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CELIAC DISEASE AND EYE DISORDERS

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ABSTRACT

Background: Celiac disease (CD) is an immune mediated enteropathy, triggered by the ingestion of gluten, in genetically susceptible individuals. CD can develop at any age and the worldwide prevalence of this condition is approaching 1%. The only available treatment today is a life-long gluten free diet. CD is associated with several disorders including malignancies, endocrine disorders and neurological symptoms. So far, there have been no studies examining the association between CD and eye disorders.

Aims: The aim of this thesis was to explore the relationship between CD and eye disorders including decreased visual acuity, cataract, uveitis and diabetic retinopathy. The overall aim was to add new knowledge in the field of CD and its complications, and to identify high-risk groups where screening may be considered.

Methods: This thesis is based on data from Swedish population based registers. In our first study we examined visual acuity in 69 patients with undiagnosed CD, 996 with diagnosed CD and 6850 controls. Data on visual acuity was obtained from the Swedish conscripts register and CD patients were identified from the Swedish national patient register (1964-2003). In studies (II-IV) we defined CD as villous atrophy and data was obtained from all pathology departments in Sweden, from 1969-2008. We identified some 29,000 individuals with CD through biopsy reports. Statistics Sweden selected five reference individuals for each CD case matched for sex, age, calendar period and county resulting in about 140,000 reference individuals. Our outcome variables were identified from the Swedish national patient register according to relevant ICD codes. By using the personal identity number of each patient we were able to link our data and calculate Hazard ratios for the outcomes. In the last study we examined the risk of diabetic retinopathy in patients with type 1 diabetes and CD as compared with patients with type 1 diabetes and no CD. We used the Swedish national patient register to identify some 40,000 patients with type 1 diabetes (1964-2010).

Results: We found no association between CD and visual acuity in our first study. On the other hand, CD was positively associated with subsequent cataract (HR=1.28; 95% CI=1.19-1.36). In addition, we found a moderately increased risk of uveitis in patients with CD compared to reference individuals (HR=1.32; 95% CI=1.10-1.58). Finally, we found that duration of CD correlates strongly with the risk of diabetic retinopathy in patients with type 1 diabetes. During the first five years of a CD diagnosis among patients with type 1 diabetes we found a low risk of diabetic retinopathy (HR=0.57; 95% CI=0.36-0.91), whereas patients with longstanding CD (≥15 years) had a threefold increased risk of diabetic retinopathy (HR=3.01; 95% CI=1.43-6.32).

Conclusions: This thesis found positive associations between CD and eye diseases. Although the risks were not very high and will by no means encourage screening for CD, clinicians should be aware of these associations and consider them when meeting with CD patients who present with eye symptoms. From the last study in this thesis, we found that longstanding CD is a strong risk factor for the development of diabetic retinopathy in patients with type 1 diabetes. Therefore, we suggest closer monitoring of diabetic retinopathy in patients with longstanding CD and type 1 diabetes.
LIST OF PUBLICATIONS


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LIST OF ABBREVIATIONS

CD    Celiac disease
HR    Hazard ratio
OR    Odds ratio
AGA   Antigliadin antibodies
TTG   Tissue transglutaminase antibodies
EMA   Endomysial antibodies
GFD   Gluten free diet
T1D   Type 1 diabetes
DRP   Diabetic retinopathy
NPR   National Patient Register
IEL   Intraepithelial lymphocytosis
1 INTRODUCTION

Celiac disease (CD) is an immune mediated enteropathy affecting some 1% of the population in the Western world\(^1\). This chronic disorder occurs in genetically susceptible individuals and is triggered by the ingestion of gluten; the main protein component of wheat, barley and rye\(^2\). When exposed to gluten an immune reaction is initiated in the upper small intestinal mucosa, which involves the innate as well as the adaptive immune system and ultimately results in inflammation and atrophy of the small intestinal villi. The only available and effective treatment today is a life long gluten free diet (GFD). The clinical presentation of CD ranges from classical symptoms such as weight loss, chronic diarrhoea, vomiting and signs of malabsorption to non-classical symptoms with few or no gastrointestinal symptoms at all\(^3\). Individuals presenting with milder symptoms such as indigestion, bloating or non-gastrointestinal symptoms are defined as having non-classical CD\(^4\), this type of CD is much more common these days\(^5\)\(^-\)\(^7\). This trend of milder symptoms or no symptoms at all has lead to an age shift at diagnosis and CD is now diagnosed at any age\(^2\). CD has been linked to a number of different complications including malignancy\(^8\), mortality\(^9\), adverse pregnancy outcome\(^10\) and neurological symptoms\(^11\). In addition, CD is linked with autoimmune diseases including type 1 diabetes (T1D) and autoimmune thyroid disease\(^12\). The diagnosis of CD is based on small intestinal biopsy with the typical appearance of intraepithelial lymphocytosis (IEL), crypt hyperplasia and villous atrophy, when the patient is on a gluten-containing diet\(^4\). With the development of serological testing there has been a remarkable improvement in identifying CD patients and serology is a great support in deciding which patient should undergo biopsy. In their consensus report from 1990; ESPGHAN (European Society for Pediatric Gastroenterology, Hepatology and Nutrition) concluded that positive CD serology alone is not sufficient for a CD diagnosis, but adds to the weight of the diagnosis if found\(^13\). However, new recommendations have recently been proposed by ESPGHAN, in which there is an option to diagnose CD without biopsy, if the CD antibody titters are very high\(^14\).

Through research there have been great improvements in the diagnostics and management of CD, however there is still a lot that is not fully understood about this complex multisystemic disorder. Many patients remain undiagnosed\(^15\) and as such, they are at risk of adverse events in the future. Although population screening is not recommended\(^16\), a case finding approach has been adopted in many parts of the world, to identify patients in high risk groups\(^17\). Much of the present research aims at finding answers to questions regarding the pathogenesis, genetics, environmental factors and complications of this perplexing condition.

The association between CD and autoimmune disorders is well established\(^18\). Likewise is the relationship between eye diseases, autoimmunity\(^19\)\(^,\)\(^20\) and inflammatory disorders\(^21\). Interestingly, there are no previous studies examining the relationship between CD and eye diseases. The purpose of this thesis was to investigate the relationship between CD and eye diseases. In the hopes of adding new and helpful knowledge in this field, for future research, for lessening the burden of eye diseases and for improving the health care of CD patients.
2 BACKGROUND

2.1 HISTORY

The word celiac is originated from the Greek word *koila* meaning belly and is related to the abdomen or abdominal cavity. Accounts of the first definition of CD date back to the 1st and 2nd centuries AD by the physician Aretaeus, where he describes the chronic nature and changed bowel functions of the disease\(^2\). The first clear description of the condition was proposed in 1888 by the English paediatrician Samuel Jones Gee, who also suggested that dietary treatment might be beneficial. Even then, Gee knew that the “coeliac affection” could affect individuals of any age and that it was an easily overlooked condition\(^3\). The great breakthrough in the definition of CD was however in the 1950s by the Dutch paediatrician Wim Dicke. Dicke showed that the exclusion of wheat, rye and oats from the diet led to improvement in the clinical condition of the patient. What was once thought to be a rare malabsorption syndrome of young age is now recognized as a multisystemic disease of all ages, with a wide range of clinical manifestations.

2.2 PREVALENCE

The complexity of CD and its broad range of clinical manifestations have resulted in many patients remaining undiagnosed. With this background CD has historically been considered a rare condition. The matter of underdiagnosis of CD was suggested in the 1980’s and explained to be associated with the diversity of clinical presentation of the disease (both mild and severe symptoms), but also due to the lack of simple diagnostic tools\(^4\). Shortly thereafter, the development of serologic testing was introduced on the market, which made it possible to screen for CD in the population\(^5\). With the increased awareness and the use of more specific and sensitive tests, it has been estimated that CD affects approximately 1.5% of the Western population. The highest prevalence (5.6%) of CD has been found in the Saharawi people in North Africa, an Arab and Berber people living as refugees in Algeria. This elevated prevalence may very likely be due to both genetic and environmental factors. The Saharawi people have a very high frequency of the DR3-DQ2 haplotype. Furthermore, they have changed their dietary habits in the last few decades, with early introduction of gluten in the diet (because of humanitarian aids from Western countries) and reduced breastfeeding\(^6\). With the exception of the Saharawi people the highest prevalence rates have been found in Ireland, Sweden and Finland\(^1\). The variations presented by studies in prevalence rates can be due to the definition of CD (histopathology or serology), age differences and populations (adults or children). Depending on which Marsh grades that have been chosen as the definition of CD, the prevalence rates can vary a great deal. Some argue that Marsh I and II should be classified as CD, which leads to higher estimates. Furthermore, defining the CD diagnosis solely on serologic tests will also affect the estimates with higher prevalence figures.
2.2.1 Unselected populations

The Swedish CD epidemic in the mid 1980’s first described by Ivarsson et al\textsuperscript{27}, increased our understanding of the relationship between CD and environmental factors. Consequently, the feeding recommendations in Sweden changed. Introduction of gluten is now recommended at 4-6 months of age, under protection of breastfeeding. The population born during the Swedish epidemic was followed up (at 12-years of age) to determine the prevalence of CD\textsuperscript{28}. In that screening study the prevalence of CD was 2.9%, however the study may have overestimated the prevalence of CD, since they accepted high IEL counts in patients with positive serology as a definition of CD diagnosis. Furthermore, patients with a previous diagnosis of CD were probably more likely to participate in this study and the presented prevalence is based on both previously diagnosed CD and newly identified cases\textsuperscript{28}. Another study from the Northern part of Sweden found a prevalence of 1.6% of CD in 1000 adults from the general population\textsuperscript{29}. The diagnosis of CD in that study was based on serologic analysis and duodenal biopsies (although increased IEL’s were enough for diagnosis in this study as well)\textsuperscript{29}. In the United States, CD has traditionally been regarded as a rare condition with prevalence rates ranging from 1/5464 to 1/4857\textsuperscript{30}. However, more recent population based screening studies in both adults and children have reported a prevalence of 0.75% (1/133)\textsuperscript{31}, as well as an increasing incidence of CD from 0.9/100,000 person-years in the 1950’s to 3.3/100,000 person-years in the 1990’s\textsuperscript{32}. In the large systematic review by Dubé et al, 133 North American and Western European studies, on CD prevalence in unselected and selected populations, were examined\textsuperscript{1}. In unselected populations, Italian studies show similar prevalence estimates as in the American studies (0.2%-0.94%)\textsuperscript{1}. As mentioned earlier higher prevalence rates were found in UK, Finland, Sweden and Ireland (between 1.0%-1.5%). However, the authors emphasize that both the highest and lowest prevalence of CD were reported by small sized studies. Therefore, the countries that presented higher prevalence of CD could show lower estimates in other studies\textsuperscript{1}. By looking at large studies only (12 out of 18) the authors found prevalence rates of CD (defined by serology only) ranging between 0.5%-1.26% compared to small sized studies 0.1%-2.67%\textsuperscript{1}. In a recent study by Mustalahti et al, four populations (both children and adults) from Finland, Italy, Germany and UK (altogether 29,212 participants) were tested for anti-TTG and EMA\textsuperscript{33}. Participants with positive results were then recommended to do small intestinal biopsies\textsuperscript{33}. The overall prevalence (both previously diagnosed and serology positives) was 1% (95% CI=0.9-1.1)\textsuperscript{33}. Because there was an under-representation of children in some countries, the prevalence of CD between countries comparison was restricted to the age range (30-64 years). The highest prevalence was found in Finland (2.4%, majority were previously diagnosed CD) and the lowest prevalence in Germany (0.3%)\textsuperscript{33}. It is also worth noting that out of 401 serology positive individuals only 147 agreed to do a small intestinal biopsy and in 100/147 the histological appearance showed CD (Marsh II and III)\textsuperscript{33}.

Increased prevalence and incidence of diagnosed CD in Denmark\textsuperscript{34} suggest that CD awareness has improved and more cases are found. The differences in prevalence between close neighbouring countries like Sweden, Finland and Denmark are also a puzzling phenomenon\textsuperscript{35}. Swedish and Danish children should be similar in ethnical, cultural and geographic aspects. On the other hand, there have been some differences in
the dietary recommendations in these countries (higher contents of gliadin in the diet in Sweden and earlier introduction to infants)\textsuperscript{36}. Danish children have low intake of wheat in their diets\textsuperscript{37}. The prevalence among Finnish schoolchildren has been estimated to be 1/99\textsuperscript{38}. Furthermore the prevalence of CD among elderly in Finland has also been reported to be quite high (around 2\%\textsuperscript{39, 40}). Dietary differences may also explain the differences in clinical presentation and trends of incidence between Sweden and Finland\textsuperscript{41}.

Traditionally research on CD has been focused on Western populations, which without doubt has contributed to the lower degree of awareness of CD in other parts of the world. However, in recent years reports on CD prevalence have emerged from China (based on serology, at risk group 7\%\textsuperscript{42}), India (schoolchildren, only positive symptoms 1/310\textsuperscript{43}), Iran (blood donors, 0.88\%, based on serology\textsuperscript{44} and South America (blood donors, biopsy-proven 0.14\%\textsuperscript{45}). Although the prevalence of CD may seem somewhat lower in other parts of the world, one must keep in mind that there are differences in study design, definition of CD and serology tests used in these countries. Blood donors are not exactly comparable with the general population and some were screened for anemia. AGA is still used in greater extent in many countries. Infant mortality is higher in these countries, presumably to some degree due to undiagnosed CD (since the most frequent causes are thought to be parasitoses, malnutrition and infectious disease). Furthermore, there are of course great differences in the diet between these countries.

2.2.2 Selected populations

CD is more prevalent in some at risk populations\textsuperscript{1}. In Sweden some well known risk groups undergo screening for CD in clinical practice, including patients with type 1 diabetes, relatives of individuals with CD, patients with iron deficiency anemia, individuals with Down’s syndrome, Turner syndrome, Williams syndrome and selective IgA deficiency\textsuperscript{46}.

The prevalence of CD is increased in both first-degree and second-degree relatives. Fasano et al screened 4508 first-degree relatives of CD patients and found a prevalence of CD (CD defined as EMA positivity) at 4.55\%. The prevalence of CD in 1275 second-degree relatives was 2.59\%\textsuperscript{31}. Rubio-Tapia et al found slightly higher prevalence of CD (11\%) in 344 first-degree relatives of 111 index CD cases (CD was defined as Marsh II-III and also potential CD were included in the overall prevalence)\textsuperscript{47}.

Another well-known risk group in CD is patients with iron deficiency anemia. The cause of iron deficiency anemia in CD is primarily due to impaired absorption and damaged mucosal surface\textsuperscript{48}. The prevalence of asymptomatic CD in patients with iron deficiency anemia has been estimated to be between 6-8.5\%\textsuperscript{49, 50}. In fact, anemia has been described as the most common mode of presentation of CD in primary care settings (15/30)\textsuperscript{17}.

Other conditions that are frequently encountered in patients with CD are vitamin deficiencies (due to malabsorption). This has been illustrated by Saiinen et al’s prospective study, where the levels of folate and vitamin B12 were lower in patients
with CD compared to controls. Vitamin deficiencies are however not only a problem at CD diagnosis, it may be that the GFD lacks sufficient amounts of nutrients.

CD is associated with an increased risk of osteoporosis. Stensson et al screened 840 individuals (266 with and 574 without osteoporosis from a bone clinic) and found a prevalence of biopsy proven CD of 3.4% in the osteoporotic group compared to 0.2% in the non-osteoporotic group. The risk of osteoporosis seems to be elevated both before and after CD diagnosis, although there are contradicting reports (smaller study). The GFD often lacks sufficient amounts of calcium and vitamin D, which could potentially be a reason for osteoporosis development even after CD diagnosis. Moreover, research suggests that individuals with CD have higher levels of PTH and lower levels of 25-hydroxyvitamin D compared to controls.

There is also an established association between CD and endocrinological disorders. A positive association between CD and thyroid disease (any type of thyroid disease) has been shown both before and after CD diagnosis. Thyroid disease occurs in 10-15% of the CD population and therefore these patients should be screened for this condition.

CD has long been known to be closely associated with type 1 diabetes, in particular by paediatricians. The association is most likely due to similar genetic background. The prevalence of CD in patients with type 1 diabetes is approximately 3-5%, however higher figures have been demonstrated as well (12%).

Irritable bowel syndrome (IBS) occurs in 5-20% of the general population and these patients have similar symptoms to those seen in CD. In the meta-analysis by Ford et al, the pooled prevalence of biopsy verified CD in patients with IBS was 4%.

A subject frequently discussed in the scientific community regarding CD occurrence, is whether or not there has been an actual increase in CD prevalence over the last decades. Some of the increase could be explained by greater awareness and the availability of low cost and reliable serologic tests. Another factor contributing to an increase is the definition of CD; with wider criteria for CD diagnosis more CD will be diagnosed. However, there are reports indicating a true increase in the prevalence of CD. From Finland, Lohi et al described an increase in total prevalence of screened CD in two cross-sectional population based cohorts. In the cohort from 1978-80 the total prevalence was 1.05% compared to 1.99% in the cohort from 2000-01. Such an increase in total prevalence, defined as both diagnosed and undetected CD, could not be explained by a change in detection rate or a rapid change in genetic traits. The authors discuss the possibilities of a general increase of CD comparable with the increase of several other autoimmune diseases (such as type 1 diabetes, multiple sclerosis). In addition, American screening studies have shown similar findings of an increasing prevalence of CD over time. Without disregarding a potential true increase of CD over time due to environmental factors (breastfeeding, diet, infections), there are limitations in these studies that should be considered, including the definition of CD based on serology without biopsy, cohort effect and the stability of serum autoantibodies after long-term storage.
2.3 PATHOGENESIS

CD is defined as an immune mediated enteropathy. Some argue that CD is an autoimmune disorder, others refer to it as a chronic inflammatory disorder and none of these statements are incorrect. The reason for the different definitions is primarily due to the lack of knowledge and the complicated nature of CD. There are still gaps in our understanding of the pathogenesis and etiology of CD. This field of research is constantly evolving.

Immune mediated diseases are a large group of diseases that are characterized by the direct and/or indirect association with the immune system. Disorders such as immunodeficiencies, immunoproliferative diseases (malignancies), autoimmune and hypersensitivity disorders (allergies, asthma) are included in the entity of immune-mediated disorders. CD is a complex multifactorial disorder involving HLA and non-HLA genes, the innate and adaptive immune system and environmental factors. Although some things remain unclear in the pathogenesis of the disease, research has come a long way, in particular regarding the genetic findings. In the future, when more is clarified regarding the pathogenesis, CD could serve as a model for many other diseases with unknown features.

2.3.1 Gluten

CD is triggered by the ingestion of gluten, which is derived from wheat, barley and rye. Although we refer to gluten as the collective main protein component activating CD, this is not entirely true. Gluten proteins are the disease activating proteins in wheat and consist of two protein fractions gliadins and glutenins. In barley and rye the corresponding disease activating proteins are called hordeins and secalins. These protein fractions are closely related and have high levels of glutamine and proline contents, which allow them to be relatively resistant to the degradation by gastric, pancreatic and intestinal brush border enzymes. Consequently, relatively large undigested proline/glutamine-rich peptides remain in the intestinal lumen and together with other factors they play a role in activating CD2, 71.

2.3.2 Genetics

The role of genetics in CD has been demonstrated in family- and twin studies, where the incidence of CD has been shown to be higher in first-degree relatives and twins compared to individuals from the general population. Studies show high concordance rates (75%) for CD in monozygotic twins, which evidently supports the genetic component in CD72.

The human major histocompatibility complex region (MHC) is localized on chromosome 6 and encodes for MHC molecules (referred to as HLA molecules in humans) that are active in the function of the immune system73. CD almost solely develops in individuals carrying HLA class II molecules, which have been estimated to account for approximately 35% of the genetic risk in CD74. The HLA class II molecules consist of two chains, alfa and beta chains, which make up the heterodimeric molecule expressed on antigen presenting cells. The outer parts of the alfa and beta chains form a cleft where it binds the peptide that will be presented to T-cells. Some 95% of CD individuals carry the HLA-DQ2 allele (DQ2.5-DQA1*0501-
DQB1*0201 or DQ2.2-DQA1*0201-DQB1*0202) and the remainders carry HLA-
DQ8 (DQA1*0301-DQB1*0302). Since approximately 30% of the general population
carry these CD risk genotypes and only 1-3% develop the disease, it is clear that HLA-
DQ2/DQ8 is necessary but not sufficient to develop CD. Through Genome Wide
Association Studies (GWAS) new non-HLA genes have been identified as genetic risk
factors involved in CD susceptibility. Around 39 new CD associated loci containing
some 115 different genes have been identified so far, and interestingly many of these
genes are associated with other autoimmune diseases. This may further support the
similarities in the pathogenesis of CD and other immune-mediated diseases. The search
for additional explanatory genetic factors in CD pathogenesis continues and is
advancing more than ever.

2.3.3 Immunology

Once the gluten peptides encounter the small intestinal mucosa there is a complex
activation of both the innate and adaptive immune defence. The gluten peptides' 
journey over the epithelium has been explained by various theories, and remains to be
elucidated. Nevertheless once the gluten peptides are in the lamina propria they are
deamidated by the enzyme tissue transglutaminase. The deamidation creates negatively 
charged residues in the gluten structure, which will then bind with high affinity to 
the positively charged pockets present in both HLA DQ2 and HLA DQ8 molecules on
antigen presenting cells. The adaptive response mediated by CD4+ T-cells (which
are activated by recognition of the presented gluten peptides) will start producing
interferon gamma as well as other proinflammatory cytokines. An inflammatory
cascade is now activated and as part of this defence, activated metalloproteinases and
other tissue damaging mediators will lead to villous atrophy. The production of anti-
transglutamases antibodies by B-cells is possible in the presence of CD4+ T-cells and
HLA DQ2/DQ8. It has been suggested that gluten peptides form complexes with tissue
transglutaminases and are then internalized by B-cells, which in turn will produce
antibodies against tissue transglutaminases with the assistance of CD4+ T-cells.
The gluten peptides will also activate an innate response involving secretion of IL-15 and activation of cytotoxic IEL’s. Gluten will stimulate enterocytes and macrophages to secrete IL-15 and express the epithelial ligand MICA, leading to an upregulation and binding of NKG2D receptors on cytotoxic IEL’s. This activates the cytotoxic lymphocytes and induces a process of epithelial apoptosis and further stimulation of other cytotoxic lymphocytes (CD8+, natural killer cells). Gluten peptides can also directly stimulate macrophages and dendritic cells through Toll-like receptor 4, in order to secrete inflammatory cytokines and potentiate both the innate and adaptive response.
2.3.4 Environmental factors

Apart from the obvious presence of gluten, other environmental factors have been suggested to influence the development of CD as well. The fetal and neonatal period is important for the growth and development of the immune system. Researchers have examined whether events during this period can affect the onset of CD. In a Swedish population based study Sandberg-Bennich et al found that low birthweight for gestational age and exposure to neonatal infections was associated with an increased risk of developing CD later in life. The association between cesarean section and CD has also been a focus for research, however studies have produced somewhat inconsistent results. The theory supporting this association is that children born by cesarean delivery differ in their intestinal bacterial flora compared to children born vaginally, which may influence the susceptibility to CD. In their case control study Decker et al found an increased risk of being born by cesarean delivery in children with CD as compared with controls. Roberts et al on the other hand found no significant association between perinatal risk factors and CD, which was also confirmed in the largest study so far by Mårild et al. However, Mårild et al found a positive association between elective cesarean delivery and later risk of CD.
Moreover, studies have shown that the intestinal microbiota in children with CD differs from controls\(^{31, 84}\) and this may influence the functioning of the immune system\(^{85}\). Smoking during pregnancy and social class could also be associated with CD development\(^{82}\).

The role of infection in CD pathogenesis derives from similar theories explaining the pathogenesis of type 1 diabetes through viral infection. It has been proposed that cross-reactivity between shared epitopes on adenovirus serotype 12 and gliadin peptides could be important in the pathogenesis of CD, as it has also been shown that many untreated CD patients have evidence of previous adenovirus infection\(^{86}\). Some have found that high frequency of rotavirus in childhood leads to an increased risk of CD\(^{87}\), whereas others have found no association between future diagnosis of CD and any infection at time of gluten introduction\(^{88}\).

The risk of CD has also been examined in relation to season of birth\(^{89}\). Ivarsson et al found an increased risk of CD in children born during the summer\(^{89}\). This risk could only be demonstrated in children younger than 2 years of age at diagnosis. In their study, Ivarsson et al also demonstrated a gender difference in the risk for CD, where girls were at higher risk than boys (RR=2.1)\(^{89}\).

The role of breastfeeding and its influence on CD development has also been studied, and results are somewhat inconsistent. Ivarsson et al found that breastfeeding had a protective effect against CD in children younger than 2 years during gluten introduction (OR=0.59; 95% CI=0.42, 0.83) and this effect was even greater if the breastfeeding continued after gluten introduction (OR=0.36; 95% CI=0.26, 0.51)\(^{89}\). Others have emphasized the timing of gluten introduction into the infant diet. An increased risk of CD was found when gluten was introduced in the first 3 months of life\(^{91}\). In the meta-analysis by Akobeng et al\(^{92}\), the risk of CD was low in infants in whom gluten introduction occurred during breastfeeding and this association was also significant with increasing duration of breastfeeding\(^{92}\). However, studies have also shown that the duration of breastfeeding and time of gluten introduction has no effect on CD development\(^{92, 88}\) and that children with CD have been breastfed more often than controls\(^{83}\). The amount of gluten has also been examined as a risk factor for CD. Larger amounts of gluten is associated with higher risk of CD\(^{90}\), which has also been the argument for the difference in CD incidence in Sweden compared to Denmark\(^{36, 37}\). The consensus in Sweden is now to introduce gluten with protection of breastfeeding at 4-6 months.

Other risk factors that have been studied for CD are smoking and body mass index (BMI). Underweight has been shown to be a risk factor for undiagnosed CD both in women and in men\(^{95}\). Most studies regarding smoking and CD have found that patients with CD are less likely to smoke than controls\(^{94-96}\). Others have found larger proportions of former smokers in patients with CD compared to controls\(^{87}\), whereas Ludvigsson et al, with the largest study found a positive association between smoking and CD but not statistically significant\(^{98}\). In conclusion, it might be that smoking has little effect on future CD.

### 2.4 CLINICAL PRESENTATION

The clinical manifestations of CD have changed over time and to describe this, symptoms have been divided into classical and non-classical symptoms. Originally, CD has been associated with classical symptoms like diarrhoea, weight loss/growth failure
and malabsorption, commonly starting between 6-24 months of age in children when gluten has been introduced. Non-classical symptoms include anemia, osteoporosis, dyspepsia, neurological symptoms and unusual intestinal complaints such as nausea, vomiting and constipation. In other words, classical CD presents with signs of malabsorption, whereas non-classical CD lacks such signs and symptoms of malabsorption. Larger proportions of CD individuals today are found through screening of family members or by belonging to risk groups; therefore many patients may lack symptoms at diagnosis. The clinical symptoms have changed both in the adult and pediatric populations, much due to the use of serologic testing but also increased awareness among clinicians. It is interesting to know whether the available serologic tests today can find CD patients presenting with non-classical symptoms. In a population based cohort study from UK, 1% of children aged 7 years were screened EMA positive. Fifty percent of the EMA positive children reported diarrhoea compared with 34% of antibody negative children (OR=1.96). The EMA positive children were both shorter and lighter than the antibody negative children. Diarrhoea is a common symptom of CD presentation in primary health care settings, however on its own it is insufficient for diagnosing CD. In an adult population the combination of TTG and EMA antibodies and abdominal symptoms showed high sensitivity and specificity for CD diagnosis. In a Swedish study, symptoms were compared in children with biopsy verified CD and children with negative biopsy. Abdominal distension was the most frequently reported symptom in patients with a later diagnosis of CD. Vomiting and diarrhoea were of borderline significance for a later diagnosis of CD. The broad variation in symptoms presented has also been described by a joint Swedish and Finnish study, where the clinical presentation of Swedish children with CD was compared to Finnish children. Finnish children were diagnosed at older age (8 years of age compared to 2 years in Sweden) and presented with milder symptoms compared to Swedish children. Some 79% of the Swedish children had classical symptoms compared to 3% of the Finnish children. Furthermore, Ludvigsson et al described the symptoms and signs of patients with biopsy verified CD in Sweden, and the most common symptom was diarrhoea (36%), with anemia occurring in 35%. According to the recommendations by North American Society for Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN), CD should be considered in children with chronic diarrhoea, failure to thrive, vomiting and abdominal pain. The non-classical symptoms of CD have been described in both pediatric and adult populations, however they may be more frequent in the adult population. An Italian study examined the prevalence of fatigue in CD, and found that fatigue was more frequent in patients with both treated and untreated CD compared to controls. CD has also been associated with depression, which may be due to the ongoing inflammation, malnutrition or the GFD. Bottaro et al describes iron deficiency anemia as the most common extraintestinal symptom in both children and adults (although higher in adults (46.3%) than in children (34.8%)), followed by short stature in children and cutaneous lesions in adults.

Neurological symptoms are suggested to occur in some 10% of patients with CD, most often presenting with peripheral neuropathy and ataxia, many times in the absence of gastrointestinal symptoms. As part of the division into classical and non-classical symptoms, CD patients are also referred to as being asymptomatic or subclinical in this context. Subclinical CD is
described as patients having symptoms but not enough to seek medical care. Interestingly, the female/male ratio and mean age at diagnosis is lower in this group of patients, presenting with symptoms like amenorrhea, short stature or anemia. The prevalence of subclinical CD in 11-15 year old subjects has been found to be 3.28 per 1000 subjects.

Asymptomatic patients with CD are defined as having no symptoms at all, but they do have mucosal changes compatible with CD. These groups of patients are usually found by screening of at risk groups or of families of affected CD patients. A central issue in this context, which still remains to be determined is; are undiagnosed CD patients at an increased risk of developing complications and can early diagnosis prevent these outcomes?

2.4.1 Complications

Since CD is associated with numerous disorders it is interesting to know whether having a diagnosis of CD increases the risk of death in these patients or not. There have been a few studies examining the risk of mortality in CD and the majority of studies have found increased relative risks between 1.3-2.0. The study by Peters et al found an increased RR of 2.0 for all causes of death in CD, however they used data on CD from the inpatient register, which therefore could reflect the mortality risk of much sicker CD patients. Ludvigsson et al on the other hand defined CD according to small intestinal histopathology (villous atrophy) and still found an increased risk of mortality (HR=1.39) in patients with CD. Another population based study by West et al found similar risk estimates for mortality as in the Swedish study (HR=1.31), compared with the general population.

The Italian study by Corrao and colleagues presented a two fold increased risk of overall mortality in the first 3 years after CD diagnosis, however they did not find an excess risk of mortality in patients with mild symptoms or those identified through screening. In contrast, Rubio-Tapia et al found that patients followed during 45 years with undiagnosed CD (14 patients) had a 4-fold increased mortality risk compared to seronegative individuals. These findings were later disputed in a larger British study, presenting no difference in mortality risk among undetected CD (EMA positive patients) compared to EMA negative participants. Positive EMA participants did not report worse health than EMA negative participants at recruitment, perhaps this reflects the lower risk of mortality found in patients with mild symptoms described earlier.

In line with examining the risk of mortality, studies have also evaluated the risk of malignancy in CD. Apparently, CD is particularly associated with an increased risk of lymphoproliferative malignancies and perhaps gastrointestinal cancers. The overall risk of malignancies in CD has been reported to be moderately elevated (HR 1.29) but not during childhood. Lymphoproliferative cancers have been extensively studied in CD and although increased risk estimates have been reported, different study designs, inclusion criterions and study sizes have yielded inconsistent results. West et al found an increased risk of lymphoproliferative cancers (HR 4.80) in the celiac cohort compared to the general population. In the largest study so far, Elfström et al found an increased risk of any type of lymphoproliferative cancers (HR=2.82) in CD, which remained statistically significant even 5 years after CD diagnosis. Elfström et al found an increased risk of Non Hodgkin lymphoma (HR=4.26) similar to the case control study by Gao et al (HR=5.35). Although West et al found an increased risk of
gastrointestinal cancer (HR=1.85)\textsuperscript{114}, Elfström and colleagues could not confirm these findings, because the increased risk of gastrointestinal cancer was only noticed the first year after biopsy and not during follow up\textsuperscript{118}.

The risk of breast cancer and lung cancer on the other hand has been shown to be low in patients with CD. West et al reported low risk of breast cancer (HR=0.35) and lung cancer (HR=0.34) in CD\textsuperscript{114}, whereas the Swedish study by Askling et al found no difference in the risk of lung cancer among patients with CD and controls\textsuperscript{116}.

Another complication well examined in CD is the risk of heart disease. Studies that have presented increased risk of cardiovascular disease in CD are; Wei et al (HR=1.9)\textsuperscript{119} and the Swedish study by Peters et al (HR=1.5)\textsuperscript{113}. Peters and colleagues based their CD data on hospitalized patients and Wei et al defined CD patients according to positive EMA antibody or positive small bowel biopsy, and had data on some 370 CD patients. Ludvigsson et al with the largest sample size (28,000 CD patients) found a modestly increased risk of ischemic heart disease (HR=1.19) in patients with CD compared to the general population\textsuperscript{20}. The mean total cholesterol level has been found to be lower in CD patients compared to the general population\textsuperscript{211}. Furthermore, others have described that patients with CD are less likely to have a diagnosis of hypertension and hypercholesterolemia\textsuperscript{122}, and as such they have also been found to have a lower risk of myocardial infarction compared to controls (HR=0.85)\textsuperscript{122}.

CD has been associated with infections, in particular pneumococcal infections. The reason for a higher risk of pneumococcal infections has been described as related to hyposplenism, which can be found in CD\textsuperscript{23}. A Swedish study assessed the risk of sepsis in CD and found that compared to reference individuals (consisting of both the general population and other hospitalized patients without CD) patients with CD were at an increased risk of sepsis (HR=1.6)\textsuperscript{124}, the highest risk was found in pneumococcal sepsis (HR=2.5)\textsuperscript{124}. CD has also been linked to an increased risk of tuberculosis\textsuperscript{125}, possibly as a result of vitamin D deficiency, which may affect the immune response, through the activation of macrophages\textsuperscript{125}.

2.5 DIAGNOSTICS

2.5.1 Diagnostic criteria

Since the first diagnostic criteria for CD were proposed in 1969 (called the Interlaken criteria) until its revision in 1990 by the European Society for Pediatric Gastroenterology and Nutrition (ESPGHAN), the gold standard for CD diagnosis has been and still is; an abnormal small intestinal biopsy while consuming a gluten containing diet\textsuperscript{13}. The gluten challenge, which was recommended by the Interlaken criteria, is not mandatory for diagnosis anymore, however it may be useful in cases where there are doubts about the initial diagnosis. The ESPGHAN statement also requires a clear-cut clinical remission (symptomatic relief) on a strict gluten free diet, which can be evaluated by a control biopsy in asymptomatic patients. The histological changes include intraepithelial lymphocytosis, crypt hyperplasia and villous atrophy. ESPGHAN further points out that the finding of positive serology is supportive for the diagnosis but not necessary\textsuperscript{13}. The NIH consensus from 2004\textsuperscript{112} is in line with the ESPGHAN statement. Since there have been a great deal of progress in the understanding of the complexity of CD, the NIH consensus describes new
recommendations regarding the diagnostic work up for CD (with serology and genetic testing) in more detail, but in the end, the diagnosis of CD is confirmed by a small intestinal biopsy. In the recent revision of the ESPGHAN criteria\textsuperscript{14} it is however suggested that duodenal biopsies can be avoided in patients (children or adolescents) with very high titers of TTG in combination with symptoms suggestive of CD\textsuperscript{14}.

2.5.2 Serology

The gold standard for CD diagnosis is based on small intestinal biopsy with the appearance of villous atrophy. To help determine which of the patients should undergo endoscopy and biopsy there are serological tests available. There has been a lot of research conducted, to assess the sensitivity and specificity of the available serological tests, which include: the older antigliadin antibodies (AGA, both IgA and IgG), antiendomysial antibodies (EMA) and anti transglutaminas antibodies (TTG). Although the older AGA antibodies are more or less replaced by other serologic tests nowadays (such as anti deamidated gliadin peptides: anti-DGP) and many claim that the sensitivity of AGA is too low to be used at all, it is still very much utilized in the identification of younger patients (<2 years of age)\textsuperscript{126}. IgA deficiency is more common among patients with CD than in the general population\textsuperscript{127}, therefore total level of serum IgA should be tested before using IgA based antibody tests. The alternative test for patients with IgA deficiency is measurement of IgG TTG/EMA\textsuperscript{77}. Although Hill et al concluded that EMA and TTG are the most sensitive and specific serologic tests available today, the reported sensitivity for AGA-IgG and AGA-IgA were not as disappointing as would be expected\textsuperscript{128}. The majority of studies in AGA-IgG and AGA-IgA described a sensitivity of 80-100\% and specificity of 70-90\%, but the results were highly variable across different studies\textsuperscript{128}. Ever since the identification of tissue transglutaminase as the autoantigen of CD by Schuppan and colleagues\textsuperscript{129}, studies have demonstrated high sensitivity (98\%), specificity (94\%) and objectivity in interpretation (compared to EMA) of TTG. Consequently, TTG is one of the most commonly used serologic tests in the diagnosis of CD\textsuperscript{130, 131}.

With these inexpensive, highly sensitive and specific tests it became possible to screen populations, and as previously suspected it became clear that CD was indeed underdiagnosed. Mäki et al screened a cohort of 3600 schoolchildren in Finland for EMA and TTG antibodies (1.5\% were positive) and found a prevalence of CD in 1/99 children (verified by small intestinal biopsy)\textsuperscript{38}. The specificity and sensitivity of EMA and TTG are essentially the same. EMA can however be subjected to interobserver variability when interpreting the immunofluorescent results using rat or monkey esophagus as substrate (which is not the case in the ELISA method used in TTG). In the studies by Hopper et al\textsuperscript{132, 133} the evaluation of TTG and EMA demonstrated a positive predictive value (proportion of CD confirmed by the test) of 28\% by TTG and 64\% by EMA. By adopting a two-step approach (first TTG and then EMA) the PPV increased at the expense of the sensitivity. Therefore, the authors concluded that irrespective of the antibody results, clinicians with high suspicion of CD should always perform a small intestinal biopsy\textsuperscript{132}. Serologic tests have also been used as an assessment of the compliance and recovery of the mucosal damage in the intestine of CD patients. Hopper et al evaluated TTG and EMA levels in patients on GFD for more than 1 year\textsuperscript{132}. They found that EMA was normalized in patients that still had villous atrophy (7/16 with villous atrophy were EMA positive)\textsuperscript{132}, however the authors point
out that the proportion of patients with positive EMA or TTG was higher in those with villous atrophy compared to those without villous atrophy. Despite the fact that it is generally accepted that the serologic tests are not suitable for assessing the compliance or mucosal healing, it is widely performed by clinicians, even in Sweden. An additional intestinal biopsy could be inconvenient for the patient but more informative. However, there are no guidelines as to when such a control biopsy should be performed, since many patients have mucosal changes even after two years with GFD. In practice, if there are no immediate indications (ongoing or worsening symptoms) for a control biopsy, they are rarely performed.

2.5.3 Genetic testing

Other than the serologic tests it is also possible to use HLA genotyping for CD (HLA DQ2/DQ8). However since the HLA test has high negative predictive value it should merely be used to rule out CD if the result is negative. Hadithi et al reported a positive predictive value for HLA DQ2/DQ8 of 6% and negative predictive value of 96%. This is due to the fact that some 30% of the general population carry these alleles without developing CD (in other words, a positive HLA result says little about the risk of developing/having CD). Therefore the HLA test is not sufficient to diagnose CD, not even in the context of positive serology.

2.5.4 Small intestinal biopsy

According to a survey conducted by our research group, to evaluate the clinical management of CD and the use of small intestinal biopsy in Sweden, 96-100% of Swedish gastroenterologists and pediatricians perform a small intestinal biopsy prior to diagnosing CD. This means that the sensitivity of small intestinal biopsy is high for diagnosed CD. Because of the somewhat invasive method of performing a small intestinal biopsy, many researchers have advocated a replacement of the intestinal biopsy with serological tests for CD diagnosis. Because of the poor positive predictive value of most serologic tests, such a development is scarcely supported. The biopsy specimens are obtained in the proximal part of the small intestine (duodenum/jejunum), either with biopsy forceps or a suction capsule during endoscopy. The histological classification most commonly applied is that described by Marsh with an advancement of the histopathology from milder forms to severe and complete villous atrophy. Villous atrophy (VA) is when the height of the villi is less than three times the crypt’s height (normally villi are 3-5 times taller than the crypts). Intraepithelial lymfocytosis (IEL) is defined as >30 lymphocytes per 100 enterocytes cells. Marsh described a four stage grading of the celiac lesions. Marsh grade 0 is described as normal mucosa (pre-infiltrative stage some may never develop CD, and some may). Marsh grade 1 is the infiltrative stage with increased count of IEL’s but normal mucosa. Grade 2 is the hyperplastic stage with increased IEL’s and crypt hyperplasia. Grade 1 and 2 represent early changes in patients who are likely to develop CD. Marsh grade 3 is the destructive stage characterized by villous atrophy and crypt hyperplasia. In the original Marsh classification there is also a stage 4 (hypoplastic), which is a rare finding characterized by flat mucosa and probably represents a long-standing irreversible inflammation. The Marsh classification as we know it today is
actually modified by Oberhuber and colleagues. In the modified Marsh classification by Oberhuber grade 3 (villous atrophy) is subdivided into; 3a partial villous atrophy; 3b subtotal villous atrophy and 3c total villous atrophy. Although the Marsh classification (modified by Oberhuber) is widely used there have been other proposals to simplify the classification system. Many categories may result in lower interobserver agreement and consequently reduced reproducibility. Corazza et al proposed a 3-stage classification (A (Marsh1-2), B1 (3a-3b), B2 (3c))138. According to the Italian researchers, pathologists have difficulties in distinguishing between the intermediate conditions, but not for the extreme ones (villous atrophy and normal mucosa). This was also demonstrated in our validation study where Swedish pathologists correctly classified 90% of biopsies with villous atrophy and 96% of biopsies with normal mucosa. The merged grade A is actually identical to “small intestinal inflammation” which is used in Swedish biopsy registers. Although our validation study showed that pathologists classify 56% of biopsies with small intestinal inflammation correctly, this may be higher than what it would have been using the traditional Marsh classification. In Sweden all biopsies are classified according to the SnoMed system (Systemized Nomenclature of Medicine) for diagnostic and register purposes. The SnoMed system follows the same histopathology stages as the Marsh classification. The mucosal changes in CD can be patchy, which is why the number of biopsy specimens taken during endoscopy is of great importance to detect the pathology. Ideally 4-6 biopsy specimens should be taken from the proximal small intestine, including a duodenal bulb biopsy. Sanders et al demonstrated that taking three biopsies (one from the duodenal bulb and two from the proximal duodenum) would detect villous atrophy in all patients with suspected CD. However to determine the severity of the villous atrophy five biopsies would be required. The bulb biopsy is important since many patients have villous atrophy only at this site, although the interpretation of it may be somewhat difficult because many other pathologies usually presents at this site (duodenitis, peptic inflammation). As described by Ludvigsson et al, the majority of clinicians in Sweden send a median number of 3 biopsy samples to the pathology department for evaluation. Other than the number of biopsy samples obtained it is also important that the samples are of sufficient size and that they are carefully oriented before sectioning so that tangential cuts of normal villi can be avoided (misleads the interpretation). Even though the methods of obtaining biopsy samples of good quality is influenced by several factors as mentioned, it seems that the specificity of VA for diagnosis of CD is high. Not only do Swedish pathologists classify 90% of biopsies with villous atrophy correctly, but also it has been shown from patient chart reviews that 95% (108 of 114) of patients with villous atrophy have CD (PPV=95%). However, CD is not the only cause of villous atrophy and increased IEL’s, other conditions causing these features include; inflammatory bowel disease, drugs, T-cell lymphoma, helicobacter pylori and more. Still, in Sweden other causes of villous atrophy than CD are rare. Through manual examination of biopsy reports, our research group found the most common comorbidity in samples with villous atrophy to be inflammatory bowel disease, which occurred in 0.3% of the samples (helicobacter pylori in 0.2%, microscopic colitis in 0.2%). Combining the high sensitivity, specificity and positive predictive value of VA for CD, it is quite clear that identification of CD patients by biopsy reports, is superior to what has traditionally been used in most large Swedish studies on CD, i.e. the Swedish NPR, which has a positive predictive value of 86% for CD (compared with 95% by biopsy reports).
While villous atrophy has been the focus of the studies included in this thesis, we have used individuals with milder histopathology in the small intestine (Marsh 0 and Marsh 1-2) as reference groups in a few separate analyses. It seems that small intestinal inflammation without VA is a heterogeneous condition, which is illustrated by the differences in characteristics of the study participants in other studies (gender, age, risk of death). Even though Ludvigsson et al found few other causes of small intestinal inflammation in their validation study, this could partly be explained by the rarity of other conditions in Sweden (Giardiasis, Kwashiorkor, Tropical Sprue). Figure 3. Histopathology of the small intestine according to Marsh classification. From left to right. A) Normal mucosa. B) Marsh I. C) Marsh II. D) Marsh III. Pictures obtained from Dr Marjorie Walker, Reader and Honorary Consultant, Histopathology, Faculty of Medicine, Imperial College London.
2.6 TREATMENT

2.6.1 Gluten free diet

The treatment of CD consists of a life long gluten free diet (GFD). This means that wheat, rye and barley are excluded from the diet. Rice, corn and potatoes are the most common substitutes for gluten containing grains. The inclusion of oats into the GFD has been somewhat controversial, since the oat products can occasionally be contaminated by wheat, barley and rye. However, studies in both adults and children have shown that consumption of oats can safely be included in the GFD. The symptoms of CD improve quite rapidly (days to weeks) after the initiation of GFD, but the normalization of the intestinal mucosa takes longer. It is of course desirable to maintain a strict gluten free diet but not necessarily possible because gluten contamination is very common in food. Catassi et al described a safety threshold for ingestion of contaminating gluten lower than 50mg per day (small study participants). Even though the authors stated a harmful effect at 50mg gluten/day and not at 10mg/day, it is unclear what is acceptable in between these thresholds. Another Finnish study described a safety threshold for gluten contamination in gluten free products of 100ppm with a daily intake of flour at about 300g (equals 30mg gluten). The study by Catassi et al demonstrated that individual patients respond differently to small amounts of gluten, which further emphasizes the importance of an individually modified treatment. Although a second biopsy is not needed for establishing the diagnosis of CD, it is important in the follow up of the patient. More studies are warranted in order to settle on a safe limit of gluten contamination in gluten free products in both children and adults.

The gluten free diet is often low in important vitamins and nutrients including vitamin D, calcium, iron, zinc, vitamin B and magnesium. In addition, few gluten free products are fortified or enriched, which adds to the risk of nutrient deficiencies in these patients. Vitamin deficiencies have been detected in patients with CD on GFD for 10 years. In a clinical trial by Hallert et al CD patients were given vitamin B supplementation and improved in their vitamin status as well as their general well being. Therefore it is important to at least consider the option of giving treated CD patients vitamin supplementation as well as following their nutritional status over time, especially since CD is a chronic condition. Furthermore, in monitoring and managing CD patients, dieticians have an important role to play in the health care team. With regular visits to a dietician it is possible to evaluate the nutritional status and compliance of the patient in more detail.

With the troubles of keeping a strict GFD there is of course a need for non-dietary therapy in CD. Potential non-dietary therapies include; oral proteases for gluten detoxification, zonulin antagonists (decreasing the intestinal permeability) and gluten tolerization (“gluten vaccine”). With the increased understanding of CD pathogenesis these potential therapies have been discovered and some are even in phase 1 and 2 clinical trials. Without doubt we will hear more about these therapies in the future.

2.6.2 Compliance

Adhering to a strict GFD is challenging and requires ongoing support and education from a multidisciplinary health team. Patients diagnosed in childhood have better
dietary compliance than patients diagnosed later in life\textsuperscript{158}. Ludvigsson et al found that the majority of Swedish patients with villous atrophy receive dietary information and that there were signs of low dietary adherence in 17\% of patients with villous atrophy, according to patient chart data\textsuperscript{103}. These data are consistent with that of Ljungman et al (compliance rate 81\%)\textsuperscript{159}. The reasons for low dietary adherence include the economic burden, difficulties finding GFD in a social context and contamination of gluten free products\textsuperscript{160, 161}. Moreover, there is no single superior method of assessment of dietary adherence in CD. Some argue that evaluation of compliance by interview is the best marker\textsuperscript{162, 163}, however self-reported compliance is often overrated and may not correlate with mucosal damage. Often serology is used to follow dietary compliance but they are not reliable in monitoring adherence or histological response to GFD, because patients with a decline in antibody titers may still have manifest mucosal injury\textsuperscript{151, 164}.

It is also important to realize that the quality of life in patients on GFD is affected. These include social factors such as difficulties when dining out, financial considerations (GFD costs 500SEK more per month compared to a normal diet), difficulties when travelling and the impact on family life\textsuperscript{165}. When patients do not respond to the GFD all these factors should be considered and the diagnosis should be re-evaluated to rule out other conditions. To assess the compliance a combination of dietary interview, serologic testing and new biopsy should be performed.

In order to give CD patients the necessary information needed to adhere to the GFD, it is important that both the clinician and the patient understand the protective effect of the treatment. In symptomatic CD there is a rapid change with clinical improvement, but other than that, there are studies showing improvement in bone density\textsuperscript{166}, iron deficiency anemia\textsuperscript{167} and growth in children\textsuperscript{168}. The effect of GFD in patients with CD and type 1 diabetes is still under debate and not conclusive\textsuperscript{169, 170}. As for the long term effects of the GFD, studies have suggested a reduction in the risk of mortality\textsuperscript{115} and malignancy\textsuperscript{171} based on the increased risk that was restricted to the first years after diagnosis. Others have shown an increased risk of lymphoma despite a GFD\textsuperscript{172}. There have also been studies on the risk of autoimmune diseases later in life related to GFD (protective effect and no effect)\textsuperscript{173, 174}. The results of the long-term effects are somewhat contradicting and should be interpreted with caution since the direct effect of GFD has not been examined. When in fact GFD has been examined, data have been collected retrospectively, which increases the risk of recall bias. Finally, most studies have focused on the effect of GFD on “classic CD” and not on the “non-classical” cases. Nevertheless, although evidence of the direct effect of GFD is insufficient with regards to associated disorders, strict adherence does result in healing of the intestinal mucosa in the majority of patients\textsuperscript{151}.
2.7 SCREENING

The issue of screening for CD has been and is still an ongoing debate. CD does in fact fulfil many of the WHO screening criteria (stated below)\(^{175}\).

<table>
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<th>WHO criteria for mass screenings</th>
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<td>(1) Early detection of the disease could be difficult on a clinical basis.</td>
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<td>(2) The disease must be a common disorder causing significant morbidity in the general population.</td>
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<td>(3) The screening tests must be highly sensitive and specific for the target disease.</td>
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<td>(4) A treatment for the disease must be available.</td>
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<td>(5) If not recognized, the disease could result in severe complications difficult to manage.</td>
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But to fully understand the concept of screening it is important to define the meaning of the word. Screening is the attempt to identify a disease or pre-disease in apparently healthy individuals\(^{176}\). CD is a common disorder, and up to two thirds of individuals with CD are asymptomatic\(^{177}\). There is treatment for CD as well as available screening tests of good quality\(^{178}\). Despite many of the criteria being fulfilled the benefits of mass screening for CD is highly questionable\(^{179}\). There are still many things that are unknown about CD, for instance; the natural history of the disease, risk of future complications in CD patients, risk of future complications in undiagnosed CD, the low positive predictive value in some serologic tests and the expected low compliance to GFD in asymptomatic patients. Ultimately, the question we are trying to answer is: will we do more benefit or harm to the population? Furthermore, is it cost-effective to screen? At this point mass screening for CD is not recommended\(^{179, 180}\). The alternative to mass screening is a case finding strategy, which allows for screening in high-risk groups of CD. This method is widely accepted today and therefore screening for CD is recommended in children with type 1 diabetes, first-degree relatives and Down's syndrome\(^{46}\).

2.8 ASSOCIATED DISORDERS

CD is associated with a number of different disorders. Some conditions, such as osteoporosis, iron deficiency anemia etc, are believed to be caused by the malabsorption found in these patients. Other associations are more complex and not yet fully understood.

2.8.1 Diabetes Mellitus

The prevalence of CD in type 1 diabetes (T1D) ranges from 3-12\(^{\%}\)\(^{31, 62, 65}\). The association between CD and T1D is most likely due to shared genetic factors\(^{181}\). There is also an increased risk of subsequent T1D in patients with CD\(^{82}\). However, in the majority of cases, CD is diagnosed after the clinical onset of T1D\(^{21, 183}\). This could be the reason that many children with T1D have subclinical CD and these cases are thus detected through serologic screening. Screening for CD in T1D is in use in clinical settings, but the suitable interval for re-examinations is not decided. Some propose
screening at the onset of T1D and then annually for at least 2 years\textsuperscript{184}, however these approaches varies in between clinical settings.

2.8.2 Refractory Sprue

Refractory sprue (refractory CD) is a condition defined as persistent symptomatic villous atrophy not responding to a strict GFD (for at least 6-12 months) and cannot be explained by other causes than CD\textsuperscript{185}. Refractory sprue is classified according to two types: type 1 with normal intraepithelial lymphocyte phenotype and type 2 with abnormal clonal intraepithelial lymphocyte population. Type 2 refractory sprue has poor prognosis (5-year survival 40-58\%), due to the frequent progression to enteropathy-associated T cell lymphoma (EATL)\textsuperscript{186}. Type 1 refractory sprue usually improves on aggressive treatment with nutritional support, strict GFD and pharmacological therapies (corticosteroids/immunosuppressive drugs). The prevalence of refractory sprue is unknown, but probably rare\textsuperscript{186}. In many studies, data have been collected from major CD referral centres in which the prevalence ranges between 10\%\textsuperscript{187} to 18\%\textsuperscript{188}. In the UK, a population based cohort found five cases of refractory sprue out of 713 individuals with CD (0.7\%), from 1978-2005\textsuperscript{189}.

2.8.3 Dermatitis Herpetiformis

Dermatitis herpetiformis is defined as a cutaneous manifestation of CD and diagnosed by identifying IgA deposits in skin lesions with biopsy\textsuperscript{190}. The skin blisters are distributed on the elbows, knees, buttocks and scalp\textsuperscript{191}. The treatment consists of GFD and pharmacotherapy (Dapsone). In fact, research show that the skin lesions will relapse if challenged with gluten\textsuperscript{192}. A recent study from Finland found a prevalence of dermatitis herpetiformis of 75.3/100,000 (eight times lower than CD prevalence in the same area) and an annual incidence of 3.5/100,000 between 1980-2009\textsuperscript{193}. Both CD and dermatitis herpetiformis are closely connected to the HLA DQ2/DQ8 genotypes. Dermatitis herpetiformis is also associated with a number of autoimmune diseases (T1D, autoimmune thyroid disease)\textsuperscript{194}. However, unlike CD, patients with dermatitis herpetiformis are at no increased risk of fractures, malignancy and mortality\textsuperscript{195}(with the exception of malignant lymphomas\textsuperscript{116}). This could be related to the fact that not all patients with dermatitis herpetiformis have mucosal changes, and the majority of those who have mucosal changes are of milder types\textsuperscript{196}. 
2.9 ASSOCIATED DISORDERS STUDIED IN THIS THESIS

2.9.1 Study I: Visual acuity

According to WHO some 285 million people are visually impaired worldwide, 39 million people are blind and the remaining have low vision\textsuperscript{197}. Approximately 153 million people (range of uncertainty: 123-184) have visual impairment due to uncorrected refractive errors, of these 8 million people are blind\textsuperscript{198}. Globally, uncorrected refractive errors are the main cause of visual impairment and cataract is the leading cause of blindness\textsuperscript{197}. Refractive errors include myopia, hyperopia and astigmatism\textsuperscript{197}. The etiology of refractive errors is not completely understood, however research suggests that both environmental and genetic factors are involved in the development\textsuperscript{199-201}. Environmental factors include socioeconomic factors, education, geographic differences, visually intensive occupations, near work activity and outdoor activity\textsuperscript{199}. CD has been associated with neurological conditions, although results are somewhat conflicting\textsuperscript{109, 202, 203}. Furthermore, there is a well-known connection between CD and autoimmune diseases including T1D\textsuperscript{204}. Research shows that diabetes mellitus (in particular T1D), can influence the thickness and shape of the lens and thereby cause refractive errors\textsuperscript{205}. It is however unclear why this effect is more prevalent in T1D and not as profound in type 2 diabetes\textsuperscript{205, 206}. There have been no earlier studies examining visual acuity in patients with CD. For this reason we examined visual acuity in patients with CD by using data from the Swedish national conscripts register and the Swedish NPR.

Figure 4. Examination of visual acuity. With permission from Dr Maria Kugelberg, St Erik Eye Hospital, Stockholm, Sweden.
2.9.2 Study II: Cataract

Cataract is defined as an opacification of the lens, which interrupts the passage of light. Although cataract usually develops with ageing, this condition can also occur in childhood\textsuperscript{207}. Cataract affects some 20 million people worldwide and it is the leading cause of blindness, particularly in developing countries\textsuperscript{208}. Risk factors include malnutrition, exposure to ultraviolet light, smoking, side effects of medications (steroids) and dehydration\textsuperscript{209}. The formation of cataract is believed to be caused by oxidative stress, however the etiology is likely multifactorial\textsuperscript{210}. There are no previous studies examining the relationship between CD and cataract (other than case reports\textsuperscript{211, 212}). We hypothesized that the risk of cataract would be increased in patients with CD either through oxidative stress, inflammatory processes or vitamin deficiencies.

Figure 4. Cataract. With permission from Dr Maria Kugelberg, St Erik Eye Hospital, Stockholm, Sweden.
2.9.3 Study III: Uveitis

Uveitis is a heterogeneous group of conditions causing inflammation against the uvea (the part between sclera and retina). Causes of uveitis include autoimmune diseases, infections, malignancies, trauma and many cases (30-60%) of unknown etiology. The idiopathic forms of uveitis are believed to be of autoimmune origin. There have been several case reports suggesting a positive association between CD and uveitis, but we are not aware of any large-scale studies examining this relationship. Therefore, we assessed the risk of uveitis in patients with biopsy verified CD compared to reference individuals.

Figure 5. Uveitis. With permission from Dr Maria Kugelberg, St Erik Eye Hospital, Stockholm, Sweden.
2.9.4 Study IV: Diabetic retinopathy

Diabetic retinopathy (DRP) involves vascular changes in the retinal circulation and is the primary cause of blindness among middle-aged people in the United States. As the incidence of diabetes is increasing it is likely that DRP will affect more people worldwide. Consequently, if not treated and managed correctly, a larger proportion of the worldwide population will suffer from permanent eye damage. The major risk factors for DRP development include the duration of diabetes, hyperglycemia, hypertension, hyperlipidemia, pregnancy and nephropathy. Research suggests that DRP development involves inflammatory and autoimmune mechanisms. The relationship between CD and T1D is well established, despite this, few studies have examined the role of CD in relation to T1D complications. We examined the risk of DRP in patients with T1D and biopsy verified CD compared to reference individuals with T1D and no CD.

Figure 6. Diabetic retinopathy. With permission from Dr Maria Kugelberg, St Erik Eye Hospital, Stockholm, Sweden.
3 AIMS

The overall aim of this thesis was to examine the association between CD and eye diseases.

As this subject is new in the area of CD research, we found it appropriate to assess the association through population-based registers in Sweden. This enabled us to use high quality data, without contacting or causing inconvenience to the study participants involved, including children. By exploring the association between CD and eye diseases we will hopefully add to the knowledge of CD complications, increase awareness among clinicians and patients, and identify risk factors for eye disease development.

Specific aims:

To examine visual acuity in patients with CD. More specifically, we investigated if an inpatient diagnosis of CD was associated with decreased visual acuity in young men.

To examine if there is an association between biopsy-verified CD and cataract.

To examine if there is an association between biopsy-verified CD and uveitis.

To examine if biopsy-verified CD affects DRP development in patients with T1D.
4 SUBJECTS AND METHODS

4.1 SETTING

The included studies in this thesis were all conducted in Sweden. With its high quality health care system, the existence of longitudinal population-based registers, the unique personal identity number and health care access to all; Sweden offers extraordinary opportunities for epidemiological research.

4.2 DATA-SOURCES

4.2.1 The Swedish national patient register

The Swedish national patient register (NPR) (Swedish: Patientregistret) is managed by the National Board of Health and Welfare and contains data on inpatient care (since 1964), hospital based outpatient care (since 2001) and day surgery care (since 1997). Data are collected once a year and since 2001 both private and publicly funded health care givers are required to deliver data to the NPR (except for primary care givers). The Swedish national inpatient register (also called the Hospital Discharge register) was launched in 1964 but has complete national coverage since 1987. Currently, 99% of all somatic and psychiatric hospital discharges are registered in the inpatient register. The data in the inpatient register are structured around the information on the hospital discharge/admission of each patient, so that each row in the dataset delivered to the researcher represents a hospital discharge/admission. Some of the variables included in the inpatient register are; the personal identity number, sex, age, admission date, discharge date, hospital, type of department and diagnoses according to the international classification of disease system (ICD adapted from WHO). The accuracy of diagnoses in the Swedish inpatient register is regarded as high. In a recent validation study Ludvigsson et al reported a positive predictive value of 85-95% for most diagnoses in the inpatient register. We have used the Swedish NPR to identify individuals with CD (study I), individuals with T1D (study IV) and the outcome measures (studies II-IV). Regarding the accuracy of eye disease diagnoses in the NPR we conclude that it is high with respect to the findings in study III. We reviewed some 200 patient charts of individuals with a diagnosis of uveitis, to characterize the uveitis patients in more detail and to validate the ICD codes for uveitis in the NPR. We found a positive predictive value for uveitis of 93% in that sample.

4.2.2 The personal identity number

The personal identity number (PIN) is a unique ten-digit identifier, issued to all Swedish residents. The PIN was introduced in 1947 in Sweden (then a nine-digit combination) and now consists of six digits representing the birth date (year-month-day), followed by three digits to identify the individual and a tenth digit, which is a check digit. The PIN is now extensively used not only for the purpose of identifying individuals but also for population statistics, medical research, health care, taxation and
social security. Data linkage between registers in this thesis was made possible through the usage of the PIN.

4.2.3 The Swedish medical birth register

We used data on pregnancy duration from the medical birth register (study IV), to exclude women who received their first T1D diagnosis 0-9 months before giving birth (potential gestational diabetes) from the study population. The medical birth register was established in 1973 and contains information on antenatal, perinatal and neonatal medical care. The register also contains data on smoking, snuffing, maternal weight and height.

4.2.4 The prescribed drug register

We used data from the prescribed drug register (study IV), to exclude individuals with a record of previous oral antidiabetic medication (such patients might have type 2 diabetes with a later need of insulin) from the study population. The prescribed drug register was launched in 2005 and contains outpatient data on medication according to Anatomical Therapeutic Chemical Classification System codes (ATC codes). This register contains records of some 8 million individuals, and according to the National Board of Health and Welfare there are 100 million prescriptions administered each year. The prescribed drug register does not include data on medication administered to inpatients, or drugs sold without a prescription (over-the-counter drugs).

4.2.5 The total population register

The total population register (TPR) contains information on all Swedish residents with regards to demographic variables such as sex, marital status, area of residence, personal identity number, emigration, immigration, internal migration, births and deaths. The TPR is maintained by Statistics Sweden and started in 1966 (computerized in 1968). The data in the TPR are received and updated daily by the national tax authorities. We used data from the TPR in all studies (I-IV) to determine the end of follow up. Statistics Sweden also maintains data on educational level, which is reported continuously from schools and educational authorities in the country. The different levels of education are divided into seven categories based on the duration (years) and type of education (high school, college, doctoral education).

4.2.6 The Swedish national conscripts register

The Swedish Defence Recruitment Agency (in Swedish “Rekryteringsmyndigheten”) maintains the conscripts register. The register contains data on military and medical information, on all men born in 1983-2010, that have enrolled or conducted an entrance assessment at the National Service Administration. The Military Archives (“Krigsarkivet”) founded in 1805 has been responsible for the keeping of military records since early 1950. However, accessible and computerized records of Swedish conscripts are only available since 1983. The variables in the register are both of military and medical interest and include weight, height, visual acuity, cognitive test results, personal identity number, year (before 1997) and year, month, day (after 1997)
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of conscription, measures of physical strength and endurance. The conscripts register also contains data on women volunteering for conscription. Until the mid 1990’s all Swedish men had to attend conscription. Thereafter political priorities and decisions changed the Swedish defence system. The proportion of Swedish men attending conscription declined between 1995-2000 (98% of all men conscripted in 1996, some 80% in 2000) according to chief statistician Ahlstrand at the Swedish conscripts register. The coverage and quality of the conscripts register is regarded as high until 1995 (<1% data irregularities and <6% missing data in all variables). Since July 2010 it is not compulsory to attend conscription (except for at time of war). Instead there is a voluntary military education available for all 18-year-old men and women in Sweden.

4.3 STUDY DESIGN

4.3.1 Study I

In the first study we investigated whether or not CD had an impact on visual acuity, in a cohort of some 8000 men. Data on visual acuity was obtained from the Swedish national conscripts register. By using the personal identity number of each individual we could link data from the Swedish national conscripts register to the Swedish national inpatient register, to identify all conscripts with an inpatient diagnosis of CD between 1964-2003. For each male conscript with CD, Statistics Sweden selected five reference individuals from the total population register (with available data from the eye examination at conscription), matched for sex, age, calendar year and county. We then evaluated visual acuity in three different exposed cohorts consisting of a) 69 males with an inpatient diagnosis of CD after conscription (undiagnosed CD) b) 996 males with an inpatient diagnosis of CD before conscription (diagnosed CD) and c) 6850 males without a diagnosis of CD. We compared the prevalence of decreased visual acuity between the groups. We also performed a case-control study, to examine the association between diagnosed/undiagnosed CD and decreased visual acuity.

4.3.2 Studies II-III

The second and third studies were historical cohorts with prospectively collected data. Individuals with CD were defined as villous atrophy (Marsh 3) according to biopsy reports obtained from all pathology departments in Sweden (n=28). The outcomes were defined according to relevant ICD codes in the Swedish NPR. For each individual with CD, Statistics Sweden selected five reference individuals from the total population register matched for sex, age, calendar period and county. The personal identity number enabled us to perform data linkage. Follow up started on the date of first positive biopsy (CD diagnosis, villous atrophy) and continued until the date of first diagnosis of the outcome in interest, death, emigration or end of study period (December 31, 2008), whichever occurred first. Follow up started at the same time for reference individuals (CD diagnosis) and ended at the same time as the CD individuals, it could also end if the reference individual underwent a small intestinal biopsy. We used Cox regression analysis to determine hazard ratios for the outcome in CD. Even though the main focus of these studies was to examine the risk of the outcomes in biopsy verified CD (Marsh
3) we also collected data on small intestinal inflammation (Marsh 1-2) and normal mucosa with positive CD serology (Marsh 0). Consequently, in separate analyses we were able to use those with a lesser degree of pathology in the small intestine (Marsh 0-2) as secondary reference groups. This was done to determine whether the risk of the outcome was specific to CD (Marsh 3) or not. We also carried out case-control studies within these cohorts. We used conditional logistic regression to estimate the odds ratio for CD in individuals with a prior diagnosis of the outcome of interest. The case-control studies were performed to evaluate if the association between CD and the outcome was restricted to those with CD preceding the outcome. As part of study III, patient charts were reviewed by the authors (KM and LT) in order to characterize individuals with the outcome (uveitis) and to validate the definition of the outcome through ICD codes in the NPR. The patient chart review was performed without prior knowledge of CD or control status.

4.3.3 Study IV

This study is a historical cohort similar to studies II-III, however there are some differences. In this study we examined the risk of DRP in patients with T1D and CD compared to reference individuals with a diagnosis of T1D only. The exposure is defined as individuals with a diagnosis of T1D and coexisting CD. T1D was defined as a diagnosis of diabetes mellitus according to relevant ICD codes in the Swedish NPR (both inpatient and outpatient data) in individuals aged ≤30 years. This age definition was necessary because the earlier versions of Swedish ICD (7-9) did not distinguish between type 1 and type 2 diabetes. Individuals with CD were identified as previously described (small intestinal biopsy reports (Marsh 3) from all pathology departments in Sweden). The outcome (DRP) was defined according to ICD codes in the NPR. Censoring information (including information on death, emigration, country of birth) was obtained from the total population register at Statistics Sweden. Follow up started on the date of T1D diagnosis and continued until the first DRP diagnosis, emigration, death or end of study period (December 31, 2009), whichever occurred first. We used Cox regression analysis with CD modeled as a time dependent covariate to calculate hazard ratios for DRP in patients with T1D and CD.

4.4 STATISTICAL ANALYSES

4.4.1 Logistic regression (studies I-III)

In papers I-III we perform logistic regression analysis using case-control designs. What distinguishes a logistic regression from a linear regression model is that the outcome variable in a logistic regression is binary (dichotomous/ CD=yes or no). The goal is indeed the same as in a linear regression model: to find the best fitting model to describe the relationship between an outcome variable (dependent variables) and a set of explanatory variables (independent variables). The independent variables can be of any type (continuous/categorical). In linear regression least of squares are used to estimate the unknown parameters ($\beta$’s), in logistic regression, maximum likelihood functions are used instead. Moreover, in contrast to assuming the normal distribution for the error variances in linear regression, a logistic distribution is assumed in logistic regression.
To assess the significance of the variables in the model, the likelihood ratio test is used, which is basically comparing the full model (with the new variables) with the reduced model (without the new variables). Many of the principles of the linear regression models are similar to those in the logistic models.

The impact of the predictor variables is expressed in terms of odds ratios. In contrast to the risk, which is defined as the number of patients developing the disease divided by the persons at risk, the odds is defined as the number of patients who develop the disease divided by the patients who do not develop the disease.

In our matched case-control studies we are using conditional logistic regression to estimate the association between for instance cataract (study II) and a subsequent diagnosis of CD. When using a conditional approach to the logistic regression model each case is compared to his/her matched control, which minimizes the effect of the matching variables. If one uses an unconditional logistic regression in a matched analysis instead, this will overestimate the odds ratio. The unconditional approach is preferred when the number of parameters is small in relation to the sample size.

4.4.2 Survival Analysis (studies II-IV)

In survival analysis we estimate the time until an event occurs. In contrast to linear regression where the outcome is a continuous variable like weight/blood pressure, and a binary variable like CD (yes/no) in logistic regression, the outcome in survival analysis is “time to an event”. In linear and logistic regression modeling we usually do not have follow-up time available (or more precisely the information is not used), which we do in survival analysis. The event can be anything from incidence of disease (i.e. cataract) to for instance death. Time (also known as survival time) is defined as the time passing from start of follow-up until the event occurs. In survival analysis we also have the ability to deal with study participants in whom we do not know the exact survival time, this is known as censoring. Reasons for not knowing the exact survival time could be the following: the patient does not experience the event before the study ends, the patient is lost to follow up or the patient withdraws from the study (i.e. death). Even though we do not know the censored person’s complete survival time we can still utilize the observed information we have on that person until we lose track of them (that is if we assume that the censoring is non-informative).

The general aim of all these statistical methods (linear/logistic/survival) is to measure the effect between exposure and outcome, usually also with adjustment for other covariates. In survival analysis we work with two basic terms the survivor function and the hazard function. The survivor function is a measure of survival; whereas the hazard function is a measure of failure at a given time, given you have survived until a certain time.

The measure of effect in survival analysis is the hazard ratio. The Cox proportional hazard model is a semi-parametric model and expressed as the hazard at time $t$ for an individual. In the Cox model the variables are independent of time, but it is also possible to use time-dependent variables (as we do in study IV). The reason for the name “semi-parametric model” is that the baseline hazard in the Cox model is unspecified, compared with parametric models where the functional parts are all specified (except for the unknown parameters).
The coefficients are estimated much in the same way as in a logistic model, through maximizing the likelihood function. When all the likelihood functions at each failure time have been calculated they are multiplied to form a likelihood function product. Once we have the maximized likelihood function we can make statistical inferences in terms of hazard ratios, confidence intervals etc. The hazard ratio is defined as the hazard of one individual divided by the hazard of another individual, the only thing differing is their set of explanatory variables.

To use a Cox model it must meet with the proportional hazard assumption, which means that the hazard of an individual is in proportion to the hazard in another individual, or in other words the hazard ratio is constant over time. There are different methods for checking the proportional hazard assumption. One approach is plotting the -log-log survival curves, which is taking the natural log of an estimated survival function twice. This method is what we use to check for proportionality in our hazards.

In our analyses we stratify for several variables. In a stratified Cox model the estimates of the coefficients are obtained by maximizing the likelihood function of each stratum and then we can obtain a summarized hazard ratio for each stratum. This means we will obtain the hazard of all females experiencing the event (cataract/uveitis/DRP) compared to the hazard of the female subjects not experiencing the event in CD and reference individuals (adjusted for all matching variables).

After adjustment the next step is to check for interaction in the model. When statistical interaction is present it means that not just the baseline hazards differ across strata (which is normal) but also the coefficients (β’s) differ. There are many different ways of defining the interaction term, but it usually involves a product term. Once interaction has been defined it is through a likelihood ratio test we can decide whether the difference between the reduced (no interaction) and full (with interaction) model is significant.

In the last study we are using CD as a time dependent variable in our Cox regression model. The difference between a time-dependent and time independent variable is their consistency over time. Some variables may be consistent over time such as race or gender and other variables may change over time such as treatment or disease status. Based on the fact that disease status (CD) may be different over time in study participants, their risk of developing the outcome DRP may also be different. Patients with T1D since the beginning of follow up and CD diagnosed in the last two years of the study period may differ from patients with CD and T1D since the beginning of the study in terms of DRP risk.

When using the extended Cox model the proportional hazard assumption is no longer satisfied.4.4.3 Comparing group statistics (study I)

In study I, we examine visual acuity in individuals with diagnosed CD, undiagnosed CD and reference individuals (without CD). To compare the median visual acuity across these groups we used the Kruskal Wallis test. Kruskal Wallis test is a non-parametric method, used to compare values of an outcome measurement between more than two groups. In the same study we used chi-2 test to compare proportions of
decreased visual acuity between the groups, to assess whether there is an association between exposure and disease. The chi-2 test compares the difference between observed (proportion of individuals with decreased visual acuity) and expected (if there was no association between visual acuity and CD) data and is expressed as a p-value.
5 RESULTS

5.1 CD AND VISUAL ACUITY (STUDY I)

There was no association between diagnosed CD and decreased visual acuity (OR=1.03; 95% CI=0.90-1.19) or between undiagnosed CD and decreased visual acuity (OR=1.04; 95% CI=0.64-1.70). Adjustment for diabetes mellitus, calendar period and socioeconomic index did not change the risk estimates. The mean (±SD) visual acuity score was similar in all three groups; reference individuals: 8.03±1.46; undiagnosed CD: 8.04±1.37; diagnosed CD: 8.02±1.50. Furthermore, the prevalence of decreased visual acuity did not differ according to CD status (reference individuals: 35.3%; undiagnosed CD: 36.2%; diagnosed CD 36.0%) (chi square test, p=0.890).

In a separate analysis the risk of very decreased visual acuity was evaluated between the groups. In reference individuals 5.4% (n=371) had very decreased visual acuity compared with 1.4% (n=1) in undiagnosed CD and 6.3% (n=63) in diagnosed CD; these differences were not statistically significant (p=0.167). There was no association between undiagnosed CD and very decreased visual acuity (OR=0.26; 95% CI=0.04-1.86) or between diagnosed CD and very decreased visual acuity (OR=1.18; 95% CI=0.90-1.56).

5.2 CD AND CATARACT (STUDY II)

CD was associated with a moderately increased risk of cataract development (HR=1.28; 95% CI=1.19-1.36). The absolute risk of cataract among patients with CD was 397/100,000 person-years; the excess risk was 86/100,000 person-years. The risk estimate did not change after adjustment for educational level and type 1 diabetes.

When we specifically examined the risk of cataract recorded as a main diagnosis in the NPR we found that CD patients had an even higher risk of cataract (HR=1.81; 95% CI=1.40-2.36). In a separate analysis we examined the risk of cataract in patients with CD compared to those with a lesser degree of histopathology (Marsh 1-2 and Marsh 0) in the small intestine. From that analysis we found that patients with CD were at no increased risk of cataract compared to those with milder changes in the small intestine. Finally, we also analyzed the risk of CD in patients with a previous diagnosis of cataract. The OR for a later diagnosis of CD in patients with a previous diagnosis of cataract was 1.14 (95% CI=1.02-1.30).

5.3 CD AND UVEITIS (STUDY III)

During follow-up we identified 148 individuals with CD that developed uveitis (HR=1.32; 95% CI=1.10-1.58). The absolute risk of uveitis in patients with CD was 50/100,000 person-years, and the attributable risk percentage was 24%. Neither age at CD diagnosis nor sex influenced the risk of uveitis. The HR was still significantly increased and did not change with adjustment for type 1 diabetes, autoimmune thyroid disease and rheumatoid arthritis (HR=1.30; 95% CI=1.08-1.56).
In a separate analysis we examined the risk of uveitis in patients with CD and compared with reference individuals consisting of patients with small intestinal inflammation but no villous atrophy (Marsh 1-2) and patients with normal mucosa and positive CD serology (Marsh 0). Patients with CD were at no increased risk of uveitis compared to individuals with inflammation (Marsh 1-2) (HR=0.91; 95% CI=0.69-1.19) or to those with normal mucosa and positive CD serology (Marsh 0) (HR=0.78; 95% CI=0.50-1.21).

From the patient chart review we found that the majority of patients (both cases and controls) had unilateral acute anterior uveitis. The most common form of comorbidities found in patients with both CD and uveitis were type 1 diabetes (8%) and inflammatory bowel disease (14%). In controls the most common comorbidity was ankylosing spondylitis (8%).

### 5.4 RISK OF DRP IN CD AND T1D

We found that duration of CD diagnosis in patients with T1D was a strong predictor for DRP development. Patients with T1D and CD were at a low risk of DRP during the first 5 years after CD diagnosis (adjusted for sex, age, calendar period) (adjusted HR, aHR=0.57; 95% CI=0.36-0.91). In years 5-<10 years after CD diagnosis the aHR was neutral 1.03 (95% CI=0.68-1.57) in patients with T1D and CD. With longer duration of CD diagnosis in T1D patients the HR grew higher: 10-<15 years of follow-up: aHR=2.83 (95% CI=1.95-4.11) and ≥15 years of follow-up: aHR=3.01 (95% CI=1.43-6.32). The absolute risk of DRP in patients with T1D and CD was 2769/100,000 person-years after more than 15 years of follow-up, with an excess risk of 1849/100,000 person-years.

In subanalyses we found that the overall risk of DRP in patients with T1D and CD during the first 10 years after CD diagnosis was low (aHR=0.75; 95% CI=0.55-1.03), whereas the overall risk of DRP was increased (aHR=2.87; 95% CI=2.06-4.02) beyond 10 years after CD diagnosis. The same pattern of aHRs (lower aHRs <10 years after CD diagnosis and higher aHRs ≥10 years after CD diagnosis) were found in stratified analyses for sex, age at T1D diagnosis and calendar period.

In sensitivity analyses we examined the risk of DRP in T1D and CD by; 1) restricting the analysis to those with an inpatient diagnosis of T1D; 2) excluding women who received their T1D during pregnancy (0-9 months before delivery); 3) excluding patients with oral anti-diabetic medication; and 4) restricting the outcome to severe DRP requiring retinal laser therapy. In analyses: 1-3 the aHRs did not change (see table). In analysis 4 we found the same lower initial aHR during short time of follow-up after CD diagnosis (<5 years with CD: HR=0.56 (95% CI=0.18-1.74); 5-9.99 years: HR=0.43 (95% CI=0.11-1.73) followed by an increased aHR with longer follow-up time after CD diagnosis (10-14.99 years: HR=2.49 (95% CI=1.18-5.25); ≥15 years: HR=2.01 (95% CI=0.50-8.06)).
<table>
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<th>Sensitivity analyses (study IV)</th>
<th>CD Duration (&lt;10 years)</th>
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<td>First T1D diagnosis not during pregnancy</td>
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<td>No oral anti-diabetic medication</td>
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<td>Inpatients with T1D</td>
<td>0.77; 0.56-1.06</td>
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*Adjustment for sex, age at T1D diagnosis and calendar period
6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

6.1.1 Study design

Randomized controlled trials are considered to be the gold standard in study design, because of their possibility to identify causal associations. However, randomized trials have some crucial disadvantages including ethical considerations, cost, lack of generalizability and feasibility. For these reasons observational studies are needed. In fact some have demonstrated that if properly designed, it is possible to find causality in observational studies as well235.

The main types of epidemiologic design are the cohort study and the case-control study. The choice of study design is mainly based on the aim of the study (what question are we answering?) and efficacy (time and financial considerations).

6.1.1.1 Cohort study

In a broad definition a cohort study is a group of people that are followed for a period of time. The assembled group of people should not have experienced the outcome of interest but all of them should be “able” to experience it (for instance, in order to examine the risk of uveitis, those included in the study should have a pair of eyes). In the cohort, members are then divided according to certain risk factors into exposed and unexposed members (patients with CD=exposed, patients without CD=unexposed). During follow-up it is then possible to measure the occurrence of disease and to compare the measure of association between the cohorts (exposed and unexposed).

Often cohort studies are referred to as expensive and time-consuming, which is the case in large-scale prospective studies (like the Nurses health study236), but that can be avoided in historical/retrospective cohort studies based on registered data (i.e. hospital registers). Because the registers are seldom designed for research purposes the historical cohorts are often limited in detailed information about exposure and outcome, restricting the assessment of confounding factors and hence the strength of the association. On the other hand, the independent registration of data without knowledge of the potential associations to be studied in the future minimizes the risk of measurement bias. Furthermore, data on exposure and outcome are registered prospectively, which reduces the risk of recall bias. The studies included in this thesis are population-based (historical) cohort studies, based on prospectively registered data on CD as the exposure and other conditions as outcome (studies I-IV).

6.1.1.2 Case-control study

When the outcome of interest is infrequent in the population, a large number of people must remain under observation for a long time in a cohort study in order to obtain results. One way of dealing with this issue is to conduct a case-control study. The goals are the same in cohort studies and case-control studies (to measure risk/rate of disease), however case-control studies do this more efficiently by using sampling. Two samples are identified: individuals who have developed the disease (cases) and individuals (otherwise similar to cases) without the disease (controls). By looking back in time we
can then determine the frequency of exposure to a certain risk factor (CD) in each
group, and calculate the relative risk (odds ratio). The validity of the case-control study
is largely based on the sampling of controls. In case-control studies the control group
act as representatives of the source population from which the cases have been
identified. Therefore it is crucial that controls be sampled independently of exposure
status and be selected from the same source population which generated the cases\textsuperscript{237}. In
our case-control study (study II) the cases and controls were selected from the same
cohort (also known as nested case-control study) and to ensure the comparability
between cases and controls, they were matched for important variables related to both
exposure and disease (sex, age and calendar period).

6.1.2 Measure of association

Epidemiology is defined as the study of disease occurrence\textsuperscript{237}. The most common and
widely understood concept is risk, but there are several measures of disease occurrence.

6.1.2.1 Incidence

Incidence is defined as the number of new cases of disease occurring during a period of
time in a defined population initially free from the disease. Absolute risk has the same
value as the incidence and the terms are used interchangeably\textsuperscript{238}. When we include the
measure of time the measure is called incidence rate; number of subjects developing
disease divided by total time experienced by the subjects that were followed. The time
unit is calculated by summing the time (during which the subject is at risk of the
disease) that each subject has contributed with during follow-up. Often the time unit is
expressed in 100,000 or 1000 person-years.

6.1.2.2 Attributable risk

It may be interesting to know how much of the risk increase/decrease that was
contributed by the presence of the exposure. This is called the attributable risk. In our
studies we have presented the attributable risk (percentage), which is calculated by 1-
1/HR or by dividing the excess risk with the absolute risk. Another way of comparing
risks, is by calculating the difference in absolute risks between the exposed group and
the unexposed group, presented as excess risk/risk difference.

6.1.2.3 Interpretation of risk estimates

The relative risk or risk ratio are commonly described in epidemiologic studies, they
describe the strength of the association between exposure and disease. However they do
not describe the degree of the attributable or absolute risk. A large relative risk can be
accompanied by a small attributable risk if the disease is uncommon (which is often the
case)\textsuperscript{238}. In such circumstances both expressions of risks should be presented.

6.1.3 Internal validity

Internal validity can be defined as the quality of the study, including everything from
study design, data analysis to the assessment of bias. In other words, is it possible to
draw conclusions from the study? In epidemiologic studies there are two major types of
errors that should be considered: systematic error (bias) and random error. The latter can be dealt with by increasing the study size.

6.1.3.1 Selection bias
Selection bias is a systematic error related to the selection of study participants. The differences between the groups that are compared will lead to risk estimates that are biased.

In the present thesis (studies I-IV), the cohort studies are based on all individuals in the Swedish population through the national health registers. All individuals in Sweden have the same access to health care and through continuously updated population-based registers and the personal identity number; all Swedish residents had the same probability of being sampled.

In study I we examined visual acuity in young men identified through the Swedish conscripts register. It could be argued that young men with CD would not have been included in the conscript register to the same extent as the general population. Between 1964-1994 all Swedish men were conscripted (except for those with a serious handicap or living in institutions), including men with a diagnosis of CD. From the mid 90ies conscription rates started to decline, consequently we excluded conscripts from 2001 and onwards.

Other potential sources of selection bias in study I were the definition of CD and missing data. CD was defined according to relevant ICD codes in the Swedish NPR (inpatient data). This definition may lead to a selection of more severe CD cases and the risk of not including all patients with CD in the population. There is also a risk that the slightly higher proportion of missing data on visual acuity in CD individuals represented a selection of those CD individuals with extremely decreased visual acuity. On the other hand, the trained staff would probably make an effort of examining those with very impaired visual acuity since it affects the conscript’s future military training. In addition, we found in a subanalysis that the risk of very impaired visual acuity among the groups (CD, undiagnosed CD and reference individuals) was not statistically significantly different. Moreover, we had data on visual acuity in 85.4% of CD individuals and 94.3% of reference individuals; therefore the missing data should not have a major effect on our results.

In studies II-IV we defined CD according to histopathologic features (villous atrophy, Marsh 3) as stated in biopsy reports from all pathology departments in Sweden. This enabled us to identify all diagnosed CD individuals in Sweden, including those who have not been admitted to a hospital. With this approach the risk of selection bias was minimized.

In study IV we defined T1D according to ICD codes in the Swedish NPR (both inpatient and outpatient data). Individuals with a diagnosis of diabetes mellitus and age ≤30 years were defined as having T1D. With the characteristics of this disease and the onset in young age, most T1D individuals will seek medical care and thereby be included in our study.
6.1.3.2 Surveillance bias/detection bias
With a diagnosis of CD there may be an increased risk of undergoing various investigations and testing, which could lead to other conditions being detected. This is probably more likely with an inpatient diagnosis of CD since such patients might have more severe symptoms and investigations may be more forceful. However, with the increasing knowledge about CD and its associations with other conditions, an outpatient diagnosis may be just as likely to be at risk for surveillance bias as an inpatient diagnosis. We cannot rule out that surveillance bias influenced our risk estimates. To minimize the risk of surveillance bias, we evaluated the risk of the outcomes by excluding the first year of follow-up (when the risk of detecting other conditions should be higher) and by evaluating the risk over years of follow-up.

6.1.3.3 Misclassification
Misclassification refers to a systematic error that can occur when collecting information about exposure or outcome. This is also called information bias. The misclassification of exposure or outcome can be differential or non-differential. When the misclassification of exposure is unrelated to the outcome it is non-differential, if it on the other hand is related to the outcome (different for those with and without outcome) it is differential. The same goes for misclassification of outcome, it is non-differential if unrelated to the exposure; and differential if related to the exposure. The differential misclassification can exaggerate or underestimate an effect, whereas the non-differential misclassification leads to a diluted effect.

6.1.3.3.1 Misclassification of exposure

6.1.3.3.1.1 Celiac disease and T1D
The identification of CD through biopsy reports from all pathology departments in Sweden has been validated and proved to have a high positive predictive value for the diagnosis of CD\textsuperscript{103}. The accuracy of histopathologic classification of small intestinal biopsies is high by Swedish pathologists. In fact, 90% (95% CI=87-94%) of small intestinal biopsies with villous atrophy are correctly classified by Swedish pathologists, as defined by the Swedish National Steering Group for Small Intestinal Pathology\textsuperscript{103}. Our research group manually reviewed some 1,500 biopsy reports to assess the specificity of villous atrophy. Villous atrophy was rarely caused by other conditions than CD (the most common comorbidity was inflammatory bowel disease occurring in 0.3% of biopsies with villous atrophy)\textsuperscript{103}. Through patient chart review our research group found that 95% (95% CI=91-99%) of individuals with villous atrophy in the biopsy report had CD\textsuperscript{103}. Sometimes villous atrophy can be patchy and if not enough biopsy samples are obtained the samples may be misinterpreted. Swedish pathologists report that almost 9 out of 10 gastroenterologists/paediatricians send at least two biopsy samples when CD is investigated\textsuperscript{103}. Out of 114 evaluated patient charts n=6 individuals' diagnosis of CD was rejected, 4 biopsies had been misclassified and 2 samples were re-evaluated\textsuperscript{103}. Although we chose not to use CD serology for the diagnosis of CD, we found through the patient chart review that 88% (95% CI=80-95%) of those with villous atrophy had positive CD serology prior to the biopsy. From these analyses we can conclude that villous atrophy has high specificity for CD.
The sensitivity of diagnosed CD according to biopsy reports should be close to 100% since the gold standard for CD diagnosis is small intestinal biopsy with villous atrophy. In addition, more than 96% of Swedish gastroenterologists and 100% of paediatricians perform a small intestinal biopsy in 9 out of 10 patients before diagnosing CD\textsuperscript{103}. The risk of misclassification of CD should thus be less through biopsy reports than by using the Swedish NPR to identify CD patients. Although the Swedish NPR has a high validity for many diagnoses\textsuperscript{222} there are no specific validation studies on the diagnosis of CD in the NPR. However, the diagnosis of CD according to ICD codes in the NPR has been evaluated and confirmed through patient chart review\textsuperscript{143}. The positive predictive value was 86% for CD in the NPR as compared with 95% from biopsy reports\textsuperscript{141}.

Through biopsy reports we identified some 30,000 CD patients, the prevalence of CD in Sweden is approximately 1\%\textsuperscript{1}. Therefore there will be some undiagnosed CD in our reference population. However, with a population of 9 million inhabitants in Sweden the undiagnosed CD in the reference population should have a marginal effect on our findings, and if so a reduction/dilution of the differences would occur.

In study IV we defined T1D according to ICD codes in the Swedish NPR. In earlier ICD versions (ICD 7-9) the Swedish NPR did not distinguish between type 1 and type 2 diabetes. Therefore, we defined T1D as a diagnosis of diabetes mellitus in individuals younger than ≤30 years of age. The sensitivity for this definition of T1D should be high. Because of the nature of the disease (develops in young age, fatal if not treated), all patients are expected to seek medical care and thereby be included in our study. The age algorithm in the definition of T1D has been used previously\textsuperscript{240, 241} and has a positive predictive value for insulin-dependent diabetes of 95%\textsuperscript{240}. However, with this definition of T1D we cannot rule out that some patients with type 2 diabetes have been misclassified as T1D (advanced type 2 diabetes may require treatment with insulin). To reduce the risk of differential misclassification, we increased the specificity of T1D by performing subanalyses in which we excluded those with a record of oral anti-diabetic medication (most likely type 2 diabetics), and those who received a diagnosis of T1D 0-9 months before giving birth (gestational diabetes). These subanalyses did not influence our results. Even if the specificity of T1D is not as high as the sensitivity, we believe that we have identified nearly all individuals with T1D in Sweden during the study period.

6.1.3.3.2 Misclassification of outcome

6.1.3.3.2.1 Visual acuity

We obtained data on visual acuity from the Swedish conscripts register. The examination of visual acuity at conscription was performed by trained personnel (nurses), which minimizes the risk of misclassification. However, there are no validation studies performed regarding the measurement and registration of visual acuity in these settings.
6.1.3.3.2.2 Cataract

Cataract was defined according to relevant ICD codes in the Swedish NPR and surgical codes in the Swedish National Day-Surgery Register. Through this definition we believe that the majority of cataract cases were identified. Cataract is a condition that is not managed in primary health care; it is rapidly referred to specialist care (ophthalmology clinic) and in that way the risk of misclassification should be reduced. In order to increase the sensitivity for cataract, we chose a broad definition including unspecified ICD codes for cataract. With increasing sensitivity the specificity of cataract decreases, therefore we carried out subanalyses where cataract was restricted to a main diagnosis of cataract and to those undergoing cataract surgery. With this slightly narrower definition of cataract the relative risks increased. Consequently, the hazard ratio for cataract in CD may be higher than 1.28. There should be no difference in misclassification between those with CD and those without CD, and so differential misclassification is not likely to have affected our results.

6.1.3.3.2.3 Uveitis

Uveitis was defined according to ICD codes in the Swedish NPR. As part of study III we (KM and LT) manually reviewed 165 patient charts, blinded to case-control status, to validate the diagnosis of uveitis. We found a positive predictive value for uveitis of 93.3% (154/165). Of the 11 patients with false-positive uveitis, one patient had keratitis and wrongly received a code for uveitis, in three patients uveitis was not mentioned during 7 years of follow up in eye clinics. Another seven patients had no data on uveitis in their patient charts and were considered unlikely to have uveitis. In difficult cases regarding the characterization of the uveitis KM received help from LT (ophthalmologist specialized in inflammatory disorders of the eye). We believe that both sensitivity and specificity for uveitis is high in the Swedish NPR, and the risk of misclassification low.

6.1.3.3.2.4 Diabetic retinopathy

In study IV, we defined DRP according to relevant ICD codes in the Swedish NPR. DRP patients are managed by ophthalmologists, which minimizes the risk of misclassification. According to the national guidelines for diabetes care provided by the Swedish National Board of Health and Welfare, all individuals with T1D should be screened for DRP by retinal fundus imaging from the time of diagnosis and every other year thereafter. With these screening routines the majority of DRP patients should be identified through ICD codes in the NPR. On the other hand, these guidelines were implemented as routine care during the 1990ies. There is a risk that DRP cases were not identified in the same extent during the start of the study (1964) as compared with the later part of the study, but such misclassification of the outcome will only affect the results if it differs between exposed (T1D and CD) and unexposed (T1D), which is unlikely. To increase the specificity for DRP we also evaluated the risk of severe DRP defined as having a DRP code and requiring retinal laser therapy according to surgical codes in the NPR. The risk of severe DRP followed the same pattern of HRs as in the main analyses.
6.1.3.4 Confounding

Confounding can be defined as a confusion of effects\textsuperscript{237}. A confounding factor is by definition associated with the exposure and should have an effect on the outcome\textsuperscript{237}. The confounding factor should not be an effect of the exposure. There are several methods to prevent/remove confounding: randomization, restriction, matching and stratification.

In this thesis we have considered several confounding factors and dealt with them in various ways. In study I potential confounders such as diabetes mellitus, socioeconomic index and calendar period were considered in the regression model. In studies II-III confounding was dealt with through matching, all reference individuals were matched for sex, age, calendar period and county to the index individual with CD. These matching variables were then considered through the internal stratification of the Cox model, thus eliminating confounding by the matching variables. In study IV, confounding such as sex, age, and calendar year were adjusted for in the Cox regression model. In studies II-IV, we also carried out stratified analyses with regards to years of follow-up, sex, age at study entry and calendar year at study entry.

In studies III-IV we adjusted our data for country of birth (Nordic vs. non-Nordic), because both exposure (CD and T1D) and outcome (uveitis, DRP) have different prevalences in different countries. Other potential confounders were educational level, because education has been associated with seeking medical care\textsuperscript{243} and other autoimmune diseases that are associated with both exposure and outcome such as diabetes mellitus, autoimmune thyroid disease and rheumatoid arthritis\textsuperscript{244, 245}. We lacked information on smoking, weight and height, however studies show that both smoking and obesity are inversely related to CD\textsuperscript{93, 98, 246}.

Effect-measure modification (statistical interaction) occurs when the effect of exposure on the disease is modified by the presence of a third variable (effect modifier). In the presence of effect-measure modification the association is different across subgroups (after stratification), we considered this through interaction analyses.

6.1.3.5 Random error

In all observations one must be prepared that random error (resulting from chance) is present. Since we are not working with the (original) entire population but instead sampling from it, we may always encounter results that are there by chance. Therefore it is important to consider how likely it is that chance accounts for the findings. It is possible to minimize the effects of chance but never entirely avoid them. The main approaches for increasing precision (reduce chance) in studies are through sample size, design of the study, use of appropriate statistical methods (stratification of data) and in the choice of significance levels. There are ways to measure random error, preferably by confidence intervals but also by p-values. The confidence interval can be set at different cut offs/limits (95\%, 99\%) and the interval gives us a range of values within which we are confident that the population difference lies. The p-value tells us the strength of the evidence against the null hypothesis that the true difference in the population is zero. The p-value can also be set at different significance levels, so that we would be more or less willing to reject the null hypothesis. Significance levels are totally arbitrary thresholds, therefore it is more important to look at the range and
different values of the confidence interval and relate the magnitude of the difference with reality and clinical importance. In our studies we have large numbers of study participants, which enhances precision. However, in study III the cases were few compared to the source population, which yielded some non-significant results probably due to chance (but the confidence intervals were narrow). In study IV, there were too few cases in some subgroups that we were unable to calculate HRs. Performing many statistical analyses will also increase the risk of type 1 error (rejecting the null when we should not), however this is less likely in our studies because we have pre-planned all subanalyses and in all studies we have one main analysis (“overall risk of cataract/uveitis”). In case of many post-hoc analyses it is suitable to consider adjustment for multiple comparisons.

6.1.4 External validity

External validity is the degree of generalizability in a study. In other words, how well can the results of a certain study be applied to other patients?238

Using biopsy reports with villous atrophy as definition for CD we can be confident that the average CD patient is identified. CD patients with subclinical or asymptomatic disease might not be found in the same extent, unless they for some reason are screened for because the gold standard for CD diagnosis is still a small intestinal biopsy in Sweden. Since small intestinal biopsy is still a part of the diagnostic work-up for CD our findings in studies II-IV can be applicable to individuals with diagnosed CD. Still, in the future this definition of CD may not be sufficient. Efforts have been made to avoid the small intestinal biopsy in the diagnostic work-up, at least in patients with high titers of CD-antibodies14. As for the definition of CD according to ICD codes in the NPR (study I), there might be a risk that those individuals suffer from a more severe form of CD, requiring inpatient care. Although in the earlier part of the study period most CD individuals would have been admitted to the hospital as part of the gastrointestinal investigations (endoscopy), particularly children. Another issue with generalizability in study I is of course that the study is restricted to conscripts (men), therefore the results may not be applicable to women, younger boys and older men.

6.2 FINDINGS AND IMPLICATIONS

There are no previous studies addressing the risk of decreased visual acuity in CD. However, numerous studies have found an increased risk of neurological symptoms in CD109, 202, 247. The mechanism of neurological symptoms in CD has been described to be mediated either through cross-reactivity between antigenic epitopes on Purkinje cells and CD antibodies109, 248 or through increased levels of homocysteine51. Hyperhomocysteinemia has been associated with retinal artery and vein occlusion249. We hypothesized that through these mechanisms CD would be associated with decreased visual acuity. We found no difference in visual acuity according to CD status. There was no association between either diagnosed CD or undiagnosed CD and
decreased visual acuity. There was an increased proportion of missing data on visual acuity in patients with CD, which might have introduced selection bias and influenced our results. However, we still had data on visual acuity in 85.4% with CD and 94.3% in reference individuals, therefore that should not have a major effect on our findings. Since this study was restricted to young men only, the results may not be applicable to women, however we can conclude that CD does not affect visual acuity in young men.

Cataract is the leading cause of visual impairment worldwide. We hypothesized that cataract would be more prevalent in CD due to vitamin deficiencies, inflammation and oxidative stress. Other than case reports there are no previous studies examining the risk of cataract in CD. We found that individuals with CD had a moderately increased risk of cataract compared to reference individuals. There was also a slightly increased risk of CD in patients with a previous diagnosis of cataract. The positive association between CD and cataract could be explained by shared risk factors for both conditions, since the association was directed in both ways. The findings of this study will lead to clinical awareness and add to the existing knowledge about CD and cataract.

Uveitis is an inflammatory disorder of the eye and it is associated with several autoimmune diseases. This study is the first (other than previous case reports) to describe the risk of uveitis in patients with CD. We found a moderately increased risk of uveitis in CD, which might be due to shared immunological factors. From our patient chart review we found that the most common comorbidity present among patients with CD and uveitis, was inflammatory bowel disease followed by type 1 diabetes. Therefore, there is a risk that some of the increased risk is due to unmeasured confounding by inflammatory bowel disease.

Uveitis can respond to a GFD according to case reports. Our validation study shows that the majority of CD patients are likely to comply with a GFD, therefore a GFD could have concealed some uveitis cases in CD and lead to an underestimation of the association. Nevertheless, we found a moderately increased risk of uveitis in patients with CD. Although CD is not a major risk factor for the development of uveitis, we believe that CD should be considered in cases of unknown uveitis, particularly since the etiology of uveitis is unknown in some 35-57% of cases.

In the largest study performed at present on T1D and CD (study IV) we found for the first time that CD duration in patients with T1D correlated strongly with risk of DRP. Previous studies have addressed the risk of DRP in patients with T1D and co-existing CD, however results have been conflicting. Earlier studies on this topic have been limited in size and follow-up time. We speculate that the lower risk of DRP found during early CD is associated with better compliance with GFD and/or lower levels of cholesterol and blood pressure that have been described in the CD population. The reason for an increased risk of DRP found with longstanding CD is unknown, but we hypothesized that it may be associated with deterioration in GFD compliance or an acceleration of autoimmune/inflammatory mechanisms influencing the DRP development in the presence of both diseases (T1D and CD).

The increased risk of DRP with CD duration was also present when we restricted the study outcome to severe DRP (requiring retinal laser therapy). Furthermore, stratification analyses according to age at T1D diagnosis, sex, calendar period at study entry showed the same pattern of risk with lower DRP risks <10 years duration of CD.
diagnosis and higher risks ≥10 years of CD duration. To increase the specificity of our T1D definition we performed subanalyses where individuals with T1D were restricted to an inpatient diagnosis of T1D, and individuals with oral anti-diabetic medication as well as those with a potential gestational diabetes (T1D diagnosis 0-9 months before delivery) were excluded from the analyses. The risk estimates did not change in these subanalyses. From this study we conclude that longstanding CD in patients with T1D require intense monitoring of DRP, thus screening routines should change for DRP in patients with T1D and CD.

6.3 FUTURE RESEARCH

The gold standard for the diagnosis of CD has been a small intestinal biopsy with villous atrophy throughout the study periods included in this thesis. However, the diagnostic work-up for CD might change in the future, particularly in children. With this in mind, future studies regarding CD and associated conditions in children might require CD serology and or genetic markers (HLA genotypes), as well as data on small intestinal biopsy in the definition of CD.

Research regarding the effect of a high/low compliance to a GFD in CD complications is still warranted. Further studies are needed on a detailed level on the mechanisms mediating a relationship between CD and other conditions.

Research about the management of asymptomatic CD is required, with a focus on the natural history of those patients and whether or not they are at the same risk of developing complications as symptomatic/classical CD.
7 CONCLUSIONS

I) CD does not affect visual acuity in young men. We cannot exclude that CD affects visual acuity in women. However, we find it unlikely since we saw no trend of decreased visual acuity in our study and most other studies on complications in CD have shown similar risks in men and women.

II) We found a moderately increased risk of cataract in individuals with CD. These findings might lead to increased awareness among clinicians managing CD patients with eye symptoms.

III) We found a positive association between uveitis and CD. Although the risk increase for uveitis in CD was moderate, we suggest that CD could be considered in patients with uveitis of unknown etiology.

IV) There is an increased risk of DRP in patients with T1D and longstanding CD. The risk increase was also associated with severe DRP. This study is of clinical importance. We suggest closer monitoring of patients with T1D and longstanding CD with regards to DRP development.
8 SAMMANFATTNING PÅ SVENSKA


I vår första studie länkade vi det svenska patientregistret med värmpliktsregistret för att studera förekomsten av synfel hos patienter med celiaki. Vi identifierade 69 unga män med odiagnostiserad celiaki, 996 med diagnostiserad celiaki och 6850 referensindivider utan celiaki (och tillgängliga syndata). Vi fann ingen skillnad i förekomst av synfel mellan dessa tre grupper. Synfel var inte associerat med varken diagnostiserad (odds ratio (OR)=1.03; 95% KI=0.90-1.19) eller odiagnostiserad (OR=1.04; 95% KI=0.64-1.70) celiaki. Celiaki påverkar således inte synen hos unga män.

Katarakt (grå starr) är den vanligaste orsaken till synnedsättning och blindhet i världen. I studie II undersökte vi risken för kataraktutveckling hos patienter med celiaki jämfört med matchade referensindivider från svenska befolkningsregistret. Celiaki visade sig vara kopplat till en måttligt förhöjd risk för senare katarakt (hazard ratio (HR)=1.28; 95% KI=1.19-1.36). Likaså fann vi en något förhöjd risk för senare celiaki hos patienter som redan haft katarakt (OR=1.14; 95% KI=1.02-1.30). Riskökningen kan vara orsakad av gemensamma riskfaktorer för båda tillstånden. Vår studie kommer förhoppningsvis leda till en ökad kunskap och medvetenhet hos läkare som träffar celiaki-patienter med ögonsymtom, och därmed snabbare handläggning.

I vår tredje studie jämförde vi risken för uveit hos celiakipatienter med matchade referensindivider. Vi noterade en moderat riskökning för uveit bland patienter med biopsi-verifierad celiaki (HR=1.32; 95% KI=1.10-1.58). Den absoluta risken för uveit...
bland celiakipatienter var 50/100,000 person-år. Som del av denna studie genomförde vi även en journalgranskning av 165 patientjournaler. Syftet med journalgranskningen var att validera ICD diagnosen för uveit i patientregistret men även att karakterisera uveitpatienterna i Sverige. Journalgranskningen visade att den vanligaste formen av uveit hos celiakipatienter och referensindivider (utan celiaki) var ensidig, främre, akut uveit. Vi fann ett högt positivt prediktivt värde (93.3%) för uveitdiagnosen i patientregistret. Trots att celiaki inte verkar vara en allvarlig riskfaktor för uveit, menar vi att man ska vara medveten om att det finns en måttlig ökad risk för uveit hos celiakipatienter, särskilt med tanke på att större delen av uveitfallen är av oklar etiologi. I vår sista studie jämförde vi risken för diabetesretinopati hos patienter med typ 1 diabetes och samtidigt celiaki med patienter som endast hade typ 1 diabetes (ingen celiakiagnos). Denna studie baserades på ca 40,000 individer med typ 1 diabetes samt drygt 1000 individer med både typ 1 diabetes och celiaki. Vi fann att risken för diabetesretinopati korrelerade starkt med durationen av celiakiagnosen hos patienter med typ 1 diabetes. Risken för diabetesretinopati var låg under tidig celiakiagnos (0-<5 år efter celiakiagnos HR=0.57; 95% KI=0.36-0.91) följt av en neutral risk vid 5-<10 år av celiakiagnos (HR=1.03; 95% KI=0.68-1.57). Därefter steg risken för diabetesretinopati påtagligt med mer än en fördubblad risk efter 10-<15 år av samtidig celiakiagnos (HR=2.83; 95% KI=1.95-4.11) samt en tredubblad risk vid ≥15 år av samtidig celiaki (HR=3.01; 95% KI=1.43-6.32). Risken för svår diabetesretinopati (som kräver laserbehandling) visade sig följa samma risk mönster. Med dessa resultat drar vi slutsatsen att en långvarig celiakiagnos hos patienter med typ 1 diabetes är en betydande riskfaktor för utvecklingen av diabetesretinopati. Dessa patienter behöver därför tätare kontroller med ögonbottenfotografering för att i möjligaste mån kunna förhindra/senarelägga framtida synskador.
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REFERENCES

46. Sjoberg K, Carlsson A. [Screening for celiac disease can be justified in high-risk groups]. Lakartidningen 2004;101:3912, 5-6, 8-9.


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231. Ahlstrand. In. Chief statistitian at the Swedish Conscripts Register (Phone: +46 (0)771-24 40 00 or +46 (0)54-146551) ed; 2011.


239. Ahlstrand. In. Chief statistician at the Swedish Conscripts Register (Phone: +46 (0)771-24 40 00 or +46 (0)54-146551) ed; 2007.


