five patients with active disease continued the treatment throughout the third trimester and received their last infusion between gestational weeks 30 and 34. After delivery, all five patients improved and did not necessitate further therapy adjustment; one patient was able to reduce the dose of infliximab from 10 to 5 mg/kg.

**Resumption of treatment**

Fourteen patients resumed treatment after an average IFX-free interval of 18 weeks (range 8 to 27 weeks). Nine patients started IFX within one month after delivery, 4 patients within 2 months and one patient postponed treatment until month 3 post partum. Of these 14 patients, 4 resumed treatment within 12 weeks (time from the last infusion of 8, 9, 9 and 11 weeks, respectively). Mean follow-up after delivery was 12 months (range 2 to 25 months). During this follow-up, one patient experienced an allergic reaction at the resumption of IFX treatment after a drug holiday of 22 weeks. The allergic reaction occurred at second infusion of IFX which was postponed to week 11 from the first infusion due to mastitis. Two patients developed side-effects to IFX after 10 and 12 months of the treatment, respectively, and had to switch to adalimumab. All patients who resumed treatment remained in remission during the follow-up.

The reasons not to resume IFX in the remaining three patients were as follows: one patient developed auto-immune hepatitis with liver failure post partum, with presumable etiology of IFX-induced hepatitis, this case has been reported elsewhere; two patients remained stable and decided not to resume the treatment. All three patients had quiescent disease during the follow-up of 11, 12 and 13 months, respectively; one without treatment and two on mono-therapy with azathioprine.
Pregnancy outcomes
There was one spontaneous miscarriage at week 6 and 17 live births with average gestational age at delivery of 39 weeks (range 32 to 42). Average birth weight was 3361 grams ranging from 2200 to 4210. In sixteen children no congenital malformations were found. One child born to the mother using co-medication with methotrexate peri-conceptionally had polydactyly. This case was reported previously as stated in the Patients & Methods section.

Effect of the timing of IFX discontinuation on the IFX levels in the newborns
The cord blood was collected from 12 newborns. Overall mean IFX level was 6.4±SEM 1.6 µg/mL. The mean cord blood IFX level in the early discontinuation group was significantly lower than in the group with 10 or less weeks from the last infusion to delivery (2.8±SEM 1.1 µg/mL and 10±SEM 2.3 µg/mL, respectively, p=0.02), Figure 2. The levels of IFX in the cord blood correlated significantly with the gestational week of IFX discontinuation (Spearman’s rho=0.71, p=0.01), Figure 3.

Table 1. Basic demographic characteristic of pregnant infliximab-using IBD patients

<table>
<thead>
<tr>
<th>Nr of patients</th>
<th>17</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age (range)</td>
<td>29 years (18-37)</td>
</tr>
<tr>
<td>Average duration of the disease (range)</td>
<td>7 years (1-13)</td>
</tr>
<tr>
<td>Crohn’s disease/Ulcerative colitis</td>
<td>12/5</td>
</tr>
<tr>
<td>Co-medication</td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>6</td>
</tr>
<tr>
<td>5-aminosalicylates</td>
<td>1</td>
</tr>
<tr>
<td>thiopurines</td>
<td>9</td>
</tr>
<tr>
<td>corticosteroids</td>
<td>2</td>
</tr>
<tr>
<td>methotrexate*</td>
<td>1</td>
</tr>
<tr>
<td>Dose infliximab</td>
<td></td>
</tr>
<tr>
<td>5 mg/kg every 8 weeks</td>
<td>14</td>
</tr>
<tr>
<td>5 mg/kg every 6 weeks</td>
<td>1</td>
</tr>
<tr>
<td>10 mg/kg every 8 weeks</td>
<td>1</td>
</tr>
<tr>
<td>10 mg/kg every 6 weeks</td>
<td>1</td>
</tr>
</tbody>
</table>

*peri-conceptionally, treatment stopped immediately after pregnancy was confirmed

Figure 1. Effect of the infliximab discontinuation during pregnancy on disease activity and complications at the re-treatment

1 spontaneous miscarriage (gestational week 6)

17 live births (94%)

Disease in remission? 17 pts

YES
12 pts (71%)

NO
5 pts (29%)

• infliximab discontinued (gestational weeks 18 to 27)
• infliximab continued (till gestational weeks 30 to 34)

• no relapse
• one patient developed allergic reaction at the resumption of the treatment post partum*

*allergic reaction at the second infusion of IFX after drug holiday of 22 weeks; second infusion postponed to week 11 due to mastitis
**Figure 2.** IFX levels in the cord blood are significantly reduced in the group with more than 10 weeks time to delivery from IFX discontinuation (early discontinuation) with average cord blood IFX level in early discontinuation group 2.8±SEM 1.1 µg/mL vs. 10±SEM 2.3 µg/mL in late discontinuation group.

*Two-sided, unpaired t-test

**Figure 3.** The IFX levels in the cord blood positively correlate with the gestational week of the IFX discontinuation. Spearman’s rho=0.71, p=0.01

---

**DISCUSSION**

In this study, we prospectively evaluated the impact of infliximab discontinuation during pregnancy on the course of inflammatory bowel disease. We found that discontinuation prior gestational week 30 in patients with quiescent disease is feasible and safe in terms of disease control. In addition, this approach significantly reduces the neonatal exposure to infliximab.

As in all procedures related to the pregnancy, there are considerations to be made in the mother’s as well as child’s interest. With respect to the mother, the main concerns are disease control and risk of side effects at the treatment resumption after being off IFX for more than 12 weeks. Interestingly, three patients in our cohort did not resume treatment and remained in remission during the remaining 11 and 13 months of the follow-up, respectively. This is in line with previous observations that pregnancy may in some patients modify the disease course with lower risk of flares in the years after pregnancy. In addition, there are clear indications that pregnancy in general represents a specific condition down-regulating the inflammatory responses in order to induce transient tolerance of the foreign body of the foetus. Thus, with a little exaggeration, one may think of pregnancy as ‘a natural immunomodulator’ with stabilizing effect on the disease on-going for several months after delivery. With this concept, the question arises whether it is beneficial for the patient to resume the treatment with anti-TNF post partum while being in remission instead of continuing the drug holiday started during pregnancy and to resume the treatment only when the disease flares again. Currently, there are no data to favour either of the approaches but keeping in mind the risks related to long-term anti-TNF use this opportunity of drug holiday may seem attractive for some female IBD patients. In addition, as shown recently, the titers of antibodies against infliximab formed during the treatment decrease with the longer duration of the drug holiday and become undetectable within one year after cessation of the treatment. Thus, prolonging the drug holiday might also be beneficial for the patient in terms of immunogenicity.

This immunogenic complication of the treatment resumption represents a second concern on the maternal side. We have observed one allergic reaction that could have been co-generated by a delayed administration of a second infusion at week 11. Probably, the right approach would be to try to minimalize the risk of allergic reaction at the treatment resumption by tight infusion schedule with conservation of the interval of eight weeks and by starting with an induction scheme as it is also the case with the use of infliximab in general.

Third, another risk of the treatment resumption related also to immunogenicity is a gradual loss of response resulting from antibody formation against IFX. During the mean follow-up of one year, we did not observe any case of loss of response, with all patients resuming the treatment remaining in remission.
Concerning the child, we found that IFX infusion ten weeks or less prior delivery led to cord blood drug levels considered therapeutic in adults. It has been shown that IFX can persist in the cord blood up to ten weeks after delivery if IFX had a normal antibody response to vaccinations with non-viable antigens. Two small case series together including 10 children reported that children born with therapeutic levels of IFX had a normal antibody response to vaccinations with bacterial antigens, but larger studies in adults showed that antibody formation to some viral antigen vaccines may be hampered by anti-TNF treatment. It is unknown whether this is the case for children vaccinated in the presence of IFX.

Second, therapeutic IFX levels may interfere with induction of a normal antibody response to vaccination of non-viable antigens. Two small case series together including 10 children reported that children born with therapeutic levels of IFX had a normal antibody response to vaccinations with bacterial antigens, but larger studies in adults showed that antibody formation to some viral antigen vaccines may be hampered by anti-TNF treatment. It is unknown whether this is also the case for children vaccinated in the presence of IFX.

Third, the theoretical concerns of late consequences of the early exposure of an immature immune system to the modulatory effect of TNF-α blockade are, in our view, justified. The follow-up of children born from pregnancies exposed to anti-TNF is still too short to allow firm conclusions on the (lack of) oncogenic, immunogenic and allergic potential of this interference of the developing immune system with a foreign protein blocking one of the main signalling pathways of immune cells. In addition, a long-term imprinting of the specific B cell repertoire by maternal antibodies has been demonstrated in mice, which, if true for the humans as well, would have important consequences for the shaping of humoral immune response repertoire in children with intra-uterine exposure to IFX.

Although the levels of IFX assessed in the cord blood were significantly reduced by discontinuation of IFX more than 10 weeks prior delivery, they were still detectable. Thus, one must keep in mind that even with this approach of discontinuing IFX during the second trimester, children are exposed to IFX in utero and in the early postnatal period. Therefore, the next step should probably be to test the feasibility of discontinuing IFX even earlier, i.e. at the beginning of the second trimester, around week 16, in order to completely eliminate the early postnatal exposure to IFX.

In conclusion, this study shows that discontinuation of IFX treatment early during pregnancy substantially limits postnatal exposure of the child to IFX and that this approach is safe for the mother in terms of disease control and risks related to resumption of the treatment. Therefore, keeping concerns about the early exposure to IFX related risks for the child in mind; we recommend considering the discontinuation of IFX treatment in patients with quiescent disease during the second trimester. The exact protocol of the resumption of the treatment remains to be determined.

References

Chapter 10

Pregnancy and AntiTNF Use: Safety is not yet Assured

Christine J van der Woude & Zuzana Zelinkova

Adapted from Inflamm Bowel Dis Monit 2011; 12(2):55-56
IBD typically affect patients in their reproductive years and the reproductive issues represent an important concern to female IBD patients. IBD patients choose to remain childless more often than non-IBD controls, mostly owing to the fear of the side-effects of the medication on the unborn child. The decision to continue the medication that is maintaining these chronically ill patients in remission during the periconceptional period and pregnancy is difficult as the data on some drugs used in IBD in this setting are limited, and evidence-based counseling is scarcely possible. It should be emphasized that a quiescent disease state is key to a favorable pregnancy outcome; therefore, a careful appreciation of the safety of the medication necessary to maintain the remission is crucial in the management of IBD patients with reproductive intentions. The data gathered thus far suggest that most of the drugs used to maintain IBD in remission are safe with regards to teratogenicity, except for methotrexate. However, the situation regarding the use of anti-TNF agents during pregnancy is more complicated compared with the use of small molecules.

Of the various anti-TNF agents available in IBD, infliximab has been used the longest; thus, most experience with anti-TNF use during pregnancy is with this biologic. Short-term data have found no important teratogenicity issues but it is important to emphasize that the size of the studied population is small, the reports are mostly retrospective, and the majority of children born from these pregnancies were exposed to infliximab only during the first trimester. The pharmacokinetics of infliximab and adalimumab differ substantially, with different macodynamic and pharmacokinetic properties of anti-TNF agents, the simple demonstration of non-teratogenicity is not sufficient to consider these agents as safe for use by mothers-to-be. Therefore, in the current scenario of a lack of long-term follow-up data, the limitation of placental transfer with infliximab at around week 32. However, in our case series of four patients, we have documented that this approach still leaves the children with significant levels of infliximab measured in cord blood. In order to limit the placental transfer to zero, the discontinuation of the treatment should take place much earlier, probably prior to gestational week 24. Therefore, we suggest limiting the use of infliximab during pregnancy to only the first two trimesters. This approach has been demonstrated to be feasible and safe in terms of disease control and no allergic reactions have been observed at the resumption of the treatment after a period of more than 12 weeks.

For adalimumab, data are even more limited. Again, in the registry data available so far, no teratogenicity issues have been observed; however, as expected based on the molecular structure of adalimumab with a functional Fc component, placental transfer has been documented. The pharmacokinetics of infliximab and adalimumab differ substantially, with different dosing regimens and biological half-times. Therefore, no advice can be given on the timing of discontinuation of anti-TNF agents in general; data for each agent need to be collected in order to make specific estimations. Currently, there are no such data available for adalimumab and therefore at this time no evidence-based recommendations can be made with regards to the timing of its discontinuation during pregnancy.

In conclusion, the use of anti-TNF agents in the periconceptional period and during pregnancy does not seem to carry a risk of congenital malformations. However, given the basic pharmacodynamic and pharmacokinetic properties of anti-TNF agents, the simple demonstration of non-teratogenicity is not sufficient to consider these agents as safe for use by mothers-to-be. The major concern regarding the use of anti-TNF agents during pregnancy is their placental transfer leading to therapeutic levels in the newborns. This early exposure of the immature immune system to the potent effects of TNF-α blockade might result in as yet unpredictable long-term complications; therefore, in patients with quiescent disease, these agents should be discontinued early in the pregnancy in order to limit this exposure to the lowest possible level.
REFERENCES


Chapter 11

Summary
Nederlandse Samenvatting
General Discussion
SUMMARY

Central to this thesis is the notion that inflammatory bowel disease (IBD) is different between females and males in important ways influencing genetics and transmission to the next generation, manifestation of the disorder, perception of disease severity whereas the different roles of males and females in human reproduction has important consequences for treatment. A theoretical foundation for the notion that IBD has important gender and/or sex specific aspects is provided in Chapter 1, which includes apart from clinical intuition, an extensive review of the existing reports in this field, and an appreciation of the physiological differences between the immune systems of males and females. The aim of this thesis is to explore these gender and/or sex specific aspects over the entire width of the field, with the main objective to provide a comprehensive study of all aspects involved.

First, in a patient empowerment study (Chapter 2), an assessment of the differences between men and women in the perception of health-related quality of life (HRQOL) is provided that confirmed previous observations of a lower HRQOL in women in general. The additional value of this study relies in its patient empowerment character with selected questions generated by the patients themselves, enabling a different view on this issue compared with studies using standardized questionnaires. This design enabled us to further characterize the respective domains in which men and women with IBD report different concerns. More specifically, as a result of the disease, women feel unable to meet their expectations with regards to study/work, household, partnership, and parenting. In order to translate these observations into a personalized approach used in everyday practice, these gender-specific differences cannot be ignored when an overall assessment of patients’ well-being is attempted and thus prompt the development of a gender-specific quality of life questionnaire. In addition, the root causes leading to these gender-specific differences should be further explored in detail taking into account both social (i.e. gender-related) and biological (sex-determined) determinants possibly involved. Together with the more theoretical framework provided in chapter 1, this part of the thesis makes likely that gender and/or sex is an important factor when considering IBD the consequences of this phenomenon are subsequently explored.

Biological factors underlying sexual dimorphic disease presentation are studied in part II of this thesis. In particular, in Chapter 3 the issue of female predominance in familial IBD is addressed by studying the imprinting pattern in families where both the parent and the child/children are affected. In this study, we show that female predominance in familial IBD is at least partially due to maternal imprinting, a phenomenon with an unclear mechanism, suggesting the existence of epigenetic rather than genetic factors in sex-specific IBD pathogenesis. The existence of this sex-specific pathogenicity suggested by maternal imprinting might have important consequences for the understanding of the disease genesis itself and necessitates further exploration. The role of sex hormones on one side, as well as the direct involvement of X-chromosome abnormalities on the other side, are areas worth investigating further in order to define in detail how sex influences the immune responses leading to IBD and may well have consequences for other diseases as well.

Chapter 4 deals with the sexual dimorphism of immune responses that is not related only to disease pathogenesis, but also presumably influences therapy outcomes once the disease is manifest. More specifically, we show that female patients are at higher risk of developing allergic reactions to treatment with monoclonal antibodies against tumour necrosis factor alpha (anti-TNF), which in turn limits the portfolio of treatment modalities available for female IBD patients. This female-specific increased risk of anti-TNF immunogenicity provides evidence for the existence of sex-related differences in immune regulation and thus has important consequences for the management of immune-mediated diseases, females requiring probably a more aggressive approach. Therapeutic possibilities in the field of IBD are still very limited; therefore, maintaining the ability to continue effective treatment by preventing adverse drug reactions is crucial for individual patients. Therefore, further research in this field on options to limit these reactions in females through sex-specific therapeutic approaches and the mechanism of this dimorphism is urgently needed.

The third part of this thesis is dedicated to a specific area of gender research; reproduction. As shown in Chapter 5, reproductive wish represents an important factor interfering with the management of IBD patients. Two thirds of IBD patients with active reproductive wish use a medication with an unclear safety profile in this setting. In addition, one third of these patients need a change in their therapeutic approach because their medication is contra-indicated for the mothers/fathers-to-be or because of uncontrolled disease. Thus, the physicians caring for IBD patients should take active role in providing counselling for these patients in order to limit the possible complications resulting from negative impact of the medication and/or uncontrolled disease on the unborn child. On the other hand, data on the safety of particular medications used peri-conceptually and during pregnancy and lactation are limited and both, the researchers and clinicians need to be creative in their attempts to safeguard the safety of a particular medication use during procreation. Such an approach is demonstrated on the case of azathioprine, one of the agents used for the maintenance of IBD. This drug has questionable safety during lactation. In Chapter 6, we show that based on drug levels monitored in the newborns, azathioprine can be used safely during lactation.

Of all the drugs used in IBD, the use of anti-TNF agents during the peri-conceptual period and pregnancy is the most complicated issue given the lack of data and specificities on the pharmacokinetics of IgG class antibodies to which these agents belong. In Chapter 7, it is demonstrated that the use of one of the anti-TNF agents, infliximab, beyond the second trimester leads to significant placental transfer of the agent, with neonatal exposure to levels of this agent that would be considered therapeutic in adults. The concerns about the potential
consequences of this early exposure of an immature immune system to powerful blockade of one of the key signalling pathways in the immune system are substantial, as summarized in Chapter 10. Therefore, in order to limit exposure, early discontinuation of infliximab during pregnancy is recommended. However, no data on the safety of this approach for the mother are available. In Chapter 9, the outcomes are shown of the prospectively-followed pregnancies of mothers using infliximab who discontinued treatment in a controlled manner during the second trimester. We demonstrate that in the case of quiescent disease, discontinuing infliximab during the second trimester does not lead to higher risk of relapse and/or adverse reactions when treatment is resumed, and is safe for the mother. Additionally, this approach helps significantly reduce neonatal exposure to infliximab. These observations provide the basis for recommending infliximab for use during pregnancy in Chapter 10.

The use of anti-TNF agents by male patients who wish to procreate represents an important and controversial issue as well and available data on this subject are even more limited than for female patients. In the case of another anti-TNF agent, adalimumab, the data on its safety for fathers-to-be are lacking. To address the need for data on this issue, a prospective assessment of the influence of adalimumab on semen composition was performed and pregnancies with indirect exposure to adalimumab in the outpatient clinic population were identified retrospectively. The results of this study, which showed no influence of adalimumab on semen composition and favourable pregnancy outcomes of men treated with adalimumab, are discussed in Chapter 8.

In summary, differences based on gender and/or sex of various aspects of IBD were assessed. Surprisingly, in an attempt to achieve the original aim, i.e. to identify issues specific to men and women, respectively, we ended by accumulating evidence almost exclusively for female issues. The results reported in this thesis support the idea that male and female IBD is not completely the same disease, maybe with the most dramatic differences when considering reproduction, but also regarding the quality of life, disease pathogenesis, and complications of therapy. Thus, using slightly exaggerated philosophical jargon, based on the observations gathered in this hypothesis-generating research, further basic and clinical research in IBD is called for to address the under-investigated notion of an IBD ‘du deuxième sexe’.

In this thesis we aimed to investigate whether the sex of the IBD patient has an influence on the pregnancy outcome. In the first part of this thesis (Sex, Hoofdstuk 3 en 4), we presented results on the differences in pregnancy outcomes between male and female IBD patients. In the second part of this thesis (Sex, Hoofdstuk 3), we focused on the differences in pregnancy outcomes between male and female IBD patients. We demonstrated that the use of anti-TNF agents by male patients who wish to procreate represents an important and controversial issue as well and available data on this subject are even more limited than for female patients. In the case of another anti-TNF agent, adalimumab, the data on its safety for fathers-to-be are lacking. To address the need for data on this issue, a prospective assessment of the influence of adalimumab on semen composition was performed and pregnancies with indirect exposure to adalimumab in the outpatient clinic population were identified retrospectively. The results of this study, which showed no influence of adalimumab on semen composition and favourable pregnancy outcomes of men treated with adalimumab, are discussed in Chapter 8.

In summary, differences based on gender and/or sex of various aspects of IBD were assessed. Surprisingly, in an attempt to achieve the original aim, i.e. to identify issues specific to men and women, respectively, we ended by accumulating evidence almost exclusively for female issues. The results reported in this thesis support the idea that male and female IBD is not completely the same disease, maybe with the most dramatic differences when considering reproduction, but also regarding the quality of life, disease pathogenesis, and complications of therapy. Thus, using slightly exaggerated philosophical jargon, based on the observations gathered in this hypothesis-generating research, further basic and clinical research in IBD is called for to address the under-investigated notion of an IBD ‘du deuxième sexe’.

NEDERLANDSE SAMENVATTING

In dit proefschrift wordt nader ingegaan op de invloed die het geslacht kan hebben op het verloop van inflammatoire darmziekten. Het overzicht van de huidige literatuur in Hoofdstuk 1 geeft onderbouwing voor de keuze van de in dit proefschrift bestudeerde aspecten ten aanzien van de relatie geslacht en IBD.

Hoofdstuk 2 laat de verschillen zien in kwaliteit van leven tussen mannelijke en vrouwelijke IBD patiënten. Het onderzoek is opgezet als een ‘patient empowerment’ studie: patiënten hebben de gebruikte vragenlijst zelf ontwikkeld. We tonen aan dat vrouwelijke IBD patiënten significant meer negatieve invloed van de ziekte op hun kwaliteit van leven ervaren vergeleken met mannelijke IBD patiënten. Dit is in overeenstemming met eerder gepubliceerde studies. Deze kwaliteit van leven bij vrouwen wordt vooral negatief beïnvloed door beperkingen op het terrein van studie/werk, huishoudelijke bezigheden, relatie met de partner en het moederschap. Wij beschrijven in deze studie voor het eerst dat geslachtsgerelateerde verschillen in deze domeinen bepalend zijn voor de kwaliteit van leven van IBD patiënten. Onze conclusie is dan ook dat het bepalen van de kwaliteit van leven bij IBD patiënten moet worden aangepast door introductie van geslachts specifieke domeinen. Daarnaast moet de aard van de ziekte-gelijketerelateerde verlaagde kwaliteit van leven bij vrouwen verder onderzocht worden om te kunnen bepalen of deze puur door socio-economische factoren wordt beïnvloed (gender-related) dan wel of door het verschil is te verklaren in de biologie van vrouwen en mannen.

Deze laatste vraag is verder bestudeerd in het tweede gedeelte van dit proefschrift (Sex, Hoofdstuk 3 en 4). In Hoofdstuk 3 laten we zien dat in families waarbij IBD voorkomt bij ouder(s) en kind(eren), dit significant vaker het geval is als er een IBD moeder is vergeleken met families met een IBD vader. Deze vinding suggereert dat er voor de overerving van IBD de klassieke Mendelien regels niet gelden. Waarschijnlijk spelen epigenetische factoren een belangrijke rol in de overerving bij IBD. Het is bekend dat IBD vrouwen een meer gecompliceerd beloop van de ziekte hebben in vergelijking met IBD mannen. Of hierbij dezelfde epigenetische factoren die ook een rol spelen in het ontstaan van de ziekte belangrijk zijn moet uit toekomstig onderzoek blijken.

Specifieke ziekte karakteristieken bij vrouwen, zoals een meer gecompliceerd beloop en het vaker voorkomen van extra-intestinale manifestaties bij IBD vrouwen duiden, samen met onze resultaten beschreven in Hoofdstuk 3, op vrouw specifieke pathogenetische mechanismen wat in theorie ook invloed zou kunnen hebben op de effectiviteit en bijwerkingen van de medicamenteuze behandeling. Deze geslachts specifieke uitkomsten van therapie zijn verder onderzocht in Hoofdstuk 4. In dit hoofdstuk wordt beschreven dat er geslachtsspecifieke verschillen zijn in het bijwerkingpatroon van medicatie voorgeschreven voor de behandeling van IBD. Het blijkt dat vrouwen vaker allergische reacties vertonen op tumor necrosis factor
alpha inhiberende medicatie (anti-TNF). Deze reacties leiden uiteindelijk tot het staken van deze medicatie. Hierdoor worden de therapeutische mogelijkheden voor IBD vrouwen significant gelimiteerd. Om de therapie effectiviteit te maximaliseren is het dus van belang onze bevinding in een prospectief onderzoek te bestuderen. Bij bevestiging van onze resultaten zal vervolgens een vrouwenspecifieke strategie voor de behandeling van IBD ontwikkeld kunnen worden.

IBD en reproductie is onderdeel van het derde gedeelte van dit proefschrift. In Hoofdstuk 5 wordt beschreven dat kinderwens in deze groep patiënten van invloed is op de therapeutische beslissingen. Echter deze beslissingen zijn soms moeilijk te nemen wegens het ontbreken van voldoende informatie over de veiligheid van het gebruik van bepaalde medicatie tijdens bevruchting, zwangerschap en borstvoeding. In Hoofdstuk 6 worden eerste resultaten getoond van monitoring van kinderen die borstvoeding krijgen van IBD moeders die azathioprine gebruiken. Wij vonden bij deze kinderen bij volledige borstvoeding onmeetbare azathioprine spiegels.

Veel informatie is er vooral niet over de veiligheid van het gebruik van anti-TNF tijdens conceptie en zwangerschap. Hoofdstuk 7 laat zien dat er duidelijke blootstelling is van de kinderen geboren van IBD moeders die tijdens zwangerschap behandeld zijn met een van deze middelen, infliximab. Onze metingen in het navelstrengbloed tonen aan dat deze kinderen blootgesteld worden aan significante hoeveelheden infliximab. Om deze blootstelling te beperken, zou infliximab tijdig tijdens zwangerschap gestopt moeten worden. Het is echter belangrijk voor de uitkomst van het kind om de ziekte tijdens zwangerschap in remissie te houden. In Hoofdstuk 9 laten we zien dat bij vrouwen met een rustige IBD het veilig is om infliximab in de tweede trimester te staken en dat deze aanpak significant de blootstelling van kinderen aan dit middel verminderd. Samenvattende opmerkingen met overzicht van alle tot nu toe gepubliceerde data over het gebruik van biologicals tijdens zwangerschap bij patiënten met IBD en het voorstel van een behandelschema zijn in Hoofdstuk 10 beschreven.

De informatie over de veiligheid van het gebruik van biologische middelen door mannen met kinderwens is nog beperker. Slechts case series zijn gepubliceerd voor infliximab en data over het effect van adalimumab, ontbreken geheel. Hoofdstuk 8 bevat een analyse van de veranderingen in het zaad bij mannen met IBD die tijdens de behandeling met adalimumab optreden. Uit onze analyse blijkt dat adalimumab geen of nauwelijks effect heeft op de samenstelling van het zaad, echter zal nog verder onderzoek moeten tonen of er ook geen DNA schade optreedt.

Samenvattend, de resultaten van de studies beschreven in dit proefschrift laten zien dat het geslacht een belangrijke rol speelt bij inflammatoire darmziekten. Dit geldt voor verschillende aspecten die effect hebben op de pathogenese, ziekte beloop en de therapeutische strategie die significant beperkt is in de periode van bevruchting, zwangerschap en borstvoeding.

Onverwacht is dat ook in de reproductie niet gerelateerde aspecten vooral vrouwelijke factoren naar voren komen die van invloed zijn. Om op maat gemaakt therapeutische strategie te ontwikkelen voor vrouwen en mannen is verder onderzoek noodzakelijk om de biologische basis voor deze geslachtgerelateerde verschillen te definiëren.
GENERAL DISCUSSION

In the recent years, a significant effort has been invested in the research on the contribution of gender/sex to human health. This effort resulted in a publication explosion of the papers dealing with the sex differences in various areas of (bio)medical research and several new journals have appeared devoted specially to gender/sex-related issues.

In general, new insights have been gained into the role of gender/sex in physiological and pathophysiological processes such as pain perception, immune responses, metabolism and pharmacokinetics and pharmacodynamics. In addition, in several areas of clinical medicine, the specific contribution of the gender/sex to the disease presentation, pathogenesis, and course as well as therapy outcomes has been recognized. However, despite of all this considerable development in the field of gender-related research, the concept of gender-specific medicine is hardly visible in clinical practice.

The implementation of the results of gender/sex-related studies in the clinical practice is hampered by several factors related to specific methodological issues of this area of research. From the public health care point of view, establishing of the concept of gender-specific care needs to be justified by clear pathogenetic explanation of a given gender/sex-related phenomenon. Without understanding the underlying mechanism by which gender/sex contributes to the respective pathogenetic processes, no gender-specific interventions can be successful. Yet, for most of the clinically observed differences between male and female patients, the explanation of molecular mechanism is lacking.

Most of the studies dealing with the sexual dimorphism in clinical medicine are descriptive population studies. In these studies, specific health outcomes, such as health-related quality of life, complicated disease course or unfavourable therapy outcomes are studied in male and female patients, respectively; and relative risks for each particular outcome are calculated for both sexes. The observed differences can be determined by both, gender- and sex-related factors. Gender-related factors are derived from societal and cultural context in which women and men deal with the burden of the disease, are exposed to different environmental factors, have gender-specific socio-economic status and occupations. Sex-determined differences in various health outcomes result from sex-specific biological processes, e.g. X-chromosome abnormalities, sex-steroids sensitive physiology of. Once the difference between male and female patients for a particular health outcome is described and, ideally, validated in several independent populations the attribution to gender-related and/or sex-determined determinant represents a crucial step for further mechanistic research. In addition, development of proper therapeutic intervention is also highly dependent on the recognition of the relative contribution of gender and sex to the particular health outcome. The distinction between gender relations and sex-linked biology is, however, not always methodologically possible as they can represent co-founder for each other or, depending on a particular study context, can act as sole, independent or synergistic determinants. Therefore, for the creation of gender- and sex-specific concept of a particular disease, a description of one isolated phenomenon is not sufficient. Instead, the evidence gained from comprehensive studies dealing respectively with the role of gender/sex in the pathogenesis, disease presentation, course and therapy outcome must be integrated in the formulation of the hypothesis that will further be explored in the mechanistic studies. In the present work, we showed that female IBD patients have a lower health-related quality of life (HRQOL). By analysing the differences between men and women with IBD in the perception of the respective domains determining HRQOL we found that the lower HRQOL in women was related to societal factors as well as to biologically-determined restrictions in physical performance. In further studies on sex-specific genesis and therapy outcome of IBD, we found additional arguments for the biological contribution of female sex to lower HRQOL and previously reported differences in the disease phenotype between male and female patients. Thus, by studying these at the first sight unrelated subjects, we aimed at proceeding towards an integrated view on the gender and sex-specific concept of IBD.

One of the specific areas greatly influenced by gender and sex is related to reproduction. Family planning of chronically ill patients represents an important factor interfering with the medical management of these mothers- and fathers-to-be. Despite the obvious need for evidence-based approach in this specific clinical setting, the available body of evidence in this area is extremely limited. For the use of medication during conception and pregnancy, compromise between the risks of the medication versus the risks of uncontrolled disease for the unborn child is sought. The final opinion on the medication safety in this setting is formed based on the animal studies and data from pregnancy registries and case-control studies. Animal models have a low predictive value for the teratogenicity in human situation and the pregnancy registries and case-control studies are prone to important selection and reporting bias. In addition, considering the incidence of chronic disorders among females in reproductive age, an adequate sample size in order to detect unfavourable effects can hardly be achieved.

On the other hand, in this situation of the limited possibilities to gather quality data allowing development of evidence-based guidelines, risks for the mother-to-be and for the unborn child can both be minimalized using different methodological approach. First, good quality pharmacokinetic studies, although performed on the limited number of patients can provide important tool to safeguard the safety of the medication use during pregnancy for the child. In the part III (Reproduction) of this thesis, we show the results of such an approach. By using the drug monitoring in children born to mothers with IBD treated with immunosuppressive and biological therapy during pregnancy and lactation, we were able to minimize the risk of the exposure of the (unborn) child to these drugs. Second, in immune-mediated disease, such as IBD, the immunological changes related to the pregnancy may result in a modulation of the disease course and therapy response. Therefore, the need for the immune suppression during
pregnancy and in the early post partum period might substantially differ from the pre-pregnancy state. In this work, we show that it is possible to safely discontinue the treatment with one biological agent, infliximab, minimizing thereby the exposure of the unborn child to this drug. In addition, the patients were able to discontinue the treatment for an unusually long period which suggests that the changes in immune response induced by pregnancy might extend their effect beyond the gestational period. Concluding, a proper use of the pharmacological armamentarium and understanding the specificities of the disease course during pregnancy can provide a valuable input for the development of therapeutic approach for pregnant women with chronic illness. In addition, in immune-mediated disease, the immunological changes during pregnancy and their impact on the disease course need to be evaluated as they may influence the management of the patient beyond the pregnancy as well.

In conclusion, in order to create a personalized medicine with gender-specific approach as its integrated part, the relative contribution of gender and sex to the observed male- and female-specificities of a particular health outcome needs to be determined. For this purpose, multidisciplinary research that will analyse the role the gender/sex play in all aspects of particular disease, including the management of the pregnant patient, is necessary.
In 2003 kwam ik naar Nederland op zoek naar ‘adventures of expat life’. Ik hoopte dat ik misschien op een dag de gelegenheid zou krijgen om mee te mogen doen in de wereld van translationeel onderzoek op het gebied van IBD dat in dit land zo sterk is. Deze luxe werd mij ook aangeboden en ik heb elke dag kunnen genieten van de combinatie van onderzoek en kliniek. Dit proefschrift is een verslaglegging van mijn Nederlandse en medisch-wetenschappelijke avontuur wat zonder de hulp van veel mensen niet mogelijk zou zijn geweest; slechts een aantal van deze mensen zal ik hier persoonlijk kunnen bedanken.


Beste Ernst, je hebt me altijd je vertrouwen gegeven en mij ondersteund in al mijn klinische en wetenschappelijke ondernemingen. Jouw bijdrage aan alle papers die we tot nu toe samen hebben gepubliceerd is priceless. Het lezen van een door jouw gecorrigeerde paper met je scherpe blik en constructieve opmerkingen was altijd een zeer plezierige leermoment voor mij. Daarnaast was je ook altijd bereid om een klinisch probleem met mij te nemen wanneer ik er met mijn geringe klinische ervaring in de MDL er niet uit kon komen. Bedankt voor alles, zonder jouw was mijn nederlandse escapade heel anders gelopen.

Beste Maikel, je was een van de eerste mensen die ik in wetenschappelijk Nederland heb ontmoet. Jouw beroemde experiment schema’s geschetst op servetjes van de koffiekamer waren altijd bereid om die rare aanvragen van Zuzana te accepteren voor weer een scopie bij een zwangere die ook nog dezelfde dag moest, of een extra buis bloed af te nemen, of even een opname te regelen. En al die poli’s die altijd uitliepen en de ontevredenheid van patiënten die door jullie professionally beantwoord werden. En dit allemaal met een glimlach. Bedankt voor deze zeer prettige samenwerking!

Dan mijn co-promoter, dr. C. Janneke van der Woude. Beste Janneke, hier komt het, onderzoek over ‘die vrouwen’, het idee dat ons door de jaren heen heeft achtervolgd. Ik hoop dat jij bij dit boekje ook veel ander werk samen gedaan. Ik heb van deze samenwerking veel geleerd en er bovendien ook veel plezier aan beleefd. Nogmaals bedankt, ik mis jullie!

Allereerst mijn co-promoter, dr. C. Janneke van der Woude. Beste Janneke, hier komt het, onderzoek over ‘die vrouwen’, het idee dat ons door de jaren heen heeft achtervolgd. Ik hoop dat jij bij dit boekje ook veel ander werk samen gedaan. Ik heb van deze samenwerking veel geleerd en er bovendien ook veel plezier aan beleefd. Nogmaals bedankt, ik mis jullie!

In het bijzonder wil ik mijn sparingpartners van de kinder MDL bedanken, dr. Lissy de Ridder en prof. Hankje Escher. Beste Lissy en Hankje, het was altijd een genoegen om met jullie medische en wetenschappelijke maar ook persoonlijke discussies te houden. We hebben los van dit boekje ook veel ander werk samen gedaan. Ik heb van deze samenwerking veel geleerd en er bovendien ook veel plezier aan beleefd. Nogmaals bedankt, ik mis jullie!

Tijdens het tot stand komen van dit boekje is er een geweldig initiatief ontwikkeld, The Dutch Delta IBD group. Zonder de inzet van alle deelnemers waren de studies naar de zwangerschapssituaties bij de behandeling met antiTNF niet mogelijk geweest. Bedankt, iedereen voor jullie actieve bijdrage en ook jullie vertrouwen.

Dit boekje is tot stand gekomen tijdens mijn opleiding tot MDL-arts. In de jaren van mijn opleiding hebben veel mensen om me heen bijgedragen aan mijn gedachternvorming over bepaalde onderwerpen die beschreven staan in dit proefschrift. De informele discussies met de stafleden en assistenten in de koffiekamer of het verslagruimte van de endoscopie hebben me vaak geïnspireerd om mijn onderzoeksvraag beter te formuleren. Naast deze genoemde brainstorm sessies was het een zeer plezierige tijd waarbij ik ook nog op jullie steun kon rekenen bij het regelen van de lastige logistiek van veel van mijn studies. Hiervoor veel dank aan alle stafleden en assistenten.

Mijn paranimfen, Rachel en Claudia. Ik ben zo blij dat jullie deze taak hebben geaccepteerd! Ik neem hier de gelegenheid om jullie te bedanken niet alleen maar voor jullie steun bij mijn promotietraject maar ook voor alle gezellige koffies, biertjes, uitjes en kletsavonden die we tijdens onze opleidingstijd samen hebben doorgebracht. Ik miss jullie en hoop echt dat we binnenkort weer samen gaan borrelen, waar dan ook!

Niks in dit boekje was mogelijk geweest zonder onze geweldige poli assistenten en de verpleegkundigen op de afdelingen endoscopie, dagbehandeling en verpleegafdeling. Jullie waren altijd bereid om die rare aanvragen van Zuzana te accepteren voor weer een scopie bij een zwangere die ook nog dezelfde dag moest, of een extra buis bloed af te nemen, of even een opname te regelen. En al die poli’s die altijd uitliepen en de ontevredenheid van patiënten die door jullie professionally beantwoord werden. En dit allemaal met een glimlach. Bedankt voor deze zeer prettige samenwerking!

In het bijzonder wil ik mijn sparingpartners van de kinder MDL bedanken, dr. Lissy de Ridder en prof. Hankje Escher. Beste Lissy en Hankje, het was altijd een genoegen om met jullie medische en wetenschappelijke maar ook persoonlijke discussies te houden. We hebben los van dit boekje ook veel ander werk samen gedaan. Ik heb van deze samenwerking veel geleerd en er bovendien ook veel plezier aan beleefd. Nogmaals bedankt, ik mis jullie!

Tijdens het tot stand komen van dit boekje is er een geweldig initiatief ontwikkeld, The Dutch Delta IBD group. Zonder de inzet van alle deelnemers waren de studies naar de zwangerschapssituaties bij de behandeling met antiTNF niet mogelijk geweest. Bedankt, iedereen voor jullie actieve bijdrage en ook jullie vertrouwen.

Dit brengt me bij de patiënten. Heel veel dank aan alle patiënten die vertrouwen in ons hadden en actief mee hebben gewerkt aan onze studies. Zonder jullie bereidheid om mee te werken aan het onderzoek was onze huidige kennis veel beperkter geweest.

Een bijzondere positie in dit bedanktijde is voor onze IBD onderzoeksgroep zowel de klinische onderzoekers als de onderzoekers op het lab. Lauran, Alison, Cheima, Jasper, Hans,
Ayala, Evelien, Veerle, Yi en Judith, jullie hebben een enorme bijdrage geleverd aan dit boekje door jullie kritische opmerkingen bij onze meetings maar ook praktisch door jullie hulp bij de logistiek van de studies. Speciaal dank aan Colin, bedankt voor al je werk met de infliximab ELISA’s en actieve input bij het analyseren van de resultaten. Hierbij hoort ook een speciale dank aan Hanneke van Vuuren en Buddy Roovers voor de organisatie en het keurig verwerken van de navelstrengsamples.

De administratie is nooit mijn sterke punt geweest. Dat deze promotie eindelijk plaats vindt, mijn diplomas evaluatie rond is, alle formulieren op tijd zijn ingeleverd en het boekje in de brievenbus van iedereen beland is, daarvoor hoort mijn bijzondere dank aan Leonie. Bedankt voor alles Leonie, je bent super efficent en georganiseerd, zonder jouw was dit mij nooit gelukt.

En dan last but not least, onze bijzondere partners van de afdelingen Andrologie en Gynecologie. Dr. G.R. Dohle en prof. dr. E.A.P. Steegers, bedankt voor jullie steun bij de zorg voor onze IBD patiënten met een kinderwens in de dagelijkse praktijk.

Dan is er nog een groep mensen die niet direct aan dit boekje hebben bijgedragen maar wel bepalend zijn geweest voor mijn Nederlandse avontuur. Als eerste prof. Sander J. van Deventer en prof. Daan W. Hommes. Beste Sander en Daan, wanneer jullie mij niet hadden aangenomen in het AMClab was er waarschijnlijk niks van dit boekje tot stand gekomen. De twee jaren op het lab in het AMC waren een fantastische tijd voor mij. Ik hoop ook dat hetgeen wat ik van jullie heb mogen leren ook tot uiting is gekomen in dit proefschrift.

Dan wil ik nog in het bijzonder een andere inmiddels ook ex-amc-er bedanken, dr. Pieter C Stokkers. Beste Pieter, de IBD-ology wordt nog uitgewerkt en ik blijf je erbij betrokken houden, of je dat wil of niet! Bedankt voor het meedenken, de brainstormsessies maar vooral gezelligheid die je overal mee naartoe brengt.

And then the expat group, I feel that all the negative aspects of globalisation have been compensated by the fact that it brought you guys into my life. Sharon, Rob, Carlos, Megan, and (not so expats) Arjan and Oliver thanks for the great time we had and still have together.

A nakoniec moja malá rodinka, Meťko a Oli. Podľa starých Egyptianov je najváčším šťastím muža mať ženu peknú, milú a múdru. Najváčším šťastím ženy je... mať vás.
Zuzana Zelinková, née Detková, was born on April 18, 1974 in Bratislava, Czecho-Slovakia. She attended the grammar school of Laco Novomeský in Bratislava from which she graduated in 1992. In Bratislava, she continued her university studies at the Faculty of Medicine, Comenius University. After obtaining her medical diploma in 1998, she started her internal medicine training at the 3rd Department of Internal Medicine of University Hospital in Bratislava together with her PhD study at this department, under supervision of Prof. Viera Kupčová.

During her postgraduate medical training, she received a scholarship from L’Association Le Pont Neuf at the Department of Infectious Diseases, Hopital Bichat-Claude Bernard, Paris, France (head, Prof. Jean-Louis Vildé). In 2001, she took a ten months elective clerkship at the Department of Gastroenterology & Hepatology, Centre Hospitalier Universitaire Montpellier, France (head, Prof. Dominique Larrey). She returned back to Slovakia in 2002 and in the same year she successfully defended her thesis Cytokines in chronic viral hepatitis and inflammatory bowel disease at the Faculty of Medicine, Comenius University in Bratislava.

In 2003, Zuzana moved to the Netherlands where she worked in the Laboratory of Experimental Internal Medicine of Academic Medical Center, Amsterdam under supervision of Prof. Daan W. Hommes and Prof. Sander J. van Deventer. During this two years fellowship, she investigated the role of pathogen-associated molecular patterns in the pathogenesis of inflammatory bowel disease.

In 2006, she started her residency in gastroenterology, first at the Department of Internal Medicine, Ikaia Ziekenhuis, Rotterdam and subsequently, in 2007 at the Department of Gastroenterology & Hepatology, Erasmus MC, Rotterdam. During her residency, she conducted the studies described in this thesis under supervision of Prof. Ernst J. Kuipers, Prof. Maikel P. Peppelenbosch and Dr. C. Janneke van der Woude. In April 2011, she finished her residency in Gastroenterology and moved back to Slovakia where she currently works as gastroenterologist at the 5th Department of Internal Medicine, Faculty of Medicine, Comenius University in Bratislava (head, Prof. Juraj Payer).


