Tampere University

VALMA FUCHS

Earlier and Less Invasive Diagnosis of Celiac Disease

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ACADEMIC DISSERTATION
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ABSTRACT

Celiac disease is a common immune-mediated disease with a variable clinical picture. Treatment with a gluten-free diet (GFD) is simple and efficient. Due to heterogenous phenotypes, the diagnosis is often made after years of persistent symptoms. Undiagnosed celiac disease predisposes patients to impaired quality of life and risk of complications. The heterogenous clinical picture has been suggested to be a reason for the diagnostic delay, but the evidence of the causes and consequences of the delay is insufficient.

Mucosal damage established in small-intestinal biopsies has long been the gold standard of the diagnosis of celiac disease. However, similar histological lesions can also be seen in many other conditions. Moreover, pathologists vary in their interpretations, and the handling and cutting of the biopsies markedly affects the final reading. High serum levels of transglutaminase 2 antibodies (TG2-ab) have been shown to be highly specific for celiac disease. In 2012, the first European pediatric criteria allowed omitting biopsy in the diagnostics if TG2-ab exceeds the upper limit of normal (ULN) at least 10-fold, endomysium antibodies (EMA) are positive, the disease-associate genotype is confirmed, and symptoms are present. The criteria have been shown to be accurate in clinical pediatric research and have recently been suggested for adult use only in Finland.

The aim of this dissertation was to elucidate factors that predispose or result from diagnostic delay in celiac disease. Another aim was to ascertain whether the pediatric serology-based criteria are accurate in diagnosing adults across a range of pretest probabilities of the disease.

The dissertation consists of three sub-studies. In Study **I,** factors associated with a long, > 10 years', diagnostic delay of celiac disease were retrospectively investigated in 825 previously diagnosed adults. In Study **II,** 611 celiac disease patients diagnosed in 2007-2008 were surveyed at diagnosis and after one year on a GFD, and possible factors associated with a delay of ≥ 3 years were explored. Study **III** evaluated whether celiac disease can be accurately diagnosed in adults without biopsies with TG2-ab ≥ 10x ULN, positive EMA, and correct genotype. These "triple criteria" were tested in three cohorts with different pretest probability: 421 high-risk

individuals with clinical suspicion, 2,358 moderate-risk family members of coeliac disease patients, and 2,722 low-risk subjects from general population.

It was observed in Study **I** that delayed celiac disease diagnosis of > 10 years declined over time and particularly after 1997, when the first Finnish Current Care Guidelines for celiac disease were issued. The proportion of diagnoses made in primary health care increased over time, but no association between the site of diagnosis and risk of delay was found. A long diagnostic delay was associated with classical celiac disease symptoms such as diarrhea and malabsorption, and with concomitant neurological or musculoskeletal disease, whereas the risk of the delay was reduced in screen-detected patients.

In Study **II**, a diagnostic delay of ≥ 3 years was associated with poorer quality of life and increased use of primary health care services and use of medications both before and one year after diagnosis. The risk due to delay was not associated with most of the socio-economic factors explored but was reduced in students and homemakers compared to employed patients.

In Study **III**, the positive predictive value of the "triple criteria" for biopsy-proven celiac disease was 100%. The accuracy was not affected by pretest probability for the disease or by the presence of symptoms. Genotyping did not improve the accuracy of the criteria. Of the 274 newly diagnosed celiac disease patients in Study **III**, the "triple criteria" were fulfilled in 33%, who thus could have been spared the biopsy.

The findings of this dissertation show that although a long diagnostic delay in celiac disease of over ten years has become rarer, it still occurs in one-fifth of patients. The presence of typical symptoms of celiac disease does not increase the probability of a prompt diagnosis. As a delay of three years is already associated with impaired quality of life and increased use of healthcare services, the delay should still be shortened. The shift in diagnostics towards primary health care has proven useful, which motivates to further educate general practitioners. This dissertation demonstrates that celiac disease can be accurately diagnosed based on high level of TGA-ab and positive EMA without biopsies, which may shorten the diagnostic delay and save the resources of the health care system.

TIIVISTELMÄ

Keliakia on yleinen ravinnon gluteenin ylläpitämä immuunivälitteinen sairas, joka ilmenee hyvin vaihtelevin oirein. Hoito gluteenittomalla ruokavaliolla on tehokas ja suhteellisen yksinkertainen. Moninaisesta taudinkuvasta johtuen diagnoosi kuitenkin tehdään usein vasta vuosia kestäneen oireilun jälkeen. Diagnosoimaton sairaus heikentää potilaiden elämänlaatua ja altistaa pitkäaikaiskomplikaatioille. Monimuotoista taudinkuvaa on esitetty syyksi diagnoosiviiveelle, mutta tieteellinen näyttö viiveen taustoista ja seurauksista on puutteellista.

Keliakiadiagnoosin kulmakivi on ollut pitkään ohutsuolen koepalassa näkyvä suolinukan vaurioituminen. Keliakialle tyypillistä histologista limakalvovauriota voi ilmetä kuitenkin monissa muissakin tiloissa. Lisäksi patologien tulkinnat koepalan vauriosta eroavat, ja näytteiden käsittely sekä leikkaussuunta vaikuttavat merkittävästi tulkintaan. Korkeiden veren transglutaminaasivasta-aineiden (TG2-ab) pitoisuuksien on osoitettu olevan spesifisiä keliakialle. Vuonna 2012 eurooppalaiset lastenlääkärit julkaisivat ensimmäistä kertaa diagnoosikriteerit, joiden mukaan ohutsuolinäytettä ei tarvita lapsilta keliakian diagnosoimiseksi, jos oireisella lapsella TG2-ab lukema ylittää normaalin ylärajan vähintään 10-kertaisesti, endomysiumvasta-aineet (EMA) ovat positiiviset ja todetaan keliakialle altistava genotyyppi. Vasta-aineisiin perustuvat kriteerit ovat osoittautuneet hyvin tarkoiksi lasten kliinisissä tutkimuksissa, mutta aikuisille vastaavat kriteerit on esitetty vasta hiljattain ja vain Suomessa.

Tässä väitöskirjatutkimuksessa oli tavoitteena etsiä keliakian diagnoosiviiveelle altistavia tekijöitä ja viiveen seurauksia. Lisäksi tavoitteena oli selvittää, soveltuvatko serologiaan perustuvat lasten keliakian diagnoosikriteerit myös aikuiskäyttöön riippuen siitä, mikä henkilön ennakkotodennäköisyys keliakialle on.

Tutkimus koostuu kolmesta erillisestä osatyöstä. Osatyössä **I** tutkittiin retrospektiivisesti tekijöitä, jotka voisivat olla yhteydessä pitkään, yli 10 vuotta kestäneeseen keliakian diagnoosiviiveeseen 825 aikuiskeliaakikolla. Osatyössä **II** selvitettiin keliakialiittoon liittyneeltä 611 potilaalta vähintään 3 vuotta kestävään diagnoosiviiveeseen mahdollisesti liittyviä tekijöitä sekä diagnoosihetkellä että vuoden kuluttua gluteenittoman ruokavalion aloittamisesta. Osatyössä **III** tutkittiin, voidaanko keliakia todeta aikuisilla luotettavasti ilman tähystyksessä otettavaa koepalaa, jos ”triplakriteerit” täyttyvät eli TG2-ab ylittää viiterajan vähintään

kymmenkertaisesti ja EMA sekä geenitesti ovat positiiviset. Diagnoosikriteereitä tutkittiin kolmessa keliakian ennakkotodennäköisyyden suhteen erilaisessa ryhmässä: 412 oireisella korkean riskin henkilöllä, 2357 keliakiaa sairastavan potilaan sukulaisella eli keskisuuren riskin henkilöllä sekä 2722 väestöseulotulla matalan riskin henkilöllä.

Osatyössä **I** todettiin, että pitkä, > 10 vuoden diagnoosiviive lyheni ajan mittaan ja erityisesti vuonna 1997 julkaistun ensimmäisen suomalaisen keliakian Käypä hoito-suosituksen jälkeen. Perusterveydenhuollossa tehtävien diagnoosien osuus lisääntyi suositusten julkaisemisen jälkeen, mutta viive ei ollut yhteydessä siihen, millä terveydenhuollon tasolla diagnoosi oli tehty. Pitkä diagnoosiviive oli yhteydessä klassisiin keliakiaoireisiin kuten ripuliin ja imeytymishäiriöihin, sekä yhtäaikaiseen neurologiseen tai tuki- ja liikuntaelimistön sairauteen, kun taas viiveen riski oli vähentynyt keliakiaseulonnalla löydetyillä potilailla.

Osatyössä **II** keliakian diagnoosiviive oli yhteydessä heikentyneeseen elämänlaatuun ja lisääntyneeseen perusterveydenhuollon palveluiden ja lääkkeiden käyttöön sekä diagnoosia edeltävänä että seuranneena vuotena. Riski diagnoosiviiveeseen ei ollut yhteydessä useimpiin tutkittuihin sosioekonomisiin tekijöihin, mutta riski oli pienentynyt opiskelijoilla ja kotiäideillä verrattuna työssäkäyviin.

Osatyössä **III** serologiaan perustuvien ”triplakriteereiden” täyttymisellä oli 100 % positiivinen ennustearvo sille, että myös ohutsuolen koepalassa todettiin villusatrofia. Kriteerit toimivat samalla tavalla riippumatta keliakian ennakkotodennäköisyydestä tai siitä, oliko potilaalla keliakiaan sopivia oireita. Myöskään geenitesti ei lisännyt kriteereiden diagnostista tarkkuutta. Tutkimuksessa todetusta 274 uudesta keliakiapotilaasta ”triplakriteerit” täyttyivät 33 prosentilla, joilla tähystys olisi siis voitu jättää tekemättä.

Tämän väitöskirjatutkimuksen tulokset viittaavat siihen, että kelaikian diagnoosi viivästyy yli 10 vuotta aiempaa harvemmalla, mutta edelleen viidesosalla potilaista. Keliakialle tyypillisten oireiden esiintyminen ei nopeuta diagnoosin tekemistä. Koska jo vähintään kolmen vuoden diagnoosiviive on yhteydessä heikentyneeseen elämänlaatuun ja lisääntyneeseen terveyspalveluiden käyttöön, viivettä tulisi yhä pyrkiä lyhentämään. Keliakiadiagnostiikan siirtyminen perusterveydenhuoltoon on todistetusti tehostanut diagnostiikkaa, joten yleislääketieteen edustajien kouluttamiseen kannattaa jatkossakin panostaa. Väitöskirja osoittaa, että keliakia voidaan luotettavasti diagnosoida korkeiden TGA-ab:n ja positiivisten EMA:n perusteella ilman koepalaa aikuisilla, mikä voisi lyhentää keliakian diagnoosiviivettä monilla potilailla ja säästää terveydenhuollon resursseja.

CONTENTS

ABBREVIATIONS

AGA	anti-gliadin antibodies
ARA	anti-reticulin antibodies
BMD	bone mineral density
CI	confidence interval
DGP	deamidated gluten peptide antibodies
DH	dermatitis herpetiformis
EATL	enteropathy-associated T cell lymphoma
ELISA	enzyme-linked immunosorbent assay
EMA	endomysium antibodies
ESPGAN	European Society for Paediatric Gastroenterology and Nutrition
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
FODMAP	fermentable oligosaccharides, disaccharides, monosaccharides and polyols
GFD	gluten-free diet
GI	gastrointestinal
HLA	human leukocyte antigen
HRQoL	health-related quality of life
IBD	inflammatory bowel disease
IBS	irritable bowel syndrome
IEL	intraepithelial lymphocyte
IgA	immunoglobulin A
IgG	immunoglobulin G
IF-γ	interferon gamma
IL	interleukin
NCGS	non-celiac gluten sensitivity
ND	no data
NHL	non-Hodgkin lymphoma
NRCD	non-responsive celiac disease

OR	odds ratio
PGWB	Psychological General Well-Being questionnaire
POCT	point of care test
PPI	proton pump inhibitors
PPV	positive predictive value
QoL	quality of life
RCD	refractory celiac disease
RCT	randomized controlled trial
TG2	transglutaminase 2
TG2-ab	transglutaminase 2 antibodies
Th	helper T cell
UPSTF	United States of America Preventive Service Task Force
UK	United Kingdom
ULN	upper limit of normal
USA	United States of America
Vh/CrD	villus height to crypt depth ratio
WHO	World Health Organization

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by Roman numerals **I-III**:

I Fuchs V, Kurppa K, Huhtala H, Collin P, Mäki M, Kaukinen K (2014): Factors associated with long diagnostic delay in celiac disease. Scandinavian Journal of Gastroenterology 49:1304-10.

II Fuchs V, Kurppa K, Huhtala H, Mäki M, Kekkonen L, Kaukinen K (2018): Delayed celiac disease diagnosis predisposes to reduced quality of life and incremental use of health care services and medicines: A prospective nationwide study. United European Gastroenterology Journal 6:567-575.

III Fuchs V, Kurppa K, Huhtala H, Laurila K, Mäki M, Collin P, Salmi T, Luostarinen L, Saavalainen P, Kaukinen K (2019): Serology-based nonbiopsy criteria for adult coeliac disease have excellent accuracy across the range of pretest probabilities. Alimentary Pharmacology and Therapeutics 49:277-284.

INTRODUCTION

Celiac disease is a gluten-induced immunological disorder with a prevalence of 1-2% in the Western world (Fasano et al. 2003; Lohi et al. 2007; Mustalahti et al. 2010). In genetically susceptible individuals, celiac disease is driven by dietary gluten, leading to damage in the small intestinal mucosa (Green et al. 2007). Classical symptoms include diarrhea and poor growth or weight loss, but a variety of other gastrointestinal and extraintestinal presentations are common, and no clinical picture is specific for the disease (Lindfors et al. 2019). At present, up to 90% of affected individuals remain undiagnosed (Lohi et al. 2007; Rubio-Tapia et al. 2012). Long-term untreated celiac disease might increase the risk of severe complications such as infertility, osteoporotic fractures and lymphoma (Holmes et al. 1989; Gasbarrini et al. 2000; Heikkilä et al. 2015).

Currently the only treatment for celiac disease is a life-long strict gluten-free diet (GFD), which soon after initiation leads to alleviation of symptoms and, eventually, healing of the mucosa (Murray et al. 2004; Haere et al. 2016). An early initiated diet reduces excess visits to health care and the risk of complications and improves quality of life (Green et al. 2001; Norström et al. 2011; Paarlahti et al. 2015). However, the duration of symptoms before the eventual diagnosis is often very long, with little understanding about the reasons and consequences of such a delay (Gasbarrini et al. 2001; Norström et al. 2011; Violato et al. 2019).

With such a common and life-long disease, practical, a cost-effective and accurate diagnostic policy is a necessity. So far, the diagnosis of celiac disease has been based on the identification of a small-bowel mucosal damage in biopsies collected in endoscopy. However, patchy lesions and poorly orientated or inadequate biopsy samples may cause misdiagnosis (Ravelli et al. 2010; Taavela et al. 2013). Moreover, although duodenal lesion is characteristic for celiac disease, it is not specific, and may also be caused by other diseases and medicines (Owen and Owen 2018).

In order to ensure that the right individuals proceed to endoscopy, tests for serum autoantibodies against tissue transglutaminase 2 (TG2-ab) and endomysium (EMA) are used, having become widely available in clinical use. Especially EMA and high values of TG2-ab show excellent diagnostic accuracy (Salmi et al. 2010; Alessio et al. 2015). In 2012, this led the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) to propose new diagnostic criteria allowing omission of biopsy in symptomatic children with TG2-ab $\geq$ 10x upper limit of normal, positive EMA, and correct genotype (Husby et al. 2012). The criteria have been proven to be accurate in

clinical use (Werkstetter et al. 2017; Wolf et al. 2017). No similar biopsy-omitting criteria have been issued for adults, and the possibility has invoked contradictory opinions (Vivas et al. 2008; Marks et al. 2018). Moreover, the role of symptoms in the serology-based algorithm is not clear, although the pretest probability based on clinical susceptibility has been proposed to affect the accuracy of serological testing (Fernandez-Banares et al. 2012; Tortora et al. 2014). However, if an accurate and safe non-invasive diagnosis could be established for a greater proportion of patients, the costs of many unnecessary endoscopies and individual burden could be spared. At the same time, a new policy could simplify and speed up the diagnostics of celiac disease.

REVIEW OF THE LITERATURE

1 PATHOGENESIS OF CELIAC DISEASE

Celiac disease is a chronic immune-mediated disease driven by exposure to dietary gluten in individuals with genetic predisposition. The pathogenesis is not yet fully understood, but genetic as well as environmental factors are needed. Currently, the only officially approved treatment is a lifelong strictly gluten-free diet (Lindfors et al. 2019).

1.1 Genetics

The prevalence of celiac disease in first-degree relatives varies between 2% and 38% across studies (Singh et al. 2015), and monozygotic twins have a concordance of over 80% (Greco et al. 2002). As in most autoimmune diseases, human leukocyte antigen (HLA) molecules have an important role in celiac disease pathogenesis (Sollid et al. 1989). Practically all patients carry genes encoding the HLA types DQ2 or DQ8 (Karell et al. 2003). HLA-DQ2 and -DQ8 molecules are expressed on the surface of antigen presenting cells and their role in celiac disease is to recognize and present specific gluten-derived peptides to CD4+ T-helper 1 (Th1) lymphocytes.

In detail, 90% of celiac disease patients carry the HLA DQ2.5 heterodimer composed of α and β chains encoded by the alleles DQA1*05 and DQB1*02 (Sollid et al. 1989; Djilali-Saiah et al. 1994). Around 8% of patients have been reported to carry DQA1*03-DQB1*0302 alleles encoding the heterodimer serologically denoted as HLA-DQ8 (Karell et al. 2003). Over 90% of the remaining patients have been found to carry only half of the risk heterodimer, either DQA1*05 or DQB1*02 alone (Margaritte-Jeannin et al. 2004). Of patients lacking HLA-DQ2.5 and -DQ8, the majority carry the haplotype DQ2.2 (HLA-DQA1*02:01 and -DQB1*02:02) (Sollid et al. 1989). Patients without HLA DQ2.5, DQ2.2 or DQ8 are extremely rare and their diagnosis of celiac disease mostly erroneous (Anderson et al. 2013).

Even though lack of the genes encoding HLA-DQ2 and -DQ8 molecules has an exceptionally high negative predictive value (Kaukinen et al. 2002; Karell et al. 2003), the positive predictive value (PPV) is low since most individuals carrying these molecules will never develop celiac disease. The prevalence of HLA-DQ2 or DQ8 differs to some extent across populations, being approximately 40% in Caucasians (Mäki et al. 2003). Eventually, only 1.5-4% of HLA-DQ2 or DQ8-positive individuals develop celiac disease in childhood (Mäki et al. 2003; Björck et al. 2016), the risk being greatest in those

homozygous for DQ2 and smallest in those with one copy of DQ8 (Liu et al. 2014; Liu et al. 2017).

Even though the specific HLA genotypes are necessary for the development of celiac disease, they only explain approximately 40% of the genetic predisposition (Trynka et al. 2011). Other genetic factors have been identified in genome-wide association studies recognizing 39 potential non-HLA loci with 57 candidate variants involved in the immunity (Dubois et al. 2010; Trynka et al. 2011). For example, variants affecting the expression of IL1/IL21, which are present in T cell activation, and genes playing key roles in thymic T cell selection have been associated with celiac disease (van Heel et al. 2007; Dubois et al. 2010). Most of the non-HLA variants identified are also present in other autoimmune diseases, supporting their role in celiac disease pathogenesis (Zhernakova et al. 2009).

1.2 Immunopathogenesis

In the 1950s, a Dutch pediatrician Willem Dicke recognized a component of wheat as the environmental driver of celiac disease, and that removal of wheat from the diet led to prompt clinical recovery (Dicke et al. 1953). Later, the component was identified and called gluten, consisting of storage peptides glutenins and prolamins. These storage peptides are also found in rye and barley, but not in oats, and are toxic and immunogenic for celiac disease patients. Prolamins in wheat, barley, and rye are known respectively as gliadins, hordeins, and secalins. These peptides are resistant to proteolysis of digestive enzymes (Shan et al. 2002) and are capable of activating innate and adaptive immune responses in the intestine (Maiuri et al. 2000; Gianfrani et al. 2005).

When gluten peptides enter the small intestine of patients with celiac disease, they can provoke immune reactions in a variety of ways. In healthy individuals, gluten binds to secretory immunoglobulin A (IgA) on the intestinal membrane. To protect enterocytes from toxins and pathogens, immune cells destroy IgA-marked peptides. In celiac disease, several possible transport mechanisms have been suggested to drive gluten peptides into the lamina propria. One explanation is that increased amounts of IgA-antigliadin bind to a transferrin receptor, which is overexpressed in celiac disease patients, enabling transcytosis through enterocytes to the lamina propria (Matysiak-Budnik et al. 2008; Lebreton et al. 2012). Another theory is that binding of gliadin to the CXCR3 receptor expressed in $CD4^+$ Th1 cells increases the release of a protein called zonulin leading to impaired mucosal integrity, which could create a paracellular pathway for gluten (Fasano 2000).

When gliadin enters the lamina propria, a specific enzyme called transglutaminase 2 (TG2) deamidates the gliadin peptide charging it negatively, which increases its affinity

to the DQ2/DQ8 antigen-binding groove at the surface of antigen presenting cells, activating the adaptive immune system (Esposito et al. 2003; Kim et al. 2004). Next, HLA DQ2 and DQ8 present gluten particles for CD4+ Th1. When Th1 cells recognize the gluten antigen, they release interferon-gamma (IF-γ) and tumor necrosis factor alfa (TNF-α), which initiate inflammation. Th1 cells also stimulate B-cells to produce IgA-type antibodies against dietary gliadin (anti-gliadin antibodies, AGA) and against the host in the form of TG2 antibodies (TG2-ab). TG2-ab in particular may have several pathological functions maintaining inflammation and leading to mucosal injury (Korponay-Szabo et al. 2004; Kalliokoski et al. 2017). TG2-ab are also used as a diagnostic tool in celiac disease, either by measuring serum antibodies against TG2 by an enzyme-linked immunosorbent assay (ELISA), or by indirect immunofluorescence detecting antibodies against TG2 of the endomysium (EMA) (Chorzelski et al. 1983; Dieterich et al. 1997), further discussed in Chapter 5.2.2.

The mucosal inflammation in celiac disease typically includes increased intraepithelial lymphocyte (IEL) count, usually rising over the level of 25/100 cells (Corazza et al. 2007; Walker et al. 2010). IELs are a heterogeneous T cell population that eliminates infected cells and promotes epithelial repair to maintain epithelial integrity. In celiac disease, the function of IELs is dysregulated. T-helper cells activated in the adaptive immune response stimulate type CD8+ killer T cells to destroy enterocytes undergoing inflammation, which leads to increased permeability of the intestinal wall and the subsequent development of villous atrophy (du Pre and Sollid 2015).

Another mediator contributing in celiac disease immunopathology is interleukin (IL) 15. With the induction of gluten, IL-15 has been observed to be overexpressed both in the gut epithelium and in the lamina propria in celiac disease (Mention et al. 2003). IL15 is further associated with inhibition of growth factor β, macrophage maturation, and epithelial stress, leading to characteristic mucosal damage (Jabri and Abadie 2015). A recent transgenic mouse model elucidated the key mechanisms of IL-15 in the development of mucosal damage in genetically susceptible mice (Abadie et al. 2020).

1.3 Environmental contributors

Besides gluten, environmental cofactors contributing to the development of celiac disease have been investigated, but only few, still somewhat controversial, associations have been found. In theory, intestinal infections may increase small-bowel permeability and up-regulate the release of TG2. There is evidence that a high frequency of rota-, entero- and reovirus infections in the first years of life may increase the risk of celiac disease (Stene et al. 2006; Bouziat et al. 2017; Kemppainen et al. 2017; Kahrs et al. 2019;

Lindfors et al. 2019). The intestinal microbiota is known to be altered in celiac disease, but it is not clear if dysbiosis is a cause or consequence (Wacklin et al. 2014; Bonder et al. 2016; Bascuñán et al. 2020). The effect of antibiotics on disease risk is also disputed (Kemppainen et al. 2017; Dydensborg Sander et al. 2019).

When it comes to dietary factors, it has been suggested that a large amount of gluten in infancy could increase the risk of celiac disease among at-risk children (Andren Aronsson et al. 2019). The effect could be cumulative in infants having enterovirus infections in the first two years of life (Lindfors et al. 2019). Neither the time of introduction of gluten nor breast-feeding has been shown to modify the disease risk among susceptible infants (Lionetti et al. 2014; Vriezinga et al. 2014). Furthermore, the current evidence is against an association between celiac disease and cesarean sections (Lionetti et al. 2017; Koletzko et al. 2018).

2 EPIDEMIOLOGY

Until the 1960s, celiac disease was regarded as a rare pediatric disease hardly ever diagnosed in adulthood but is nowadays acknowledged to be common and present in all age groups. The global prevalence has been reported to be 1.4% in a recent meta-analysis (Singh et al. 2018), varying between countries (Table 1). The reasons for the regional differences are partly unclear. One explanation is the genetic HLA-type variance between ethnic groups (Kang et al. 2013). However, in Europe the differences in the prevalence occur between countries despite similarities of gluten intake and predisposing HLA haplotypes (Mustalahti et al. 2010).

In most countries, the prevalence has increased in recent decades (Lohi et al. 2007; Rubio-Tapia et al. 2009). In Finland, the prevalence of recognized celiac disease increased from 0.03% in 1978-1980 to 0.7% in 2012 (Lohi et al. 2007; Ilus et al. 2014). The heightened awareness and development of useful non-invasive diagnostic tools are likely the main reason for the increasing clinical prevalence (Collin et al. 1997; Murray et al. 2003). However, there also seems to have been a true increase of the prevalence, at least in some countries, because simultaneously with increased clinical yield, the prevalence of undiagnosed celiac disease has risen from 1.03% to 1.47% in Finland and from 0.2% to 0.8% in the USA (Lohi et al. 2007; Rubio-Tapia et al. 2009; Catassi et al. 2010). Some critics claim the prevalence to be overestimated (Biagi et al. 2010). Nevertheless, the rise in the incidence of celiac disease has been simultaneous with type 1 diabetes and other autoimmune diseases (DIAMOND Project Group 2006). A rapid increase like this has been attributed to environmental factors rather than to genetic changes (Gillespie et al. 2004; Steck et al. 2011). One suggested explanation is the worldwide increase in wheat consumption, but, for example, in Finland the intake of gluten containing cereals per capita has actually decreased in the last century (Kasarda 2013; Kortesmaa and Salo-Kauppinen 2018). Other environmental exposures are currently a target of keen research (Agardh et al. 2015).

Table 1. Prevalence of celiac disease in different countries and age groups, based on population-based screening.

	Data collected	Sample	Diagnostic criteria	Prevalence, %	Reference
Africa					
Algeria	1998	989 children	EMA	5.6	Catassi et al. 1999
Libya	ND	2,920 children	Biopsy	0.8	Alarida et al. 2011
Egypt	2001-2004	1,500 children	Biopsy	0.5	Abu-Zekry et al. 2008
Asia					
China	2010-2013	19,778 adults	TG2-ab	0.4	Yuan et al. 2017
India	2001	23,331 adults	TG2-ab	0.1-1.2[1]	Ramakrishna et al. 2016
Iran	2003-2015	36,833 all ages	Biopsy	2.0	Mohammadibakhsh et al. 2017
Japan	2014-2016	2,008 adults	Biopsy	0.05	Fukunaga et al. 2018
Russia	ND	1,740 adults	TG2-ab + biopsy	0.6	Stroikova et al. 2006
Russia	1997–2001	1,988 children	TG2-ab + biopsy	0.2	Kondrashova et al. 2008
Saudi-Arabia	2014-2016	7,930 children	Biopsy	1.5	Al-Hussaini et al. 2017
Australia & Oceania					
Australia	ND	3,011 adults	Biopsy	0.4	Hovell et al. 2001
New Zealand	1996	1,064 adults	Biopsy	1.2	Cook et al. 2000
North and South America					
Argentina	1998-2000	2,000 adults	Biopsy	0.6	Gomez et al. 2001
Brazil	2003-2004	3,000 adults	Biopsy	0.5	Oliveira et al. 2007
USA	2009-2014	22,277 adults	TG2-ab + EMA	1.0	Unalp-Arida et al. 2017
Europe					
Finland	1989-1990	6,993 adults	TG2-ab + EMA	1.1	Lohi et al. 2007
Finland	1994	3,654 children	Biopsy	1.0	Mäki et al. 2003
Finland	2000-2011	6,402 adults	TG2-ab + EMA	2.0	Lohi et al. 2007
Finland	2005	2,216 elderly	Biopsy	2.3	Vilppula et al. 2009
Germany	1999-2001	4,633 adults	TG2-ab + EMA/biopsy	0.3	Mustalahti et al. 2010
Germany	2003-2006	12,741 children	TG2-ab	0.8	Laass et al. 2015
Hungary	2005	2,690 children	Biopsy	1.4	Korponay-Szabo et al. 2007
Italy	2000–2002	4,781 adults	TG2-ab + EMA/biopsy	0.7	Mustalahti et al. 2010
Sweden	1998-2001	1,000 adults	Biopsy	1.8	Ludvigsson et al. 2013
Sweden	2005	7,567 children	AGA + mucosal inflammation	2.9	Myleus et al. 2009
UK	1990	5,470 children	EMA	1.0	Bingley et al. 2004
UK	1990-1995	7,550 adults	EMA	1.2	West et al. 2003

[1] Regional differences

AGA, anti-gliadin antibodies; EMA, endomysium antibodies; ND, no data; TG2-ab, transglutaminase 2 antibodies; UK, United Kingdom; USA, United States of America

Celiac disease is slightly more common in women than men, which is typical for most autoimmune diseases. Studies have reported male to female ratios from 1:1.1 to 1:1.8 in adults and from 1:1.4 to 1:2 in children (West et al. 2003; Bingley et al. 2004; Lohi et al. 2007; Kivelä et al. 2017). Evidence of the age distribution gives somewhat contradictory results. In a global meta-analysis, the prevalence was significantly greater in children than in adults, 0.9% vs. 0.5% respectively (Singh et al. 2018). Two large British studies have observed similar celiac disease prevalence (approximately 1%) in pediatric and adult populations (West et al. 2003; Bingley et al. 2004). In Finland, however, the prevalence would appear to increase from childhood to adulthood (Table 1). These comparisons are, however, complicated by the variability of methods and populations studied at different time points (Mäki et al. 2003; Lohi et al. 2007; Vilppula et al. 2009). Based on a recent birth cohort study carried out on at-risk children, presence of celiac disease antibodies seems to be greatest before ten years of age, peaking at the age of 33 months (Hagopian et al. 2017). No such follow-up studies have been presented on adults, but the point prevalence seems to slowly increase towards older age groups, indicating the appearance of new cases, also among adults (Lohi et al. 2007; Vilppula et al. 2008; Vilppula et al. 2009; Kang et al. 2013).

3 CLINICAL PICTURE

3.1 Classical presentation

Until the 1970s, suspicion of celiac disease was based solely on symptoms and signs of malabsorption (Cooke 1984). Nowadays such a clinical picture is called classical, defined as diarrhea, steatorrhea, weight loss or growth failure (Ludvigsson et al. 2013). Currently, approximately 13% to 50% of celiac disease patients present with the classical form (Volta et al. 2014; Spijkerman et al. 2016; Dominguez Castro et al. 2017). In the past 50 years, the proportion of patients with classical disease has clearly decreased while the proportion of non-classical forms has increased (Spijkerman et al. 2016). This is likely mostly due to recognition of the wide spectrum of clinical manifestations. Nevertheless, there is evidence that the clinical picture has also truly changed in recent decades, at least in children (Kivelä et al. 2015).

In the 1970's, the understanding of the various clinical manifestations of celiac disease improved as the introduction of serological tests simplified screening for cases without classical symptoms (Seah et al. 1971; Carswell and Ferguson 1972). Non-classical gastrointestinal (GI) manifestations were found to include symptoms similar to those of irritable bowel syndrome (constipation, abdominal distention, bloating), gastroesophageal reflux disease, and dyspepsia (Sanders et al. 2001). Celiac disease was also detected to appear in extraintestinal and even asymptomatic forms (Bottaro et al. 1999). It is noteworthy that the classical presentation is not a specific finding for celiac disease nor more typical than non-classical forms (Spijkerman et al. 2016; Irvine et al. 2017).

3.2 Extraintestinal manifestations

Approximately 60% of both adult and pediatric celiac disease patients have one or more extraintestinal manifestations at diagnosis, usually in addition to GI symptoms (Jericho et al. 2017; Nurminen et al. 2018). The prevalences of common extraintestinal manifestations in adult patients are listed in Table 2. The proportion of celiac disease diagnoses made due to mainly extraintestinal symptoms varies 9-16% in adults and 18-

24% in children (Ukkola et al. 2011; Paarlahti et al. 2013; Jericho et al. 2017; Nurminen et al. 2018).

Malabsorption may be the underlying cause in many symptoms, both extraintestinal and classical. For example, osteoporosis in celiac disease is likely driven by calcium malabsorption, which stimulates parathormone secretion, which consequently increases cortical bone loss (Walters 1994). However, other pathological mechanisms, such as release of proinflammatory cytokines, may play an important role here (Fornari et al. 1998; Abu Daya et al. 2013). Even though histological severity at diagnosis correlates e.g. with folate and iron deficiency and decreased bone mineral density (BMD) (Thomas et al. 2009; Zanini et al. 2013), these may also be present in patients with normal mucosa (Mustalahti et al. 1999; Repo et al. 2017). Moreover, the degree of mucosal damage has been shown to have only a weak association with severity of symptoms (Brar et al. 2007; Rubio-Tapia et al. 2010; Taavela et al. 2013; Zanini et al. 2013).

As one plausible player among extraintestinal manifestations, TG2-ab have been explored as a pathophysiological cause in celiac disease, being deposited extracellularly in the duodenal mucosa even before macroscopic mucosal damage or increased serum antibodies (Kaukinen et al. 2005). Additionally, deposits of TG2-ab have been identified in the liver, lymph nodes, kidneys, muscles and thyroid tissue of patients with incipient celiac disease, indicating humoral immunity (Korponay-Szabo et al. 2004). Subclinical thyroid disease, possibly driven by TG2-ab, has been suggested to be a mediator in psychiatric symptoms common at celiac disease diagnosis (Carta et al. 2002). Besides TG2-ab, other transglutaminase antibodies have been associated with extraintestinal manifestations. For example, in patients with gluten ataxia, autoantibodies against transglutaminase 6 in cerebellar cells have been identified and may be implicated in the development of neurological symptoms (Luostarinen et al. 2001; Hadjivassiliou et al. 2013). In dermatitis herpetiformis (DH), the cutaneous form of celiac disease, patients develop antibodies against TG2 and against epidermal transglutaminase 3 (Sardy et al. 2002).

DH is one of the most common extraintestinal manifestations of celiac disease, presenting in one out of eight Finnish patients (Salmi et al. 2011). This is a blistering skin disease usually occurring in the knees, elbows, and buttocks, and characterized by pathognomonic granular IgA deposits in the upper dermis layer of the skin (Zone et al. 1996). GI symptoms are rare in DH patients even though duodenal lesions often occur (Mansikka et al. 2018; Salmi 2019). Recently, a Finnish study showed a decrease in the prevalence of severe villous atrophy from 42% to 29% over a time span of 45 years (Mansikka et al. 2017). Lifelong GFD is essential for all patients with DH, but as the rash may take months or years to recover on diet alone, most patients need additional treatment with dapsone during the first years (Salmi 2019). Patients with incomplete GFD have been shown to be at risk of developing DH, which has led to a hypothesis

that DH is a complication of long-term untreated celiac disease rather than an independent variation (Kurppa et al. 2008; Salmi et al. 2015). These findings suggest that earlier celiac disease diagnoses and treatment could prevent the development of DH (Salmi et al. 2011; West et al. 2014).

Table 2. Prevalence of extraintestinal manifestations in adult celiac disease patients at diagnosis.

Extraintestinal manifestation	Prevalence, %	Reference
Anemia	20-21	Harper et al. 2007; Jericho et al. 2017
Aphthous ulcers	4-21	Campisi et al. 2007; Jericho et al. 2017
Dental enamel defects	4-23	Bottaro et al. 1999; Campisi et al. 2007
Dermatitis herpetiformis	10-13	Salmi et al. 2011; West et al. 2014
Elevated liver enzymes	2-11	Korpimäki et al. 2011; Jericho et al. 2017
Fertility problems[1]	2-16	Lasa et al. 2014; Jericho et al. 2017
Joint pain	7-8	Bottaro et al. 1999; Jericho et al. 2017
Neurological symptoms[2]	11-23	Luostarinen et al. 2003; Jericho et al. 2017
Osteoporosis	10-26	Lucendo et al. 2013; Jericho et al. 2017

[1] Miscarriages, infertility, preterm labor; [2] Cerebellar ataxia, peripheral neuropathy, epilepsy, migraine

3.3 Clinically silent celiac disease

Of adult celiac disease patients, 10-18% are reportedly asymptomatic at diagnosis (Paarlahti et al. 2013; Mahadev et al. 2016). These patients are usually found by screening risk groups, such as individuals with family history of celiac disease (Kivelä et al. 2017). The prevalences of celiac disease in different risk groups are listed in Table 3 and discussed further in Chapter 6. Categorization of patients to symptomatic or asymptomatic can be challenging or even arbitrary because screen-detected, apparently asymptomatic patients have often suffered from symptoms not recognized before the diagnosis (Ukkola et al. 2011; Agardh et al. 2015) and derive clinical benefit from the GFD (Kurppa et al. 2014). On the other hand, GI symptoms are frequent in general population and their association with even established celiac disease is not always clear (Rosen et al. 2014).

Currently, evidence is sparse as to whether patients with asymptomatic celiac disease have the same risks for complications and whether they always benefit from the GFD like symptomatic patients (Tursi et al. 2009; Tio et al. 2012). According to the only randomized controlled trial (RCT) on the issue, also seemingly asymptomatic patients respond positively to the GFD in clinical, serological and histological measures (Kurppa

et al. 2014). Thus, many asymptomatic patients may be relabeled as symptomatic once the effects of dietary treatment have been established. Furthermore, clinically detected and screen-detected, even asymptomatic patients, have been reported to be comparable regarding the severity of histologic damage and the level of TG2-ab, as well as in adherence and response to dietary treatment (Mahadev et al. 2016; Kivelä et al. 2017). Longitudinal studies are still scarce, but when Finnish patients diagnosed in childhood were examined in adulthood, measures of health, quality of life (QoL), and dietary adherence of screen-detected and clinically detected patients were comparable (Kivelä and Kurppa 2018). Furthermore, GFD seems to improve QoL and to decrease mortality risk even in patients with symptomless celiac disease (Mustalahti et al. 2002). Despite the many benefits of a GFD in asymptomatic patients, some of these patients have reported increased anxiety or impaired QoL on a GFD (Ukkola et al. 2011; Kurppa et al. 2014). Thus, while the evidence supports active screening of celiac disease in high-risk groups, more long-term evidence is called for before guidelines can with confidence recommend the restrictive, life-long GFD to all asymptomatic patients (Bibbins-Domingo et al. 2017).

Table 3. Prevalence of celiac disease in different risk groups.

Risk group	Study cohort	Celiac disease, %	Reference
Addison's disease	109 children and adults	2.7	Betterle et al. 2006
	925 adults	0.3	Krishnareddy et al. 2014
Autoimmune thyroid disease	302 children	2.3	Sattar et al. 2011
	952 adults	10.2	Krishnareddy et al. 2014
Down syndrome	105 children	3.8	Pueschel et al. 1999
	72 children	5.6	Nisihara et al. 2005
First-degree family members	4,508 children and adults	4.5	Fasano et al. 2003
	14,225 children and adults	5.6	Singh et al. 2015
IgA deficiency	126 children	8.7	Lenhardt et al. 2004
	34 children and adults	6.0	Fahl et al. 2015
IgA nephropathy	168 adults	3.6	Collin al. 2002
	827 adults	8.2	Nurmi et al. 2018
Sjögren's syndrome	111 adults	4.5	Szodoray et al. 2004
	925 adults	10.5	Krishnareddy et al. 2014
Turner syndrome	87 children	4.6	Ivarsson et al. 1999
	389 children and adults	6.4	Bonamico et al. 2002
Type 1 diabetes mellitus	4,322 children	6.8	Cerutti et al. 2004
	1,151 children	9.1	Bybrant et al. 2013

4 COMPLICATIONS AND SOCIETAL BURDEN

4.1 Osteoporosis and infertility

Distinguishing between extraintestinal symptoms and complications can be difficult, but symptoms, unlike complications, are considered to decrease on adequate treatment (Laurikka et al. 2018). Although BMD usually increases on a GFD, not all adult celiac disease patients achieve full bone recovery (Szymczak et al. 2012). Decreased BMD turns into a complication by progressing to fractures (Vasquez et al. 2000; West et al. 2003). A recent meta-analysis reported an overall increased risk of 30% for all fractures and 69% for hip fractures in celiac disease patients compared to general population (Heikkilä et al. 2015). The risk of fractures has also been elevated in individuals with unrecognized celiac disease (Agardh et al. 2009; Vilppula et al. 2011). According to the clinical guidelines in Finland, BMD is recommended to be measured one year after diagnosis in patients with severe symptoms, refractory celiac disease (RCD), or not adhering to a strict GFD. Investigations may also be valuable in patients with other risk factors for fractures such as older age or being postmenopausal (Scott et al. 2000).

Female infertility has been associated with untreated, but not with diagnosed and treated celiac disease according to one systematic review (Lasa et al. 2014). On the other hand, in a recent study of women with unexplained or identifiable infertility, celiac disease was not more common than among general population (Gunn et al. 2017). Nevertheless, the risk of spontaneous abortion seems to be increased in untreated compared to treated celiac disease, and initiation of the GFD has been reported to reduce this risk significantly (Ciacci et al. 1996; Tursi et al. 2008; Moleski et al. 2015).

4.2 Refractory celiac disease

Even after years on a strict GFD, up to 25% celiac disease patients continue to suffer from some GI symptoms (Paarlahti et al. 2013; Laurikka et al. 2016; Stasi et al. 2016). There are signs that treated celiac disease patients suffer from GI symptoms more often than general population, although comparative studies are scarce (Laurikka et al. 2016). The situation where there is some response to the GFD must, however, be differentiated from non-responsive celiac disease (NRCD) in which patients have persistent or

recurring symptoms and/or villous atrophy. Etiologies for NRCD most often include gluten cross-contamination, but also irritable bowel syndrome (IBS), lactose intolerance, and microscopic colitis (Leffler et al. 2007; Hollon et al. 2013). Other mechanisms and alternative causes of recurrent villous atrophy are discussed in detail in Chapter 5.2.1. When other causes of NRCD have been excluded, refractory celiac disease (RCD) is considered.

RCD is defined by persistent or recurrent symptoms of malabsorption together with villous atrophy after 6-12 months of a verified strict GFD and exclusion of other possible etiologies (Rubio-Tapia and Murray 2010; Ilus et al. 2014). The distinction between a slow response to a GFD, accidental gluten intake, and RCD may be difficult. Also, malignancies must be excluded before setting the diagnosis of RCD, and the initial celiac disease diagnosis must be indisputable (Rubio-Tapia and Murray 2010). In primary RCD, patients have never responded to a GFD, and in secondary RCD they have relapsed despite initial response and adherence to the GFD. According to studies conducted in tertiary centers, RCD has been diagnosed in 10-20% of celiac disease patients suffering from persistent symptoms (Leffler et al. 2007; Dewar et al. 2012). The prevalence of RCD in these studies has been overrepresented due to the concentration of selected unresponsive patients. In a Finnish study, 0.3 of all adult celiac disease patients eventually developed RCD, with an RCD prevalence of 0.002% in general population (Ilus et al. 2014).

Histopathologically, RCD is further divided into type I, where the phenotype of IELs is normal, and type II, where the IELs have lost their normal surface markers (Cellier et al. 1998). Type II RCD is considered as a precursor of enteropathy-associated T cell lymphoma (EATL) (Cellier et al. 2000). However, both subtypes are associated with increased mortality (Daum et al. 2009). Of RCD patients, about 70% have RCD I, and 30% RCD II, but the distinction between these is not always easy to make (Ilus et al. 2014). Symptoms of RCD resemble those in celiac disease except usually being more severe and debilitating (Dewar et al. 2012). Alarming symptoms for EATL comprise elevated body temperature, nocturnal sweating, weight loss, GI bleeding, and abdominal pain (Gale et al. 2000). Older age, symptoms of malabsorption, negative serology at celiac disease diagnosis, and poor dietary adherence have been reported to predispose to subsequent development of RCD (Biagi et al. 2014; Ilus et al. 2014).

4.3 Malignancies and mortality

Malignancies are a rare but feared complication of celiac disease. The risk of cancer has varied across studies depending on when and with what kind of cohort the study has been conducted. In early studies, which mainly included patients with classical and severe

symptoms, the overall risk of malignancies was up to two-fold that of general population (Holmes et al. 1989). In more recent studies also including patients with milder and atypical presentations, the risk has not been significantly elevated (Card et al. 2004; Ilus et al. 2014) and in screen-detected patients the risk has even been lower than in general population (Anderson et al. 2007; Lohi et al. 2009a). A meta-analysis combining clinically diagnosed and screen-detected patients reported an odds ratio of 1.07 (Tio et al. 2012).

Even though the overall risk of malignancies does not appear to be elevated in celiac disease, several studies have reported increased risk of small bowel cancer, esophageal carcinoma, and especially lymphoma (Lohi et al. 2009a; Elli et al. 2012) There is great variability in the risk ratios of non-Hodgkin lymphoma (NHL) between clinically detected cohorts, having maximally been 43-fold according to Holmes et al., probably reflecting selection bias (Holmes et al. 1989). One large study observed the risk of NHL to decline from 13- to 4-fold from 1975 to 2004, resulting in a 5-fold risk on average (Gao et al. 2009). Lately, smaller risks of up to 6-fold have been reported, and in some studies the lymphoma risk has not even been increased (Smedby et al. 2006; Lohi et al. 2009a; Elli et al. 2012).

The increased risk of certain malignancies in celiac disease has been suggested to result from mechanisms that enable carcinogens to enter the immune system: chronic inflammation that impairs immune functions, nutritional deficiencies, and increased gut permeability (Green et al. 2003). The majority of patients with already diagnosed celiac disease developing lymphoma have not kept to a strict GFD, suggesting that the diet protects against future malignancies (Holmes et al. 1989; Viljamaa et al. 2006), but there are also contradictory results (Olen et al. 2011; Elfström et al. 2012). The risk of cancer seems to be highest within the first years after diagnosis and to decline later, which may reflect an ascertainment bias created by finding malignancies coincidentally while investigating celiac disease related issues or, conversely, by discovering the disease in the course of cancer examinations (Askling et al. 2002; Card et al. 2004; Tio et al. 2012).

Studies of overall mortality risk in celiac disease patients show inconsistent results. Risk estimates between 1.3 and 4 have been shown in earlier studies (Cottone et al. 1999; Viljamaa et al. 2006; Rubio-Tapia et al. 2009), but according to more recent evidence, overall mortality is not increased in previously inidentified patients compared to healthy controls (Lohi et al. 2009b; Godfrey et al. 2010). Among already diagnosed patients, the mortality risk has been emphasized in patients with poor response to the GFD and in those with delayed diagnosis or severe symptoms before diagnosis (Nielsen et al. 1985; Corrao et al. 2001), but appears otherwise to be comparable to that in general population (Abdul Sultan et al. 2015). In patients with DH, the risk of mortality has even been decreased compared to that in general population (Hervonen et al. 2012; Viljamaa et al. 2006).

4.4 Quality of life

The World Health Organization (WHO) has defined QoL as individuals' perception of their position in life in the context of their culture and value systems and in relation to their goals, expectations, standards, and concerns (WHO 2017). It has been recognized that interventions and management of chronic diseases must be evaluated with meaningful measures of health-related quality of life (HRQoL) (Read et al. 1987). The Psychological General Well-Being questionnaire (PGWB), EuroQol-5D and the Short-Form 36-Item QoL measure are commonly used instruments to assess QoL in celiac disease, PGWB being one of the most frequently used scales (Dupuy 1984; Ludvigsson et al. 2018). These tools are not specific for celiac disease, but their advantage is the possibility for comparison to other diseases. Relevant celiac disease specific instruments for assessing QoL in adult patients include among others the Celiac Disease Questionnaire and the Celiac Disease Quality of Life Survey (Ludvigsson et al. 2018).

Untreated celiac disease has repeatedly been associated with impaired QoL (Johnston et al. 2004; Viljamaa et al. 2005), in both symptom- and screen-detected patients compared to non-celiac controls (Ukkola et al. 2011). GFD usually achieves an improvement in QoL after 12 months of strict adherence, at least in symptomatic patients (Ukkola et al. 2011; Borghini et al. 2016). Long-term impaired QoL on a GFD has been associated with long duration of symptoms before diagnosis and presence of comorbidities (Paarlahti et al. 2013; Violato and Gray 2019). An important factor improving QoL is likely the alleviation of symptoms, but in the majority of studies, QoL has also improved in asymptomatic patients on a GFD (Mustalahti et al. 2002; Johnston et al. 2004; Viljamaa et al. 2005; Kurppa et al. 2014). However, this has not been observed in all studies and some asymptomatic patients may even do worse on a GFD (Johnston et al. 2004; Ukkola et al. 2011). Thus, the benefits of the GFD in asymptomatic patients remain to some extent unresolved. Not all aspects of QoL are necessarily associated with symptoms, for example concern about health, contentment, and well-being (Ukkola et al. 2011; Mahadev et al. 2016). Moreover, females with treated celiac disease tend to experience poorer QoL than males (Hallert et al. 1998; Roos et al. 2006; Violato and Gray 2019).

4.5 Societal burden

Untreated celiac disease has been associated with increased use of health care services (Long et al. 2010; Ukkola et al. 2012). On average, 3-5 health care visits due to related symptoms precede the eventual suspicion of celiac disease (Ukkola et al. 2012; Mattila et al. 2013). Moreover, use of medicines such as painkillers and antibiotics before

diagnosis has been reported to be increased in celiac disease patients compared to healthy controls (Ukkola et al. 2012). A possible explanation is the presence of a variety of unspecific symptoms not immediately recognized as signs of celiac disease (Nachman et al. 2011; Canavan et al. 2014).

After diagnosis, there are controversial results as to whether celiac disease related visits to health care increase costs. It has been reported that either health care visits already diminish in the first year on a GFD (Long et al. 2010), or first increase in the first year on treatment due to follow-up, after which the number of consultations decreases (Green et al. 2008). Reducing health care costs implies an economic benefit of the early diagnosis and treatment of celiac disease, although this estimate does not include the impact of potentially decreased work productivity or the additional costs of gluten-free products (Long et al. 2010). A female predominance in the excess use of health care services on a GFD has been observed, mostly resulting from GI and musculoskeletal symptoms or mental disorders (Roos et al. 2011), but another study found increased costs particularly in males (Long et al. 2010).

5 DIAGNOSIS

5.1 Diagnostic criteria

In 1969, the European Society of Paediatric Gastroenterology and Nutrition (ESPGAN) issued the first diagnostic criteria for celiac disease. According to these, the diagnosis was based on the finding of intestinal biopsies at three time points: first, total or subtotal villous atrophy on a gluten-containing diet, secondly, recovery of mucosal structure in a follow-up biopsy on a GFD, and thirdly, recurrent histological damage during a gluten challenge (Meeuwisse 1970). A major revision took place in 1990, when ESPGHAN (European Society of Paediatric Gastroenterology, Hepatology and Nutrition) excluded the need for gluten challenge in most cases and alleviated the follow-up biopsy recommendations (Walker-Smith et al. 1990). Clinical recovery was then deemed sufficient to confirm the diagnosis in symptomatic patients, while histological recovery on a GFD had still to be demonstrated in asymptomatic patients. In cases of unclear diagnosis, for example when the initial diagnostic biopsy was lacking or inadequate, an additional gluten challenge was still recommended (Walker-Smith et al. 1990). The gluten challenge recommendations continue to apply today, but only after confirming the presence of HLA DQ2 or DQ8 (Husby et al. 2012; Rubio-Tapia et al. 2013).

The role of serology has increased enormously in celiac disease diagnostics since the identification of TG2 as the autoantigen and the subsequent development of practical and quantitative assays. Traditionally, antibody tests have been used as a screening tool before confirmation of the diagnosis with biopsies. However, in 2012, serology-based diagnostic criteria were for the first time proposed as an accurate alternative to biopsies in children, as discussed further in Chapter 7 (Husby et al. 2012).

5.2 Diagnostic methods

5.2.1 Small-bowel mucosal biopsy

The first signs of villous damage in the small-bowel mucosa of celiac disease patients were obtained by autopsies at the beginning of the 20th century (Manson-Bahr 1924). Further proof of the characteristic histological damage came from laparotomy samples

in the 1950s, after which perioral biopsy methods with rigid endoscopy and later with biopsy capsule were developed (Shiner 1956). The modern flexible endoscopes further improved the situation in the 1980s (Demling and Hagel 1985). Availability of biopsies cleared the way for specific examination and classification of small-bowel mucosal injury. Common practice is to report the quantitative villus height crypt/depth ratio (Vh/CrD), which decreases in untreated celiac disease (Kuitunen et al. 1982). Moreover, it was typical to categorize the gradual villous atrophy to partial, subtotal, or total (Kuitunen et al. 1982). In 1992, Michael Marsh presented the widely used grouped classification based on the gradual mucosal damage developing from inflammation characterized by IEL infiltration (Marsh I), crypt hyperplasia (Marsh II), and finally villous atrophy (Marsh III) (Marsh 1992). Nowadays these two are often combined, and Marsh III is further described as partial (IIIa), subtotal (IIIb), or total (IIIc) villous athropy (Oberhuber et al. 1999). The gradual development of the mucosal injury causes a challenge in designating the point of definite celiac disease, further discussed in Chapter 7.

Before the discovery of serological markers, small-bowel biopsy was the only way to examine for and set a reliable celiac disease diagnosis. Until today, the biopsy has been the gold standard of the diagnosis, but it has several limitations. First, morphological mucosal injury and inflammation can have several other causes, often mimicking celiac disease, also clinically. Especially mild histological lesions have low specificity and can be caused, for example, by *Helicobacter pylori* infection or non-steroidal anti-inflammatory drugs (Biagi et al. 2008; Aziz et al. 2010). Even completely flat mucosa can sometimes be attributed to something other than celiac disease (Table 4). Especially in seronegative patients, villous atrophy is more likely due to other causes, such as GI infections, immunodeficiencies, malignancies, and inflammatory bowel disease (IBD) (Ludvigsson et al. 2009; Aziz et al. 2017). As a curiosity, use of angiotensin II blockers commonly used in the treatment of hypertension, is associated with various degrees of mucosal damage (Rubio-Tapia et al. 2012; Owen and Owen 2018).

Another challenge in histology-based diagnostics is to obtain representative biopsies. Celiac disease can cause patchy lesions, and the severity of the damage may vary throughout the duodenum or even within a single biopsy (Ravelli et al. 2010). To obtain representative samples, the recommendation is to take at least four biopsies from the second or third part of the duodenum and one or two from the duodenal bulb, even though here injuries due to causes other than celiac disease are common, increasing the risk of false-positive diagnoses (Lebwohl et al. 2011; Taavela et al. 2016). Assuming the biopsy is adequate, appropriate handling of the specimen is equally important, as wrong orientation may result in misdiagnosis (Ravelli and Villanacci 2012; Taavela et al. 2013). Pathologists' interpretations of histology have also shown substantial intra- and interobserver variability, a risk of which can be reduced if standard operating procedures in specimen cutting and analysis are followed (Corazza et al. 2007; Taavela et al. 2013).

Table 4. Non-celiac conditions that may cause small intestinal villous atrophy.

Chronic immunological or allergic conditions
Autoimmune enteropathy
Cow´s milk allergy
Common variable immunodeficiency
Enteropathy-associated T cell lymphoma
Eosinophilic gastroenteritis
Inflammatory bowel disease
Iatrogenic causes
Olmesartan and other angiotensin II blockers
Non-steroidal anti-inflammatory drugs
Radiation and chemotherapy
Infections
Cryptosporidiosis
Giardiasis
Helicobacter pylori
Human immunodeficiency virus
Viral gastroenteritis
Other
Malnutrition
Peptic duodenitis
Small intestine bacterial overgrowth
Vitamin B12, folic acid or zinc deficiencies

Adapted from Aziz et al. 2010; DeGaetani et al. 2013; Owen and Owen 2018

5.2.2 Antibodies

Utilization of serology in celiac disease diagnostics began in the early 1970s with the introduction of anti-reticulin (ARA) and anti-gliadin (AGA) antibodies (Seah et al. 1971; Carswell and Ferguson 1972). The problem with AGA was poor accuracy (Grodzinsky et al. 1992), with various studies reporting sensitivities of 61–96% and specificities of 79–94% for celiac disease (Giersiepen et al. 2012). ARA, by contrast, have a high accuracy in children, but suboptimal sensitivity in adults (Seah et al. 1971; Seah et al. 1973; Mäki et al. 1988). These two tests have later mostly been replaced by improved tests, even though AGA are still used today in some countries despite its weaknesses (Sharma et al. 2015).

In the early 1980s, EMA were detected to react with IgA in the endomysium from the smooth muscle of monkey esophagus (Chorzelski et al. 1983). Later, human umbilical cord was discovered to be a more ethical alternative as an antigen (Ladinser et al. 1994). EMA are determined by indirect immunofluorescence, in which a typical staining pattern can be seen under a microscope if positive. The method may produce laboratory and performer dependent results, which is reflected in the reported variability of test sensitivity (83–100%) and specificity (95–100%) for celiac disease (Giersiepen et al. 2012).

In 1997, TG2 was identified as the autoantigen of celiac disease (Dieterich et al. 1997). While EMA are detected by the reaction with extracellular TG2, TG2-ab bind to celiac disease patients' TG2 expressed in the cells of the small bowel or other tissues (Korponay-Szabo et al. 2004). An enzyme-linked immunosorbent assay (ELISA) was established for the detection of IgA and immunoglobulin G (IgG) class TG2-ab, becoming an effective diagnostic tool (Sulkanen et al. 1998), demonstrating 90-100% sensitivity and specificity in most studies (Giersiepen et al. 2012). Besides ELISA, a radioligand binding assay can be used in the assessment of TG2-ab (Seissler et al. 1999).

The PPV of TG2-ab is typically slightly lower than that of EMA (Carroccio et al. 2002). There are also some non-celiac disease conditions in which TG2-ab may occasionally be elevated even with negative HLA DQ2/DQ8 genotype, such as liver disease (Villalta et al. 2005), GI infections (Ferrara et al. 2010) and heart diseases (De Bem et al. 2006; Di Tola et al. 2008). Low or borderline positive antibody values may also fluctuate or normalize even on a gluten-containing diet in both adults and children (Simell et al. 2007; Kurppa et al. 2011; Mahadev et al. 2011). However, high TG2-ab titers are highly specific for celiac disease and have not been seen in other diseases (Lo Iacono et al. 2005; Di Tola et al. 2008). Studies evaluating multiples of the upper limit of normal (ULN) of TG2-ab have given high PPV for celiac disease, depending on the ULN factor and test kit used (Table 5). Biopsy-proven celiac disease has not been established in 100% of individuals with positive EMA and elevated TG2-ab, but the presence of only mild mucosal findings does not automatically imply false positive antibody results (Katz et al. 2011; Mustalahti et al. 2010). Instead, Marsh 1 or even 0 mucosal findings in EMA-positive individuals could represent early stage celiac disease later developing into more severe damage (Kurppa et al. 2009; Kurppa et al. 2010). Also, seropositivity with non-diagnostic histology can result from inappropriate handling of the mucosal samples or erroneous interpretation of the pathology (Ravelli and Villanacci 2012; Taavela et al. 2013).

One challenge in serological testing is that IgA deficiency is overrepresented in celiac disease. The prevalence of IgA deficiency varies globally 0.005-0.7% (Kanoh et al. 1986; McGowan et al. 2008), but among celiac disease patients the variation is as high as 2-9% (Lenhardt et al. 2004; Pallav et al. 2016). These individuals receive false negative results in IgA-based antibody testing. Thus, it is recommended to assess the total IgA level in susceptible patients and to perform IgG-based antibody testing in IgA deficient cases (Ludvigsson et al. 2014). Although IgG class EMA and TG2-ab have high specificity for celiac disease, their poor sensitivity in patients with normal IgA levels prohibits their use as a universal screening tool (Rostom et al. 2005).

Point of care tests (POCTs) for the diagnosis of celiac disease are also available. Because these do not require a laboratory or experienced staff, and they have a quick procession time, they can be used immediately in a physician's consulting room once the

suspicion of the disease arises. Hence, POCTs have theoretical potential to increase diagnostic yield and facilitate early diagnosis. The pooled sensitivity and specificity for IgA-TG2-ab-based POCTs are respectively 91% (range 70-97%) and 95% (range 79-97%) (Singh et al. 2018). However, the role of POCTs in the diagnostics remains indeterminate and currently the POCT result should always be confirmed with laboratory-based testing (Ludvigsson et al. 2014). A rapid, automatized TG2-ab test providing a numerical outcome has recently been developed to challenge POCTs, which only give a polar response of either `positive` or `negative` (Rusanen et al. 2019).

Antibodies against deamidated gliadin peptides (DGP) have been discovered to react to peptides that TG2 cuts from native gliadin, the target of AGA, and have thus been considered promising biomarkers for celiac disease (Schwertz et al. 2004). DGP may afford better sensitivity than TG2-ab and EMA in detecting early-stage disease when villous morphology has not yet been affected (Kurppa et al. 2011). Nevertheless, DGP positivity in TG2-ab negative individuals has low PPV, which makes the former an unsuitable screening marker (Gould et al. 2019). At present, the role of DGP in celiac disease diagnostics is therefore unclear, and it is not recommended in basic diagnostics apart from perhaps the IgG based DGP test in IgA deficiency (Husby et al. 2020).

Table 5. Positive predictive value of high TG2-ab values in celiac disease diagnostics.

Cohort	TG2-ab threshold	Test kit	PPV, %	Reference
Asymptomatic patients				
157 children[1]	10x ULN	ND	100	Paul et al. 2018
74 children	10x ULN	Euroimmun®	98	Wolf et al. 2017
56 children	10x ULN	Eurospital®	93	Trovato et al. 2015
Symptomatic patients				
234 adults	10x ULN	2 kits, ND	96-100	Efthymakis et al. 2017
17,505 children	10x ULN	Euroimmun®	98	Gidrewicz et al. 2015
230 children	10x ULN	Eurospital®	91	Trovato et al. 2015
310 adults	8.9x ULN	Delta Biologicals®	100	Tortora et al. 2014
166 adults and 36 children	11x ULN	Inova®	100	Beltran et al. 2014
79 children and adults	5x ULN	Inova®	96	Donaldson et al. 2008
All clinical presentations				
707 children	10x ULN	8 separate kits[2]	99-100	Werkstetter et al. 2017
898 children	10x ULN	Euroimmun®	99	Wolf et al. 2017
945 adults	5x ULN	Celikey® and Eurospital®	100	Zanini et al. 2012

[1] At-risk groups: Celiac disease in family; type 1 diabetes; Down, Turner and William syndrome
[2] EiA Celikey®, Varelisa Celikey®, Inova Quanata Lite®, Inova Quanta Flash®, Eurospital®, Euroimmun®, 2 different tests from R-Biopharm/Zedira
TG2-ab, transglutaminase 2 antibodies; PPV, positive predictive value; ULN, upper limit of normal; ND, no data

5.3 Pathway to diagnosis

The three main approaches to identifying individuals with untreated celiac disease are active case finding based on clinical suspicion, screening of at-risk groups, and population-based screening (Kivelä and Kurppa 2018; Lindfors et al. 2019). At present, only the first two are applied in clinical practice, with some rare exceptions. According to these two approaches, either presence of the signs and symptoms discussed previously in Chapter 3 or belonging to a risk group (Table 3) should raise suspicion of celiac disease. Before further testing, it must be confirmed that the individual is on a gluten-containing diet. Next, serum samples to assess TG2-ab with or without EMA are collected, and in children, one measurement of total IgA is also recommended (Husby et al. 2012; Ludvigsson et al. 2014). In patients with mild symptoms and normal gluten consumption, negative antibody levels are considered to rule out celiac disease and no further investigations are needed unless otherwise clinically indicated (Ludvigsson et al.

2014). Alternative diagnoses with symptoms mimicking celiac include particularly lactose intolerance, irritable bowel syndrome, pancreatic insufficiency, microscopic colitis, small-intestinal bacterial overgrowth, lymphoulcerative jejunitis, T-cell lymphoma, and fructose intolerance (Abdulkarim et al. 2002; Leffler et al. 2007; Dewar et al. 2012).

If TG2-ab and/or EMA are elevated or the suspicion of celiac disease is otherwise high, biopsies must be performed, with an exception of European children (Husby et al. 2012) or Finnish adults (Celiac Disease Current Care Guidelines 2018) fulfilling the ESPGHAN serology-based criteria. With severe symptoms such as anemia, weight loss or constant diarrhea, seronegative celiac disease must be considered, particularly in adults (Gustafsson et al. 2019; Ludvigsson et al. 2014). Also, obtaining biopsies is always indicated to rule out malignancies in the presence of so-called "red flag symptoms" such as substantial weight loss, bloody stools or dysphagia (Marshall et al. 2011). The establishment of villous atrophy, crypt hyperplasia, and elevated lymphocyte count leads to celiac disease diagnosis (Chapter 5.2.1), although it must again be realized that the lesion is not 100% specific (Table 4).

Individuals without villous atrophy but with positive serum antibodies are currently considered to have potential celiac disease (Ludvigsson et al. 2014). Several studies have shown that such patients may suffer from symptoms or even complications before the development of villous atrophy (Collin et al. 1994; Salmi et al. 2006; Kurppa et al. 2012; Volta et al. 2016). Moreover, seropositive patients with normal mucosal structure may benefit from early treatment with a GFD (Kaukinen et al. 2001; Paparo et al. 2005). One study has observed that virtually all subjects with positive EMA eventually develop mucosal damage (Kurppa et al. 2010). However, the current guidelines do not agree with setting the diagnosis and starting a GFD until damage to the duodenal mucosa is identified. Instead, patients without clear histological changes but confirmed EMA or TG2-ab should be monitored closely (Husby et al. 2020).

6 MANAGEMENT

6.1 Dietary treatment

Since its discovery in the 1950s, the only officially accepted treatment for celiac disease has been a lifelong strict GFD (Dicke et al. 1952). This means total elimination from the diet of wheat, rye, barley, and products with additional gluten. Most studies have reported that purified oats are safe in the diets of children and adults with celiac disease and DH (Janatuinen et al. 1995; Reunala et al. 1998; Högberg et al. 2004; Aaltonen et al. 2017). However, there are observations that some patients using oats may have increased numbers of inflammatory cells in the duodenal mucosa, even though the majority eventually tolerate oats (Lundin et al. 2003; Peräaho et al. 2004). A recent large long-term cohort study reported that oat-consuming patients may even have better QoL than those avoiding it (Aaltonen et al. 2017).

The effectiveness of a strict GFD is usually shown by rapid alleviation of GI symptoms, which can occur within days to weeks (Murray et al. 2004). The diet has also been shown to have a beneficial impact on extraintestinal manifestations such as BMD, neurological symptoms, and liver function (Volta et al. 1998; Mustalahti et al. 1999; Gabrielli et al. 2003). Moreover, 12 months on GFD has led to improvement in QoL and mental health, at least in symptomatic patients (Ukkola et al. 2011; Kurppa et al. 2014; Borghini et al. 2016). The median recovery time of the mucosa is longer, approximately two to four years (Lanzini et al. 2009; Haere et al. 2016; Newnham et al. 2016; Pekki et al. 2017). In some patients, however, symptoms may persist even after full mucosal recovery (Paarlahti et al. 2013).

Despite its effectiveness, GFD may also have some nutritional disadvantages. Gluten-free products tend to contain more fat, sugar, and salt and less fiber and protein than gluten-containing products (Fry et al. 2017). Furthermore, as limiting gluten intake often simultaneously decreases the intake of whole grains, the risk of cardiovascular events may be increased in celiac disease (Lebwohl et al. 2017). Thus, GFD without a clear medical reason is not advisable. Besides, the products are more expensive than regular foods (Fry et al. 2017).

A substantial number of non-celiac individuals around the world also avoid gluten, for example due to wheat allergy, IBS, and non-celiac gluten sensitivity (NCGS). In NCGS, patients experience intestinal and extraintestinal symptoms that maybe triggered

by gluten, but allergic or autoimmune mechanisms are not involved in these (Biesiekierski et al. 2011). Overlapping of NCGS and IBS has been suggested, but unlike in IBS, NCGS patients self-report gluten as the specific cause of their symptoms (Catassi et al. 2017). Other substances such as fructan and wheat amylase-trypsin inhibitors have also been suggested to induce symptoms besides or instead of gluten (Zevallos et al. 2017; Skodje et al. 2018), but treatment with a diet avoiding fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) recommended in IBS has not resulted in comparable effects in NCGS patients (Biesiekierski et al. 2013). Before setting the diagnosis of NCGS, it is important to rule out celiac disease and then to prove the gluten dependency of symptoms by a double-blind gluten challenge in order to avoid an unnecessary, potentially even harmful GFD (Sapone et al. 2012). Recently, increased zonulin levels have been suggested to differentiate NCGS from IBS (Barbaro et al. 2020).

6.2 Novel treatment options

Celiac disease patients are interested in therapies supplementing or replacing the burdensome GFD (Ukkola et al. 2012; Tomal et al. 2016). Along with increasing knowledge of the pathogenesis, new alternative treatment modes have been explored, but not yet accepted in clinical use (Caio et al. 2019). These aim specifically to help patients with persistent symptoms and those with compliance difficulties (Agarwal et al. 2016). Molecules under current research e.g. degrade gluten before its interaction with the intestinal mucosa (Lähdeaho et al. 2014; Kaukinen and Lindfors 2015), inhibit digestion of gluten into harmful immunogenic peptides (Liang et al. 2009), or directly inhibit TG2 (Molberg et al. 2003; Rauhavirta et al. 2013), but none of these have so far demonstrated unequivocal benefits. Immunomodulation, for example anti-IL-15 treatment, has also been explored with no effects on regular celiac disease (Lähdeaho et al. 2019), and possibly modest results on RCD (Cellier et al. 2019). Also, "vaccines" using gluten peptides to induce tolerance are under development, but very recent results were disappointing (Goel et al. 2017; Truitt et al. 2019). Challenges in drug development have included a lack of a good animal model mimicking celiac disease and a lack of sensitive non-invasive surrogate markers for the biopsy (Ju et al. 2015; Ludvigsson et al. 2018), although recently a mouse model of celiac disease succeeded in representing the induction of immune tolerance to gliadin with gliadin nanoparticles (Freitag et al. 2020).

6.3 Follow-up

The main objective of following-up celiac disease patients is supporting the patient in strict adherence to the GFD, and subsequently promoting normalization of the duodenal mucosa and prevention of complications (Vivas et al. 2008). Soon after diagnosis, all patients ought to be referred to dietary counseling, which has been reported to improve treatment compliance (Rajpoot et al. 2015). There is inconsistent evidence as to whether regular long-term follow-up further enhances dietary adherence (Hall et al. 2009; Pekki et al. 2018). Besides interventions by a dietitian, membership of an advocacy group and cognitive, emotional, and socio-cultural influences may be related to adherence (Hall et al. 2009).

There are a few methods to monitor mucosal recovery on a GFD, all of which have their limitations. Normalization of TG2-ab values is a rather insensitive marker and does not confirm the presence of healthy mucosa or strict GFD adherence (Leonard et al. 2017). This is demonstrated e.g. by the level of antibodies decreasing rather quickly on the GFD, whereas normalization of the intestinal mucosa can take up to eight years (Haere et al. 2016). DGP are proposed to be more closely associated with biopsy results during follow-up and may therefore be a more sensitive marker of mucosal healing (Agardh 2007; de Chaisemartin et al. 2015), but more evidence is needed. As a promising novel method, measuring gluten immunogenic peptides from feces or urine can be utilized to monitor dietary adherence (Comino et al. 2016; Moreno et al. 2017).

Some of the current guidelines recommend a follow-up biopsy for all patients after one year on a GFD (Lebwohl et al. 2011), while others do not consider it to be essential in case of unequivocal clinical recovery (Ludvigsson et al. 2014). Noteworthy, as already mentioned, only 37-50% patients achieve full histological remission at 12 months even on a strict diet, this figure being approximately 90% after five years (Haere et al. 2016; Newnham et al. 2016; Pekki et al. 2017). In clinical practice, repeat endoscopy is often performed after one year on patients with severe presentation at diagnosis, who are also those with the slowest mucosal recovery (Pekki et al. 2017). Therefore, the result may not be very informative (Pekki et al. 2017). Moreover, as incomplete mucosal recovery at this point is not associated with increased risk of mortality or impaired long-term well-being, the need for endoscopic follow-up for all patients can be questioned overall (Pekki et al. 2015; Pekki et al. 2017). There are nevertheless contrasting results as to whether incomplete mucosal recovery after one year on a GFD increases the risk of lymphoma (Lebwohl et al. 2013; Pekki et al. 2017). Based on the aforesaid results, it has been proposed that repeat endoscopies could be performed, for example, about two years after the diagnosis on selected patients depending on age, severity of the disease presentation at diagnosis, and response to the GFD (Rubio-Tapia et al. 2013; Pekki et al. 2017). The recent Finnish guidelines recommend repeat biopsies on patients with

negative celiac disease serology or severe clinical picture at diagnosis, and those without dietary response, especially in elderly patients who have increased risk for RCD (Duodecim 2018).

Although histological recovery may take years, only 0.31 of adults eventually develop RCD (Ilus et al. 2014). Risk for RCD has been reported to be associated with elderly age, seronegative celiac disease, and male gender (Ilus et al. 2014). As regards the diagnostic biopsy, Elfstöm et al. suggested that severity of the histological damage could have prognostic value for lymphoproliferative malignancies; however, hampering the conclusions, the authors compared individuals with potential and not conclusive celiac disease to those with flat mucosa (Elfström et al. 2011). Thus, baseline histopathology may not be helpful when selecting patients for repeat endoscopies. Instead, if TG2-ab are absent in serum but deposited in the small bowel mucosa, celiac disease may be more advanced since the avidity of the antibodies to the mucosa increases over time, and the risk for EATL has been associated with negative serum antibodies (Salmi et al. 2006; Ilus et al. 2014). Thus, seronegativity at diagnosis could be considered a strong indicator for histological follow-up (Duodecim 2018).

7 CHALLENGES AND NEW APPROACHES IN DIAGNOSING CELIAC DISEASE

7.1 Weaknesses of current diagnostic approaches

Globally, diagnosing of celiac disease still depends mostly on clinical case-finding (White et al. 2013; Barada et al. 2014). Despite increased awareness, even optimal case finding only reaches patients who actively seek medical advice. Simultaneously, up to 85% of patients eventually found by screening are observed to have suffered from unrecognized symptoms (Hansen et al. 2006; Kinos et al. 2012; Kivelä et al. 2017). Since both patients and experts seem to have problems recognizing celiac disease behind the vast variety of clinical presentations, case finding does not appear to serve as a sufficient diagnostic strategy (Barada et al. 2014). For example, up to 90% of patients in the USA are estimated to go undetected despite the availability of effective diagnostic methods (Fasano et al. 2003; Rubio-Tapia et al. 2012).

Celiac disease is overrepresented in certain syndromes and many autoimmune diseases, which offers an opportunity to screen these risk groups (Table 3). Besides screening in first-degree relatives of already diagnosed celiac disease patients, the European and North American pediatric guidelines recommend celiac disease antibody testing of children with type 1 diabetes, autoimmune thyroidal disease, autoimmune liver disease, IgA deficiency, Trisomy 21, and Williams and Turner syndrome (Hill et al. 2005; Husby et al. 2012). Correspondingly, many adult guidelines also recommend serological testing of at-risk individuals (Rubio-Tapia et al. 2013; Ludvigsson et al. 2014; Downey et al. 2015). In clinical practice, these recommendations do not necessarily actualize (Pavlovic et al. 2017). Even if risk-group screening is optimal, it only reaches 30-50% of all celiac disease patients (Myleus et al. 2009; Kivelä et al. 2015).

In order to increase diagnostic yield and to identify apparently asymptomatic patients, screening of general population has been suggested (Catassi and Fasano 2014). WHO has issued criteria for screening of a medical condition (Table 6). In celiac disease, most of these criteria are fulfilled, but unclear issues remain in the prognosis of untreated celiac disease in asymptomatic patients, and whether possible benefits of the GFD outweigh the social and economic burden of the strict and life-long dietary restriction (Wilson and Jungner 1968; Ludvigsson et al. 2015; Chou et al. 2017). Moreover, one

important question would be the frequency and optimal time window for screening, since celiac disease can develop at any age (Vilppula et al. 2009).

The United States Preventive Services Task Force (UPSTF) recently commissioned a systematic review of the evidence on the benefits and harms of screening for celiac disease at population level (Chou et al. 2017). The review found insufficient evidence and called for more research in order to understand optimal screening strategies and the effectiveness of screening and treatment (Chou et al. 2017). However, most screen-detected patients seem to benefit from the GFD as do clinically detected patients (see Chapter 3.3).

Studies evaluating the cost-effectiveness of screening are rare. One survey estimated that mass screening would be cost-effective if the diagnostic delay is longer than six years and GFD adherence exceeds 98% (Hershcovici et al. 2010). One alternative strategy suggested has been to test all children for HLA at birth and measure antibodies from genetically susceptible children at least at the age of ten years (Catassi and Fasano 2014). It must, however, be realized that before future studies provide stronger evidence about the effects of screening, most patients will go undetected and likely have increased risk for future complications (Kivelä and Kurppa 2018).

Table 6. Summary of criteria for mass screening of a medical condition according to WHO.

Category	Criteria
Condition	The condition sought should be an important health problem whose natural history is adequately understood, including development from latent to declared disease. The condition should have a recognizable latent or early symptomatic stage.
Diagnosis	Diagnostic tests should be available, safe and acceptable to the population concerned. There should be an agreed policy, based on respectable test findings and national standards, as to whom to regard as patients, and the whole process should be a continuing one.
Treatment	There should be an accepted and established treatment or intervention for individuals identified as having the disease or pre-disease condition and facilities for treatment should be available.
Cost	The cost of case-finding (including diagnosis and treatment) should be economically balanced in relation to possible expenditure on medical care.

Adapted from Holland et al. 2006.

7.2 Diagnostic delay

Diagnostic delay in celiac disease refers to the time from the onset of first disease-related symptoms or signs until the diagnosis is set. In many reports, the average delay has been very long, up to 13 years, with wide variation between studies (Table 7). The diagnostic delay can be divided into patients' delay, referring to the duration of symptoms prior to the first visit to a physician, and doctors' delay referring to the time from the first physician contact until the final diagnosis (Norström et al. 2011; Vavricka et al. 2016). The concepts of undetected disease and delayed diagnosis may overlap regarding their consequences, but differ by delay being a quantitative, temporary, and avoidable period while undetected is a qualitative term.

Delayed diagnosis in celiac disease has been shown to impair HRQoL (Norström et al. 2011; Paarlahti et al. 2013). Besides, the delay may increase the risk of associated complications, such as osteoporosis (Corazza et al. 1995), fertility problems (Gasbarrini et al. 2000), malignancies (Silano et al. 2007), and even mortality (Corrao et al. 2001; Rubio-Tapia et al. 2009). The delay is also associated with incremental medical costs before diagnosis (Long et al. 2010). An early initiated GFD has been reported to reduce this economic burden on health care, the risk of complications, and to improve HRQoL (Green et al. 2001; Norström et al. 2011).

The reasons for the delay are complex and not adequately known (Norström et al. 2011). One reason may be the heterogenous and unspecific clinical presentation. For example, celiac disease has considerable overlap with IBS-type symptoms such as abdominal pain, abdominal distention, and change in bowel habit (Sanders et al. 2001; Rampertab et al. 2006). These symptoms may be overlooked, mislabeled as IBS, and lead to a diagnostic delay until celiac disease is finally detected (Canavan et al. 2014). There is also evidence that risk of delay is increased in patients with atypical presentation (Choung et al. 2017; Paez et al. 2017) and among the elderly (Vivas et al. 2008; Norström et al. 2011). In DH, which usually has a characteristic clinical picture, the median diagnostic delay in a Finnish study was only 10 months, although in 30% of patients it was two years or more (Mansikka et al. 2017). Female sex and presence of duodenal lesion at diagnosis have been found to increase the risk for delay in DH (Mansikka et al. 2017).

Median diagnostic delay appears to have decreased over in the last few decades in Europe and the USA (Rampertab et al. 2006; Norström et al. 2011; Vavricka et al. 2016). This phenomenon may be associated with a simultaneously observed decrease of the incidence of severe complications and mortality in celiac disease (Holmes et al. 1989; Corrao et al. 2001; Ilus et al. 2014). Although further evidence is needed, these findings support aiming at early diagnosis and further elucidation of factors predisposing to delay (Corrao et al. 2001).

Table 7. Diagnostic delay[1] of celiac disease in different studies.

Country	Study period	Mean age at diagnosis, years	Cohort, n	Delay, years		Reference
				Mean	Median	
Canada	2002	56	2,681	12	5	Cranney et al. 2007
Finland	2007-2008	49	698	3	3	Ukkola et al. 2011
Germany	ND	37	446	4	ND	Hauser et al. 2006
India	2000-2005	28	45	ND	2.5	Makharia et al. 2007
Italy	1997-1998	37[2] / 69[3]	1,293 / 60	14 / 17	ND	Gasbarrini et al. 2001
Saudi-Arabia	2009-2015	ND[4]	59	2	ND	Saeed et al. 2017
Spain	2000-2006	36	54	8	ND	Vivas et al. 2008
Sweden	2009	52	1,031	10	4	Norström et al. 2011
Switzerland	ND	41	1,689	7	2	Vavricka et al. 2016
UK	2006	41	788	13	ND	Gray and Papanicolas 2010
UK	2012-2015	44	1,584	12.8	ND	Violato and Gray 2019
USA	1996-1997	53	1,611	11	ND	Green et al. 2001
USA	1993-2001	46	1,032	ND	1	Zipser et al. 2003
USA	1952 2004	43	590	11 4	ND	Rampertab et al. 2006
Wales	1996–2005	50	347	6	2	Hurley et al. 2012

[1] Duration of symptoms before celiac disease diagnosis; [2] 18-64 years; [3] At least 65 years; [4] Range 1-16 years
ND, no data; UK, United Kingdom; USA, United States of America

7.3 Serology-based criteria

As endoscopy is burdensome and expensive, handling and interpretation of biopsies is challenging (Chapter 5.2.1.), and contemporary serological tests have excellent accuracy, biopsy-omitting diagnostic criteria have logically been called for. In 2012, ESPGHAN issued new guidelines allowing the omission of duodenal biopsy in symptomatic at-risk children with high TG2-ab titers (≥ 10 times ULN), a positive EMA test and the correct HLA genotype (Husby et al. 2012). This approach has been shown to be valid if applied correctly (Werkstetter et al. 2017). Besides ESPGHAN and the recent update of the Finnish recommendations (Celiac disease: Current Care Guideline 2018), no other guidelines have proposed situations where biopsy could be omitted either in pediatric or adult diagnostics (Hill et al. 2005; Rubio-Tapia et al. 2013; Ludvigsson et al. 2014; Downey 2015).

One limitation of the biopsy-omitting strategy is that there is no standardization between TG2-ab assays (Egner et al. 2012). All commercially available kits report arbitrary units with their own method-specific reference ranges. It is unclear whether some kits do not achieve a sufficiently high PPV with any ULN multiple (Werkstetter et

al. 2017). Also, for some kits, there has been poor concordance in diagnostic accuracy between different centers, perhaps partly due the pitfalls of biopsy as a diagnostic reference (Beltran et al. 2014) The celiac disease specificity of TG2-ab ≥ 10x ULN applies for the range of tested of antibody kits, but with lower TGA-ab values, supplementary diagnostic tools are essential (Beltran et al. 2014; Werkstetter et al. 2017).

The non-biopsy approach has been criticized due to the risk of missing complications or other concomitant GI conditions, particularly in adults (Efthymakis et al. 2017; Marks et al. 2018). Another debated issue, even in pediatric application, was the feasibility of the criteria in populations with presumably lower pretest probabilities, including screen-detected or asymptomatic subjects (Fernandez-Banares et al. 2012; Tortora et al. 2014). Since it is theoretically impossible for a diagnostic test to achieve 100% sensitivity and specificity, pretest probability affects the validity of the result (Fagan 1975). Excluding asymptomatic patients from the 2012 ESPGHAN criteria was explained by the possibly higher risk of false-positive TG2-ab (Vecsei et al. 2009; Husby et al. 2012). One study found the serology-based criteria to be extremely accurate in high-risk individuals but not in those with pretest probability below 10% (Fernandez-Banares et al. 2012). However, this study lacked follow-up, and it is possible that patients with high TG2-ab, but initially normal intestinal mucosa had early-stage celiac disease and would subsequently have developed diagnostic villous atrophy during continuing gluten intake (Fernandez-Banares et al. 2012).

A cheaper, more effective, and straightforward serology-based approach would likely accelerate the diagnostics. Also, the opportunity to obtain a diagnosis without necessarily undergoing the unpleasant endoscopy may lower some patients' threshold to approach health care with suspicion for celiac disease. Consequently, the biopsy-omitting strategy may also shorten the diagnostic delay.

THE PRESENT STUDY

8 AIMS

Early diagnosis of celiac disease and the subsequent initiation of a GFD are perquisites to reduce the harm caused by untreated disease. Nevertheless, the diagnosis is often delayed. In addition, the current biopsy-based diagnostics is burdensome, expensive, and has many pitfalls. The first main aim of the present study was to find underlying factors for and elucidate the burden of diagnostic delay among celiac disease patients and in society. The second aim was to ascertain if serology-based diagnostics could be reliably used to simplify and expedite the diagnostic process in adults.

The specific aims in Studies I-III were:

I. To investigate timely changes in celiac disease diagnostics and factors associated with very long (> 10 years) diagnostic delay

II. To identify associated risk factors and consequences of at least a median diagnostic delay (≥ 3 years) in celiac disease

III. To ascertain if the serology-based diagnostic criteria for celiac disease are accurate in adults with different pretest probabilities

9 MATERIALS AND METHODS

9.1 Participants

9.1.1 Patients in Study I

Celiac disease patients for Study **I** were recruited from all parts of Finland by newspaper advertisements and via local celiac societies. Only adults with a biopsy-proven diagnosis verified from the medical records were included. Altogether 922 volunteers ≥18 years were willing to participate. However, uncertain diagnosis in 21 participants, lack of information regarding the date of diagnosis in 14, and uncertain duration of symptoms before diagnosis in 62 led to exclusion from the study. Eventually, 825 eligible subjects formed the final study cohort.

9.1.2 Patients in Study II

A validated, self-report questionnaire was sent to all 1,864 newly diagnosed patients joining the Finnish Celiac Disease Society between February 2007 and May 2008. In total, 1,062 (57%) responded, representing approximately 40% of all new celiac disease patients in Finland as 70% of them join the Society soon after diagnosis (Ukkola et al. 2011). Confirmation of celiac disease was based on patients' reports on the presence of a diagnostic small-bowel mucosal biopsy or skin biopsy (DH). Of the responders, 451 were excluded: 157 for not being diagnosed within a year, 132 for being under 16 years, 73 for not having a confirmed diagnosis, and 89 for missing information on the duration of symptoms. A follow-up-questionnaire was sent to all participants after one year. Altogether, 611 subjects were eligible and 559 (91%) of them also completed the follow-up questionnaire.

9.1.3 Participants in Study III

The study comprised 5,497 participants who had different pretest probabilities for celiac disease. Only subjects ≥18 years old and with no previous celiac disease or DH diagnosis and who were on a gluten-containing diet were included.

The participants were divided into three cohorts based on the estimated likelihood of celiac disease. The high pretest probability cohort consisted of 421 consecutive adults who had been referred for upper GI endoscopy by physicians of all health care levels due to various clinical symptoms and signs suggestive of celiac disease. The literature has shown the prevalence of celiac disease to vary 5-50% in such cohorts (Collin et al. 2002; Hopper et al. 2007). Fifty percent of the subjects had been tested for some celiac antibodies before, but seronegative subjects were also referred for endoscopies due to clinical suspicion. Serological and endoscopic investigations were performed at the Department of Gastroenterology, Tampere University Hospital, between 1995 and 2009.

The moderate pretest probability cohort was collected following the same nationwide recruitment as in Study **I**. The original cohort had comprised 3,268 first- and second-degree family members of 895 known celiac disease patients. Their pretest probability for celiac disease is known to be around 8% (Singh et al. 2015). For the present study, 911 subjects were excluded: 895 subjects with age below 18 years, and 16 being on a self-initiated GFD despite no verified celiac disease or DH diagnosis. Eventually, 2,357 adult family members were eligible.

The low pretest probability cohort was initially collected for a research project on ageing and well-being, presumably with a celiac disease prevalence similar to that of Finnish general population, approximately 2% (Lohi et al. 2007; Vilppula et al. 2008). Recruitment and interviews of the participants and serum sampling were performed in 2002. Altogether, 4,272 individuals born in the years 1946–1950, 1936–1940 and 1926–1930 and living in the Päijät-Häme Hospital District were randomly selected at The Finnish National Institute for Health and Welfare to represent general population in the respective age groups. Of these, 2,815 (66%) agreed to participate, but 26 subjects with previous celiac disease or DH diagnosis, three with a self-initiated GFD, and 64 with insufficient data were excluded. The present study thus comprised 2,722 unselected subjects.

9.2 Definitions

9.2.1 Diagnostic delay (Studies I-II)

The information on diagnostic delay was based on the patient's report of the duration of celiac disease-related symptoms before diagnosis. In Study **I**, patients were asked about the duration of symptoms in five categories: no symptoms or symptoms for less than one year, 1-5 years, 5-10 years or more than ten years. The diagnosis was considered substantially delayed if symptoms had lasted more than ten years. This cutoff was justified by a report showing elevated risk of malignancies in patients with such a delay (Green et al. 2001). In Study **II**, the duration of symptoms was elicited from the patients at diagnosis and the diagnosis was considered delayed if the symptoms had lasted ≥ 3 years, according to the median diagnostic delay in Finland (Ukkola et al. 2011).

9.2.2 "Triple positivity" as diagnostic criteria (Study III)

According to the 2012 ESPGHAN criteria, the presence of symptoms was one prerequisite for the non-biopsy diagnosis (Husby et al. 2012). This was not the case here, since the study aimed to evaluate the criteria among subjects with different diagnostic approaches, including asymptomatic patients. Thus, "triple criteria" for celiac disease was defined as TG2-ab ≥ 10x ULN, positive EMA and correct genotype as stated previously in Chapter 7.3, as in the term "triple test" used previously (Klapp et al. 2013). For the TG2-ab test used here, 10x ULN meant 50 U/ml for Celikey® and 200 U/ml for Quanta Lite® (see 9.3.5).

In all three cohorts, the proportion of new celiac disease patients who could be diagnosed based on positive "triple criteria" with no need for biopsy was evaluated. As all subjects in the high-risk group were biopsied, the total prevalence of celiac disease in this cohort was calculated. In the moderate and low risk cohorts, only seropositive patients were biopsied. Thus, the number of possible seronegative celiac disease patients could not be assessed.

9.3 Methods

9.3.1 Demographic and clinical data (Studies I-III)

Participants in Study **I** completed self-administered questionnaires and were interviewed by a physician or a study nurse with expertise in celiac disease. Subjects were asked about the duration and type of symptoms before diagnosis and the date and place of diagnosis. According to these, changes in celiac disease diagnostics over time were examined. Moreover, questions included family history of the disease and presence of celiac disease -associated co-morbidities, including type 1 diabetes, thyroidal disease, malignancy, psychiatric diseases, and neurological (transient ischemic attacks, dementia, neuropathy, migraine, and epilepsy), GI (lactose intolerance, food allergy, gastroesophageal reflux, diverticulosis, and diaphragmatic hernia) or musculoskeletal disease (osteoporosis, osteopenia, arthritis, fibromyalgia, and prolapsed disc).

A structured and validated questionnaire was sent to the participants of Study **II** at diagnosis and after one year on a GFD. The questionnaire was developed in co-operation with celiac disease patients, the Finnish Celiac Society and Tampere Celiac Disease Research Center. Data was collected on gender, marital status, occupational and working position, site of first suspicion and diagnosis of celiac disease (primary, secondary or tertiary care), family history of the disease, clinical picture, use of pharmaceuticals and health care services, and on self-perceived health and psychological well-being. Moreover, patients were categorized geographically into subjects living in the southern/western and those living in the northern/eastern areas of Finland and based on urban or rural residence.

In Studies **I** and **II**, symptoms were categorized into three groups of clinical detection according to patients' information: presentation of GI symptoms (abdominal pain, diarrhea, abdominal distention, constipation, reflux, nausea, malabsorption, weight loss), extraintestinal symptoms (e.g. dermatitis herpetiformis, tiredness, neurological symptoms), and screening of at-risk groups (first-degree relatives of celiac disease patients, patients with autoimmune disorders such as type 1 diabetes mellitus or autoimmune thyroid disease). Malabsorption was defined as weight loss and presence of characteristic laboratory abnormalities, such as anemia, hypoalbuminemia, low folate or low vitamin B12. In Study **I**, all self-reported retrospective information was verified from the medical records.

In Study **III**, all participants in the high and moderate-risk cohorts were interviewed on their clinical presentation and family history of celiac disease. In the low-risk population-based cohort, only subjects with positive celiac disease serology were invited to corresponding interviews and the interview was carried out with volunteer

seropositive subjects. All newly diagnosed celiac disease patients were invited to a follow-up one year after the diagnosis to assess symptoms, serology, histology, and adherence to the GFD. Adequate response was defined as normalization or consistent decrease in celiac antibody values, recovery from the intestinal damage, and alleviation of (possible) symptoms.

9.3.2 Health-related quality of life (Study II)

To assess HRQoL, a structured Psychological General Well-Being (PGWB) questionnaire was used at diagnosis and after one year on a GFD (Dupuy 1984), see Appendix. The questionnaire is widely used in celiac disease research and was chosen here to enable comparison to other studies (Hallert et al. 1998; Mustalahti et al. 2002; Usai et al. 2002). It measures self-perceived health-related well-being and distress and comprises 22 questions, which can be divided into six sub-dimensions: anxiety, depressed mood, positive well-being, self-control, general health, and vitality. Each item is scored on a six-point Likert scale, measuring the patients's opinion on the issue in question. The Likert scale ranges from one extreme to the other, higher scores indicating better psychological well-being. The PGWB questionnaire was translated into Finnish (Dimenäs et al. 1996, see Appendix) after which the feasibility of the study questionnaire was pre-tested by a group of celiac disease patients. The questionnaire has also been translated into Swedish, but only the Finnish version was used in the present study as all patients were using Finnish.

Self-estimated burden of celiac disease was measured with a qualitative scale at diagnosis and after one year. The questions have been formulated in collaboration with Finnish celiac disease patients and medical experts. Self-perceived health was rated on a 4-point Likert scale as excellent, good, fair, or poor; in analysis excellent and good were combined into "good". Concern about personal health at diagnosis and after one year and burden of symptoms at diagnosis ranged from "not at all" to "extremely" on a 3-point Likert scale. The reaction to the diagnosis was rated on a 5-point Likert scale as "upset", "confused", "relieved", "no effect", and "hard to say". In analysis, upset or confused were combined and compared against relieved; "no effect" and "hard to say" were excluded from the analysis as being equivocal. To measure re-test reliability, 11 patients completed the questionnaire again after one week. Intraclass correlation coefficient was used to establish test-retest reliability. The observed kappa values ranged from 0.84 to 1.00, where values above 0.70 are regarded as excellent (Ukkola et al. 2011). In addition, a celiac disease focus group and gastroenterologists reviewed the face and content validity of the tested items (Ukkola et al. 2011).

9.3.3 Use of pharmaceutical agents and health care services (Study II)

Participants reported the number of all-cause visits to healthcare providers during the last 12 months both at diagnosis and after one year on a GFD, including visits to primary care doctors (including both private and public sectors), nurses, physiotherapists, dentists, laboratory tests, X-ray examinations, and hospital inpatient periods. Number of days absent from work during the year before and after the diagnosis was also elicited. Consumption of selected pharmaceutical agents during these same periods was reported by average number of pills per month, but antibiotics were reported as regimens per year. Besides antibiotics, the medication groups surveyed included any analgesics, drugs for dyspepsia (antacids and proton pump inhibitors, PPI), sleeping pills, herbals, vitamins or micronutrients, and psychopharmaceutical drugs referred to as antidepressants.

9.3.4 Serological tests (Study III)

For celiac disease serology, TG2-ab and EMA were assessed. The TG2-ab level was measured with EiA Celikey® ELISA (Thermo Fisher, Freiburg, Germany). According to the manufacturer, the measuring range of Celikey® is 0.1 to 128, and cut-off values < 7 U/ml are considered negative and ≥ 7 U/ml positive (Phadia 2006). However, the manufacturer recommends calibrating cut-offs in local laboratory settings, and thus, the ULN was set at 5 U/ml corresponding to previous research settings (Mäki et al. 2003; Fernandez et al. 2005; Vilppula et al. 2011).

In the moderate-risk group, TG2-ab had first been measured with Quanta Lite® ELISA test (INOVA diagnostics, San Diego, CA). To make the results comparable to the high and low risk cohorts, all 403 samples positive for Quanta Lite® and 450 randomly chosen negative samples were re-tested with Celikey®. Both kits use human recombinant TG2 as antigen and the results are given in arbitrary units. Quanta Lite® is semi-quantitative and the instructed ULN of 20 U/ml was used (FDA 2008).

For the determination of EMA, expert laboratory technicians used an indirect immunofluorescence method using human umbilical cord as antigen (Ladinser et al. 1994; Sulkanen et al. 1998). Detection of a characteristic staining pattern at a serum dilution of 1: ≥ 5 was considered positive as were further dilutions of 1:50, 1:100, 1:200, 1:500, 1:1000, 1:2000, and 1:4000.

9.3.5 Genetics (Study III)

Celiac disease associated genetics were analyzed at the Haartman Institute, Department of Medical Genetics, University of Helsinki, Finland. Participants were genotyped for HLA-alleles DQB1*02 and DQQA1*05 corresponding to HLA DQ2, and for the allele DQB1*0302 corresponding to HLA DQ8. Genotyping was performed by the DELFIA Celiac Disease Hybridization Assay (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland) or with the Olerup SSP DQ low-resolution kit (Olerup SSP AB, Stockholm, Sweden) (Koskinen et al. 2009).

9.3.6 Histological verification of celiac disease (Studies I- III)

The final celiac disease diagnosis was based on duodenal biopsy. In Study **I**, the self-reported information of the diagnosis was verified from medical records. In Study **II**, confirmation of celiac disease was based solely on the participants' information about biopsy-proven diagnoses. In Study **III**, detailed biopsy results were available. Endoscopies in the high-risk cohort of Study **III** were performed at Tampere University Hospital, at the endoscopy units of all health care levels in the family-risk cohort, and at Päijät-Häme Central Hospital in the population-based cohort. At least four biopsy samples were taken from distal duodenum. The specimens were paraffin-embedded, oriented, stained by hematoxylin–eosin, and studied under a light microscope (Taavela et al. 2013).

The Marsh-Oberhuber classification was used to evaluate the degree of villous damage (see 5.2.1). Marsh grade ≥ 2 was considered a diagnostic finding for celiac disease (Husby et al. 2012). Subjects who had only celiac-type mucosal inflammation (Marsh 1) continued on a gluten-containing diet and the diagnosis was set if Marsh ≥ 2 emerged in a follow-up biopsy.

9.3.7 Statistical analysis (Studies I-III)

For all studies statistical analysis was carried out using Statistical Package for the Social Sciences Statistics, version 20.0 (IBM, Armonk, NY). All data were blindly coded before statistical analysis. The distribution of general characteristics of the subjects was presented as percentages, medians, and ranges as appropriate. A p-value < 0.05 was considered significant.

In Studies **I** and **II,** binary logistic regression analysis was used to identify category factors associated with diagnostic delay. The results are shown as odds ratios (OR) with

95% confidence intervals (CI). Quantitative data were analyzed by Independent-Samples T-test for normally distributed variables and by Mann-Whitney U-test for skewed variables. The use of pharmaceuticals in Study **II** was divided to any use or no use of certain medicines and analyzed by Pearson's Chi-square test (Coakes 2012).

In Study **III**, the PPV of the "triple criteria" for biopsy-proven celiac disease was assessed by the equation PPV = a / (a + b), where 'a' is 'true positives', referring to biopsy proven celiac disease and 'b' is 'false positives', referring to histology without evident celiac disease. A 95% CI for PPV was calculated for each cohort according to the number of "triple positive" patients. Moreover, the lowest TG2-ab level giving a 100% PPV was determined. Independent-Samples T-test was used to compare the median TG2-ab levels of biopsied and non-biopsied "triple positive" subjects.

9.4 Ethical considerations

The study design and patient recruitment of Study **I** and high and moderate-risk cohorts in Study **III**, were approved by the Regional Ethics Committee of the Expert Responsibility area of Tampere University Hospital. The original study to collect the low-risk cohort in Study **III** was approved by the Ethics Committee of Päijät-Häme Central Hospital. The research ethics of the study followed the World Medical Association Declaration of Helsinki (WMA 2018). All participants gave written informed consent.

In Study **II**, no ethical committee approval was needed, but informed consent was obtained from all subjects after a written explanation of the objectives of the study, including ethical considerations, data protection, and assurance of the anonymous handling of the questionnaires.

10 RESULTS

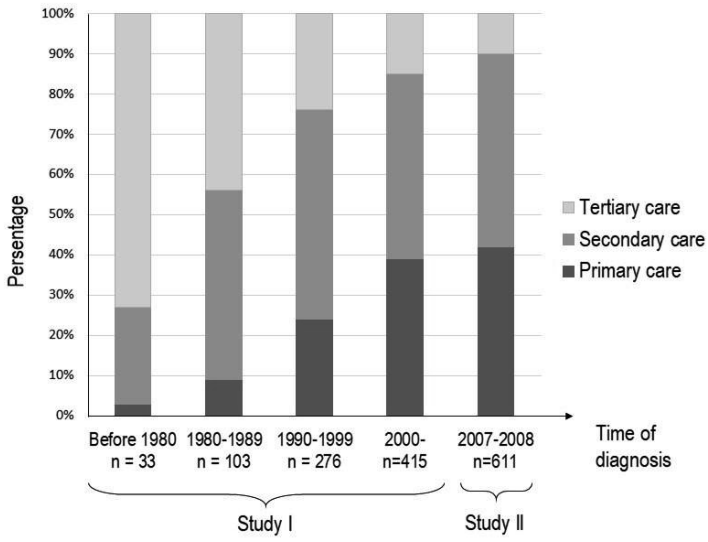
10.1 Demographic data and clinical picture (Studies I-II)

In both Studies **I** and **II**, 76% of the eligible participants were females. Median age at diagnosis was 44 years in Study **I,** and 49 years in Study **II**. In Study **I**, the main clinical presentation at diagnosis was GI in 68%, extraintestinal in 13%, and screen-detected in 19%. In Study **II,** the corresponding figures were 71%, 7% and 22%.

10.2 Changes in celiac disease diagnostics over time (Studies I-II)

In Studies **I** and **II**, 28% and 42% of the patients were diagnosed at the level of primary health care, 47% and 48% in secondary care, and 24% and 10% in tertiary care. A secular shift in the site of celiac disease diagnosis from tertiary health care towards secondary and primary care was observed in Study **I**, and this shift continued at the later time point observed in Study **II** (Figure 1).

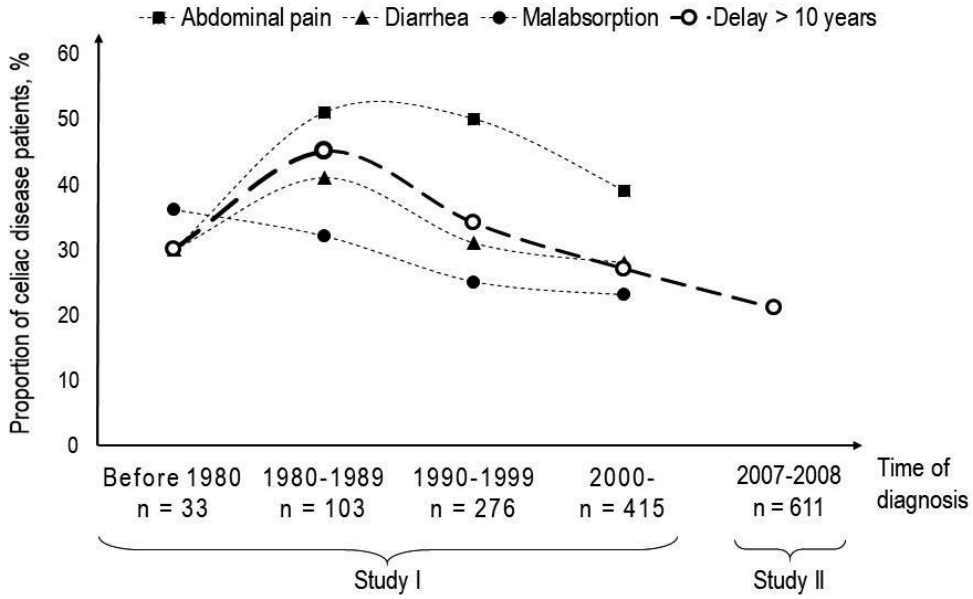
Figure 1. Shift in the proportion of celiac disease patients diagnosed at different health care levels in Studies **I** and **II** over time.



10.3 Diagnostic delay (Studies I-II)

Altogether, 32% of celiac disease patients in Study **I** reported a diagnostic delay of > 10 years. This percentage was 30% before 1980, then increased to 45% in 1980-1989 but declined again in 1990-99 and further in 2000, when it was 27% (Figure 2). In Study **II**, 54% had a delay of ≥ 3 years based on an interview at diagnosis and in 21% the delay was > 10 years (Figure 2).

Figure 2. Change in the proportion of long diagnostic delay (> 10 years) over time in Studies I and II and simultaneous decrease of classical symptoms at celiac disease diagnosis in Study I.



10.3.1 Association of diagnostic delay and socio-demographic factors (Studies I-II)

Of the socio-demographic factors investigated in Study **I**, female gender and diagnosis before the year 1997 were associated with increased risk, and family history of celiac disease with decreased risk of diagnostic delay (Table 8), whereas there was no association between delay and age at or site of diagnosis (Table 1 in original publication **I**).

In Study **II**, being a student or homemaker compared to being employed were associated with reduced risk of diagnostic delay (Table 8). By contrast, gender, age at diagnosis, marital or occupational status, position at workplace, geographical residence,

and site of first recorded suspicion or eventual diagnosis of celiac disease had no effect on the delay risk (Table 1 in original publication **II**).

10.3.2 Association of diagnostic delay and clinical picture and presence of concomitant diseases (Studies I-II)

Being diagnosed by screening versus by GI symptoms was associated with reduced risk of diagnostic delay in Studies **I** and **II** (Table 8). Extraintestinal compared to GI clinical presentation at diagnosis was a protective factor against delay in Study **II** (Table 8) but not in Study **I** (Table 1 in original publication **I**).

In the detailed analysis of GI symptoms in Study **I**, diarrhea, abdominal pain, and malabsorption were associated with long diagnostic delay in univariate analysis (Table 8). In multivariable analysis, there was still an association with malabsorption and abdominal pain, but not with diarrhea (Table 3 in original publication **I**). As a supplementary analysis for the thesis, patients were stratified according to diagnosis before or after the year 1997, when the first Finnish guidelines for celiac disease were published, and diarrhea was found to be associated with the delay before but not after this year (Table 9). The association of the delay with abdominal pain and malabsorption remained statistically significant before and after 1997 (Table 9). Of note, the proportion of classical symptoms in Study **I** and the proportion of diagnostic delay of over ten years in Studies **I** and **II** was observed to decline simultaneously over time (Figure 2). Weight loss, abdominal distension, constipation, nausea, and reflux were not associated with the delay (Table 3 in original publication **I**).

Of the concomitant chronic conditions explored in Study **I**, neurological and musculoskeletal diseases were associated with long diagnostic delay of celiac disease in univariable analysis and musculoskeletal diseases also in multivariable analysis (Table 8). There was no significant association between the long delay and malignancy, psychiatric disease, gastroenterological disease, osteoporotic fracture, type I diabetes or autoimmune thyroidal disease (Table 2 in original publication **I**).

Table 8. Factors associated with diagnostic delay of celiac disease in Studies I and II.

	OR	95% CI	P value
Long diagnostic delay (> 10 years) (Study I)			
Malabsorption[1]	2.27	1.64-3.10	< 0.001
Abdominal pain[2]	1.98	1.47-2.67	< 0.001
Female gender	1.79	1.23-2.59	0.002
Musculoskeletal disease[3]	1.61	1.12-2.20	0.003
Diagnosis before the year 1997[4]	1.55	1.15-2.09	0.004
Neurologic disease	1.54	1.02-2.34	0.043
Diarrhea	1.53	1.12-2.09	0.008
Screen-detected vs. clinical diagnosis	0.46	0.30-0.70	< 0.001
Family history of celiac disease	0.68	0.50-0.93	0.014
At least median diagnostic delay (≥ 3 years) (Study II)			
Burden of symptoms at diagnosis			
Not at all	1		
Moderate	2.99	1.18-4.55	0.015
Extreme	3.58	1.81-7.08	< 0.001
Concern about health before diagnosis			
Not at all	1		
Moderate	2.99	1.62-5.50	< 0.001
Extreme	4.20	2.10-8.39	< 0.001
Concern about health after diagnosis			
Not at all	1		
Moderate	1.70	1.17-2.26	0.005
Main clinical presentation			
Gastrointestinal	1		
Extraintestinal	0.32	0.16-0.61	0.001
Screen-detected	0.63	0.43-0.93	0.020
Reaction to diagnosis			
Confusion or devastation	1		
Relief	1.55	1.12-2.15	0.008
Self-perceived health before diagnosis			
Good	1		
Fair	1.64	1.16 – 2.33	0.005
Poor	1.70	1.03 – 2.79	0.037

[1] In multivariable analysis OR 2.19, 95% CI 1.59-3.03, P < 0.001; [2] In multivariable analysis OR 1.91, 95% CI 1.41-2.58, P < 0.001; [3] In multivariable analysis OR 1.50, 95% CI 1.09-2.06, P = 0.014; [4] Issue of first national Current Care Guidelines for celiac disease in Finland
CI, confidence interval; OR, odds ratio

Table 9. Symptoms associated with long diagnostic delay (> 10 years) in 825 adult celiac disease patients, classified by diagnosis before or after year 1997[1].

	Diagnosis before 1997 n = 347				Diagnosis from 1997 onwards n = 478			
	Delay, %	OR	95% CI	P value	Delay, %	OR	95% CI	P value
Abdominal pain								
No	28	1			34	1		
Yes	47	2.28	1.46-3.56	< 0.001	23	1.67	1.12-2.51	0.013
Diarrhea								
No	33	1			26	1		
Yes	45	1.69	1.08-2.66	0.022	31	1.26	0.80-1.98	0.312
Malabsorption								
No	29	1			25	1		
Yes	52	2.78	1.76-4.40	< 0.001	36	1.68	1.07-2.56	0.024

[1] Issue of first Finnish Current Care Guidelines for celiac disease
OR, odds ratio; CI, confidence interval

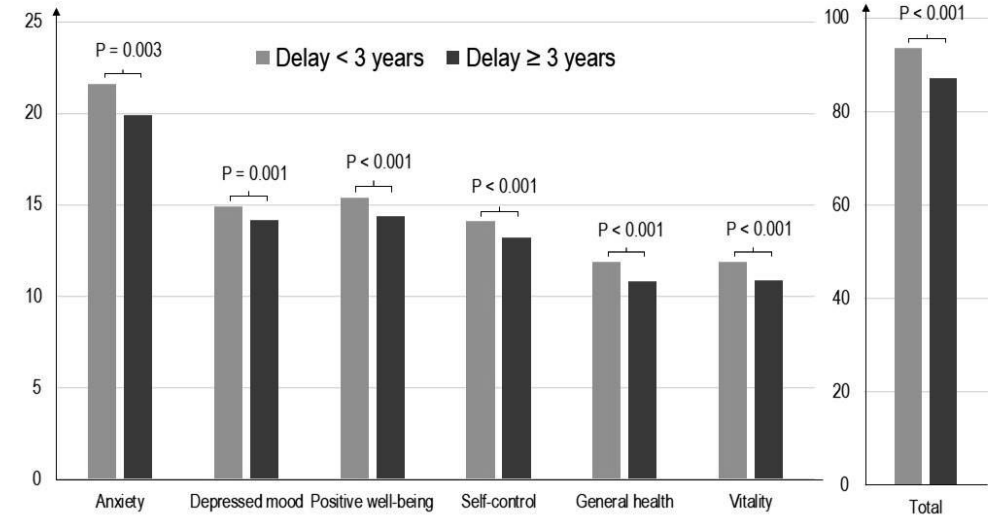
10.3.3 Association of delay and perceptions of health and well-being (Study II)

Diagnostic delay of ≥ 3 years was associated with the risk of poor or only fair self-estimated health and concern about health in Study **II** (Table 8). Furthermore, patients with delayed diagnosis more often reported moderate or extreme burden of symptoms at diagnosis, as well as relief (compared to confusion or devastation) at the diagnosis in comparison to subjects with no delay (Table 8). At follow-up one year after diagnosis, there was no longer a difference between the groups in self-perceived health, but concern about health remained more frequent in patients with the delay (Table 2 in original publication **II**).

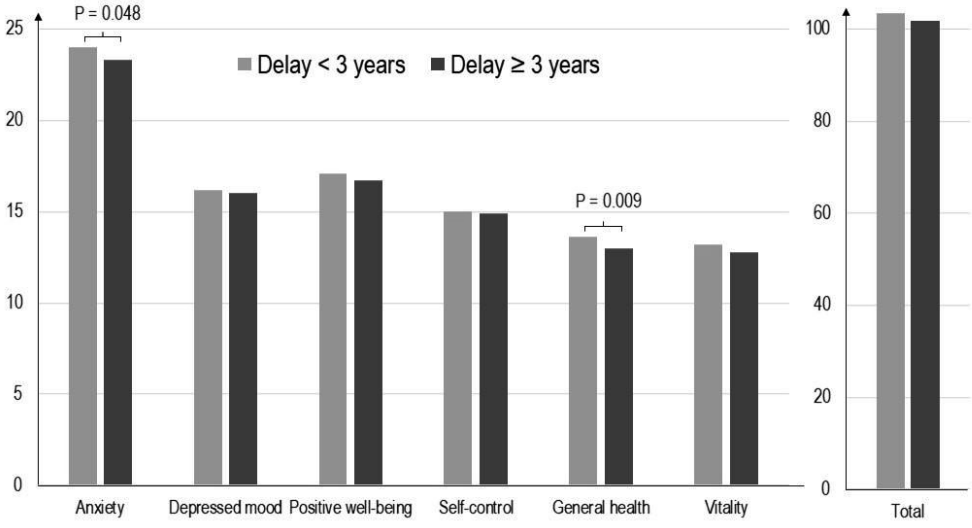
Both PGWB total and all sub-scores were significantly lower at diagnosis in patients with delayed diagnosis than in those without delay (Figure 3a). After one-year follow-up, the scores improved in both groups, but anxiety and general health scores remained poorer in the delay group (Figure 3b).

Figure 3. Mean sub-scores and total score on the Psychological General Well-Being (PGWB) questionnaire in patients according to duration of diagnostic delay of celiac disease at diagnosis (a) and after one year on a gluten-free diet (GFD) (b) in Study II. Difference between groups was compared with Independent sample T-test. Higher score indicates better well-being. A histogram is used here for clarity, but exact numbers are presented in Table 3 of original publication **II**.

a) PGWB scores at celiac disease diagnosis



b) PGWB scores one year after celiac disease diagnosis on a GFD



10.3.4 Association of delay and use of health care services and pharmaceutical agents (Study II)

Before diagnosis, visits to primary health care doctors, dentists, and physiotherapy were more common, and X-ray examinations less common among those with delay of ≥ 3 years (Table 10). There was no association between delay and the number of nurse visits, laboratory tests or hospital treatment periods prior to diagnosis. After diagnosis, the frequency of visits to primary health care doctors decreased in both groups but remained significantly more frequent in those with delayed diagnosis (Table 10). Dentist and physiotherapy visits and X-ray and laboratory examinations were increased in those with delay in the year following the diagnosis. Hospital treatment periods and nurse visits were still not associated with delay. The median number of visits to other health care providers than primary health care was zero in both groups. During the year prior to and following celiac disease diagnosis, days of sickness were more frequent in subjects with diagnostic delay, but on a GFD, the number of sickness days increased in both groups.

The proportion of subjects using analgesics, drugs for dyspepsia, and antidepressants was increased in patients with diagnostic delay compared to those without delay during the year before diagnosis. (Table 11) The increased use of antidepressants and drugs for dyspepsia persisted when on a GFD.

10.4 Response to GFD (Study II)

All 559 patients attending follow-up in Study **II** were on a GFD, but 64 (11%) reported occasional lapses. On GFD, symptoms disappeared completely in 130 (23%), diminished in 337 (60%), remained unchanged in 71 (13%), and increased in three (0.5%) subjects. The risk of symptoms persisting on a GFD was increased in those with diagnostic delay (OR 1.61, 95% CI 1.08-2.42, P = 0.022).

Table 10. Use of health care services in the year prior to and following the diagnosis of celiac disease in 611 adults with celiac disease, categorized by length of diagnostic delay in Study II.

	Delay ≥ 3 years		Delay < 3 years		
	Median	Mean (range)	Median	Mean (range)	P value[1]
Before diagnosis, n=609					
Days of sickness	0	9 (0-200)	0	5 (0-180)	0.020
Dentist visits	1	1 (0-20)	0	1 (0-10)	< 0.001
Hospital treatment periods	0	0 (0-3)	0	0 (0-4)	0.644
Laboratory tests	0	2 (0-20)	0	2 (0-30)	0.075
Visits to nurses	0	1 (0-12)	0	0 (0-8)	0.101
Visits to primary care doctors	3	4 (0-31)	2	3 (0-30)	0.002
Physiotherapy visits	0	2 (0-30)	0	1 (0-23)	0.001
X-ray examinations	0	0 (0-10)	0	0 (0-38)	0.014
After diagnosis, n=557					
Days of sickness	0	10 (0-356)	0	7 (0-365)	0.021
Dentist visits	0	1 (0-12)	0	1 (0-8)	0.019
Hospital treatment periods	0	0 (0-3)	0	0 (0-15)	0.797
Laboratory tests	0	2 (0-14)	0	1 (0-12)	0.003
Visits to nurses	0	1 (0-15)	0	0 (0-20)	0.751
Visits to primary care doctors	2	3 (0-20)	2	2 (0-15)	< 0.001
Physiotherapy visits	0	1 (0-30)	0	1 (0-70)	0.002
X-ray examinations	0	0 (0-10)	0	0 (0-3)	< 0.001

[1] Mann-Whitney U-test
CI, confidence interval

Table 11. Use of pharmaceutical agents (number of pills per month) during the year before and after the diagnosis of celiac disease diagnosis in Study II.

	Delay ≥ 3 years		Delay < 3 years		
	Median	Mean (range)	Median	Mean (range)	P value[1]
Before diagnosis, n = 609					
Drugs for dyspepsia	0	5 (0-60)	0	2 (0-50)	< 0.001
Antidepressants	0	3 (0-90)	0	1 (0-30)	0.015
Analgesics	2	8 (0-250)	2	5 (0-100)	0.005
Sleeping pills	0	2 (0-30)	0	1 (0-31)	0.300
Vitamins, micronutrients, herbal products	3	19 (0-150)	0	16 (0-120)	0.250
Antibiotics[2]	0	1 (0-10)	0	1 (0-10)	0.080
After diagnosis, n = 559					
Drugs for dyspepsia	0	3 (0-120)	0	2 (0-30)	0.006
Antidepressants	0	3 (0-120)	0	1 (0-60)	0.027
Analgesics	2	7 (0-300)	2	4 (0-140)	0.139
Sleeping pills	0	2 (0-31)	0	1 (0-30)	0.243
Vitamins, micronutrients, herbal products	10	24 (0-210)	10	22 (0-150)	0.818
Antibiotics[2]	0	1 (0-6)	0	0 (0-10)	0.315

[1] Mann-Whitney U-test; [2] Reported as number of courses per year
CI, confidence interval

10.5 Serology-based diagnostic criteria (Study III)

10.5.1 Demographic data

In Study **III,** the proportion of females was higher (71%) in the high-risk cohort compared to 57% and 53% in the moderate and low-risk cohorts respectively. Due to the original study design exploring elderly population, participants in the low-risk cohort were older than those in the moderate or high-risk cohorts. Of the 90 newly detected celiac disease patients fulfilling the "triple criteria", 80% in the high-risk cohort, 65% in the moderate-risk cohort and 64% in the low-risk cohort were females. The prevalence of celiac disease in family members was 22% in the high-risk cohort, 100% in the moderate-risk cohort, and 29% in the low-risk cohort. In the clinically suspected cohort, all new celiac disease patients were symptomatic. When new patients in the moderate and low risk cohorts were interviewed at biopsy, 71% and 57% respectively reported some clinical symptoms despite being screen-detected.

10.5.2 Accuracy of "triple positivity" for celiac disease

In the high-risk cohort in Study **III**, 133 out of the 421 participants had positive TG2-ab with Celikey® (Figure 4). Altogether, 60 (45%) out of these 133 had TG2-ab ≥ 10x ULN and correct HLA genotype. EMA was negative in one, thus 59 fulfilled the triple positivity criteria. Celiac disease was found in 56 (95%) of them in the initial endoscopies (Figure 4). The three "triple positive" subjects having initially Marsh I lesions continued one further year on a gluten-containing diet, during which all of them developed Marsh III lesions and received a celiac disease diagnosis. Hence, all 59 were eventually diagnosed, giving the serology-based criteria a PPV of 100% (95% CI 94-100%) in subjects with high pretest probability (Figure 4).

Out of the 2,357 family members in the moderate-risk cohort, 93 had TG2-ab positivity with Celikey® and 24 (26%) of these fulfilled the "triple criteria" (Figure 4). Altogether seven out of these 24 did not attend the endoscopy and were excluded from further analysis: five declined the procedure, one deceased, and one had reduced dietary gluten consumption. Celiac disease was confirmed in all remaining 17 subjects and the PPV of the criteria was thus 100% (95% CI 82-100%) in this cohort, too (Figure 4). TG2-ab values at baseline did not vary between biopsied and non-biopsied triple positive subjects (Celikey® median 83 vs. 90 U/ml, $p = 0.658$). In comparison, when the family members were first tested with the other TG2-ab assay used in the study, QuantaLite®, 93 subjects were found to be positive and in 29 out of the 93 the values were ≥ 10x ULN (≥200 U/ml). All these 29 had positive EMA and correct HLA. Twenty "triple positive" patients had biopsy results available, all Marsh III, also resulting in a PPV of 100% (95% CI 84-100%) for the "triple positivity" with QuantaLite®.

In the population-based low-risk cohort, 49 out of the 2,722 subjects had elevated TG2-ab with Celikey®, the value exceeding 10x ULN in 16 (33%) (Figure 4). All 16 had positive EMA, but two subjects did not to want to proceed to HLA testing and endoscopy. Correct HLA and histologically confirmed celiac disease was established in the remaining 14 subjects, and PPV for "triple positivity" was again 100% (95% CI 78-100%) (Figure 4). The TG2-ab values of the two non-biopsied subjects with high TG2-ab were 100 and 82 U/ml, which were comparable with the values of those undergoing endoscopy (median 91 U/ml).

All non-biopsied subjects with TG2-ab ≥ 10x ULN and positive EMA had correct HLA in the high and moderate-risk cohorts. In the low-risk cohort HLA was not determined from subjects refusing to undergo biopsies.

10.5.3 Lowest TG2-ab values reaching 100% PPV for celiac disease

Besides testing the accuracy of 10x ULN, the lowest TG2-ab value resulting in 100% PPV for histologically proven celiac disease was calculated in all three cohorts in Study **III**. In the high and moderate-risk cohorts, this was the case in all biopsied subjects with TG2-ab ≥ 7 U/ml, equaling 1.4x ULN Celikey®. In the low-risk cohort, 100% PPV was achieved at 17 U/ml (3.3x ULN with Celikey®). With positive EMA, the 3.3x ULN threshold would have allowed setting a biopsy-free diagnosis in 113 (71%) celiac disease patients of the high-risk group, in 59 (69%) of the moderate-risk group and in 22 (76%) of the low-risk group respectively. Also, all (100%) of the biopsy-proven patients with TGA < 10x ULN had correct HLA genotype.

Participants in the moderate-risk cohort were initially tested with QuantaLite®, before running the serum with Celikey®. In this pre-selected at-risk cohort, QuantaLite® gave the lowest TG2-ab level for 100% PPV at 106 U/ml, equaling 5.3x ULN. Again, these subjects had positive EMA and all who had their genotype tested had either HLA DQ2 or DQ8. Thus, 54 (64%) of the new celiac disease patients could have been diagnosed by QuantaLite® 5.3x ULN.

10.5.4 New celiac disease patients

The total number of newly detected biopsy-proven patients in Study **III** was 274. In the high-risk cohort all were biopsied, and celiac disease was found in 160 (38%) subjects. Only subjects with positive TG2-ab in the family risk and population cohorts were further referred to endoscopies, and 85 (3.6%) and 29 (1.1%) of the total cohorts were found to have biopsy-proven celiac disease respectively. Of all patients with newly diagnosed celiac disease, the "triple criteria" were fulfilled in 90 (33%) (Table 3 in original publication **III**). These 90 "triple positive patients" had no other endoscopic or histological findings than uncomplicated celiac disease in the diagnostic biopsies.

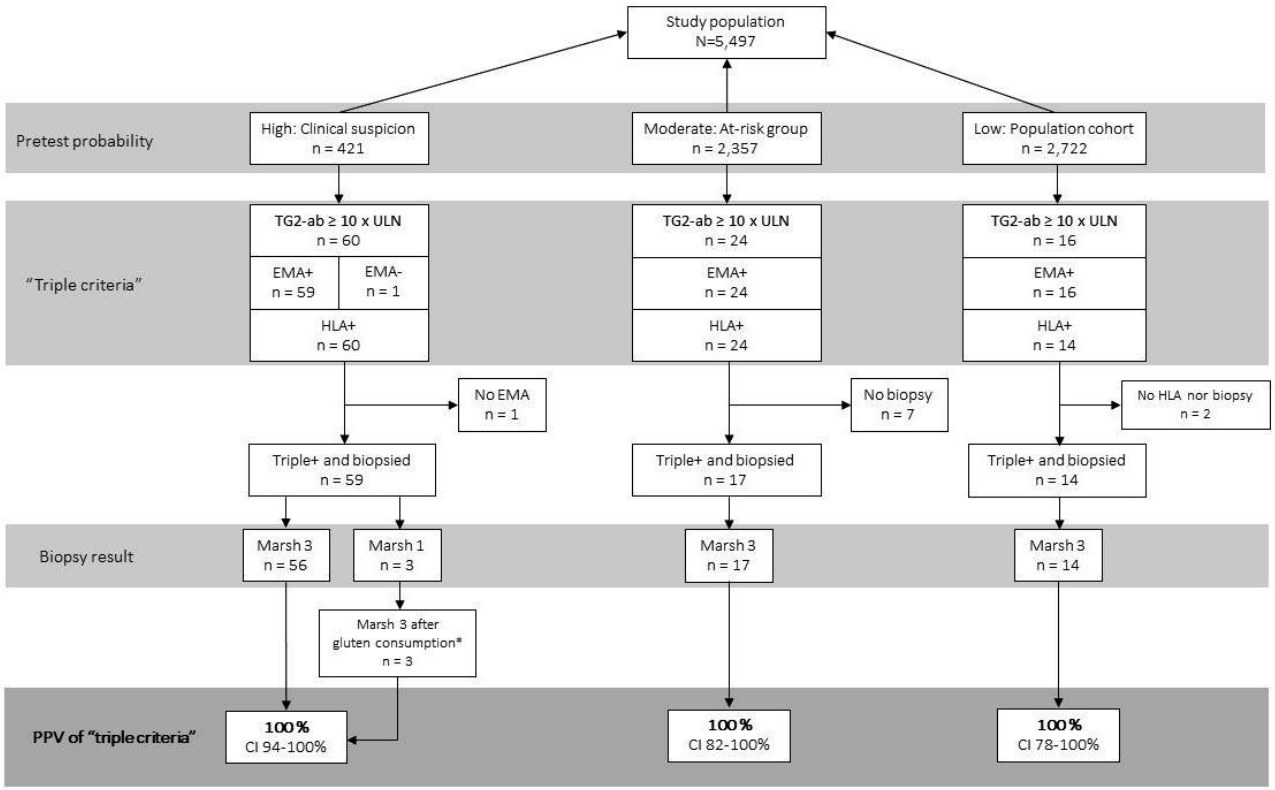
10.5.5 Follow-up

Altogether, 67 (74%) out of the 90 "triple positive" subjects in Study **III** underwent follow-up assessment as a part of the study protocol one year after the diagnosis, and the remaining 28 patients were followed-up elsewhere by their local health care providers. All but one (99%) of the 67 followed-up patients reported strict adherence to the GFD and showed a clear histological and serological response. Two (3%) of the histologically and serologically responsive patients reported occasional abdominal pain

on the diet. In the follow-up biopsies of the 66 dietary compliant patients, no signs of RCD, malignancies or other intestinal pathology were found, but all had a mucosal morphology of healing celiac disease. The sole non-compliant patient did not undergo follow-up biopsies in the present study.

Of new celiac disease patients who did not fulfil the "triple criteria", one was found to develop RCD and one had lymphoma. Both were from the symptom-based high-risk cohort and had negative TG2-ab. In the moderate and low risk groups no such complications were identified.

Figure 4. Study design and main results of the positive predictive value (PPV) for "triple positive" non-biopsy diagnostic criteria of celiac disease in three adult cohorts with different pretest probability. Modified from the original publication **III**.
Abbreviations: EMA+, positive endomysium antibodies; GFD, gluten free diet; HLA, human leukocyte antigen; TG2-ab, transglutaminase 2 antibodies; ULN, upper limit of normal. *Patients continued normal gluten intake until follow-up biopsies after one year were performed.



11 DISCUSSION

11.1 Diagnostics of celiac disease over time

One major observation of the present study was that the proportion of celiac disease diagnoses increased in primary health care and decreased in tertiary care from the 1960s to the 21st century. Meanwhile, a vast rise in the diagnostic yield has been observed (Virta et al. 2009). These shifts have been partially simultaneous with the issue of the first Finnish national guidelines for diagnostics and management of celiac disease in 1997 (Celiac disease: Current Care Guideline 1997). The guidelines particularly aimed to improve diagnostic yield in primary health care (Collin et al. 2007; Virta et al. 2009), and the present findings indicate that they have been successful.

During the same period, the proportion of patients with long diagnostic delay decreased markedly. Although this cannot be attributed with certainty to the guidelines, regular training of health care professionals has very likely contributed to the awareness of celiac disease at all health care levels (Collin et al. 2007; Virta et al. 2009). Another likely contributor to the reduced delay is the introduction of accurate serological tests in the 1980s and 1990s, lowering the threshold to seek for celiac disease among patients with milder symptoms and to screen the at-risk groups (Chorzelski et al. 1983; Dieterich et al. 1997). The discovery of sensitive antibodies may also explain the paradoxically lower proportion of long diagnostic delays before 1980 compared to 1980-1989: in the earlier era the heterogenous clinical presentation was not recognized and only patients with severe symptoms were diagnosed. In line with this, Singh et al. have proposed that limited availability of serological tests may account for missed or delayed diagnoses (Singh et al. 2014). Then again, in the UK the diagnostic delay was not reduced from 2006 to 2015 despite improved access to serological and endoscopic services and increasing awareness of celiac disease (Violato and Gray 2019). Over a longer period from the 1950s to 2006 in the USA, a decrease in mean diagnostic delay was observed (Rampertab et al. 2006). In light of these inconsistent results, more studies from different countries are still needed.

11.2 Factors associated with diagnostic delay

11.2.1 Underlying aspects

Studies **I** and **II** offered a few possible explanations for the diagnostic delay in celiac disease. Females were at greater risk for long delay (> 10 years) in Study **I**, but not for delay ≥ 3 years in Study **II**. In line with this, one study has reported longer delay in females (Vavricka et al. 2016). Mislabeling of celiac disease as IBS has previously been proposed to cause the increased delay in females (Card et al. 2013). The gender association here may also be explained by most males having been found by screening, which was associated with reduced risk for delay. The proportion of males was much lower than in the known gender distribution in celiac disease (Lohi et al. 2007). With such selection, it can be assumed that a substantial part of males with celiac disease remain undiagnosed or have a lower research participation rate.

Importantly, most socio-demographic factors had no association with delay, among them marital status, employment status, or geographical area of residence. In the USA, limited access to endoscopies and lower clinical prevalence in rural compared to urban areas has been observed (Haakenstad et al. 2019), and lower socio-economic status has been discovered as a diagnostic barrier (Hafner-Eaton 1993). Here, the modest associations between delay and social factors may demonstrate more equal access to health care services and alertness to celiac disease throughout Finland. One opposing explanation, however, could be that unemployed people, who typically have a higher threshold to access health care services (Leung and Caplan 2016), did not participate in the study. This assumption is emphasized by the finding that 70% of unemployed participants had delayed diagnosis, but as their number was small, their effect remained statistically insignificant. The finding of homemakers or students having reduced risk for delay may imply that they, by contrast, may have better access to health care providers (Kunttu 2013).

The risk for delayed diagnosis did not differ between health care levels, which is encouraging and may again support the usefulness of nationwide diagnostic guidelines aimed particularly at primary health care. At global level, despite generally increasing awareness, the knowledge of and adherence to guidelines is variable and often insufficient among general practitioners in Europe and Asia (Assiri et al. 2015; Jinga et al. 2018; van Gils et al. 2018; Malik et al. 2019). Hence, arrangements to raise awareness and to train professionals are needed, and communication between different health care levels is essential, especially in the case of updates to existing guidelines (Fueyo-Diaz et al. 2019). Such actions to shorten the delay may not only benefit the patient, but also

lead to significant savings for the health care system (Green et al. 2008; Hershcovici et al. 2010).

Presence of co-existing neurological or musculoskeletal diseases was associated with delay. Due to the retrospective data collection, it is difficult to determine whether celiac disease preceded the other disease or vice versa. Musculoskeletal and neurological manifestations are identified signs of untreated celiac disease (Jackson et al. 2012), and it is possible that delayed diagnosis may exacerbate these. Indeed, delay was recently found to be associated with non-GI symptoms and reduced BMD (Paez et al. 2017). Conversely, co-existing chronic illnesses may mask celiac disease and predispose to delayed diagnosis (Lauret and Rodrigo 2013). Other concomitant conditions were not associated with long delay here, possibly due to low-threshold screening for celiac disease in other autoimmune diseases. There were some trends for delay, particularly with psychiatric disease, but no statistically significant differences were found. However, patients with any psychiatric disease were underrepresented (4%), as e.g. the prevalence of clinical depression is 10% in the general population of Finland (Markkula et al. 2015) and 16% in USA (Garud et al. 2009). The reported prevalence of deperession in celiac disease patients varies widely, from 6 to 69%, depending on study methods, the risk being increased by comorbid diseases (Garud et al. 2009). Maintaining a GFD > 5 years has been associated with lower risk for depressive symptoms (van Hees et al. 2013). Thus, those with psychiatric disease in the present study may actually be at risk for delayed celiac disease diagnosis, especially as the use of psychopharmaceuticals before diagnosis was increased in the delay group, but more studies on this issue are needed.

11.2.2 Consequences of delay

Diagnostic delay was found to be associated with impaired HRQoL both before and after diagnosis, as in earlier research (Norström et al. 2011; Paarlahti et al. 2013; Zingone et al. 2015). Pathophysiological mechanisms of impaired psychological health could include, for example, poorer QoL due to malabsorptive symptoms (Addolorato et al. 2001), TG2-ab-induced subclinical thyroid disease (Carta et al. 2002), impaired central monoamine metabolism caused by tryptophan malabsorption (Hallert et al. 1982), and cerebral hypoperfusion (Addolorato et al. 2004). These mechanisms may be enhanced by long-term untreated celiac disease. Delay-associated psychological morbidity could accordingly explain the increased use of antidepressants before diagnosis.

Even after one year on a GFD, patients with delayed diagnosis experienced more anxiety and concerns about their health. Previous studies have shown that deteriorating QoL in untreated celiac disease is comparable to that with stroke (Clarke et al. 2002; Gray and Papanicolas 2010) but improves to the level of general population on a GFD

(Gray and Papanicolas 2010; Ukkola et al. 2011). Here, the alleviation of symptoms was insufficient in patients with delayed diagnosis. Also according to previous evidence, delay has been associated with persistent symptoms at least one year after diagnosis (Vavricka et al. 2016) or even on a long-term GFD (Paarlahti et al. 2013). Because long duration of symptoms before diagnosis may slow down the alleviation of symptoms (Vavricka et al. 2016), it is possible that the delay may also prolong psychological recovery. However, the association of delay and impaired well-being after diagnosis may also be due to depression acting as a confounding factor (Roos et al. 2011).

Visits to primary health care were increased in those with delay before and after diagnosis. The true significance of this finding remains somewhat unclear since the mean numbers of visits were rather low and the difference between groups in numbers was quite small. Repeated visits to doctors and increased use of analgesics, drugs for dyspepsia and psychopharmaceuticals may still reflect unspecific and vague symptoms that have not been recognized as celiac disease (Stasi et al. 2016). Because use of PPI's has been reported to increase the risk of fractures, the burden of excess use of drugs is not only financial but may also have unwanted long-term health effects (Pasternack et al. 2018). The increased primary health care visits and use of medicines after the diagnosis may also be connected to persistent symptoms or other chronic diseases associated with delay (Rubio-Tapia et al. 2009; Paarlahti et al. 2013). Besides primary care, visits to other health care providers were increased in the delay group, but as the number of these was very low, their clinical relevance remains equivocal.

Delay was associated with increased days of sickness both during the year before and the year after celiac disease diagnosis. In comparison, other chronic GI diseases, particularly if involving diarrhea, have also been associated with impaired work ability (Kim et al. 2017; Dasari et al. 2019). It has been suggested that once celiac disease patients initiate a GFD, fewer days are missed from school and work (Mearns et al. 2019). Here, the median number of sickness days increased in both groups in the year following the diagnosis, but the figures were skewed by some individuals being absent from work for the whole year. Although the reasons for this severe work disability remain unclear, ongoing symptoms associated with delay may impair the ability to work (Paarlahti et al. 2013).

Even though no significant relation between malignancies and diagnostic delay was seen here, one study has reported an association between delay and increased risk of mortality, mostly due to NHL (Corrao et al. 2001). Simultaneously with the decrease of long diagnostic delays, the risk of severe complications of celiac disease such as NHL and RCD have decreased when comparing earlier studies to more recent ones (Holmes et al. 1989; Rampertab et al. 2006; Ilus et al. 2014). The association with delay remains speculative, and there may be many other factors affecting the reduced risk of severe complications. Predictors for RCD, including older age, male gender, and seronegative

celiac disease have previously been assumed to be associated with long diagnostic delay (Ilus et al. 2014). Here, however, these risk factors of RCD were not related to delay. Of note, however, the present study was not specifically designed to assess malignancies or mortality, and the number of patients with severe complications remained low.

11.3 Serology-based diagnostic criteria in adults

11.3.1 Accuracy

Study **III** found serology-based "triple criteria" to have a 100% PPV for celiac disease regardless of the clinical presentation or assumed pretest probability for the disease. This shows that a significant part of adult celiac disease patients could be accurately diagnosed without biopsy, in this study 33%. The serology-based criteria have been fulfilled in approximately 50% of pediatric patients having symptoms at diagnosis (Gidrewicz et al. 2015; Werkstetter et al. 2017). Hence, the persentage of possible biopsy-omitting patients is similar at least in symptomatic adults and children, as 48% of celiac disease in the high-risk cohort were "triple positive".

Although the manufacturer gives an ULN of 7 U/ml for Celikey® , the low ULN of 5 U/ml used here was based on earlier research, aiming to test the accuracy of the triple criteria also at the lower limit of the 10x ULN in the population in question (Fernandez et al. 2005; Vilppula et al. 2009; Werkstetter et al. 2017). In the moderate-risk cohort, 100% PPV was already achieved at 1.4x ULN with Celikey® and at 3.3x ULN with QuantaLite®. The difference between kits reflects the varying quality, specificity, sensitivity, and affinity of the TG2 antigens used in the commercial assays (Phadia 2006; FDA 2008). Currently, TG2-ab tests are not standardized and their calibration curves and optimal ULNs vary, but test-specific thresholds would make diagnostic criteria too complicated (Husby et al. 2012; Husby et al. 2020). Thus, the 10x ULN together with the confirmation of positive EMA has been chosen as a safe limit for the non-biopsy approach to avoid false positive diagnoses resulting from less specific TG2-ab tests and technical errors (Husby et al. 2012; Werkstetter et al. 2017). It is not certain whether the accuracy of the triple criteria observed here can be extrapolated to all commercial antibody assays. However, the cut-off levels for the majority of TG2-ab tests throughout Europe have been explored in laboratories with well-described test norms (Husby et al. 2012; Murch et al. 2013), and at least in pediatric use the serology-based criteria have proven to be accurate in broad clinical use (Werkstetter et al. 2017; Wolf et al. 2017). On the other hand, since the tests have been validated mostly in Western populations, the

results may not be directly generalizable to other populations and geographical areas (Barada et al. 2010).

As all patients with high TG2-ab also had positive EMA, which thus does not seem to add diagnostic accuracy, one might question the role of this non-automatized test in non-biopsy diagnosis. The high specificity of positive EMA has its important function in confirming the high TG2 value among the vast variability of test kits (Husby et al. 2012; Husby et al. 2020). However, TG2-ab measurement can be considered sensitive enough as a first-line screening tool for celiac disease without the need to assess EMA before confirming positive TG2-ab values (Husby et al. 2012; Celiac disease: Current Care Guideline 2018).

Testing for HLA had no additional diagnostic value in adults with high TG2-ab and positive EMA. Similar concordance of high antibody values and presence of HLA DQ2 or DQ8 has been seen throughout pediatric studies (Werkstetter et al. 2017; Wolf et al. 2017). Accordingly, in the very recently updated ESPGHAN criteria HLA verification is no longer a part of the diagnostic algorithm (Husby et al. 2020). Besides not adding to diagnostic value, obligatory HLA typing is prone to misuse, and is not even always available. However, HLA-genotyping will preserve its high negative predictive value in excluding celiac disease in unclear cases, e.g. subjects with fluctuating TG2-ab positivity or unequivocal histology, or if a new TG2-ab test comes on the market (Egner et al. 2012; Werkstetter et al. 2017; Husby et al. 2020).

11.3.2 Benefits and risks of omitting biopsies

The results of Study **III** support an important move forward in celiac disease diagnostics. Harnessing the serology-based criteria to clinical practice would allow at least 30% of adult patients to avoid the burden of endoscopy. These patients would also be released from the symptoms caused by the ongoing gluten-containing diet while waiting for the endoscopy. The greater availability of serological tests compared to endoscopy may also lead to shorter diagnostic delay in celiac disease. Besides, many of those who would refuse to undergo biopsies could be diagnosed, further increasing the diagnostic yield.

The costs per one positive biopsy proven celiac disease diagnosis vary between 900 and 45,000 euros, depending on the diagnostic strategy, and using endoscopies ineffectively as a screening tool increases the costs tremendously (Mearns et al. 2019). The costs of a TG2-ab test are only 5-15 euros and those of EMA around 25 euros, compared to approximately 1,200 euros attributed to the endoscopy and handling of biopsies (Mearns et al. 2019). Therefore, omitting the biopsy would save approximately 95% of the diagnostic costs of one single celiac disease diagnosis, and much more through not using biopsy to exclude celiac disease (Paul et al. 2018; Mearns et al. 2019).

Instead, endoscopic resources could be prioritized for investigations having diagnostic or surveillance value such as those needed for patients with IBD (Molodecky et al. 2012).

Some gastroenterologists are worried that omitting endoscopies would result in missing serious complications (Efthymakis et al. 2017; Marks et al. 2018). No concomitant diseases besides celiac disease were found here, neither at diagnosis nor in the follow-up biopsies. Likewise, co-morbidities in the diagnostic endoscopy have been very rare in the few existing adult studies, although there have been no studies designed particularly to investigate this issue (Salo et al. 2008; Tortora et al. 2014; Efthymakis et al. 2017). Regarding the most serious conditions, histopathological findings of the baseline biopsy do not predict development of RCD or lymphomas. Instead, the diagnosis of RCD begins from clinical non-response to the GFD after 6-12 months and a subsequent endoscopy (Rubio-Tapia and Murray 2010; Ilus et al. 2014). Systematic follow-up to ensure dietary adherence and alleviation of symptoms is still needed, and any concerns in clinical recovery on a GFD should lead to further investigations. Moreover, in every case with alarming symptoms or other atypical characteristics, the clinician should always proceed to endoscopy since celiac disease may also co-exist with another GI disease.

As Study **III** was conducted retrospectively, it was not possible to survey the patients' attitudes towards the serological diagnosis. One concern about the diagnostics among general practitioners has been that patients may not accept the diagnosis (Marks et al. 2018). Possible lack of trust in the diagnosis may reflect a more widespread phenomenon of patients blaming primary health care, especially for not finding correct diagnoses in time (Kostova et al. 2014). Therefore, in the ESPGHAN criteria it is emphasized that the physician in charge should discuss with the parents and patient to ensure mutual understanding and acceptance of the serology-based diagnosis (Husby et al. 2012; Husby et al. 2020). An accepted diagnosis is largely a product of communication, beginning from physicians themselves having confidence in the diagnosis.

It is also feared that removing diagnostics from specialized care would lead to both under- and overdiagnosis (Biagi et al. 2009; Marks et al. 2018). An Italian study observed that a substantial number of patients referred to a tertiary center had previously been erronoeusly diagnosed with celiac disease (Dewar et al. 2012; Ianiro et al. 2016). Such overdiagnosis represents limited awareness and adherence to guidelines and happens irrespective of the site of diagnosis (Assiri et al. 2015; Jinga et al. 2018; van Gils et al. 2018; Malik et al. 2019). Besides poor knowledge, there can be financial reasons for underdiagnostics. The decision not to undergo diagnostic testing may be affected by the refusal of insurance company coverage, at least in the USA (Fasano et al. 2003; Fasano 2005). With accurate and simple non-biopsy criteria being under development, fear of ignoring guidelines should not be the reason to restrict the diagnostics to gastroenterology units. The cost-effective and reasonable way to minimize the burden

of celiac disease misdiagnosis is to strive for greater adherence to guidelines. There is evidence that effective and correct diagnostics depends on education and that case-finding can be improved by an active role of primary care (Hin et al. 1999; Holmes et al. 2017).

11.4 Role of clinical presentation in celiac disease diagnostics

11.4.1 Clinical presentation and diagnostic delay

GI presentation compared to being screen-detected was associated with increased risk of long (> 10 years) or at least median (≥ 3 years) diagnostic delay in Studies **I** and **II** respectively. Interestingly, particularly abdominal pain and classical symptoms diarrhea and malabsorption were more frequent. When explored in more detail, both the proportion of patients having diarrhea and of those with long delay decreased from earlier decades to the present. The association of diarrhea and delay was only seen before 1997. Recent studies have demonstrated that celiac disease has become milder and classical presentation less frequent than before (Rampertab et al. 2006; Gray and Papanicolas 2010; Kivelä et al. 2015; Violato and Gray 2019). The discovery of sensitive antibodies has enabled the detection of the disease at earlier stages together with discovering the wide range of symptoms. Thus, serological testing and increased awareness may be accountable for both the change in the clinical picture and decreasing diagnostic delay. The association of diagnostic delay and diarrhea before but not after 1997 may also be explained by recall bias, as the duration of symptoms before diagnosis may be overestimated in patients with difficult symptoms. Malabsorption and abdominal pain were associated with delay across the decades studied. Being unspecific, these symptoms may have been mislabeled as some other conditions, such as IBS or menorrhagia (Irvine et al. 2017; Spencer et al. 2017). One study has found non-GI clinical presentation to be associated with diagnostic delay, but the most common "non-GI" sign there was anemia, which here was regarded as a malabsorptive GI symptom (Paez et al. 2017).

In Study **III**, 38% of patients with high clinical suspicion were eventually found to have celiac disease. In comparable clinical settings the prevalence of the disease has varied substantially from 5% to 50% (Collin et al. 2002; Hopper et al. 2007; Sugai et al. 2010). As the definition of "high risk" is not unanimous even among researchers, it is even less so among practitioners without expertise in celiac disease (Hujoel et al. 2018). Many of the detected patients in the moderate or low-risk cohorts had in fact "typical" symptoms, perhaps already for several years, but had not approached health care

personnel due to them. Conversely, a large part of the moderate and low risk patients was asymptomatic and detectable only by screening. These patients represent the global reality, as only a minority of celiac disease patients are detected worldwide (West et al. 2003). The present results further emphasize that symptom-based case finding is a poor approach to the early detection of celiac disease (Rosen et al. 2014; Hujoel et al. 2018).

11.4.2 Role of clinical presentation in diagnostic accuracy

For children, the accuracy of non-biopsy criteria was originally proven in symptomatic patients (Husby et al. 2012). Here, the "triple criteria" worked precisely regardless of the diagnostic approach or assumed pretest risk for celiac disease. Specifically, the criteria functioned equally in adults with and without obvious symptoms, even though the number of asymptomatic "triple positive" patients was small. Restriction of the criteria to symptomatic patients was originally thought to improve diagnostic accuracy (Husby et al. 2012), but recent evidence supports the efficacy of the criteria equally across the range of clinical presentations (Trovato et al. 2015; Werkstetter et al. 2017; Paul et al. 2018). Accordingly, the revised ESPGHAN no-biopsy guidelines allow an option for diagnosis to be extended to asymptomatic children (Husby et al. 2020). The prospective studies which included asymptomatic patients found the criteria also to apply in these, although they mostly belonged to risk groups (Lionetti et al 2014; Vrieznga et al. 2014; Werkstetter et al. 2017; Wolf et al. 2017). However, the recommendation was left conditional, stating that the PPV of the criteria may be lower in asymptomatic children, which should be considered while making the decision to omit the biopsy (Husby et al. 2020).

Altogether, the separation of patients to symptomatic and asymptomatic is rather artificial. Only few people are always completely symptomless, and besides, Study **III** and previous evidence show that screen-detected patients frequently have unrecognized symptoms (Agardh et al. 2015). On the other hand, asymptomatic signs such as anemia or osteoporosis often lead to the diagnosis of clinically detected patients (Mustalahti et al. 1999; Kurppa et al. 2014). Because the clinical and histological presentation correlate poorly and symptoms may fluctuate or not be recognized until their alleviation on a GFD, the presence of symptoms and their association with celiac disease is challenging to define (Kurppa et al. 2014). "Classical" symptoms are also common in general population and their PPV is low (Rosen et al. 2014). Especially in "triple positive" subjects, the requirement of symptoms does not add to the diagnostic accuracy and can thus be considered unnecessary (Husby et al. 2020).

11.5 Strengths and limitations of the study

On main strength of the study was the availability of large and well-defined patient cohorts. Studies **I–II** included considerable numbers of celiac disease patients who had been diagnosed at all health care levels with various clinical presentations. In Study **I**, the diagnoses and relevant medical data were confirmed from medical records. Study **II** was carried out prospectively and included questions with a retrospective data collection time of one year, which is an unlikely period to create recall errors (Longobardi et al. 2011). In Study **III**, an important benefit was having three cohorts with varying clinical approaches and pretest probabilities for celiac disease, enabling the evaluation of the accuracy of the "triple criteria" in a unique setting resembling clinical reality.

There were also limitations. In Studies **I–II**, recruitment of patients was carried out mainly through celiac societies which, although increasing the participation rate, predisposes to selection bias. It is possible that some of the poorly coping patients did not participate, possibly explaining the low prevalence of psychiatric diseases and unemployed patients in Studies **I** and **II** respectively. Moreover, the participation rate of males in Study **I** was low, which reduces the representativeness of the cohort. Being retrospective, Study **I** was prone to recall bias. The retrospective data collection moreover made it impossible to assess whether celiac disease preceded a concomitant disease or vice versa.

In Study **II**, all data was self-reported, making diagnostic data less reliable. On the other hand, the patient-based outcomes gave important information on on-demand medications and HRQoL. In Study **II**, the numbers of health care visits and sickness days were small, increasing the possibility of chance in the association with delay. Also, the follow-up time of one year was rather short and afforded no opportunity to evaluate long-term associations.

Despite large initial sample sizes in each cohort of Study **III**, the eventual number of celiac disease patients in the moderate and low-risk cohorts was quite modest. As 30% of "triple positive" subjects from the moderate risk cohort and 13% from the low risk cohort refused to undergo biopsies, selection bias may also have occurred. In addition, although the PPV of "triple criteria" was 100% in all cohorts, the small number of patients resulted in wide 95% confidence intervals of the PPV, particularly in the population cohort. The study was also conducted with a well-performing TG2-ab test kit that turned out to be very reliable which, although a positive issue as such, may hamper the generalizability of the results to all tests on the market. Finally, there was only a short follow-up time for those fulfilling the triple criteria and thus the long-term consequences of omitting biopsies could not be evaluated.

12 SUMMARY

A continuous shift of celiac disease diagnostics from tertiary and secondary health care centers towards primary care was observed. Simultaneously, the proportion of patients with long diagnostic delay declined. These shifts were likely driven by the increasing awareness and availability of diagnostic tools and education of primary care professionals and are also reflected in increased diagnostic yield (Lohi et al. 2007; Virta et al. 2009).

Of the factors possibly associated with diagnostic delay, only limited associations between socio-economic factors were found. These results are somewhat contradictory with the findings of the few studies presented so far (Hafner-Eaton 1993; Barbero et al. 2014), possibly due to differences in socio-economic and health care systems. Being screen-detected and having celiac disease in the family were associated with decreased risk and coexisting neurological and musculoskeletal diseases with increased risk of delay. Manifestations of celiac disease may have been masked by these simultaneous chronic illnesses, which thus might have predisposed to delay (Lauret and Rodrigo 2013). Alternatively, musculoskeletal and neurological symptoms may have been signs or even complications of delayed and untreated celiac disease but mistaken for independent disease (Jackson et al. 2012).

Of self-perceived health-related factors, delay related to impaired psychological well-being before diagnosis and after one year on a GFD. Patients with delay also experienced excess primary health care visits, more days absent from work, and extensive use of pharmaceutical agents. The burden prior to diagnosis may be due to mislabeled celiac disease symptoms before correct diagnosis (Stasi et al. 2016) or due to another disease predisposing to delay (Lauret and Rodrigo 2013). After the diagnosis, slow recovery on a GFD may explain the poorer psychological well-being and excess healthcare visits in those with delay (Vavricka et al. 2016; Tovoli et al. 2018).

This study demonstrated the possibility of accurate serology-based diagnostics of celiac disease in adults irrespective of their clinical presentation or pretest probability. All 90 "triple positive" patients were found to have celiac disease, giving a PPV of 100%. Verification of HLA did not add to the diagnostic accuracy, showing that this relatively expensive and error prone examination is not needed in serology-based diagnostics. These findings are in line with the very recently updated pediatric no-biopsy criteria, in which presence of symptoms and HLA verification are no longer a necessity (Husby et al. 2020).

None of the "triple positive" celiac disease patients revealed complications or concomitant findings at diagnosis or in the follow-up after one year. This implies that the serology-based diagnosis could safely be adopted for clinical use without fear of missing severe complications or coexisting conditions. Previously, the option to omit the biopsy has been offered only to children (Husby et al. 2012). Already before this dissertation, the Study **III** has affected the current care guidelines of celiac disease in Finland allowing patients of all ages with TG2-ab ≥ 10x ULN and positive EMA to be diagnosed without biopsies (Celiac disease: Current Care Guideline 2018).

13 CLINICAL IMPICATIONS AND FUTURE PROSPECTS

This dissertation demonstrates a successful gradual shift of celiac disease diagnostics from tertiary and secondary to primary health care in Finland. Even though the nationwide guidelines have been efficacious in increasing diagnostic yield and reducing delay, much work still lies ahead as many patients remain undiagnosed or suffer from the consequences of delayed diagnosis. With such a highly prevalent disease, the harms of delay also cause a substantial economic and social burden to society. These findings underline the importance of early diagnosis and extending the awareness about the broad spectrum of celiac disease among the population and health care professionals.

Only a small number of underlying factors were found to offer instruments to detect patients at risk for delay. More research, including qualitative studies, would be needed in order to elucidate the mechanisms behind such delay. In any case, the burden of delay on individuals and on society prompts us to find more effective diagnostic approaches to replace inefficient case finding. According to the present study, clinical presentation is a poor predictor for celiac disease, as it does not increase diagnostic accuracy or help to set an early diagnosis. Instead, long diagnostic delay was more frequent in patients with abdominal symptoms and malabsorption, which are labeled as characteristic signs of untreated celiac disease. Theoretically, screening would be a powerful tool to reduce the delay and its consequences. It remains uncertain, however, what kind of a screening strategy would be most efficient and whether this approach would be cost-effective.

Serology-based criteria were found to possess excellent accuracy in diagnosing celiac disease, also in adults. It must, nevertheless, be realized that the study was conducted with a well-performing TG2-ab assay and the results may not be generalizable to all tests on the market. Thus, more evidence comparing the performance of different kits at 10x and also at lower multiples of ULN in adult use is called for. Applying the non-biopsy approach would markedly reduce the need for endoscopies and health care costs without compromising precise diagnostics, and probably simultaneously decrease diagnostic delay. Even though none of the "triple positive" patients had any complications in the diagnostic or follow-up biopsies, further research in different clinical settings and different countries is needed to evaluate whether biopsy-omitting diagnostics increases the risk of missing severe complications of celiac disease. HLA genotype or pretest probability for celiac disease did not add to the diagnostic value and, as already done in the revised ESPGHAN guidelines (Husby et al. 2020), could be omitted as a diagnostic prerequisite.

As the first country in the world, the option to omit biopsies to diagnose celiac disease in adults with sufficient antibody values has recently been established in Finland (Celiac disease: Current Care Guideline 2018). The present study presents important proof of the accuracy of the new diagnostic policy. Now that a new serology-based era in diagnosing celiac disease has begun, primary health care and individual practitioners will bear even more responsibility for setting adequate diagnoses. This dissertation encourages trusting in primary care diagnostics even with new diagnostic criteria if education is carried out consistently. In the future, it may be possible to extend non-biopsy criteria to an even greater group of celiac disease patients by lowering the multiplier of the ULN of TG2-ab. Until such more liberal steps are taken, standardization of TG2-ab assays and automatization of EMA testing would be beneficial (Caetano Dos Santos et al. 2019; Penny et al. 2020).

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Valma Fuchs

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APPENDIX: PGWB QUESTIONNAIRE

R_____

PGWB INDEX

Nimi_____

Tutkimuksen tämä osa sisältää kysymyksiä siitä, miltä Teistä tuntuu ja kuinka Teillä on mennyt VIIMEKSI KULUNEEN VIIKON AIKANA. Jokaisen kysymyksen osalta rastittakaa (X) se vaihtoehto, joka parhaiten sopii Teidän kohdallenne.

1. Miltä Teistä on YLEISESTI ottaen TUNTUNUT viimeksi kuluneen viikon aikana?
   - ☐ Mielialani on ollut erinomainen
   - ☐ Mielialani on ollut oikein hyvä
   - ☐ Mielialani on ollut enimmäkseen hyvä
   - ☐ Mielialani on vaihdellut paljon
   - ☐ Mielialani on ollut enimmäkseen huono
   - ☐ Mielialani on ollut hyvin huono

2. Kuinka usein Teitä on VAIVANNUT JOKIN SAIRAUS, RUUMIILLINEN VAIVA, SÄRYT tai KIVUT viimeksi kuluneen viikon aikana?
   - ☐ Joka päivä
   - ☐ Melkein joka päivä
   - ☐ Noin puolet ajasta
   - ☐ Silloin tällöin, mutta vähemmän kuin puolet ajasta
   - ☐ Harvoin
   - ☐ Ei koskaan

3. Tunsitteko itsenne MASENTUNEEKSI viimeksi kuluneen viikon aikana?

   ☐ Kyllä – niin paljon, että minusta tuntui siltä, että ottaisin itseni hengiltä

   ☐ Kyllä – niin paljon, etten välittänyt mistään

   ☐ Kyllä – hyvin masentuneeksi melkein joka päivä

   ☐ Kyllä – melko masentuneeksi useita kertoja

   ☐ Kyllä – lievästi masentuneeksi silloin tällöin

   ☐ Ei – en ole kertaakaan tuntenut itseäni lainkaan masentuneeksi

4. Oletteko pystynyt HALLITSEMAAN KÄYTTÄYTYMISTÄNNE, AJATUKSIANNE, MIELIALOJANNE tai TUNTEITANNE viimeksi kuluneen viikon aikana?

   ☐ Kyllä, ehdottomasti

   ☐ Kyllä – useimmiten

   ☐ Yleensä

   ☐ En kovin hyvin

   ☐ En, ja se häiritsee minua jonkin verran

   ☐ En, ja se häiritsee minua kovasti

5. Onko Teitä vaivannut HERMOSTUNEISUUS tai LEVOTTOMUUS viimeksi kuluneen viikon aikana?

   ☐ Erittäin paljon, jopa niin, että en ole voinut tehdä työtä tai huolehtia asioista

   ☐ Hyvin paljon

   ☐ Melko paljon

   ☐ Jonkin verran, niin että se on vaivannut minua

   ☐ Vähän

   ☐ Ei lainkaan

6. Kuinka paljon TARMOA, PIRTEYTTÄ tai ELINVOIMAA Teillä on ollut viimeksi kuluneen viikon aikana?
   - ☐ Hyvin täynnä tarmoa – erittäin pirteä
   - ☐ Melko tarmokas suurimman osan ajasta
   - ☐ Tarmokkuuteni on vaihdellut melkoisesti
   - ☐ Yleensä vähän tarmoa tai pirteyttä
   - ☐ Hyvin vähän elinvoimaa tai tarmoa suurimman osan ajasta
   - ☐ Ei lainkaan tarmoa tai elinvoimaa – olen tuntenut itseni loppuun ajetuksi tai loppuun kuluneeksi

7. Olen tuntenut itseni ALAKULOISEKSI JA SYNKKÄMIELISEKSI viimeksi kuluneen viikon aikana?
   - ☐ En kertaakaan
   - ☐ Vähän tänä aikana
   - ☐ Jonkin verran tänä aikana
   - ☐ Melkoisen osan tästä ajasta
   - ☐ Suurimman osan tästä ajasta
   - ☐ Koko ajan

8. Oletteko yleisesti ollut KIREÄ tai tuntenut itsenne JÄNNITTYNEEKSI viimeksi kuluneen viikon aikana?
   - ☐ Kyllä, erittäin jännittyneeksi suurimman osan ajasta tai koko ajan
   - ☐ Kyllä, hyvin jännittyneeksi suurimman osan ajasta
   - ☐ En ole ollut koko ajan kireä, mutta olen tuntenut itseni melko jännittyneeksi useita kertoja
   - ☐ Olen tuntenut itseni vähän jännittyneeksi muutamia kertoja
   - ☐ En ole yleensä tuntenut itseäni jännittyneeksi
   - ☐ En ole lainkaan tuntenut itseäni jännittyneeksi

9. Kuinka ONNELLINEN, TYYTYVÄINEN tai MIELISSÄNNE olette ollut viimeksi kuluneen viikon aikana?

   ☐ Erittäin onnellinen, en olisi voinut olla tyytyväisempi tai enemmän mielissäni

   ☐ Hyvin onnellinen suurimman osan ajasta

   ☐ Yleensä tyytyväinen ja mielissäni

   ☐ Joskus melko onnellinen ja joskus melko onneton

   ☐ Yleensä tyytymätön ja onneton

   ☐ Hyvin tyytymätön tai onneton suurimman osan ajasta tai koko ajan

10. Oletteko tuntenut itsenne riittävän TERVEEKSI tekemään asioita, joita haluatte tehdä tai Teidän on ollut pakko tehdä viimeksi kuluneen viikon aikana?

    ☐ Kyllä, ehdottomasti

    ☐ Suurimman osan ajasta

    ☐ Terveysongelmat ovat merkittävästi rajoittaneet minua

    ☐ Olen ollut vain niin terve, että olen voinut huolehtia itsestäni

    ☐ Olen tarvinnut jonkin verran apua itseni huolehtimisessa

    ☐ Olen tarvinnut toista henkilöä auttamaan itseäni useimmissa tai kaikissa asioissa, joita minun on täytynyt tehdä

11. Oletteko tuntenut itsenne niin SURULLISEKSI, LANNISTUNEEKSI tai TOIVOTTOMAKSI, että olette miettinyt, onko millään mitään merkitystä viimeksi kuluneen viikon aikana?

    ☐ Erittäin paljon – niin paljon, että olen ollut valmis luovuttamaan

    ☐ Hyvin paljon

    ☐ Melko lailla

    ☐ Jonkin verran – sen verran, että se on vaivannut minua

    ☐ Vähän

    ☐ En lainkaan

12. Oletteko herännyt PIRTEÄNÄ ja LEVÄNNEENÄ viimeksi kuluneen viikon aikana?

- ☐ En kertaakaan
- ☐ Muutaman harvan kerran
- ☐ Joitakin kertoja
- ☐ Aika monta kertaa
- ☐ Useimmiten
- ☐ Joka kerta

13. Oletteko ollut HUOLISSANNE tai LEVOTON TERVEYDESTÄNNE viimeksi kuluneen viikon aikana?

- ☐ Erittäin paljon
- ☐ Hyvin paljon
- ☐ Melko paljon
- ☐ Jonkin verran, mutta en kovin paljon
- ☐ Käytännöllisesti katsoen en koskaan
- ☐ En lainkaan

14. Onko Teistä tuntunut siltä, että olisitte ”MENETTÄMÄSSÄ JÄRKENNE” tai KONTROLLINNE siitä, miten TOIMITTE, PUHUTTE, AJATTELETTE, TUNNETTE tai MITÄ MUISTATTE viimeksi kuluneen viikon aikana?

- ☐ Ei lainkaan
- ☐ Vain vähän
- ☐ Jonkin verran, mutta ei niin paljon, että olisin ollut huolissani tai levoton siitä
- ☐ Jonkin verran ja olen ollut vähän huolissani
- ☐ Jonkin verran ja olen ollut melko huolissani
- ☐ Kyllä, hyvin paljon ja olen ollut hyvin huolissani

15. Päivittäinen elämäni on ollut TÄYNNÄ minua KIINNOSTAVIA ASIOITA viimeksi kuluneen viikon aikana?

☐ Ei lainkaan tänä aikana
☐ Vain pienen osan tästä ajasta
☐ Joskus
☐ Melkoisen osan tästä ajasta
☐ Suurimman osan tästä ajasta
☐ Koko ajan

16. Oletteko tuntenut itsenne AKTIIVISEKSI/TARMOKKAAKSI tai TYLSÄKSI/VELTOKSI viimeksi kuluneen viikon aikana?

☐ Hyvin aktiiviseksi/tarmokkaaksi joka päivä
☐ Enimmäkseen aktiiviseksi/tarmokkaaksi – en koskaan tylsäksi/veltoksi
☐ Melko aktiiviseksi/tarmokkaaksi – harvoin tylsäksi/veltoksi
☐ Melko tylsäksi/veltoksi – harvoin aktiiviseksi/tarmokkaaksi
☐ Enimmäkseen tylsäksi/veltoksi – en koskaan aktiiviseksi/tarmokkaaksi
☐ Hyvin tylsäksi/veltoksi joka päivä

17. Oletteko ollut HUOLESTUNUT, HARMISSANNE tai AHDISTUNUT viimeksi kuluneen viikon aikana?

☐ Erittäin paljon – niin paljon, että olen tuntenut itseni melkein sairaaksi huolestuneisuudesta
☐ Hyvin paljon
☐ Melko lailla
☐ Jonkin verran – sen verran, että se on vaivannut minua
☐ Vähän
☐ En lainkaan

18. Olen tuntenut itseni TASAPAINOISEKSI ja VARMAKSI viimeksi kuluneen viikon aikana?

- ☐ En lainkaan tänä aikana
- ☐ Pienen osan tästä ajasta
- ☐ Joskus
- ☐ Huomattavan osan tästä ajasta
- ☐ Suurimman osan tästä ajasta
- ☐ Koko ajan

19. Oletteko tuntenut itsenne LEVOLLISEKSI/HUOJENTUNEEKSI vai PINGOTTUNEEKSI/KIREÄKSI viimeksi kuluneen viikon aikana?

- ☐ Olen tuntenut itseni levolliseksi ja huojentuneeksi koko viikon
- ☐ Olen tuntenut itseni levolliseksi ja huojentuneeksi suurimman osan ajasta
- ☐ Yleensä olen tuntenut itseni levolliseksi, mutta ajoittain olen tuntenut itseni melko pingottuneeksi
- ☐ Yleensä olen tuntenut itseni pingottuneeksi, mutta ajoittain olen tuntenut itseni melko levolliseksi
- ☐ Olen tuntenut itseni pingottuneeksi/kireäksi suurimman osan ajasta
- ☐ Olen tuntenut itseni hyvin pingottuneeksi/kireäksi koko ajan

20. Olen tuntenut itseni ILOISEKSI/HUOLETTOMAKSI viimeksi kuluneen viikon aikana?

- ☐ En lainkaan tänä aikana
- ☐ Pienen osan tästä ajasta
- ☐ Joskus
- ☐ Melkoisen osan tästä ajasta
- ☐ Suurimman osan tästä ajasta
- ☐ Koko ajan

21. Olen tuntenut itseni VÄSYNEEKSI ja LOPPUUN KULUNEEKSI viimeksi kuluneen viikon aikana?

- ☐ En lainkaan tänä aikana
- ☐ Pienen osan tästä ajasta
- ☐ Joskus
- ☐ Melkoisen osan tästä ajasta
- ☐ Suurimman osan tästä ajasta
- ☐ Koko ajan

22. Oletteko tuntenut itsenne ”STRESSAANTUNEEKSI”, RASITTUNEEKSI tai PAINEEN ALAISEKSI viimeksi kuluneen viikon aikana?

- ☐ Kyllä, melkein enemmän kuin voin sietää tai kestää
- ☐ Kyllä melko lailla
- ☐ Kyllä, jonkin verran – enemmän kuin tavallisesti
- ☐ Kyllä, jonkin verran – kuten tavallisesti
- ☐ Kyllä, vähän
- ☐ En lainkaan

TARKISTAKAA, ETTÄ OLETTE VASTANNUT KAIKKIIN KYSYMYKSIIN!

KIITOS HYVÄSTÄ YHTEISTYÖSTÄ.

PUBLICATIONS

Factors associated with long diagnostic delay in celiac disease

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ABSTRACT

Objective. We here investigated the factors associated with long diagnostic delay in celiac disease and the impact of the national Current Care Guidelines in reducing the delay.

Material and methods. This population-based study involved 825 adult celiac disease patients. The diagnosis was considered delayed when the interval between first symptoms and diagnosis was more than 10 years. The patients were asked about the duration and type of symptoms before diagnosis, time and site (tertiary, secondary or primary care) of the diagnosis, family history of the disease and presence of significant co-morbidities. Analysis was performed by binary logistic regression.

Results. Altogether 261 (32%) out of 825 participants reposted a diagnostic delay of more than 10 years. Female gender, neurologic or musculoskeletal disorders and presence of diarrhea, abdominal pain and malabsorption were associated with prolonged delay. Male gender, diagnosis after the introduction of the first Current Care Guidelines in 1997 and being detected by serologic screening and family history of celiac disease were associated with a lower risk of delayed diagnosis. Factors not associated with the delay were site of diagnosis, age, presence of dermatitis herpetiformis, type 1 diabetes or thyroidal disease.

Conclusions. The number of long diagnostic delays in celiac disease has decreased over the past decades. The shift of diagnostics from secondary and tertiary care to primary care has not been detrimental. National guidelines for the diagnosis and active screening in at-risk groups are important in these circumstances.

Key Words: adults, celiac disease, current care guidelines, diagnostic delay

INTRODUCTION

In recent years the diverse clinical presentation of celiac disease has been recognized. Classical symptoms are diarrhea and poor growth or weight loss, but a variety of extraintestinal and atypical presentations are becoming increasingly common [1]. The heterogeneous clinical picture constitutes a challenge to physicians, and the average diagnostic delay is indeed very long, up to 12 years [2-7]. Besides the burden inherent in the ongoing symptoms, unrecognized celiac disease is associated with excessive use of health care services and on-demand medications [4, 8, 9]. Further, untreated disease predisposes to complications such as osteoporotic fractures [10], infertility [11, 12] and intestinal lymphoma [13, 14]. An early initiated gluten-free diet reduces the incremental burden to health care and the risk of complications and also improves health-related quality of life [2, 5, 8, 15].

In Finland, nationwide guidelines for the diagnosis and treatment of celiac disease were published in 1997 and are regularly updated [16]. The primary aims of the guidelines were to increase the diagnostic yield and to shift diagnostics from secondary and tertiary centres to primary care. General practitioners in primary care are systematically trained to maintain a low threshold for celiac disease suspicion and recognize patients with mild or atypical symptoms [17]. A decrease in diagnostic delay is one expected consequence of the revised clinical practice [16, 18]. Indeed, since the guidelines were launched, the prevalence of biopsy-proven celiac disease has increased, now being up to 0.7 % in Finland [17, 19]. There is also evidence that the median diagnostic delay has shortened compared with the approximately 10 years seen in many other countries, but even in Finland up to 25 % of patients have an unacceptable lag of 7-59 years [2-7]. Causes for the delay in diagnosis are complex and inadequately known [2].

We therefore set out to explore the factors underlying the long diagnostic delay in celiac disease. Particular attention was devoted to the connection between the national guidelines for celiac disease and the delay.

METHODS

Participants and study design

The study was conducted at Tampere University Hospital and the University of Tampere. First, adult patients diagnosed with biopsy-proven celiac disease were recruited by a nationwide search using newspaper advertisements and via local celiac disease societies. All celiac disease diagnoses had to be verified from the medical records. Exclusion criteria were uncertain diagnosis, diagnosis before the age of

18 years and lack of information regarding date of diagnosis or duration of symptoms leading to it. Next, the eligible participants filled self-administered questionnaires and consented to phone interviews by a physician or a study nurse with expertise in celiac disease. Particular attention was paid to the duration and type of symptoms before diagnosis, date and place of diagnosis, family history of the disease and presence of celiac disease-associated co-morbidities such as type 1 diabetes and thyroidal disease, or other significant co-morbidities such as malignancy and neurological, psychiatric and musculoskeletal disease. According to baseline presentation, the patients were further categorized into 1. subjects with gastrointestinal (abdominal pain, diarrhea, abdominal distention, constipation, reflux, nausea, malabsorption, weight loss) symptoms; 2. subjects with extraintestinal (e.g. dermatitis herpetiformis, tiredness, neurological symptoms) symptoms, and 3. those who were detected by screening in at-risk groups. All self-reported retrospective information was verified from the subjects´ medical records. The diagnosis was considered substantially delayed if the disease-related symptoms had lasted more than ten years before diagnosis [5].

The study protocol was approved by the Ethics Committee of Tampere University Hospital. All study participants gave written informed consent.

Statistics

Binary logistic regression analysis was used to identify factors associated with delay as follows: First, univariable analysis was conducted with each variable in question. Next, multivariable analysis was performed with variables found to have significant association in the univariable analysis. The results are presented as percentages and odds ratios (OR) with 95 % confidence intervals. A P value less than 0.05 was considered statistically significant. All statistical calculations were performed using IBM SPSS Statistics 19 (New York, NY, USA).

RESULTS

Of the altogether 922 volunteers willing to participate, 825 were eligible and included in further analyzes. Of those who were excluded, 62 lacked information regarding the duration of symptoms before celiac disease diagnosis and 14 date of diagnosis; in 21 cases the diagnosis could not be verified. Median age at diagnosis was 44 (range 18-81) years, and 76% of the 825 participants were females. Gastrointestinal symptoms remained the main clinical presentation in 52% of males and 73% of females, while 24% and 9% evinced some extraintestinal presentation and 24% and 18% were detected by screening in at-risk groups (Table 1). Dermatitis herpetiformis was present in 23 % of males and 8 % of females. There was a

strong secular trend in the site of diagnosis, as it has markedly shifted from tertiary centres to primary and secondary health care over time (Figure 1).

In total, 261 (32 %) out of 825 patients had diagnostic delay of 10 years or more. In univariable analysis female gender, celiac disease diagnosis before the year 2000 and diagnosis before the first national Current Care Guidelines was significantly associated with diagnostic delay (Table 1). In contrast, significant protecting factors were male gender, celiac disease detected by screening and family history of celiac disease. There was no association between long diagnostic delay and age at diagnosis, site of diagnosis or presence of dermatitis herpetiformis (Table 1).

The association between concomitant clinical conditions and diagnostic delay in celiac disease is shown in Table 2. A significant association was found between long delay and the presence of musculoskeletal or neurologic disease. In contrast, there was no association between long delay and presence of concomitant psychiatric or gastroenterological disease, osteoporotic fracture, malignancy, type 1 diabetes or thyroidal disease (Table 2).

Altogether 559 (68%) out of the 825 subjects reported gastrointestinal symptoms before the diagnosis. Presentations significantly associated with long delay were diarrhea, abdominal pain and malabsorption. In contrast, no association was seen between the delay and weight loss, abdominal distention, constipation, reflux and nausea (Table 3).

In multivariable analysis, a statistically significant association was observed between long diagnostic delay and the presence of some musculoskeletal disorder ($p = 0.014$), abdominal pain ($p < 0.001$) and malabsorption ($p < 0.001$). There was also a non-significant trend towards a reduced proportion of delayed diagnoses after the introduction of the first Current Care Guidelines in 1997 ($p = 0.063$).

DISCUSSION

An important finding in the present study was that the proportion of subjects with a diagnostic delay of 10 years or more in celiac disease has been significantly reduced within the past decades and also since the introduction of the first national guidelines for celiac disease in 1997. At the same time, the site of the diagnosis shifted markedly from secondary and tertiary to primary care. These findings, together with the fact that there was no significant difference in the prevalence of long delay between the different health-care sites, indicate that the regular and systematic training of primary care physicians in early recognition of celiac disease has been successful and encourages to continue [17, 18]. We believe that such a wide-scale decentralization of the diagnostics is a necessity, as the number of new celiac disease diagnoses is on a steep increase in most Western countries [20]. Furthermore, intensified awareness among health-care

professionals and subsequent active case-finding has proved to be a cost-effective alternative to population-based mass-screening in celiac disease [3]. Somewhat contradictory to our findings, some recent studies in other countries have indicated that non-gastroenterologists and other physicians with less expertise with celiac disease may have significantly poorer adherence to the published diagnostic guidelines for the condition than experts [21, 22]. These findings emphasize that the re-organization with possible decentralization of celiac disease diagnostics must be implemented in conjunction with a systematic education of physicians likely to encounter undetected celiac disease patients in their daily practice.

In univariable analysis here we found several factors which were associated with long diagnostic delay in celiac disease, some of them being rather surprising. Quite opposite to our expectations, males were significantly less at risk of long delay than females. One explanation for this might be that a greater proportion of males than females were detected by serological screening in at-risk groups for celiac disease. Also, extraintestinal symptoms were almost three times more common in males and, even though there was no statistically significant difference here, can be easier to find. This can be due for example to the rather straightforward diagnosis of skin symptoms of dermatitis herpetiformis, which is more common among males [23]. Also, males in general seek less medical advice until disease-related symptoms become very severe [24]. Thus, even though males had long diagnostic delay less often than females, it is possible that a higher proportion of males remain unrecognized. Moreover, particulary in female celiac disease patients the former irritable bowel syndrome diagnosis is common, this often in fact being unrecognized celiac disease with delayed diagnosis [25, 26].

Of concomitant medical conditions, the presence of a neurologic or musculoskeletal disease was significantly associated with a long diagnostic delay. As one plausible explanation here, it is likely that the presence of a previous serious disease distracts from diagnosis of another. In addition, several recent studies have shown that different neurological manifestations, such as ataxia and neuropathy are frequently implicated with celiac disease [27]. It is important that neurologists and general practitioners learn to know celiac disease as a possibility behind a patient's unspecific neurological symptoms. Likewise, physicians should recognize that various musculoskeletal symptoms, such as joint pains and osteoporosis, are also common and possible the sole finding in celiac disease patients [28, 29].

It was surprising that one of the most characteristic and classic signs of celiac disease, malabsorption, increased the risk for long delay. Iron deficiency anemia is the most common form of malabsorption in celiac disease [1]. As it is often the only sign of untreated celiac disease, the presence of unexplained anemia should always lead to a suspicion and prompt exclusion of the disorder [30, 31]. Similarly to malabsorption, other particulary typical symptoms of untreated celiac disease, diarrhea and abdominal pain,

were associated with excessive delay in the present study; abdominal pain remained significant even after multivariable analysis. This rather unexpected result might be partly explained by a recall bias in the retrospective design, as the duration of these burdensome and typical symptoms of celiac disease is easily overestimated compared to milder or atypical symptoms. More research is evidently needed to confirm these unexpected associations.

Strengths of the present study were the nationwide approach and the large number of participants with well-verified biopsy-proven celiac disease. Further, the clinical data were collected meticulously both by expert interviews and from medical records. The variable clinical presentation of celiac disease was also taken into account. Nevertheless, there were also certain limitations. First, the majority of study subjects were members of patient support organizations, which might have caused selection bias. On the other hand, approximately 70 % of all celiac disease patients in Finland are members of such organizations; we thus believe our results to be representative [17, 33]. Second, the retrospective design and long period of time covered by the study made it vulnerable to a number of confounding factors; also only a few associations remained significant after multivariable analysis. The threshold of 10 years or more used here for considerably delayed diagnosis is also somewhat artificial; however, we would maintain that such a long delay is in any case unacceptable. There is moreover evidence that a delay this long is associated with the development of celiac disease-associated malignancies [5]. The precise impact of the national Current Care Guidelines in this progress could not be evaluated even though the directives are a possible factor in reducing the diagnostic delay. Nevertheless, these guidelines are intended mainly for the general practitioners, and we believe that they will augment the diagnostics at population level.

To conclude, an unacceptably long diagnostic delay in celiac disease has become less common in Finland over the past decades. It was surprising that the classic diagnostic clues to celiac disease, gastrointestinal symptoms and malabsorption, did not reduce but on the contrary were associated with a higher risk of long diagnostic delay. The shift of diagnostics from secondary and tertiary to primary care has not resulted in longer delays. National guidelines for the diagnosis of celiac disease are important in these circumstances.

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Table 1. Association between clinical characteristics, place and time of diagnosis, presence of risk factors and delayed diagnosis[a] in 825 patients with celiac disease

	n	Delay[a], %	Odds ratio	95 % CI	P value
Gender					
Male	198	23	1		
Female	627	34	1.79	1.23-2.59	0.002
Clinical presentation					
Gastrointestinal[b]	559	35	1		
Extraintestinal[c]	106	30	0.80	0.51-1.25	0.316
Screen-detected[d]	160	20	0.46	0.30-0.70	<0.001
Dermatitis herpetiformis					
Yes	95	28	1		
No	730	32	1.19	0.74-1.91	0.474
Site of diagnosis					
Primary care	234	30	1		
Secondary care	389	30	1.00	0.69-1.41	0.944
Tertiary care	199	36	1.30	0.87-1.95	0.198
Calendar period of diagnosis					
2000-	415	27	1		
1990 – 1999	276	34	1.44	1.03-2.00	0.031
1980 – 1989	101	45	2.20	1.41-3.45	0.001
Before 1980	33	30	1.19	0.55-2.58	0.658
Diagnosis after 1997[e]					
Yes	478	28	1		
No	347	37	1.55	1.15-2.09	0.004
Celiac disease in family					
No	283	37	1		
Yes	537	28	0.68	0.50-0.93	0.014

[a] Symptoms lasting 10 years or more before diagnosis of celiac disease
[b] Diarrhea, abdominal pain or constipation, reflux, nausea, malabsorption
[c] Dermatitis herpetiformis, tiredness, joint pains, neurological symptoms
[d] Celiac disease in first-degree relatives, presence of autoimmune disorder
[e] After the first national Current Care Guidelines for celiac disease
CI, confidence interval

Table 2. Association between presence of concomitant clinical condition and delayed diagnosis[a] in 825 patients with celiac disease

	n	Delay[a], %	Odds ratio	95 % CI	P value
Any malignancy					
No	781	32	1		
Yes	42	26	0.75	0.37-1.52	0.431
Psychiatric disease					
No	790	30	1		
Yes	34	40	1.75	0.78-3.49	0.115
Neurologic disease[b]					
No	718	30	1		
Yes	107	40	1.54	1.02-2.34	0.043
Gastroenterological disease[c]					
No	290	28	1		
Yes	535	39	1.28	0.95-1.74	0.108
Musculoskeletal disease[d]					
No	570	28	1		
Yes	254	39	1.61	1.12-2.20	0.003
Osteoporotic fracture					
No	792	31	1		
Yes	31	45	1.83	0.89-3.77	0.102
Type 1 diabetes					
No	808	32	1		
Yes	17	29	0.90	0.31-2.58	0.842
Thyroidal disease					
No	688	31	1		
Yes	137	34	1.16	0.79-1.71	0.462

[a] Symptoms for 10 years or more before diagnosis of celiac disease

The most common presentations were; [b] Transient ischemic attacks, dementia, neuropathy, migraine, epilepsy; [c] Lactose-intolerance, food allergy, gastro- esophageal reflux, diverticulosis, diaphragmatic hernia; [d] Osteoporosis or osteopenia, arthritis, fibromyalgia, discus prolapse

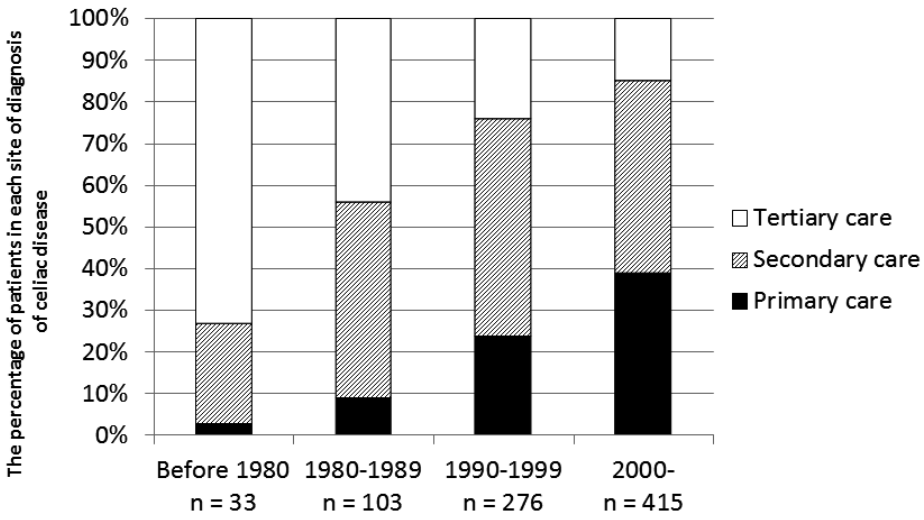
CI, confidence interval

Table 3. Association between the presence of different gastrointestinal symptoms before diagnosis and delayed diagnosis[a] in 825 patients with celiac disease

	n	Delay[a], %	Odds ratio	95 % CI	P value
Diarrhea					
No	579	29	1		
Yes	246	38	1.53	1.12-2.09	0.008
Weight loss					
No	701	31	1		
Yes	124	33	1.08	0.72-1.62	0.711
Abdominal pain					
No	462	25	1		
Yes	363	40	1.98	1.47-2.67	<0.001
Abdominal distension					
No	625	35	1		
Yes	200	31	1.19	0.85-1.67	0.317
Reflux					
No	772	32	1		
Yes	53	26	0.76	0.41-1.43	0.399
Constipation					
No	750	31	1		
Yes	75	36	1.24	0.76-2.04	0.395
Malabsorption					
No	593	27	1		
Yes	232	45	2.27	1.64-3.10	<0.001
Nausea					
No	52	32	1		
Yes	773	31	0.96	0.52-1.76	0.890

[a] Symptoms for 10 years or more before diagnosis of celiac disease
CI, confidence interval

Figure 1. Development of the site of celiac disease diagnosis by time



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Delayed celiac disease diagnosis predisposes to reduced quality of life and incremental use of health care services and medicines: A prospective nationwide study

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Abstract

Background: Celiac disease is challenging to recognize, predisposing to long diagnostic delay. Currently, associated factors and significance of the delay remain obscure.

Objective: The objective of this article is to investigate associated sociodemographic risk factors and health consequences of diagnostic delay in celiac disease.

Methods: Altogether 611 patients were surveyed at diagnosis and after one year on a gluten-free diet regarding sociodemographic variables, well-being and use of medicines and health care services. Quality of life was measured by a validated Psychological General Well-Being (PGWB) questionnaire. The results were compared between patients with and without delayed ($\geq$3 years) diagnosis.

Results: A total of 332 (54%) individuals reported a delay of $\geq$3 years. Associated with the delay were being a student or homemaker, but not gender, marital or occupational status, site of diagnosis or place of residence. Patients with the delay also had decreased self-perceived health and poorer PGWB scores compared to those without delay; in anxiety and general health this was seen even on a gluten-free diet. Days of sickness and doctor visits as well as use of drugs for dyspepsia and antidepressants were increased in the delay group both before and after diagnosis.

Conclusion: A delay in celiac disease diagnosis predisposes to reduced well-being and incremental use of medicines and health care services, both before diagnosis and one year after diagnosis.

Keywords

Celiac disease, diagnostic delay, sociodemographic, quality of life, health care services

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Key summary

Established knowledge on this subject:

- Celiac disease is a very common but markedly unrecognized condition.
- Median diagnostic delay of the disease is as long as from three to 13 years.
- At present, factors underlying and consequences of the delay remain mostly obscure.

New findings of this study:

- A diagnostic delay of only three years is associated to decreased quality of life and excess doctor visits, days of sickness and use of pharmaceutical agents before diagnosis.
- Many of the detriments associated with the delay may remain overrepresented even during the year after diagnosis.
- Being a student or homemaker is associated with reduced risk of delay, whereas no other associations with socioeconomic factors were found.

Introduction

Celiac disease is a gluten-induced immunological disorder with an estimated prevalence of as high as 1%–2% in Western countries.[1] The diverse clinical picture of the disease is a challenge to physicians, and at present approximately 75%–90% of affected individuals remain unrecognized.[2,3] In clinical practice, a mean diagnostic delay of up to even 13 years has been reported.[4–8] Long-term untreated celiac disease predisposes to severe complications such as osteoporosis, infertility and lymphoma,[9–11] and there is also evidence to suggest that in undiagnosed but symptomatic patients incremental use of health care services and pharmaceutical agents is likely.[4,8,12,13]

Another concern possibly associated with a delay in diagnosis is poor quality of life, as many untreated celiac patients suffer reduced psychological well-being, which improves on a gluten-free diet.[14–16] Currently it remains unclear what factors are associated with the delay, and whether the delay affects patients' self-perceived health. In addition, it is not known whether the delay predisposes to long-term increased consumption of medicines and consultations with physicians, which could be prevented by early diagnosis and dietary treatment of celiac disease.

In this prospective study, we investigated a number of patient-centered factors associated with diagnostic delay in celiac disease, and the effect of one year on a gluten-free diet on these. In particular, we aimed to evaluate the role of variable sociodemographic factors in the delay, and whether the delay is associated with individual health burden and increased use of health care services and pharmaceutical agents.

Methods

Participants and study design

The study was conducted in collaboration with the Finnish Celiac Society, which approximately 70% of new celiac disease patients in Finland join soon after diagnosis.[14] During a nationwide enrollment, a structured and validated questionnaire was sent to all new members joining the society in years 2007 and 2008. The patients were diagnosed at all health care levels from primary to tertiary care. Respondents older than 16 years of age with biopsy-proven celiac disease diagnosed within one year were considered eligible and continued in the study. Exclusion criteria were uncertain celiac disease diagnosis and lack of information on the duration of symptoms leading to the diagnosis. A follow-up questionnaire was sent to all participants after one year on a gluten-free diet. No ethical committee review was obtained because this was a questionnaire-based survey. However, informed consent was obtained from all participants after a written explanation of the aims of the study, including considerations regarding ethics and data protection and the anonymous deposition of the questionnaire.

Celiac disease diagnosis was considered delayed ("delay group") if the disease-related symptoms had lasted at least three years before diagnosis, according to the previously shown median diagnostic delay in Finland.[8] Specific symptoms leading to celiac disease diagnosis have been defined elsewhere in detail.[14] Participants were further categorized on the basis of place of residence into individuals living in the South/West and those living in the North/East areas of the country, and also into those living either in urban or in rural areas. The South/West region of Finland has a markedly higher population density (41.7 inhabitants/km^2) than the North/East (6.4 inhabitants/km^2).[17] An urban area was defined as a population center with more than 15,000 inhabitants according to the Finnish Environmental Administration.[18]

Questionnaires

The baseline and follow-up questionnaires were designed in cooperation with celiac disease patients

and the Finnish Celiac Society. They comprised questions on a variety of sociodemographic aspects and the patients' perceptions of the impact of the diagnosis on their overall health and well-being. Particular attention was paid to the duration of symptoms before diagnosis, occupational and working position, place of residence, site of first suspicion and diagnosis of celiac disease (primary, secondary or tertiary care), and self-rated health, concern for health, burden of symptoms and reaction to the celiac disease diagnosis both at the time of diagnosis and after one year on a gluten-free diet. Self-estimated health was rated on a four-point Likert scale as excellent, good, fair or poor; in analysis excellent and good were combined. Concern for personal health and burden of symptoms ranged from "not at all" to "extremely" on a three-point Likert scale. The participants also reported the number of all-cause visits to health care providers, consumption of pharmaceutical agents and days of absence from work during the year before diagnosis and in the first year on a gluten-free diet. Moreover, patients were asked about adherence to the gluten-free diet after one year on the diet.

Health-related quality of life

In addition to the above survey, self-estimated quality of life was measured by the structural Psychological General Well-Being Questionnaire (PGWB) both at diagnosis and after one year of a gluten-free diet. PGWB is a well-validated and widely used questionnaire in general and also in celiac disease research.[15,16,19] It consists of 22 items, each using a six-grade Likert scale, with higher scores indicating better psychological well-being. The questionnaire is further subdivided into six subdimensions, each containing three to five separate items: anxiety, depression, well-being, self-control, general health and vitality. The total PGWB score is the sum of all 22 items and may thus range from 22 to 132 points.

Statistics

The feasibility of the study questions was pretested by a group of celiac disease patients as previously described in detail.[20] Briefly, for test-retest reliability, 11 treated patients repeated the questionnaire one week after the first contact and the intraclass correlation coefficient was measured. The kappa values ranged from 0.84 to 1.00, being thus considered excellent (> 0.70). Statistical analysis was carried out using the Statistical Package for the Social Sciences Statistics, version 20.0 (IBM, Armonk, NY, USA). Binary logistic regression analysis was used to identify category factors associated with diagnostic delay. The results are shown as odds ratios (ORs) with 95% confidence interval (CI). A p value ≤ 0.05 was considered significant. Quantitative data were analyzed by independent-samples t test for normally distributed variables and by Mann-Whitney U test for skewed variables. The use of pharmaceuticals was divided into any use or no use of certain medicines and analyzed by Chi-square test. In order to make the results more comprehensive, both range and medians with quartiles are shown in Table 4. All data were blindly coded before statistical analysis.

Results

Altogether 1062 (57%) of the 1864 new members joining the Celiac Society during the study period responded. Of these, 451 were excluded: 157 as not being diagnosed within one year, 132 for being younger than 16 years of age, 89 for a lack of information regarding the duration of symptoms or otherwise substantially missing data and 73 owing to unclear celiac disease diagnosis. Of the 611 eligible individuals, 559 (91%) also completed the follow-up questionnaire. Seventy-six percent of the final study cohort were women.

The median duration of symptoms before celiac disease diagnosis was three (range 0–50) years and in 332 (54%) cases diagnosis was delayed by at least three years. Median age at diagnosis was 50 (16–75) years in patients with a delay and 48 (17–82) years in those without a delay ($p = 0.363$).

Of the various sociodemographic characteristics, being a student or homemaker was associated with reduced risk of diagnostic delay compared with being employed (Table 1). In contrast, gender, marital or occupational status, position at workplace, geographical residence and site of first suspicion or eventual diagnosis of celiac disease had no association with the risk of delay (Table 1).

All 559 individuals who returned the follow-up questionnaires were on a gluten-free diet, but 64 (11%) reported occasional lapses. On the diet the symptoms disappeared completely in 130 (23%), were alleviated in 337 (60%), remained unchanged in 71 (13%) and increased in three (0.5%) people. The likelihood of symptoms persisting on a gluten-free diet was increased in those with diagnostic delay (OR 1.61, 95% CI 1.08–2.42, $p = 0.022$).

Diagnostic delay was associated with the risk of poor or only fair self-estimated health and concern about health at celiac disease diagnosis (Table 2). After one year on a gluten-free diet, there was no longer a difference between the groups in self-perceived health, but concern about health remained higher in patients with the delay. Further, these individuals

Table 1. Association between diagnostic delay[a] and sociodemographic characteristics at diagnosis in 611 adults with celiac disease.

	n	Delay, %	Odds ratio	95% CI	*p* value
Gender					
Male	262	56	1		
Female	467	49	0.82	0.93–1.97	0.232
Marital status					
Married/with partner	460	55	1		
Single	151	53	0.93	0.64–1.34	0.700
Occupational status					
Employed	388	56	1		
Student or homemaker	56	36	0.43	0.24–0.78	**0.005**
Unemployed	15	73	2.14	0.67–6.85	0.198
Retired	138	53	0.88	0.59–1.29	0.876
Position at workplace					
High	156	60	1		
Middle	145	55	0.83	0.53–1.32	0.436
Low	279	52	0.74	0.50–1.11	0.143
Geographical residence					
North and East[b]	159	52	1		
South and West[c]	452	55	1.16	0.81–1.67	0.416
Urban or rural residence					
Urban	351	56	1		
Rural	260	52	0.82	0.60–1.13	0.232
First suspicion of disease					
Secondary/tertiary care	75	51	1		
Primary care	289	51	0.99	0.60–1.65	0.982
Oneself, friend, family	214	58	1.37	0.81–2.32	0.245
Site of diagnosis					
Secondary/tertiary care	283	52	1		
Primary care	325	56	1.16	0.84–1.60	0.361

[a]Celiac disease-related symptoms for three years or more before diagnosis. [b]Population density 6.4/km^2. [c]Population density 41.7/km^2. CI: confidence interval.

more often reported a moderate or extreme burden of symptoms at diagnosis and experienced feeling relief (compared to upset or confused) after the diagnosis in comparison to patients with no delay (Table 2).

PGWB total and all subscores were significantly lower at diagnosis in patients with delayed diagnosis compared to those without (Table 3). On dietary treatment, the scores improved in both groups, but anxiety and general health scores remained lower in the delay group (Table 3).

The numbers of outpatient visits in primary health care and days of sickness during the year prior to celiac disease diagnosis were higher in individuals with diagnostic delay compared to those without (Table 4). The frequency of visits decreased in both groups during the year following diagnosis, but the difference remained significant. In contrast to outpatient visits, the number of days of sickness increased in both groups on a gluten-free diet (Table 4).

The proportion of patients using analgesics, drugs for dyspepsia and antidepressants was increased in patients with diagnostic delay compared to those without during the year before diagnosis, and the difference in the two latter remedies remained significant on a gluten-free diet (Table 5). There was a similar but nonsignificant trend with antibiotics in the year before diagnosis (Table 5). Comparable differences between the delay group and controls were seen when the use of pharmaceutical agents was analyzed according to amount of pills per month (data not shown).

Table 2. Associations between diagnostic delay[a] and self-rated perceptions of health at diagnosis and one year after diagnosis in 611 adults with celiac disease.

	n	Delay %	Odds ratio	95% CI	*p* value
At diagnosis					
Self-perceived health					
Good	242	47	1		
Fair	278	59	1.64	1.16–2.33	**0.005**
Poor	87	60	1.70	1.03–2.79	**0.037**
Concern about health					
Not at all	55	29	1		
Moderate	436	55	2.99	1.62–5.50	**<0.001**
Extreme	117	63	4.20	2.10–8.39	**<0.001**
Burden of symptoms					
Not at all	44	32	1		
Moderate	287	52	2.31	1.18–4.55	**0.015**
Extreme	259	62	3.58	1.81–7.08	**<0.001**
Reaction to the diagnosis					
Upset or confused	300	49	1		
Relieved	291	60	1.55	1.12–2.15	**0.008**
One year after diagnosis					
Self-perceived health					
Good	411	53	1		
Fair	130	58	1.22	0.82–1.82	0.329
Poor	17	53	1.01	0.38–2.66	0.990
Concern about health					
Not at all	164	45	1		
Moderate	371	58	1.70	1.17–2.26	**0.005**
Extreme	24	50	1.22	0.52–2.87	0.654

[a]Celiac disease-related symptoms for three years or more before diagnosis.
CI: confidence interval.

Discussion

Our main finding was that as little as three years' diagnostic delay in celiac disease is associated with reduced health and well-being and increased use of health care and medicines. In fact, many of these detriments remained overrepresented in the delay group even during the year after diagnosis. Since in many countries the median delay is as high as 9–13 years,[4–7] the morbidity observed here in patients with a substantially shorter period is alarming. Although part of these problems may eventually be alleviated on a gluten-free diet, it seems that a considerable number of celiac patients suffer from an excess health and economic burden avoidable by earlier diagnosis.

One of the key findings here was the reduced self-perceived health and psychological well-being in patients with a diagnostic delay. This is in accord with a previous Swedish study likewise showing poorer quality of life at diagnosis in those with a long delay.[7] Here, some of these important clinical outcomes remained poorer even after one year on a gluten-free diet, indicating that recovery from the psychological burden associated with long-term unrecognized celiac disease takes some time. Moreover, although the matter remains somewhat controversial,[15,21] there is previous evidence that a subgroup of patients may continue to suffer from persistent poor health and mental problems even after years on a gluten-free diet.[7,16] It is therefore essential that physicians and other health care professionals devote particular attention and support to those with a markedly delayed celiac disease diagnosis.

Somewhat surprisingly, we found no association between different socioeconomic factors and diagnostic delay except for a lower risk in students and homemakers compared to those who were employed. The lack of other associations might be related to the long-term political goal to reduce inequalities in health and health care in Finland.[22] Here, inexpensive and easily accessible public health care diagnoses and treats the great majority (in the present study 89%) of

Table 3. Psychological General Well-Being scores of 592 celiac disease patients[a] at diagnosis and one year after diagnosis, categorized by length of diagnostic delay.

	Delay ≥ 3 years	Delay < 3 years	
	Mean (95% CI)	Mean (95% CI)	*p* value[b]
At diagnosis			
Total	87.1 (84.9–89.4)	93.6 (91.2–96.0)	<**0.001**
Anxiety	19.9 (19.3–20.5)	21.6. (20.9–22.2)	**0.003**
Depressed mood	14.2 (13.9–14.5)	14.9 (14.5–15.2)	**0.001**
Positive well-being	14.4 (14.0–14.9)	15.4 (14.9–15.9)	<**0.001**
Self-control	13.2 (12.8–13.5)	14.1 (13.7–14.4)	<**0.001**
General health	10.8 (10.5–11.1)	11.9 (11.6–12.3)	<**0.001**
Vitality	10.9 (10.5–11.2)	11.9 (11.5–12.3)	<**0.001**
One year after diagnosis			
Total	101.6 (99.6–103.5)	103.5 (101.5–105.6)	0.132
Anxiety	23.3. (22.8–23.8)	24.0 (23.5–24.5)	**0.048**
Depressed mood	16.0 (15.7–16.3)	16.2. (16.0–16.5)	0.220
Positive well-being	16.7 (16.3–17.1)	17.1 (16.6–17.4)	0.339
Self-control	14.9 (14.7–15.2)	15.0 (14.7–15.3)	0.628
General health	13.0 (12.7–13.4)	13.6 (13.3–14.0)	**0.009**
Vitality	12.8 (12.5–13.2)	13.2 (12.8–13.5)	0.070

[a]592 patients at diagnosis and 580 after one year. [b]Independent-samples *t* test.
CI: confidence interval.

Table 4. Use of health care services in the year prior to and following diagnosis in 611 celiac disease patients, categorized by length of diagnostic delay.

	Delay ≥ 3 years		Delay < 3 years		
	Median (Q1,Q3)	Range	Median (Q1, Q3)	Range	*p* value[a]
Before diagnosis					
Doctor visits[b]	3 (1, 5)	0–31	2 (1, 4)	0–30	**0.002**
Days of sickness	0 (0, 5)	0–200	0 (0, 3)	0–180	**0.020**
After diagnosis					
Doctor visits[b]	2 (1, 4)	0–20	1 (0, 3)	0–15	<**0.001**
Days of sickness	0 (0, 6)	0–356	0 (0, 4)	0–365	**0.021**

[a]Mann-Whitney *U* test. [b]In primary care.
Q1, Q3: lower and upper quartiles; CI: confidence interval.

celiac disease patients. Because of the differences in health care systems, some caution is needed before extrapolating our findings to other countries. For example, more variability exists in terms of health care accessibility in the United States, where low income has been shown to be a major barrier to celiac disease diagnosis.[23] Significant regional and socioeconomic variation in the prevalence of celiac disease has also been observed in the United Kingdom, possibly reflecting disparities in health-seeking behavior and/or access to correct diagnostic pathways.[24] The somewhat counterintuitive lower risk of delay in students and homemakers noted here might be explained by the well-organized student health care and maternity clinics in Finland.[25,26] Although there are no other similar studies, Vavricka and colleagues[27] have previously shown age younger than 30 years, the typical age for students and homemakers, to be associated with reduced risk of diagnostic delay.

Neither place of residence nor level of health care at which the celiac disease diagnosis was made was associated with the risk of diagnostic delay. This is compatible with our previous findings in patients with a delay of 10 years diagnosed mostly in the area of one tertiary center,[28] whereas the earlier mentioned British study reported significant regional differences in the

Table 5. Proportion of patients using pharmaceutical agents in the year prior to and following diagnosis in 611 celiac disease patients, categorized by length of diagnostic delay.

	Delay ≥ 3 years %	Delay < 3 years %	
	$n = 330$	$n = 279$	p value[a]
Before diagnosis			
Analgesics	69.4	60.9	**0.029**
Dyspepsia drugs	34.1	20.1	**<0.001**
Antidepressants	11.0	5.4	**0.014**
Sleeping pills	13.9	10.8	0.236
Antibiotics	34.9	27.6	0.055
Other[b]	51.8	48.2	0.308
After diagnosis	$n = 301$	$n = 258$	
Analgesics	68.1	67.4	0.867
Dyspepsia drugs	23.6	14.0	**0.004**
Antidepressants	9.7	5.0	**0.039**
Sleeping pills	14.0	11.2	0.337
Antibiotics	28.9	26.5	0.520
Other[b]	55.5	56.6	0.793

[a]Chi-square test.
[b]Vitamins, micronutrients, herbal products.

diagnostic delay.[24] The low regional variation in Finland is very likely affected by the frequently updated nationwide Current Care Guidelines for celiac disease.[29] The guidelines aim to increase awareness and diagnostic efficacy in celiac disease particularly in primary care.

As a result of this decentralization the prevalence of diagnosed celiac disease in Finland is among the highest in the world.[28,30] There are no studies from other countries evaluating the effect of such a reorganization of celiac disease diagnostics, but the issue has been investigated for instance in inflammatory bowel disease and chronic lung diseases, with somewhat less promising results.[31,32] We believe that primary care diagnostics can be very successful, but only if combined with practical diagnostic tools and continuous education of physicians.

Patients in the delay group reported more primary health care visits and days of sickness both in the year prior to and following the diagnosis. One reason leading to excess visits and ill health could be the often vague and unspecific symptoms not being recognized as celiac disease.[33] The higher use of health care even after the diagnosis might be related to our previous observation that diagnostic delay predisposes individuals to persistent symptoms on a gluten-free diet.[16] The increased number of days of sickness probably occurs for the same reasons as the excess health care visits. Interestingly, a similar association between delayed diagnosis and increased work absence has been reported in endometriosis patients,[34] further demonstrating difficulties encountered in cases of chronic diseases with a diverse clinical picture. The increased work absence in both study groups in the year following diagnosis can be explained for example by a severe infection season.

There was also incremental use of analgesics, antidepressants and medicines for dyspepsia in the delay group in the year prior to the diagnosis. Previous studies have already shown excessive use of pharmaceuticals preceding celiac diagnosis,[8,35] and this problem would appear to be further aggravated by delay. We could not trace the indications for these drugs, but they might have been prescribed for example in an attempt to ameliorate persistent gastrointestinal and depressive symptoms caused by unrecognized celiac disease.[13,36] A parallel association between delay and excessive analgesic use before diagnosis has again been observed in endometriosis patients.[37] In line with the lower quality of life and excess health care visits, the increased drug use continued even after the diagnosis. Besides slow resolution of symptoms, this might be due to patients' reluctance to discontinue drugs they have used with some benefits perhaps for several years.

The main strengths of the study were its prospective design, the large nationwide patient cohort, validated questionnaires and broad range of relevant study outcomes. There was also an excellent response rate in the follow-up survey. On the other hand, questionnaire-based studies are prone to overrepresentation of healthy individuals who feel well, the risk of which is further aggravated by participants being members of celiac societies. It is also noteworthy that, although the treatment response was followed prospectively, outcomes and duration of symptoms before diagnosis were assessed retrospectively and are thus prone to recall bias. However, a recall period covering a maximum of one year in self-reported use of health care services and pharmaceutical agents has previously been shown to be reliable.[38] The fact that the patients were enrolled almost 10 years ago might in theory have an effect, but there have been no major changes in our health care system or celiac disease diagnostics, and we believe that the results are still representative. Finally, because of a lack of original patient records, we were unable to verify the self-reported medical information including celiac disease diagnosis, and to evaluate the possible impact of different comorbidities on results.

Conclusions

We found even a relatively short diagnostic delay in celiac disease to be associated with increased health

burden both at the individual and society level. Improved awareness of the diversity of the disease among physicians and at-risk group screening could be an effective means to reduce the delay at the population level.

Declaration of conflicting interests

None declared.

Ethics approval

No ethical committee review was obtained because this was a questionnaire-based survey.

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Informed consent

Informed consent was obtained from all participants after a written explanation of the aims of the study, including considerations regarding ethics and data protection and the anonymous deposition of the questionnaire.

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Serology-based nonbiopsy criteria for adult coeliac disease have excellent accuracy with different pre-test probabilities

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SUMMARY

Background: The revised paediatric criteria for coeliac disease allow omission of duodenal biopsies in symptomatic children who have specific serology and coeliac disease-associated genetics. It remains unclear whether this approach is also applicable for adults with various clinical presentations. Aim: To evaluate the accuracy of serology-based criteria in adults with variable pretest probabilities for coeliac disease.

Methods: Three study cohorts comprised adults with high-risk clinical coeliac disease suspicion (n = 421), moderate-risk family members of coeliac disease patients (n = 2357), and low-risk subjects from the general population (n = 2722). Serological and clinical data were collected, and "triple criteria" for coeliac disease comprised transglutaminase 2 antibodies >10 × the upper limit of normal, positive endomysium antibodies, and appropriate genetics without requirement of symptoms. The diagnosis was based on intestinal biopsy.
Results: Coeliac disease diagnosis was established in total 274 subjects. Of these, 59 high-risk subjects, 17 moderate-risk subjects, and 14 low-risk subjects fulfilled the "triple criteria". All had histologically proven coeliac disease, giving thus the criteria a positive predictive value of 100%. Altogether, 90 (33%) of all 274 newly diagnosed patients could have avoided the biopsy, including 37% among high-risk, 20% among moderate-risk, and 48% among low-risk patients. No histological findings other than coeliac disease were found in the biopsies of "triple positive" subjects.

Conclusions: Coeliac disease can reliably and safely be diagnosed without biopsy in adults fulfilling the "triple criteria" regardless of the pre-test probability. Revised criteria would enable to reduce the number of endoscopies by one-third.

INTRODUCTION

The true prevalence of coeliac disease is known to be as high as 1%-2%, emphasising the importance of practical and cost-effective diagnostic policy. On the other hand, since the treatment consists of a life-long and restrictive gluten-free diet, the diagnosis should be highly accurate. Demonstration of small-bowel mucosal damage has been the gold standard for the diagnosis for a long time. This invasive histology-based approach contains, however, some limitations. The required duodenal lesion is a characteristic but not specific finding, as it can be caused also by other conditions and medicines (1). In addition, gradual development or patchy mucosal damage and inadequate or poorly orientated biopsy specimen may result in misdiagnosis (2,3).

Tests for serum autoantibodies against tissue transglutaminase 2 (tTG-ab) and endomysium (EMA) have become widely available for first line screening of coeliac disease. These tests, especially EMA and high positive values of tTG-ab, have been found to possess excellent diagnostic accuracy (4,5). Due to this and the aforesaid problems with the histology-based diagnosis, the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) established in 2012 new criteria stating that the biopsy could be avoided in

symptomatic children with tTG-ab value more than 10 times the upper limit of normal (ULN), positive EMA, and coeliac-type genotype (6). There is increasing evidence to support the accuracy of these guidelines for paediatric coeliac disease if applied meticulously (7,8).

Whether the nonbiopsy approach could be applicable also in adult coeliac disease remains controversial (9). An unsolved issue even with the paediatric criteria is their feasibility in populations with variable pre-test probabilities, including screen-detected and asymptomatic subjects, as this might affect the accuracy of serological testing (10,11). We investigated the applicability of the nonbiopsy approach and its impact on reducing the number of endoscopies in three large adult cohorts, including high-risk subjects with clinical suspicion of coeliac disease, moderate-risk subjects with family history of the disease, and low-risk individuals participating in population-based screening

2. MATERIALS AND METHODS

2.1 Participants and study design

The study comprised altogether 5 500 adults who had no previous coeliac disease or dermatitis herpetiformis diagnosis and were on a gluten-containing diet. The whole cohort was formed by evaluating retrospectively the data of three, originally prospectively collected subgroups with different pre-test probabilities for coeliac disease:

1. The high-risk cohort comprised 421 adults referred to the Department of Gastroenterology and Alimentary Tract Surgery, Tampere University Hospital, for further serological and endoscopic investigations due to variable clinical symptoms and signs compatible with coeliac disease such as diarrhoea, loose stools, abdominal pain, dyspepsia, flatulence, or malabsorption. Based on previous literature, the prevalence of coeliac disease in such pre-selected patients varies approximately between 5% and 50% depending on the setting and population in question (12,13). Even though about one half of high-risk subjects had been pre-tested for coeliac disease serology, clinical presentation was the defining characteristic as also subjects with negative antibody results were referred for endoscopies. All subjects underwent routine clinical evaluation, determination of coeliac disease serology, and disease-associated genetics. Furthermore, upper gastrointestinal endoscopy with duodenal biopsies were offered regardless of serology results.

2. The moderate-risk cohort (at-risk family members) was collected by nationwide recruitment of 2357 family members of 730 previously diagnosed coeliac disease patients via newspaper advertisements and from the Finnish coeliac society as described elsewhere (14). According to a recent meta-analysis, the pooled prevalence of coeliac disease is 7.5% in this at-risk group (15). The family study was coordinated by the Tampere Celiac Disease Research Center. Coeliac disease-associated serology and genetics were measured from all voluntary family members and endoscopy was offered to seropositive subjects.

3. The low pre-test probability cohort comprised 4272 randomly selected 51 to 76-year-old individuals living in the Päijät-Häme Hospital district. The cohort representing the ageing Finnish general population was originally collected for a research project aiming to improve health and well-being, not especially for coeliac disease research (16). Of them, coeliac disease autoantibodies were screened from altogether 2722 non-selected subjects who had no previous contact to health care due to coeliac disease related symptoms. The prevalence of coeliac disease in this cohort (2%) has been shown to be comparable with the general Finnish population (17). Seropositive subjects were offered determination of genotype and endoscopy.

2.2 Clinical data

All subjects with a clinical suspicion of coeliac disease and at-risk family members were interviewed for their clinical presentation and family history of coeliac disease. In the low-risk population cohort, the interview was carried out only with volunteered seropositive subjects. In addition, all newly diagnosed coeliac disease patients underwent assessment of adherence to the gluten-free diet and of clinical, serological, and histological response 1 year after the diagnosis. Adequate response was defined as normalisation or marked decrease in antibody levels, recovery from the intestinal mucosal damage, and symptom alleviation.

2.3 Serological tests and genotyping

In the high-risk and low-risk study groups, serum tTG-ab was detected by Celikey® ELISA (Phadia, Freiburg, Germany) having ULN of 5 U/mL to indicate tTG-ab positivity (18). In the moderate-risk group, tTG-ab was first measured with a sensitive Quanta Lite®ELISA test (INOVA diagnostics, San Diego, CA, USA). To unify the results, all 403 samples positive (>20 U/mL) for Quanta Lite®and450 additional randomly chosen negative samples were re-tested with Celikey®. EMAs were determined by an indirect immunofluorescence method using human umbilical cord as antigen as previously described (19). Dilution of 1:≥5 was considered positive.

Coeliac disease-associated HLA genotyping was performed bythe DELFIA Celiac Disease Hybridization Assay (PerkinElmer Life and Analytical Sciences, Wallac Oy, Turku, Finland) or with the Olerup SSP DQ low-resolution kit (Olerup SSP AB, Stockholm, Sweden).

“Triple criteria” were defined as tTG-ab value > 50 U/mL which is equal to Celikey® >10×ULN, positive EMA and presence of HLA DQ2/DQ8, regardless of the clinical presentation. For the moderate-risk cohort, the accuracy of the triple criteria was tested also with QuantaLite®, where tTG-ab > 10×ULN was attained at > 200 U/mL.

2.4 Histology

According to our clinical routine, a minimum of four representative small-bowel mucosal biopsies are taken upon oesophagogastroduodenoscopy from the distal duodenum. Well-orientated samples are paraffin-embedded, stained by haematoxylin-eosin and studied under a light microscope. In the present study, the reference standard for coeliac disease diagnosis was considered Marsh grade ≥ 2 (6). In cases having only coeliac-type mucosal inflammation (Marsh 1), the diagnosis was established if the disease was clinically and histologically aggravated on a gluten-containing diet (20,21).

2.5 Occurrence of coeliac disease

The proportion of new coeliac disease patients that could be diagnosed with the "triple criteria" was evaluated for each cohort. All in the high-risk group underwent endoscopy and the total prevalence of coeliac disease was calculated. In the family-risk and population-based cohorts, only seropositive patients were biopsied and the number of possible seronegative coeliac disease patients could not be evaluated.

2.6 Statistics

Statistical analysis was carried out using SPSS version 20.0 (IBM, Armonk, NY, USA). The distribution of general characteristics of the subjects was presented as percentages, medians, and ranges as appropriate. For all cohorts, the positive predictive value (PPV) of the "triple criteria" for biopsy-proven coeliac disease was calculated as follows: PPV = a/(a+b), where "a" is the "true positives", refer-ring to biopsy-proven coeliac disease and "b" is the "false positives", referring to histology without evident coeliac disease. A 95% CI (confidence interval) for PPV was assessed in all three cohorts according to the number of "triple positive" patients. Additionally, the lowest tTG-ab level giving a 100% PPV was determined. All data were coded and analysed blinded.

2.7 Ethical aspects

The study design and patient recruitment were approved by the Regional Ethics Committees of Pirkanmaa Hospital District and Päijät-Häme Central Hospital. All participants gave written informed consent.

3 RESULTS

Clinical characteristics of the 5 500 enrolled participants are shown in Table 1. There were more women in the clinically investigated high-risk cohort and, by definition, higher median age in the low-risk population cohort compared to the other groups (Table 1).

3.1 PPV of the “triple criteria” for coeliac disease

3.1.1 High-risk cohort: clinical suspicion

Altogether 133 of 421 clinically suspected participants had positive tTG-ab, with a value of >10×ULN in 60 (45%) of the 133 (Figure 1). All 60 had coeliac-type HLA and all but one positive EMA. At endoscopy, coeliac disease was initially found in 56 (95%) of 59, but also the remaining three “triple positive” subjects with only Marsh I lesion were subsequently diagnosed with coeliac disease since they developed Marsh III lesion during one further year on a gluten-containing diet. Thus, eventually all 59 patients received coeliac disease diagnosis, giving a PPV of 100% (CI 94%-100%) for “triple positivity” (Figure 1).

3.1.2 Moderate-risk cohort: at-risk family members

TTG-ab positivity with Celikey®was seen in 93 of the 2 357 family members; 24 (26%) of these fulfilled the “triple criteria”(Figure 1).However, seven of 24 were not biopsied and were excluded from further analysis: five refused, one deceased, and one had already initiated a gluten-free diet by himself before endoscopy. All remaining 17 subjects were found to have biopsy-proven coeliac disease (PPV 100%, CI 82%-100%). TTG-ab values did not differ between biopsied and nonbiopsied subjects (median 83 vs 90 U/mL, P= 0.658). With QuantaLite®,>10×ULN (>200 U/mL) was achieved in 29 of the 93 subjects, all of whom were triple positive. Biopsy was available from 20 patients who all had Marsh III lesions, resulting in a PPV of 100% (CI 84%-100%) for the triple criteria.

3.1.3 Low-risk cohort: screened general population

Forty-nine (2%) of the 2722 screened subjects had elevated tTG-ab. Sixteen (33%) of these had tTG-ab >10×ULN and positive EMA, but two subjects withdrew from the study before HLA testing and endoscopy. The remaining 14 were “triple positive” and had histologically confirmed coeliac disease, resulting in PPV of 100% (CI 78%-100%) (Figure 1). The two nonbiopsied subjects had comparable tTG-ab values with those undergoing endoscopy (100 and 82 U/mL vs median 91 U/mL, P= 0.883).

3.2 Clinical characteristics of the triple positive subjects

In detailed analysis of the 90“triple positive” subjects, as in the whole study cohort, there were more women among the high-risk subjects and higher median age among the low-risk subjects (Table 2). Despite of being screen-detected, most family members and population-based subjects reported some clinical symptoms when requested and only 43% and 29%, were eventually asymptomatic respectively. Family history for coeliac disease was common also in clinically detected and population-screened patients (Table 2). No clinically significant endoscopic or histological findings other than those related to coeliac disease were exposed in either diagnostic or follow-up biopsies.

3.3 Prevalence of coeliac disease and proportion of triple positive patients

The total number of new biopsy-proven coeliac disease patients detected in our three cohorts was 274, of whom the "triple criteria" were fulfilled in 90 (33%) (Table 3). All subjects in the high-risk cohort were biopsied and 160 (38%) of them were found to have coeliac disease. In the family risk and population cohorts, only seropositive subjects were biopsied and 85 (3.6%) and 29 (1.1%) were found to have coeliac disease respectively.

3.4 Lowest tTG-ab value resulting in 100% PPVfor triple criteria

All biopsied subjects with tTG-ab ≥ 7U/mL in the high-and moderate-risk cohorts had histologically proven coeliac disease. The corresponding value in the low-risk cohort was 17 U/mL (3.3×ULN with Celikey®), which was thus the lowest value for 100% PPV in the whole study cohort. Subjects in the moderate-risk cohort were initially tested with QuantaLite®, which gave the lowest tTG-ab level for 100% PPV at 106 U/mL, equalling 5.3×ULN.

DISCUSSION

We found that accurate non-invasive coeliac disease diagnosis can be established in "triple positive" adults regardless of the pre-test probability. The paediatric criteria are currently restricted to clinically suspected subjects (6). Recent evidence suggests they could be extended to asymptomatic children (7,22,23), although this has also been questioned (10). Here, the criteria worked equally well in adults with and without apparent symptoms, and while our study was not designed for asymptomatic patients, we consider such a dichotomous categorisation problematic. As was seen here and also previously (24), screen-detected patients often have unrecognised symptoms and, vice versa, some patients are clinically detected due to asymptomatic signs such as anaemia or osteoporosis (25,26). Definition of symptoms and their association with coeliac disease are challenging, as the clinical and histological presentation may not correlate and symptoms can fluctuate or not be recognised until their alleviation on a gluten-free diet (26). Abdominal complaints are also frequent in the general population, have low PPV for coeliac disease, and case finding based on them is ineffective (27,28). Thus, inflexible grouping of patients to "asymptomatic" and symptomatic corresponds poorly to the clinical reality and does not improve diagnostic accuracy, particularly in EMA-positive subjects (21). Based on this, categorising the clinically suspected cohort as "high risk" due to symptoms is somewhat debatable, especially as many subjects had been serologically pre-tested. Nevertheless, 38% of the cohort eventually had coeliac dis-ease, demonstrating successful labelling as "high risk".

We believe that a major contributor for the 100% PPV for the "triple criteria" was the use of validated serological and histopathologic methods as recommended (6). For example, some studies reporting lower PPV have used arbitrary cut-offs such as 100 U/mL for tTG-ab instead of >10×ULN (29). Currently there is no standardisation for tTG-ab tests and their optimal ULN varies (6), as demonstrated by the differences between the two kits in the present study. In fact,

even the 10×is rigid and was chosen more to be on a “safe side” (6,7), as setting test-specific thresholds would be challenging. In Finland, public laboratories use certificated quality control by outside accreditors to evaluate the performance of test kits and their application (30). The ESPGHAN criteria require disease-specific EMA partly due to the non-standardisation and variable performances of the tTG-ab assays (6). Unfortunately, not all studies evaluating the criteria have included EMA (10). In line with paediatric studies (7,8), we observed excellent agreement between EMA positivity and tTG-ab>10×ULN, giving further credibility for the results. One might ask whether laborious EMA was required in all cases, but currently it could be considered as inexpensive quality control. In contrast, HLA testing seems to add minimal value in adults with high tTG-ab values and positive EMA, similarly as recently shown in children (7). Therefore, genotyping could be restricted to exclude coeliac disease in unclear cases (7,31).

Another explanation for suboptimal PPV for serology in some studies could lie in the use of error-prone biopsy results as the gold standard (3,32). Accordingly, Werkstetter et al (7) observed remarkable variability in histopathological analyses between local and centralised providers even in a pre-planned research setting. Only a few studies evaluating the nonbiopsy criteria have given satisfactory data on this issue, including the number and location of biopsies, handling and orientation of the samples, and histological interpretation. Hence, some cases considered to have “false-positive serology” might actually have false-negative histology (3,33), giving thus misleading PPVs. In fact, objective serology could offer more accurate diagnostics in clinical routine where it is challenging to apply laborious and expertise-requiring histopathology with the increasing number of patients.

Altogether 33% of new coeliac disease patients could have been diagnosed applying the “triple criteria”, which might be even a conservative estimation as some subjects with a high likelihood for coeliac disease withdrew before the endoscopy. In the population-based low-risk cohort, the figure (48%) was close to that seen in paediatric studies (7,34). Besides being easier for patients, reduced endoscopies could provide substantial healthcare savings, as it is estimated that up to 95% of diagnostic expenses could be spared by omitting the biopsy (22). The released healthcare resources could be redirected for example to the follow-up of the increasing number of inflammatory bowel disease patients (35). It is feared that ceasing referrals for biopsy would lead to missing coeliac disease, or that patients might not approve a serology-based diagnosis (9,36). On the contrary, there is evidence that an active role of primary care actually improves case finding, and effective and acceptable diagnostics is more a matter of education and close collaboration with primary health care (37,38).

There are also other non-diagnostic reasons why retaining the biopsy has been advocated (39), including fear of missing a concomitant disorder (9) or complication such as refractory coeliac disease and malignancy (40). Evidently, coexistence of two conditions is possible, but performing endoscopy to all “triple criteria” positive individuals does not seem justified. None of the patients who could have avoided the biopsy were found to have any comorbidities in the diagnostic endoscopy, and these have been extremely rare also in previous studies (11,40,41). Further investigations are obviously indicated in case of red flag symptoms such as bloody

stools, dysphagia, or severe weight loss, with extra caution in elderly who are at greater risk for malignancies (11,42). As a comparison, patients with gastrointestinal reflux are rarely referred directly to endoscopy without red flag symptoms (43). The diagnosis of refractory coeliac disease is based on poor clinical response and severe histopathologic findings despite the gluten-free diet, and baseline biopsy results would not be helpful (44). Elfström et al suggested that the biopsy could have prognostic value for lymphoproliferative malignancies, but they compared patients having potential coeliac disease with normal mucosal architecture to those with flat mucosa (45). Elsewhere, the severity of established villous atrophy at diagnosis did not affect the complication risk (46). Further, to emphasise, the aim was not to entirely abandon the biopsy but to provide easier and more cost-effective diagnostics, and if any concerns arise, endoscopy should be per-formed with a low threshold.

Our main strength was the utilisation of three large cohorts comprising patients with varying diagnostic approaches and pre-test probabilities. Moreover, serology was used as recommended, validated histopathological methods were used, and subjects not fulfilling the "triple criteria" were carefully excluded. However, there were also limitations. Serology was not measured from two separate samples as ESPGHAN instructs, although currently there are no instructions how to operate with possible conflicting results and it remains unclear if this would be necessary (6). The prevalence of coeliac disease in moderate and low-risk cohorts was lower than expected as subjects with a previous diagnosis were excluded. In theory, such exclusion might cause some selection bias, as also could 30% of moderate risk and13% of low risk "triple positive" patients who were not biopsied. Even though there are no indicators to suspect selection in these screen-based cohorts, applicability of the criteria to nonbiopsied subjects is not 100% sure. Due to the withdrawals among subjects who did and also those who did not fulfil the "triple criteria", estimating percentages for avoidable biopsies was not possible. Altogether, the number of triple positive subjects in the moderate and low-risk cohorts was quite small, giving wide theoretical confidence intervals. Moreover, exact clinical information was available only for biopsied subjects in these cohorts. Finally, it must be stressed that our results can be generalised only to centres using accredited labs and test kits with linear calibration curves allowing to use multiples of ULN.

To conclude, we demonstrated that reliable nonbiopsy diagnosis of coeliac disease is possible in adults regardless of their clinical presentation or assumed pre-test probability for the disease. Applying such serology-based approach would lead to substantially reduced number of endoscopies and subsequent healthcare savings without affecting the diagnostic accuracy. Our findings of the applicability of tTG-ab>10×ULN with positive EMA are a promising start, but we believe that extending biopsy-omitting diagnostics to even more patients could be expected in the future.

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AUTHORSHIP

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Authors'contributions: Katri Kaukinen and Kalle Kurppa designed and supervised the study. Kaija Laurila, Teea Salmi, Markku Mäki, Pekka Collin and Liisa Luostarinen organised the data collection. Päivi Saavalainen performed genetic testing. Heini Huhtala provided expertise in statistical analysis and interpretation. Valma Fuchs drafted this paper, which was revised for important intellectual content by all the other authors. All authors approved the final version of the manuscript.

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Table 1. Clinical characteristics of the three study cohorts with different pre-test probabilities for coeliac disease.

Pre-test probability	High: Clinical suspicion n = 421	Moderate: At-risk group† n = 2,357	Low: Population cohort n = 2,722
Age, median (range), years	46 (18-83)	45 (18-96)	63 (51-76)
Female, %	71	57	53
Family history for coeliac disease, %	14	100	no data

† First and second degree relatives of coeliac disease patients

Table 2. Clinical characteristics of 90 biopsied study subjects fulfilling triple criteria† for coeliac disease diagnosis in different pre-test probability cohorts.

Pre-test probability	High: Clinical suspicion n = 59	Moderate: At-risk group‡ n = 17	Low: Population cohort n = 14
Age, median (range), years	47 (18-74)	46 (21-59)	62 (54-75)
Female, %	80	65	64
Main clinical presentation, %			
Gastrointestinal	73	71	57
Malabsorption	34	24	0
Extraintestinal	19	12	0
Asymptomatic	0	29	43
Family history of coeliac disease, %	22	100	29

†Transglutaminase 2 antibodies >10 x upper limit of normal, positive endomysium antibodies and coeliac disease-associated genotype; ‡First and second degree relatives of coeliac disease patients

Table 3. New biopsy-proven coeliac disease patients in three study cohorts with different pre-test probabilities and the proportion of "triple positive" cases[†].

Pre-test probability	High: Clinical suspicion n=160	Moderate: At-risk group[‡] n=85	Low: Population cohort n=29	Total coeliac disease patients n=274
"Triple positive", n	59	17	14	90
Positive tTG-ab not fulfilling "triple positivity", n	68	48	15	166
Negative tTG-ab, positive EMA, n	16	18	0	34
Negative tTG-ab, negative EMA, n	17	1	0	18
"Triple positive" out of total patients	37%	20%	48%	33%

[†]Transglutaminase 2 antibodies (tTG-ab, Celikey®) >10 x upper limit of normal, positive endomysium antibodies (EMA) and coeliac disease-associated genotype; [‡]First and second degree relatives of coeliac disease patients

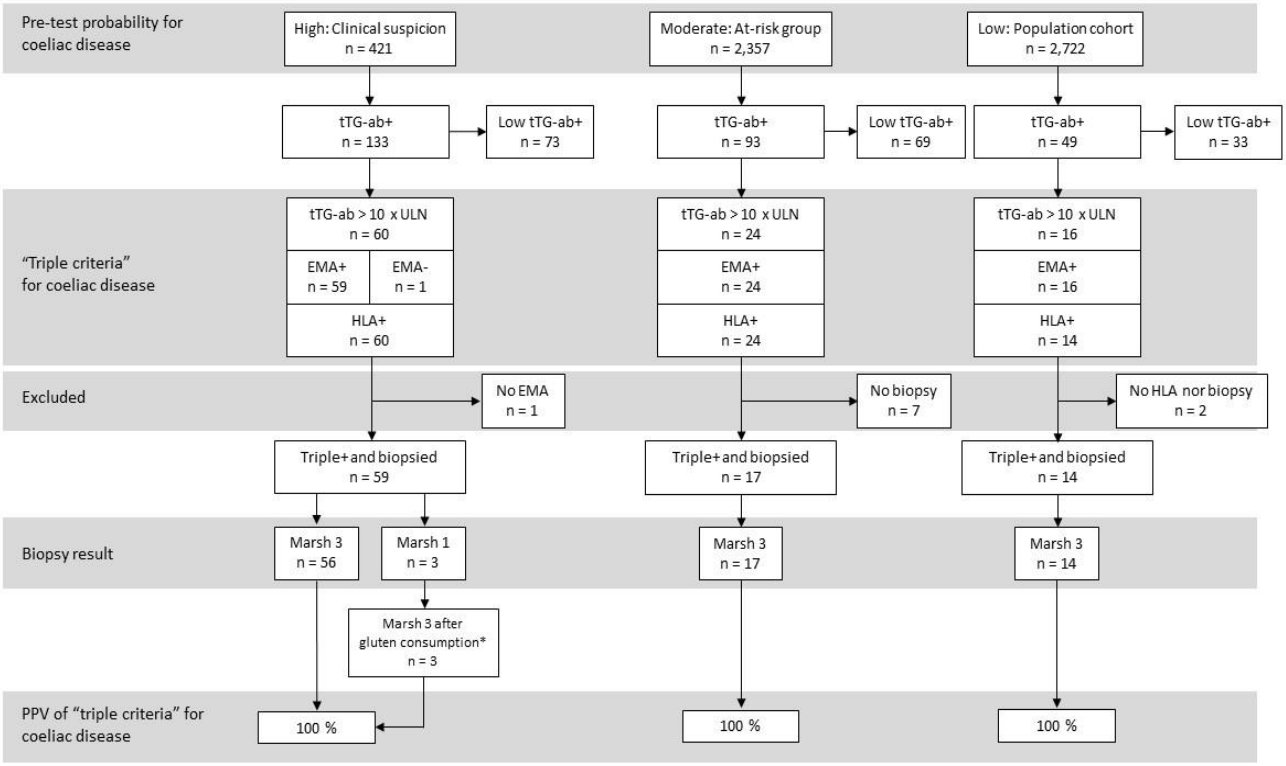

Figure 1. Study design and main results of the positive predictive value for "triple positive" non-biopsy diagnostic criteria of coeliac disease in three adult cohorts. "Triple positivity" comprises tTG-ab >10 x ULN, positive EMA and HLA genotype DQ2/DQ8. Abbreviations: tTG-ab+, positive tissue transglutaminase antibodies; ULN, upper limit of normal; EMA+, positive endomysium antibodies; HLA, human leukocyte antigen; GFD, gluten free diet. *Patients continued normal gluten intake until follow-up biopsies after one year were performed.