Screening Detected Celiac Disease in Children

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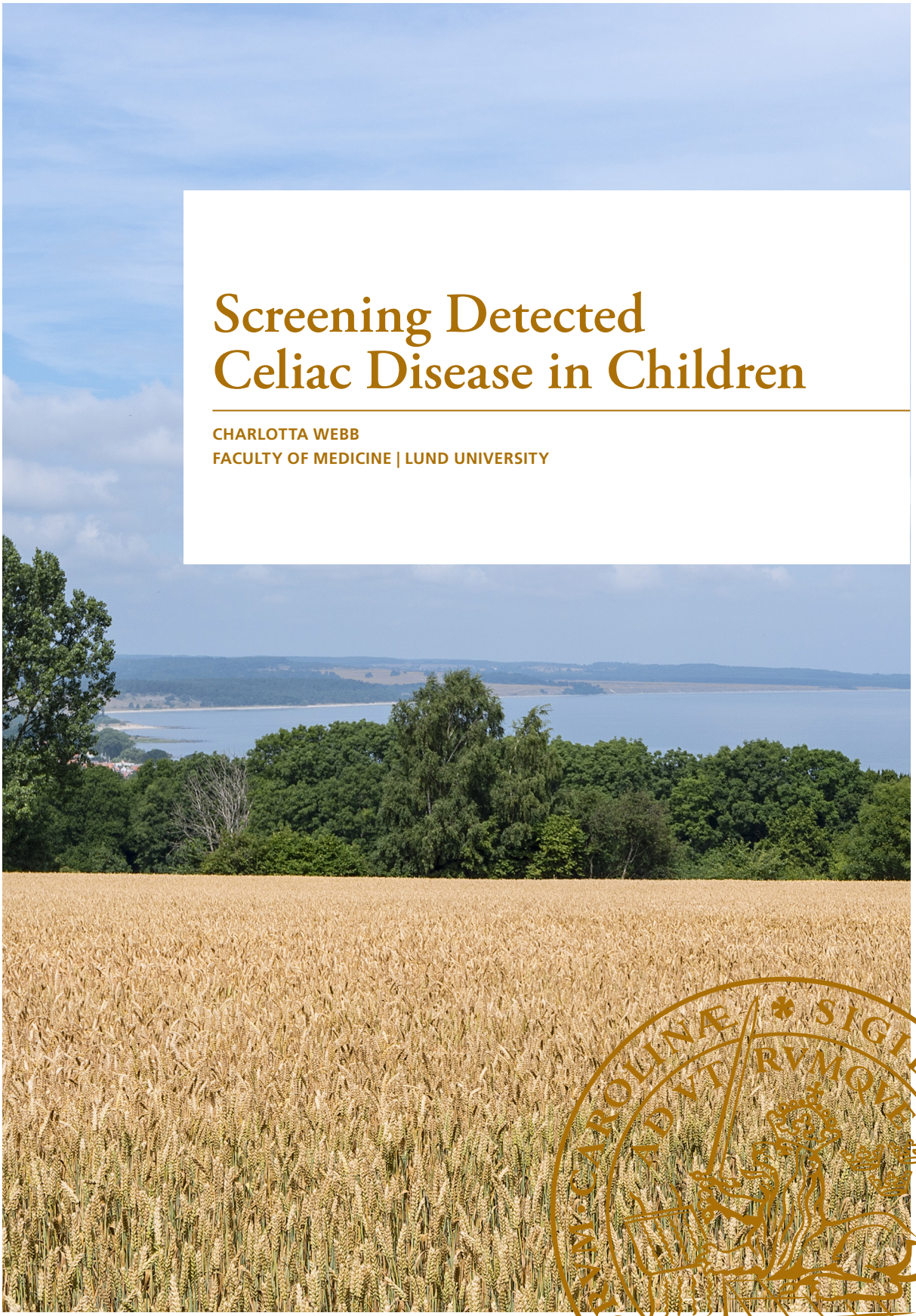

Screening Detected Celiac Disease in Children

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Abstract

Background:

The prevalence of celiac disease (CD) is estimated to be around 1%, but most CD cases are undiagnosed. Sweden experienced an epidemic of clinically detected celiac disease in children younger than 2 years of age, partly due to changes in infant feeding practices, where the amount of gluten and the age of the child at the time of gluten introduction were changed. However, it was not clear if the increase in clinically detected children was due to more CD cases being detected due to symptoms and thus previously undiagnosed, or if it was a true change in CD prevalence.

In the revised 2012 ESPGHAN criteria for CD diagnosis, a small intestinal biopsy is no longer mandatory in symptomatic patients, when the tTG-IgA levels exceed ten times the upper limit of normal (> 10xULN) in combination with positivity for EMA and HLA- DQ2 and/or DQ8.

Biopsy is still recommended in asymptomatic screening detected cases.

The main distinction in the approach to the diagnostic process is the presence of symptoms suggestive of CD. Recent studies have shown that screening also detects symptomatic cases, since many are unaware of their previous symptoms until after they have started with GFD. Furthermore, there are many pitfalls in the diagnostic process, where the choice of biopsy method and the histopathological evaluation have a significant impact on the diagnostic outcome. To use a capsule device to obtain mucosal specimens was previously considered to be gold standard, but the lesions may be patchy and missed if only one mucosal specimen is obtained. In a clinical setting there can be an advantage of using endoscopy due to the ability to take multiple biopsies. The treatment with a gluten-free diet is a life-long challenge and entails social sacrifices, which may affect the adherence. To be a teenager at the time of diagnosis and being screening detected, can be negative factors to adherence.

Objectives:

To study the total prevalence of clinical and screening detected celiac disease in children born during the Swedish epidemic, and to investigate the effect and accuracy in each step of the diagnostic process in obtaining CD diagnosis. Furthermore, to investigate the correlation between the level of the serological markers (tTG-IgA) and

the degree of gluten induced enteropathy and applying the revised 2012 ESPGHAN guidelines on screened CD cases.

Additionally, to evaluate the adherence to the GFD in adolescents with screening detected CD.

Method:

We performed a two-phased screening study, ETICS (Exploring the Iceberg of Celiacs In Sweden). A total of 13 279 twelve year old children were investigated, belonging to two different birth cohorts. The first birth cohort was born during the epidemic period in 1993 and the second cohort was born in the post-epidemic period in 1997. The prevalence of clinically detected CD was gathered from the National Swedish Childhood Celiac Disease Register and the total prevalence was estimated together with the ETICS screening study. Screening for CD was conducted by using a serological marker, anti-tissue transglutaminase antibodies (tTG-IgA). The CD diagnosis was confirmed by a small intestinal biopsy using either a suction capsule or endoscopy according to local routine in the study sites. The clinical diagnostic procedure was reviewed by performing endoscopic re-biopsies in children who hade normal or inconclusive primary biopsy. All of the mucosal specimens were re-evaluated by an expert pathologist and if there was disagreement with the local pathologist, a second expert pathologist re-evaluated the specimens to reach a diagnostic consensus.

The correlation between the degree of enteropathy and the level of the serological markers (tTG-IgA) was investigated by comparing the level of tTG-IgA at the time of biopsy to the degree of gluten-induced enteropathy. The revised ESPGHAN guidelines for symptomatic CD were applied to the screening cases in order to evaluate if the biopsy could have been omitted.

The adherence to the GFD was measured both by the change of tTG-IgA levels after 12 months and by self-reported questionnaires where the response alternatives were: *always*, *often*, *sometimes* and *never*.

Results:

The total prevalence of CD in children born 1993 was 2.9%, with two thirds of the cases being unrecognized. Endoscopic biopsies were inconclusive in 0.6% compared to 7.4% of the capsule biopsies and patchy enteropathy was found in 9% of the children who had conclusive fractions from both proximal and distal duodenum. By controlling the diagnostic process several CD cases were found, re-biopsy resulted in 8 new cases and re-evaluation of all mucosal specimens by an expert pathologist, resulted in additional 6 CD cases. In our screened population all cases with tTG-IgA levels exceeding ten times upper limit of normal (> 10xULN) at a cut-off of 5 U/mL, got CD diagnosis and all except for one child got CD diagnosis at a cut-off of 3

U/mL. The majority of these children had Marsh 3 lesions. The adherence was high in the screened population where 83% had tTG-IgA levels <5 U/mL after 12 months on GFD. Most of the children reported to always (75%) or often (14%) be adherent. There were children who reported to always be adherent, but the serological markers were not yet normalized after 12 months. All of these children had halved their initial values, whereof the majority (85%) of these children initially had high serological markers >50 U/mL.

Conclusions:

The prevalence of 3% was unexpectedly high, whereof two thirds were previously unrecognized. This finding underlines the fact that many CD cases remain undiagnosed, but also that the exposure to an unfavourable infant feeding in this birth cohort may have affected the high total prevalence.

The accuracy in the diagnostic procedure is dependant on various factors, where biopsy method and the pathological evaluation are crucial in finding the CD cases. The preferable method for biopsies to be recommended is by using endoscopy and to sharpen the diagnostics to perform re-biopsies and/or re-evaluating of normal or inconclusive biopsies. The revised ESPGHAN guidelines for symptomatic cases seems to be applicable even on screening detected cases, due to the correlation between high levels of tTG-IgA and degree of enteropathy in this group. Screening detected children have a high adherence to the treatment with GFD, which can be measured by using self-reported questionnaires in combination with the change of the serological markers.

Keywords:

Celiac disease; screening; prevalence; small-bowel biopsy; enteropathy; transglutaminase; gluten-free diet; adherence;

Original papers

Anna Myléus, Anneli Ivarsson, Charlotta Webb, Lars Danielsson, Olle Hernell, Lotta Högberg, Eva Karlsson, Carina Lagerqvist, Fredrik Norström, Anna Rosén, Olof Sandtsröm, Lars Stenhammar, Hans Stenlund, Stig Wall, Annelie Carlsson.
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Submitted, J Pedatri Gastroenterol Nutr., 2014. Accepted with revisions.

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High Adherence to a Gluten-Free Diet in Adolescents With Screening-Detected Celiac Disease.
J Pedatri Gastroenterol Nutr. 2014 September 17th; Published ahead of print, open access.

List of abbreviations

CD	Celiac Disease
EMA	Endomysial antibodies
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
ETICS	Exploring the Iceberg of celiacs in Sweden
GFD	Gluten free diet
HLA	Human leukocyte antigen
IEL	Intraepithelial lymphocytes
tTG	Anti-tissue transglutaminase , referral both to antibodies and enzyme
> 10xULN	Exceeding ten times the upper limit of normal

Introduction

Celiac disease (CD) is one of the most common chronic diseases in children and has emerged as a worldwide public health problem [1]. The first modern description of symptoms suggestive of CD in children was presented in 1888 by the British physician Samuel Gee in a lecture entitled "The coeliac affection". He came to the conclusion that the cause of the disease was dietary after observing a patient: "the boy was fed upon a quart of the best Dutch mussels daily, throve wonderfully, but relapsed when the season for mussels was over" [2]. However, it was not until 1950 that the link between CD and the ingestion of dietary gluten proteins was established by Willem-Karel Dicke, a Dutch paediatrician. During World War II, when there was a shortage of flour, he noticed that the children with chronic diarrhoea and malabsorption improved drastically and additionally that the mortality decreased from 30% to less than 1% in these patients. After crucial experiments with standardised diets excluding or adding wheat or rye, he concluded that the cause of the disease was dietary. Hence, the treatment with gluten-free diet (GFD) was based on these observations. Subsequent studies in collaboration with the biochemist van der Kamer and co-workers revealed that the toxic agent was the gliadin component, the alcohol-soluble fraction of gluten [3, 4].

In 1954, John W Paulley using a forceps instrument at gastroscopy provided the first descriptions of the characteristic histological features of the intestinal lesions. These lesions include various degrees of villous atrophy, crypthyperplasia and increased intraepithelial lymphocytes, commonly graded according to the Marsh-Oberhuber classifications[5, 6].

There is a strong genetic predisposition which is associated with alleles at the human leukocyte antigen (HLA) DQ loci, located on chromosome 6 .The majority, 95% of the CD cases have DQ2 and 3% have DQ8 [7].The risk increases depending on whether the alleles are expressed in a homozygous or heterozygous state (gene-dose effect). In addition there are more than 50 non-HLA regions identified by genome-wide association studies (GWAS), which are associated with CD [8].

The genetic influence is illustrated by the increased prevalence of CD in first-degree relatives, 15% and in homozygotic twins, where the risk is as high as 75%, if one twin is affected [9, 10]. Additionally there is a gender difference. The incidence is twice as high in girls as in boys [11, 12]. Around 30% of the Swedish population have the genetic predisposition with DQ2, and or DQ8 but only a minority develop CD,

which implies that environmental and lifestyle factors have a casual role in the development of the disease [13-15].

The symptoms of CD can vary from being mild to severe and different organs can be affected. Gastrointestinal manifestations are common especially in younger children. In older children and adults the symptoms vary from fatigue, anaemia, infertility to depression ([16-18]. Many cases are asymptomatic, however some screening detected patients improve on GFD, revealing symptoms they were not fully aware of [19, 20].

Epidemiology

In the past CD was considered to be a rare disease, which mostly affected individuals of European origin, but later CD has also been found to be a common disease in North Africa, the Middle East and India. The occurrence of CD is often described as an "Iceberg", where those who are diagnosed are the ones visible above the waterline and those with silent or latent CD are below the waterline. The silent cases may be asymptomatic, but have positive serology and small intestine lesions suggestive of CD, whereas the latent or potential CD cases only have positive serology [21, 22].

Screening for CD has become more common due to highly sensitive and specific serological markers, which has revealed clinically atypical and asymptomatic forms[23]. The highest prevalence in the world (5,6%) has been found in a screening study of the Saharawi population, living in refugee camp in Algeria [24]. In the rest of the Western World the prevalence of clinical detected CD cases has been estimated to be 0,5-1% [10, 25-27], while the prevalence in the developing countries are not yet known. Notable is the increased prevalence in specific subgroups of the population e.g. patients with autoimmune diseases, especially in type 1 diabetes, where the average prevalence is around 4-10% [28], but also in patients with thyroiditis. Other subgroups with an increased prevalence are patients with genetic diseases, Mb Down, Turner and Williams syndrome [29, 30]. These at-risk groups are often screened for CD by using serological markers and sometimes also using genetic tests for HLADQ2/DQ8.

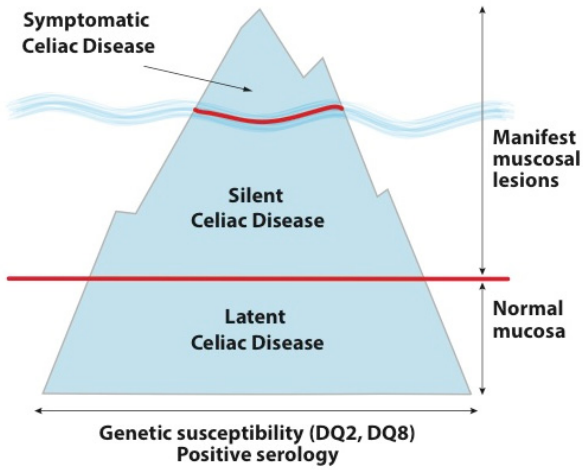

Figure 1. Model of the Celiac disease Iceberg. Adapted from Fasano et al. Coeliac disease in children. Best Pract Res Clin Gastroenterol 2005;19:467-78

Etiology

A genetic predisposition and the exposure to dietary gluten are prerequisites for CD. However, all factors that influence the immunological response have not yet been revealed. There are several environmental and life-style factors that have been identified, including infant feeding. Introducing small amounts of gluten between four and seven months of age during on-going breastfeeding has been reported by several studies to be protective for CD development [13, 31, 32] and is currently a recommendation by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) [33].

A high amount of gluten in the diet may also have a significant impact. The Saharawi people have a high genetic susceptibility, but also had a drastic change of diet. Their previous diet was based on smaller amounts of cereal, camel milk, dates and legumes, whereas in the refugee camp the staple food was made with wheat flour. CD is also more common along the silk road where wheat was used for trading [1].

A seasonal difference has been noted, whereas being born in the summer increases the risk for CD [14, 34]. An explanatory theory to these findings is that the children born in the summer will be around four to six months of age during the autumn and winter period, when many of them stop breastfeeding and are introduced to gluten. This is also the time of year when there is an increased risk of infections. Rotavirus, which affects the gut permeability, is one of the most common causes of childhood

gastroenteritis and has been shown to be an independent risk factor for CD development [35]. It has been reported that there is an increased risk if the child has had three or more infectious episodes during the first six months of life, regardless of type of infection [15]. Most reports indicate a higher risk for CD in areas with better socioeconomic conditions [36]. In a study of schoolchildren with different living standards but the same genetic susceptibility, it was reported that the children from the more prosperous area had an increased risk to develop CD [25]. The factors that might be protective of CD related to lower socioeconomic standards are not fully understood, however the speculations are that the risk is dependent on variation in gut flora, diet and infections [37].

The role of the intestinal microbiota is demonstrated by the fact that children born via Caesarean section have a different microbiota and an increased risk for CD, compared to children born vaginally [38].

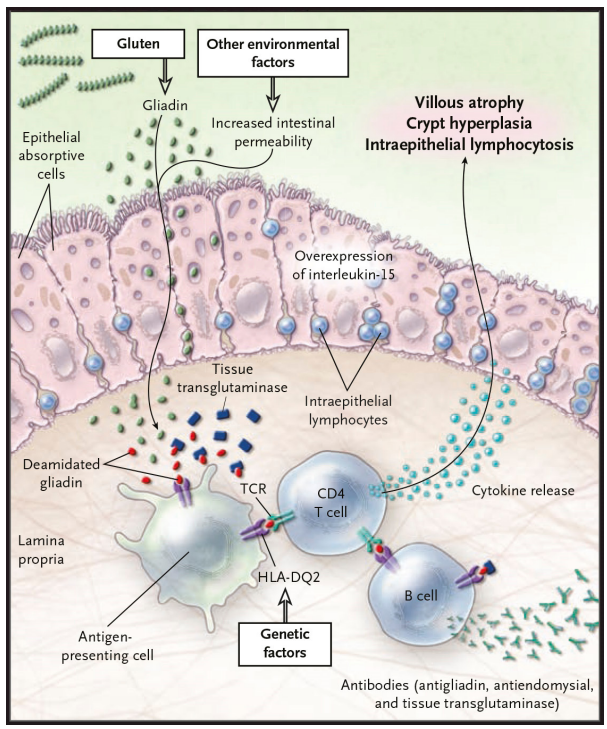

Figure 2. Overview of the celiac disease pathogenesis. Gluten proteins (e.g. gliadin) cross the epithelium into the lamina propria where it becomes deamidated by tissue transglutaminase (tTG). The HLA DQ2/DQ8 forms a complex with the deamidated gluten which is recognized by CD4+ T-cells. Cytokines are released and atnibodies are produced. The Inflammatory responses, by the intraepithelial lymphocytes (IEL) resuls in the mucosal damage.
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Gluten is rich in glutamine and proline residues, which cannot be fully digested in the human intestine since they are resistant to gastric and pancreatic enzymes and also to intestinal brush-border membrane proteases. As a result, intact gluten peptides are left in the gut lumen, where some may cross the intestinal barrier and gain access to the submucosal compartment. The peptides come in contact with the enzyme tissue transglutaminase (TG2) which deamidates the peptides thereby generating highly negatively charged peptides, ideally suited to interact with the HLA-DQ2 and/or DQ8 molecules, which are positively charged.

HLA-class II genes (HLA DQ2/DQ8) are molecules expressed on the surface of the antigen-presenting cell in the gut lamina propria, where they are responsible for presenting gluten peptides to CD4+ T-lymphocytes. The CD4+ cells then trigger the inflammatory reaction, which secondarily leads to the activation of cytotoxic CD8+ intraepithelial lymphocytes.

The subsequent infiltration by CD4+ T-lymphocytes into lamina propria and CD8+ into the intestinal epithelium are hallmarks for active CD. [39, 40]. The inflammatory process causes the formation of antibodies against both gliadin and TG2 and the determination of the titers of the antibodies are used in clinical practice.

The expression of interleukin 15 cytokine appears to play a central role in driving various processes that lead to the increased number of intraepithelial lymphocytes (IELs) as well as in the destruction process of the epithelial cells and the mucosal damage[41].

The Swedish epidemic of celiac disease

In 1982, Swedish paediatricians recommended postponing the gluten introduction to babies from four months to six months of age. The change was an attempt to postpone the development of CD in infants and was also in line with the European recommendations [42]. The new recommendations coincided with a change in the contents of industrially produced milk-cereal based formulas and porridges. In order to reduce the protein load in the products, the milk protein was replaced with wheat and therefore the amount of gluten increased significantly. However, paediatricians all through Sweden reported an increasing number of young children who were diagnosed with CD due to severe symptoms of malnutrition. The incidence rate was approximately four-fold [43]. During a ten year period from 1984, Sweden experienced an epidemic in children below two years of age with clinically detected CD[44]. Many of these children were malnourished with growth impairment during the period of life when growth acceleration is most prominent. There was an on-going debate during this period in the Swedish Paediatric Society, whether it was beneficial that the children developed symptoms of CD at an early age, and therefore were diagnosed. It was claimed that these children would probably develop CD later

on and due to the present infant feeding practise they emerged from the iceberg at an earlier stage. The opposite side claimed that the change in infant feeding was not beneficial and actually had caused CD in children who otherwise would have been healthy. In order to study the incidence of childhood CD in Sweden, the section of Paediatric Gastroenterology within the Swedish Paediatric Association formed a working group. The results from a case-referent study conducted by Ivarsson et al. at Umeå University indicated that, to decrease the risk for CD development the gluten should be introduced in smaller amounts and preferably during on-going breastfeeding [13]. In 1996 the national recommendation of infant feeding was revised due to the working group and the epidemic came to an end.

The celiac epidemic was unique for Sweden and many lessons could be learnt. A group of paediatricians with special interest in CD felt a responsibility to find out the true cause of the epidemic. If the timing and the amount of gluten were crucial for the development of CD, is primary prevention of CD possible? Was the increase during the epidemic a true change in CD prevalence or just an increase in the clinically detected children? A screening study conducted by Carlsson et al. at Malmö University Hospital [45], showed a high prevalence of clinically detected CD in children that were 2.5 years old (1%) and born during the epidemic (1993) but also a high percentage of children with undetected CD (2%) in this birth cohort. The study supported the hypothesis that the change in feeding recommendation in 1986 might have increased the CD morbidity in these young children.

Later the group repeated a CD screening in children, of the same age, but born after the epidemic (1997) [46]. They found a lower number of children with clinical- and screening detected CD compared to children born before the epidemic, but there was no statistical significant difference between the two birth cohorts. One hypothesis for the lack of significance was that the number of children in the two screening studies was too small, i.e. 690 children in each cohort.

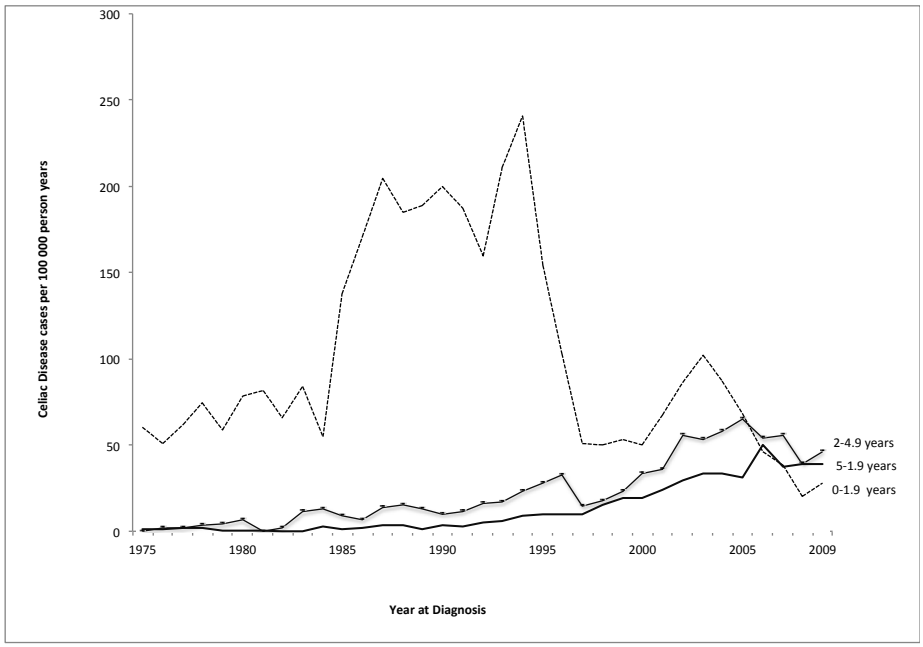

Figure 3. The Swedish Epidemic. The incidence rate stratified in three age groups. Reproduced with kind permission of F. Namatovu, BMC Gastroenterology 2014, 14:59

Clinical presentation and complications

CD is an autoimmune disease which not only manifests itself through classic symptoms of the gastrointestinal tract, but may also involve other organs. CD was earlier known to be presented either very early in life, between 9-24 months or in the third or fourth decade[22, 47]. It is now known that the development of CD can occur at any age, during childhood, adolescence and also in adulthood [48]. The very wide variation of symptoms and signs of CD often leads to missed or delayed diagnosis. There are also many cases who have no symptoms (silent) but due to active case finding through screening more of these cases are presently being diagnosed. However, many of these screening detected patients are not aware of any symptoms, but do report improved physical and psychological health after following a GFD and are therefore not truly asymptomatic. The symptoms can be divided into gastrointestinal manifestations (typical symptoms) or extra-intestinal manifestations (atypical symptoms).

Intestinal manifestation – typical

There is a wide spectrum of gastrointestinal presentations that range from mild abdominal symptoms to generalised malabsorption. The classic symptoms of celiac disease, such as chronic diarrhoea, failure to thrive, abdominal distension and vomiting, are mainly seen in young children [21]. In older children the clinical presentation can vary from abdominal pain to short stature and delayed puberty [49-51]. In adults, diarrhoea, steatorrea, weight loss and fatigue have been classic signs of CD. However many may have anaemia and vague symptoms. The damage in the small intestinal mucosa leads to different degrees of malabsorption, depending on the length of the intestine that is affected. Nowadays, severe cases of malabsorption are rarely seen in infants (celiac crisis) but can occur in children with coexisting adenovirus infection. The clinical presentation has also changed over time. Whereas diarrhoea was previously the most common symptom it is now found in only 50% of the cases and constipation and abdominal pain have become more common [52, 53]. Weight loss is more uncommon and presently around 30% are even overweight at the time of diagnosis [54].

External-intestinal manifestations-atypical

CD patients can present with a variety of extra-intestinal manifestations, without gastrointestinal manifestations e.g. enamel hypoplasia, recurrent aphteous ulcerations and cardiomyopathy. Anemia due to iron, folate and/or vitamin B12 deficiency is a common presentation in adult patients[55].

Dermatitis herpetiformis is a dermatological expression of the disease, presenting with itchy blisters. There are patients with neurological symptoms e.g. epilepsy with cerebral calcifications or cerebellar ataxi, who respond well to the GFD .The liver can be affected with unexplained transaminase elevation [56]. In women, unexplained infertility and an increased risk of miscarriages have been reported [57].

Complications

The bone mineral density can be low both in adults and children with untreated CD and are also found in asymptomatic cases, due to reduced reabsorption of vitamin D and calcium [58, 59].

The osteopenia reverses on GFD, but in some cases the process has already advanced to osteoporosis and increased risk for fractures [60]. CD subjects also have a higher risk for other autoimmune diseases, such as diabetes type 1 and thyroiditis [28, 61].

The incidence of certain types of cancer is increased among CD patients e.g. non-Hodgkin lymphoma at any site and enteropathy-associated T-cells lymphoma (EATL), which is a rare high-grade T-cell non-Hodgkin lymphoma of the small intestine with poor prognostic outcome [62]. The incidence of adenocarcinom in the

small intestine and esophagus is also increased. Evidence that treatment with GFD might reduce the risk of malignancy was reported by Holmes et al. [63].

Diagnostics

The ESPGHAN guidelines

The European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) published in 1970 the criteria for diagnosing CD in children .The recommendation included three small bowel biopsies in order to obtain CD diagnosis: a structurally abnormal jejunal mucosa whilst on gluten containing diet, clear improvement in mucosal structure following removal of gluten from the diet and subsequent mucosal deterioration following re-introduction of gluten into the diet.

The criteria were revised in 1990, stating that biopsy was still mandatory for CD diagnosis but follow-up biopsy after GFD and subsequent gluten challenge were recommended only in children in whom there was doubt about the initial diagnosis or in children younger than two years at first biopsy [64]. The criteria were recently revised in 2012 [33], making a biopsy no longer mandatory in all symptomatic children. The criteria to obtain CD diagnosis are: symptoms indicative of CD, tTG-IgA levels exceeding ten times the upper limit of normal and positivity for EMA and HLA DQ2/DQ8. Biopsy is still recommended in children with lower levels of the serological markers and in asymptomatic, screening detected children.

Serological markers

The development of the celiac serological markers over the last decades has facilitated diagnosing and screening for CD. Initially, reticulin antibodies (ARA) and anti-gliadin antibodies (AGA) were used as diagnostic tools [65, 66]. AGA was introduced in the early 1980s with a sensitivity and specificity of about 80%. The test is used in children under the age of 18 months since younger children can lack both EMA and tTG-IgA [67].

EMA came in use in the early 1990s. Monkey oesophagus or umbilical cord smooth muscle are used as antigens. The sensitivity and specificity is very high at about 96-98%. However, the test uses an immunoflourescense staining technique which needs manual evaluation and experienced laboratory personnel [68]. In 1997 the antigen for EMA, anti- tissue transglutaminase was identified and the anti-tTGA test was introduced. In the beginning, guinea pig liver was used as substrate, but the test has been further developed and human recombinant tTGA is used. The sensitivity is 96% (86%-100%) and the specificity 97% (95% -100%) [69-71].

The test for tTG and EMA are dependent on IgA antibodies and levels of total serum IgA need to be determined to rule out IgA deficiency, which is a common disorder in CD and in the general population [72]. New serological markers are being tested, e.g deaminated gliadin, which does not seem to be as sensitive in children as in adults [73].

Small bowel biopsy

Capsule biopsy

Biopsy was introduced by Margot Shiner in 1956 and was a major step forward in the diagnostics of CD [74]. In the beginning, the small intestinal biopsy was performed by using a per oral capsule device. Crosby and Kugler developed a capsule for adults constructed to prevent the rather frequent loss of the cap. [75]. The capsule was further improved by Read and was named the Watson capsule on the market. The size of the adult capsule was 9.5x19mm and the paediatric version was 6.5x15mm. This method was for a long time considered to be gold standard, as only one mucosal sample could be obtained with the standard Watson capsule and multi-biopsy instruments were developed later. The capsule method is able to get biopsies from more distal parts of the upper small intestine and the specimens obtained are usually bigger in size compared to the samples obtained via endoscopy[76]. A disadvantage of this method is that radiography is used to locate the capsule. Loss of parts of the capsule is a complication that can occur, but usually the parts will be recovered in the stools within days.

Endoscopic biopsy

Biopsy using a small-diameter, flexible and fiberoptic instrument has many advantages, including that the mucosa of the upper gastrointestinal tract can be visualised and multiple biopsy samples can be obtained .The current recommendation is four-six specimens from different locations, including the duodenal bulb, which can be the only affected part [77]. Endoscopic features of CD include the absence of folds, scalloped folds, visible mucosal blood vessels and a mosaic pattern of the mucosa between the folds, but are only seen in patients with subtotal/total villous atrophy. Endoscopic evaluation without biopsies is therefore inadequate to confirm or exclude a diagnosis. The disadvantages compared to the capsule technique are the need for heavy sedation or general anaesthesia and that the mucosal samples are smaller and only located from more proximal duodenum. Complications to both biopsy procedures are rare, but include perforation, bleeding and intra-duodenal haematomas [78].

Histopathology

There are many pitfalls in the histopathological evaluation. A good collaboration between the pathologist and the clinician will make the difficult pathway to a correct diagnosis easier.

The first challenge is the size and numbers of the mucosal specimens, which vary depending on which method is being used, endoscopy or capsule. The next challenge is to decide whether CD causes the enteropathy, since the histopathological changes are not patognomonic for CD. For example, villous atrophy can be caused by food-allergies, such as cow milk or soy protein or by other autoimmune diseases or other conditions (ref). An increased number of IEL in the mucosa is seen in many various conditions; inflammatory bowel disease, giardiasis, viral enteritis or drug induced enteropathy [79, 80]. There is a normal histological variation of the proximal small intestinal mucosa where the villi can have different structures, such as; finger, leaf, tongue or ridge like structures (ref). These variations can also be seen within a single individual. In the proximal duodenum the villi are usually broader and shorter, especially the villi overlying Brunners glands.

IEL

The number of IEL is an indication of immunological activity in the lamina propria. There has been an on-going debate on how many IELs should be considered to be normal and the latest proposal is up to 30 IEL/100 enterocytes [81-83]. To count of the number of IELs is very time consuming and other methods have been developed, such as quantifying villous tip IELs [84]. Staining methods, using haematoxylin and eosin can be used to enhance IEL diagnostics.

Crypthyperplasia

Crypthyperplasia denotes elongation of the crypts of Lieberkuhn, which usually precedes villous atrophy [85, 86]. The elongation may be caused by the expansion of the lamina propria and an influx of inflammatory cells. The normal ratio of villous height to crypt depth is generally assumed to be 3:1 to 5:1, but in children a ratio of 2:1 is considered to be normal.

Villous atrophy

The height of the villus is generally three times the base width. A mild atrophy is indicated by minor villi blunting, more severe atrophy include the presence of remnants of blunted villous and a total atrophy implies the complete absence of villi.[6].

Marsh-Oberhuber classifications

To grade the enteropathy there are several different grading systems. Nowadays, the most commonly used is the Marsh-Oberhuber classification, starting with intraepithelial lymphocytosis > 30 IEL/100 enterocytes (Marsh 1) to crypthyperplasia (Marsh 2) and various degrees of villous atrophy, (Marsh 3a-c) (Figure 1) [86].

According to the ESPGHAN criteria, a Marsh 1 classification is considered to indicate uncertain cases, where a false positive serology, false negative biopsy or potential celiac disease should all be considered. There is an on-going debate whether Marsh 1 lesions can be considered to be indicative of CD or should be grounds for the classification as potential CD [87-89].

In Sweden small biopsies are often classified according to the SnoMed system, which is based on the same histopathology parameters as the Marsh classifications[90].

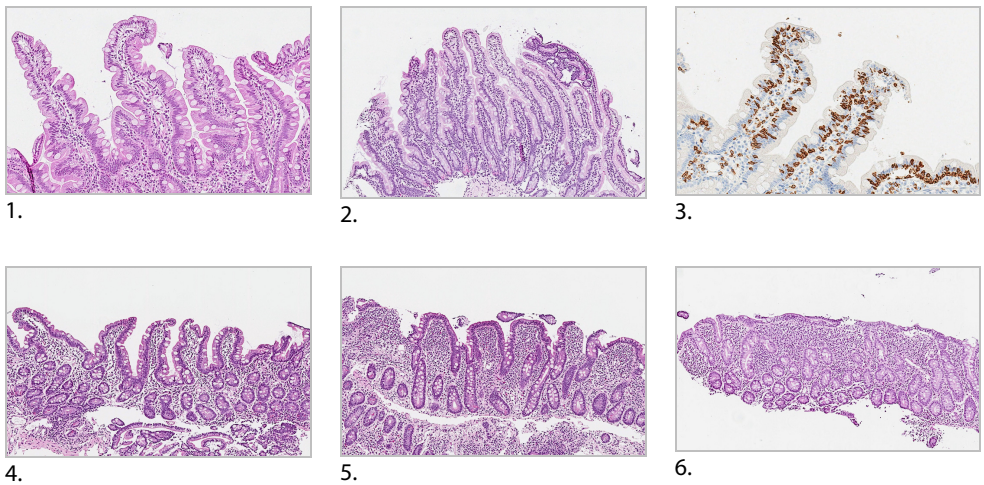

Figure 4. Intestinal biopsies, showing various degrees of mucosal damage.
1. Normal mucosa, Marsh 0, **2.** IEL, Marsh 1, **3.** IEL, CD3+
4. Partial. villous atrophy, Marsh 3a, **5.** Subtotal villous atrophy, Marsh 3b,
6. Total villous atrophy, Marsh 3c
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Treatment and Adherence

The only treatment available today is strict adherence to a GFD diet. The name gluten refers to the protein fraction of wheat (gliadin and glutenins). The gliadin is a prolamine and the corresponding prolamins in other cereals are secalin in rye and hordeins in barley. Oat contains avenin, which was previously considered to be harmful but has lately been proven to be safe for the majority of people with CD [91-93], although some concerns have recently been raised [94]. Gluten-free products can contain smaller amounts of gluten (20-100 ppm/parts per million) which some patients react to and are therefore treated with a natural GFD e.g. rice, potatoes. A threshold for safe daily gluten intake has been determined and a total daily intake of up to 10 mg of gluten daily is in most cases harmless, whereas an intake of > 50 mg daily seems to initiates the inflammatory process [95].

Previously the GFD has been described to be low in fibre, minerals and vitamins, but a recent study has not shown any nutritional difference when compared to a gluten containing diet [96].

To adhere to a GFD is a life-long challenge and there are several different factors affecting the outcome.[97-99]. Many patients describe poor palatability, especially in bread, which is due to the fact that gluten gives the dough its elasticity and viscous properties. The availability of gluten free products is another factor, even though there has been an increase of gluten-free products in general stores over the last years. Eating out in restaurants and cafés can still be a problem. Some studies suggest that access to a dietician with special interest in GFD might be the most important help to adhere to the GFD [100], However, other studies have reported that the lack of dietary counselling does not affect the adherence [97, 98], suggesting that information on GFD is easily accessible on the internet and via support groups It is often reported that the adherence in adults is generally low and the information on long-time adherence in children is scarce [101]. Age at the time of diagnosis and age at current time are other factors of importance, suggesting that to get the diagnosis as a teenager and also currently being a teenager decreases the adherence [97, 102]. There are studies reporting poorer adherence in screening detected cases compared to clinically detected cases. However, other studies show varying results on the effect of being screening detected and few studies are conducted on children.

In a foreseeable future there may be alternative treatments to CD, such as oral enzymes that would degrade gluten into non-toxic parts, gluten free grains and vaccines preventing CD development by blocking the immunological reaction [103].

Screening

Screening for CD in the general population is controversial [104, 105], whereas screening at-risk groups is common practice in many countries. The at-risk groups include e.g. first-degree relatives, patients with type I diabetes or thyroid disease and patients with genetic disorders such as Downs and Turners syndrome.

A two-phased screening can be conducted by starting with HLA-DQ genotyping and as a second step by serological markers indicative of CD such as tTG-IgA and EMA.

Screening detected cases have been designated to be asymptomatic (silent) to distinguish them from clinically detected, symptomatic patients. However, many of these screened cases are not truly asymptomatic (silent) but simply unrecognized, either because the patients are not aware of their symptoms or the physician has not considered CD [19, 20]. The natural history of screening detected CD is not known. However, there are follow-up studies of children with CD identified by mass screening which show beneficial effects on symptoms and quality of life [106]

The World Health Organization (WHO) has established requirements for implementation of medical mass screening, and most of these criteria are fulfilled for celiac disease since it is: a fairly common disease, the negative health consequences are extensive, reliable screening tools are widespread and an effective treatment is available [107].

There are studies that evaluate the health economic aspect of mass screening. One study found a reduction in health care consumption after the treatment was initiated and another study reported a reduction of the medical cost after CD diagnosis compared to the years preceding the diagnosis [108, 109]. However, the economic cost of screening and treatment versus the morbidity prevention has not been fully evaluated.

Objectives

The main objectives of this thesis were to investigate the total prevalence of celiac disease in children born during the Swedish epidemic; furthermore the effect of each step of the diagnostic process to obtain CD diagnosis and the adherence to GFD.

Specific objectives

- To investigate the total prevalence of celiac disease in clinical and screening detected children born 1993, during the Swedish epidemic.
- To evaluate the accuracy of CD diagnosis by using different biopsy methods; capsule or endoscopy and to evaluate the impact of the pathological evaluation in the diagnostic process.
- To hypothetically apply the revised ESPGHAN guidelines on screening detected children by evaluating any potential correlation between anti-tissue transglutaminase antibodies and the degree of gluten induced enteropathy.
- To evaluate the adherence after one year on gluten-free diet in children with screening detected CD.

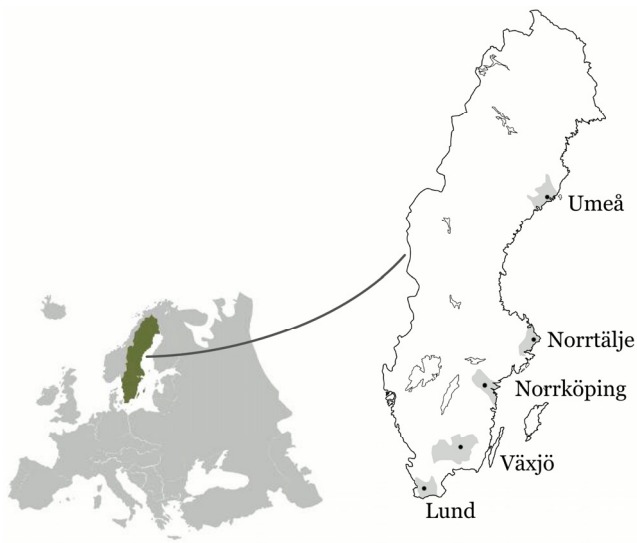
Overview of objectives

	Paper I	**Paper II**	**Paper III**	**Paper IV**
Study design	Quantitative Cross-sectional	Quantitative Cross-sectional	Quantitative Cross-sectional	Quantitative Cross-sectional
Aim	To investigate the total prevalence of celiac disease in clinical and screening detected children born 1993, during the Swedish Epidemic	To evaluate the accuracy of the CD diagnosis by using different biopsy methods; capsule or endoscopy and to evaluate the impact of the pathological evaluation in the diagnostic process	To apply the revised ESPGHAN guidelines on screening detected children by evaluating any potential correlation between tTG-IgA levels and the degree of gluten induced enteropathy	To evaluate the adherence after one year on gluten-free diet in children with screening detected celiac disease
Participants	ETICS I	ETICS I	ETICS I+II	ETICS I+II
Material	Blood samples Clinical data	Blood samples Small bowel biopsies	Blood samples Small bowel biopsies	Blood samples Questionnaires

Material and Methods

The Etics study - Exploring the Iceberg of Celiacs in Sweden

We performed a two-phased screening study in order to investigate the true prevalence of celiac disease in children exposed to different infant feeding practices. The first cohort included children born in 1993, during the epidemic and the second cohort, children born in 1997, after the epidemic. Five study sites were involved in the study where each site included a major city with municipalities in the surrounding suburbs. The study sites; Umeå, Norrtälje, Norrköping, Växjö and Lund/Malmö are representative of different geographical areas from the very north to the south of Sweden. The first phase was performed in 2005-2006 and the second phase in 2009-2010. The children were enrolled at the age of twelve years and a letter of invitation was sent home, requesting parental consent to participate. The screening was performed by research nurses in cooperation with school nurses by collecting blood samples from the children at their schools. The children who were suspected of CD were referred to their local paediatric department for further investigation with a small intestinal biopsy. The study was part of the Prevent CD European project [125]



The national Swedish childhood celiac disease register

The Celiac Disease Register became a nationwide incidence register for clinically detected CD in 1998. All 47 of the paediatric departments in Sweden report new CD cases. The reports are based on a standardized form which includes the personal identity, place of residence, gender, date of the small intestinal biopsy and the basis for diagnosis; serological markers, symptoms and mucosal evaluation. The cases included in the register at the present time are both clinically and screening detected. The criteria for CD diagnosis is achieved according to the current ESPGHAN guidelines.

Screening strategy

Laboratory Analyses

All blood samples were analysed for tTG-IgA. The values of tTG-IgA was determined by enzyme-linked immunosorbent assay, using a commercial kit(Celikey,Phadia GmbH,Freiburg,Germany).Intermediate values of tTG-IgA (2-4 U/mL) were additionally analysed for EMA-IgA by indirect immunofluoresence technique using monkey esophagus. (The Binding Site, Birmingham, UK). Sera yielding fluorescent binding to the endomysial structure were diluted to determine the lowest titer detectable and values with 1:5 dilution were considered to be positive.To increase the sensitivity of the test the cut-off for positivity was lowered from the manufacturers recommendation of 5 U/mL to 4 U/mL. Total s-IgA was measured in all blood samples in the cohort of 1993, but due to low-yield it was not repeated in the cohort of 1997. When the levels of S-IgA was low (<0,5g/L) the blood samples were further analysed for tTG-IgG where the cut-off for positivity was >6 U/mL.Intermediate values (3-6U/mL) were further analysed for EMA-IgG. Total s-IgA was measured using a routine nephelometric method (BN Pro Spec System). IgA deficiency was defined as <0,06 g/L.

HLA DQ2/DQ8 genotyping

All of the screening detected children were genotyped for HLA-DQ2/DQ8.

Small intestinal biopsy

All children were recommended further investigation with a small intestinal biopsy. The biopsies in the first phase of the study were performed using either a suction capsule or endoscopy, according to local routine. The children, who had a normal or inconclusive biopsy, were re-biopsied by using endoscopy. The recommendation was to take 4-6 biopsies from both the proximal and the distal duodenum including the bulb.

Histopathological evaluation

The preparation of the mucosal specimens took place locally at the five different pathology departments, where they were stained with hematoxylin and eosin. Staining for CD3+ cells was only performed as a routine at one of the study sites. The specimens were initially evaluated by a local pathologist according to recommendation from the Swedish Society of Pathology. These recommendations correspond to the Marsh classifications, except for that Marsh 3b and Marsh 3c are grouped together in one group and named subtotal/total villous atrophy. All specimens were re-evaluated by an expert pathologist, blinded of the previous results. If there was diagnostical divergence between the local and expert pathologist, a third expert pathologist re-evaluated the specimens to reach a diagnostic consensus

Study Population

A total of 18 325 children were invited, 10 041 from the 1993 cohort and 7567 from the 1997 cohort. The two cohorts invited represented 8,8% vs. 9,2% of the total population in the birth cohorts born in 1993 (n=117 997) and 1997 (n= 90 502). In both cohorts, 95% of the children were born during the intentional year 1993 or 1997. The remaining children were born either during the preceding year or the year thereafter. The proportion of girls participating in the two birth cohorts was 48% vs. 49%, respectively. There were 7567 (75%) children in the first cohort and 5712 (69%) in the second cohort who participated; from these children 7208(72%) vs. 5424(69%) blood samples were obtained. Children with clinically detected celiac disease diagnosed within clinical care prior to the study, were reported by the parents at enrolment and ascertained through the National Swedish Childhood Celiac Disease Register and/or the child's medical record.

Paper I,

The study population in this paper were children from the 1993 cohort: (phase 1) and children with clinically detected CD within clinical care. The screening was performed by using tTG-IgA as a serological marker indicative of CD. For further information about the screening strategy and the diagnostic process, see Methods.

Paper II,

The study population in this paper were screening detected children from the 1993 cohort (phase 1) in whom a small intestinal biopsy was performed. The small bowel biopsy was conducted by using a Storz or Watson suction capsule, or by using endoscopy according to local routine at the different study sites. Children who had a normal or inconclusive biopsy were re-biopsied by using an endoscopy. The histopathological preparation and initial evaluation took place at the local pathology department at each of the five study sites. To stain for CD3+ cells as a routine was only performed at one of the study sites, otherwise staining using hematoxylin and eosin were performed at all sites.The re-evaluation of the mucosal specimen was performed by an expert pathologist who was blinded to the previous results. If there was any diagnostic divergence between the local and the expert pathologist a second expert pathologist re-evaluated the mucosal specimens to reach a diagnostic consensus.

In Paper III,

The study population in this paper were children from the 1993 and the 1997 cohorts (phase 1 & 2). The children included were those who had a small intestinal biopsy performed, a mucosal specimen, which could be evaluated and a second serum sample drawn at the time of biopsy.

Paper IV,

The study population in this paper were children from the 1993 and the 1997 cohorts (phase 1 & 2). The children who were included were those who had serum samples (tTG-IgA) taken both at the time of biopsy and after 12 months. They also had a completed self-reported questionnaire at the one year follow-up.

Statistical analyses

Descriptive statistics were used to present the data. Mean values, standard deviations, cross tabulations, frequency tables, medians and quartiles were used where appropriate. The proportions for categorical variables were presented when suitable. In Paper I, the prevalence of celiac disease was presented as cases per 1000 individuals with a 95% confidence interval, both for the whole population and for subgroups of celiac disease (screening detected from ETICS first field phase and previous cases), gender and study site. Relative risk was used to determine if there was any difference between subgroups.

In Paper II and Paper IV, the Pearson's $\chi 2$ test was used for cross-tabulated data, unless the required condition of cell counts was not fulfilled when instead Fisher´s

exact test was used. In Paper III, Student's t-test was used to compare the different degrees of mucosal lesions in the small intestine.

To perform our analysis Microsoft Access 2010 was used for data handling, Stata 11.2 and IBM SPSS (versions 15, 16 and 20) for statistical analysis and Microsoft Excel 2003-2010 was used to create graphs.

Table 1

Statistical method	Statistical test	Paper I	Paper II	Paper III	Paper IV
Non-parametric test	Fisher's Exact test		X		X
Non-parametric test	Pearson's $\chi 2$ test		X		X
Parametric test	Student's T-test			X	

Results

Main findings are presented in two parts, based on the different screening phases. The first phase was conducted in 2004-2005, where 10 041 children from the 1993 cohort were invited, 7567 (75%) children participated and 7208 (72%) blood samples were obtained. The total number of children who were invited was representative of 8,8% of the total birth cohort of children born in 1993 (n=117 997).

The second phase was conducted in 2009-2010, where 8 284 children children form the 1997 cohort were invited, 5712 (69%) children participated and 5 434 blood samples were obtained. The total number of children who were invited was representative of 9,2% of the total birth cohort of children born in 1997 (n= 90 502) (Statistics Sweden; www.scb.se). In Paper I and II, the results are based on the first phase, and in Paper III and IV, on the first and second phase, of the screening study.

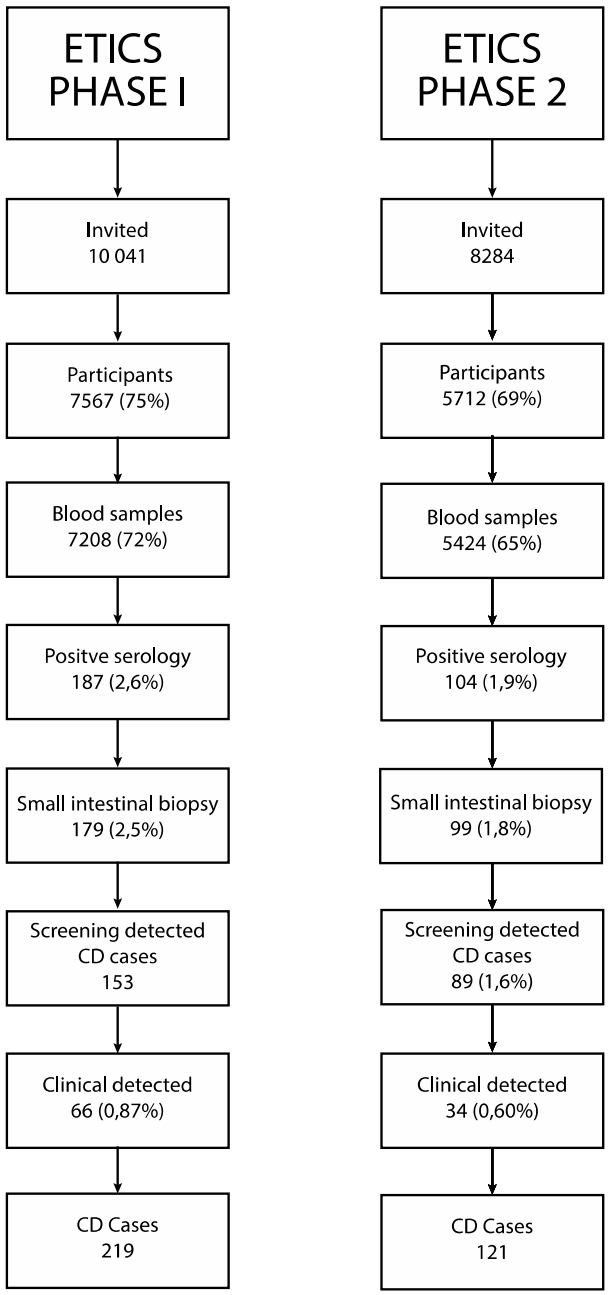


Figure 5. Results from the two screening phases of the ETICS study.
Adapted with kind permission from A. Myléus.

Paper I

Analyses of the serum samples yielded a total of 192(2,7%) children with positive markers who were recommended further investigation with a small intestinal biopsy, which was performed in 180 children, either by using a suction capsule or an endoscope. Celiac disease was verified in 145 children 20/1000 (95% CI 17-23).

Clinically detected CD cases

Previously diagnosed celiac disease was found in 66 children 8.9/1000 (95% CI 6,7-11). The CD diagnosis was ascertained in 66 of the children, whereof 65 children had biopsy verified diagnosis and in one child where the diagnosis was based on EMA-IgA 1/320, family history of CD, and clinical response to a GFD. There was one child who initially was included but later shown to be misclassified. The total prevalence of celiac disease was 2,9%, 29/1000(95% CI 25-33).

Prevalence of celiac disease in the different study sites

Table 2. CI=confidence interval . Adapted with kind permission from A. Myléus et al.

STUDY SITE	LUND	VÄXJÖ	NORRKÖPING	NORRTÄLJE	UMEÅ
Participants clinically detected	3241	1108	954	869	1395
CD cases	29	14	8	5	11
Blood samples Participants screening detected	3078	1017	912	845	1355
CD cases	65	29	17	18	16
Prevalence	30	41	27	27	20
Relative risk	1.5	2.1	1.4	1.4	1.0
95% CI	1.0-2.3	1.3-3.4	0.80-2.3	0.79-2.4	-

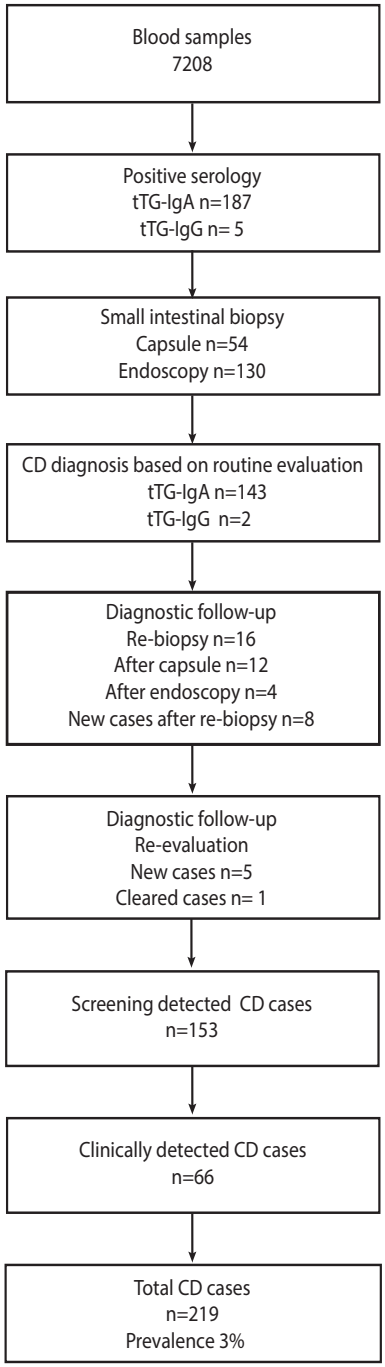


Figure 6. Results from the phase 1, screening of the 1993 cohort. Adapted with kind permission from A. Myléu

Table 3. Study population variables of the 1993 and 1997 cohorts.
Adapted with kind permission from A. Myléus.

STUDY POPULATION VARIABLES	**1993 cohort**	**1997 cohort**
SEROLOGICAL MARKERS	**n / %**	**n / %**
tTG-IgA > 4 U/mL	167 (87)	85 (82)
tTG-IgG> 6 U/mL	5 (3) Analyses performed if s-IgA <0.5g/mL	Analyses of s-IgA not performed
tTG-IgA 2-4 U/mL + EMA-IgA >1:5	20 (10)	19 (18)
Total	192	104

MARSH CLASSIFICATIONS	**n / %**	**n / %**
Marsh 3a-c	141 (77)	88 (89)
Marsh 2	2 (1)	0
Marsh I+ symptoms and signs	9 (4,9)	1 (1)
Marsh+no symptoms and signs	3 (1,6)	0
Marsh O	26 (14)	10 (10)
Non-interpretable	3 (1,6)	0
Total number CD cases	153 (83)	89 (90)

HLA GENOTYPING	**n / %**	**n / %**
DQ2	115 (76)	71 (80)
DQ8	15 (9,9)	9 (10)
DQ2/DQ8	23 (15)	8 (9)
Non DQ2/DQ8	0	0
Not available	0	1 (1)
Total	153	89

Case ascertainment symptoms and/or signs

Children with Marsh 1 lesions were considered to be CD cases if they had symptoms and/or signs suggestive of CD, positivity for HLA- DQ2/DQ8 and a clinical response to GFD. There were 12 children with Marsh 1 lesions, whereof 3 children had no symptoms and signs of CD. The other children showed a variation of symptoms and signs, which are presented in the table below.

Table 4. Characteristics of children with mucosal lesions with >30 IEL/ 100 enterocytes. Adapted with kind permission from A. Myléus.

Gender	Symptoms and signs	tTG-Ig A (U/mL)	EMA
F	Tiredness	98	
F	Stomach ache	4,7	EMA-IgA 1:10
F	Tiredness	23	
F	Tiredness, nausea, underweight	2.6	EMA-IgA 1:5
F	Constipation, underweight Hypothyroidism	12	EMA-IgA 1:20
F	Secondary lactose intolerance	6.6	EMA-IgA 1:5
F	Tiredness, flatulence	44	
M	Tiredness, flatulence	14	EMA-IgA 1:5
M	Stomach ache, heredity for autoimmune disorders	36	
F	Flatulence	>100	
M	Stomach ache	31	
M	Tiredness, flatulence, heredity for CD	97	
F	Stomach ache, loose stool	4.6	EMA-IgA 1:5
M	Alternate constipation and loose stools, underweight.	50	
F	Tiredness, nausea, iron deficiency	32	
F	Constipation, tiredness, short for age, heredity for CD	51	
F	Nausea, hypothoridism	7.0	EMA-IgA 1:5
F	None	32	

Paper II

A small biopsy was performed in 184 children. Four additional biopsies were performed at a later stage, the results were therefore not included in Paper I.

The biopsies were conducted with two different biopsy methods – 130 of the biopsies were conducted with endoscopy and 54 by suction capsule.

Endoscopic biopsies were inconclusive in 0.6%, compared with 7.4% of the biopsies performed by suction capsule.

There were 16 children who were re-biopsied due to the first biopsy being evaluated as normal or inconclusive. Of these 16 children 12 of them were re-biopsied after an initial suction capsule biopsy and four children after an initial endoscopic biopsy. By performing re-biopsies, the total number of CD cases increased to 153.

The diagnostic process was initiated by a local pathologist, this was then enhanced by re-evaluation of all mucosal specimens by an expert pathologist and when disagreement occurred, a second expert pathologist evaluated the specimens to reach diagnostic consensus.

In six children (3.3%) the diagnosis was changed after the re-evaluation and increased the total number of CD cases to 145 children. In five children the diagnosis was changed from normal to CD. The expert pathologist cleared one child who was diagnosed with CD.

Due to the additional cases being diagnosed the CD prevalence increased to 30/1000 (95% CI 26-34) but it was no statistical significant difference from the CD prevalence previously reported in Paper I.

Patchy enteropathy was found in 9.1%.

Results Re-biopsy

There were 16 children who had a first biopsy that was evaluated as being normal or inconclusive. The children were re-biopsied by endoscopy.

However, four of these children received their CD diagnosis after the re-evaluation of the first biopsy and not through the second biopsy and are excluded as new cases due to performing a re-biopsy.

There were eight children who had an initial capsule biopsy performed who received CD diagnosis after being re-biopsies using an endoscopic method. None of the four children with an initial endoscopic biopsy obtained CD diagnosis.

Table 5. Results different biopsymethods.
*130 primary biopsies and 16 re-biopsies.

Biopsy method	Suction capsule	Endoscopy
Total	n=54	n=146*
Inconclusive	n=4 (7,4%)	n=1 (0,6%)
Re-biopsies	n=12	n=4
Conclusive fractions from proximal and distalduodenum	Not performed	110

Results of the Re-evaluation

In the majority of the cases, 96% (177/ 184) the expert pathologist was in agreement with the local pathologist and verified the diagnosis. However, the re-evaluation changed the diagnostic outcome in six (3,3%) of the children, where five children with a normal biopsy according to the local pathologist got CD diagnosis and one child was cleared of the CD diagnosis. Additionally there was one child with Marsh 1 lesions according to the primary evaluation, which was considered to be not interpretable by the expert pathologist. The re-biopsy failed and the family did not want to perform an additional biopsy. This child still obtained CD diagnosis due to increased levels of the serological markers and reoccurring symptoms on gluten challenge.

Table 6. Results from the re-evaluation.

Marsh classifications	Local pathology evaluation	Expert pathology evaluation
Marsh 3	123	133
Marsh 2	0	2
Marsh 1	19	15
Marsh 0	41	29
Non interpretable	1	5

Patchy Enteropathy

Biopsies were recommended to be taken both from the proximal and distal duodenum, including the bulb. There were conclusive fractions from both locations in 75% /110/146) of the endoscopic examinations, whereof ten (9,1%) children had patchy distribution, where one of the locations had mucosal damage and the other location was normal.

The different histopathological combinations were as follow;

Table 7. Patchy enteropathy, Marsh classifications in different fractions.

Marsh classifications in different fractions of duodenum	**Children n=10**
Marsh 1/ Marsh 0	n=3
Marsh 3a / Marsh 0	n=3
Marsh 3b / Marsh 0	n=2
Marsh 3c/ Marsh 0	n=2

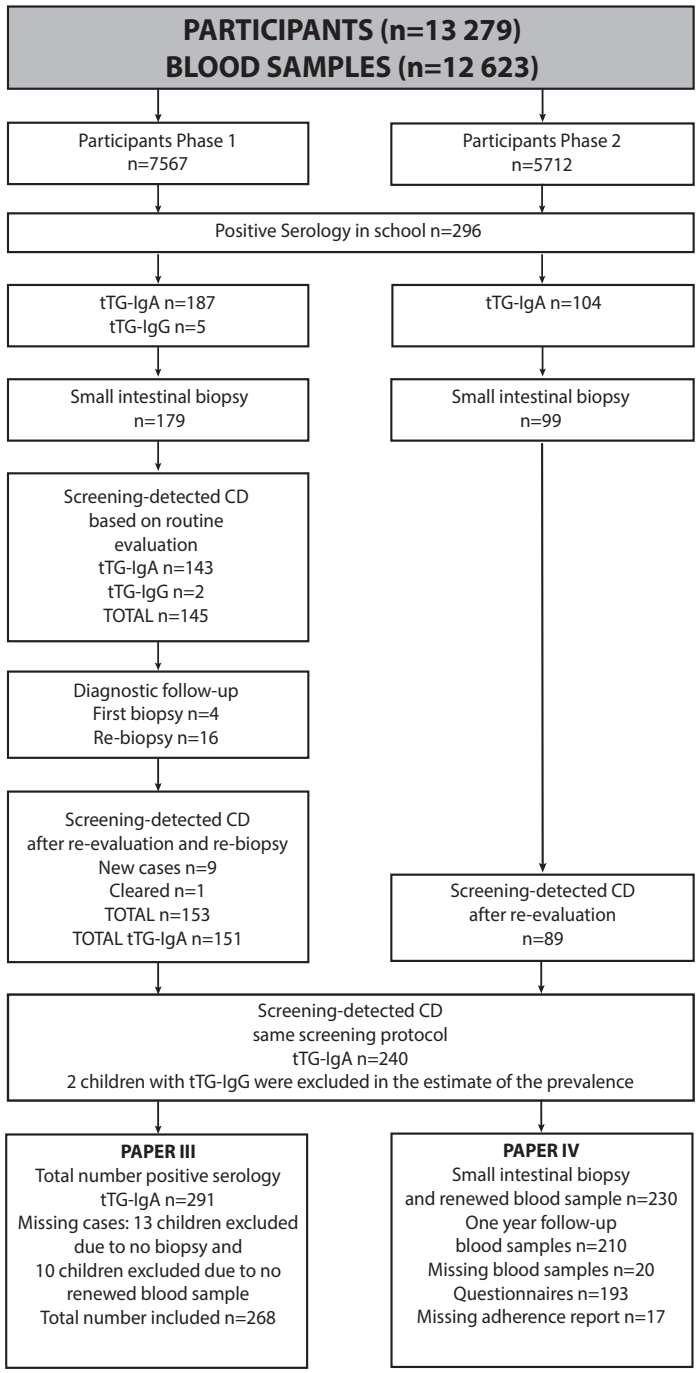


Figure 7. Results Paper III & IV– phase 1 & 2.

Paper III

Out of the 267 children with increased levels of tTG-IgA, 230 children were diagnosed with CD. There was a widespread discrepancy of the tTG-IgA levels in relation to the degree of gluten-induced enteropathy, which was more apparent in the groups with lower levels of tTG-IgA (<5 U/mL), where 55% had Marsh 3 lesions. Notable, though was a gradual increase of the tTG-IgA levels predicting mucosal damage. The manufacturer's cut- off for positivity is 5 U/mL, however several recent studies have suggested that a lower cut-off of 3 U/mL would be more correct for predicting enteropathy [118, 119], therefore we adjusted the cut-off in this sub-study, including both the level of 3 U/mL and 5 U/mL. By assessing the ESPGHAN criteria when serological markers exceeded ten times the upper limit of normal (>10xULN), all biopsies could have been omitted when using >5 U/mL (50 U/mL) as the cut-off level and almost all, (with the exception of one child), at the cut-off level at >3 U/mL.

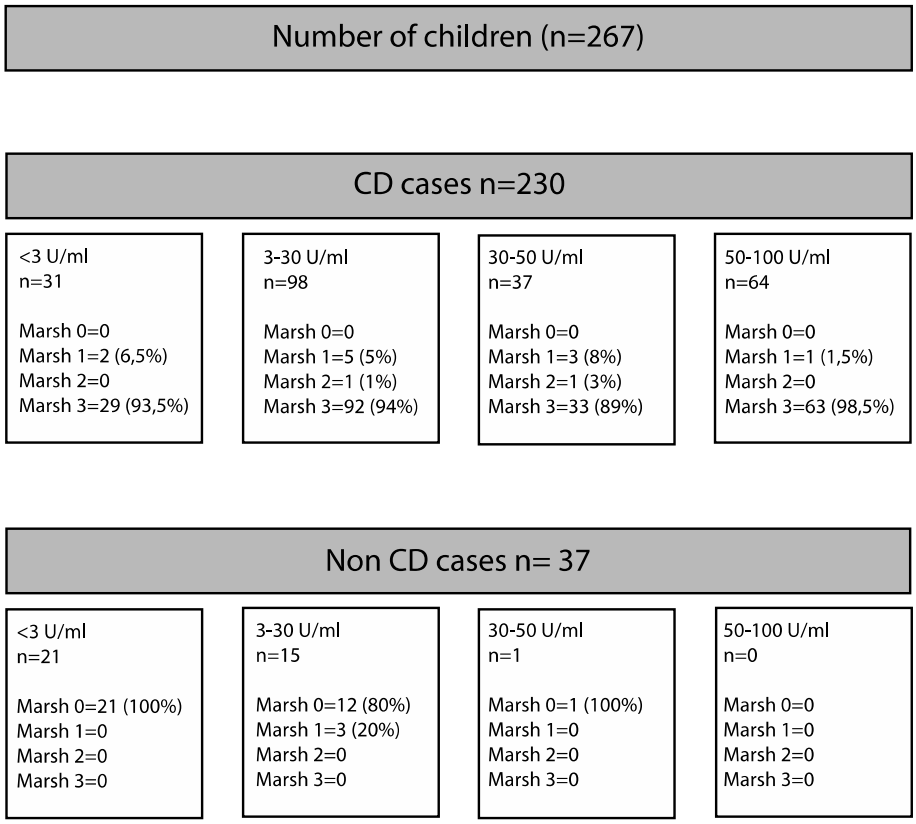
In the first group (tTG-IgA > 50 U/mL), 98% (63/64), of the children had Marsh 3 lesions vs. 95% (96/101) in the latter group (>30U/mL). There was no statistical significant difference in Marsh 3 lesions between boys and girls (85% vs. 79%, p = 0.23).

Additionally, there were four children who had Marsh 1 lesions and serological markers at a level of > 30 U/mL who obtained CD diagnosis based on additional criteria according to the study design. On the contrary, there were three children with Marsh 1 lesions who were not considered to be CD cases who had tTG-IgA levels between 5 U/mL and 30 U/mL

There were 34 cases who had normal biopsies and 76% (26/34) of these children had tTG-IgA levels < 5 U/mL. Seven children, 21% (7/34) had tTG-IgA levels between 5 U/mL and 30 U/mL.

The only child in the group with tTG-IgA > 30 U/mL who was not considered to have CD, had a tTG-IgA level of 44 U/ mL and a normal biopsy (Marsh 0). However, this child had a strong genetic susceptibility for CD and was followed up by an annual serology test and clinical control. Over time the serological markers normalized and no clinical signs suggestive of CD appeared, thus the possibility of the child to have transient serological markers and to be a potential CD case.

Table 8. Mucosal damage in different serology groups.



Paper IV

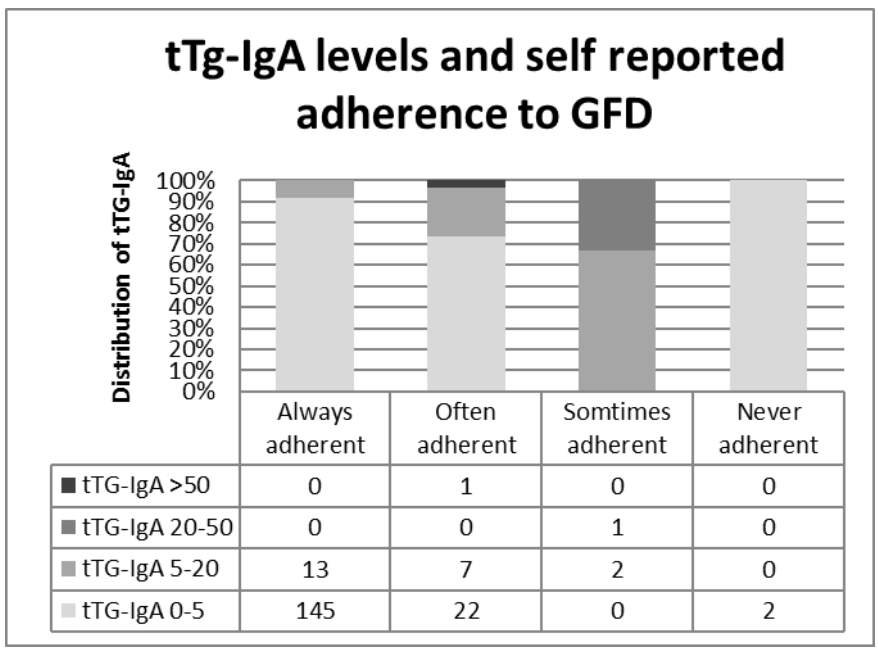
To measure the adherence we used serology tests in combination with self-reported questionnaires. Of the children with screening detected CD, 85% (179/210) had normalized their tTG-IgA levels (<5 U/mL) after one year of treatment with GFD. There were no significant statistical difference between gender – 120 girls and 90 boys. (P=0.911) The majority of the children (94%) were classified with Marsh 3 a-c lesions. Two children had Marsh 2 lesions (1%) and 10 children (4.8%) had Marsh 1 lesions in combination with symptoms/signs of CD.

The self- reported questionnaire, where the response alternatives were: *always*, *often*, *sometimes* and *never*, were completed by 193 (92%) of the children. The majority, 82%(158/193), reported to always be adherent. Similar adherence was seen for both boys and girls, (P=0.691).

When comparing the serological markers and self-reported adherence at the one-year follow-up with GFD, 75 % (145) of the children who reported to always be adherent had normalized tTG-IgA levels. There were 13 children (6.7%) who reported to always be adherent, but the serological markers were not yet normalized (mean tTG-IgA 8.1 U/mL, range 5.2-10.9 U/mL) However, a majority of these children (85%) had initial high values and all of them had halved their values. There were 3 adolescents who reported to only follow the GFD sometimes, who had elevated tTG-IgA levels. Additionally there were two of the adolescents who had normal tTG-IgA levels at the follow-up, but reported to never adhere to the GFD.

There were 35 children who had normal tTG-IgA levels at the time of the biopsy. In the blood samples obtained at school 16 (46%) of these children had intermediate levels of tTG-IgA and positivity for EMA and 19 (54%) children had lower levels of tTG-IgA (4-10 U/mL). However, the correlation between the gluten induced enteropathy and the level of tTG-IgA was not coherent in this group, 31 out of 35 children had Marsh 3 lesion and the remaining four children had Marsh 1 lesions. The self-reported adherence in this group was high (88%). and all of the children responded to the treatment with GFD.

The children with tTG-IgA <5 U/mL at the time of the biopsy remained at this level after 12 months and the majority (90%) of the children with tTG-Ig A 5-20 U/mL normalized their values.



	Always adherent	Often adherent	Somtimes adherent	Never adherent
tTG-IgA >50	0	1	0	0
tTG-IgA 20-50	0	0	1	0
tTG-IgA 5-20	13	7	2	0
tTG-IgA 0-5	145	22	0	2

Figure 8. tTg-IgA levels and self reported adherence.

Methodological considerations

In the first paper, concerning the prevalence of CD in twelve- year-olds born during the epidemic, there may have been a risk of selection bias since individuals experiencing health problems may be more prone to participate. However, assuming that there were no further cases among non-participants, the prevalence would still be high, 21/1000. We considered children who had Marsh 1 lesions to be CD cases under the conditions that they also had symptoms and /or signs of CD, positivity for HLA DQ2/DQ8 and a clinical response to the GFD. To obtain CD diagnosis according to the ESPGHAN criteria the degree of mucosal damage has to be graded as Marsh 2 or Marsh 3. However, when restricting the diagnostic criteria to only include Marsh 3 lesions it did not effect the results.

In the second paper, concerning the diagnostic process, the biopsies were performed according to local routine in the five different study sites, thus the material was heterogenic. To sharpen the diagnostics, all biopsies which were considered to be normal or inconclusive, were endoscopically re-biopsied. The advantage of the heterogenic material was the ability to compare two different biopsy methods,which resulted in that all biopsies in the second phase were performed by using endoscopy. Furthermore, the CD3+ staining did not increase the probability to diagnose Marsh 1 lesions, which could be due to a small number of biopsies being investigated.

In the third paper, concerning the correlation between the level of the serological markers and the degree of mucosal damage, a reason for the wide variation in tTG-IgA levels and the degree of gluten induced enteropathy could be due to a patchy distribution of the mucosal lesion or a poor quality of the mucosal specimen.

In the fourth paper concerning the adherence to the GFD, there were missing cases due to questionnaires not being fulfilled or serum samples not being obtained at the one year follow-up, which could be due to poor adherence. However, assuming that everyone without data was non-adherent to the diet the adherence still would be as high as 70%.We also included children who had <5 U/mL at the time of biopsy, where the majority of the children had Marsh 3 lesions. The changes in serological markers after one year on GFD could not be evaluated in this group, interestingly, they reported a high adherence and remained on the level of <5 U/mL.

A strength of this study is that the study population invited was representative of nearly 10% of the total Swedish birth cohort. The diagnostic process was conducted according to the same study protocol in all study sites. The same serological test was used in both study phases. All of the specimens were re-evaluated by an expert pathologist.

Discussion

Main findings

One of the main findings in this thesis was the unexpectedly high prevalence of celiac disease in 12- year-old children born during the Swedish epidemic, where two thirds were screening detected. Another main finding was the benefit of using endoscopy to increase the diagnostic accuracy and revealing a patchy distribution of the mucosal lesions. These findings are supportive of the recent shift in clinical practice, where previously the gold standard was using the suction capsule as the biopsy method. Another interesting finding was that all screened children with tTG-IgA >10xULN had a severe degree of gluten-induced enteropathy. By applying the ESPGHAN guidelines for screening detected children, the biopsy could have been omitted in a quarter of the children in our study. A positive and unexpected finding was the high adherence to the GFD in screening detected children.

The Swedish population experiment

The Swedish CD epidemic, 1984-1996, was preceded by changes in infant feeding recommendations, implemented at the well-baby clinics, where almost all Swedish children attend. In 1982, the recommendations were changed by postponing the introduction of food containing gluten, from four to six months of age. This was in accordance with new European recommendations that were made with the ambition to postpone the development of CD through encouraging delayed gluten introduction. Concurrently, the gluten content was increased two-fold in industrially manufactured cereal based formulas in order to reduce the total amount of protein given to infants. Thus the milk content was decreased and the amount of cereal increased. These independent factors played a significant role in the four-fold increase of clinically detected CD in children below two years of age.

The recommendations were revised in 1996 to the pre-epidemic recommendations of gradual introduction of smaller amount of gluten while still breastfeeding. In addition the amount of gluten was decreased in the baby formulas. After the change of the recommendations, the incidence rate of symptomatic CD in children younger than 2 years of age abruptly declined to the pre epidemic levels. The 12- year-old children who were investigated in this thesis were born in 1993 during the epidemic period

and in 1997 in the post-epidemic period, respectively. Our finding of the unexpectedly high prevalence of 3%, in the 1993 cohort where two thirds of the CD cases were undiagnosed prior to screening suggest that early infant feeding practices affects the risk of CD development, at least up to the age of 12 years [110], We have later published that the CD prevalence in the 1997 cohort was significantly lower than in the 1993 cohort with no significant difference between the cohorts regarding age at diagnosis in the clinically detected cases [111].

A pilot screening study of children (2.5 year old), born in 1993 and 1997, respectively, reported that 0.7% of the children in the 1993 cohort were clinically detected and 1.3% screening detected children compared to 0.4% clinically detected and 0.7% screening detected, respectively in the1997 cohort [45, 46]. This difference was not statistically significant, but the findings are supportive of our results when screening the same birth cohort at the age of 12 years. By using the national register, the two birth cohorts were followed through childhood and symptomatic cases successively continued to emerge.

At the age of six years, there was still a difference in the cumulative incidence of CD, 5.4% in the 1993 cohort compared to 2.9% in the 1997 cohort [112].

The main difference between the 1993 and 1997 cohorts was the difference in infant feeding practice, specifically regarding the age at gluten introduction and the amount of gluten given and also whether gluten was introduced while still breast- feeding. There are several studies reporting the importance of each of these factors in relation to the risk of CD development. These studies show that introducing large amounts of gluten compared to smaller amounts increase the risk by 50% and breast-feeding continuing beyond gluten introduction more than halves the risk [13], Additionally, introducing gluten during the first three months of life is associated with an increased risk. Breast-milk contains several immune-modulating factors and affects the microbiota and breastfeeding has also been associated with reduced risk of gastroenteritis [113]. It has also been proven that the microbiota is different between formula-fed and breast-fed infants. Repeated rotavirus infections increase the CD risk [35]. The seasonal pattern, where children born in the summer have an increased risk of CD and also that children experiencing three or more infections episodes before six months of age have an increased risk [14, 34]. This exemplifies the fact that CD is a multifactorial disease where infectious agents can trigger the development. Unfortunately, there is no data as to whether or not the infectious panorama changed in Sweden during the epidemic period. The Swedish epidemic was unique in its scale and evidently the new recommendations in 1996 affected the decline of CD incidence in children below two years of age. It appears that the manner of gluten introduction influences the risk of developing CD early in life. Several epidemiological studies by Ivarsson et al and newly published data from the TEDDY study are supportive of these findings [43]. The TEDDY study is a prospective international study following children with a high genetic susceptibility to develop

Type 1 Diabetes and CD. The results showed that the risk to develop CD was nearly double compared to children in the USA and also that Swedish children are given gluten-containing cereals at an earlier age compared to other countries [114]. However, the CD incidence seems to fluctuate over time, and except for a temporary decline in 1995 to 1997, there has been an overall increase in the CD risk for the entire child population. The median age at diagnosis has increased and there are almost no CD cases in children below two years of age [112]. The epidemic cohorts of 1984-1994 maintained the highest cumulative incidence at comparable ages over a period until 2009 compared to the post-epidemic cohorts of 1995-1999, but the difference between the two cohorts was less obvious as the children grew older. However, for unknown reasons the cumulative incidence in the birth cohorts of 2000-2002 exceeded that of the epidemic cohorts, despite a favourable infant feeding, which suggests additional environmental factors affecting the CD development [115].

Challenges in diagnosing and finding CD cases

Histology can be questioned as the predominant CD diagnostic tool, since the biopsy can be falsely negative .The intestinal mucosa lesions may be patchy and missed if only one biopsy is taken. In our study we had the opportunity to compare different biopsy methods due to different local routines in the five study sites. By sharpening the diagnostic process and performing endoscopic re-biopsies in children who had a normal or inconclusive primary biopsy, additional CD cases were found. With the ability to take multiple biopsies from various locations including the duodenal bulb, endoscopy is superior to the suction capsule method. By using endoscopy, it was also revealed that 9% of the children with conclusive fractions from both proximal and distal duodenum showed a patchy distribution of the mucosal lesions. However, a disadvantage with endoscopy is that the mucosal specimens are smaller and can also be of poorer quality, compared to specimens obtained by capsule, which makes the histopathological preparation and evaluation more difficult.

Staining for CD3+ is important to perform in milder forms of enteropathy, but is unnecessary in cases with subtotal/total villous atrophy. The ESPGHAN criteria for CD diagnosis require a more severe degree of enteropathy (Marsh 2 or Marsh 3 lesions) than we did in our study, where children with increased levels of IELs in combination with symptoms and/or signs suggestive of CD and HLA positivity were considered as new CD cases. The process of having three pathologists evaluating the mucosal specimens is not applicable in a normal clinical situation. However, it is important to remember that the pathologist is a key player in the diagnostic work up. In our study the agreement between the local and expert pathologist was fairly high, nevertheless the re-evaluation of the mucosal specimens generated additional CD

cases. It is therefore important to consider re-biopsy, staining and re-evaluation in unclear cases.

In our study we used the commercial kit, Celikey, Phadia to determine the level of tTG-IgA. The cut-off for positivity, recommended by the manufacturer was >5 U/mL, based on their validation study [116]. To increase the sensitivity of the test, we decided to lower the cut-off to > 4 U/mL. However, the decision of correct cut-off for positivity is complicated. Several studies report that a lower cut-off of >3 U/mL would be justified [117-119]. Nevertheless, we considered levels between 2-4 U/mL to be intermediate and if accompanied by positive EMA, CD was suspected .CD. Retrospectively, the level for tTG-IgA positivity could have been lowered, since almost all children with tTG-IgA > 30 U/mL had severe mucosal damage. In the 2012 ESPGHAN guidelines, biopsy confirmation is still mandatory for CD diagnosis in screened children [33]. The first suggested step for CD screening in risk groups, e.g. patients with Type 1 Diabetes or Down syndrome is to perform genotyping for HLA DQ2/DQ8.This procedure aims at avoiding repetitive blood tests in children who have a genetic susceptibility.

Screening with HLA-genotyping to exclude patients without risk for CD development would be unsuitable in screening of a general population in Sweden, since around 50% carry the HLA DQ2/DQ8 genotype [120].

The 2012 ESPGHAN guidelines concerning the correlation between mucosal damage and level of serological markers are based on a systematic review and meta-analysis of studies [73]. High levels of tTG-IgA predict villous atrophy but EMA should be used as a confirmatory test. Noteworthy, is that the technique used to evaluate EMA has some difficulties with inter-observer variability. This has to be taken into consideration. The main criteria for obtaining CD diagnosis without biopsy confirmation are symptoms suggestive of CD. However, a sub-study of the ETICS study revealed that such symptoms CD were as common in children with screening-detected CD as in children without CD [121]. Thus, symptoms are a poor diagnostic tool. It is also evident that screening detected celiac children have symptoms they are not fully aware of until starting the treatment with GFD [19]. The main distinction in the approach to the diagnostic process is the presence of symptoms suggestive of CD, however if the ESPGHAN guidelines for symptomatic cases were applied on our screened population all cases with tTG-IgA levels exceeding ten times upper limit of normal (> 10xULN) obtained CD diagnosis and the biopsy could have been omitted in a quarter of all children.

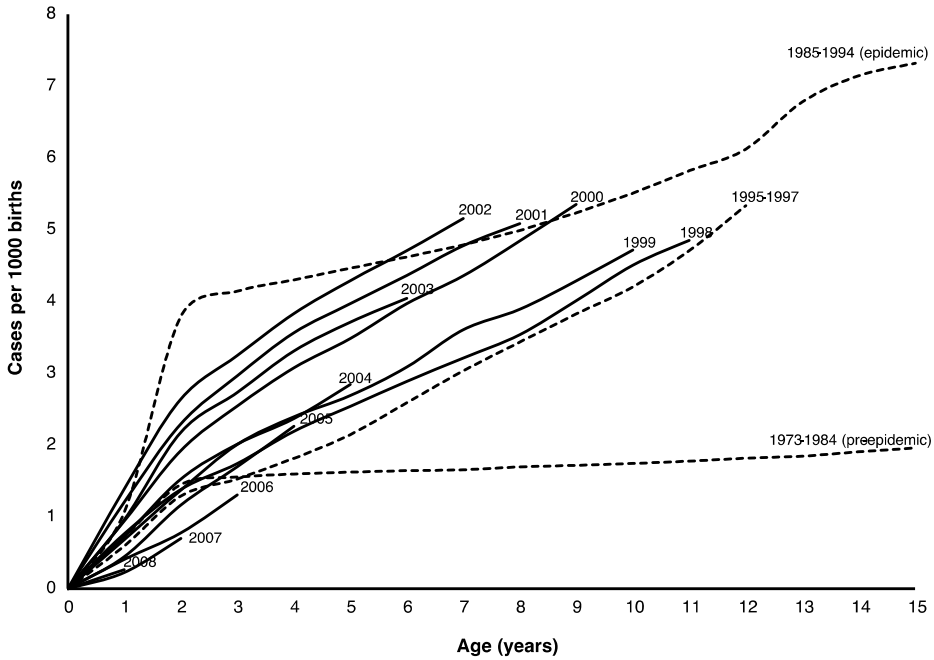


Figure 9. Cumulative incidence of CD for children born between 1973 and 2009 and followed from birth until 2009 or to an age of 14-9 years.
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Adherence to the gluten-free diet

The GFD is an effective treatment which leads to healing of the small intestinal mucosa, symptom reduction and prevention of negative health consequences [49]. Previous studies are reporting that being screening detected and currently being a teenager can be negative factors to adherence [97]. Our results showing high adherence in screening detected children were thus unexpected and also very positive. The age at diagnosis or to currently being a teenager did not seem to affect the adherence, since the children were diagnosed at the age of 12 and followed up as adolescents. Swedish CD patients are entitled to subsidised gluten-free products until 16 years of age, which could have affected the adherence since manufactured gluten-free products usually are more expensive. Swedish CD patients are also offered consultation with a dietician for dietary advice as part of standard care.

The dietician has an important role in helping to achieve adherence by providing support and advice on alternative gluten-free products. In some studies the dietician is even the most important factor for adherence [122]. However, information about the GFD has become more accessible through the Internet and patient support groups.

Changing to a GFD can be a challenge in everyday life and often entails social sacrifices. Bread, pasta and pizza are common food products that contain gluten. In ordinary flour, gluten gives the flour its elasticity and viscous properties, which make the bread fluffy, while the gluten-free bread often has a crumbling texture. To be an adolescent can be a challenge in itself and to obtain a CD diagnosis at this time of life can be difficult, not only because of the need to change previous eating habits, but also due to extra practical difficulties such as fewer food options when eating outside home. There has also been a change in social behaviour spending time eating outside the home e.g cafés is common and due to the latest trend of eating GFD without having CD, the availability of GFD products may be more varied. Usually, CD is a chronic disease with few or no physical signs, but in some situations the condition becomes very visible e.g. when GFD food is not available unless requested or the only food available contains gluten. Assessing the adherence by using both tTG-IgA measurements and self-reported questionnaires seems like a sufficient combination. The majority of the children, 75%, reported to be always adherent and also had normalized tTG-IgA levels after one year of GFD. There are limitations in using this model, since the correlation between the level of serological markers and the mucosal damage is not always coherent. Moreover if the patient does not want to reveal voluntary gluten intake, the self-reported adherence might not be accurate. Even though the observation time was only one year, primary results from our five-year follow-up show a high long-term adherence. There are several studies suggesting that clinically detected children have better adherence due to symptom reduction than screening detected children [123, 124]. Another reason for the high adherence may be symptom reduction in children who were unaware of symptoms prior to the screening or concerns about secondary complications.

Conclusion and future remarks

The prevalence of screening detected CD in children was high in both birth cohorts, thus showing that many children are still undiagnosed. Our data support that gluten introduction has an impact in the risk of developing CD, but there are many unknown factors, which needs further exploration. We know that 1-2% of all children in the birth cohorts born during the epidemic period and also a number of children in the cohorts thereafter, have unrecognised and subsequently undiagnosed CD. Many of these children may benefit in improved health and quality of life if they were diagnosed. However it is still controversial to screen for CD in a general population though we do not know if these children will benefit from GFD and there are many uncertainties as to what tools to use in a screening situation.

A first step to find CD cases would be to increase the awareness among professionals working in the healthcare system and be generous with tTG-IgA testing. The clinical presentation of CD can vary and thus CD patients can seek medical care at the dentist, dermatologist, and gynaecologist or at the general practitioner due to a wide range of symptoms.

The next challenge finding undiagnosed CD cases is the diagnostic process, where all patients should be biopsied by using endoscopy and if that is not possible at the local clinic, all unclear cases should be referred to a clinic with better resources, after an initial negative capsule biopsy. Good collaboration with pathologists will also increase the possibility of finding all CD cases by enhancing the diagnostics with immunostaining and performing re-biopsy in unclear cases.

The development of the serological markers may in the foreseeable future make it possible to avoid biopsy confirmation in all groups. It would therefore be beneficial to further explore whether the ESPGHAN guidelines can be applied on screening detected cases to avoid unnecessary biopsies and save resources for unclear cases. Endoscopy requires general anaesthesia which can be a risk factor and should perhaps be avoided in certain group of patients e.g. patients with Mb Down. Furthermore many children in the at-risk groups are screened annually for CD and it should be emphasized that as a first step of screening genotyping for HLA DQ2/DQ8 could be performed. This would rule out around 50% of all children with Mb Down and may be a first step towards screening in the general population. On the other hand in children with type 1 diabetes it would not be beneficial since 90% also have the risk genotype for CD.

Even though the adherence amongst our study population was high it has to be considered that this was a homogenous group of twelve-year-old children who were still living at home and were just on the brink of establishing independence from their parents. How will the adherence be affected at the age of 16 years, when these adolescents are no longer entitled to subsidised products, and at 18 when they move away from home and are socialising in bars drinking beer? Future studies are needed to evaluate what factors are of importance to adherence and thereby making everyday life easier for children and adolescents with CD.

Svensk sammanfattning

Bakgrund och syfte med avhandlingen

Glutenintolerans, celiaki är en av de vanligaste kroniska sjukdomarna bland barn och innebär att man inte tål gluten. Gluten är ett protein som finns i vete, råg och korn. Tunntarmen har en veckad slemhinna med fingerlika utskott, tarmludd och hela tarmen har en yta på 250 kvadratmeter, vilket är lika stort som en tennisplan. Denna stora yta är viktig för att tunntarmen ska kunna ta upp näring. Hos de personer som har celiaki skadar gluten tarmluddet. Det första som händer är att antalet försvarsceller, så kallade T-lymfocyter, ökar i tarmslemhinnan, därefter minskar tarmluddets i höjd och tarmen blir sedan helt slät. Det leder i sin tur till att tunntarmens yta som suger upp näring minskar och kan medföra olika symtom och bristsymptom hos de individer som drabbas av sjukdomen. För att man ska kunna få celiaki måste man ha vissa ärftliga förutsättningar, vilket innebär att man har generna (HLA-DQ2/DQ8). Dessa gener förekommer vanligen hos 25 % av normalbefolkningen, men i Sverige finns det rapporter om att det är så många som 53 % av befolkningen som har detta arvsanlag. För att sjukdomen ska utvecklas är det också nödvändigt att man äter livsmedel som innehåller gluten t.ex. bröd, pasta och pizza. Vilka andra faktorer som är av betydelse för att sjukdomen ska utvecklas hos den enskilde individen är fortfarande till stor del okända. Celiaki finns hos 1-2 % av den svenska befolkningen och är dubbelt så vanlig hos kvinnor som hos män. Symtomen på celiaki varierar, men hos små barn är det ofta besvär ifrån tarmen, såsom diarré eller förstoppning, dåligt näringsupptag och dålig tillväxt som dominerar. Många med celiaki har inga tydliga symtom och en del har inga symtom, i alla fall är de inte medvetna om dem. Detta innebär att om man inte letar aktivt efter dessa patienter så förblir de oupptäckta. Mörkertalet är stort och många är fortfarande odiagnostiserade.

Det har rapporterats att personer med celiaki i medeltal har väntat i 10 år från första symtom till diagnos. I mitten av 1980-talet började svenska barnläkare hitta oväntat många fall av celiaki bland småbarn. Den svenska epidemin, varade under åren 1984-1996 och ökningen av kliniskt diagnosticerade barn var nästan fyrfaldig hos barn under 2 år, men sjönk sedan åter till samma nivåer som tidigare. Det framkom att det fanns en rad olika faktorer som eventuellt kunde förklara epidemin. I början av1980-talet hade det kommit nya rön om att det var lämpligt att minska mängden protein i spädbarnsmaten, som välling, gröt och burkmat. Det ledde till att industrin minskade mängden mjölk i produkterna, men för att få en smaklig produkt tillsattes mjöl, vilket

i vissa produkter ledde till att halten mjöl mer än dubblerades. Ungefär samtidigt kom en ny rekommendation till föräldrar att de skulle vänta med att introducera gluten i spädbarnskosten tills sex månader, i stället för fyra månaders ålder. Syftet var att försöka skjuta upp insjuknandet i celiaki hos de riktigt små barnen. Detta råd innebar dock att fler barn började äta högre halter mjöl i samband med att de slutade ammas, eller efter 6 månaders ålder, vilket senare har visat sig vara en riskfaktor. Rekommendationerna ändrades och man gav rådet till föräldrar att föra in gluten mera gradvis, gärna parallellt med amning, samtidigt minskade industrin halten vetemjöl i sina produkter, vilket resulterade i att epidemin fick ett slut. Epidemiologiska studier har visat att mängden mjöl i spädbarnkosten hade stor betydelse för risken att utveckla celiaki och att introducera gluten under pågående amning har skyddande effekt.

Celiaki beskrevs på 1880 talet av en engelsk läkare vid namn Samuel Gee. Han noterade att det fanns ett samband mellan sjukdomen och kosten då barnen blev bättre på en kost som enbart bestod av musslor, för att sedan bli sämre när musselsäsongen var slut.

Det var först på 1950-talet, som en holländsk barnläkare vid namn Willem-Karel Dicke gjorde kopplingen mellan celiaki och intag av vetemjöl. Han noterade att barnen mådde bättre när det var brist på vetemjöl under andra världskriget, för att sedan åter bli sjuka när tillgången på vetemjöl förbättrades. Han var också med i den forskargrupp som upptäckte att barn med celiaki saknade tarmludd, vilket blev ett viktigt diagnostiskt tecken.

Under 1970-talet, då tunntarmskapslarna infördes som klinisk rutin, fastställdes diagnostiska kriterier för celiaki. Dessa innebar att diagnosen ställdes genom att man utförde tre på varandra följande tunntarmsbiopsier, en initial biopsi vid utredning av symtom, en efter en period med behandling med glutenfri kost och en efter en period då barnet hade ätit normalkost. När de serologiska markörerna för celiaki (blodprover) introducerades i kliniken reviderades diagnoskriterierna 1991 till att gälla en enda biopsi för att fastställa tunntarmsskada. ESPGHAN, den europeiska barngastroenterologiska föreningen (*European Society for Paediatric Gastroenterology, Hepatology and Nutririon*) kom 2012 med reviderade riktlinjer, där man föreslog att om barnet har symtom som gör att man får en stark misstanke om celiaki och har 10-faldigt förhöjda serologiska markörer i blodet, så kan man avstå ifrån att göra en tunntarmsbiopsi. Tunntarmsbiopsi innebär att man undersöker tarmen, antingen med ett kapselinstrument eller ett endoskopiskt instrument. Dessa kriterier gäller dock bara barn med symtom. Tunntarmsbiopsi är fortfarande obligatoriskt för barn som hittas vid screening. Även metoden för att göra tunntarmsbiopsier har utvecklats. Tidigare var standardmetoden att använda kapsel, men den metoden innebär att man bara kan bedöma en enda liten bit av tarmen. Det har visat sig att barn kan ha fläckiga förändringar i tarmen och om man bara tar en enda biopsi så kan man missa

fall. Fördelen med den endoskopiska metoden är att man kan ta flera tunntarmsbitar ifrån olika synliga områden av tarmen.

Behandlingen av celiaki går ut på att helt utesluta gluten ur maten. I Sverige får man upptill 16-års ålder ekonomisk hjälp i form av subventionerade glutenfria livsmedel och man har ofta även tillgång till dietistkontakt. Följsamheten är beroende av en rad faktorer, såsom ålder vid diagnos och om man har blivit upptäckt via screening. Andra faktorer som påverkar är bristande tillgänglighet på glutenfria alternativ, svårigheter att acceptera smak och konsistens på den glutenfria maten. Gluten är det som ger brödet en fluffig konsistens, vilket gör att glutenfritt bröd ofta är smuligare och hårdare i konsistensen. Ökad kunskap om celiaki hos omgivningen och socialt stöd är faktorer som stärker följsamheten, vilket är speciellt viktig för ungdomar med celiaki som ofta upplever många praktiska och sociala problem relaterade till den glutenfria kosten. Café-livet är utbrett, men sortimentet av glutenfria alternativ är relativt begränsat, vilket eventuellt kan förbättras av trenden att äta glutenfritt, även om man inte har celiaki.

Syftet med denna avhandling var att ville vi undersöka förekomsten av odiagnostiserad celiaki hos barn som var födda under den så kallade celiaki-epidemin, för att ta reda på om det var fler barn med oupptäckt celiaki som hade upptäckts eller om det faktiskt var fler barn som hade insjuknat med celiaki. Vi ville också jämföra två olika biopsimetoder; kapsel och endoskopi, för att se om valet av biopsimetod påverkar diagnostiken. Vi ville också studera om korrelationen mellan nivån på de serologiska markörerna och graden av tarmskada var överensstämmande och därmed kunna utvärdera om ESPGHANs nya riktlinjer, som innebär att man kan avstå biopsi, även skulle kunna omfatta screenade barn.

Slutligen ville vi också undersöka följsamheten till den glutenfria kosten och se om tonåringar som får diagnosen celiaki genom screening, följer den glutenfria kosten ett år efter de har fått diagnos.

Deltagare och metod

Ett av syftena med screening studien ETICS (*Exploring the Iceberg of Celiacs in Sweden*) var att se hur stort mörkertalet var av oupptäckt glutenintolerans bland friska 12 åriga barn, födda både före och efter epidemin. Genom att jämföra två grupper av barn med olika spädbarnkost var det också möjligt att utforska om glutenintolerans kan förebyggas genom att introducera gluten gradvis under pågående amning, eller om det bara medför att insjuknandet senareläggs

Denna avhandling är baserad på ETICS studien som var indelad i två etapper (2005-2006, 2009-2010) och utfördes på fem studieorter; Lund, Växjö, Norrköping, Norrtälje och Umeå. Alla barn i årskurs 6 inbjöds till testning för glutenintolerans genom ett blodprov. Den första etappen omfattade barn födda under toppen av

epidemin, 1993, och 10 041 barn inbjudna, varav 7 567 (75 %) barn deltog. Under den andra etappen omfattande, barn födda efter epidemin, 1997, blev 8 284 barm inbjudna varav 5712 (69 %) deltog. Sammantaget, utgjorde de 18,000 inbjudna barnen 10 % av alla barn födda under åren 1993 och 1997. Studien var skolbaserad och forskningssjuksköterskor åkte ut till skolorna för att ta blodproverna i samarbete med skolsköterskorna. De barn som hade förhöjda värden av de serologiska markörerna i blodprovet, kontaktades och fick komma till respektive barnklinik för fortsatt utredning med tunntarmsbiopsi. De lokala rutinerna varierade på studieorterna, vilket gjorde att en del barn fick göra en tunntarmsbiopsi där man använde ett kapselinstrument och en del av barnen fick göra en endoskopisk tunntarmsbiopsi. De barn som hade en tunntarmsbiopsi som blivit bedömd som normal eller som inte var bedömbar, fick genomgå ytterligare en tunntarmsbiopsi, där man enbart använde endoskopi.

I den andra fasen av ETICS studien utfördes alla tunntarmsbiopsier endoskopiskt. Tunntarmsbitarna bedömdes i första hand av patologläkaren på respektive sjukhus och sedan av en expertpatolog, som var ovetandes om resultaten. Om bedömningen mellan den lokala och expertpatologen inte överensstämde, gjordes ytterligare en bedömning av en andra expertpatolog. Följsamheten till den glutenfria kosten bedömdes genom en kombination av frågeformulär där svarsalternativen var följande; alltid, ofta, ibland, sällan eller aldrig och nivån på de serologiska markörerna efter 1 år med glutenfri kost.

Resultat

I screening av barn födda 1993 under epidemin upptäckte vi att 2/3 av alla barnen med celiaki var tidigare oupptäckta. Den totala förekomsten av glutenintolerans i denna åldersgrupp var 3 %, vilket är den högsta förekomsten som hittills påvisats i Europa och USA. Celiaki var vanligare bland flickor än pojkar.

Genom att skärpa den diagnostiska processen hittade vi fler fall med celiaki, dels genom att biopsera om alla barn som hade en första normalbiopsi, vilket resulterade i att ytterligare tre barn upptäcktes och dels genom att låta en expert patolog bedöma alla tunntarmsbitarna, vilket resulterade i att fem barn fick diagnosen celiaki.

Av de barn som hade tarmbitar som var bedömbara ifrån olika delar av tarmen var det 9 % som hade fläckig utbredning av tarmskadan.

När vi jämförde korrelationen mellan nivån på de serologiska markörerna med graden av tarmskada, fann vi att alla barn som hade 10 ggr över de normala nivåerna också hade en högre grad av tarmskada. Om ESPGHAN:s riktlinjer även gällde för screenade barn, skulle de innebära att 25 % av de barnen som undersöktes inte skulle ha behövt genomgå en tunntarmsbiopsi för att erhålla diagnos. Det har tidigare rapporterats att följsamheten till den glutenfria kosten kan påverkas negativt om man

har blivit upptäckt via screening och även att bli diagnosticerad som tonåring. Det visade sig dock att följsamheten till den glutenfria kosten var hög (75 %) bland de screening-upptäckta ungdomarna. Majoriteten (82 %) svarade i enkäten att de alltid åt glutenfri kost, vilket överensstämde med att de serologiska värdena hade normaliserats. Det fanns dock en liten grupp som svarade att de alltid följde den glutenfria kosten, men som inte normaliserade värde efter ett år med glutenfri kost. Det var dock så att majoriteten (85 %) av dessa barn hade höga värden initialt och deras värde halverades efter 1 år. Bland barn som rapporterade att de oftast följde den glutenfria kosten hade de flesta normaliserade värde, medan de barn som rapporterade att de bara följde den glutenfria kosten ibland, hade förhöjda värden.

Slutsatser

Celiaki är vanligare bland barn än vad som tidigare var känt och majoriteten av fallen är odiagnostiserade. Varje steg i den diagnostiska processen är av betydelse för hur många fall med glutenintolerans man hittar. Genom att skärpa den diagnostiska processen, med att utföra biopsier i oklara fall och att låta en expertpatolog bedöma tunntarmsbitarna, hittar man fler fall med celiaki.

ESPGHAN:s rekommendationer, där man inte behöver göra en tunntarmsbiopsi på barn med höga värden av de serologiska markörerna, verkar överensstämma även på screening- upptäckta barn. Detta fynd gör att man behöver ifrågasätta huruvida kriterierna för ställa celiaki diagnos bör ändras för de screenade barnen.

Följsamheten till den glutenfria kosten var hög även hos ungdomar som var screeningupptäckta, vilket kan ha påverkats av många faktorer, såsom subventionerade glutenfria livsmedel och god tillgång till dietist. Uppföljningen var dock efter endast ett år, och det krävs fler studier för att värdera om följsamheten kvarstår efter en längre tids behandling med glutenfri kost.

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