Summary and General discussion
Chapter 5.

The diagnostic algorithm for children differs, it is shown that the CD diagnosis differed between males (52 years, IQR 41-61) and females (44 years, IQR 32-56). In the whole group, the female to male ratio was 2.4:1. Although a majority (52%) was diagnosed before the age of 4 years. In adults, median age at diagnosis was 64 years (IQR 48-76). During childhood, CD was diagnosed up to the age of 88 years, with 36% diagnosed before the age of 4 years. In children, the majority (52%) was diagnosed before the age of 4 years. In adults, median age at diagnosis differed between males (52 years, IQR 41-61) and females (44 years, IQR 32-56). In the whole group, the female to male ratio was 2.4:1. Although a potentially biased cohort of the Dutch CD Society was used, these age- and gender distributions are comparable with current literature. This showed that physicians should be aware of CD at all ages, as CD is by many physicians still considered to be a disease diagnosed in children and young adults and not in adults (Chapter 5).

The currently used CD diagnostic algorithm for adults consists of measuring CD antibodies (especially antibodies against tissue transglutaminase, TTG) as first step and, when positive, or when there is a high clinical suspicion, followed by duodenal biopsies to investigate the presence of villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis. The diagnostic algorithm for children differs significantly. The European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) proposed a guideline in which intestinal biopsies in a subset of children can be avoided. This is only possible in children who meet the following criteria: characteristic symptoms of CD, TTG IgA levels >10 x upper limit of normal (confirmed with a positive EMA in a different blood sample), and positive HLA-DQ2 or HLA-DQ8. Although this diagnostic approach is not yet integrated in the diagnostic guidelines for adults, some studies hypothesized that a subset of adult CD patients could be diagnosed using the same method (i.e. without duodenal biopsies), based on the positive correlation between the level of TTG IgA and the presence of villous atrophy and CD.

The follow-up of CD is currently not standardized. This thesis focused on extraintestinal manifestations, such as oral health problems, and complications of CD, such as refractory CD and malignancies. Besides these topics, the management of CD as it is currently performed by general practitioners: the cornerstones of the Dutch health care system, is explored as well.

The individual novel insights described in this thesis are summarized and discussed in this chapter.

Coeliac disease: diagnosis

Dicke described the classical form of CD in young children. Nowadays, it is known that CD is a clinical chameleon, diagnosed at all ages, with abdominal pain (28%) and diarrhea (35%) present in the minority of patients. Oral manifestations, especially enamel defects and aphthous stomatitis, are less known, but however frequently observed in CD as described in Chapter 6. In Chapter 2, it is shown that in the cohort, CD was diagnosed up to the age of 88 years, with 36% diagnosed during childhood and the remaining 64% during adulthood. In children, the majority (52%) was diagnosed before the age of 4 years. In adults, median age at CD diagnosis differed between males (52 years, IQR 41-61) and females (44 years, IQR 32-56). In the whole group, the female to male ratio was 2.4:1. Although a recently described potential new diagnostic test with a high sensitivity and specificity for CD is the HLA-DQ-gluten tetramer-based assay in blood, detecting gluten-reactive T-cells in CD patients in both patients on a GFD and on a gluten-containing diet. This test may be used to diagnose CD without biopsy and may facilitate differentiation between CD and non-coeliac gluten (or wheat) sensitivity (NCGS) without performing a gluten re-challenge (NCGS is described elsewhere in this chapter).
Even using the “classical” pathway of diagnosing CD (i.e. antibodies and histology), there are some pitfalls in the diagnosis of CD. Here, three of these pitfalls are discussed: 1) the absence of some of the diagnostic criteria 2) other causes of villous atrophy and 3) self-reported gluten sensitivity in the absence of CD.

The absence of some of the diagnostic criteria
In some patients, it can be difficult to diagnose CD. This applies for example to patients with positive CD antibodies and Marsh I (intra-epithelial lymphocytosis in the absence of villous atrophy and crypt hyperplasia). In these patients, an additional CD marker could be helpful. Such markers could be a HLA-DQ-gluten tetramer-based assay, tissue transglutaminase deposits in the duodenum analyzed by immunohistochemistry or the presence of an increased population T-cell receptor γδ positive (TCRγδ+) intra-epithelial lymphocytes (IEL) 21,22. In Chapter 3, the presence of TCRγδ+ IEL in different groups, including controls, active CD, CD in remission and potential CD, was evaluated. The latest group was defined as having positive CD serology in the presence of HLA DQ2 and/or DQ8 in combination with intraepithelial lymphocytosis without villous atrophy. In this selected patient group, a cut-off of ≥14% TCRγδ+ IEL of the total IEL led to a sensitivity of 66% and a specificity of 97% for diagnosing CD in the active CD group versus controls; useful to diagnose CD with a low percentage of false positive results. The presence of the TCRγδ+ IEL population in the context of CD diagnosis should be interpreted very carefully, as recent literature showed that increased number of TCRγδ+ IEL are for example also seen in olmesartan-induced enteropathy, another CD-like entity 23.

Since TCRγδ+ IEL could potentially be used as an extra diagnostic test for diagnosing CD, the potential function of these cells was studied in Chapter 4, using in vitro polyclonal stimulation (CD2/CD3/CD28) of these cells, focusing on T-helper and T-regulatory functions. No significant cytokine production by duodenal TCRγδ+ lymphocytes could be detected by the experimental approach used. It is rather unexpected that lamina propria derived TCRγδ+ T-cells do not produce any (pro-inflammatory or regulatory) cytokines upon T-cell receptor triggering. Possible explanations for this lack of cytokine production may be that important mediators of regulatory CD8+ TCRγδ+ lymphocyte function (such as TGF-β1 release 24) were not analyzed or that not the right stimulation pathway was used. Further studies analyzing a more broad panel of cytokines upon different stimulation pathways such as Toll-like receptor triggering are needed to unravel the function of duodenal TCRγδ+ in CD 25.

In the same chapter, the role of another cell subset potentially involved in CD has been investigated: the CD4+CD8+ double positive (DP) T-cells. Similar to TCRγδ+ lymphocytes, CD4+CD8+ double positive (DP) lymphocytes are suggested to play a role in the pathophysiology of CD with pro-inflammatory and regulatory functions attributed to both cell subsets 26,28,30. Whereas TCRγδ+ IEL are elevated in most CD patients, CD4+CD8+ DP IEL are observed to be decreased in CD patients 31,30. The results suggest that CD4+CD8+DP T-cells have a more-like Th1 function compared to CD4+ single positive T-cells. This indicates a predominantly pro-inflammatory rather than immunosuppressive role of these cells. Furthermore, these cells have an increased cytokine production profile in active CD patients compared to CD in remission. Why the CD4+CD8+DP T-cells are decreased in CD compared to controls despite their pro-inflammatory role, is unknown. One of the possible explanations is that their number is relatively decreased (in terms of percentages), but that absolute numbers, due to intra-epithelial lymphocytosis, is increased in CD versus healthy tissue. In the future, new techniques such as organ-on-chip models 31, may provide more information regarding the exact role and the interaction between different cell subsets, and other factors such as the microbiome, (potentially) involved in CD.

Other causes of villous atrophy
Most diagnostic guidelines recommend to take always duodenal biopsies when there is a high clinical suspicion of CD. Once CD-related antibodies remain negative in the presence of villous atrophy, other causes of villous atrophy should be taken into account. Some of these are described in Chapter 8. Although rare, these other entities should be excluded before diagnosing seronegative CD, which is an uncommon condition as well (approximately 2% or less of all CD patients 32), but nevertheless a frequent diagnosis when CD-related antibodies are absent in the presence of villous atrophy. Two conditions of non-CD villous atrophy are described more in detail in this thesis.

The first is collagenous sprue. Evidence-based treatment strategies for collagenous sprue are currently lacking 33. Several treatment strategies are used, mainly based on small observational studies. One of the treatments described are steroids and azathioprine 34. In Chapter 13, four patients treated with thiouguanine, another thiouiprine, are described of whom three were also treated with budesonide (steroid). Three of the four patients improved histologically and all patients improved clinically. One of these patients had reoccurring symptoms after stopping thiouguanine. These observations suggested that thiouguanine with or without budesonide might be a therapeutic option in collagenous sprue. The exact working mechanism of thiouguanine in collagenous sprue is unkown.
The second condition is losartan-induced enteropathy [Chapter 14]. Of all angiotensin receptor blockers, olmesartan is the best known to cause villous atrophy in some patients 35. In this chapter the second patient with losartan-induced enteropathy ever described was presented. This patient had a very fast and total clinical and histological recovery after discontinuing the drug. Potentially, more patients using losartan, having diarrhea as side effect, could have undiagnosed villous atrophy. This is relevant since 1% of the Dutch population uses this drug according to the Dutch drug information system of national health institute (www.gipdatabank.nl) and since villous atrophy could potentially lead to malabsorption in these patients.

Self-reported gluten sensitivity
Since 1976, it has been described that gluten could cause symptoms in the absence of CD 36,37. For the diagnosis of this so-called non-coeliac gluten (or wheat) sensitivity (NCGS), a proposal has been formatted by an expert group 38. Currently, there is still no diagnostic marker for NCGS available and it is unclear whether the symptoms are caused by gluten or other parts of grains, such as Fermentable Oligo-Di- and Monosaccharides and Polysols, Wheat Germ Agglutinin or alpha-Amylase/Trypsin Inhibitors 39. The expert group proposed to use a double-blind placebo-controlled re-challenge with gluten to diagnose NCGS after excluding CD and wheat allergy. A meta-analysis confirmed that the diagnostic approach using these ‘Salerno criteria’ is useful to be more strict in diagnosing NCGS (despite the absence of a gold standard) 40. They reported that 40% of the patients tested had symptoms on gluten versus 24% on placebo.

In Chapter 12, the prevalence and the characteristics of self-reported gluten sensitivity (srGS) in the Netherlands is analyzed. This entity is a not well-described medical entity, but frequently encountered in daily clinical practice. Six percent of the participants of this study on srGS reported symptoms related to the ingestion of gluten-containing food. These individuals were younger, predominantly female and lived more frequently in urban regions compared to the other respondents. Main symptoms were bloating, abdominal discomfort and flatulence. Half of the srGS individuals tried a gluten-free or gluten-restricted diet. Thirty-five percent of the srGS respondents reported a reduction of clinical signs when consuming spelt bread, which precludes gluten sensitivity as spelt grains contain gluten.

Other studies showed similar prevalence of srGS. An UK study, using a similar questionnaire as chapter 12, showed a prevalence of 13% of srGS 41, a prevalence of 8% in Argentina 42 and 12% in an Italian high school population 43. In all these studies, the minority consumed a GFD.

After reviewing current literature it could be concluded that it is difficult to diagnose NCGS in the absence of knowledge about the pathophysiological mechanism. The two most important conclusions are that NCGS should be seen as a different entity than CD and that it is unknown if a life-long GFD is benefit for NCGS individuals. Future research should focus on the epidemiology and etiology of NCGS. Furthermore, evaluation of the usefulness and practical implications of expert group guidelines regarding NCGS is necessary.

Future perspectives regarding the diagnosis of CD
The incidence of CD patients is still rising 4. However, it is doubtful whether general practitioners, the “gatekeepers” of the Dutch health care system, have enough knowledge when and how to test for CD [Chapter 5]. Education and adjusting current general practitioners guidelines, including awareness that CD could present with different symptoms, could improve the detection of the undiagnosed but symptomatic patients.

Another issue is, that it is disputable whether asymptomatic CD patients have to be diagnosed, taking into account the low absolute risk of serious adverse events like cancer caused by CD [Chapter 11]. It has been shown that undiagnosed CD patients more frequently have hypothyroidism 44 and additionally, there is some evidence that screening could protect undiagnosed CD patients from osteoporosis and osteopenia 45, 46. Whether undiagnosed CD patients have an elevated mortality risk is still debatable 44, 47, 48. Once diagnosed, screen-detected CD patients have the same compliance to a GFD as symptom-detected CD patients 46, although the treatment burden of CD is high 49. A large CD screening study randomizing screen-detected CD patients between a GFD and gluten-containing diet with standardized follow-up could help to clarify whether detecting and treating asymptomatic CD patients leads to benefits.

Coeliac disease; follow-up
Two cornerstones in treatment and follow-up of CD are the adherence to a GFD and the prevention of complications. Most of the CD follow-up recommendations in current guidelines are based on expert-based statements.

The adherence to a gluten-free diet
The adherence to a GFD could be assessed by 4 different methods with each of them having both advantages and disadvantages 44. These 4 different methods include symptom evaluation, dietetic review, serology and follow-up biopsies.
First, the follow-up of symptoms, is an inferior method to check the adherence to a GFD and villi recovery, but is for the patient probably the most important parameter. For the second method, a dietetic review, a GFD expert is recommended. In the best situation, this should be an (expert) dietician who could give individualized advise and knows the pitfalls of the adherence to a GFD. It is likely that medical doctors, especially general practitioners, do not have enough knowledge regarding all the aspects of a GFD [Chapter 5]. Another additional method to perform dietetic review is the organization of group-based workshops, supervised by dieticians, where patients cook together to learn how to prepare gluten-free food. The third method is the follow-up of serology. This is in clinical practice the most frequently used method of monitoring the adherence to a GFD. It has been shown that levels of antibodies drop after starting a GFD 54, although low titer antibodies are not always a good marker for villi recovery 51,52. The last and most invasive method to assess the compliance of a GFD is taking follow-up biopsies. Since this is an invasive method for follow-up and since CD antibodies also decrease after starting a GFD, this method is in general only used when symptoms do not recover despite a GFD in combination with low antibody levels or as follow-up method of seronegative CD.

Complications

(Pre-)malignant conditions

There are several malignant and non-malignant complications of CD. The best known malignant complications are two rare malignancies: the enteropathy-associated T-cell lymphoma (EATL), and the adenocarcinoma of the small bowel 51,54. In daily clinical practice, it can be useful to provide newly diagnosed CD patients risk estimates: what is the actual chance to develop associated malignancies? In Chapter 11, it is shown that the CD-associated relative risk (RR) to be diagnosed with malignancies simultaneously or after CD diagnosis was elevated for T-cell lymphoma (RR 35.8), small bowel adenocarcinoma (RR 12.7) and esophageal squamous cell carcinoma (RR 3.5). Absolute risks were relatively low with a highest absolute risk of 4.3 % between age 50 and 80 years to be diagnosed with a T-cell lymphoma for a male diagnosed with CD at age 50 years. This risk rapidly decreases to 1.8 %, 3 months after CD diagnosis in the same person. Other types of lymphomas and gastro-intestinal carcinomas were not associated with CD.

Besides the two best known associated malignant conditions the squamous cell carcinoma of the esophagus was also associated with CD. This association was also observed in a meta-analysis of 8 studies including 79,365 CD patients. The pooled odds ratio was 3.7 59. Although it is unknown why CD and esophageal cancer are associated, the authors suggested that esophageal dysmotility, chronic gastroesophageal reflux, and subsequent chronic esophagitis, could contribute to the association. They did however not differentiate between squamous cell and adenocarcinoma.

Based on these results, symptom driven cancer case finding in CD patients (EATL, small bowel adenocarcinoma and squamous cell carcinoma of the esophagus) is recommended, but insufficient support for routine standardized screening for these malignancies during follow-up is found.

A rare form of CD is called refractory CD, or RCD [Chapter 8]. Refractory CD is a rare condition and affects 0.83 / 10,000 CD patients in the Netherlands 56, most often diagnosed above the age of 50 years. It is important to distinguish between RCD type I (RCD I) and type II (RCD II) since the first is considered as a benign condition and the second as a low-grade non-mass lymphoma based on the presence of aberrant IEL with a characteristic phenotype (lacking surface CD3 despite the presence of intracellular CD3). Although it is also possible to develop EATL in CD patients in the absence of aberrant IEL, RCD II is strongly associated with EATL since 50 % of RCD II patients develop an EATL with a dismal prognosis 57. There is evidence that EATL develops from RCD II based on the same clonality of cells 54. Therefore, and since the association with EATL is so strong, some experts consider more and more to rename RCD II as ‘pre-EATL’. Another reason to rename RCD II is that in some CD de novo patients on a gluten-containing diet, a significant population of aberrant IEL is found (data not published), in whom it is unknown how this entity is related to the classical form of “RCD II”. In daily practice these patients will be frequently treated as RCD II patients.

Topical steroids and thiopurines are the main cornerstone in the treatment of RCD I. The treatment of RCD II remains more challenging, with topical steroids, Cladribine (2-chlorodeoxy-adenosine), but also other treatment strategies, followed by an autologous stem cell transplantation when possible. Chapter 15 reports on a RCD II patient in whom villi restored after fecal microbiota transfer. This observation is a co- incidental observation of an Clostridium difficile positive patient treated with fecal microbiota transfer due to recurrent infections. After fecal microbiota transfer, duodenal histology completely recovered including the absence of intra-epithelial lymphocytosis. One of the hypothesis is that dysbiosis could function as a driver for the IL-15 response which could be influenced by changing the microbiome 59. The role of IL-15 in the pathophysiology of RCD II is a.o. based on IL-15 mediated survival of, and anti-apoptotic signaling in, (aberrant) IEL56,57.
In this light, the first randomized, double-blind, placebo-controlled study on the treatment of RCD II using anti-IL-15 (AMG 714) took place in 2016 and 2017. This is an unique project since research regarding pathophysiology and treatment remains challenging due to the low prevalence of RCD II.

In the AMG 714 study, the use of anti-IL-15 in RCD II patients from both the United States of America and Europe was evaluated. The study reports no statistically difference in the percentage of aberrant IEL and histology in patients treated with anti-IL-15 versus placebo, although anti-IL-15 improved diarrhea and had a positive effect on T-cell receptor clonality (both statistically significant). The main question remains whether anti-IL-15 could prevent the progression of RCD II into EATL.

Other complications and manifestations
There are several non-malignant complications, or manifestations, of CD. These include a.o. skin manifestations (dermatitis herpetiformis), neurological manifestations (for example gluten ataxia), other auto-immune disorders (thyroiditis, type I diabetes), oral manifestations (enamel defects, aphthous stomatitis), reviewed in Chapter 6 and hyposplenism.

This thesis focused on two of these manifestations: oral manifestations and the association with the splenic volume.

Since oro-dental manifestations of CD have previously been described, self-reported oral health and xerostomia in CD have been investigated. This study showed that oral health problems, including aphthous stomatitis, painful mouth and gingival problems, were more frequently reported by CD patients. The impact on oral health, measured by the Oral Health Impact Profile 14 (OHIP-14), was significantly higher in CD patients compared to controls (4.9 versus 2.6, p<0.001) as well as the presence of xerostomia, measured by the xerostomia inventory score (22.2 versus 17.2, p<0.001). In patients diagnosed with CD, the time on a GFD did not influence these scores. The etiology of xerostomia in CD patients is unclear. However, this could a.o. be explained by a decreased salivary production in CD patients.

Based on the results of Chapter 6 and 7, it could be proposed that dentists should be more aware of CD when several CD-associated (oral) conditions are present and should consider to refer these patients to a general practitioner to evaluate the presence of CD. On the other hand, general practitioners, gastroenterologists and other medical professionals treating CD patients should ask their CD patients for oral symptoms since these are more often present in these patients than in the general population. They should consider to refer CD patients to a dentist for evaluation and treatment once symptoms are present.

The spleen, an important organ in immunological processes, has been described to be atrophic in CD patients. Additionally, impairment of splenic function in CD patients has been recognized over the years. Chapter 10 showed however that splenic volume is rather enlarged in uncomplicated CD versus controls, although there is a large inter-individual variation. On the other hand, the median splenic volume in RCD II patients was smaller than in controls, which may be of clinical relevance considering the immune-compromised status of these patients. Since impaired splenic function and splenic atrophy are the cause of an increased risk of infection by encapsulated bacteria, especially pneumococcal infections, it is recommended to vaccinate these hyposplenic patients. Since it is unclear which CD patients are functional hyposplenic, it is unclear who to vaccinate. Based on the finding of splenic atrophy in RCD II patients, there seems to be some evidence to vaccinate at least these patients against Streptococcus pneumoniae. Both the conjugated and the polysaccharide vaccine are recommended since the conjugated vaccine leads to immunological memory, but the polysaccharide vaccines currently covers more serotype subsets. This polysaccharide vaccine should be repeated every five years and may be less effective in hyposplenic patients as these responses are primarily induced in the spleen. Vaccination against other the encapsulated bacteria Haemophilus influenza type B and Neisseria meningitidis should also be considered in hyposplenic CD patients.

Future perspectives of follow-up
Little evidence is available regarding the exact recommended follow-up of diagnosed CD patients. Is follow-up always recommended? Which parameters should be included in the follow-up? Who has to perform the follow-up? Currently, most of the follow-up statements in guidelines are based on expert opinions: it is recommended to visit a doctor two times during the first year after diagnosis, monitoring antibodies and deficiencies, with a decrease of CD antibodies as marker for gluten-free diet adherence. The current agreement is that there is no indication for duodenal biopsy follow-up in all patients, with exceptions of those patients who are diagnosed with seronegative CD or patients who are suspected to have RCD. Due to the frequent prevalence of osteoporosis, a bone density index is also indicated, as well as the evaluation of deficiencies of several vitamins and minerals and the liver transaminases. There is no evidence that screening for...
specific malignancies in CD is recommended. However, physicians should be alert on the development of EATL, small bowel carcinoma and squamous cell carcinoma of the esophagus [Chapter 11], especially in patients diagnosed above the age of 50 years. The follow-up should be performed by someone who is aware of the possible complications of CD, including RCD. Therefore, it is questionable whether a general practitioner who has low exposure to CD patients, should manage the follow-up of CD.

Regarding the adherence to the only effective, accepted, well-tolerated and safe therapy of CD, a GFD, it is stated that cognitive, emotional and sociocultural influences, membership of an advocacy group and regular dietetic follow-up are the factors most strongly associated with the adherence to the diet 22. Therefore dieters are the cornerstone in treatment and follow-up of CD.

In conclusion, although the current knowledge regarding diagnosis and treatment of CD is high, awareness of CD by non-experts, including dentists and general practitioners, and the knowledge regarding the follow-up of patients should be improved. Another issue is the development of more sophisticated non-invasive methods to diagnose CD without taking duodenal biopsies. Last but not least, the development of a safe and effective treatment strategy for RCD II patients is needed, which remains however challenging because of the rarity of the condition.

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