Part V

Chapter 10

Summary, Discussion and Future Perspectives
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This thesis concerns the epidemiologic, diagnostic, and therapeutic aspects of uncomplicated celiac disease (CD), complicated CD (including refractory celiac disease and enteropathy associated T-cell lymphoma), and non-celiac gluten sensitivity. In this chapter, we summarize and discuss these aspects and provide possible directions for future research.

Celiac disease

Celiac disease (CD) is defined as a chronic enteropathy caused by a state of heightened immunological responsiveness to ingested gluten or related proteins in patients with a genetic predisposition\(^1\)\(^-\)\(^3\). With a prevalence of 1% in the Western population, CD is one of the most common intestinal disorders\(^1\). CD can be acquired at any age and clinical presentation varies widely. The past fifty years remarkable progress has been made in unraveling the pathogenesis of CD and the development and improvement of diagnostic tools\(^4\); serological markers are widely used\(^5\), many genetic risk factors are identified\(^6\) and histopathological features and criteria are defined\(^7\). In chapter 2 we proposed to use intra-epithelial lymphocytes (IEL) bearing the T cell receptor gamma-delta chain (TCR\(\gamma\delta\)) as an additional diagnostic test in those cases where the diagnosis is not straightforward. TCR\(\gamma\delta\)-lymphocytes might, for example, be of diagnostic value in patients with minimal histological abnormalities (i.e., Marsh I) or individuals with positive serology in the absence of histological abnormalities (i.e., potential or latent celiac disease). In this chapter, we have proposed a clear cut-off value for this IEL subset (\(\geq 14\)% of total IEL) by generating a receiver operating characteristic (ROC) curve comparing active CD patients with controls. A cut-off value of \(\geq 14\)% has 97% specificity and 66% sensitivity for CD diagnosis. Therefore the TCR\(\gamma\delta\)+ cut-off value is mainly useful to determine true CD negatives with a low percentage of false positives. In other words, TCR\(\gamma\delta\)+ IEL can be used to diagnose CD with a high degree of confidence. This was confirmed by implementing the cut-off value in the subgroup with potential CD which showed \(\geq 14\)% TCR\(\gamma\delta\)+ IEL in the majority patients (92%). It can be discussed if this cut-off value needs to be corrected for age, since controls were significantly younger at baseline compared to active CD patients. We choose not to, since the clinical usability of a cut-off value would be much higher without the necessity to make logistic corrections. Furthermore, the cut-off value did not change upon correction for age; the specificity after correction was even
higher (98%). For the future, an external validation cohort would be valuable to confirm our established cut-off value.

The TCRγδ+ IEL subset appears to remain elevated even after the introduction of a gluten-free diet and can possibly be used in patients already compliant to a GFD without the need for a gluten-challenge. Furthermore, this IEL subset might be helpful in cases where the diagnosis of CD may be difficult. One such an example is villous atrophy associated with common-variable immunodeficiency syndrome (CVID). This condition is difficult to distinguish from CD since both conditions share the same histological abnormalities. Moreover, comorbidity with CD in CVID has been described. Therefore, TCRγδ might be helpful to distinguish CD from CVID, by selecting cases in which a gluten free diet (GFD) might be helpful.

So far, the role of these TCRγδ cell subsets in the pathogenesis of CD is not completely understood. After partial digestion, gluten fragments trigger a cascade of immune reactions in the small intestine of CD patients. It has been suggested that TCRγδ-IEL are involved in mucosal repair, a hypothesis which is supported by mice experiments which showed an essential role of CD3+TCRγδ+ IEL in promoting epithelial reconstitution following mucosal injury. The observation that TCRγδ-IEL are depleted in complicated, pre-malignant CD, supports the notion that TCRγδ-IEL might play a crucial role in regaining homeostasis in CD and possibly even tumor surveillance. On the other hand, it cannot be excluded that these TCRγδ-IEL play a pro-inflammatory role in CD. Recently, these regulatory and proinflammatory hypotheses were both confirmed. Distinct subsets TCRγδ-IEL can accumulate during the various stages of CD; in active CD a IL-21 producing effector TCRγδ-IEL subset predominates, in contrast with CD on a GFD where a regulatory TCRγδ-IEL subset under the stimulus of transforming growth factor-β1 predominates.

This might also be the explanation of the persistent high percentage of TCRγδ-IEL despite the absence of the triggering agent in patients on a GFD. In these patients, the regulatory TCRγδ-IEL subset might contribute to recovery from epithelial damage and maintenance of mucosal homeostasis. Future research will hopefully shed light on the exact roles and stimulants of this lymphocyte subset. Besides gluten and the identified genetic risk factors being involved in disease development, additional environmental factors may be of importance in CD. This is reflected by the age of CD onset; it is still unclear why some individuals develop CD during infancy while others acquire the disease in adulthood. Bacterial or viral infections may be contributing factors to CD development. It is hypothesized that specific infections, i.e. reovirus, could result in loss of tolerance to
gluten and amplify the response to gluten in predisposed individuals and therefore trigger the onset of CD\textsuperscript{17-19}. In the last years, the role of the human microbiome has received a lot of interest. Different studies indicate that CD patients have imbalances in the intestinal microbiota (dysbiosis), which are not fully normalized despite adherence to a gluten-free diet\textsuperscript{20, 21}. Microbial dysbiosis may contribute to loss of gluten-tolerance\textsuperscript{22}, intestinal barrier defects and promote inflammatory responses to gluten\textsuperscript{17, 23, 24}. Nevertheless, these theories are speculative, and more direct evidence is needed to understand how dysbiosis or specific infections interact with the host’s immune system to promote celiac disease.

Complicated celiac disease
In a small minority of CD patients, villous atrophy and clinical deterioration occurs despite a strict GFD. When inadvertent gluten contamination or a (concomitant) small intestinal bowel disorder resembling CD, have been excluded, patients are diagnosed with refractory celiac disease (RCD). RCD is an extremely rare disorder with an annual incidence of 0.031 per 100,000 Dutch inhabitants and an annual incidence of 0.83 per 10,000 CD patients\textsuperscript{25}. It is believed that in RCD the intestinal immune reaction initially induced by gluten has evolved into an autonomous (auto)immune reaction. According to the phenotype of intra-epithelial lymphocytes, RCD is divided into two types. RCD type I (RCDI) presents with villous atrophy and increased IEL with a normal phenotype. RCD type II (RCDII) on the other hand, shows an expansion (>20%) of a usually clonal, IEL population with an aberrant phenotype\textsuperscript{26}. As stated in chapter 3, RCDI and RCDII differ substantially in clinical presentation, endoscopic characteristics and pathogenesis with a generally benign course and good prognosis in the former and a poor prognosis in the latter. This can be attributed to a high risk to develop Enteropathy associated T-cell Lymphoma (EATL) in RCDII and therefore, recognition of this complicated form of CD is of the utmost importance.

RCDII and EATL patients show overlapping clinical characteristics with ‘classical’ or untreated uncomplicated CD; ongoing weight loss, abdominal pain, diarrhea and steatorrhea\textsuperscript{27}. In gastrointestinal malignancies, weight loss is negatively correlated to quality of life, morbidity and mortality\textsuperscript{28}. Analyzing different aspects of nutritional status, intake and total energy use in RCDII and EATL patients might provide a rationale for dietary interventions in these patients. Therefore, we described and compared the
nutritional status of uncomplicated CD, RCDII and EATL patients in chapter 4, focusing on anthropometrics, nutritional intake, energy expenditure and fecal losses. Generally, nutritional status and energy balance are affected in all types of CD patients at diagnosis. In complicated CD, nutritional status was seriously affected at presentation. In EATL patients involuntary weight loss (defined as a loss of >10% in the past six months or >5% in the past month) was a frequent clinical feature at presentation (60% of the patients). In these patients, resting energy expenditure was higher than predicted in the majority of patients (89%). This may be interpreted as ‘hypermetabolism’. In contrast, RCDII patients were more often characterized by a more chronic form of malnutrition, i.e. a low BMI (33% of RCDII patients presented with a BMI <18.5). This observation may be attributed to an increased fecal energy loss due to malabsorption (<85% absorption; 44% of RCDII patients) or severe malabsorption (<75% absorption; 18% of RCDII patients). These observations underline the importance of an extensive nutritional assessment and subsequent restoration of nutrients in complicated CD.

Given the high percentage of RCD II patients that develop an EATL, the treatment goal in RCD is to improve clinical course and prevent or delay progression to an overt EATL. Unfortunately, RCDII is, at least in part, resistant to most evaluated therapies. To date there is no standardized treatment approach. Classic immunosuppressive drugs, such as thiopurines are ineffective and do not prevent the development of EATL. In fact, they might even accelerate the onset\textsuperscript{29-31}. Therefore, more experimental and intensified therapies have been evaluated in the past decades, including cladribine and autologous stem-cell transplantation (auSCT). Cladribine is a synthetic purine nucleoside homologue which induces lymphocytic apoptosis and is supposed to be especially active against low-grade malignancies\textsuperscript{32}. It has been proven effective in for example hairy cell leukemia and selected autoimmune disorders\textsuperscript{33-36}. AuSCT is an increasingly accepted and effective treatment option for patients with severe autoimmune diseases refractory to conventional treatment and has been used successfully in patients with multiple sclerosis, rheumatoid arthritis, systemic sclerosis, systemic lupus erythematosus and Crohn’s disease\textsuperscript{37}. The rationale for this strategy is based on the concept of immunoablation using high-dose chemotherapy, with subsequent regeneration of the immune system from reinfused hematopoietic progenitor cells. Although relatively large series described a preventive effect of both abovementioned treatment strategies on EATL development compared to other treatment strategies in RCDII\textsuperscript{38-40}, they do not
prevent EATL in all cases. Clinical features associated with EATL development have never been identified. The early recognition of patients at risk of developing EATL provides a rationale for more aggressive and uniform treatment strategies to ultimately prevent this malignant lymphoma transformation. Therefore, we retrospectively evaluated RCDII patients receiving cladribine with or without auSCT in chapter 5 and described factors associated with EATL development. In this retrospective study with a median follow-up of 64 months, 22% of RCDII patients developed an EATL and 44% died despite (intensive) therapy. One- and five-year survival rates were 82% and 63% respectively. EATL development was low and median overall survival was significantly better in the group treated with the most intensive treatment strategy (auSCT); one out of fifteen patients developed an EATL (7%) and one- and five-year survival rates were 100% and 85% respectively. It should be noted that patients receiving the most aggressive therapeutic regimen were significantly younger compared to those treated with cladribine monotherapy (p=0.003). Besides this, patients who underwent auSCT had less comorbidity and therefore survival differences should be placed in perspective. Selection of patients for the most aggressive treatment strategy was non-standardised and, with the above mentioned factors as age and comorbidity, a certain selection bias for more eligible patients could have contributed to the displayed survival advantage. Unfortunately, the small cohort of patients makes multivariate analysis impossible.

Evaluation of factors associated with EATL development in RCDII patients treated with cladribine revealed that histological response determines EATL development; when histological remission is achieved after cladribine monotherapy, the frequency of EATL development is low and survival is comparable with the patients treated with an intensive step-up strategy including both cladribine and auSCT.

Remarkably, persisting high percentages of aberrant IEL was not associated with EATL development or survival. As confirmed in other studies, the presence of aberrant IEL above 20% seems to determine the risk of EATL; higher percentages do not result in a higher EATL risk. It can be hypothesized that a small percentage, of phenotypically different heterogenous aberrant IEL, might be cladribine resistant and result in EATL development over time although there are no data yet to substantiate this hypothesis.

Thus, cladribine, with or without auSCT, is effective in the treatment of signs and symptoms of RCDII when histological normalization is achieved. When histological
response is absent a more aggressive step-up strategy is needed to prevent EATL development. Whether the benefits from auSCT outweigh the costs and the risks of this aggressive treatment strategy needs to be evaluated in a larger cohort with longer follow-up. Unfortunately, cladribine-failures with persistent histological abnormalities who are non-eligible for auSCT still bear an extremely high risk of malignant transformation and moreover, aggressive treatment with auSCT could not prevent EATL in all patients. This underlines the need for new, advanced treatment strategies for RCDII.

In the last years, the nature of these phenotypical aberrant IEL has been further unravelled and involved cytokines have been identified. This may provide clues for future treatment strategies. RCDII is characterized by a continuous gluten-independent duodenal immune-activation with an abnormal intra-epithelial lymphocyte population. This cell population is found under physiological circumstances in the intestine and may expand as a consequence of a lack of apoptotic control. It has been described that the presence of IL-15 in RCDII is likely to contribute to the expansion and survival of aberrant IEL through its anti-apoptotic effect. Moreover, enterocyte-derived IL-15 might exert cytotoxicity against epithelial cells and be responsible for the severe enteropathy observed in RCDII patients. Recently, a randomized controlled trial consisting of anti-IL15 therapy has been rolled out in Europe and results are pending. Hopefully, this immunotherapy can effectively resolve villous atrophy and eliminate the aberrant IEL population in RCDII patients in order to prevent development of EATL. However, aberrant IEL show a heterogenous nature with respect to receptor expression: CD56-CD127-aberrant IEL are characterized by IL2/15 receptor B-chain (CD122) expression and CD56-CD127+ aberrant IEL showed co-expression of IL21-receptor and IL15 receptor alpha-chain. Due to this heterogenous nature of aberrant IEL, anti-IL15 therapy might not be effective in all RCDII patients and alternative treatment strategies may have to be sought in common downstream signalling molecules of aberrant IEL. This proposed hypothetical strategy is supported by a recently published genetic analysis of EATLs which showed JAK-STAT mutations to be the most commonly mutated signalling pathway.

There are no validated and standardized treatment protocols for EATL due to the rarity of this disease. Treatment strategies consist of different successional steps, including surgery, chemotherapy and stem cell transplantation (SCT). Each step is applied depending on the eligibility of the patient. Besides the aggressive and often
chemotherapy-refractory nature of the T-cell lymphoma itself, the deprived nutritional status of most EATL patients at diagnosis (chapter 4) negatively affects clinical condition and therefore prevents the use of aggressive treatment strategies. This results in an extremely variable five-year survival of EATL patients between 8% and 50%\(^{49-54}\). So far, treatment of EATL is only centralized in a minority of cases. Due to the risk of bowel perforation during chemotherapy the preferred first step in the treatment of EATL is surgical debulking, whether or not followed by subsequent chemotherapy. In chapter 6 we described indications of surgery, resectability of the lymphoma and morbidity and mortality of EATL patients who underwent surgical debulking. Furthermore, to confirm the additive value of surgery, we compared the outcome of this group with the patients who underwent chemotherapy without debulking. As described in this chapter, the frequency of early- and late post-operative complications is relatively high after surgical debulking (35% and 13% respectively). Early complications mainly include anastomotic leakage and sepsis and more than half of these patients underwent a second surgery. Stenosis at the site of the anastomosis is the most frequently reported late complication. Postoperative mortality occurred in 3 patients (8%) with a median of 20 days after initial surgery, all due to sepsis after anastomotic leakage. In our small cohort, mortality was higher when the resection was performed in the acute setting compared to the elective setting, without a significant difference in Ann Arbor stage, age, primary vs. secondary EATL and presence of RCDII at diagnosis between these groups. This stresses the importance of early diagnosis of EATL.

When comparing the outcome of patients who received chemotherapy without surgical debulking to the patients who underwent surgical debulking, the latter group showed a significantly better median overall survival (5 months vs. 14 months respectively). It should be noted that almost one-third of patients (30%) who underwent primary resection were unable to receive adjuvant chemotherapy due to poor clinical condition. This group did not significantly differ from the group who was able to receive adjuvant chemotherapy on patient characteristics as age (p=0.24), primary vs. secondary EATL (p=0.17) and presence of RCDII at EATL diagnosis (p=0.39). There is however a trend for a higher Ann-Arbor stage in those who were unable to receive adjuvant chemotherapy (p=0.07). This trend was also seen in patients receiving chemotherapy alone compared to those who underwent surgical debulking, and moreover, the chemotherapy-alone group more often included RCDII patients (p=0.005). Although median overall survival is significantly better in the total group of patients who underwent surgical debulking, median overall survival
in patients who underwent resection alone was not significantly different from those receiving chemotherapy alone (p=0.36). Taking into account the limitations of statistical comparison in these small groups, this places the results described in chapter 6 in perspective; the displayed survival advances for patients who underwent surgical debulking versus those who did not, might be more the consequence of the ability to receive both therapeutic steps (surgical debulking and chemotherapy) due to a less advanced disease stage and a lower frequency of underlying RCDII. Nevertheless, although the frequency of early- and late post-operative complications is high, we believe surgery should the preferred first step due to the risk of bowel perforation described in our chemotherapy-alone group. When the patient is eligible, surgical debulking should be followed by adjuvant chemotherapy for better overall survival.

Standard induction chemotherapy regimens applied in EATL are usually anthracycline-based, consisting of CHOP in most cases (cyclophosphamide, doxorubicin, vincristin, prednisolon)\(^51, 53-55\). Five-year survival percentages following surgical debulking and standard induction chemotherapy vary from 10% to 28%\(^51, 53, 56\). The most common reason for withholding chemotherapy after surgical debulking is a poor condition of the patient after surgery. Furthermore, a considerable proportion of EATL patients are unable to complete chemotherapy due to poor nutritional status, rapid progression of disease during treatment, and local or systemic complications\(^52\). As standard induction chemotherapy (CHOP) combined with surgical debulking showed persisting dismal survival percentages, several novel strategies are being assessed in EATL treatment. In the last decades, treatment intensification using high-dose chemotherapy and consolidation with haematopoietic stem-cell transplantation (SCT) as upfront therapeutic option has become an increasingly accepted treatment option in aggressive T-cell Non-Hodgkin Lymphomas. In chapter 7 we retrospectively reviewed the outcome of a large multicentre-cohort of EATL patients in order to compare differences in treatment response, relapse rate and survival between different therapy-regimens. With an overall five-year survival of only 10%, this study reiterates the dismal prognosis of EATL patients. Patients treated with the most aggressive treatment strategy, i.e. resection, chemotherapy and auSCT, showed the most favourable outcome with complete remission in all patients, the lowest relapse rate and one- and five-years overall survival of 100% and 33% respectively. Other studies have also demonstrated that consolidation therapy with auSCT combined with resection and intensive chemotherapy (CHOP combined with
etoposide [CHOEP]^{49,50} or methotrexate [IVE/MTX]^{49,51} is feasible and shows the most favourable outcome. Outcome in this group improved to a 5-year overall survival of 50-60\%^{50,51}. It should be noted that these studies included a variable EATL population consisting of only primary EATL^{51}, various types of peripheral T-cell non-Hodgkin lymphomas (NHL)^{50} or EATL-patients in whom various chemotherapy schemes preceded the auSCT^{49}. These results clearly support the idea that patients who can tolerate these more intensive approaches may benefit. Again, it should be noted that selection of patients eligible for transplantation is non-standardised in most studies (including our study) and a certain selection bias for more eligible patients could have contributed to the displayed survival advantages.

Besides intensification of chemotherapy and consolidation using auSCT, the evidence for the effectiveness of targeted therapy with monoclonal antibodies (mAbs) is increasing. Owing to their excellent potential for specific detection, delineation and selective treatment of systemic diseases, mAbs are broadly used as treatment-strategy in diseases such T-cell lymphomas. The rationale for this strategy is based on the utilization of the antigens expressed by tumour cells. Cytotoxic agents are delivered inside the malignant cell based on this antigen-expression using antibody-drug conjugates. CD30 is an ideal target for mAb-therapy due to its expression on EATL in the majority of cases^{57,58} and due to limited expression on normal tissues. Brentuximab vedotin is an anti-CD30 antibody^{59}. Following binding to CD30, brentuximab vedotin leads to cell-cycle arrest and apoptosis of the tumour cell. Several studies showed this agent to be well-tolerated and highly active in patients with relapsed Hodgkin’s Lymphoma^{60}, systemic anaplastic lymphoma or primary cutaneous T-cell NHL^{59}. The most frequent reported adverse event is peripheral neuropathy^{61}. This anti-CD30 mAb has been described to be effective for EATL in a case-description^{62}. Preliminary data in a limited number of patients with EATL shows multi-agent therapy consisting of intensive-chemotherapy combined with anti-CD30 followed by consolidation with BEAM and auSCT to be safe and effective, although longer follow-up is required. These results show promise for the future. Several other mAbs have been used for EATL in the past, for example the anti-CD52 mAb alemtuzumab. Although, this therapy might be effective in some studies^{63}, CD52 expression in EATL is usually low, relapse after initial response is high^{64} and this mAb is frequently associated with severe CMV infections and other complications^{65}.
Recent genetic analysis of EATLs may create opportunities for treatment strategies targeting mutations. Most commonly mutated signaling pathway in EATL, with frequent activating mutations, was found in the JAK-STAT pathway. This pathway is involved in survival and differentiation of immune cells. JAK-STAT inhibitors are approved for the treatment of various autoimmune disease (i.e. rheumatoid arthritis and myelofibrosis). Although the current available inhibitors show clinical activity, the ability to induce remission in hematologic malignancies seems limited. It would be interesting to explore possible new mutation-targeting treatment strategies for the future.

The most favourable treatment results are achieved in those who started treatment early in the disease course. Therefore, stringent strategies for early EATL diagnosis in patients with uncomplicated CD or RCDII should be implemented. One of the options would be to perform radioactive labelling of mAbs followed by PET-assisted detection of EATL.

Non-celiac gluten sensitivity
Non-celiac gluten sensitivity (NCGS) is characterized by intestinal (diarrhoea, abdominal discomfort or pain, bloating and flatulence) and/or extra intestinal symptoms (including fatigue, headache, lethargy) that occur after the ingestion of gluten and improve after gluten withdrawal in patients in whom CD and wheat allergy are excluded. In chapter 8 we reviewed current knowledge on NCGS and outlined different causal theories. While NCGS has been encountered with a great deal of skepticism for decades (the first case description was already published in 1978), there are several recent well-conducted studies that support the concept of gluten containing food causing symptoms in the absence of CD. While these initial positive observations lend strong support to the notion that gluten may induce symptoms in individuals without CD, these results are under debate. First of all, in some of the studies wheat instead of gluten was used as causative agent. It can be hypothesised that other proteins in grain, for example lectins or pest resistance molecules known as α-amylase/trypsin inhibitors (ATIs), might induce the adverse symptoms displayed in NCGS. Secondly, other studies suggests that short-chain carbohydrates (i.e., fermentable oligo-, di-, monosaccharides, and polyols [FODMAPs], which are found widely in grains) rather than gluten-peptides itself are responsible for the symptoms found in NCGS. It must be considered that the induction of symptoms by gluten in these studies might be a wheat- or carbohydrate-specific rather than a gluten-specific phenomenon. This theory is supported by the notion...
that both CD and NCGS are characterised by different immune activating mechanisms; in NCGS rather an innate than an adaptive immune response has been identified\textsuperscript{75, 87, 88}. Moreover, a recent study showed systemic innate immune activation in NCGS which was absent in CD and in healthy controls, suggesting translocation of microbial products due to compromised epithelial barrier integrity\textsuperscript{75, 89}. Contrary to that, other studies showed increased anti-gliadin antibody reactivity\textsuperscript{87, 90}, increased intraepithelial lymphocytes\textsuperscript{77, 89, 91, 92} and a higher incidence of HLA-DQ\textsuperscript{77, 93-95} which suggests a shared (genetic) predisposition with CD, although literature on these observations is conflicting\textsuperscript{89}. It cannot be excluded that some of these patients, in fact, had latent CD. Also, it has been suggested that these CD-mimicking observations may be the consequence of ongoing intestinal epithelial barrier defects rather than a primary immune response in NCGS\textsuperscript{89}.

Although all the above mentioned observations have not been consistently reproduced in the studies performed so far, all studies point towards an, albeit mild, immune activation in NCGS which deserves further crystallization. More clinical and translational studies are warranted to elucidate the immunostimulatory effect of different types of wheat proteins and to differentiate between gluten-sensitivity, grain-sensitivity and short-chain carbohydrate-sensitivity.

Despite the controversy regarding the causative agent and involved immune mechanisms in NCGS, there is an overwhelming interest in this condition both in the scientific community and the mass media. Unfortunately, the actual prevalence is difficult to establish. In some countries the number of individuals embracing a gluten-free diet is already well above 10\% of the population. A substantial part of this group consists of people who avoid gluten-containing food in the light of a healthier lifestyle. This theory (outlined in a bestselling book\textsuperscript{96}), holds wheat responsible for many negative health aspects including obesity, due to its high carbohydrate content. The theory that grains by means of their composition are unhealthy, should be distinguished from the question as to whether grains can cause clinical relevant symptoms in the absence of CD. In chapter 9 we evaluate the population prevalence and the clinical characteristics of self-reported gluten-sensitivity (srGS) in adults in the Netherlands. Among the 785 questionnaire respondents, forty-nine (6.2\%) reported symptoms related to the ingestion of gluten-containing food. The most frequently reported symptoms were bloating (74\%), abdominal discomfort (49\%) and flatulence (47\%). Tiredness and headache were the most frequent
extra-intestinal symptoms reported (35% and 17% respectively). Individuals reporting intestinal or extra-intestinal symptoms following gluten ingestion were younger and predominantly female. The frequency of symptoms varied widely from always (12%) to almost every day (33%) and a few days a month (31%). Dietary changes were initiated by twenty-three of srGS respondents; two (4%) followed a strict gluten-free diet (GFD) and 21 (43%) a gluten-restricted diet. It cannot be excluded that FODMAPs are in part responsible for the reported symptoms since abdominal discomfort related to these short-chain carbohydrate-containing food was more often reported in srGS individuals compared with the other respondents (73.5% vs. 21.7%). Comparing these results with recently worldwide conducted studies displays a high variety in srGS prevalence ranging from 13% in the UK to our reported 6.2%.\textsuperscript{97-100} These differences may be related to media attention, but data to support this hypothesis are lacking. Compared to the prevalence of NCGS in referral centers (with an objectified prevalence ranging between 3.2%\textsuperscript{91} and 6.0%\textsuperscript{69}) self-reported prevalence is two to four times higher. This might be the result of a limited number of individuals seeking medical assistance or to the fact that only a minority of self-reported NCGS patients ultimately clinically responds to gluten peptides in double blind placebo controlled trials\textsuperscript{72-80}. As mentioned above, other peptides or carbohydrates in wheat might be responsible for the signs and symptoms caused by wheat and this requires further study. The number of individuals embracing a strict gluten-free diet in srGS individuals is relatively low in most studies (around 4%\textsuperscript{97-100}) but higher compared to GFD adherence in the general population (0.6%)\textsuperscript{101}. This relatively low dietary adherence in srGS might be related to costs and availability of gluten-free products\textsuperscript{102}, social restrictions or low frequency of gluten-related complaints as described in a subset of our population.

To conclude, due to doubt on the role of gluten as the culprit food component, an ill-defined line between NCGS and latent CD in some studies and the absence of established biomarkers and immunological knowledge, accurate figures for epidemiological details are yet unavailable. Future research will certainly shed light on this whole spectrum of non-celiac gluten/grain sensitivity.
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