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ACADEMIC DISSERTATION
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ABSTRACT

The gold standard of celiac disease diagnosis has for a long time been the demonstration of small-bowel mucosal villous atrophy with crypt hyperplasia in an endoscopic sample. Even if the biopsy is useful in diagnostics, the question whether it is mandatory also in the follow-up is more complex. Endoscopic follow-up one year after diagnosis has been seen essential to ensure treatment response, but evidence is scarce. Inadequately treated or non-responsive celiac disease may increase the risk of severe complications, further supporting the histological follow-up. Then again, a repeat biopsy taken after one year on gluten-free diet might not pick these rare cases among the non-complicated patients who have not reached full mucosal recovery at this point. Altogether, the significance of a repeat biopsy and the histological recovery after one year on diet to the long-term health outcomes have been scarcely studied. This is the case also on studies on the overall value of regular follow-up in celiac disease.

This dissertation comprised three studies investigating the above-mentioned follow-up issues. Study **I** was a prospective cohort study involving 263 adult celiac disease patients. Comprehensive clinical, serological and histological data were collected both at diagnosis and after one year on treatment, and the participants were divided into two groups based on the presence or lack of villous recovery in the repeat biopsy. In addition, long-term medical record data were collected to assess the possibly increased frequency of severe clinical outcomes such as mortality, malignancies and comorbidities. The results showed that more severe disease at diagnosis in terms of villous atrophy, symptoms and serology predicts incomplete mucosal recovery one year after the diagnosis. However, the lack of full histological recovery did not affect patients' self-experienced symptoms or quality of life at the time of repeat biopsy or increase the risk of poor long-term health outcomes.

Study **II** comprised 760 previously diagnosed celiac disease patients participating in a follow-up study between 2006 and 2010. Medical records and personal interviews were used to collect clinical, histological and serological findings both at the time of diagnosis and later. During the study visit the participants fulfilled validated questionnaires for current gastrointestinal symptoms and quality of life and blood sample for serology was drawn to evaluate dietary adherence. For the subsequent study analyses, the participants were divided into two groups depending on whether or not they had undergone a repeat biopsy one year after diagnosis, and the biopsied patients further into two groups based on the degree of villous recovery as in Study **I**. More severe disease at diagnosis predicted

both the existence of a repeat biopsy and incomplete histological recovery in it. There were no differences between re-biopsied and not re-biopsied patients in the prevalence of long-term complications or in current dietary adherence, quality of life and gastrointestinal symptoms. However, patients without repeat biopsy were more insecure about their gluten-free diet and more often seropositive. As in Study **I**, the degree of histological recovery was not associated with any of the long-term variables used in the study.

Patients for study **III** were extracted from the same cohort as in study **II**. Altogether 648 long-term treated participants were divided into two groups based on whether they had received a long-term (>2 yr) follow-up or not, and the groups underwent comparisons of the same variables as in Study **II**. The median duration of follow-up was 10 years (range 2-38 years). Only 15% of the patients were found to have received long-term follow-up, depending partly on the presence of coexisting chronic diseases. However, even if the majority of patients wished regular healthcare visits, the follow-up and no follow-up groups did not differ in the long-term management of their disease, positivity for celiac disease serology or quality of life. There was also no difference in current gastrointestinal symptoms when evaluated by structured questionnaire, although those without a follow-up reported more overall symptoms.

The results of studies **I-III** indicate a need to re-evaluate the current follow-up strategies for celiac disease. More severe disease at diagnosis predicted both the presence of a repeat biopsy and incomplete villous architecture one year after diagnosis. However, ~~but~~ neither the biopsy and no biopsy nor the histological recovery and no recovery groups differed in the main long-term health and treatment outcomes, including quality of life, dietary adherence and frequency of complications. Similarly, although the prevalence of regular follow-up was markedly lower than recommended in the guidelines, again this did not affect important treatment outcomes such as dietary adherence and quality of life in the long term. Altogether, more personalized approach to follow-up in celiac disease is needed.

TIIVISTELMÄ

Keliakian diagnostisena kriteerinä on pitkään ollut ohutsuolesta otettu koepala, josta pystytään todentamaan suolinukan vaurio samanaikaisesti suolikuopakkeiden syventymisen kanssa. Vaikka koepalan rooli on vakiintunut diagnostiikassa, on sen asema keliakian seurannassa epäselvempi. Histologisen seurannan on yleisesti ajateltu olevan avainasemassa hoidon toteutumisen kannalta, mutta aiheesta on vain vähän tieteellistä näyttöä. Kultaisena standardina pidettyä ohutsuolikoepalaa vuoden gluteenittoman ruokavaliohoidon jälkeen puoltaa huonosti hoidettuun tai hoitoon reagoimattamaan keliakiaan liittyvä riski myöhempiin lisäongelmiin. Toisaalta vuoden kohdalla otettu koepala ei välttämättä pysty erittelemään näitä harvinaisia tapauksia siitä populaatiosta joilla suolinukan korjaantuminen tapahtuu luonnostaan hitaasti onnistuneesta hoidosta huolimatta. Paitsi seurantatähytyksestä, keliakiaseurannan toteutumisesta ja merkityksestä ylipäätään on niukasti tutkimuksia.

Tutkimus perustui kolmeen erilliseen osatyöhön, joissa selvitettiin seurannan toteutumista, sen vaikutusta hoidon onnistumiseen ja merkitystä pitkällä aikavälillä. Osatyöhön **I** sisältyi 263 aikuiskeliaakikkoa. Potilaista kerättiin laajat kliiniset, serologiset ja histologiset tiedot sekä diagnoosihetkellä että vuoden ruokavaliohoidon jälkeen, ja heidät jaettiin kahteen ryhmään perustuen suolinukkavaurion parantumisen asteeseen vuoden kohdalla otetussa seurantakoepalassa. Lisäksi myöhempiä sairaskertomustietoja kerättiin mahdollisesti lisääntyneen kuolleisuuden ja liitännäissairauksien esiintymisen arvioimiseksi. Epätäydellistä suolinukan korjaantumista ennusti diagnoosivaiheessa suolinukkavaurioltaan, oireiltaan ja serologialtaan vaikeampi taudinkuva. Keskeneräisellä parantumisella vuoden kohdalla ei kuitenkaan ollut vaikutusta tutkittuihin kliinisiin muuttujiin, kuten potilaiden elämänlaatuun ja oireisiin, eikä myöhempään kuolleisuuteen tai liitännäissairauksien esiintyvyyteen 15 vuoden seurannassa.

Osatyö **II** koostui 760 aiemmin diagnosoidusta aikuiskeliaakikosta, jotka osallistuivat seurantatutkimukseen vuosina 2006-2010. Kaikilta osallistujilta kerättiin haastatteluiden ja sairauskertomusten avulla keliakiaan liittyvät kliiniset, histologiset ja serologiset löydökset sekä sairastumishetkellä että myöhemmin. Lisäksi tutkimuskäynnillä mitattiin kyselyiden avulla oireita ja elämänlaatua, arvioitiin ruokavaliohoidon onnistumista ja mahdollisten komplikaatioiden ja liitännäissairauksien esiintyvyyttä, sekä otettiin keliakiavasta-aineet ruokavaliohoidon toteutumisen arvioimiseksi. Analyyseja varten osallistujat jaettiin kahteen ryhmään sen perusteella, oliko seurantakoepalaa otettu vuoden ruokavaliohoidon jälkeen. Lisäksi seurantatähystyksen läpikäyneitä potilaita

vertailtiin koepalalöydöksen perusteella kuten osatyössä **I**. Sekä seurantakoepalan ottoa että keskeneräistä histologista paranemista ennustivat diagnoosihetken vaikeampi tauti. Pitkäaikaisseurannassa tähystämättättömät potilaat noudattivat gluteenitonta ruokavaliota yhtä tarkasti kuin seurantakoepaloissa käyneet, eikä ryhmien välillä ollut juurikaan eroja potilaiden elämänlaadussa, oireissa, liitännäissairauksien ja komplikaatioiden määrässä. He kuitenkin kokivat itsensä epävarmemmiksi ruokavalion noudattamisessa ja olivat seurantatähystettyjä potilaita useammin seropositiivisia. Histologisen paranemisen aste vuoden kohdalla ei vaikuttanut tässäkään osatyössä pitkäaikaisseurannan tulokseen.

Osatyössä **III** käytettiin osin samaa aineistoa ja analysoitavia parametrejä kuin osatyössä **II**. Kaikkiaan 648 seurantatutkimukseen osallistunutta hoidossa olevaa keliakiapotilasta jaettiin vertailuja varten kahteen ryhmään sen perusteella, oliko heillä ollut sairauden pitkäaikaisseurantaa (>2 vuotta) vai ei. Keskimääräinen seuranta-aika oli 10 vuotta (vaihteluväli 2-38 vuotta). Osallistujista kerättiin jälleen laajat diagnoosihetken ja seuranta-ajankohdan tiedot. Tulokset osoittivat pitkäaikaisseurannan toteutuneen vain 15 % keliaakikoista, osin riippuen muista samanaikaisista sairauksista. Vaikka suurin osa potilaista toivoi seurantaa, ryhmien välillä ei ollut eroja seurantahetken ruokavaliohoidon osaamisessa tai tiukkuudessa, seropositiivisuudessa, elämänlaadussa tai strukturoidusti arvioiduissa suolisto-oireissa, joskin ilman seurantaa olleet raportoivat kokonaisuutena hieman enemmän oireita.

Osatöiden **I-III** tulokset osoittivat, että keliakian seurantaa on tarve kehittää. Sekä vuoden kohdalla otetun seurantakoepalan ottamista että sen tulosta voidaan ennustaa sairauden vakuvuudella diagnoosihetkellä. Kuitenkaan pitkällä aikavälillä seurantakoepalan tuloksella tai edes kontrollitähystyksen poisjättämisellä ei näyttäisi olevan suurta vaikutusta potilaiden ruokavaliohoitoon sitoutumiseen, elämänlaatuun ja oireisiin, tai merkittäviin pitkäaikaismuuttujiin kuten kuolleisuuteen ja oheissairauksiin. Lisäksi, vaikka keliaakikoita seurataan selvästi suositeltua vähemmän, tälläkään ei ollut merkittävää vaikutusta ruokavaliohoidon onnistumiseen tai elämänlaatuun pitkällä aikavälillä. Seurantakoepalan roolia tulee tulevaisuudessa pohtia ja kehittää yksilöllisempiä tapoja keliakian seurantaan.

TABLE OF CONTENTS

ABBREVIATIONS

ACG	American College of Gastroenterology
AGA	Anti-gliadin antibody
ALAT	Alanine aminotransferase
APC	Antigen-presenting cell
ARA	Anti-reticulin antibody
BMD	Bone mineral density
BMI	Body mass index
BSG	British Society of Gastroenterology
CCG	Current Care Guideline
DGP	Deaminated gluten peptide
DH	Dermatitis herpetiformis
ELISA	Enzyme linked immunosorbent assay
EmA	Endomysium antibodies
ESPGAN	European Society of Pediatric Gastroenterology and Nutrition
ESPGHAN	European Society of Pediatric Gastroenterology, Hepatology and Nutrition
FBC	Full Blood Count
GFD	Gluten-free diet
GIP	Gluten Immunogenic Peptide
GSRS	Gastrointestinal Symptom Rating Scale
H&E	Hematoxylin and eosin
HLA	Human leucocyte antigen
IELs	Intraepithelial lymphocytes
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IL-15	Interleukin-15
NK	Natural killer
PGWB	Psychological General Well-Being
QoL	Quality of life
RCD	Refractory celiac disease
SF-36	Short-Form 36
TCR	T cell receptor
TG2	Transglutaminase 2

TG2-ab	Transglutaminase 2 antibodies
TSH	Thyroid stimulating hormone
ULN	Upper limit of normal
Vh/CrD	Villous height crypt depth ratio
WGO	World Gastroenterology Organization
WHO	World Health Organization

INTRODUCTION

Celiac disease is a chronic immune-mediated disorder caused by the ingestion of gluten in genetically predisposed individuals (Green and Cellier 2007). In adults, the diagnosis of celiac disease has thus far required the demonstration of villous atrophy and crypt hyperplasia in the small-bowel biopsy, whereas in children it is possible to establish the diagnosis in certain cases without biopsies (Husby 2012). Currently there is increasing trend towards less invasive diagnostics in adults too and, for the first time, serology-based diagnosis has been made possible in the very recent Finnish guidelines (Rubio-Tapia et al. 2013, Husby et al. 2012, Current Care Guideline 2018). The classical symptoms are diarrhea, stunting and malabsorption in childhood (Gee 1888, Visakorpi et al. 1970), but the disease can appear with varying symptoms at any age with an increasing prevalence through aging (Vilppula et al. 2009). During the last decades, with improved diagnostics and better understanding of the disease, the clinical presenation has become more heterogenous (Mäki et al. 1988a, Murray et al. 2003).

Histological injury in celiac disease develops gradually. Elimination of dietary gluten by means of a gluten-free diet usually leads to rapid alleviation of symptoms and slower recovery of villous structure (Järvinen et al. 2003, Lähdeaho et al. 2011). However, in most literature complete mucosal recovery has been reached only in a minority of patients (Wahab et al. 2002, Tursi et al. 2006, Lanzini et al. 2009, Sharkey et al. 2013). The most common reason for incomplete mucosal recovery has usually been imperfect dietary adherence (Abdulkarim et al. 2002, Leffler et al. 2007, Sharkey et al. 2013).

The main aims for follow-up in celiac disease are to ensure response to treatment and to find the rare cases with refractory celiac disease (RCD). In RCD, mucosal damage persists despite a strict gluten-free diet, increasing the risk of serious complications. A repeat biopsy one year after the celiac disease diagnosis is thus recommended in most guidelines, at least in patients with persistent symptoms. In Finland, the prevalence of strict dietary adherence is over 90% (Ukkola et al. 2011) and long-term histological recovery rate as high as 96% (Ilus et al. 2012). As as result, frequency of RCD was recently shown to be as low as 0.3% (Ilus et al. 2014) of all celiac disease patients, which calls for more personalized histological follow-up strategies.

On the other hand, even without actual RCD, incomplete mucosal recovery in the repeat biopsy has been associated with adverse outcomes, such as osteoporosis and increased risk of lymphoma and mortality, although these results are controversial (Lebwohl et al. 2013b). Overall, the significance of a repeat biopsy in celiac disease

remains scarcely studied and is under discussion in expert panels (Ludvigsson et al. 2014).

Strict adherence to gluten-free diet is vital for mucosal healing and symptom alleviation. It has been argued that having access to healthcare and regular follow-up improves the adherence to gluten-free diet (Bardella et al. 1994, Haines et al. 2008), but these studies have been carried out on small samples and in populations with often poor overall adherence. Altogether, evidence on the optimal timing, actual content end even the necessity of follow-up remains scarse (Bai et al. 2013, Ludvigsson et al. 2013c, Rubio-Tapia et al. 2013, Current Care Guideline 2010).

The main aim of the present study was to investigate the implementation and significance of routine repeat biopsy one year after celiac disease diagnosis. This was accomplished by comparing a variety of short- and long-term health and treatment outcomes between large cohorts of celiac disease patients. We focused on the features predicting the aquiring of a repeat biopsy after one year on gluten-free diet. If the endoscopy was conducted, we investigated the features predicting the result of the repeat biopsy as well as its effect on long term health between those with and without histological recovery. Furthermore, the effect of a regular long-term (>2 yr) follow-up of celiac disease to the aforesaid health and treatment outcomes was investigated.

LIST OF ORIGINAL PUBLICATIONS

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

I Pekki H, Kurppa K, Mäki M, Huhtala H, Sievänen H, Laurila K, Collin P, Kaukinen K (2015): Predictors and Significance of Incomplete Mucosal Recovery in Celiac Disease After 1 Year on a Gluten-Free Diet. Am J Gastroenterol. 110:1078-1085.

II Pekki H, Kurppa K, Mäki M, Huhtala H, Laurila K, Ilus T, Kaukinen K (2017): Performing Routine Follow-up Biopsy 1 Year After Diagnosis Does Not Affect Long-term Outcomes in Coeliac Disease. Aliment Pharmacol Ther. 45:1459-1468.

III Pekki H, Kaukinen K, Ilus T, Mäki M, Huhtala H, Laurila K, Kurppa K (2018): Long-term Follow-up in Adults with Coeliac Disease: Predictors and Effect on Health Outcomes. Dig Liv Dis. 50:1189-1194.

REVIEW OF THE LITERE

1 History

First alleged description of celiac disease was written by Aretaeus of Cappadocia in the 1st century B.C. (Adams 1856). Since the causative dietary agent, gluten, is present only in wheat, rye and barley, at its earliest celiac disease could have evolved only after the development of agriculture in Mesopotamia approximately 10 000 years ago (Harlan 1966). Up to the last few centuries grains contained relatively low amounts of gluten (Belderok 2000, Kasadra 2013), which may explain the lack of medical reports of celiac disease before the beginning of the 19th century (Baillie 1815). The earliest scientific report was written by Samuel Gee (1888). A variety of dietary treatments were attempted during the first half of the 20th century, but the actual cause of celiac disease remained unknown. The main disese driver, gluten, was found after World War II by Dicke et al. (1953), which finally enabled targeted treatment of celiac disease.

A year later, in 1954, the characteristic histopathologic changes, i.e. small-bowel mucosal villous atrophy and crypt hyperplasia, were discovered (Paulley 1954). Endoscopic biopsy techniques were introduced during the next few years (Royer 1955 and Shiner 1956). The first serum antibodies were described in 1958 (Berger 1958), and the main autoantigen of the disease in 1997 (Dieterich et al. 1997). The suggestion that celiac disease as an autoimmune disease was also made in the 1990's (Mäki 1991 ja Mäki 1994). Finding serological markers paved the way to wide-scale screening studies, which revealed the wide clinical spectrum and

high prevalence of the disease. Nowadays celiac disease is recognized to be one of the most common chronic gastrointestinal conditions in the Western world (Mäki et al. 2003, Fasano et al. 2003, Lohi et al. 2007).

2 Epidemiology

For a long time, celiac disease has apparently been a very rare condition (Guandalini 2008). One of the oldest epidemiological reports approximated the incidence of celiac-like sprue syndrome to be between 1:10000 and 1:5000 in Great Britain (Davidson and Fountain 1950). Reports since have varied depending on the diagnostic criteria and nation in question, but a considerable increase in the prevalence took place in the 1980s, as the understanding of the diverse presentation increased and celiac disease was found also in adults (Logan et al. 1983, Mäki et al. 1988). The main contributor to this change was improvements in diagnostic methods, but studies indicate also a rise in the true prevalence of celiac disease (Lohi et al. 2007, Rubio-Tapia 2009, Ludvigsson 2013a, Choung et al. 2017).

Recent screening studies have shown an approximate prevalence of 1% for celiac disease in both children (Hoffenberg 2003, Mäki et al. 2003) and adults (Fasano 2003, West et al. 2004, Walker et al. 2010). In Finland, the prevalence has been found to be even higher, reaching approximately 2% in adults (Lohi et al. 2007). The prevalence increases through aging and is up to 2.7% in the elderly Finnish population (Vilppula et al. 2009). The highest prevalence in the world thus far, 5.6%, has been found among Saharawi children in Algeria (Catassi 1999). Due to the rarity of the predisposing genes in the population (Chapter 3.2), the disease is rare in China and virtually absent in Japan (Cummins and Roberts-Thompson 2009).

3 Pathogenic Background

3.1 Genetics

The prevalence of celiac disease varies between 3% and 10% in first-degree relatives of the patients (Mäki et al 1991, Singh et al. 2015). The genetic risk is largely associated with gene areas encoding human leucocyte antigens (HLA), which are necessary but not sufficient for celiac disease to develop. HLA genes are polymorphic genes located in a gene cluster called the major histocompatibility complex. One of these genes on chromosome 6p21.3 has been associated with celiac disease already in 1970s (Stokes 1972). The HLA locus and, more specifically, its alleles found in 1972 were later defined to encode HLA-DQ2 and DQ8 molecules on the antigen-presenting cells (Sollid et al. 1989, Sollid 2000). HLA-DQ2 homozygosity increases the disease risk of five-fold compared to heterozygotes (Murray 2007, Koskinen 2009). Approximately 90% of celiac disease patients express the HLA-DQ2 haplotype (DQA1*0501/DQB1*0201). Five percent of patients have the HLA-DQ8 haplotype (DQA1*0301/DQB1*0302) and almost all remaining subjects have at least one of the two genes encoding the DQ2 αβ-heterodimer (DQB1*0201 or DQA1*0501) (Karell et al. 2003).

Approximately a third of the population carry the DQ2 or DQ8 alleles thus being at genetic risk for celiac disease, but for yet unknown reasons only about 3-4% percent of people with these haplotypes develop the condition (Jabri and Sollid 2009). In addition to the HLA alleles, a large number of non-HLA susceptibility loci have been proposed to be involved in the pathogenesis of celiac disease (Dubois et al. 2010, Romanos et al. 2014), and also non-coding DNA may participate at some level (Vaira et al. 2014).

3.2 Environmental Factors

Environmental factors are needed for the development of celiac disease, particularly gluten without which the disease cannot develop. The term gluten is a general heading for insoluble prolamine peptides, namely gliadin in wheat, hordein in barley and secalin in rye (Platt and Kasarda 1971). In wheat, prolamins function as storage proteins (Anand et al. 1978). Gluten is rich in proline (15%), making it resistant to luminal degradation

by gastrointestinal enzymes and thus increasing its pathogenicity (Frazer et al. 1959, Jabri 2009). The three-dimensional structure of gliadin enhances the baking properties of dough so that, unfortunately for celiac disease patients, cereals containing it are favored in the food industry (Molberg et al. 2003).

In addition to the necessary gluten as a disease driver, it seems evident that there are also other environmental triggers. This would explain why only a part of the genetically predisposed people get the disease, as well as the increase in the true prevalence (Lohi et al. 2007). Also differences in the prevalence between genetically similar populations with different living environments support the hypothesis of additional triggers (Khondrasova et al. 2009).

Breastfeeding has been studied in this context and has been thought to offer protection against the development of celiac disease (Ivarsson et al. 2002), but this matter is still under debate (Lionetti et al. 2014, Vriezinga et al. 2014). The effect of age at gluten introduction is ambivalent, but the amount of gluten used at the time seems to be connected to an increased risk (Ivarsson et al. 2002, Aronsson et al. 2016).

Adeno- and rotavirusinfections have been suggested to increase the risk of celiac disease, possibly through molecular mimicry (Stene et al. 2006). The role of rotavirus is supported by the reduced risk in vaccinated children. (Kemppainen et al. 2017). In the viral hypothesis, it is thought that particularly the age in which an infant gets certain types of infections affect the likelihood to develop celiac disease. This is somewhat supported by the observed seasonal pattern in celiac disease, as the incidence varies depending the month of birth. (Ivarsson et al. 2003). A recent study found reovirus infections to suppress certain types of T-cells, thus potentially triggering celiac disease (Bouziat et al. 2017).

Interestingly, the prevalence of celiac disease has increased more in developed countries than in developing countries (Kang et al 2013). Consequently, a concept known as the "hygiene hypothesis" has gained popularity as a possible cause for this phenomenon (Lohi et al. 2007, Kondrashova et al. 2008). In this hypothesis, the lack of microbial exposure in developed countries increases the prevalence of autoimmune diseases, as the human immune system reacts to self-antigens in the lack of infectious agents (Strachan 1989, Bodansky et al. 1992, Lohi et al. 2007).

3.3 Pathogenesis

Celiac disease is thought to be one of the best understood autoimmune diseases as regards to pathogenesis (Lindfors et al. 2010, Sollid and Jabri 2011). Gluten plays a key role in the development of mucosal inflammation in affected patients. The ensuing immune reaction from the ingestion of gluten eventually leads to villous atrophy with

crypt hyperplasia. Adaptive immunity was thought to be the main pathway to mucosal destruction, but more recent studies have found the process to be a combination of adaptive and innate immunity (Sollid and Jabri 2011). In active celiac disease the barrier between the gut lumen and the rest of the body is though to be damaged. The immunogenic peptides cross the epithelial barrier to the lamina propria either through paracellular route due to increased epithelial permeability, or via transcellular route with the help of secretory immunoglobulin A (IgA) (Sapone et al. 2008, Rauhavirta 2014).

After the entrance to the lamina propria, the peptides are deamidated by transglutaminase 2 (TG2), which further activates the adaptive T-helper cell 1 type pro-inflammatory response (Sollid 2000). This causes various pro-inflammatory cytokines, particularly interferon γ, to begin a cascade resulting in the disruption of small-bowel mucosal structure (Sollid et al. 1989). TG2 is a multifunctional enzyme present mainly intracellularly in the gut, although the enzyme can be located also in other places around the body (Sollid and Jabri 2011). Under tissue-damaging conditions, such as gastrointestinal infections, the tolerance to native gluten peptides breaks and TG2 is released extracellularly (Siegel et al. 2008). The gliadin peptides are next processed and presented by the HLA-DQ8 or DQ2 molecules on antigen-presenting cells (APCs) and recognized by the gluten-specific CD4+ T cells (Molberg et al. 1998). In the end, crypt hyperplasia and villous atrophy develop through direct cytotoxicity and by activation of matrix metalloproteinases (Pender et al. 1997, Jabri 2009).

For yet unclear reasons, specific autoantibodies are developed against TG2 (TG2-ab). These antibodies are assumed to cause extraintestinal manifestations by depositing in different tissues such as the liver, skeletal muscle and brain (Korponay-Szabo et al. 2004, Hadjivassilou et al. 2006). These effects may in part be due to disrupted angiogenesis by TG2-ab (Myrsky et al. 2008). Additionally, TG2-ab may promote celiac disease progression by inhibiting the differentiation of epithelial cells and ~~thus~~ increasing their proliferation (Halttunen and Mäki 1999 Barone et al. 2007). Nowadays TG2-ab also have a major role in the diagnostics of celiac disease (Chapter 6.1.1)

Gliadin may also induce mucosal damage via a non-T-cell-dependent innate pathway, particularly by activating interleukin-15 (IL-15) (Maiuri et al. 2003). There is a variety of intraepithelial lymphocytes (IELs) in the small-intestinal mucosa, in celiac disease most importantly the CD8+ T cells presenting T cell receptor (TCR) αβ+. Many of these TCR αβ+ cells also express natural killer (NK) cell receptors, which act by lowering the threshold for T cell activation at stressful times (Bauer et al. 1999). IL-15 up-regulates IELs to express high levels of activating NK receptors (Meresse et al. 2004). In enterocytes, IL-15 up-regulates the expression of their ligands resulting in an increase in the production of proinflammatory cytokines and cytolytic enzymes leading to apoptosis and mucosal damage (Figure 1) (Hüe et al. 2004, Jabri et al. 2000). IELs have also recently

been shown to gain malignant features in some patients with untreated celiac disease (Ettersperger et al. 2016).

4 Clinical Features

4.1 Classic Manifestations

Historically, as celiac disease was thought to be a solely pediatric condition, the classic signs and symptoms were poor growth, persistent diarrhea, and signs of malabsorption (Visakorpi and Mäki 1994). Due to malabsorption, patients often had deficiencies of fat-soluble vitamins D, E, A and K, as well as important trace elements iron, calcium, zinc, folate and vitamin B12, and subsequently complications such as anemia, rickets, poor growth, short stature and delayed puberty (Visakorpi and Mäki 1994). The classic manifestations became less frequent in the 1980s as more patients with milder or non-classical symptoms were found (Logan et al. 1983, Mäki et al. 1988, Kivelä et al 2015). Nowadays celiac disease patients often suffer only from mild abdominal symptoms, such as loose stools, abdominal discomfort and flatulence or have only extraintestinal symptoms (Volta et al. 2014, Agardh et al. 2015).

4.2 Extraintestinal Manifestations and Complications

Celiac disease can manifest with a variety of extraintestinal symptoms. The line between an extraintestinal manifestation and a complication is often obscure and depends on how these are defined. Due to the changing clinical spectrum of celiac disease the term 'atypical' is no longer considered valid in patient with only extraintestinal symptoms (Collin et al. 1999, Kaukinen et al. 2010, Ludvigsson et al 2012).

4.2.1 Extraintestinal Manifestations

One of the most common extraintestinal symptoms of celiac disease, which is sometimes even seen as its own disease entity, is dermatitis herpetiformis (DH), which manifests itself on the skin as a polymorphic blistering rash on elbows, knees, buttocks and scalp (Duhring 1884). The condition is rare in childhood, the mean age of onset being usually between 30 and 40 years with a slight male predominance (Salmi et al. 2014).

Neurological symptoms include gluten ataxia (Hadjivassiliou et al. 1996), peripheral neuropathy, encephalopathy, myopathy (Hadjivassiliou et al. 2010) and migraine

(Gabrielli 2003). An increased prevalence of epilepsy has also been associated with celiac disease (Cooke and Smith 1966, Ranua et al. 2009). Alzheimer's disease and celiac disease overlap genetically and thus may be associated to some extent, even if patients with celiac disease do not seem to have significantly increased risk for dementia (Lebwohl et al. 2016)

Celiac disease patients may also suffer from severe psychiatric problems such as anxiety, depression and schizophrenia (Kalaydjian et al. 2009). Particularly depression seems to be more prevalent in both adults and adolescents with untreated celiac disease (Pynnönen et al. 2004, Ludvigsson et al. 2007). A gluten-free diet may alleviate both depression and anxiety (Addolorato et al. 2001).

Untreated celiac disease can contribute to gynecological maladies such as unexplainable infertility (Morris et al. 1970), delayed menarche, secondary amenorrhea and early menopause (Moleski et al. 2015). These risks significantly decrease on a gluten-free diet (Tersigni et al 2014). In recent studies no differences on pregnancy outcomes were found in patients with treated celiac disease when compared to the general population (Tersigni et al. 2014, Saccone et al. 2016).

Hepatic conditions are common in celiac disease (Farre et al. 2002). The hepatic injury seems to be gluten-sensitive and its severity may vary from only slightly elevated transaminases, which decrease after the introduction of a gluten-free diet, to severe liver failure requiring transplantation (Kaukinen et al 2002). In Finland, the latest estimate of elevated liver enzymes in untreated patients is between 10% and 15% (Korpimäki et al. 2011, Äärelä et al. 2016), but globally this percentage is as high as 40-50% (Castillo et al. 2015, Vajro et al. 2013).

Another frequently observed extraintestinal manifestation of celiac disease is permanent damage of dental enamel ~~defects~~, which, like many other extraintestinal symptom, can also be seen as a complication (Aine et al. 1996). Other extraintestinal manifestations possibly associated with celiac disease are for example recurrent aphthous ulcerations (Ferguson et al. 1976), arthritis (Mäki et al. 1988b, Collin et al. 1992a) and alopecia areata (Corazza et al. 1995a).

4.2.2 Complications

Of the conditions considered more often as a complication than extraintestinal manifestation, the association between bone disorders and celiac disease has been long known (Salvesen and Boe 1953). However, the formerly common osteomalacia and rickets have given way to osteoporosis at least in developed countries (Valdimarsson et al. 1994, Tikkakoski et al 2007). Both children and adults with celiac disease may present lower bone mineral density (BMD) than healthy controls (Tau et al. 2006, Corazza et al.

1995b), although in a recent study this was not seen in women diagnosed over at 40 years of age (Kamycheva et al 2017). In a recent meta-analysis, the risk of osteoporotic fractures is significantly increased in celiac disease (Heikkilä et al. 2015). The evident cause for osteoporosis in celiac disease would be malabsorption, and indeed more advanced histological disease at diagnosis is associated with lower BMD (Abu Daya et al. 2013). However, low BMD can be present even when the villous structure is still morphologically normal (Kurppa et al. 2010a), and it has been suggested that mucosal inflammation causing chronic release of proinflammatory cytokines would disturb the balance of bone remodeling (Riches et al. 2009, Hård et al. 2018).

Some malignancies have been found more prevalent in patients with celiac disease, especially in older studies. However, this feared complication may have been overrepresented due to detection bias, as only symptomatic patients were actively sought. In more recent studies the malignancy risk has decreased, the highest risk ratio being 1.9-5.8 of non-Hodgkin lymphomas (Grainge et al. 2012, Ilus et al 2014). The risk of lymphoma is greatest during the first years after the diagnosis of celiac disease, but it has been reported to remain elevated for up to 15 years (Grainge et al. 2012). The elevated risk may still at least partly be due to selection bias, as the clinical detection rate of celiac disease in those studies was much lower than the previously reported serological prevalence, and the patients thus could have been those with the most severe presentation (Catassi et al 2002, Lohi et al. 2009).

Of other malignancies, the risk for esophageal cancer is up to 4-fold and for stomach cancer up to 3-fold in untreated celiac disease (Askling et al. 2002, West et al. 2004a). As regards to colon and hepatic cancers, the results are controversial (Askling et al. 2002, Viljamaa et al. 2006, Elfström et al. 2012). Interestingly, the risk of all malignancies among celiac disease patients has been seen to decrease after the year 2000, which may be due to increased detection rates of patients with milder symptoms and/or because of shorter diagnostic delay and improved adherence to dietary treatment (Eigner et al. 2017, Ludvigsson et al 2012).

Recent studies have shown the mortality of untreated celiac disease patients to be comparable with the general population (Lohi et al. 2009, Godfrey et al. 2010, Chounq et al. 2017). This is contradictory to earlier findings in which especially disease patients with malabsorptive symptoms were reported to have increased mortality (Corraro et al. 2001, Viljamaa et al. 2006). However, the mortality has remained elevated among patients with RCD (Biagi et al. 2014).

4.3 Associated Diseases

In certain diseases an unusually high prevalence of celiac disease has been observed; most notably approximately 20-30% of celiac disease patients have a coexisting autoimmune disorder (Viljamaa et al. 2005b, Cosnes et al. 2008). Common autoimmune comorbidities are type 1 diabetes (Visakorpi 1969), autoimmune thyroidal diseases (Kuitunen et al. 1971) and Sjögren´s syndrome (Viljamaa et al. 2005a). Autoimmune diseases share genetic risks, but it is possible that other pathogenetic mechanisms are also involved (Collin et al. 1994, Viljamaa et al. 2005b). Celiac disease is also linked with selective IgA deficiency (Collin et al 1992), which is clinically important as IgA-class antibodies are routinely used for the screening of celiac disease (Meini et al. 1996).

Celiac disease has been associated also with several other gastrointestinal diseases and dermatological diseases. The former includes particularly microscopic colitis (Matteoni et al. 2001), autoimmune liver diseases and atrophic gastritis. Of dermatological diseases, for example alopecia areata, dermatomyositis, scleroderma, psoriasis and vitiligo have been reported to be overrepresented in celiac disease (Peña 1987, Abenavoli et al. 2010). Oral diseases such as geographic tongue and lichen also seem to be more common in celiac disease patients than in the population (Ciglic et al 2015, Ciglic et al. 2016), and this could be the case also with Down´s and Turner´s syndromes and myasthenia gravis (Book et al. 2003, Sagodi et al. 2006, Freeman et al. 2009).

4.4 Silent Celiac Disease and Screening

Clinically silent celiac disease with positive celiac disease serology and small-bowel mucosal atrophy but no apparent symptoms is nowadays a frequent phenomenon (Kurppa et al 2014, Ludvigsson et al. 2013b). This phenotype is more often seen in screen-detected at-risk patients (Current Care Guideline 2010, Rubio-Tapia et al. 2012, Ludvigsson et al. 2013c, Bai and Ciacci 2017).

There are contradictory findings on whether the symptoms correlate with the other disease features; i.e. milder symptoms do not automatically mean less severe histological and serological disease (Brar et al. 2007, Murray et al. 2008, Taavela et al 2013a). On the other hand, asymptomatic subjects seem to have lower risk of intestinal lymphomas than clinically detected patients, and the restricting gluten-free diet may decrease quality of life (Ukkola et al. 2011, Mearin et al. 2006, US Preventive Services Task Force 2017). When applying WHO standards, screening in high risk groups might be appropriate, but further evidence for mass screenings of the general population is needed (Viljamaa et al. 2005b, Ludvigsson et al. 2015). However, data suggest that, on a general level, screen-detected patients benefit from a gluten-free diet similarly to symptomatic patients,

although individual differences exist (Kurppa et al. 2014, Mustalahti et al. 2002). Interestingly, when assessed systematically by validated questionnaires, screen-detected patients may in fact have as severe symptoms as clinically detected patients (Paavola et al. 2012). Based on these findings, prediction of the risk of celiac disease on basis of symptoms is difficult (Kårhus et al. 2016, Kivelä et al. 2017)

4.5 Potential Celiac Disease

Owing to the increasing serological screening, patients with positive celiac autoantibodies but normal mucosal architecture are increasingly found, such situation having been common already for a long time among DH patients (Reunala 2001, Mäki et al. 2003, Kurppa et al. 2010b). Sometimes the antibodies may be false positive especially in low titres, but in many cases seropositivity appears to be an early phenomenon in the disease continuum (Korponay-Szabo et al. 2004, Ferrara et al. 2010, Simell et al. 2010). In a randomized trial, most participants with positive endomysium antibodies (EmA) developed duodenal atrophy within one year on a gluten-containing diet, showing that the serology can be elevated before the appearance of morphological mucosal damage (Kurppa et al 2009). Patients may also present with symptoms and sings of celiac disease and benefit from a gluten-free diet before the development of evident villous damage (Repo et al. 2017, Volta et al. 2016).

5 Quality of Life and Persistent Symptoms

5.1 Quality of Life

WHO defines health-related quality of life as an individual's overall satisfaction with life in the context of the culture in which he lives and as a sense of general personal well-being comprising physical, social and psychological aspects and also general sense of well-being and somatic sensations affected by one's health status (WHO 1997). Reproducible and validated methods should be used when quantifying the effect of a disease on the quality of life. In celiac disease research, frequently used quality of life questionnaires include Psychological General Well-Being (PGWB) (Dupuy et al. 1984) and the 36-item short-form (SF-36) (Ware and Sherbourne 1992). Studies assessing long-term quality of life in celiac disease patients have been mostly cross-sectional (Lohiniemi et al. 2000, Nachman et al. 2009) (Table. 1)

In most studies, the quality of life of untreated celiac disease patients has been decreased compared to both healthy controls and the general population (Johnston et al. 2004, Viljamaa et al. 2005b, Nachman et al. 2009). It seems that concomitant gastrointestinal disorders predispose to more severe symptoms and poorer quality of life at diagnosis (Usai et al. 2002, Nachman 2009, Kurien et al. 2011). As previously mentioned, a substantial part of the so called 'asymptomatic patients' found through screening actually have symptoms when systematically inquired. However, these symptoms seem to impair quality of life only in clinically-detected patients, possibly because of their overall more severe presentation (Paavola et al. 2012). Furthermore, the psyche seems to play a significant role on quality of life, as depression has a stronger correlation with reduced quality of life than gastrointestinal symptoms (Sainsbury et al. 2013a). In untreated patients, female gender and psychiatric, neurologic or gastrointestinal comorbidities seem to increase the risk for reduced quality of life (Usai et al. 2002, Hallert et al. 2002a, Paarlahti et al. 2013). Long diagnostic delay also seems to affect quality of life adversely (Norström et al. 2011, Fuchs et al. 2018).

Most studies have shown improved quality of life on a gluten-free diet (Lohiniemi et al. 2000, Nachman et al. 2009, Borghini et al. 2017, Hughey et al. 2017), although this is more prominent with originally symptomatic patients (Ukkola et al. 2011, Mahadev et al. 2016). Long-term gluten-free diet does not seem to decrease the quality of life even in screen-detected patients (Viljamaa et al. 2005b, Kurppa et al. 2012), giving further

justification to recommend the diet to this patient group. Female gender seems to be connected with lower quality of life also in treated celiac disease, females expressing more concern about the impact on socializing with friends and having to abstain from important things in life (Hallert et al. 2002). For many patients, celiac disease diagnosis is a relief, but some experience problems adjusting to a chronic illness and the diagnosis has been reported to be even a shock to 6% of patients (Ukkola et al. 2012). Besides the burden of the diet, the quality of life in long-treated celiac disease patients may be somewhat impaired due to increased anxiety related to possible accidental ingestion of gluten (Leffler et al. 2017).

Table 1. Long-term cross-sectional studies on the quality of life (QoL) and symptoms in treated adult celiac disease patients

Author	Country	N:o of patients	N:o of controls	Method(s)	GFD, yrs	Findings
Hallert et al. 1998	Sweden	89	-	SF-36, GSRS	10	Women with celiac disease had lower QoL compared to general population
Lohiniemi et al. 2000	Finland	58	110	GSRS, PGWB	10	No differences in QoL between patients and non-celiac controls
Usai et al. 2002	Italy	66	136	SF-36	>2	Celiac disease patients had lower QoL compared to non-celiac controls
Fera et al. 2003	Italy	100	100	SF-36, IBQ, SCID, M-SDS, STAI	9	Celiac disease patients had more depression and anxiety than controls; no association with dietary compliance
O'Leary 2004	Ireland	50	-	SF-36	28	No difference in QoL between celiac disease patients and general population
Viljamaa 2005	Finland	53	110	SF-36, PGWB, GSRS	14	No difference in QoL between celiac disease patients and controls
Häuser 2006	Germany	446	-	SF-36, HADS, GSCL	9	Celiac disease patients had lower QoL compared to the general population
Häuser 2007	Germany	516	-	HADS. SF-36	No data	Mental disorders, dietary lapses and dissatisfaction with physician predicted reduced QoL in celiac disease
Nachman 2010	Argentina	53	-	SF-36	4	QoL increased after diagnosis and remained higher than at diagnosis even if decreased in a 4-year follow-up
Barratt 2011	UK	573	-	SF-36, HADS	8	Difficulties in dietary adherence predicted reduced QoL
Zampieron 2011	Italy	187	-	CD-QOL	10	Women and symptomatic celiac disease patients had reduced QoL compared to asymptomatic patients
Paavola 2012	Finland	366	110	GSRS, PGWB	7-9	Symptomatic celiac disease patients had reduced QoL; no difference between screened patients and controls
Paarlahti 2013	Finland	596	-	GSRS, PGWB	11	Long-lasting and severe symptoms before diagnosis predicted persistent symptoms and reduced QoL
Mahadev 2016	USA	211	-	CD-QOL, PGWB,CDAT	4	Screen-detected patients do not differ from symptom-detected in QoL or long-term GFD adherence
Hughey 2017	USA	1832	-	CSI, CD-QOL	No data	Reduced QoL improved and symptoms alleviated on a long-term GFD

GFD, gluten-free diet; SF-36, Short Form 36 Health Survey; GSRS, Gastrointestinal Symptoms Rating Scale; PGWB, Psychological General Well-Being Index; IBQ, Illness Behavior Questionnaire; SCID, Structured Clinical Interview for DSM; M-SDS, modified version of the Zung Self-Rating Depression Scale; STAI, State and Trait Anxiety Inventory; HADS, The Hospital Anxiety & Depression Scale; GSCL, Giesen Symptom Check List; CSI, Celiac Disease Specific Symptoms; CDAT, Celiac Disease Adherence Test; CD-QOL, Celiac Disease and Quality of Life

5.2 Persistent Symptoms

Failure to reach expected clinical response to gluten-free diet is most commonly defined as persistent gastro-intestinal symptoms (Abdulkarim et al. 2002, Dewar et al. 2012). Several cross-sectional studies have examined the long-term symptoms in celiac disease (Table 1). An identifiable cause can be found in over 90% of cases, the most common being dietary lapses (Abdlukarim et al. 2002, Dewar et al. 2012, Stasi et al. 2016). Symptoms may also be caused for example by coexisting lactose intolerance, irritable bowel syndrome, eating disorders, gastroesophageal reflux, microscopic colitis, giardiasis, inflammatory bowel disease, small intestinal bacterial overgrowth, thyroid impairment and pancreatic insufficiency (Abdulkarim et al. 2002, Leffler et al. 2007, Stasi et al. 2016). Even without any of the aforementioned diseases, celiac disease patients may have more gastrointestinal symptoms than the general population despite a strict gluten-free diet (Laurikka et al. 2016).

In recent studies, the prevalence of persistent symptoms has been approximately 20% (Paavola et al. 2012, Laurikka et al. 2016, Stasi et al. 2016), although this may be overestimation as the symptom alleviation can continue a rather long time (Hughey et al. 2017). Diagnosis at working age, lower socioeconomic status, long duration and severe symptoms before diagnosis, along with the presence of coexisting thyroidal disease, non-celiac food intolerance or gastrointestinal morbidities, have been associated with the persistence of symptoms (Paarlahti et al. 2013, Oza et al. 2016). There are indications that patients who reach national recommended fiber intake have less often persistent symptoms (Laurikka et al. 2019).

6 Diagnosis

The suspicion of celiac disease can be based on several issues, including clinical symptoms, existence of associated comorbidities and family history for the disease. As serology is quite sensitive and specific, it can be used to target patients into further invasive studies, but, if clinical suspicion is high, even seronegative patients should be endoscopied (Current Care Guideline 2010, Salmi et al. 2006). In Finland patients who belong to at-risk groups are recommended to be screened comprehensively and, if necessary, repeatedly (Current Care Guideline 2010). The antibodies are also recommended to be measured with low treshold even from individuals with mild or extraintestinal symptoms.

6.1 Celiac Disease Specific Antibodies

Antibodies against dietary gluten and certain tissue structures were discovered from the sera of untreated celiac disease patients already in 1950s (Berger 1958) and were later developed into noninvasive screening tools. The first clinically relevant antibodies were called antigliadin antibodies (AGA) (Seah et al. 1971). Unfortunately, these were present in also several other conditions, such as food-allergies and post-infectious sprue (Unsworth et al. 1983). The sensitivity and specificity varied from about 30% up to 97% (Mäki et al. 1991b, Sulkanen et al. 1998). Nevertheless these were widely used before the more accurate serological test became available (Hill et al. 2005).

The first celiac autoantibodies were the R1- type antireticulin antibodies (ARA) discovered in 1971, which reacted against the reticular fibres of the connective tissue (Seah et al. 1971). Even if the sensitivity of ARA was only moderate, being more specific than AGA it was used until the late 1990's (Lock et al. 1999). More sensitive EmA were established in 1983 and thereafter gradually replaced ARA. Later an improved EmA methodology using human umbilical chord as a substract was developed (Ladinser et al. 1994). Due to their excellent accuracy, tests for EmA remain to be used in the diagnostics of celiac disease (Chorzelski et al. 1984).

The diagnostic approach of celiac disease revolutionized after the recognizion of transglutaminase as an autoantigen of EmA, as this enabled the utilization of practical

enzyme linked immunosorbent assay (ELISA) for quantitative measurement of the antibody values (Dieterich et al 1997, Sulkanen et al. 1998). As the accuracy of the serological tests has improved, it has been discussed if high levels of serum TG2-ab and EmA would be diagnostic by themselves (See 6.3).

6.1.1 Current Antibody-Based Tests

In most studies TG2-ab has been more sensitive (93-98%) than EmA (84-95%), but slightly less specific (95-98% vs. 99-100%) (Sulkanen et al. 1998). False-positive TG2-ab results may be due to inflammatory bowel disease, infections and chronic liver disease (Ferrara et al. 2010, Bizzarro et al. 2006). Possibility to use practical high-troughput method for TG2-ab measurements has made it as the first-line screening method for celiac disease. It is important to note that celiac disease patients have a higher tendency for IgA deficiency and that the total IgA should be tested with a low threshold to exclude this condition (Savilahti et al. 1971). In case of IgA deficiency, the TG2-ab should be measured in IgG class (Collin et al. 1992b, Sulkanen et al. 1998a).

The AGA were later replaced by improved antibodies against deamidated gliadin peptides (DGP), which are superior in accuracy (Kaukinen et al. 2007a). Tests measuring antibodies to DGP have proved to be almost as good as TG2-ab, both in children and in adults, and can be used in IgG class for IgA-deficient patients (Kurppa et al. 2011). However, the use of DGP still looks for its place in clinical practice, as the benefits of DGP compared to TG2-ab seem to be relatively minute.

Rapid point-of-care tests to measure celiac antibodies from a fingertip sample have also been developed to reduce costs, make testing possible without specialized laboratories and to shorten the diagnostic delay (Korponay-Szabo et al. 2005, Popp et al. 2013). Currently, however, the accury of these tests is not compatible with laboratory-based TG2-ab and EmA (Nemec et al. 2006, Popp et al. 2013), and their clinical significance remains to be seen.

6.2 Histology

The development of peroral biopsy devices in the 1950's enabled the assessment of the intestinal mucosa in living persons. It was discovered that, while in healthy patients the mucosa of the small-intestine has long finger-like or leaflet-like villi and short crypts, characteristic to untreated celiac disease is villous atrophy and crypt hyperplasia (Shiner 1957). The most seriously affected areas are usually the duodenum and the proximal part

of jejunum, but the mucosal lesion may be patchy and variable along the whole length of the small intestine (MacDonald et al. 1964, Scott and Losowsky 1976).

6.2.1 Mucosal Morphology

The first classification of small-bowel mucosal sections in celiac disease was set by Doniach and Shiner in 1957 and subsequently acknowledged by European Society of Pediatric Gastroenterology and Nutrition (ESPGAN) as the standard (Meeuwisse 1970). Properly orientated biopsy specimen are vital for correct diagnostics, as poor orientation can lead to false positive and false negative findings (Taavela et al. 2013b). In 1992 a new grouped classification was introduced, as Marsh presented three phases from normal (0) to mucosal atrophy (III) in the development of celiac disease (Marsh 1992). Type III, which represents the lesion diagnostic for celiac disease, was later divided by Oberhuber into subgroups of IIIa, IIIb and IIIc (Oberhuber et al. 1999). These are approximately equivalent to partial, subtotal and total villous atrophy.

In addition to the categorical classifications, Shiner and Doniach (1960) gave precise values for villous height and crypt depth ratio (Vh/CrD). This method was further developed by Kuitunen and associates (1982) and Taavela and associates (2013b). The cut-off for normal VH/CrD is still under discussion, as in literature the used ratios range from 1.0 to 3.0 (Chang et al. 2005, Wahab et al. 2002, Corazza and Villanacci 2005). In a Finnish study, healthy controls were found to have ratios from 1.8 to 3.3 (Kuitunen et al. 1982). As a result, ratio 2.0 has been in use as cut-off in the Tampere Celiac Disease Research Group. Supporting this treshold, it has been shown to be in line with other celiac disease markers (Holm 1993, Koskinen et al. 2010). The Vh/CrD is superior in its repeatability but more laborious than the categorized classifications.

6.2.2 Intraepithelial Lymphocytes

Small number of mucosal IELs are seen even in helathy subjects, but in celiac disease these cells are significantly overrepresented. The reference value has traditionally been defined as below 40 IELs/100 epithelial cells (EC) in hematoxyclin-eosin staining (Ferguson et al. 1971), but recent studies suggest a lower cut-off of 25 IELs/100 ECs (Hayat et al. 2002). Intraepithelial lymphocytocis has been considered to carry weight ~~also~~ in celiac disease diagnostics, even if its specifity is rather poor (Oberhuber et al. 1999).

Immunohistochemistry can be used in borderline cases and in academic and pharmacological studies, but use of this methodology requires special expertise (Järvinen

et al. 2003, Collin et al. 2005). By immunohistochemistry, IELs can ~~also~~ be counted from frozen samples as CD3+ IELs as well as αβ- and γδ- IELs (Ferguson and Murray 1971, Kuitunen et al. 1982, Järvinen et al. 2003 Veress et al. 2004, Mino et al. 2003). The abnormally high numbers of especially γδ+ IELs are quite specific for celiac disease (Mäki et al. 1991c, Holm et al. 1992, Järvinen et al. 2003).

6.2.3 Diagnostic Characteristics and Challenges in Biopsy Samples

The small-bowel mucosal deterioration in celiac disease develops gradually from a completely normal mucosa to increased density of IELs, hyperplasic crypts, and eventually to different states of villous atrophy (Marsh 1992).

Although considered diagnostic, villous atrophy is not specific for celiac disease, being possible also in disorders such as rotavirus infection, cow's milk allergy, autoimmune enteropathy, giardiasis, tuberculosis, Crohn´s disease, acquired immunodeficiency syndrome, small-intestinal bacterial overgrowth, Whipple´s disease, collagenous sprue, eosinophilic enteritis, intestinal lymphoma, infectious enteritis, graft versus host disease and acquired immune deficiency syndrome (Rubio-Tapia et al. 2013, Green and Cellier 2007, Rubio-Tapia et al. 2010). Furthermore, there is evidence that the angiotensin II receptor blockers may cause severe enteropathy resembling celiac disease (Rubio-Tapia et al. 2012).

6.3 Diagnostic Guidelines

The first diagnostic criteria for celiac disease were presented in 1969 by ESPGAN. These criteria were revised in 1990 by the follower of ESPGAN, the European Society of Pediatric, Gastroenterology, Hepatology and Nutrition (ESPGHAN) (Walker-Smith et al. 1990). No united European guidelines on adult celiac disease have been introduced to date.

The main consensus in current adult guidelines is that the suspicion of celiac disease should be based on a combination of physical signs such as diarrhea, bloating, signs of malabsorption or postprandial abdominal pain, compatible medical history such as first-degree family members with celiac disease or concomitant diseases associated with celiac disease, and positive serology. The confirmation of the diagnosis is recommended to be done upon endoscopically obtained duodenal biopsies while on normal gluten-containing diet. Multiple samples are suggested to improve sensitivity, but already one

sample presenting with villous atrophy is diagnostic (Rubio-Tapia et al. 2013, Ludvigsson et al. 2015).

Since the current tests for EmA and TG2-ab demonstrate such a good specificity celiac disease, ESPGHAN proposed in 2012 that the diagnosis of pediatric celiac disease could be set without an intestinal biopsy in symptomatic children with TG2-ab levels over ten times the upper limit of normal (ULN), positive EMA and presence of HLA DQ2 or DQ8 (Husby et al. 2012).

Finland has had its own Current Care Guidelines (Käypähoito) for celiac disease since 1997 (Collin et al. 1997). In the most recent version of the guidelines, approach to pediatric celiac disease diagnosis corresponds to that of ESPGHAN, except that symptoms and HLA-testing are not mandatory for the serology-based diagnosis. In addition, for the first time in the world, it is now possible to establish celiac disease diagnosis in a part of the adults without biopsy using similar criteria as in children (Current Care Guideline 2018).

7 Treatment

Since the early 1960's the cornerstone of the treatment of celiac disease has been a strict gluten-free diet (Collins et al. 1964). This approach is sufficient to most patients excluding those with RCD (Chapter 8). At present, gluten-free diet is the only officially accepted treatment, but there is ongoing research for additional non-dietary treatments (Mäki 2014).

7.1 Dietary Treatment

Gluten is nowadays added to a wide variety of food products. Daily intake of 10-30 milligrams of gluten is estimated to be safe for celiac disease patients, whereas normal western diet contains 15-20 grams of gluten per day (van Overbeek et al. 1997, Collin et al. 2004, Catassi et al. 2007, Lähdeaho 2011). Patients with DH seem to be particularly sensitive to trace amounts of gluten, and additive anti-inflammatory dapsone medication is often required for the first few years following the diagnosis (Kruizinga and Hamminga 1953, Reunala 2001).

The initiation of a gluten-free diet alleviates gastrointestinal symptoms usually within days or weeks and decreases antibody titers within months. Also the small-bowel histological damage starts to improve rapidly, although complete healing of the mucosa may take several years (Yardley et al. 1962, Ilus et al 2014).

Dietary treatment usually alleviates also the extraintestinal manifestations and complications of celiac disease, which is why proper treatment is important in cases of for instance gynecological and obstetric problems (Smecuol et al. 1996, West et al. 2004, Tersigni et al. 2014). A gluten-free diet also seems to reduce the risk of malignant diseases (Holmes et al. 1989) and overall mortality compared to untreated patients (Corrao et al. 2001). Decreased BMD usually increases while on diet (Valdimarsson et al. 1994, Tau et al. 2006, Zanchetta et al. 2016), but to ensure normal bone accurual the treatment should be started as early as possible (Tau et al. 2006).

Long-term adherence to the gluten-free diet has varied substantially in different studies ranging from 42% to 96% (Fabiani et al. 2000, Whitaker et al. 2009, Hall et al. 2009, Ilus et al. 2014), suggesting that there is a need for other treatment options. Notably, in Finland the percentage of strictly adherent patients is high. Adolescent and screen-detected patients are usually considered to have increased risk for low dietary

adherence, although in some studies screened patients have shown similar or even higher adherence than symptom-detected patients (Kurppa et al. 2012, Kivelä et al. 2017).

The use of oats as a part of the gluten-free diet has been under debate, but prospective studies have proven purified oats products to be safe for most patients (Sey et al. 2011, Janatuinen et al. 1995, Högberg et al. 2004). Oats could provide health benefits and can increase fiber intake in celiac disease patients (Kaukinen et al. 2013). It has also been discussed whether only naturally gluten-free products should be allowed, but several studies have shown that industrially purified products are safe for celiac disease patients (Kaukinen et al. 1999, Lohiniemi et al. 2000).

It is important to note that, despite the aforementioned benefits, lifelong gluten-free diet is expensive, difficult to maintain and may involve social restrictions (Singh et al. 2011, Villafuerte-Galvez et al 2015). In addition, along with poor fiber intake, the diet may induce an undesirable intake of fat and sugar (Wild et al. 2010). The unfavorable dietary content can eventually lead to detrimental weight gain (Dickey and Kearney 2006) and poor vitamin status (Hallert et al. 2002b).

7.2 Novel Therapies

The demand for alternative therapeutic approaches in celiac disease is high. This is especially true for patients frequently visiting restaurants, those dissatisfied with the cost of gluten-free diet and for those with decreased quality of life due to dietary restrictions (Tennyson et al. 2013). Several new drugs and devices have entered in phase 1 and 2 clinical trials (Hindryckx et al. 2018). As celiac disease already has an effective and safe dietary treatment, defining satisfactory outcomes for drugs has been under discussion. Small intestinal mucosal improvement has been suggested as a primary endpoint in phases 1 and 2, while the effect on patient-reported outcomes, such as symptom relief, should be emphasized in later stages of clinical trials (Ludvigsson et al. 2018).

Selecting, breeding and genetically modifying wheat to be safe for celiac disease patients could be one approach (Schuppan et al. 2009, Kaukinen et al. 2014) and also different ways to degrade harmful gluten peptides before they enter the gut have been considered (Gianfrani et al. 2017). Various bacteria, funghi, probiotics and parasites have been found to be capable of degrading gluten, but thus far clinical trials have been disappointing (Daveson et al. 2011, Smecuol et al. 2013, Tack et al. 2013, Tye-Din et al. 2010). Polymeric binders, TG2-inhibitors and blocking HLA-DQ2 and DQ8 are other pharmaceutical approaches which have been under research (Molberg et al 1998, Rauhavirta et al. 2013, Kaukinen et al. 2014). Furthermore, a desenzitising vaccine to restore oral tolerance is under development (Goel et al. 2017).

8 Non-Responsive and Refractory Celiac Disease

Non-responsive celiac disease is defined as continuation of symptoms or clinical manifestations suggestive of celiac disease despite a gluten-free diet (Leffler et al. 2007). Non-responsive celiac disease is globally quite common, the main reason behind it being ongoing advertent or inadvertent gluten consumption. Only a minority of non-responsive patients have actual RCD (Ilus et al. 2012), which is defined as presence of persistent symptoms, signs of malabsorption and villous atrophy despite a strict gluten-free diet for at least 6-12 months (Rubio-Tapia and Murray 2010). Other possible etiologies for villous atrophy should be excluded. The symptoms of RCD are often severe and require additional therapeutic interventions (Rubio-Tapia and Murray 2010).

Celiac disease patients homozygous for HLA-DQ2 are more susceptible to RCD (Malamut et al. 2009). RCD is divided into type I and type II, of which the latter is a severe condition predisposing to malignancies, notably enteropathy-associated T cell lymphoma (EATL) (Malamut et al. 2009). RCD I is usually treated with topical steroids and budesonide, at least if rigorously strict gluten-free diet is not enough, but also more potent treatments such as azathioprine may be needed (Maurino et al. 2002, Goerres et al. 2003). In comparison, RCD II is usually resistant to all treatment. Treatment with cytotoxic chemotherapeutic agents can be tried to delay the inevitable development of EATL (Kelly et al. 2015). Due to its malignant outcomes, the potential risk of RCD is often considered a major indication to ensure response to dietary treatment and mucosal healing by repeat endoscopies.

9 Follow-Up

Experts agree that the main goals of dietary treatment in celiac disease are the healing of the small-bowel mucosal damage, the disappearance of symptoms and the prevention of complications (Ludvigsson et al. 2014). The success of the treatment is recommended to be confirmed by histological follow-up, serological tests, dietary evaluation and clinical follow-up, but scientific evidence behind these guidelines is scarce. Furthermore, the lack of sensitive non-invasive methods to assess dietary adherence comprises a major challenge in the follow-up. Endoscopy is burdensome and uncomfortable for patients and the data on its necessity remain insufficient. In particular, there is a paucity of studies regarding optimal timing and effect of the results of follow-up biopsies on patients' long-term health and treatment outcomes.

Some studies suggest that patients without follow-up have more dietary lapses (Viljamaa et al. 2005b, Leffler et al. 2007). As regards to systematic international guidelines for celiac disease follow-up, there are currently no unified European guidelines for adults, although there are several national guidelines, including those assembled in the United Kingdom, Finland, Norway, Denmark and Russia (Fluge et al. 1997, Current Care Guideline 2010, Ludvigsson et al 2014, Parfenov et al. 2017). United States has their own quideline made by the American College of Gastroenterology (Rubio-Tapia et al. 2013). The Oslo definitions for celiac disease and related terms comes closest to a unified international statement (Ludvigsson et al 2014). The main reccomendations on follow-up in recent guidelines are shown in Table 2.

The 2010 Finnish Current Care Guidelines suggested a repeat biopsy one year after diagnosis and clinical and serological follow-up at 2-3-year intervals (Table 2). For most patients, long-term follow-up can be organized in primary healthcare with a possibility to consult a dietitian. Problematic cases, such as patients with persistent symptoms despite gluten-free diet and those with possible severe complications, are referred to secondary and tertiary centers (Current Care Guideline 2010). In the very recent revision of the guidelines, the follow-up recommendations remained otherwise similar, but repeat biopsy is not considered mandatory in clinically and serologically recoverd patients (Current Care Guideline 2018).

Table 2. Follow-up recommendations for adult celiac disease patients in recent guidelines

	Year	Country	Frequency	Clinician visits	Dietician visits	Repeat biopsy	Celiac serology	Other laboratory parameters
WGO[1]	2017	Global	Every 1-2 years	Every time	Every 1-2 years	If no clinical response	During every visits	Until normalized at the intervals of 3-6 months
AGG[2]	2013	USA	Annually	Annually	If adherence problems	After 1 year	Annually	Abnormal baseline values until normalized
BSG[3]	2014	UK	Annually	Annually	If adherence problems	If no clinical response	Annually	Annually FBC, iron status, folate, TSH, ALAT, vitamin D and B12, calcium
CCG[4]	2010	Finland	Every 2-3 years	Every time	If adherence problems	After 1 year	During every visit	If clinical suspicion arises

FBC, full blood count; TSH, thyroid stimulating hormone; ALAT, alanine aminotransferase; BMD, bone mineral density, WGO, World Gastroenterology Organization; ACG, American College of Gastroenterology; BSG, British Society of Gastroenterology, CGG, Current Care Guidelines
[1]Bai et al. 2017; [2]Rubio-Tapia et al. 2013; [3]Ludvigsson et al. 2014; [4]Current Care Guideline 2010

9.1 Clinical Follow-Up

Clinical follow-up is essential for the evaluation of dietary adherence, as well as to evaluate patients' burden of illness, quality of life and possible ongoing symptoms. Gastrointestinal symptoms alleviate within weeks in the majority of celiac disease patients who commence a strict gluten-free diet (Murray et al. 2004), whereas the improvement of extra-intestinal symptoms is more variable. Elevated liver enzymes usually normalize within a year, whereas improvement of BMD can take several years and remain incomplete (Bardella et al. 1995, Grace-Farfaglia 2015). Most guidelines suggest permanent clinical follow-up, starting approximately three to six months after the diagnosis and then continue on regular intervals of 1-2 years (Table 2).

9.2 Mucosal Recovery

Currently most celiac disease guidelines recommend a repeat biopsy to be taken at least from patients without full clinical recovery approximately one year after the diagnosis (Table 2). It has been suggested that a repeat biopsy should be done as early as after 6 months, but there are indications that complete histological normalization of the small-

intestinal mucosa can be reached only in 8-60% of the adult patients during such a short period of time, the median recovery time being from two to four years (Lanzini et al 2009, Tursi et al 2006, Sharkey et al. 2013). In countries with high dietary adherence, it is possible to reach recovery figures of as high as 94-96% in the long run (Ilus et al. 2012, Ilus et al. 2014). Some evidence suggests that particularly slow histological recovery can be expected when the mucosal damage is severe at diagnosis and there is a long history of gluten exposure (Tursi et al. 2006, Rubio-Tapia et al. 2010).

Incomplete mucosal recovery has been associated with increased morbidity and mortality in celiac disease, the most important causes of death being malignancies and infections (Rubio-Tapia et al. 2010, Catassi et al. 2002). However, in a recent study no such association was seen (Lebwohl et al. 2013a). Persistent villous atrophy may also increase the risk of osteoporosis (Kaukinen et al. 2007b).

In children, routine follow-up biopsies are no longer recommended. Interestingly, due to lack of evidence on its necessity, also recent adult guidelines have questioned the necessity of a follow-up biopsy. The World Gastroenterology Organization (WGO) gave its own guidelines in 2013, being the first guideline that did not recommend a systematic follow-up biopsy in all patients (Bai et al. 2013). Also the British Society of Gastroenterology (BSG) suggests that follow-up biopsies can be omitted if patients are asymptomatic on a gluten-free diet and there are no signs of complications (Ludvigsson et al. 2014). The WGO guideline was renewed in 2017, currently stating that follow-up biopsies should be mandatory in symptomatic patients despite strict adherence to gluten-free diet, but not in asymptomatic patients (Table 2). These recommendations, however, are based on expert opinions and have not been backed with solid scientific evidence.

9.3 Serology and Other Laboratory Parameters

Serology and other laboratory parameters are recommended to be monitored at intervals of 6-12 months after starting gluten free diet (Rubio-Tapia et al. 2013, Current Care Guideline 2010, Ludvigsson et al. 2013). Serum celiac antibodies are used primarily to reveal dietary transgressions, although they have rather poor sensitivity and may be negative despite ongoing dietary lapses and mucosal damage (Silvester et al. 2017). Altogether, studies on the utility of serology in the long-term follow-up of celiac disease are scant.

Other laboratory parameters such as full blood count, iron status and vitamin levels are mostly used to confirm the disappearance of signs of malabsorption, as well as to monitor the normalization of possible abnormal results at diagnosis (Table 2).

Various other non-invasive markers to assess dietary adherence have been developed, but most of these remain too insensitive or unspecific for clinical use (Vives-Pi et al. 2013). Measurement of gluten immunogenic peptides from urine or feces seems to be

the most promising recent technique, but additional research is needed (Moreno et al. 2017).

9.4 Long-Term Follow-Up

Short-term follow-up of celiac disease is focused mainly on ensuring treatment response and patients' competence in managing the dietary treatment. After these are achieved, in the following years the focus shifts to maintaining the results and intervening if problems occur.

Perhaps the most thorough follow-up routine is suggested by the BSG (Table 2) (Ludvigsson et al. 2014). The review by Haines and colleagues (2008) comes close, as they suggest annual follow-up of patients by physician or dietitian, regular measurement of laboratory parameters, and even bone densitometry at 3-5-year intervals in patients with high-risk for osteoporosis. American College of Gastroenterology (ACG) and WGO suggest somewhat lighter follow-up protocols (Table 2).

In Finland, long-term follow-up of adult celiac disease has been recommended to be organized at 2-3 years intervals (Table 2). In these occasions, serology can be used to monitor diet if the autoantibody values have been elevated before diagnosis. If clinical response is incomplete, the patient reports maintaing gluten-free diet problematic or serology is constantly elevated, dietician's consultation is recommended. Long-term follow-up should be organized mostly in primary healthcare Current Care Guideline 2010 and 2018).

THE PRESENT STUDY

1 Aims

A follow-up biopsy to confirm histological recovery has been considered the gold standard of celiac disease follow-up, usually recommended to be taken one year after diagnosis, or at least in clinically non-recovered patients. The procedure is invasive, uncomfortable to the patient and expensive. At present, expert opinions vary on the necessity of follow-up biopsies on all patients, and scientific evidence is scarce. In addition, optimal timing and significance of repeat biopsy, if taken, to the long-term outcomes in celiac disease remain obscure. In fact, the implementation and overall value of even regular long-term follow-up in celiac disease is scarcely studied. We aimed to investigate the significance of a repeat biopsy, short-term mucosal healing and regular follow-up to the long-term health and treatment outcomes in adult celiac disease patients.

The specific aims were to:

1. Find patient- and disease-related factors associated with and the significance of incomplete small-bowel mucosal recovery one year after the celiac disease diagnosis (studies **I-II**).

2. Study factors associated with and the significance of repeat biopsy one year after the celiac disease diagnosis on patients' long-term dietary compliance and health outcomes (**II**).

3. Investigate the overall implementation of a regular long-term follow-up and its value on the long-term health and treatment outcomes in celiac disease (**III**)

2 MATERIAL AND METHODS

2.1 Patients

All study patients were adults with a histologically verified celiac disease. They were diagnosed and repeat biopsies were taken either in the Department of Gastroenterology and Alimentary Track Surgery in Tampere University Hospital (**I**) or also in other facilities performing upper gastrointestinal endoscopies across all levels of heathcare (**II-III**). Patients were recruited to the studies directly from the hospital clinic (**I**), through newspaper advertisements, or with the help of local celiac disease societies (**II-III**).

2.2 Clinical Data

Study **I** comprised of patients selected from the Celiac Disease Research Group's prospective patient series collected between 1996 and 2009. Additional long-term data of 200 patients were collected from medical records to study whether the follow-up biopsy result affects to the risk of severe complications. Only cases with a repeat biopsy carried out after one year on a gluten-free diet were included. Patients using angiotensin receptor blockers were excluded since this class of drugs may cause severe enteropathy resembling celiac disease (Rubio-Tapia et al. 2012).

The material for the studies (**II**) and (**III**) was collected from a nationwide cross-sectional study conducted in Tampere University Hospital. The participants had a biopsy-proven celiac disease diagnosis established at least two years before the study. Subjects with unclear diagnosis or substantially lacking medical information were excluded. The possible use of angiotensin receptor blockers was again considered an exclusion criterion. In Study **III** also patients with concomitant DH were excluded.

The participants were interviewed either at the time of the celiac disease diagnosis and after one year on a gluten-free diet (**I**) or at the time of the follow-up study (**II-III**). Demographic data, family history of celiac disease and celiac disease-associated and other significant co-morbidities were recorded, as well as clinical manifestations such as anemia, malabsorption, diarrhea, and possible presence of extraintestinal manifestations such as arthritis, gynecological problems or neurological symptoms. Duration and severity of symptoms before diagnosis were also assessed and the latter further

categorized as mild (occasionally disturbing gastrointestinal or extra-intestinal symptoms), moderate (a combination of them) and severe (symptoms seriously disturbing daily life or requiring inpatient treatment).

The participants also completed validated questionnaires for current symptoms and health-related quality of life, neither at diagnosis and after one year on dietary treatment (**I**) or at the time of study (**III**) (Chapter 2.4). Dietary adherence was assessed systematically by an experienced dietitian or study nurse. In addition, order to confirm the diagnosis and to complement medical data and laboratory parameters in Studies **II** and **III**, blood samples were drawn for celiac disease serology and patient records were reviewed.

In Study **I** patients were divided into two groups based on whether they had (Recovery) or had not (Atrophy) reached mucosal recovery one year after diagnosis (Figure 1). In Study **II** patients were divided to those with and those without a repeat biopsy acquired approximately one year after the diagnosis. Patients with a repeat biopsy were further divided into two groups based on the presence or absence of mucosal recovery similarly as in Study **I**. In Study **III** patients were divided into those with regular healthcare follow-up for more than two years after the diagnosis and those with only shorter follow-up. These groups where then compared to each other, as well as those without any follow-up (Figure 1).

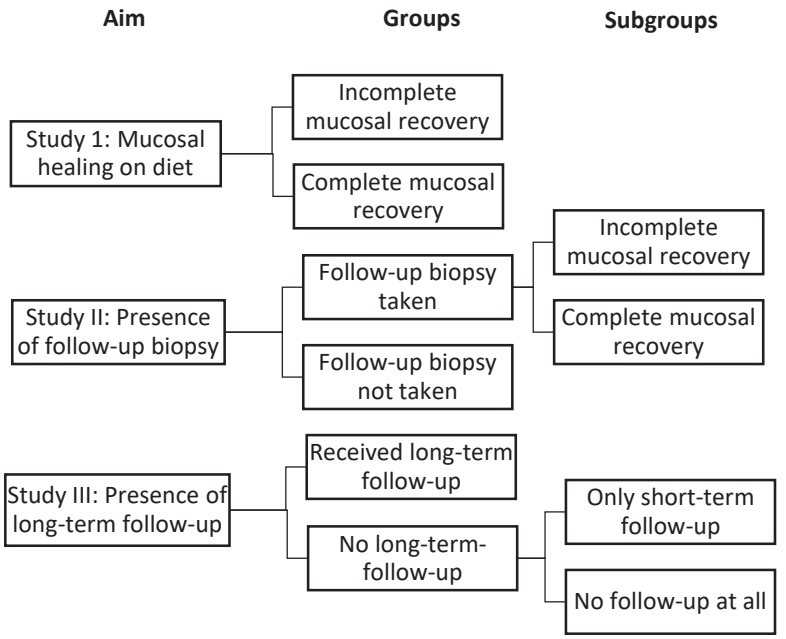

Figure 1. Categoriziation of patients for subsequent analyses in Studies **I-III**.

2.3 Small-Bowel Mucosal Biopsies

In Study **I**, a minimum of six biopsy specimens were taken from the distal duodenum upon esophagogastroduodenoscopy. Three specimens were carefully orientated, processed and stained with hematoxylin and eosin (H&E) and analyzed under light microscopy. The Vh/CrD was calculated by measuring the mean height of a villus and the adjacent crypt from at least three well-orientated villus-crypt units. The final morphological analysis was conducted from the most severely damaged specimen (Taavela et al. 2013b). Normal Vh/CrD was considered to be over 2.0 (Kuitunen et al. 1982).

The degree of small-bowel mucosal inflammation was determined by measuring the density of IELs (Taavela et al. 2013a). The IELs were counted in both H&E-stained and frozen sections with a 100× flat-field light microscope objective. Immunohistochemical studies were made from frozen 5-µm-thick sections and CD3+ IELs were stained using the monoclonal antibody Leu-4 (Becton Dickinson, San Jose, CA). The density of H&E stained IELs was expressed as cells/100 epithelial cells (reference value over 30 per 100 enterocytes) and density of CD3+ IELs as cells/mm (reference value 37 cells/mm).In addition, αβ+ IELs were stained from frozen sections using monoclonal antibody αF1 (T Cell Diagnostics, Woburn, MA), and γδ+ IELs with T-cell receptor-bearing cell γ antibody (T Cell Diagnostics) and then counted.

In studies **II** and **III**, the histopathological data were collected from patient records. At least four small-bowel mucosal biopsies should have been taken both in the diagnostic and follow-up endoscopy according to our national guidelines (Current Care Guideline 2010). All samples were evaluated in the department of pathology from well-orientated and representative cuttings.

The severity of mucosal damage was graded using either quantitative VH/CrD as stated above (**I**) or by dividing the finding into normal, partial, subtotal or total villous atrophy based on the original pathology reports (**II-III**). The latter grading corresponds approximately Marsh-Oberhuber scores I-II (normal), IIIA (partial atrophy), IIIB (subtotal atrophy) and IIIC (total atrophy). In order to see if there was a dose response to the outcome measures in Study I, the Atrophy group was retrospectively categorized also in this study into those with subtotal or total villous atrophy (Vh/CrD below 0.9) and to those with only partial atrophy (Vh/CrD 0.9-1.9).

2.4 Serology and Laboratory Parameters

Serum IgA-class TG2-ab were measured in Study **I** by ELISA (Celikey; Phadia, GmbH, Freiburg, Germany) considering values >5.0 U/L positive according to manufacturer's

instructions. In studies **II-III** the corresponding antibodies were measured by another commercial ELISA (QUANTA Lite h-tTG IgA, INOVA Diagnostics, San Diego, CA), in which values >30.0 U/ were rated positive. Serum EmA were assessed by an indirect immunofluorescence method on human umbilical cord (**I-II**) (Ladinser et al. 1994). EmA titers 1: ≥5 were considered positive and further were diluted to 1:50, 1:100, 1:200, 1:500, 1:1000, 1:2000 and 1:4000 until negative. In cases of selective IgA deficiency, the corresponding IgG-class antibodies were applied (Kurppa et al. 2014). The following laboratory parameters were measured using standard laboratory methods: blood hemoglobin (reference values: men >134 g/l; women >117 g/l) (**I-II**), mean corpuscular volume (82-98 fl), ionized calcium (1.16-1.30 mmol/l), parathyroid hormone (1.0-7.5 pmol/l), serum total iron (9-34 umol/l), erythrocyte folic acid (200-700 nmol/l) and serum vitamin B12 (>150pmol/l) (**I**).

2.5 Bone Mineral Density and BMI (**I**)

BMD was measured from the lumbar spine and femoral neck by dual energy X-ray absorptiometry following our standard procedures (Sievänen et al. 1996). Expressed values are standard deviation scores comparing individual values with either that of healthy young adults (T-score) or the age and genger-matched population (Z-score). Osteoporosis was defined according to the recommendation of the World Health Organization as T-score ≤-2.5 and osteopenia as T-score -2.4-(-1.0), respectively (World Healt Organization 1994). Body mass index (BMI) was calculated as weight divided by the square of height (kg/m^2).

2.6 Gastrointestinal Symptoms and Quality of life (**I-III**)

Gastrointestinal Symptoms Rating Scale (GSRS) (Svedlund et al 1988) was used to evaluate the self-perceived severity of patients' gastrointestinal symptoms either both at diagnosis and after one year on treatment (**I**) or after long-term follow-up (**II-III**). The scale is well-validated and widely applied in celiac disease (Dimenäs et al. 1996, Kurppa et al. 2010). GSRS consists of 15 separate items which can be counted for an average as a total score and divided into five sub-dimensions measuring abdominal pain, gastro-esophageal reflux, indigestion, diarrhea and constipation. The scoring is based on a 7-grade Likert scale where higher scores stand for more severe gastrointestinal symptoms.

The Psychological General Well-Being Index (PGWB) was used to measure self-perceived psychological well-being and quality of life at the same time points as GSRS (Studies **I-III**). There are 22 items in PGWB, which can be added together as a total

score and divided into seven sub-dimensions measuring anxiety, depression, well-being, self-control, general health and vitality. The questions use a 6-grade Likert scale where high scores indicate better well-being and quality of life. PGWB is well-validated and widely used in celiac disease (Viljamaa et al. 2005b, Ukkola et al. 2011, Nachman et al. 2009).

SF-36 was used in Studies **II** and **III**. It comprises of 36 separate questions which can be divided into eight domains measuring physical functioning, role limitations due to physical problems, bodily pain, general health, vitality, social functioning, role limitations due to emotional problems and mental health (Haere et al. 2016, McHorney et al. 1994). Items are re-scored from 0 to 100, higher scores indicating better health and quality of life.

In Studies **II** and **III** patients were also asked to evaluate the amount of overall symptoms subjectively. Patients were given four categories: no symptoms, mild symptoms, moderate symptoms and severe symptoms. For statistical analysis mild and moderate symptoms were grouped under slight symptoms.

2.7 Adherence to Gluten-Free Diet

In study **I** dietary advice was routinely given to all patients at celiac disease diagnosis by a dietitian with expertise in celiac disease. In studies **II** and **III** provision of professional dietary advice was verified by patient interview and from the patient records. Adherence to the diet was evaluated by a dietician after one year (**I)** or by an experienced study nurse at time of study (**II-III**). In all studies patients were categorized as adherent (no gluten or only a minor inadvertent gluten intake less than once per month) and as non-adherent (more lapses).

2.8 Statistical Analysis

Statistical testing was done using PASW version 18 (IBM, New York, NY, USA) (Study **I**) or SPSS version 20 (IBM) (Studies **II-III**). Results on continuous variables are shown either as medians with lower and upper quartiles, (Q_1-Q_3) or medians with range. Mann-Whitney U test was used in statistical comparisons between groups in continuous variables. Categorical variables are presented as percentages and number of patients. Chi-square test was used in comparisons between binomial and categorical variables (**I-III**). Multivariable analysis was further applied for variables with statistical significance (**I**). A p-value <0.05 was considered statistically significant in all analyses.

2.9 Ethics

Patient collection was conducted with the permission and according to the guidelines of the Ethical Committee of Tampere University Hospital and the Declaration of Helsinki (World Medical Association 1964). All participants gave written informed consent before data collection (**I-III**). Further, the collection of long-term follow-up data from the medical records (Study **I**) were conducted with the permission of the Department of Internal Medicine, Tampere University Hospital.

3 RESULTS

Study **I** comprised of altogether 263 adults with biopsy-proven celiac disease. Their median age was 45 (range 15–79) years and 68 % were female. Long-term follow-up data on the patients' mortality, malignancies other gastrointestinal diseases and osteoporosis after a median of 16 years (range 15-17 years) after the diagnosis was available from 205 (78%) out of the 263 patients, as well as the GSRS and PGWB results from a subgroup of 44 patients with a median of five years after the diagnosis.

Altogether 760 individuals fulfilled the inclusion criteria in Study **II.** The population comprised 78% females and had a median age of 44 (range 18-84) years. The median time on a gluten-free diet prior to the study enrollment was eight years (range 1-38 years).

Study **III** included 648 participants with a median age of 44 (range 22–89) years and 80% female predominance. The median follow-up time for the whole cohort was 10 years (range 2-38 years).

3.1 Incomplete Mucosal Recovery (**I-II**)

Altogether 178 (68%) subjects in Study **I** had and 85 (32%) had not reached a full histological recovery after one year on a gluten-free diet (Table 1 in the original publication **I**). The corresponding figures in Study **II** were 276 (58%) and 200 (42%) out of those 476 patients who had the follow-up histology result available. Diagnostic factors predicting incomplete mucosal healing after one year on diet in both studies were presence of malabsorption, severe mucosal damage (measured either qualitatively or quantitatively with Vh/CrD), high levels of serum TG2ab or EmA and low hemoglobin in women, and in Study **I** also low values of serum total iron and lumbar and femoral BMD T-scores (Table 3). In neither study, patients with and without mucosal recovery differed in age, gender, clinical presentation other than malabsorption, proportion of screened patients, severity nor duration of symptoms before diagnosis, family history for celiac disease and presence of coexisting chronic conditions (Table 1 in the original publication **I**).

There was a significant difference in the speed of mucosal recovery between groups in Study **I**, measured as the change in Vh/CrD between the diagnostic biopsy and the one taken after one year on a gluten-free diet. This improved more among histologically healed than the still atrophic patients (changes in median ratio on diet 2.1 vs. 0.9, p

<0.001). Six patients did not reach any increase in Vh/CrD during one year despite a strict gluten-free diet. Of these, one patient later developed RCD type I without lymphoma or signs of RCD type II. Detailed data on these six cases are presented in the original article **I**.

After one year on a gluten-free diet in Study **I**, there was still significant difference between the recovery and atrophy group in TG2ab, VH/CrD, blood hemoglobin in women and femoral T-score, and now also in CD3+ IELs, whereas the groups were comparable in the density of H&E stained IELs, other laboratory values and all GSRS and PGWB scores (Tables 2-4 in the original publication **I**). The atrophy group had significantly lower adherence to the gluten-free diet, although this was excellent in both groups (87% vs. 97%, p<0.001).

As regards to the long-term significance of mucosal recovery one year after the diagnosis in Study **I**, no differences were seen between the atrophy and recovery groups in mortality or prevalences of coexiting chronic gastrointestinal diseases, osteoporosis and malignancies, and the median GSRS and PGWB scores and hemoblobin were also comparable (Table 5 in the original publication I). In Study **II**, patients with incomplete recovery after one year had more concomitant respiratory (15% vs. 22%, P = 0.031) and dermatological conditions (17% vs. 10%, P = 0.043, whereas there were no differences in the frequency of other chronic comorbidities, strictness or capability to manage gluten-free diet, use of purified oats, presence of regular follow-up and frequence of TG2ab positivity, malignancies or fractures (Table 5 in the original publication **II**).

Table 3. Selected diagnostic factors that were either investigated both in Studies **I** and **II** or were significantly associated with incomplete mucosal recovery one year after the celiac disease diagnosis on a gluten-free diet. Results are given in percentages or in medians and quartiles, except age which is given in median and range.

	Study **I**			Study **II**		
	Atrophy n=85	Recovery n=178	P-value	Atrophy n=200	Recovery n=276	P-value
Females, %	65	70	0.420	82	81	0.951
Age, years	48 (18-77)	44 (15-79)	0.068	57 (25-84)	54 (21-77)	0.532
At-risk group[1], %	37	47	0.129	24	33	0.152
Clinical presentation, %						
Malabsorption	60	34	0.001	55	41	0.003
Screen-detected	22	29	0.543	10	10	0.733
Severity of histopathology						
VH/CrD	0.2 (0.1-0.4)	0.4 (0.1-0.8)	0.003	ND	ND	ND
TVA, %	ND	ND		32	19	<0.001
TG2ab, U/l	57 (19–100)	30 (8–71)	0.017	ND	ND	
High[2] EmA value, %	66	52	0.069	46	25	<0.001
Blood hemoglobin, g/l						
Women	119 (112–126)	131 (125–139)	<0.001	123 (112-130)	127 (114-134)	0.071
Men	142 (135-148)	149 (144-153)	0.056	Data too few	Data too few	
Serum iron, µmol/l,	12.0 (8.8–17.6)	18.0 (12.2–21.6)	0.001	ND	ND	ND
Bone mineral density						
Lumbar T-score	-1.5 (-2.2 to-0.4)	-1.0 (-1.8 to 0.2)	0.022	ND	ND	ND
Femoral T-score	-1.2 (-1.9 to -0.5)	-0.7 (-1.5 to 0.0)	0.016	ND	ND	ND

The results except percentages are shown as median and quartiles; [1]Celiac disease in relatives; [2]Titer >1:200; [3]data were available only from 286 patients
EmA, endomysial antibodies; ND, no data; TG2-ab, transglutaminase-2 antibodies; TVA, total villous atrophy; VH/CrD, villous height crypt depth ratio

3.2 The Follow-Up Biopsy (II)

Altogether 516 (68%) participants in Study **II** had and 244 (32%) had not undergone a follow-up biopsy after a median of one year. A record of repeat biopsy was significantly associated with the presence of malabsorption and more severe villous atrophy at diagnosis, along with coexisting Sjögren's syndrome and gastroenterological and musculoskeletal diseases, whereas the biopsy was omitted more often in patients detected by screening or in the private sector (Table 4). The repeat biopsy and no repeat biopsy groups were similar when it comes to the prevalence of gastro-intestinal or extraintestinal symptoms and EmA titers at diagnosis, the duration of symptoms before diagnosis, presence of symptoms in childhood, family history for celiac disease, prevalence of coexisting thyroidal, gynecological, neurological or psychiatric diseases and frequency of malignancies or fractures (Tables 1-2 in the original publication **II**).

Rebiopsied and non-rebiopsied patients had also received similar amounts of dietary advice ($p=0.683$) and were equally strict of their gluten-free diet ($p=0.374$), but subjects in the latter group felt less often capable to manage their diet ($p=0.002$) (Table 3 in the original publication **II**). The non biopsied patients also had more often current EmA positivity ($p=0.012$), while in TG2ab the difference was not significant ($p=0.139$) (Table 3 in the original publication **II**).

Patients who had undergone the follow-up biopsy reported more indigestion based on GSRS and more bodily pain and lower physical functioning based on SF-36, whereas the repeat and no-repeat biopsy groups did not differ in PGWB or other GSRS and SF-36 scores (Table 4 in the original publication **II**).

Table 4. Diagnostic characteristics and comorbidities that were significantly associated with the existence of a repeat biopsy one year after diagnosis in Study **II**.

	No repeat biopsy (N=244)		Repeat biopsy (N=516)		
	N	%	N	%	P-value
Clinical presentation					
Malabsorption	80	33	235	46	0.001
Screen-detected	39	16	49	10	0.010
Degree of villous atrophy					<0.001
Total	42	24	124	26	
Subtotal	58	34	196	42	
Partial	73	42	149	32	
Site of celiac disease diagnosis					<0.001
Primary care	41	17	79	15	
Private care	57	23	58	11	
Secondary care	108	44	250	49	
Tertiary care	38	17	125	24	
Comorbidities					
Sjögren's syndrome	1	0	14	3	0.033
Musculoskeletal disease[1]	66	27	182	36	0.023
Gastroenterological disease[2]	78	32	203	40	0.049

[1]E.g. arthritis, osteoporosis; [2]E.g. reflux, lactose intolerance, gastritis

3.3 Regular Follow-Up (III)

In total, 99 (15%) of the 677 participants in Study **III** had and 578 (85%) had not received long-term follow-up for coeliac disease. Factors predicting the follow-up were coexisting immunological (35% vs. 24%, P=0.020) and circulatory disease (20% vs. 12%, p=0.010) while it was less common in subjects who had musculoskeletal disease (23% vs. 34%, P=0.045) or did not belong to any at-risk group (Table 1 in the original publication **III**). The follow-up and no follow-up groups did not differ in the presence of other co-morbidities, site of diagnosis, smoking, family risk for celiac disease or duration of symptoms and presence of symptoms in childhood, or in demographic data, seropositivity, severity of histopathology and clinical presentation at diagnosis (Table 1 in the original publication **III**).

At the time of the study, participants in both follow-up and no-follow up groups had comparable dietary adherence and ability to manage the gluten-free diet, use of purified oats and positivity to celiac autoantibodies (Figure 2), as well as equal health-related quality of life based on SF-36 and PGWB scores (Table 3 in the original publication **III**). There was also no difference between the groups in gastrointestinal symptoms as measured by GSRS (Table 3 in the original publication), but those without follow-up had significantly more self-perceived subjective overall symptoms (Figure 2).

The majority of the patients with or without regular follow-up wished for it in the future, this percentage being significantly higher in those with follow-up (Figure 2). Most of these patients wished the follow-up to be organized in public healthcare (Figure 1 in the original publication **III**), whereas there was no major difference regarding who should be in charge of the follow-up (Figure 2 in the original publication **III**).

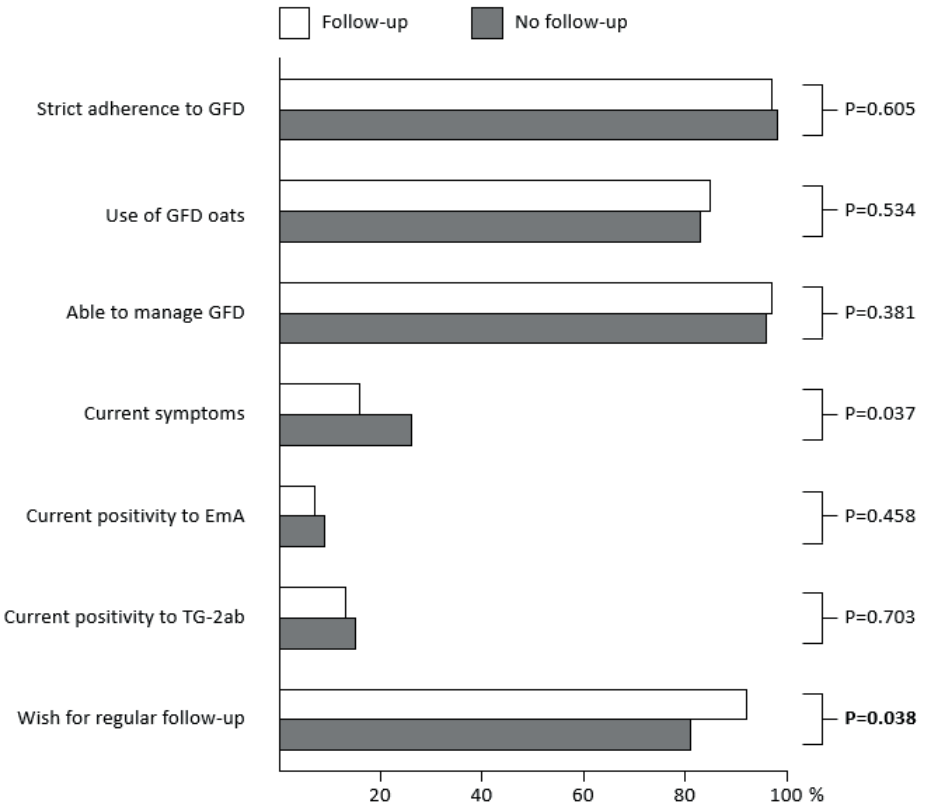

Figure 2. Comparison of major long-term outcomes between patients with and without reglular long-term follow-up in coeliac disease.

GFD, Gluten-free diet; EmA, Endomysium antibodies; TG2-ab, transglutaminase-2 antibodies. The minimum number of patients for each variable was 508 patients.

4 Discussion

4.1 Short-Term Clinical and Endoscopic Follow-up

4.1.1 Short-term Follow-up and Treatment Outcomes

Clinical evaluation of patients' early response and adherence to gluten-free diet has been emphasized in previous guidelines for celiac disease (Rubio-Tapia et al. 2010, Bai et al. 2013, Ludvigsson et al. 2014), but there is little scientific evidence on by what means and how often should short-term follow-up of the disease be organized. Perhaps an even more debated issue among experts is the signifcance of endoscopic follow-up to the treatment outcomes (Ludvigsson et al. 2014, Bai et al. 2017).

Some studies have indicated poor dietary adherence among celiac disease patients without follow-up but, on the other hand, the serological renspose to the treatment has been unsatisfactory also in regularly followed patients (Barnea et al. 2014, Zanini et al. 2009). In contrast, patients of the present study reported excellent adherence to the gluten-free diet irrespective of the existence of short-term follow-up, the accuracy of this self-assesment being further enforced by the low prevalence of seropositivity in both follow-up and no-follow up groups. It seems that, at least in the Finnish population, the association between follow-up and results of the treatment are not as straightforward as previously assumed (Zanini et al. 2010, Barnea et al. 2014, Hall et al. 2009).

4.1.2 Predictors for Repeat Biopsy One Year after the Diagnosis

In many chronic diseases, such as chronic pulmonary disease, physicians seem to favor more intense follow-up for patients with a more severe disease presentation at diagnosis, as these cases supposedly have an increased risk of long-term complications (van de Bemt et al. 2009). Accordingly, we found that the repeat biopsy one year after the celiac disease diagnosis was undertaken especially for clinically-detected patients with advanced histological damage and severe symptoms, while the biopsy was done less frequently in screen-detected cases who often have milder presentation at diagnosis. This difference might also be partially explained by the lower willingness of these patients to undergo an unpleasant repeat biopsy. Accordingly, the often screen-detected celiac disease

patients with a coexisting type 1 diabetes (Laitinen et al. 2017) were also less likely to undergo a repeat biopsy. In contrast, patients with gastro-intestinal and musculoskeletal comorbidities had higher likelihood for endoscopic follow-up. This suggests that the procedure is carried out with a lower threshold among patients who have incomplete clinical response to gluten-free diet or who have frequent healthcare visit due to the comorbidities.

The repeat biopsy was also omitted more often among celiac disease patients diagnosed in the private sector than those diagnosed in the public healthcare. One reason for this difference might be the costs of endoscopy in private care for patienst without health insurance covering the procedure. In addition, the role of the public healthcare has traditionally been strong in Finland (Fuchs et al. 2014), whereas private sector is more focused on the frontline screening than final diagnostics and follow-up of chonic diseases (Häkkinen et al. 2005). It is thus possible that different results would be obtained in countries where the private sector plays a larger role in healthcare, such as in the USA (Ridic et al. 2012).

Another important observation here was the lack of differences in the rate of re-biopsy between primary, secondary and tertiary healthcare settings. This somewhat unexpected finding might be explained by the long tradition of decentralization of celiac disease diagnostics and follow-up in Finland (Häkkinen et al. 2005, Collin et al 2010).

4.1.3 The Impact of Repeat Biopsy on Long-Term Treatment Outcomes

The omission of a repeat biopsy one year after the diagnosis did not affect long-term dietary adherence of the celiac disease patients. This surprising result suggests that the invasive follow-up does not play a major role in the commitment to the gluten-free diet. It must, however, be realized that the diet is relatively easy to maintain in Finland (Kaukinen et al. 2010, Ilus et al. 2014), and the situation might be different in countries where maintaining a strict adherence is more challenging (Bardella et al. 1994, Leffler et al. 2007, Zanini et al. 2010). In addition, despite the generally good dietary adherence, we found subjects without a repeat biopsy to be more uncertain of their capability to manage the gluten-free diet and to be more often seropositive. This indicates that a subgroup of celiac disease patients having challenges with gluten-free diet might still benefit from the endoscopic follow-up. On the other hand, it is unclear whether the repeat biopsy is in fact the main issue here, or whether other means of follow-up, such as enhanced dietary counselling, would be equally effective (See et al. 2015, Sainsbury et al. 2013b).

There was also no significant difference between the repeat biopsy and no repeat biopsy groups in psychological well-being as measured by the validated PGWB

questionnaire. The differences between follow-up and no follow-up groups in the prevalence of reported bodily pain and physical functioning can be explained to some extent by the higher prevalence of musculoskeletal comorbidities among patients with an ongoing long-term follow-up.

However, celiac disease patients who underwent a repeat biopsy had more often self-perceived symptoms on long term than those without the repeat biopsy. This could, to some extent, be explained by the higher prevalence of gastrointestinal comorbidities among the re-biopsied patients. In addition, there is evidence that celiac disease patients with a severe presentation at diagnosis, i.e. similar subjects that had undergone a follow-up biopsy more often in the present study, could have increased risk for persistent symptoms despite a strict gluten-free diet (Paarlahti et al. 2013). In previous studies the persistence of symptom has been associated with reduced self-perceived well-being and quality of life (Paarlahti et al. 2013, Paavola et al. 2012, Nachman et al. 2010). As mentioned, similar association was not seen in the present study, but here the design was different and the symptoms, in general, rather mild.

4.2 Mucosal Recovery in the Repeat Biopsy

4.2.1 Prevalence of Incomplete Mucosal Recovery

Patients with incomplete mucosal recovery one year after the celiac disease diagnosis represented 33% (Study **I**) and 42% (Study **II**) of the study populations, respectively. Althoug a seemingly large percentage, these figures are actually low in global comparison (Wahab et al. 2002, Rubio-Tapia et al. 2010, Sharkey et al. 2013), probably reflecting the generally high dietary adherence in Finland (Ilus et al. 2012). Furthemore, only 2.3% of all patients in Study **I** showed no signs of histological improvement after one year on a gluten-free diet, and only one patient developed RCD. This excellent result is in accordance with a previous Finnish study, in which 96% of celiac disease patients reached complete histological recovery in the long run, only 0.3% eventually having RCD (Ilus et al. 2012, Ilus et al. 2014). The good availability and strict labeling of gluten-free products and the (now removed) financial subsidy by the government are all likely to reduce inadvertent and advertent gluten intake (Abdulkarim et al. 2002), these being by far the most common reasons for non-responsive celiac disease globally (Rubio-Tapia et al. 2010, Leffler et al. 2007).

4.2.2. Predictors of Incomplete Mucosal Recovery

The most conspicuous factor predicting incomplete histological recovery on a gluten-free diet was the presence of more severe celiac disease at diagnosis in terms of histology, serology and signs of malabsorption. Despite the differences in histology, the clinical parameters were similary improved in both groups after one year, suggesting that it just takes longer for severily damaged mucosa to heal even on diet, which is in accord with a recent Norweigian study (Haere et al. 2016). Furthermore, in Study **I** we quantitatively assessed the actual speed (ΔVh/CrD) of mucosal recovery, and found also this to be lower in patients with incomplete than complete recovery. The slower speed of recovery, along with more severe atrophy at diagnosis, readily explain why these patients had not attained mucosal recovery in one year despite strict dietary adherence. A few previous studies have investigated this issue indirectly (Wahab et al. 2002, Rubio-Tapia et al. 2010, Lanzini et al. 2009). The results have been somewhat controversial, which might be explained by differences in dietary adherence and by the use of less precise grouped classifications for assessment of histopathology (Taavela et al. 2013).

Another indicator of the presence of more severe disease at diagnosis among those with incomplete histological recovery after one year was their higher levels of celiac disease antibodies at diagnosis, although the correlation between the antibody values and the severity of histological and clinical disease is rather low in individual patients (Sharkey et al. 2013. These patients had significantly higher levels of antibodies also after one year, but the actual difference in numbers was small, the median values in both groups were already within normal limits and the great majority had already turned seronegative. This indicates that, although in some patients with high baseline values, the normalization of the serology may take more than a year, it still occurs faster than mucosal healing. However, we cannot fully exclude the possibility for more frequent gluten intake in the histologically non-recovered group (Hollon et al. 2013).

The only clinical parameters at diagnosis associated with with incomplete histological recovery were the presence of anemia and lower BMD T-scores. In line, previous studies in both children and adults have reported an association between the presence of anemia and more severe histological and clinical presentation at celiac disease diagnosis (Taavela et al. 2013b, Abu Daya et al. 2013, Saukkonen et al. 2016). Again, despite the differences in mucosal healing, after one year on a gluten-free diet the recovery and no recovery groups were comparable in all laboratory values except for a minor difference in hemoglobin, demonstrating that, as with serology, laboratory abnormalities recover faster than the histology. In contrast, the median BMD remained lower even after one year on diet. This might be explained by the slow turnover rate of bone tissue, causing the normalization of BMD to take longer than our study period. Especially if the

absorption capacity of relevant micronutrients in the intestine remains sub-optimal due to ongoing mucosal recovery.

4.2.3 Long-Term Effects of Incomplete Mucosal Recovery after One Year

Normalization of the small-bowel mucosal damage is considered an important long-term goal in celiac disease, as incomplete histological recovery is thought to predispose patients to severe complications such as malignancies and RCD (Ilus et al. 2014, Lebwohl et al. 2013a, Kaukinen et al. 2010). As mentioned, only one subject out of the cohort of 263 patients in Study **I** later developed type I RCD, and it is likely that this case would have been anyway detected due to lack of clinical response to gluten-free diet. Furthermore, the result of histology one year after the diagnosis was not associated with the risk of malignancies in a 15-year follow-up, further indicating that prediction of forthcoming severe complications cannot justify the repeat biopsy by itself.

When considering the effect of the celiac disease diagnosis and gluten-free diet on the daily live of patients, it is essential to study not only the clinical, histological and serological improvement, but also the general quality of life, as celiac disease affects a fundamental part of patients' lives and adhering to the diet can be stressing and socially limiting (Shah et al. 2012, See et al. 2015). Particularly non-responsive disease with ongoing symptoms can have adverse effects on patients' quality of life (Paarlahti et al. 2013). In our study patients had similar quality of life and frequency of gastro-intestinal symptoms irrespective of mucosal status both one year after the diagnosis and in the long run, even if those with incomplete villous recovery had poorer GSRS and PGWB values at baseline. This is in line with the abovementioned observation of the healing of the small-bowel mucosa lagging behind clinical recovery.

Altogether, these findings indicate that the result of the repeat biopsy after one year correlates poorly with both the clinical outcome and the long-term prognosis in celiac disease. Instead, it can be seen representing only a "snapshot" rather than the end-point of the still ongoing mucosal healing, thus not giving much additional value for the long-term management of the patients. Hence, it seems that more individualized approaches for the endoscopic follow-up in celiac disease are required. In fact, based on the already published articles of this dissertation, the repeat biopsy was not anymore considered mandatory to clinically and serologically recoved patients in the very recently revised Finnish Current Care Guidelines. (Curren Care Guideline 2018)

4.3 Long-Term Management of Celiac Disease

There is a clear demand for improved non-invasive methods to evaluate dietary adherence and long-term histological recovery in celiac disease. Unfortunately, neither the current serological tests, self-reported adherence nor even a standardized evaluation by a dietitian are sensitive enough to detect all dietary lapses (Moreno et al. 2017, Leffler et al. 2007). Another problem is that the effects of long-term management and follow-up on the health outcomes have not been comprehensively studied and expert opinions vary. There has been scarcity of evidence particularly on how this follow-up is carried out in clinical practice and what factors affect its execution. Altogther, the data on how follow-up, or lack of it, affects long-term outcomes such as dietary adherence, frequency of complications and quality of life, have been insufficient (Hall et al. 2009, Leffler et al. 2008, Herman et al. 2012, Haines et al. 2014).

4.3.1 Prevalence, Predictors and Demand of Long-Term Follow-Up

The presence of a regular long-term follow-up for celiac disease was surprising low (15%), particularly when considering that it is recommended to all Finnish patients and since the majority of the study participants wished for it. This is also a very low percentage compared to previous reports (Bebb et al. 2006, Bardella et al. 1994). Although our national guidelines recommend regular follow-up for celiac disease patients, it has not been clarified on how this should be arranged. The lack of a clear follow-up protocol in the guidelines, along with the generally good knowledge of celiac disease among the patients and the easy availability of the gluiten-free products, may explain the result as it is much up to patients' own activity to arrange the control visits (Ilus et al. 2014). It could be hard to find time and motivation e.g. to annual follow-up visits for a disease that is felt to be in remission and does not cause marked stress in daily life.

Of the predictors of the presence of long-term follow-up, it was less common in patients who did not belong to any risk group of celiac disease, excluding those with family history for the disease who actually had more frequent control visits. Although there are some previous studies about the association between patient-derived factors and follow-up, thus far no systematic reports on this issue have been published (Bardella et al. 1994, Bebb et al. 2006). In a study by Bebb et al. (2006) the most popular method of follow-up among patients was a dietitian's interview with a doctor available if needed. Here patients rather wanted the follow-up to be organized in the primary care than in expert clinics, although one third of patients would want the follow-up to be arranged to an internal physician. This result could be due to the the strong position of primary

health care in Finland. In fact, this may also, to some extent, explain the low percentage of long-term follow-up, as the general practitioners treat the majority of patients' illnesses and it is thus possible that a part of the celiac disease follow-up is carried out during healthcare visits that were initially arranged due to some other chronic disease. Also, due to the generally high level of knowledge amont the patients, it may be that physicians trust them to be in contact if any problems appear. Celiac disease may also be seen as a rather bening disease without much need for follow-up when patients remain symptomless.

4.3.2 Significance of Long-Term Follow-Up

There was no difference between celiac disease patients in the follow-up group and no follow-up group in the adhererence to the gluten-free diet, and the long-term quality of life and severerity of gastrointestinal symptoms as measured by validated GSRS were also comparable. The former finding is in contrast with the previous studies, in which the presence of regular follow-up has been associated with better dietary compliance (Villafuerte-Galvez et al. 2015, Hall et al. 2009, Leffler et al. 2008). This difference might be caused by the generally high dietary adherence and easy availability of gluten-free products in Finland (Ilus et al. 2012). Nevertheless, we still found patients without long-term follow-up to report more overall symptoms and lower self-perceived capability to manage the diet compared with the followed subjects. The feeling of better competence with the dietary treatment among the followed subjects might be explained by an enhanced sense of self-capability when patients receive positive feedback during regular healthcare visits. Of note, neither the adherence nor capability to manage a gluten-free diet was affected by who gave the initial dietary information (physician, nurse, dietician). This information is helpful when planning cost-effective follow-up strategies, although possible differences between healtcare settings should be acknowledged.

4.4 Strengths and Limitations of the Study

Major strength of the present study is the large number of well-defined celiac disease patients and the high number of different outcome variables. Furhermore, the use of validated questionnaires for symptoms and quality of life makes the results more reliable and repeatable. The fact that the repeat biopsies were taken systematically after one year on a gluten-free diet, as well as utilization of quantitative morphometry in the histologic evalution, reduces the risk of misclassification bias in Study **I** (Taavela et al. 2012). Further strength of Study **I** was the comprehensive data on mortality and long-term complications. The excellent adherence to gluten-free diet observed in Studies **I** and **II** enabled us to evaluate other causes behind histological non-recovery than the often dominating high frequence of compliance problems, although this makes the results less applicable to countries where dietary lapses are more common (Oxetenko et al 2014). Another major streght of the present study is the large number of celiac disease patients diagnosed on different levels of healthcare in Studies **II** and **III**, whereas Study I was limited to tertiary care settings.

As a limitation, we were not able to compare the details of celiac disease patients who underwent a repeat biopsy and those who refused follow-up (approximately 15% of all subjects in our center). In theory, the non-compliant patients may be more prone to refuse repeat biopsy. Another limitation is the lack of systematic endoscopic follow-up in patients with incomplete mucosal recovery after one year on a gluten-free diet. This was mostly due to the decentralization of long-term follow-up in our settings; in other words patients with uncomplicated celiac disease are usually followed non-invasively in primary care (if they are followed). Nevertheless, according to our national guidelines, celiac disease patients with poor and unexplained response to gluten-free diet should be referred to secondary or tertiary centers for further evaluation.

Morever, the retrospective analysis of serious long-term outcomes is prone to survival bias, as the study patients were not categorized already at the time of the repeat biopsy. The recruitment of a substantial part of the participants via celiac societies may increase the risk of responder bias, since patients belonging to such organisations might be more committed to the treatment of their disease. The information of these studies also may not have reached all age groups equally. We also did not have data on the patients' socio-economic status, which has been considered important in some previous studies on follow-up (Ciacci et al. 2002, Artama et al. 2016). Furthermore, mortality data were lacking from studies **II** and **III** and, in all studies, the self-reported symptoms scale had only three levels, making it possibly too imprescise a measurement, even if the results were in line with the GSRS scores in Study **III.**

Finally, all three substudies of the present dissertation were carried out before the recommendation to take routine biopsies also from the duodenal bulb (Ludvigsson et al. 2014). It is therefore possible that some cases with partially incomplete histological recovery on a gluten-free diet have been missed (Evans et al. 2011). Nevertheless, at present the role of bulb biopsies in the diagnostics and follow-up of celiac disease remains controversial (Taavela et al. 2016).

5 Summary and Conclusions

A repeat biopsy one year after celiac disease diagnosis for at least symptomatic subjects, as well as regular long-term follow-up on a gluten-free diet, have been considered essential due to the possible adverse outcomes of unsuccessful treatment (Bai et al. 2017, Current Care Guideline 2010, Ludvigsson et al. 2013c, Rubio-Tapia et al 2010). However, we found neither the existence nor result of a repeat biopsy, nor even the presence of regular follow-up, to have major effects on the long-term health and treatement outcomes. In addition, one year may be too short of a time on a gluten-free diet for some patients, particularly among those with a severe form of the disease at diagnosis in terms of histology, serology and signs of malabsorption, to reach full morphological recovery of the small-bowel mucosa. This seems to be the case even if we know that the great majority of also these cases reach recovery in the long run (Ilus et al. 2012). Together these results indicate that, in contrast to most of the current recommendations, the long-term follow-up of celiac disease, including the decision to conduct a repeat biopsy, could be more personalized. I could also be based on the individual capabilities to maintain a gluten-free diet and to the short-term treatment response.

This dissertation paves the way to further studies on the short- and long-term follow-up in celiac disease. Future research should focus particularly on questions of which patients would benefit from a more systematic follow-up, and who should undergo a repeat biopsy on gluten-free diet. More accurate non-invasive markers for the dietary adherence and mucosal healing should also be and are being developed, including for example HLA-DQ:gluten tetramer test in blood (Sarna et al. 2018), although they have not yet found their place in clinical practice. These improvements could be expected to provide easier and more cost-effective approach to the follow-up of celiac disease.

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Tampere, September 2019
Henna Pekki

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APPENDIX 1

THE GASTROINTESTINAL SYMPTOM RATING SCALE
(GSRS)

Nimi_____

Lue tämä ensin:
Tutkimus sisältää kysymyksiä voinnistasi ja tilastasi kuluneen viikon aikana.
Merkitse rastilla (X)
se vaihtoehto, joka sopii parhaiten sinuun ja tilaasi.

1. Onko Sinulla ollut VATSAKIPUJA kuluneen viikon aikana? (Vatsakivuilla tarkoitetaan
kaikenlaista kipua tai särkyä vatsassa.)

☐ Ei minkäänlaisia vaivoja
☐ Vähäpätöisiä vaivoja
☐ Lieviä vaivoja
☐ Kohtalaisia vaivoja
☐ Melko pahoja vaivoja
☐ Pahoja vaivoja
☐ Erittäin pahoja vaivoja

2. Onko Sinulla ollut NÄRÄSTYSTÄ kuluneen viikon aikana? (Närästyksellä tarkoitetaan kirvelevää
tai polttavaa pahanolontunnetta rintalastan takana.)

☐ Ei minkäänlaisia vaivoja
☐ Vähäpätöisiä vaivoja
☐ Lieviä vaivoja
☐ Kohtalaisia vaivoja
☐ Melko pahoja vaivoja
☐ Pahoja vaivoja
☐ Erittäin pahoja vaivoja

GSRS (F)

3. Onko Sinulla ollut HAPPAMIA RÖYHTÄISYJÄ kuluneen viikon aikana? (Happamilla röyhtäisyillä
tarkoitetaan äkillisiä, hapanta vatsanestettä sisältäviä röyhtäisyjä.)

☐ Ei minkäänlaisia vaivoja
☐ Vähäpätöisiä vaivoja
☐ Lieviä vaivoja
☐ Kohtalaisia vaivoja
☐ Melko pahoja vaivoja
☐ Pahoja vaivoja
☐ Erittäin pahoja vaivoja

4. Onko Sinua HIUKAISSUT kuluneen viikon aikana? (Hiukaisulla tarkoitetaan vatsassa olevaa
hiukovaa tunnetta, johon liittyy tarve syödä aterioiden välillä.)

☐ Ei minkäänlaisia vaivoja
☐ Vähäpätöisiä vaivoja
☐ Lieviä vaivoja
☐ Kohtalaisia vaivoja
☐ Melko pahoja vaivoja
☐ Pahoja vaivoja
☐ Erittäin pahoja vaivoja

5. Onko Sinulla ollut PAHOINVOINTIA kuluneen viikon aikana? (Pahoinvoinnilla tarkoitetaan
pahanolontunnetta, joka saattaa muuttua kuvotukseksi tai oksentamiseksi.)

☐ Ei minkäänlaisia vaivoja
☐ Vähäpätöisiä vaivoja
☐ Lieviä vaivoja
☐ Kohtalaisia vaivoja
☐ Melko pahoja vaivoja
☐ Pahoja vaivoja
☐ Erittäin pahoja vaivoja

GSRS (F)

6. Onko vatsasi KURISSUT kuluneen viikon aikana? (Kurinalla tarkoitetaan vatsassa tuntuvaa
värinää tai ”murinaa”.)
☐ Ei minkäänlaisia vaivoja
☐ Vähäpätöisiä vaivoja
☐ Lieviä vaivoja
☐ Kohtalaisia vaivoja
☐ Melko pahoja vaivoja
☐ Pahoja vaivoja
☐ Erittäin pahoja vaivoja

7. Onko vatsaasi TURVOTTANUT kuluneen viikon aikana? (Turvotuksella arkoitetaan vatsassa
tuntuvaa pingotusta, johon usein liittyy tuntemuksia ilmavaivoista.)
☐ Ei minkäänlaisia vaivoja
☐ Vähäpätöisiä vaivoja
☐ Lieviä vaivoja
☐ Kohtalaisia vaivoja
☐ Melko pahoja vaivoja
☐ Pahoja vaivoja
☐ Erittäin pahoja vaivoja

8. Onko Sinua vaivannut RÖYHTÄILY kuluneen viikon aikana? (Röyhtäilyllä tarkoitetaan tarvetta
päästää ilmaa suun kautta, minkä yhteydessä vatsassa tuntuva pingotus usein helpottuu.)
☐ Ei minkäänlaisia vaivoja
☐ Vähäpätöisiä vaivoja
☐ Lieviä vaivoja
☐ Kohtalaisia vaivoja
☐ Melko pahoja vaivoja
☐ Pahoja vaivoja
☐ Erittäin pahoja vaivoja

GSRS (F)

9. Onko Sinulla ollut ILMAVAIVOJA kuluneen viikon aikana? (Ilmavaivoilla tarkoitetaan tässä
tarvetta päästää ilmaa, jonka yhteydessä vatsassa tuntuva pingotus usein helpottuu.)

☐ Ei minkäänlaisia vaivoja
☐ Vähäpätöisiä vaivoja
☐ Lieviä vaivoja
☐ Kohtalaisia vaivoja
☐ Melko pahoja vaivoja
☐ Pahoja vaivoja
☐ Erittäin pahoja vaivoja

10. Onko Sinua vaivannut UMMETUS kuluneen viikon aikana? (Ummetuksella tarkoitetaan
ulostuskertojen harventumista.)

☐ Ei minkäänlaisia vaivoja
☐ Vähäpätöisiä vaivoja
☐ Lieviä vaivoja
☐ Kohtalaisia vaivoja
☐ Melko pahoja vaivoja
☐ Pahoja vaivoja
☐ Erittäin pahoja vaivoja

11. Onko Sinua vaivannut RIPULI kuluneen viikon aikana? (Ripulilla tarkoitetaan ulostuskertojen
lisääntymistä.)

☐ Ei minkäänlaisia vaivoja
☐ Vähäpätöisiä vaivoja
☐ Lieviä vaivoja
☐ Kohtalaisia vaivoja
☐ Melko pahoja vaivoja
☐ Pahoja vaivoja
☐ Erittäin pahoja vaivoja

GSRS (F)

12. Onko Sinua vaivannut LÖYSÄ VATSA kuluneen viikon aikana? (Jos ulosteesi on välillä ollut
kovaa ja välillä löysää, ilmoita vain, missä määrin ulosteesi löysyys on Sinua vaivannut.)

☐ Ei minkäänlaisia vaivoja
☐ Vähäpätöisiä vaivoja
☐ Lieviä vaivoja
☐ Kohtalaisia vaivoja
☐ Melko pahoja vaivoja
☐ Pahoja vaivoja
☐ Erittäin pahoja vaivoja

13. Onko Sinua vaivannut KOVA VATSA kuluneen viikon aikana? (Jos ulosteesi on välillä ollut kovaa
ja välillä löysää, ilmoita vain, missä määrin ulosteesi kovuus on Sinua vaivannut.)

☐ Ei minkäänlaisia vaivoja
☐ Vähäpätöisiä vaivoja
☐ Lieviä vaivoja
☐ Kohtalaisia vaivoja
☐ Melko pahoja vaivoja
☐ Pahoja vaivoja
☐ Erittäin pahoja vaivoja

14. Onko Sinua vaivannut kuluneen viikon aikana PAKOTTAVA ULOSTAMISEN TARVE? (Pakottavalla
ulostamisen tarpeella tarkoitetaan äkillistä tarvetta käydä WC:ssä. Siihen liittyy usein
puutteellisen pidättämiskyvyn tunne.)

☐ Ei minkäänlaisia vaivoja
☐ Vähäpätöisiä vaivoja
☐ Lieviä vaivoja
☐ Kohtalaisia vaivoja
☐ Melko pahoja vaivoja
☐ Pahoja vaivoja
☐ Erittäin pahoja vaivoja

15. Onko Sinulla kuluneen viikon aikana ollut ULOSTAMISEN YHTEYDESSÄ TUNNE, ETTÄ SUOLI EI
OLE TYHJENTYNYT KOKONAAN? (Tällä tarkoitetaan, että suoli ei ponnistuksista huolimatta
tunnu tyhjentyneen kunnolla.)

☐ Ei minkäänlaisia vaivoja
☐ Vähäpätöisiä vaivoja
☐ Lieviä vaivoja
☐ Kohtalaisia vaivoja
☐ Melko pahoja vaivoja
☐ Pahoja vaivoja
☐ Erittäin pahoja vaivoja

16. ONKO SINULLA VIIMEISEN KUUKAUDEN AIKANA ESIINTYNYT SEURAAVIA OIREITA
(rengasta sopivat vaihtoehdot)

☐☐ kielikipuja
☐☐ haavaumia suussa
☐☐ luustokipuja
☐☐ puutumista
☐☐ muuta, mitä

_____

TARKISTA, ETTÄ OLET VASTANNUT KAIKKIIN KYSYMYKSIIN, ENNEN KUIN PALAUTAT LOMAKKEEN.
KIITOS AVUSTASI!

APPENDIX 2

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

PGWB (F)

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

PGWB (F)

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

PGWB (F)

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

PGWB (F)

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

PGWB (F)

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

PGWB (F)

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

PGWB (F)

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

ORIGINAL ARTICLES

COLON/SMALL BOWEL

Predictors and Significance of Incomplete Mucosal Recovery in Celiac Disease After 1 Year on a Gluten-Free Diet

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OBJECTIVES: **In celiac disease, a follow-up biopsy taken 1 year after diagnosis is considered important in monitoring histological recovery. In many cases, recovery is incomplete, and the clinical significance of this is poorly understood. We now investigated associated factors and the significance of imperfect histological recovery in patients in whom the follow-up had been completed.**

METHODS: **Two hundred sixty-three biopsy-proven patients were divided into two groups: histological recovery and incomplete recovery after 1 year on gluten-free diet. Serology, laboratory values, bone mineral density, and different clinical variables were measured at diagnosis and after 1 year. Gastrointestinal symptoms and quality of life were assessed by validated questionnaires. Further, long-term follow-up data on mortality, malignancies, and other severe complications were collected.**

RESULTS: **The incomplete recovery group had more severe mucosal damage (P=0.003), higher antibody values (P=0.017), and more signs of malabsorption (P<0.001) at diagnosis. There was no difference in gender, symptoms or quality of life, family history of celiac disease, or comorbidities. At follow-up, there was still a difference in antibodies (P=0.018) and femoral T-scores (P=0.024). Histologically recovered patients showed better dietary adherence, although it was excellent in both groups (97% vs. 87%, P<0.001). There was no difference in long-term outcomes between groups.**

CONCLUSIONS: **The presence of more severe disease in terms of histology, serology, and signs of malabsorption was associated with histological non-response. In patients with high dietary adherence, incomplete villous recovery after 1 year does not affect the clinical response or long-term prognosis. A personalized approach is required to decide the optimal timing of the follow-up biopsy.**

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INTRODUCTION

Celiac disease is a lifelong gluten-induced enteropathy with a prevalence of up to 2% in Caucasian populations (1,2). The only current treatment for the condition is lifelong adherence to a gluten-free diet, the effectiveness of which is shown by a high rate of symptom alleviation on a strict diet (2,3). The demonstration of gluten-induced small-bowel mucosal damage and crypt hyperplasia upon intestinal biopsy is required for the diagnosis, but current guidelines are somewhat contradictory as to whether the biopsy should be repeated after 1 year on dietary treatment (3–7). The main reasons for routine follow-up biopsy would be to monitor histological recovery and dietary adherence, as well as to exclude serious complications such as refractory celiac disease in non-responsive patients. However, a substantial proportion of celiac disease patients have not reached complete mucosal recovery after 1 year, whereas in the long run it is seen in up to 96% of patients (8–12). The challenge is thus to distinguish patients with simply a slow histological response from those with true refractory celiac disease. Ill-timed endoscopic studies comprise a major burden for patients and health care, and the

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cost-effectiveness of the second biopsy remains obscure (9). On the other hand, persistent villous atrophy predisposes to severe complications and increased mortality in the long run (6,9,11). Currently, it is unclear whether there are specific patient-related factors associated with the speed of villous recovery, whether a correlation prevails between clinical outcomes and mucosal recovery after 1 year on treatment, and whether incomplete mucosal recovery after 1 year on a gluten-free diet has a long-term impact (5,9–11). Understanding these issues would make for a more personalized approach to the follow-up of celiac disease and spare patients and limited health-care resources unnecessary invasive examinations.

The objective of this study was to identify patient-related factors possibly predicting incomplete histological recovery at follow-up biopsy 1 year after diagnosis, and to assess the clinical significance and long-term consequences of such slowly recovering mucosal damage. This was established by measuring a wide variety of histological, serological, and clinical parameters, both at diagnosis and after 1 year on a gluten-free diet, in a large cohort of adults with celiac disease.

METHODS

Patients and study design

The study was conducted in Tampere University Hospital and the University of Tampere between 1996 and 2009. Altogether, data regarding 263 adults with biopsy-proven celiac disease (179 women and 84 men, median age 45 (range 15–79) years) were collected from our prospective patient series. The present study included only cases where a first follow-up biopsy had been carried out after 1 year on a gluten-free diet. Further, as there is recent evidence that the angiotensin II receptor blocker olmesartan may cause severe enteropathy resembling celiac disease (13), the possible use of this drug in our patients was checked. All participants were interviewed at the time of the diagnosis and demographic data, family history of celiac disease, and celiac disease-associated and other significant comorbidities were enquired. Special attention was devoted at diagnosis to characteristic clinical manifestations of celiac disease, among them anemia, malabsorption, diarrhea, and to the possible presence of atypical or extraintestinal symptoms such as arthritis, gynecological problems, or neurological symptoms. Duration of symptoms before diagnosis was assessed and the symptoms further categorized as mild, moderate, or severe. Further, the proportion of subjects detected by screening in at-risk groups, such as relatives of celiac disease patients and those with type 1 diabetes or autoimmune thyroidal disease, was calculated.

All patients received comprehensive dietary guidance by a dietitian with expertise in celiac disease and started a gluten-free diet following the diagnosis. Adherence to the diet was evaluated by the dietitian after 1 year, and patients with no gluten or only a minor inadvertent gluten intake less than once per month were defined as adherent (14,15). The patients underwent a follow-up biopsy after 1 year on diet and were divided into two groups based on their morphological small-bowel mucosal recovery. Subjects evincing mucosal recovery were defined as the 'Recovery group' and those with incomplete morphological recovery as the 'Atrophy group'. Besides the above-mentioned clinical data, the following comparisons between groups were carried out both at diagnosis and at follow-up biopsy: celiac disease serology, small-bowel mucosal histology, laboratory values, bone mineral density (BMD), body mass index (BMI), gastrointestinal symptoms, and health-related quality of life. In addition, special attention was paid to patients who did not show any signs of histological recovery after 1 year on the dietary treatment. In our setting, after the first follow-up biopsy patients with good clinical and serological response are assigned to secondary or primary health care for further endoscopic and clinical follow-up, and in cases of persistent mucosal atrophy or recurrence of symptoms, subjects are referred back to a tertiary center for further investigations.

Long-term follow-up data on the patients' mortality, malignancies, other gastrointestinal diseases, and osteoporosis were collected from their medical records up to present. In addition, the degree of gastrointestinal symptoms and quality of life was assessed by validated questionnaires among a subgroup of patients with a median of 5 years after diagnosis (see below).

Small-bowel mucosal biopsies

A minimum of six biopsy specimens were taken from the distal duodenum upon upper gastrointestinal endoscopy. Three specimens were carefully orientated, processed, and stained with hematoxylin and eosin and analyzed under light microscopy. The degree of morphological small-bowel mucosal damage was measured quantitatively using the villous height-crypt depth ratio (Vh/CrD). This was calculated by measuring the mean height of a villus and the adjacent crypt from at least three well-orientated villus–crypt units. The final morphological analysis was conducted on the most severely damaged biopsy specimen (16). Normal Vh/CrD was considered to be over 2.0 (17). To ascertain a dose response reflected in outcome measures, the Atrophy group were further categorized into those with subtotal or total villous atrophy (Vh/CrD below 0.9) and those with partial villous atrophy (Vh/CrD 0.9–1.9).

The degree of small-bowel mucosal inflammation was determined from the biopsy specimens by measuring the density of intraepithelial lymphocytes (IELs), as previously described in detail (18). Briefly, the IELs were counted in both hematoxylin- and eosin-stained and frozen sections with a ×100 flat-field light microscope objective. Immunohistochemical studies were made on frozen 5-μm-thick sections and CD3+ IELs were stained using the monoclonal antibody Leu-4 (Becton Dickinson, San Jose, CA). The density of hematoxylin- and eosin-stained IELs was expressed as cells per 100 epithelial cells (reference value over 30 per 100 enterocytes) and that of CD3+ IELs as cells per mm (reference value 37 cells per mm) (18). Further, the densities of αβ+ IELs and γδ+ IELs were counted in the frozen sections as described previously (19,20).

Serology and laboratory parameters

Serum IgA-class transglutaminase-2 antibodies (TG2abs) were measured by enzyme-linked immunosorbent assay (Celikey;

Phadia, GmbH, Freiburg, Germany). Values >5.0 U/l for TG2abs were considered positive according to the manufacturer's instructions. Serum IgA-class endomysium antibodies were assessed by an indirect immunofluorescence method as described previously (21). Titers 1:≥5 were considered positive. In cases of selective IgA deficiency, the corresponding IgG-class antibodies were applied. The serological tests were subjected to meticulous quality assessment as described elsewere (22). Our laboratory is one of the six laboratories in the international quality control network (UK NEQAS) for celiac antibody testing. In our settings, the batch-to-batch variation in serology has been 10–15%. The following laboratory parameters were measured using standard methods: blood hemoglobin (reference values: men >134 g/l; women >117 g/l), mean corpuscular volume (82–98 fl), ionized calcium (1.16–1.30 mmol/l), parathyroid hormone (1.0–7.5 pmol/l), serum total iron (9–34 μmol/l), erythrocyte folic acid (200–700 nmol/l), and serum vitamin B12 (>150 pmol/l).

BMD and BMI

BMD was measured from the lumbar spine and femoral neck by dual-energy X-ray absorptiometry (Norland XR-26; Norland Corp, Fort Atkinson, WI) following our standard procedure (23). The values expressed are standard deviation scores, which compare the individual value with either that of healthy young adults (*T*-score) or the age-matched population (*Z*-score) (24,25). Both *T*- and *Z*-scores were applied here according to the recommendation of the World Health Organization (24). The individual's BMI was calculated as weight in kilograms divided by the square of height in meters (kg/m^2).

Gastrointestinal symptoms and quality of life

The Gastrointestinal Symptoms Rating Scale (GSRS) (26) was used to evaluate the self-perceived severity of patients' gastrointestinal symptoms. The scale is well-validated and widely applied in celiac disease (21,27,28). GSRS consists of 15 separate items and the total score is the sum of the mean values obtained for each separate item. Further, the questionnaire can be divided into five subdimensions measuring abdominal pain, gastroesophageal reflux, indigestion, diarrhea, and constipation. The scoring is based on a seven-grade Likert scale where higher scores stand for more severe gastrointestinal symptoms.

The Psychological General Well-Being Index (PGWB) was used to measure self-perceived psychological well-being and quality of life (29). PGWB is a 22-item questionnaire using a six-grade Likert scale where high scores indicate better well-being and quality of life. The separate items can be further divided into six subdimensions measuring anxiety, depression, well-being, self-control, general health, and vitality. PGWB is also a well-validated and widely used questionnaire in celiac disease (14,21,27,28,30–33).

Ethics

Patient collection was conducted with the permission and according to the guidelines of the Ethical Committee of Tampere University Hospital. All celiac disease patients participating in the present study gave written informed consent before data collection. Further, the collection of long-term follow-up data on the patients was conducted with the permission of the Department of Internal Medicine, Tampere University Hospital.

Statistical analysis

Statistical testing was made using PASW version 18 (IBM, New York, NY). Results are shown as medians with 25th to 75th percentiles (lower and upper quartiles, Q_1–Q_3). Mann–Whitney *U*-test was used in statistical comparisons between groups in age, serology, mucosal histology, laboratory parameters, BMD, BMI, GSRS, PGWB, and symptom duration. $\chi 2$ Test was used in comparisons of sex, clinical presentation and severity of the disease, presence of comorbidities, adherence to gluten-free diet, and family history of celiac disease. Multivariate analysis was further applied for variables with statistical significance. A *P*-value <0.05 was considered statistically significant.

RESULTS

Histology, serology, and clinical parameters

By definition, at diagnosis all patients here had biopsy-proven celiac disease with villous atrophy and crypt hyperplasia (Vh/CrD <2.0). After 1 year on a gluten-free diet, the small-bowel mucosal morphology had normalized in 178 (68%) patients (Recovery group), whereas 85 (32%) showed incomplete mucosal recovery (Vh/CrD <2.0, Atrophy group) in the follow-up biopsy. Demographic data, clinical characteristics, and comorbidities of the participants at diagnosis are shown in **Table 1**. Among these, a significant difference between the groups was seen only in the presence of malabsorption at diagnosis, this being more common in the Atrophy group. There was also a trend toward a higher prevalence of histological non-recovery in older patients and subjects with psoriasis and a lower prevalence of ongoing atrophy in autoimmune thyroidal diseases, although this was not statistically significant (**Table 1**). Altogether, 21% of the Recovery group and 25% of the Atrophy group were at least 60 years of age at diagnosis, giving an adequate representation of the elderly for the analyses. There was no significant difference between histologically recovered patients and those with ongoing atrophy in gender distribution, clinical presentations at diagnosis, severity or duration of symptoms before diagnosis, diarrhea, family history of celiac disease, or presence of celiac disease-associated and other illnesses (**Table 1**); also, the prevalence of screen-detected patients was comparable in both groups.

Subjects in the Atrophy group had significantly higher serum TG2ab levels at diagnosis (**Table 2**). This difference was also observed after 1 year on a gluten-free diet, but at that time median TG2ab values were low and within the reference range in both groups (**Table 2**). Altogether, only 11% of the histologically recovered and 16% of the atrophic patients remained seropositive at the time of the follow-up biopsy, the rates at diagnosis being 88% and 93%, respectively. In all but one initially seropositive subject antibody values decreased on diet.

At diagnosis, patients in the Atrophy group had more severe small-bowel mucosal damage as measured by the Vh/CrD

Table 1. Baseline demographic data, clinical characteristics, and associated diseases in 263 celiac disease patients with (Recovery) or without (Atrophy) a histological response[a] to 1 year on a gluten-free diet

	Atrophy, *N*=85	Recovery, *N*=178	*P* value
Age, median (range) (years)	48 (18–77)	44 (15–79)	0.068
Age over 60 years (%)	24.7%	20.6	
Females (%)	65	70	0.420
Clinical presentation at diagnosis[b] (%)			
Malabsorption	60	34	0.001
Diarrhea	20	21	0.897
Extraintestinal symptoms[c]	13	14	0.176
Screen-detected[d]	22	29	0.543
Severity of symptoms at diagnosis (%)			0.512
Mild	87	82	
Moderate	13	16	
Severe	0	2	
Duration of symptoms, median (range) (year)	2 (0–40)	1 (0–57)	0.219
Family history of celiac disease (%)	37	47	0.129
Presence of comorbidities (%)			
Autoimmune thyroidal disorders	6	26	0.080
Psoriasis	5	3	0.064
Type 1 diabetes mellitus	2	5	0.830
Lactose intolerance	5	16	0.385
Asthma	3	9	0.579
Any malignancy	3	5	0.750
Any neurological disorders	3	12	0.293
Hypertension	7	20	0.453
Coronary artery disease	4	5	0.429
Psychiatric disease	4	9	0.902

Ig, immunoglobulin.
[a]Defined as a small-bowel mucosal villous height crypt depth ratio >2.0 at the follow-up biopsy.
[b]One patient can present with more than one symptom.
[c]Arthritis, dental enamel defects, dementia, dermatitis herpetiformis, glossitis, aphthous stomatitis, gynecological problems, myopathy, neurologic symptoms, osteoporosis, Sjögren's disease, chronic eczema, and IgA nephropathy.
[d]Celiac disease in relatives, type 1 diabetes mellitus, and autoimmune thyroidal disease.

Table 2. Serological and histological parameters in 263 celiac disease patients with (Recovery) or without (Atrophy) a histological response[a] to 1 year on a gluten-free diet

	Atrophy, *N*=85	Recovery, *N*=178	*P* value
	Median (Q_1–Q_3)	Median (Q_1–Q_3)	
Transglutaminase 2 antibodies (U/ml)[b]			
At diagnosis	56.8 (19.1–100.0)	29.5 (7.8–70.8)	0.017
After 1 year	1.8 (0.6–3.4)	0.4 (0.0–2.1)	0.018
Villous height–crypt depth ratio			
At diagnosis	0.2 (0.1–0.4)	0.4 (0.1–0.8)	0.003
After 1 year	1.2 (0.8–1.6)	2.6 (2.3–2.9)	<0.001
IELs (cells per 100 epithelial cells)			
At diagnosis	46 (36–57)	43 (35–58)	0.739
After 1 year	29 (22–40)	26 (19–36)	0.056
CD3+ IELs (cells per mm)			
At diagnosis	76 (53–89)	67 (49–89)	0.353
After 1 year	38 (28–58)	35 (23–52)	0.034

IEL, intraepithelial lymphocyte.
The values are shown as medians with upper and lower quartiles (Q1 and Q3).
[a]Defined as a small-bowel mucosal villous height crypt depth ratio >2.0 at the follow-up biopsy.
[b]Upper limit of the assay is 100.0 U/ml.

compared with those in the Recovery group (**Table 2**). Further, there was a significant difference in the speed of mucosal recovery between the groups, as the Vh/CrD improved more among histologically healed (change in median ratio 2.1) compared among those still atrophic (change in median ratio 0.9) ($P<0.001$), demonstrating that more severe villous atrophy at diagnosis also predicted slower mucosal healing.

There was no difference between the groups in the levels of either hematoxylin- and eosin-stained or CD3+ IELs at diagnosis, but in the follow-up biopsies the median density of CD3+ IELs was significantly higher in the Atrophy group (**Table 2**). No significant difference was seen between the groups in the density of αβ+ or γδ+ IELs in either the diagnostic or the follow-up biopsy (data not shown).

Laboratory parameters, BMD and BMI

The median blood hemoglobin level in women was significantly lower both at diagnosis and after 1 year on diet in the Atrophy group compared with the Recovery group. A similar but nonsignificant trend was seen in men (**Table 3**). The serum total iron level was significantly lower in the Atrophy group at diagnosis but not at follow-up. There was also a trend toward higher parathyroid hormone and lower erythrocyte folic acid levels in the atrophic patients at diagnosis. In bone both lumbar and femoral *T*-scores were lower in the Atrophy group; after 1 year on a gluten-free diet, the difference was present only in the femur. No significant difference between the groups was seen either in lumbar or in femoral *Z*-scores or in BMI (**Table 3**).

Table 3. Laboratory parameters, bone mineral density, and body mass index in 263 celiac disease patients with (Recovery) or without (Atrophy) a histological response[a] to 1 year on a gluten-free diet

	Atrophy, *N*=85	Recovery, *N*=178	*P* value
	Median (Q_1–Q_3)	Median (Q_1–Q_3)	
Blood hemoglobin[1], women (g/l)			
At diagnosis	119 (112–126)	131 (125–139)	<0.001
After 1 year	127 (120–134)	133 (128–137)	0.029
Blood hemoglobin[1], men (g/l)			
At diagnosis	142 (135–148)	149 (144–153)	0.069
After 1 year	145 (134–150)	147 (147–151)	0.342
Serum total iron[2] (µmol/l)			
At diagnosis	12.0 (8.8–17.6)	18.0 (12.2–21.6)	0.001
After 1 year	19.6 (13.4–24.4)	17.6 (14.0–21.5)	0.245
Erythrocyte folic acid[5] (nmol/l)			
At diagnosis	548 (256–481)	423 (296–507)	0.060
After 1 year	548 (424–676)	508 (383–650)	0.547
Serum vitamin B12 (pmol/l)			
At diagnosis	294 (190–360)	287 (229–368)	0.414
After 1 year	383 (286–464)	341 (269–428)	0.153
Ionized calcium[4] (mmol/l)			
At diagnosis	1.24 (1.23–1.27)	1.25 (1.22–1.27)	0.664
After 1 year	1.25 (1.21–1.28)	1.24 (1.21–1,27)	0.708
Parathyroid hormone[5] (pmol/l)			
At diagnosis	6.3 (4.4–8,2)	4.4 (3.4–6.9)	0.065
After 1 year	4.6 (3.7–6.9)	4.3 (3.4–6.7)	0.645
Lumbar T-score[6] (s.d.)			
At diagnosis	−1.5 (−2.2 to −0.4)	−1.0 (−1.8 to 0.2)	0.022
After 1 year	−1.1 (−2.2 to −0.2)	−0.7 (−1.7 to 0.4)	0.117
Femoral T-score (s.d.)			
At diagnosis	−1.2 (−1.9 to −0.5)	−0.7 (−1.5 to 0.0)	0.016
After 1 year	−1.1 (−1.9 to −0.5)	−0.6 (−1.5 to 0.0)	0.024
Lumbar Z-score[7] (s.d.)			
At diagnosis	−1.2 (−1.7 to 0.1)	−0.3 (−1.1 to 1.0)	0.082
After 1 year	−0.5 (−1.3 to 0.1)	0.1 (−1.1 to 0.9)	0.376
Femoral Z-score[8] (s.d.)			
At diagnosis	−0.7 (−1.0 to 0.2)	−0.3 (−0.8 to 0.3)	0.153
After 1 year	−0.5 (−0.7 to −0.1)	−0.3 (−0.6 to 0.3)	0.323
Body mass index[3] (kg/m²)			
At diagnosis	23.4 (21.3–26.2)	24.6 (22.2–27.7)	0.119
After 1 year	23.9 (22.0–27.3)	24.3 (22.1–27.1)	0.823

The values are shown as medians with upper and lower quartiles (Q1 and Q3).
Variables were available from the following numbers of subjects: [1]153, [2]144, [3]142, [4]95, [5]67, [6]152, [7]159, [8]140 and [9]136.
[a]Defined as a small-bowel mucosal villous height crypt depth ratio >2.0 at the follow-up biopsy.

Table 4. Strictness of gluten-free diet and GSRS and PGWB total scores in 263 celiac disease patients with a histological response[a] (Recovery) or no response (Atrophy) to 1 year on a gluten-free diet

	Atrophy, *N*=85	Recovery, *N*=178	*P* value
	Median (Q_1–Q_3)	Median (Q_1–Q_3)	
Strict gluten-free diet, % (*n*)	87 (74)	97 (173)	<0.001
GSRS total score			
At diagnosis	2.4 (1.7–2.9)	2.2 (1.6–2.8)	0.138
After 1 year	1.5 (1.3–1.8)	1.5 (1.3–2.0)	0.321
PGWB total score			
At diagnosis	98 (87–108)	105 (91–115)	0.260
After 1 year	112 (102–118)	111 (100–117)	0.699

GSRS, Gastrointestinal Symptom Rating Scale; PGWB, Psychological General Well-Being Index.
The values, except strictness of the diet, are shown as medians with upper and lower quartiles (Q1 and Q3).
[a]Defined as a small-bowel mucosal villous height crypt depth ratio >2.0 at the follow-up biopsy.

Adherence to the gluten-free diet, gastrointestinal symptoms, and quality of life

Most (87%) patients, both atrophic and recovered, maintained a strict diet and only 13% reported dietary lapses, but low adherence to the diet, nonetheless, predisposed the patients to incomplete mucosal recovery in the follow-up biopsy (**Table 4**). There was no difference between the groups either at diagnosis or after 1 year on treatment in the degree of gastrointestinal symptoms and health-related quality of life as measured by the GSRS and PGWB total scores (**Table 4**). No difference was found between the groups in any of the GSRS and PGWB subdimension scores (data not shown).

A statistically significant difference in dose response between study outcomes and the severity of mucosal atrophy in the follow-up biopsy was seen in serology, as patients with subtotal or total villous atrophy had lower TG2ab values compared with those with partial atrophy (median 0.6 vs. 2.3 U/l, P=0.017) after 1 year. There was no significant difference in dose response between the degree of mucosal damage and any other study outcomes (data not shown).

Multivariate analysis

In multivariate analysis, only malabsorption at diagnosis was found to significantly increase the risk of incomplete villous recovery after 1 year on diet (odds ratio 4.9, 95% confidence interval 2.2–10.8).

Patients with no signs of histological improvement

Detailed investigation revealed that altogether only 6 (three female, median age 45 (range 35–63) years; 2.3% of all patients) out of the

Table 5. Long-term follow-up data on 205 celiac disease patients with a histological response[a] (Recovery) or no response (Atrophy) after 1 year on a gluten-free diet

	Atrophy Group, *N*=71	Recovery Group, *N*=134	*P* value
Duration of follow-up, median (range) (years)	16 (5–21)	16 (5–20)	0.136
Mortality (% (*n*))	14.1 (10)	9.0 (12)	0.259
Any malignancy[b] (% (*n*))	13.3 (8)	6.4 (8)	0.116
Lymphoma (% (*n*))	1.4 (1)	1.6 (2)	0.968
Other chronic gastrointestinal disease[c] (% (*n*))	11.3 (8)	14.2 (19)	0.455
Osteoporosis (% (*n*))	14.0 (8)	13.0 (16)	0.850
GSRS[d] total score	1.6 (1.0–4.0)	1.9 (1.0–3.0)	0.132
PGWB[e] total score	109 (52–124)	108 (62–121)	0.416
Blood hemoglobin, median (range) (g/l)	133 (116–173)	132 (111–158)	0.724

GSRS, Gastrointestinal Symptom Rating Scale; PGWB, Psychological General Well-Being Index.
[a]Defined as a small-bowel mucosal villous height crypt depth ratio >2.0 at the follow-up biopsy.
[b]Cancers of uterus, breast, lung, pancreas, urinary bladder and prostate and lymphoma, sarcoma.
[c]Reflux disease, collagenous/lymphocytic colitis, diverticulosis, Crohn's disease, ulcerative colitis, gallstones, Barrett's esophagus, pancreatitis, and cirrhosis.
[d]*N*=44.
[e]*N*=44.

87 subjects in the Atrophy group showed no signs of improvement in villous architecture after 1 year on a gluten-free diet (mean Vh/CrD at diagnosis 0.4, after 1 year 0.2). At diagnosis, four of them presented with mild abdominal symptoms and two with signs of malabsorption; four had a family history of celiac disease. Before treatment, three out of five had positive TG2ab and four out of five positive endomysium antibodies, but the antibody values were relatively low (TG2ab mean 6.6 U, range 0–15.6 U; endomysium antibody median titer 1:50, range negative—1:200). All six reported keeping to a strict gluten-free diet and were seronegative at the time of the follow-up biopsy. Five subjects showed good clinical response, whereas one presented with relapsing fever and hyposplenism after 2 years. Further investigations revealed type I refractory celiac disease with mesenteric lymph node cavitations and widespread total villous atrophy extending to the ileum, but there were no lymphomas and markers of type II refractory celiac disease were negative.

Long-term follow-up

Long-term follow-up data on mortality, malignancies, and other complications and comorbidities were available for a total of 205 (78%) study patients (**Table 5**). There were no statistically significant differences between the Atrophy and Recovery groups in any of the long-term parameters measured (**Table 5**).

DISCUSSION

In this large cohort of celiac disease patients who had undergone a follow-up biopsy after 1 year on diet, the most prominent factor predicting incomplete small-bowel recovery was the presence at diagnosis of more severe disease in terms of histology and serology and signs of malabsorption. Further, while mucosal recovery was still ongoing, most of the clinical parameters measured had improved and were already at the same level in the Atrophy and Recovery groups, and the groups also showed no difference in long-term outcomes. Although the patients with incomplete recovery represented one-third of the subjects here, we have shown that in a highly adherent population at least 96% of patients achieve complete recovery on long-term treatment and only 0.3% develop refractory celiac disease (12,34). In accord with this, only 2.3% of all patients here still showed no signs of histological improvement after 1 year, and only one presented with complications and type I refractory disease. These findings indicate that follow-up biopsy after 1 year correlates poorly with clinical outcome and long-term prognosis, and represents only a 'snapshot' rather than the end point of ongoing mucosal healing.

Altogether, 68% of the subjects evinced morphological mucosal recovery after 1 year on diet, which is in fact a relatively high percentage compared with previous reports (5,6,9). Nevertheless, it is in line with our previous studies and very likely reflects the generally high dietary adherence in Finland (4,35). The good availability and strict labeling of gluten-free products and financial subsidization by the government are all likely to reduce inadvertent gluten intake (36). This was seen in the present study, as both the histologically recovered and non-recovered patients showed, in global perspective, excellent dietary adherence. It is thus evident that, in contrast to many previous reports (6,10), dietary lapses explained only a small minority of non-responsive cases in this study.

The most conspicuous difference here was in the presence of more severe mucosal damage in the Atrophy group at diagnosis. In accord, Rubio-Tapia *et al.* (6) discovered an association between the severity of the baseline damage and slow histological recovery. Another recent study (37) showed that patients with less severe atrophy were also more likely to respond to the diet. However, this was the first time a quantitative approach has been used to assess the actual speed (ΔVh/CrD) of villous recovery and it was observed to be lower in the Atrophy group. This slower recovery combined with severe atrophy at diagnosis explains why these patients had not regained normal mucosa during 1 year despite a strict diet. Some studies have indirectly investigated whether the speed of histological recovery is associated with the severity of the baseline damage, but results have been controversial (5,6,9,15,37,38). This might be explained by differences in dietary adherence and by the use of inexact grouped classifications (e.g., Marsh) in histology (16).

Another indicator of the presence of more severe disease in the Atrophy group at diagnosis was their higher levels of TG2ab, even if these antibodies are poor predictors of the severity of histological and clinical findings in individual patients (9,15,22). TG2ab also

remained at a higher level in the incompletely recovered patients after 1 year, with the difference, however, being small and the median values in both groups falling within normal limits. Further, most of the patients in the Atrophy group also became seronegative, indicating that the disappearance of the antibodies occurs faster than the healing of the mucosa.

Of clinical presentations, the presence of malabsorption was associated with histological non-recovery. This was most evident in the lower levels of iron as well as hemoglobin in the Atrophy group. These results, together with the lower BMD *T*-scores also observed, probably reflect insufficient absorption of nutrients in patients with severe villous atrophy. Previous studies have likewise found a correlation between the presence of anemia at diagnosis and more severe histological and clinical presentation (18,39,40). Despite the differences in mucosal healing, after 1 year all laboratory values, excluding a minor difference in hemoglobin, were comparable in both groups. This demonstrates that, as with serology, on treatment laboratory values improve faster than the mucosa. In contrast, BMD, although improved, still remained lower in the Atrophy group after 1 year on diet. Obviously, normalization of BMD takes more than 1 year to complete.

Similar to most of the other clinical parameters, we found no association between self-perceived symptoms and quality of life and mucosal recovery at follow-up. In fact, there was no significant difference between the groups even at diagnosis, this probably reflecting the high individual variation in these respects. On the contrary, there was a trend toward poorer GSRS and PGWB values at baseline in the Atrophy group, whereas after 1 year the results were practically identical. These findings further indicate that histological healing lags behind clinical recovery.

The poor correlation between histological recovery and other outcomes indicates that a follow-up biopsy taken after 1 year is not an optimal approach in monitoring celiac disease. Our long-term follow-up results support this view, as there were no differences between the groups in mortality and other complications or in gastrointestinal symptoms and quality of life. Then again, although their prevalence remains unclear, the risk of severe complications in the long run has been associated with incomplete mucosal recovery and warrants careful follow-up (4,10,41). Unfortunately, there are currently no sensitive surrogate markers for ongoing mucosal damage, and endoscopic investigations are still needed (15). Nevertheless, here only 1 out of 87 subjects evincing incomplete recovery after 1 year presented with complications, suggesting that in almost all cases it is merely a question of slow mucosal healing. Our results support the need to individualize the current procedures, taking into account both the baseline severity of the disease and the dietary response. Whether a routine follow-up biopsy is mandatory for all patients who have uncomplicated celiac disease with good clinical response is a subject for future studies.

Major strengths of this study are the large number of patients and the diversity of outcomes measured, and the use of validated questionnaires. Further, the fact that follow-up biopsies were taken systematically after 1 year and were analyzed by quantitative Vh/CrD reduces the risk of misclassification bias (16). We were also able to collect a substantial body of long-term follow-up data regarding mortality and other complications (6). The high dietary adherence in our cohort enabled us to evaluate other causes behind non-recovery than the usually dominating poor compliance; however, the results may not be directly applicable to countries where dietary lapses are common (42). A limitation is that we were not able to compare subjects consenting to follow-up biopsy and those who refused (~15% of subjects in our center). This may cause bias as non-compliant patients may be more prone to refuse, and lack of follow-up biopsy has also been associated with increased mortality (43). Also, no systematic follow-up was undertaken for those with incomplete recovery, as in our settings patients with uncomplicated disease are assigned to primary health care for further follow-up. Nevertheless, according to our clinical practice patients with persistent atrophy would have been referred to us for further investigations. Finally, as the study was initiated before routine biopsies from the duodenal bulb were recommended (3), they were not systemically taken, and thus some cases with lesion only in the bulb might have been missed (44).

In conclusion, we showed that only the presence of more severe disease in terms of histology and serology were associated with incomplete histological recovery at the follow-up biopsy. Moreover, differences in the speed of mucosal healing were not reflected in the short- or long-term clinical outcomes. Based on these findings a more personalized approach should be adopted when deciding the optimal timing of the histological follow-up. One year is often too short a time for the mucosa to recover, and postponing the biopsy, e.g., for another year (45), would presumably result in lower number of cases with ongoing atrophy.

CONFLICT OF INTEREST

Guarantor of the article: Kalle Kurppa, MD, PhD.

Specific author contributions: Henna Pekki, Kalle Kurppa and Katri Kaukinen contributed to the study design, interpretation of the results, and editing the manuscript. Heini Huhtala contributed to the data analysis and interpretation of the results. Katri Kaukinen, Kalle Kurppa, and Pekka Collin collected the clinical data and biopsy samples. Henna Pekki wrote the manuscript. Markku Mäki, Harri Sievänen, and Kaija Laurila contributed to the study design and to the final editing of the manuscript. All authors have approved the final version of the manuscript.

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Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Follow-up biopsies in celiac disease are important in the monitoring of mucosal recovery and screening for complications.
- ✓ All celiac disease patients do not reach mucosal recovery in 1 year's time despite strict gluten-free diet.
- ✓ The optimal timing of the follow-up biopsy is unclear.

WHAT IS NEW HERE

- ✓ Only the presence of more severe disease at diagnosis, in terms of histology and serology and signs of malabsorption, was associated with incomplete mucosal recovery.
- ✓ The rate of mucosal recovery does not affect patient's quality of life and clinical symptoms 1 year after diagnosis or long-term prognosis.
- ✓ Personalized approach is required to decide the optimal timing of the follow-up biopsy.

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AP&T Alimentary Pharmacology and Therapeutics

Performing routine follow-up biopsy 1 year after diagnosis does not affect long-term outcomes in coeliac disease

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SUMMARY

Background
A repeat biopsy is recommended, but often omitted in coeliac disease patients on a gluten-free diet. The effect of performing or not performing repeat biopsies is currently unknown.

Aim
To identify factors associated with and the significance of lacking biopsy for long-term outcome. Predictors and the importance of incomplete histological recovery after 1 year was investigated in re-biopsied patients.

Methods
A total of 760 patients participated in a nationwide follow-up study. Medical data were gathered via interviews and patient records, and blood samples were drawn for serology. Current symptoms and well-being were assessed by validated PGWB, SF-36 and GSRS questionnaires.

Results
Malabsorption was more common among those with a repeat biopsy (46%) than those without repeat biopsy (33%), $P < 0.001$, as were severe symptoms at diagnosis (24% vs. 16%, $P = 0.05$) and concomitant gastrointestinal (40% vs. 32%, $P = 0.049$) or musculoskeletal (35% vs. 27%, $P = 0.023$) diseases such as arthritis, osteoporosis and back pain. Repeat biopsy was more rare in subjects diagnosed in private care (11% vs. 23%, $P < 0.001$) or by screening (10% vs. 16%, $P = 0.010$). The groups were comparable as to current symptoms and dietary adherence, but those without re-biopsy were less confident of their diet (89% vs. 94%, $P = 0.002$) and more often seropositive on diet (14% vs. 9%, $P = 0.012$). They reported better SF-36 physical functioning ($P = 0.043$) and less pain and indigestion ($P = 0.013$ and $P = 0.046$ respectively) and total GSRS ($P = 0.052$) score. Incomplete mucosal recovery was predicted by more advanced histological ($P < 0.001$) and serological ($P = 0.001$) disease at diagnosis, whereas the groups did not differ in long-term adherence, symptoms, seropositivity, questionnaire scores, frequency of fractures or malignancies.

Conclusions
Severe disease at diagnosis predicted the record of a repeat biopsy and incomplete mucosal recovery. Neither lacking biopsy nor incomplete recovery in a relative short time span of 1 year was associated with poorer long-term outcome, although survival bias cannot be excluded.

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INTRODUCTION

A careful follow-up of coeliac disease activity after the initial diagnosis is considered important due to the possible complications associated with incomplete healing of the small-bowel mucosa.[1, 2] Owing to the lack of sensitive surrogate markers for histological recovery, most current guidelines recommend a repeat biopsy to be considered on a gluten-free diet,[1–6] this often being executed approximately 1 year after the diagnosis, even if there is lack of evidence on the effect of such practice.[3, 7–9] On the other hand, due to its unpleasantness and resource-consuming nature, the control endoscopy is often omitted in clinical practice, the effect of this on long-term outcomes being currently unknown.[7] Altogether, due to the scarcity of evidence, this topic has been under active discussion in the expert guidelines.[1]

Even if a repeat biopsy is conducted, the significance of possible incomplete villous recovery on dietary treatment remains scantly studied. It has been linked, for example, to rare cases of refractory coeliac disease and lymphoproliferative malignancies, but the relevant results appear to be markedly dependent in a 15-year follow-up on the study population and the timing of the endoscopy.[1, 3, 8, 9, 11, 12] We have in fact recently shown that incomplete histological recovery after 1 year on a gluten-free diet is not associated with reduced short-term well-being or increased risk of cancer and mortality.[8] Furthermore, although after 1 year's diet up to 50% of coeliac patients may show signs of villous damage, in the long run this is seen in less than 10% of cases and only 0.3% have true refractory coeliac disease.[8, 10, 12]

The rapidly growing number of coeliac disease patients renders optimal targeting and timing of the endoscopic follow-up a major public health issue.[8–10, 12] To further elucidate these aspects, we investigated factors associated with the omission of routine a invasive follow-up and the value of a repeat biopsy 1 year after diagnosis with respect to long-term outcome. Simultaneously, we were able to further explore the significance of incomplete histological recovery found in the follow-up biopsy in a nationwide coeliac disease cohort.

MATERIALS AND METHODS

Patients and study design

The nationwide cross-sectional study was conducted in Tampere University Hospital and the University of Tampere. The participants were recruited via newspaper advertisements and with the help of local and national coeliac disease societies. Inclusion criteria were age ≥18 years and a biopsy-proven coeliac disease diagnosis at least 2 years before the present study. All voluntary participants completed validated questionnaires for current symptoms and health-related quality of life and were interviewed systematically by an experienced physician or study nurse. In addition, blood samples were drawn for serology and medical records reviewed to confirm the diagnosis and to complement clinical data and laboratory parameters. Subjects with unclear coeliac disease diagnosis or substantially lacking medical information were excluded. The possible use of olmesartan therapy was also checked for and considered an exclusion criterion, as it may cause severe enteropathy resembling coeliac disease.[13]

After collection of study data, the results were compared between subgroups of participants who had either undergone (Repeat biopsy) or not (No repeat biopsy) a routine follow-up biopsy approximately 1 year after the coeliac disease diagnosis. For similar comparison, subjects who had been re-biopsied while on a gluten-free diet were categorised into those with complete and those with incomplete histological recovery after 1 year on the diet.

The study enrolment and collection of personal information, blood samples and medical data were conducted with the permission and according to the guidelines of the Ethical Committee of the Pirkanmaa Hospital District. All participants gave written informed consent.

Clinical characteristics

The following clinical and demographic data was collected from all participants: gender, age at present and at diagnosis, clinical presentation at diagnosis, the type (gastrointestinal symptoms, extra-intestinal symptoms, screen-detected), duration and severity of symptoms before diagnosis and also their current persistence, family history of coeliac disease, possible symptoms in childhood, presence of coeliac disease-associated and other significant chronic comorbidities, and site (primary, secondary or tertiary care, private care) of coeliac disease diagnosis. Severity of symptoms was further categorised as mild, moderate and severe as previously described in detail[14].

Small-bowel mucosal biopsies

Data on the biopsies were collected from patient records. Our national guidelines recommend at least four small-bowel mucosal biopsies to be taken routinely from each patient upon coeliac disease suspicion and during the repeat endoscopy. The histological specimens are

forwarded to the hospitals' pathology department, where the severity of mucosal damage is evaluated in representative biopsy cuttings. In the present study, the severity of mucosal lesion was at diagnosis graded into normal, partial, subtotal or total villous atrophy based on the original pathology report. Mucosal recovery on gluten-free diet was defined morphologically based on normalised villous height crypt depth ratio.

Serology and haemoglobin

The values of serum endomysial antibodies at time of diagnosis, if available, were gathered from patient files. Further serum endomysial antibodies and transglutaminase 2 antibodies were measured in all subjects at the time of the study while on a strict gluten-free diet. Serum IgA-class serum transglutaminase-2 antibodies were tested by commercial enzyme-linked immunosorbent assay (QUANTA Lite h-tTG IgA, INOVA Diagnostics, San Diego, CA). Values >30.0 U/were rated positive according to the manufacturer's instructions. Serum endomysial antibodies were assessed by indirect immunofluorescence on human umbilical cord [15]. Serum endomysial antibodies titres 1:≥5 were considered positive and diluted until negative to 1:50, 1:100, 1:200, 1:500, 1:1000, 1:2000 and 1:4000. The values were further sub-categorised into low (titres 1:5-1:200) and high positive (1:500-1:4000). In cases of selective IgA deficiency the corresponding IgG-class antibodies were measured. Blood haemoglobin values and the possible presence of anaemia at coeliac disease diagnosis were gathered from the medical files.

Adherence to the gluten-free diet

Provision of professional dietary advice at coeliac disease diagnosis was verified by patient interview and from the patient records. Current self-reported long-term adherence to the gluten-free diet was inquired and classified as "strict" (minor inadvertent lapses less than a few times a year), "occasional lapses" (lapses less frequently than once per month) and "normal diet" (more frequent lapses).[16, 17] Alongside adherence, also the patient's overall competency to manage the diet and the possible use of purified oats and wheat starch products were asked.[18, 19] Long-term dietary adherence was further estimated on the basis of coeliac antibody positivity at the time of the present study.

Questionnaires

All questionnaires were filled in at the time of this study on a long-term gluten-free diet. Short Form 36 Health Survey (SF-36), and Psychological General Well-Being questionnaires (PGWB) and Gastrointestinal Symptoms Rating Scale (GSRS) were used to assess patients' self-perceived quality of life and gastrointestinal symptoms over time up to this study. SF-36 comprises of 36 separate questions which can be divided into eight domains as follows; physical functioning, role limitations due to physical problems, bodily pain, general health, vitality, social functioning, role limitations due to emotional problems and mental health.[10, 16, 20–23] Items are re-scored from 0 to 100, higher scores indicating better health and quality of life.

PGWB is a well-validated and widely used questionnaire both in general and in coeliac disease.[15, 16, 22] The 22 separate items can be further divided into six sub-dimensions measuring anxiety, depression, well-being, self-control, general health and vitality. All items use a 6-grade Likert scale with higher scores representing better well-being and quality of life.

Self-perceived severity of gastrointestinal symptoms was evaluated by the GSRS questionnaire.[15, 24] This comprises of 15 separate items which can be added together as a total score and divided into five sub-dimensions measuring abdominal pain, gastro-oesophageal reflux, indigestion, diarrhoea and constipation. The scoring is based on a 7-grade Likert scale, higher scores reflecting more severe gastrointestinal symptom.

Statistical analysis

Continuous variables and questionnaire findings are presented as medians with quartiles or ranges. Binominal and categorical variables are presented as number of subjects and percentages. Continuous variables were studied using Mann–Whitney test and binominal and classified variables using Chi-square test. A <0.05 was considered significant in all analyses. Statistical analyses were made using the Statistical Package for the Social Sciences version 20 (Released 2011, IBM SPSS Statistics for Windows, Version 20.0; IBM Corp., Armonk, NY, USA).

RESULTS

Altogether 791 participants (median age at diagnosis 44 years, females 78%) had confirmed coeliac disease and were enrolled in further analyses. However, 27 of them were excluded because it was unclear whether they had undergone a repeat biopsy and four due to possible use of olmesartan. Of the final cohort of 760 participants 516 (68%) had (Repeat biopsy group) and 244 (32%) had not (No repeat biopsy group) undergone a follow-up endoscopy after a median of 1 year.

Factors predicting record of a repeat biopsy

Patients with severe clinical or histological presentation or signs of malabsorption at diagnosis were more likely to undergo a repeat biopsy, whereas it was less common among those diagnosed in private care or by screening (Table 1). Record of a histological follow-up was not associated with the duration or type of symptoms and serum endomysial antibodies titres at diagnosis, gender, presence of symptoms during childhood and family history of coeliac disease (Table 1). Median age at diagnosis was also comparable in subjects with and without repeat biopsy (53 vs. 55 years, $P = 0.885$).

Long-term follow-up data on patients with and without a repeat biopsy

The median time on a gluten-free diet prior to this study was 8 years. Coeliac disease patients who had undergone a repeat biopsy were more often found to suffer from concomitant Sjögren's syndrome, musculoskeletal disease or gastrointestinal disease (Table 2). There were no significant differences between the groups in the prevalence of other chronic conditions (Table 2).

Both study groups had received comparable dietary advice at diagnosis and showed equal adherence at time of inquiry (Table 3), but those with no repeat biopsy after 1 year were less confident as to their current capability to manage a strict diet. Furthermore, the nonbiopsied subjects had significantly, even if only modestly, higher serum endomysial antibodies titres on a long-term gluten-free diet. There was, however, no difference between the groups in the presence or severity of current self-estimated symptoms (Table 3), and, based on the GSRS questionnaire, patients without repeat biopsy had even fewer overall gastrointestinal symptoms and less indigestion (Table 4). There were also no differences between the groups in respect of most current health and quality of life measurements except that subjects without a repeat biopsy reported better SF-36 physical functioning and bodily pain scores (Table 4).

Predictors of incomplete villous recovery in re-biopsied subjects

The result of the re-biopsy was available in 476 (92%) out of the 516 patients undergoing the procedure 1 year after diagnosis. Altogether 276 (58%) had reached morphological small-bowel mucosal recovery, while in 200 (42%) it remained incomplete. Factors predicting incomplete recovery were malabsorption (55% vs. 41%, $P = 0.003$), high serum endomysial antibodies titre (46% vs. 25%, $P < 0.001$) and severe mucosal damage (total atrophy 32% vs. 19%, $P < 0.001$) at diagnosis. The recovery and nonrecovery groups did not differ in gender, age at diagnosis, family history of coeliac disease, site of diagnosis, severity and duration of symptoms before diagnosis or presence of symptoms in childhood (data not shown). There was also no difference between the groups in haemoglobin at diagnosis, when analysed with both genders together, but in separate analysis the median value was lower in women evincing no recovery (12.3 g/dL vs. 12.7 g/dL, $P = 0.030$).

Long-term outcomes in re-biopsied patients with and without histological recovery

Coeliac disease patients with incomplete mucosal recovery 1 year after diagnosis had more concomitant respiratory (15% vs. 22%, $P = 0.031$) and dermatological diseases (17% vs. 10%, $P = 0.043$) at current evaluation, while there was no differences in the frequency of coeliac disease-associated and other chronic diseases. Furthermore, the recovery and nonrecovery patients showed similar severity of current gastrointestinal symptoms and quality of life as measured by the questionnaires (data not shown). The groups had also received equally much dietary advice at diagnosis and did not differ in current adherence or capability to manage the diet, in record of regular follow-up, or in use of purified oats and prevalence of serum transglutaminase-2 antibody positivity (Table 5).

DISCUSSION

The main finding in the present study was that patients with or without endoscopic follow-up did not differ in severity of symptoms or in well-being after a median follow-up time of almost a decade. In addition, the repeat biopsy and no biopsy groups showed excellent and comparable self-reported dietary adherence. Finally, even if a repeat biopsy had been conducted, incomplete mucosal recovery 1 year after diagnosis did not affect the long-term clinical outcomes.

A repeat biopsy after 1 year was undertaken especially for patients with severe presentation at diagnosis, while it was more often omitted in screen-detected cases. It would seem logical that, as also seen in other chronic diseases,[25] physicians are keener to follow sicker patients with an increased risk of long-term complications. Alternatively, those with milder or screen-detected disease are likely to be less willing to undergo an unpleasant repeat biopsy and, in turn, physicians neglect it in view of to the anticipated better prognosis. This may also explain the similar tendency to omit the repeat biopsy in type 1 diabetes

patients, whose coeliac disease is often found by screening and who might have an increased risk of endoscopic complications.[26] Repeat biopsies were also more often taken from patients with concomitant gastrointestinal and musculoskeletal illnesses, strengthening the conception that those with comorbidities and ongoing symptoms undergo the procedure with a lower threshold.

We also found patients diagnosed in the private sector to have the repeat biopsy omitted more often than those diagnosed in public healthcare. This might be because in private care patients have to pay for the second endoscopy themselves. In addition, in Finland healthcare has evolved around a strong public sector which, as also seen in the present study, treats most coeliac disease patients. In contrast, the private sector is more focused on frontline screening and refers the putative patients to public healthcare for further diagnosis and follow-up.[28] The results would very likely differ in countries where a system of private health insurances predominates, as in the USA.[27, 28] What is more, we found no differences in the rate of re-biopsy between public healthcare levels. This somewhat unexpected finding might be explained by the organised decentralisation of coeliac disease diagnostics and the use of uniform nationwide guidelines at all healthcare levels in Finland.[26, 29] In fact, nowadays up to 85% of Finnish coeliac disease patients are diagnosed and followed in primary and secondary care.[29, 30]

Table 1 | Clinical, serological and histological characteristics at diagnosis in 760 coeliac disease patients without (No repeat biopsy) or with (Repeat biopsy) a follow-up biopsy done

	No repeat biopsy *n* = 244		Repeat biopsy *n* = 516		
	n	%	*n*	%	*P*-value
Females	181	74	413	80	0.093
Clinical presentation*					
Malabsorption	80	33	235	46	**0.001**
Gastrointestinal symptoms	204	84	425	82	0.664
Extra-intestinal symptoms†	97	40	201	39	0.864
Screen-detected	39	16	49	10	**0.010**
Duration of symptoms before diagnosis					0.571
>5 year	96	42	209	43	
<5 year	119	50	245	50	
Severity of symptoms at diagnosis					**0.052**
Severe	40	16	122	24	
Moderate	100	41	216	42	
Mild	91	37	158	31	
No symptoms	13	5	18	4	
Symptoms during childhood	72	28	181	35	0.223
Coeliac disease in family	146	63	329	64	0.790
Endomysial antibody titres at diagnosis					0.461
High 1: >200	36	39	77	35	
Low 1:5–1:200	47	51	107	49	
Negative	10	11	35	16	
Severity of villous atrophy at diagnosis					**<0.001**
Total	42	24	124	26	
Subtotal	58	34	196	42	
Partial	73	42	149	32	
Site of diagnosis					**<0.001**
Primary care	41	17	79	15	
Private care	57	23	58	11	
Secondary care	108	44	250	49	
Tertiary care	38	17	125	24	

* Patient can present with more than one symptom.

† Arthritis, dental enamel defects, dementia, dermatitis herpetiformis, glossitis, aphtous stomatitis, gynecological problems, myopathy, neurologic symptoms, osteoporosis, Sjögren's disease, chronic eczema, IgA nephropathy.

Bold values are statistically significant.

Table 2 | Presence of coeliac disease-associated or other co-morbidities during long-term follow-up in 760 coeliac disease patients without (No repeat biopsy) or with (Repeat biopsy) a follow-up biopsy done

	No repeat biopsy *n* = 244		Repeat biopsy *n* = 516		
	n	%	*n*	%	*P*-value
Associated diseases					
Sjögren's syndrome	1	0	14	3	**0.033**
Type 1 diabetes	6	3	9	2	0.090
Thyroidal disease	39	16	83	16	0.973
Other conditions					
Musculoskeletal disease*	66	27	182	36	**0.023**
Gastroenterological disease†	78	32	203	40	**0.049**
Gynecological disease	39	16	103	20	0.198
Neurological disease	29	12	67	13	0.671
Psychiatric disease	11	5	25	5	0.834
Any malignancy	9	4	24	5	0.547
Any fracture	68	28	141	28	0.857
No comorbidities	38	16	58	11	0.079

* For example, arthritis, osteoporosis, back pain.

† For example, reflux, lactose intolerance, gastritis.

Bold values are statistically significant.

Table 3 | Long-term follow-up data on 760 coeliac disease patients without (No repeat biopsy) or with (Repeat biopsy) a control biopsy done while on a gluten-free diet (GFD)

	No repeat biopsy *n* = 244		Repeat biopsy *n* = 516		
	n	%	*n*	%	*P*-value
Received dietary advice	231	95	492	95	0.683
Capable of managing GFD	216	89	480	94	**0.002**
Strictness of GFD					0.374
Strict diet	231	96	499	98	
Occasional lapses	9	4	11	2	
Normal gluten intake	0	0	0.0	0	
Endomysial antibody titres on GFD					**0.012**
High 1: >200	12	5	7	1	
Low 1:5–1:200	20	9	38	7	
Negative 1: <5	189	86	486	92	
TG2-ab positivity on GFD	39	16	57	11	0.139
Current symptoms					0.268
None	155	78	199	72	
Slight	37	19	70	25	
Serious	5	3	7	4	

GFD, gluten-free diet; Tg2-ab, transglutaminase 2 antibodies.

Bold values are statistically significant.

Interestingly, based on the GSRS, coeliac disease patients with a repeat biopsy after 1 year also remained more symptomatic during the current long-term follow-up. One reason for this may be the aforementioned higher prevalence of gastrointestinal comorbidities in this group. In addition, we have shown that patients with severe presentation at diagnosis are also more likely to remain symptomatic on a long-term gluten-free diet.[32] Likewise, here those with repeat biopsy had currently more bodily pain and decreased physical functioning,

Table 4 | Gastrointestinal symptoms and quality of life during long-term follow-up in 760 coeliac disease patients with (Repeat biopsy) or without (No repeat biopsy) a follow-up biopsy done

	No repeat biopsy $n = 244$		Repeat biopsy $n = 516$		
	Median	Quartiles	Median	Quartiles	*P*-value
GSRS sub-scores					
Total	1.8	1.5–2.5	1.9	1.5–2.6	**0.052**
Indigestion	2.3	1.8–3.2	2.5	1.8–3.0	**0.046**
Diarrhoea	1.3	1.0–2.3	1.7	1.0–2.0	0.128
Abdominal pain	1.7	1.3–2.7	2.0	1.3–2.3	0.150
Constipation	1.7	1.0–2.7	1.8	1.0–2.7	0.323
Reflux	1.5	1.0–2.0	1.5	1.0–2.0	0.468
SF-36 sub-scores					
Bodily pain	78	53–90	68	48–90	**0.013**
Physical functioning	95	80–100	90	75–100	**0.043**
Role limitations, emotional	100	67–90	100	67–88	0.127
General health perception	65	45–80	60	45–75	0.196
Role limitations, physical	100	25–100	75	50–100	0.223
Vitality	75	53–85	70	55–80	0.583
Social functioning	90	75–100	88	75–100	0.634
Mental health	84	72–100	80	72–100	0.978
PGWB sub-scores					
Total	107	95–117	106	94–115	0.515
General health	14	10–15	13	11–16	0.147
Well-being	17	15–20	18	15–19	0.745
Vitality	18	16–20	18	15–21	0.746
Anxiety	25	21–27	25	21–27	0.810
Depression	17	15–18	17	15–18	0.941
Self-control	16	14–17	16	13–17	0.958

GSRS, gastrointestinal symptom rating scale, lower scores indicate fewer gastrointestinal symptoms; PGWB, psychological general well-being, higher scores indicate better well-being; SF-36, short form 36, higher scores indicate better social functioning.

Bold values are statistically significant.

again probably since they have more musculoskeletal comorbidities. In contrast, the groups did not differ in any of the PGWB sub-dimension scores, indicating that the minor differences seen in the prevalence of symptoms and comorbidities have no major effect on self-perceived well-being and quality of life.

Another important finding was that the omission of a repeat biopsy 1 year after the diagnosis did not affect long-term dietary adherence. This suggests that the invasive follow-up does not play a major role in commitment to the gluten-free diet, at least in Finland, where adherence is generally very good and the additional costs of the diet remain reasonable.[2, 31, 33] Nevertheless, patients without a repeat biopsy considered their capability to manage their diet lower and were somewhat more often seropositive, indicating that a subgroup of patients might benefit from the endoscopic follow-up.[32] Then again, it might be worthwhile investigating whether the re-biopsy

Table 5 | Long-term follow-up characteristics in 476 coeliac disease patients with (Recovery) or without (Atrophy) histological response at follow-up biopsy

	Atrophy $n = 200$		Recovery $n = 276$		
	n	%	*n*	%	*P*-value
Received dietary advice	154	77	215	78	0.683
Capable of managing GFD	184	92	264	95	0.820
Strictness of GFD					0.060
Strict	195	97	275	100	
Occasional lapses	5	3	1	0	
Normal gluten use					
Use of oats	162	81	231	82	0.947
Regular follow-up	131	66	198	71	0.426
TG2-ab positivity	45	23	79	28	0.497
Any malignancy	9	5	14	5	0.762
Fractures	53	27	80	28	0.859

GFD, gluten-free diet; TG2-ab, transglutaminase-2 antibodies.

is in fact the main issue here or whether other means of follow-up combined with enhanced dietary counselling would be equally effective.[34–37]

We also sought to identify associated factors and the long-term significance of incomplete histological recovery 1 year after coeliac disease diagnosis in a nationwide cohort. As with the repeat biopsy, incomplete recovery was predicted by more severe disease presentation at diagnosis. Similar associations between advanced disease and incomplete recovery on treatment have previously been observed in smaller studies.[3, 8, 38, 39] Furthermore, we recently showed that more ill patients need a longer time to reach complete mucosal recovery.[13] Hence, there still was villous atrophy in as many as 42% of the patients here after 1 year on gluten-free diet. Notwithstanding the differences in the speed of mucosal recovery, long-term damage is present in only 4–6% of patients, a fraction of what is seen after 1 year.[11, 31, 40, 41] Moreover, no more than 0.3% have true refractory coeliac disease.[29] It must be emphasised that incomplete recovery is not explained by dietary lapses since, in line with previous findings by the present group[9] and Haere and associates,[35] also the majority of those without mucosal healing showed excellent adherence to the gluten-free diet.

Notably, the recovery and nonrecovery groups did not differ in the prevalence of malignancies. This was in line with our previous study carried out on a different patient cohort in a tertiary centre setting[8] and provides an interesting contribution to discussion of the risk of malignancies and the necessity of a repeat biopsy. In contrast to our results, Lebwohl and colleagues[42] found an increased risk of lymphoproliferative malignancies in patients with persistent villous atrophy, even if this could only be seen in those diagnosed before the year 2000. This controversy might be explained by differences in study design and duration of follow-up and improvements in the diagnostics and management of coeliac disease during the past decades. Since what is involved is a rare complication it is also possible that statistical significance was not reached by chance only.

The only significant long-term difference between subjects with or without histological recovery was the higher prevalence of dermatological and respiratory comorbidity in the latter group. In particular, there was no difference in gastrointestinal symptoms or general well-being even in the long run, which is in accord with our previous short-term findings[8] in a tertiary centre. This strengthens the conception that slower mucosal recovery does not directly affect the improvement of symptoms and quality of life in patients on a strict gluten-free diet. The reason for poorer recovery among those with concomitant skin and respiratory diseases is unclear and remains a subject for further studies.

Our major strength here was the large number of representative patients diagnosed at different levels of health care. We also succeeded in collecting a wide variety of relevant study parameters, and the use of validated questionnaires increases the reliability and reproducibility of results. A limitation is that evaluation of the repeat biopsies was not centralised.[43] Furthermore, the recruitment of most of the participants through coeliac societies may increase the risk of selection bias. The analysis of in particular serious outcomes such as malignancy according to follow-up histology has also the potential for survival bias, as sampling was not done at the time of the repeat biopsy. Finally, we were not able to compare mortality between the groups, since we did not take a certain predefined sample but instead enrolled existing patients. Lack of histological follow-up and incomplete mucosal recovery might be associated with increased mortality,[9, 36] and more studies are needed to further elucidate this issue.

In conclusion, coeliac disease patients with severe initial presentation were more prone to undergo a repeat biopsy after 1 year on diet and were also found to lack full mucosal recovery if re-biopsied. However, neither the lack of the repeat biopsy nor histological recovery was reflected in long-term clinical outcomes and dietary adherence. On the basis of these results, performing a routine endoscopy 1 year after diagnosis is not necessarily an optimal approach. Instead, we propose a more personalised follow-up, wherein the repeat biopsy is conducted later, after 2–5 years and only for a selected group based on age, initial disease severity and response to the gluten-free diet.

AUTHORSHIP

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LINKED CONTENT

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Alimentary Tract

Long-term follow-up in adults with coeliac disease: Predictors and effect on health outcomes

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ABSTRACT

Introduction: Guidelines recommend regular follow-up in coeliac disease, but effect of this on long-term outcomes remains unclear.
Aims: To evaluate predictors and significance of long-term follow-up.
Methods: 677 previously diagnosed coeliac patients were recruited for a nationwide health survey. Medical data were gathered through interviews and patient records. Current symptoms and quality of life were assessed by validated questionnaires and blood samples were drawn for serology. All variables were compared between patients with and without long-term (>2 years) follow-up.
Results: 15% had long-term follow-up, median duration 10 years. Predictors ($p < 0.05$) for the follow-up were immunological (35% vs. 24%) and circulatory (20% vs. 12%) comorbidities, whereas it was less common in subjects with musculoskeletal (23% vs. 34%) comorbidity and those not belonging to any at-risk group (16% vs. 27%). Patients with or without follow-up had comparable age, adherence and ability to manage a gluten-free diet and frequency of seropositivity. Also questionnaire scores paralleled, but those without follow-up reported more overall symptoms (16% vs. 26%). Most patients wished for follow-up.
Conclusion: Only a minority of patients had regular follow-up. However, patients with and without the follow-up were comparable in most long-term outcomes, indicating that it might not be always necessary. The results call for more personalized follow-up policies in coeliac disease.

1. Introduction

Coeliac disease is a lifelong gluten-induced autoimmune enteropathy with a prevalence up to 2% in Caucasian populations [1]. The only treatment is a strict gluten-free diet, the initiation of which usually elicits a clear clinical and histological response and disappearance of the disease-specific autoantibodies [2]. Unfortunately, the high cost and restrictive nature of the gluten-free diet predisposes to poor adherence and subsequently to persistent enteropathy and an increased risk of serious complications such as osteoporotic fractures and malignancy [3–6]. Moreover, there is a subgroup of patients with a condition called refractory coeliac disease, who do not attain adequate clinical and histological recovery despite a strict diet and thus have particularly poor prognosis [7].

In order to ensure proper adherence and response to the gluten-free diet and to detect possible complications, most current guidelines recommend regular long-term follow-up in coeliac disease even as often as annually [2,8]. However, there is a paucity of evidence as to how the follow-up is actually implemented in clinical practice, and whether the absence of follow-up really affects the long-term coping and health of the patients [9–15]. Interestingly, in a 15-year follow-up we recently found that the lack of a repeat biopsy one year after coeliac disease diagnosis is not associated with an increased risk of adverse outcomes such as reduced well-being or malignancies [16]. This would indicate that the association between the presence of follow-up and the prognosis of coeliac disease is more complex than one might expect.

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To further elucidate the significance of regular follow-up to the treatment success in coeliac disease, we conducted a nationwide survey and compared various patient-related and other relevant factors between large cohorts of coeliac disease patients with or without long-term follow-up after diagnosis.

2. Materials and methods

2.1. Patients and study design

A nationwide cross-sectional health survey was carried out in Tampere University Hospital and the University of Tampere. The survey was created to investigate variable aspects of the treatment success in coeliac disease, not just factors associated with the long-term follow-up. The participants were recruited through newspaper advertisements and with the aid of coeliac disease societies. Inclusion criteria were age ≥18 years and a biopsy-proven diagnosis at least two years before enrolment. All participants filled validated questionnaires on current symptoms and quality of life and were interviewed systematically by a physician or a study nurse with expertise in coeliac disease. Patients unable to attain the study center were interviewed by phone and questionnaires were sent by mail. Patient records were reviewed in order to confirm the diagnosis and to complement medical data. In addition, blood samples were drawn for serological measurements, in case of phone interviews this was done at the nearest laboratory. Subjects with dermatitis herpetiformis were excluded owing to their different diagnostic and follow-up protocol, as well as patients with an unclear diagnosis or substantially incomplete medical information. Altogether 677 participants, representing approximately 2% of the whole Finnish coeliac population [4], were enrolled for further analyses.

After data collection the results were analyzed between subgroups of participants who either had or had not been systematically assessed for at least two years after the diagnosis by health care for dietary compliance and treatment success. This sorting was based on the patients' own reporting and medical record data. Also possible clinical and/or serological coeliac disease controls carried out during health care visits for other conditions were included. Subgroup analyses were carried out in patients with only a short-term follow-up (<2 years) and those without any follow-up. The findings were compared with Finnish national recommendations. In Finland, the follow-up of coeliac disease is decentralized to primary care, with the general practitioners in charge. If needed, the patient can be referred to a specialist in a reasonable time frame. To unify diagnostics and treatment, there are national Current Care Guidelines, which recommend a repeat biopsy one year after diagnosis and regular clinical and serological follow-up at 2–3-year intervals.

Patient enrolment and collection of study data were conducted with the permission and according to the guidelines of the Ethical Committee of the Pirkanmaa Hospital District. All participants gave written informed consent.

2.2. Clinical characteristics

The clinical information collected included demographic data, the type and the severity of clinical presentation before diagnosis, duration of symptoms and their current self-experienced persistence. Family history of coeliac disease, presence of coeliac disease-associated and other significant chronic comorbidities were also inquired. The latter included particularly immunological (e.g. asthma, allergies), circulatory (e.g. hypertension, coronary artery disease) and musculoskeletal (e.g. arthritis, fibromyalgia) conditions. Current or previous smoking and whether the coeliac disease diagnosis was made in primary, secondary or tertiary public care or in private care were also inquired. The self-perceived severity of clinical presentation was asked to estimate the burden of symptoms. These were further classified into (1) no symptoms, (2) mild symptoms such as occasionally disturbing gastrointestinal or extra-intestinal symptoms or a combination of them and (3) severe symptoms seriously disturbing daily life, such as recurrent awakenings because of pain or symptoms requiring acute inpatient care [17].

2.3. Small-bowel mucosal biopsies

Data on small-bowel mucosal biopsies were collected from the patient records. Our national diagnostic guidelines for coeliac disease recommend at least four duodenal biopsies to be taken routinely from each patient upon coeliac disease suspicion and during the possible repeat endoscopy after one year on a gluten-free diet. The histological specimens are forwarded to the hospitals' pathology department, where the severity of mucosal damage is evaluated in representative and correctly orientated biopsy cuttings. Demonstration of duodenal villous atrophy is required for celiac disease diagnosis. The degree of mucosal lesion has for decades been graded as partial, subtotal or total villous atrophy, these corresponding approximately grades 3a, 3b and 3c in Marsh-Oberhuber classification.

2.4. Celiac disease serology

Values for serum endomysial antibodies (EmA) at diagnosis were gathered from patient records. Furthermore, EmA and transglutaminase 2 antibodies (TG2ab) were measured in all patients at the time of the current study. Indirect immunofluorescence was used for EmA measurements as previously described [18]. Titers 1: ≥5 were considered positive and diluted until negative to 1:50, 1:100, 1:200, 1:500, 1:1000, 1:2000 and 1:4000. TG2ab were tested by commercial ELISA (QUANTA Lite h-tTG IgA, INOVA Diagnostics, San Diego, CA), considering a cut-off >30.0 U/l positive. Corresponding IgG-class antibodies were measured in cases of selective IgA deficiency.

2.5. Adherence to the gluten-free diet

Provision of dietary advice at coeliac disease diagnosis was verified by patient interview and from patient records. Current long-term dietary adherence was also inquired by an experienced study nurse/physician with an expertise in coeliac disease and classified as "strict" (minor inadvertent lapses less than a few times a year), "occasional lapses" (lapses less often than once per month) and "normal diet". Long-term adherence was further estimated on the basis of coeliac antibody positivity at the time of the present study. Alongside adherence, patient's overall competency in managing the diet and the possible use of purified oats was asked.

2.6. Questionnaires

Short Form 36 Health Survey (SF-36) and Psychological General Well-Being questionnaires (PGWB) were used to assess patients' current self-perceived quality of life and gastrointestinal symptoms at time of study.

SF-36 comprises 36 separate questions, divided into eight subdimensions as follows; physical functioning, role limitations due to physical problems, bodily pain, general health, vitality, social functioning, role limitations due to emotional problems and mental health [19–23]. Items are re-scored from 0 to 100, higher scores indicating better health and quality of life.

Table 1
Clinical, serological and histological characteristics at diagnosis in 648 celiac disease patients with or without long-term follow-up.

	Long-term follow-up (N = 99)		No long-term follow-up (N = 549)		P value
	N	%	N	%	
Women	80	80	437	80	0.783
Site of diagnosis					0.662
Primary healthcare	18	18	89	16	
Secondary/tertiary healthcare	64	65	379	70	
Private healthcare	17	17	78	14	
Symptoms in childhood	40	41	179	33	0.292
Duration of symptoms before diagnosis					0.915
>5 year	31	43	221	43	
1–5 year	29	30	165	32	
<1 year	17	18	94	18	
No symptoms	9	9	38	7	
Symptom at diagnosis[1]					
Gastrointestinal	85	82	457	84	0.590
Malabsorption	48	49	239	44	0.385
Extraintestinal symptoms	40	40	193	34	0.190
Screening	12	12	67	12	0.962
Risk group for celiac disease					
Family history	74	76	362	67	0.064
Associated condition[3]	24	24	101	19	0.186
No risk group	16	16	146	27	**0.025**
EmA positivity at diagnosis	23[2]	88	172[4]	91	0.812
tTG-ab positivity at diagnosis	18[3]	90	136[5]	90	0.852
Severity of villous atrophy at diagnosis					0.959
Total atrophy	21	27	121	26	
Subtotal atrophy	29	38	179	39	
Partial atrophy	27	35	159	35	
Smoking					0.836
Yes	7	7	48	9	
Quit	20	20	112	21	
No	71	72	379	70	

[1] Patient can present with more than one symptom.
Data were available from more than 90% of the cases in each category except: [2] 26, [3] 20, [4] 190 and [5] 152.
[3] Type 1 diabetes mellitus, Sjögren's disease, IgA deficiency, autoimmune thyroidal disease.
EmA, endomysium antibodies; tTg-ab, transglutaminase 2 antibodies.

PGWB is a well-validated and widely used questionnaire both in coeliac disease and in general [18,20,22,23]. The 22 separate items can be further divided into six sub-dimensions measuring anxiety, depression, well-being, self-control, general health and vitality. All items use a 6-grade Likert scale with higher scores representing better well-being and quality of life.

Gastrointestinal symptoms were evaluated by the Gastrointestinal Symptom Rating Scale (GSRS) questionnaire [18,24]. It includes 15 separate items which can be added together as a total score and further divided into 5 sub-dimensions measuring abdominal pain, gastro-esophageal reflux, indigestion, diarrhea and constipation. The scoring is based on a 7-grade Likert scale, higher scores reflecting more severe gastrointestinal symptom.

2.7. Statistical analysis

Continuous variables are presented as medians with quartiles or ranges. Categorical variables are presented as number of subjects and percentages. Statistical significance in differences between patients with and without long-term follow-up was studied using Mann–Whitney test. Binominal and classified variables were analyzed using Chi-square test. A p-value <0.05 was considered significant in all analyses. All analyses were carried out using SPSS 20.0 (IBM Corp., Armonk, NY).

3. Results

The median age of the whole study cohort was 44 (range 22–89) years and 80% were women. The median follow-up time for the whole study group was 10 (range 2–38) years. Out of the 677 participants, 99 (15%) had and 578 (85%) had not received long-term follow-up for coeliac disease.

3.1. Factors predicting long-term follow-up

Existence of follow-up was predicted by the presence of immunological (35% vs. 24%, p = 0.020) and circulatory (20% vs. 12%, p = 0.010) comorbidities, whereas it was less common in subjects with coexisting musculoskeletal disease (23% vs. 34%, p = 0.045). Those not belonging to any at-risk group (coexisting type I diabetes, family history for coeliac disease) were more likely to be without follow-up (Table 1). In contrast, follow-up was not predicted by other co-morbidities (data not shown), gender, site of diagnosis, seropositivity for celiac autoantibodies or severity of histological lesion at diagnosis, symptoms in childhood, clinical presentation or duration of symptoms before diagnosis, family risk or associated condition and smoking (Table 1). Also age at diagnosis (median 42 vs. 44 years, range 18–75 vs. 18–71 years, p = 0.117) or at present (median 54 vs. 54 years, range 22–81 vs. 21–89 years, p = 0.919) did not significantly differ between patients with and without follow-up.

When looking into the short-term characteristic one year after diagnosis in patients whom the data were available, there was no significant difference between the presence of long-term follow-up and short-term coeliac autoantibody positivity (regular follow-up 6.3% vs. no follow-up 15.1%, respectively, p = 0.343; n = 95). Neither did the groups differ in the prevalence of histological recovery (fully normalized 53.0% vs. 60.2%, p = 0.378; n = 398) after one year on a gluten-free diet.

Table 2
Follow-up features in 648 celiac disease patients with or without long term follow-up.

	Long-term follow-up (N = 99)		No long-term follow-up (N = 549)		P value
	N	%	N	%	
Adherence to gluten-free diet					0.741
Strict diet	94	97	527	98	
Occasional lapses	3	3	11	2	
Frequent lapses	0	0	0	0	
Use of gluten-free oats	82	85	449	83	0.534
Able to manage the diet	96	97	503	96	0.381
Symptoms at the time of study					**0.043**
No	81	83	400	75	
Slight	12	13	127	24	
Severe	3	3	10	2	
EmA positivity at study	7	7	52	9	0.458
TG2-ab positivity at study	13	13	81	15	0.703
Wish for regular follow-up	90	92	443	81	**0.038**

EmA, endomysium antibodies; TG2-ab, transglutaminase-2 antibodies.

Table 3
Current gastrointestinal symptoms and quality of life as measured by validated questionnaires in 648 celiac disease patients or without long term follow-up.

	Long-term follow-up (N = 99)		No long-term follow-up (N = 549)		P value
	Median	Quartiles	Median	Quartiles	
GSRS sub-scores					
Total	1.9	1.6–2.5	1.9	1.5–2.7	0.974
Diarrhea	1.7	1.0–2.0	1.3	1.0–2.3	0.311
Constipation	1.7	1.0–2.3	1.7	1.0–2.7	0.313
Indigestion	2.5	2.0–3.3	2.3	1.8–3.3	0.315
Abdominal pain	2.0	1.5–3.0	2.0	1.5–3.0	0.818
Reflux	1.3	1.0–2.0	1.3	1.0–2.0	0.992
SF-36 sub-scores					
General health perception	55	40–75	65	50–75	0.088
Role limitations, emotional	100	67–100	100	67–100	0.455
Vitality	70	51–80	70	55–84	0.506
Bodily pain	78	48–90	68	57–90	0.516
Social functioning	88	75–100	88	75–100	0.707
Physical functioning	93	75–95	90	75–100	0.738
Role limitations, physical	100	25–100	75	25–100	0.851
Mental health	80	68–92	80	70–88	0.867
PGWB sub-scores					
Total	108	100–117	106	93–115	0.107
General health	13	12–16	13	10–15	0.123
Anxiety	25	22–28	25	21–27	0.145
Self-control	16	14–17	16	14–17	0.150
Well-being	18	15–20	17	15–20	0.311
Vitality	19	16–20	18	16–20	0.347
Depression	17	16–18	17	15–18	0.628

GSRS, Gastrointestinal Symptom Rating Scale, lower scores indicate fewer gastrointestinal symptoms; PGWB, Psychological General Well-Being, higher scores indicate better well-being; SF-36, Short Form 36, higher scores indicate better social functioning.

3.2. Long-term outcomes in patients with and without follow-up

Participants in both follow-up and no-follow up groups had similar dietary adherence and ability to manage the gluten-free diet, as well as use of purified oats (Table 2). There was also no significant difference between the groups in current positivity for coeliac autoantibodies (Table 2) or in any of the questionnaire scores measuring health-related quality of life (SF-36, PGWB) and gastrointestinal symptoms (GSRS) (Table 3). However, patients without regular long-term follow-up suffered more from current self-reported overall symptoms (Table 2). In line, the GSRS total (median 1.8 vs. 2.3, $p < 0.001$), pain (1.7 vs. 2.0, $p < 0.014$), indigestion (2.3 vs. 2.8, $p < 0.001$) and constipation (1.7 vs. 2.3, $p < 0.001$) scores were higher in subjects with current self-reported symptoms than those without, whereas this was not seen in reflux and diarrhea.

Altogether 98% of patients with regular long-term follow-up wished for it also in the future, and this was also seen in over 80% of the patients of patients not currently under follow-up (Table 2). Most of these patients wished the follow-up to be organized in public healthcare (Fig. 1), whereas there was no major difference regarding who should be in charge of the follow-up (Fig. 2).

3.3. Sub-analysis of patients without any follow-up and short-term follow-up

Altogether 84 (12%) out of the 677 participants had not received any follow-up for coeliac disease after the diagnosis, while 465 (69%) had been subject only to short-term follow-up (<2 year). Compared to patients receiving long-term follow-up, patients without any follow-up had more current mild symptoms (27% vs. 13% $p = 0.038$). Furthermore, those without any follow-up had fewer circulatory diseases (18% vs. 35%, respectively, $p = 0.009$) and a lower desire for regular follow-up (85% vs. 92%, $p = 0.024$) than those with long-term follow-up.

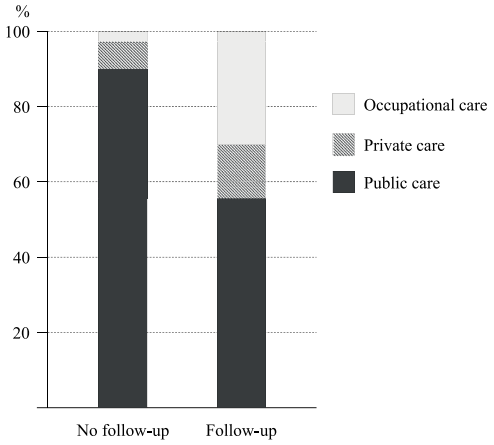

Fig. 1. Patients' opinion on where the long-term follow-up of celiac disease should be organized, divided on basis of the lack (No follow-up) or presence (Follow-up) of current regular follow-up.

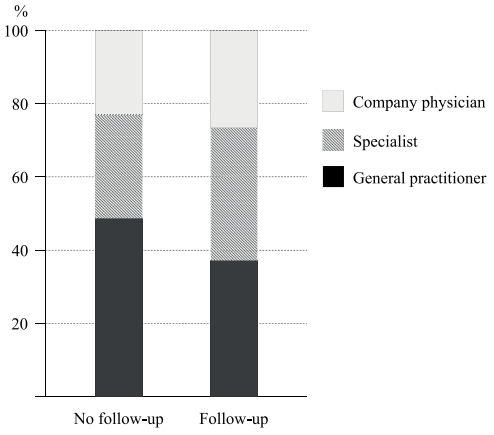

Fig. 2. Patients' opinion on who should be in charge of the long-term follow-up of celiac disease, divided on basis of the lack (No follow-up) or presence (Follow-up) of current regular follow-up.

4. Discussion

We found the prevalence of regular long-term follow-up to be only 15% in Finnish coeliac disease patients [16]. This is in stark contrast to our national guidelines which recommend systematic clinical and serological follow-up at 2–3 years intervals in all patients. It is also a markedly lower percentage than reported previously. In the early 1990s Bardella et al. [25] reported a follow-up rate of 77%. Bebb et al. found the corresponding figure to be 62%, although only 6% of the patients later transferred to primary care received follow-up [26]. In a study by Herman et al. 41% of patients received a follow-up during the first year after, this percentage increasing to 89% after five years [11]. The results by Bardella et al. could be affected by the fact that the patients were investigated during their transition from pediatric to adult care, at which time at least one visit is often scheduled. Another explanation for the different percentages could be related to our practice to refer patients in primary care soon after the diagnosis, whereas most of the previous studies were carried out in tertiary centers. We must, however, emphasize that also the majority of our patients received short-term follow-up, including a follow-up biopsy one year after the diagnosis [16].

There are also further explanations why the long-term follow-up frequency is low particularly in Finland. Most of the patients have only mild symptoms, reflecting the short diagnostic delay and good recognition of the disease by physicians [27]. This, along with the good dietary adherence, has made severe complications associated with advanced or poorly treated coeliac disease rare [4]. As a result, demonstration of initial treatment response might often be deemed sufficient by physicians. From patients' perspective, they may find it hard to motivate themselves to seek for healthcare visits when the disease does not constitute a substantial daily burden. In Finland public healthcare is accessible equally to all citizen and active patients would likely have received a follow-up, further indicating that they have not contacted the healthcare services. Thus, it is likely that, even if patients would "like" to have a follow-up, they do not vigorously seek it if not organized by the health care. In these circumstances, a more active role of the healthcare providers might be required to improve attendance in follow-up [28].

One of the main arguments for follow-up in coeliac disease is the need to monitor dietary adherence. It was thus of significance that also patients without follow-up reported excellent adherence and competence to maintain a gluten-free diet. This was supported by the low frequency of current coeliac antibody positivity, although it must be realized that serology has limited sensitivity in the detection of dietary lapses [3]. The higher prevalence of seropositivity over against self-reported adherence could be caused by unrecognized lapses or via the slow normalization of high values [13,29–31]. Previously Bardella et al. found regular follow-up to improve adherence in adults [13] and Barnea et al. reported similar finding in children [29]. The absence of such an association in Finland could be due to the high prevalence of coeliac disease and good availability of gluten-free products, and the widespread knowledge of the disease among physicians and food industry. We also wish to emphasize that previously all Finnish patients received governmental reimbursement for dietary expenses (ended in 2016). Owing to these particularities, our results may not be directly applicable to countries in which the condition is rarer and less well known.

The majority of patients were satisfied that the follow-up was arranged by general practitioners, although one third would prefer visits to a specialist. This is in line with the strong public healthcare sector in Finland and a long tradition of decentralization in coeliac disease follow-up. This aspect has not been studied using a similar approach, but Bebb et al. [26] found the preferred method for follow-up to be visits to a dietician with a possibility to consult a physician. We find such a dietician-led clinic an interesting alternative, as dietary lapses are the most common reason for non-response in coeliac disease, even in apparently compliant subjects [32]. However, this approach would likely provide no major benefits in well-doing and strictly adherent patients. The possible need for patients with persistent symptoms to consult a physician should also be taken into account in these circumstances.

Although most of the long-term outcomes were comparable between the study groups, patients without follow-up felt they had more current symptoms when asked as a qualitative estimation. We recently found persistent symptoms to be common even in well-treated coeliac disease [33], but no studies have looked into the association between ongoing symptoms and lack of follow-up. Interestingly, the difference in symptoms disappeared when evaluated by a validated questionnaire, indicating that the direct question emphasizes the subjective experience rather than the objective magnitude of symptoms. Gastrointestinal symptoms are common and not always necessarily related to coeliac disease. In fact, the median GSRS scores in our treated celiac disease patients were comparable to the Finnish population [33]. It might be that

regular healthcare contacts improve sense of self-capability and reduce excessive monitoring of symptoms. Accordingly, patients with irritable bowel syndrome have benefited from physicians' reassurance on the benignity of their symptoms [34,35]. Here it must be realized that the discordance between self-estimated symptoms and GSRS might be partly technical, as the latter measures a wide scale of symptoms that can be even opposing, such as diarrhea and constipation.

Strength of the present study was the large number of patients diagnosed on different levels of healthcare. We also succeeded in collecting a variety of clinically relevant parameters, and the use of validated questionnaires increases the reliability of results. As a limitation, patient recruitment by advertisements and via celiac societies predisposes to selection bias. For example, the study information might have reached different age groups and socioeconomic classes unequally. Furthermore, although not specifically designed as a follow-up study, patients without regular follow-up could have considered our survey as an "extra visit" and thus have been more eager to participate. Additional study limitations were the lack of data on the patients' socio-economic status [30,36], scarcity of short-term serological follow-up data and the use of inexact three-point scale in the evaluation of self-reported symptoms. The cross-sectional design also prevented us from evaluating the causality in for example the incidence of comorbidities.

In conclusion, we found follow-up in coeliac disease not to meet the current recommendations. However, the necessity for rigid follow-up scheme could be questioned, as there was no difference between the study groups in most long-term health outcomes. These findings call for more a personalized approach: patients achieving clear remission could visit healthcare only if symptoms reappear or after major life changes, whereas those facing more challenges might benefit from more systematic follow-up.

Conflict of interest

None declared.

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