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EDITORIAL

Recent advances in celiac disease

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Abstract

Celiac disease now affects about one person in a hundred in Europe and North America. In this review, we consider a number of important and exciting recent developments, such as clinical associations, HLA-DQ2 and HLA-DQ8 predispositions, the concept of potential celiac disease, the use of new imaging/endoscopy techniques, and the development of refractory disease. This review will be of use to all internists, pediatricians and gastroenterologists.

Key words: Inflammation; Infection; Malabsorption; Pathophysiology; Physiology

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INTRODUCTION

Celiac disease (CD) is an immune-mediated enteropathy characterized by intolerance to gluten. CD is usually characterized by various gastrointestinal (GI) symptoms (e.g. diarrhea, malabsorption, weight loss) associated with consumption of grains containing gluten (wheat, barley, rye). Although some CD patients may have primarily GI symptoms, CD may be detected due to associated extraintestinal disorders, even without GI symptoms, or due to screening for CD based on a positive family history. CD has a strong association with HLA-DQ2 and HLA-DQ8. Serological testing for antibodies to tissue transglutaminase (tTG) is usually positive (-95%) in the untreated patient. Endoscopic and histological damage seen in the proximal intestine is characteristic, but not diagnostic. As CD is defined to be a gluten-sensitive enteropathy, definitive diagnosis ultimately depends on a positive small bowel biopsy and demonstration of a response to a gluten-free diet (GFD)[1]. Common serological changes include the appearance of anti-tTG and other antibodies, e.g. endomysial antibodies (EMA). These antibodies have been reported in some, but not all studies, to decline or disappear in association with a clinical and/or histological response to a gluten-free diet.

The clinical spectrum of CD includes patients with classical gastrointestinal symptoms (e.g. diarrhea and weight loss), those who are detected on screening because of a family history of CD or having a CD-associated autoimmune condition, or those who have a predisposition for developing CD but at a particular time of testing, conceivably could have no symptoms, negative CD serology and a histologically normal small bowel biopsy. Now, a CD-specific quality of life instrument has also been developed and validated psychometrically[2], and may prove useful in everyday clinical practice.

EPIDEMIOLOGY

CD is highly prevalent in Caucasian populations and their descendants. The age of clinical onset (based on diagnosis) is often described by some experts as bimodal: the first peak is at 8 to 12 mo of age, and the second during the third to fourth decades of life[3]. Recent studies suggest, however, that CD should be considered as a disorder that has a risk of developing throughout life, even in the elderly. Overall, CD is twice as frequent among females as compared to males, possibly because the necessary HLA haplotypes, DQ2/DQ8, are more frequent in female than in male CD patients (i.e. 94% *vs* 85%)[4]. In addition, CD may be detected more frequently in females because females tend to seek medical care more often than males, usually at a younger age. With aging, however, this female predominant pattern disappears. In the elderly, the ratio of newly diagnosed males is equivalent to newly diagnosed females. In the few DQ2/DQ8 -negative CD patients, there is a male excess, and only inheritance of a paternal DQ2 haplotype leads to a daughter's predominance[4].

While it is estimated that CD affects up to 2% of Caucasians, the risk is higher in first-degree relatives of affected sibling pairs (17%), monozygotic twins (75%), and HLA-identical siblings (40%). Indeed, the single most important risk factor for celiac disease is having a first-degree relative with already-defined CD, particularly a sibling[5]. The estimated prevalence of CD in first-degree relatives living in Minneapolis, USA is 11% of all affected family members who carried at-risk genotypes (HLA-DQ2 in more than 90% of cases, and the remainder carrying HLA-DQ8). About half of these persons have clinically "silent disease", and yet, their small bowel biopsies may show severe architectural changes[6]. This apparent disconnection in some patients between severity of symptoms with CD and the severity of histological abnormalities (typically defined in the proximal small bowel) may reflect the variable extent of histological involvement further along the length of the small intestine.

The prevalence of CD appears to be increasing, particularly as the population ages[7]. CD in the elderly has been reviewed and also likely reflects increased recognition of undiagnosed CD in this age group[8-10]. The estimated prevalence of CD in individuals in the United Kingdom between 45 to 76 years of age is approximately 1.2%. About 20% of all newly diagnosed celiac cases are over 60 years of age. The clinical presentation in these older individuals is variable, ranging from "silent" disease, to vague abdominal complaints, to anemia. These limited symptoms could also lead to a delay in diagnosis.

Prolonged gluten exposure in undiagnosed CD is seen to increase the incidence of autoimmune diseases, such as diabetes and autoimmune thyroiditis. As far as gastrointestinal complications are concerned, it is important to rule out collagenous and lymphocytic colitis[11,12], which may be mistaken for non-compliance with a gluten-free diet (GFD). Eight percent of elderly persons in nursing homes or long-term care centers may have associated small bowel bacterial overgrowth, manifesting with malabsorption and diarrhea-like symptoms. Nutrient depletion in these individuals is common, and the incidence of osteopenic bone disease is increased. Finally, neurological disorders, such as dementia, are becoming increasingly recognized in elderly patients with CD[13].

Screening serological tests (IgA and IgG) appear to be age-independent in adults. However, the elderly seem to have an increased incidence of sero-negative CD. Interestingly, the fate of different celiac antibodies in genetically at-risk children on a normal diet has been assessed, and, remarkably, these appear to spontaneously disappear[14]. Specifically, antibodies to tTG and EMA were spontaneously lost in 49% and 45%, despite continuing gluten exposure. Although the procedural risk of endoscopy and biopsy may be marginally increased in the elderly, endoscopic biopsy remains crucial as the "gold standard" for diagnosis of CD in this age group as well as in all other age groups.

GENETICS

It is conceivable that CD could be a heterogeneous disorder, not only with differing clinical presentations, but also different degrees of pathological change in the small intestinal mucosa. Further morphometric and immunohistochemical studies from geographically and genetically diverse populations are needed to confirm observations on increased intraepithelial lymphocytes (IELs) in otherwise architecturally normal small intestine.

A small percentage of EMA-positive patients may have a small bowel biopsy in which the mucosa might be considered by some pathologists to be architecturally normal. From 409 children who were positive for celiac-related antibodies, 24 (5.9%) of the individuals were reported to have an architecturally normal small intestinal mucosa, 46% (11 of these 24 patients) had increased CD3+ intraepithelial lymphocytes, and 71% (17 of the 24) had an increased density of gamma-delta + cells[15]. In 17 of these 24 persons (70%), the number of lamina propria CD25+ cells was increased, and/or the expression of ICAM-1 and crypt HLA-DR was enhanced. Interestingly, in those persons with apparently normal jejunal histology, there appeared to be immunohistological evidence of immune activation in the epithelium, lamina propria and intestinal crypts. A GFD is usually not recommended in individuals with an architecturally-normal small bowel biopsy. Sophisticated immunohistochemical findings, such as these, raise the question as to whether definition of "normal" needs to be extended, and whether such subjects with abnormal antibodies in the intestinal biopsy may need further monitoring to determine if a GFD is indicated. In addition, duodenal mucosal tTG detection improves the sensitivity of diagnosis in CD for those with very mild histological changes, i.e. Marsh 1 lesions[16]. Thus, the definition of CD may conceivably extend to persons with an architecturally normal mucosal biopsy, but with abnormal CD-associated immunohistochemical changes. Additional studies are needed to confirm, evaluate and further elucidate these interesting observations.

Gluten has specific peptide sequences which show

HLA-DQ2 or HLA-DQ8 restrictive binding motifs across the various gluten proteins. About 40% of the heritability of CD includes the human leukocyte antigen HLA-DQ2 and HLA-DQ8 heterodimers. The HLA DQ2 and HLA-DQ8 molecules are necessary to develop CD, but are not, in themselves, sufficient for phenotypic expression of the disease. Indeed, HLA markers only explain an estimated 40% of the heritable risk for CD. Therefore, other non-HLA genes must also be involved. One of these may relate to genetic variants on chromosome 19, in the myosin IXB gene (i.e. MYO9B), and may potentially predict responsiveness to a GFD[17].

CD-associated HLA-DQ molecules bind and present gluten peptides to antigen-specific T-cells in the intestinal mucosa, and induce T-cell proliferation as well as cytokine secretion. Siblings who share HLA haplotypes have a greater likelihood of concordance with CD than the generally estimated risk for siblings. A small percentage of CD patients are DQ-2 negative, usually being DR4-positive for the class 2 antigen, DQ8. Carrying two copies of DQB1*02 is associated with an even greater risk for CD, but does not predict an earlier age of onset of disease or disease severity. This suggests that assessment of copy number of the DQB1*02 allele could permit stratification of risk[18].

In twin studies in which CD was diagnosed by small bowel biopsy and serology in one twin, monozygotic twin pairs had a high probability of being concordant with CD in the second twin: monozygotic and dizygotic co-twins had 70% and 9% cumulative probability of having symptomatic or "silent" forms of CD, respectively, within 5 years[19]. Under ACE (additive genetic, common and unshared environmental factors/models) with CD prevalences of 1/91 and 1/1000, heritability estimates were 87% and 57%, respectively[19].

The overall risk of siblings of children with CD developing CD was 10% in an Italian population, but the risk estimate ranged from 0.1% to 29% when HLA-DQ information of the proband, parents and sibling was considered[20]. The risk for the sibling developing CD was less than 1% for 40% of the sibs of the probands, 1% to 10% for 30% of the probands, and above 25% for the remaining 30% of the siblings. Thus, information about the risk for a second child to develop CD can be provided to parents with a child with CD. This antenatal estimate of the risk of CD in the child may be useful to provide early diagnosis and management, as well as providing focused and specific follow-up depending upon the risk stratification. Of importance, because CD is treated with a GFD and the resultant quality of life of the CD patient is high, the antenatal diagnosis of CD should not be used as a reason to consider termination of the pregnancy.

A double dose of DR3 (often with DQ2) is associated with an even higher risk of development of CD. HLA genotyping is thought to be useful to exclude CD in family members, or in persons in whom there is an increased risk of CD, such as those with Turner syndrome or Down's syndrome[21].

A strategy that combines gene expression profiling of intestinal biopsy specimens, linkage region information, and different bio-informatic tools for the selection of potential regulatory single-nucleotide polymorphisms (SNPs) has been used to search for novel candidate determinants of predisposition to CD in previously identified linkage regions[22]. Abnormalities in functional proteins have been observed in CD. By using genetic association analysis with a SNPs approach, the tight junction permeability barrier genes, KRD 3 (2SNPs) and MAGI 2 (2SNPs), were shown to be associated with CD in British and Dutch persons[23].

In addition to the involvement of HLA class I restricted CD8+ T-cells, the innate immune system may also be involved in CD. In the mucosa of untreated CD, there is an increase in activated CD8+ T-cells containing large granzyme-B (GrB)-positive granules, as well as cell surface expression of the Fas ligand (FasL). CD8+ T-cell cytotoxicity occurs in the mucosa of patients with active CD (through Fas and FasL-mediated killing of enterocytes). The gliadin interaction for this CD8+ T cell-mediated response (occurring through TCR/HLA class I) induces enterocyte apoptosis[24].

Variation among four closely linked genes on chromosome 4q27 represents a non-HLA genetic risk factor for CD, mapping to a region that contains IL2, IL21, TENR, and K1AA1109[25]. Also, multiple common variants for CD influencing immune gene expression have been defined with second generation genome wide association studies[26].

Genetic studies have also recently identified nine non-HLA loci that contribute to CD risk. Combining HLA and non-HLA risk genotypes increases the sensitivity of CD diagnosis by 6.2% compared with using only HLA for identification, with only a slight decrease in specificity[27]. There may be a quantitative relationship between the type and proportion of DQ heterodimers and the risk of CD[28].

PATHOGENESIS

Gliadins, derived from an alcohol soluble fraction of gluten, are storage proteins that are ingredients in wheat, barley and rye as well as other grains that contain gluten (albeit of less importance). Gliadins are characterized by a high content of glutamine and proline residues. Glutenins are insoluble in aqueous alcohol and are different in structure from the glutens. The early immune response in CD patients is directed towards several of these peptides, while the long-standing inflammatory response may be driven by gluten peptides deamidated or cross-linked by tTG and bound more tightly to HLA-DQ2 and HLA-DQ8. The tTG catalyzed modifications in gliadin are not restricted to single gliadin types or epitopes[29]. Prolamines in barley and rye are known as hordein and secalin, respectively. These barley and rye prolamines induce an mRNA interferon-gamma response in celiac mucosa[30]. The α-2 gliadin-33mer appears to cross the brush border membrane (BBM) of the jejunal enterocyte by a dose-dependent mechanism. Both the uncleaved as well as the degraded form of the 33mer translocate into the enterocyte[31]. Interferon-gamma enhances the trans-

location of the 33mer.

After passage across the BBM, gliadins trigger a Th-1 type-dependent inflammatory reaction. The effects of gliadin peptides and A-gliadin peptide P31-43 on cell lines and cultured small intestinal biopsies are mediated through epidermal growth factor receptor (EGFR) activation by interfering with EGFR endocytosis[32]. Gliadin has an immunogenic effect, but also directly affects cultured cells and intestinal preparations by way of separate peptides such as A-gliadin p31-43 (P31-43). The gliadin-induced delay of EGFR endocytosis in cultured intestinal biopsies suggests a role for EGFR activation in CD[32]. A 33 amino acid fragment of α-2 gliadin is an important trigger of the inflammatory process. In patients with active CD, there is transepithelial translocation of the attack 33mer, as well as incomplete degradation of the 33mer during intestinal transport.

In persons with active CD, there is a marked accumulation of polarized Th-1 cells that produce large amounts of interferon γ (IFNγ). T-bet, a member of the T-box family of transcription factors, is present in CD4+ and CD8+ mucosal T cells in patients with CD. Interleukin 21 (IL-21) is present in activated CD4+ T cells, as well as in natural killer T cells (NK T cells). IL-21 regulates the production of cytokines by T cell subsets. IL-21 increases the expression of Stat4 and T-bet, and stimulates the production of IFNγ in human T cells. In duodenal mucosal biopsies from patients with CD, there is enhanced IL-21 RNA and protein expression, and neutralization of IL-21 largely prevents peptic-tryptic and digest-enhanced IL-21 expression[33,34].

In persons with a genetic susceptibility to develop CD, gliadin interacts with the intestine to trigger disassembly of the inter-enterocyte tight junctions (TJs). The 2α-gliadin 20mer synthetic peptides of gliadin bind to the chemokine receptor, CXCR3. This binding induces MyD88-dependent zonulin release. In turn, zonulin release leads to increased intestinal permeability[35]. Increased intestinal permeability occurs prior to the onset of clinically apparent CD. Even on a GFD, the initially enhanced intestinal permeability does not necessarily return to normal.

Another important aspect related to the pathogenesis of CD may include the intestinal microflora that may be central to the clinical expression of the disease. In one recent report, enrichment in the mucosa-associated microbiota with rod-shaped bacteria in those that developed CD may have contributed to the so-called "epidemic" in Swedish children less than two years of age[36].

Finally, other recent studies related to CD pathogenesis have directly used intestinal biopsy specimens from CD and non-CD persons. For example, IL-15 receptor α mRNA expression is higher in duodenal biopsies from CD compared to non-CD persons, regardless of whether the CD subjects are or are not consuming gluten. IL-15 induces an intense immunological response in CD, with the production of nitrites and IFN gamma[37].

SEROLOGY

Serological markers of significance include EMA and tTG antibodies. The sensitivity of tTG is 98% and specificity 96%, whereas the EMA is 100% specific and sensitivity is greater than 90%[38]. Assays for tTG antibodies are largely based on the dominant antigen in the EMA test, however, tTG assays are more reliable and more reproducible, largely because the EMA is a qualitative assay and tTG assays are quantitative.

The antibodies to tTG and deamidated gliadin peptide (DGP) have been combined in a multiplex immunoassay of persons suspected as having CD, to potentially provide a complete antibody phenotype[39], and thereby to improve the performance characteristics of the serological testing. A meta-analysis has shown that the tTG antibody test out-performs the DGP antibody test, with a 5.2% greater sensitivity (93.0% *vs* 87.8%) and a 2.4% greater specificity (96.5% *vs* 94.1%), respectively[40].

DGP has been suggested to possibly be a better diagnostic test for CD before institution of a GFD than is the conventional gliadin antibody testing: the sensitivity, specificity, and accuracy of deamidated gliadin-IgA (74%, 95%, and 86%), deamidated gliadin-IgA (65%, 98%, and 84%), and deamidated gliadin-IgA + IgG (75%, 94%, and 86%), were superior to gliadin-IgA (63%, 90%, and 79%) ($P > 0.05$) and gliadin-IgG (42%, 90%, and 60%) ($P > 0.01$), and were similar to tTG-IgA (78%, 98%, and 90%)[39]. Further comparative evaluation with more modern serological assay methods would be useful, including tTG antibodies.

Because the small bowel biopsy in the person with CD does not have a pathognomic histological feature, serological testing may have an important supportive role in providing added information for the diagnosis of CD. Tissue transglutaminase (tTG) catalyzes the Ca^{2+}-dependant formation of cross links between protein-bound glutamine and glycine residues. The glutamine residue can be deamidated to glutamic acid by tTG, including specific glutamines in gluten-containing proteins. Deamidated gluten proteins have enhanced affinity for the HLA-DQ heterodimer of antigen-presenting cells. This activates T-lymphocytes and produces a T-helper type 1 response in the mucosa of celiac patients. The tTG-gluten complex is processed by B-cells, and presented to gluten-specific T cells, that give rise to tTG antibody T-helper type 2 response[41]. The tTG autoantibodies interact with extracellular membrane-bound transglutaminase, and thereby play an important role in proliferation of epithelial cells in persons with predisposition to CD.

The tTG is responsible for post-translational modification of proteins by introduction of lysine crosslinks, as well as deamidation. The IgA anti-tTG responses in CD and in dermatitis herpetiformis are focused on the region of tTG responsible for its transamidation and deamidation reactions, whereas the IgG response targets other regions of the enzyme[42].

The performance of serum anti-tTG may depend on clinical presentation of CD; e.g. classic symptomatic disease or silent asymptomatic disease. In patients estimated to have Marsh-Ⅲ A, B, or C degree of villous atrophy,

the sensitivity, specificity, positive and negative predictive values of the anti-tTG antibody test were 71%, 65%, 91%, and 30%, respectively[43]. The sensitivity was 90% for subjects with total villous atrophy, and only 42% for those with partial villous atrophy. In persons thought to have a high pretest probability of having CD based on symptoms such as weight loss, anemia, or diarrhea, 9.1% were anti-tTG negative[44], indicating that serological testing may miss a substantial number of cases of untreated CD that are antibody negative. Strongly positive tTG assay results without CD biopsy changes have also been recorded[45]. In the latter, it is not known if further biopsies at a later date will reveal the typical morphological changes of untreated CD.

The supply sources for EMA are limited to monkey esophagus or umbilical cord, and many assays are done "in-house" that may not be readily duplicated in other laboratories. EMA are considered to be highly sensitive and specific for serological changes seen in untreated CD. However, EMA assays are expensive, qualitative, and therefore subjective. EMA is increasingly being replaced by serological testing for antibodies to tTG, especially since the anti-tTG assay can be more precisely quantitated.

The EMA binding patterns and serum samples from CD patients are tTG-2 targeted, and the humoral response against tTG occurs at the level of the intestinal mucosa. tTG-2 targeted extracellular IgA deposits have been demonstrated by immunofluorescence in the small bowel mucosa in untreated celiac subjects, even when they are absent from the serum. In those subjects suspected of having CD but who are EMA and anti-tTG negative, finding the tTG-2 targeted antibody in the jejunal mucosa may help to make the diagnosis of CD[46].

Frozen sections of small bowel specimens were evaluated by immuno-fluorescence using rabbit antibody against human IgA. Although at best, semi-quantitative, these immunofluorescent deposits may be better initial markers for gluten sensitivity than small bowel mucosal IEL densities[47]. While architectural changes, such as villous atrophy, may lead to suspicion of untreated CD, tTG-2 specific IgA deposits may potentially be more useful. Further studies are needed.

Seronegative (EMA or tTG) CD occurs in less than 10% of celiacs, particularly in those with lesser degrees of villous atrophy. The presence of EMA in subjects with an architecturally-normal small bowel biopsy could indicate early developing CD. Serum and intestinal celiac anti-autobodies and intra-epithelial lymphocytes have been assessed as possible indicators of developing CD. Celiac autoantibody deposits have been recorded to provide a sensitivity and specificity of 93% and 93%, respectively, in detecting subsequent CD; this is compared to 59% and 57% for CD3+; 76% and 60% for gamma-delta+, and 88% and 71% for villous tip intra-epithelial lymphocytes[48].

Simple "in the office" anti-tTG tests have been developed commercially, and the blood drop-based assay for IgA anti-tTG was reported to have a sensitivity of 90% and a specificity of 95%[49]. The sensitivity and specificity of serum anti-tTG is laboratory-dependent, and assay results may differ for clinical compared to research laboratories. Because CD does not have a pathognomic histological feature, serology may have a supportive role in making the diagnosis. As CD is defined as a gluten-sensitive enteropathy, a clinical or serological response to a GFD is essential to establish a diagnosis of CD. Sometimes, re-biopsy after a GFD is necessary, or even further evaluation after a gluten challenge may be required.

A normal tTG level does not predict recovery of villous atrophy in celiac subjects on a GFD. For example, 16 of 48 (33%) subjects with CD on a GFD had persistent villous atrophy, but 7 of these 16 (44%) had a normal tTG[50]. In a multicenter, prospective study involving adult subjects attending one of several primary care practices, and in individuals not having symptoms or a condition known to be associated with CD, initial testing was done with anti-tTG. Those with elevated anti-tTG were tested for EMA (IgA), and then those in turn who were positive for EMA underwent an intestinal biopsy and HLA typing[51]. A positive anti-tTG was found in 3.1%, and the prevalence of CD in the serologically screened sample was 2.3%. When a similar study was performed in a university hospital, the prevalence of CD was 3.5%, and a negative HLA-DQ type excluded the diagnosis[52]. However, the "addition of HLA-DQ typing to TGA and EMA testing, and the addition of serological testing to HLA-DQ typing, provided the same measures of test performance as either testing strategy alone"[52].

Because the EMA and anti-tTG responses may remain elevated in CD on a GFD, it may be useful to measure soluble CD163, a scavenger receptor shed by tissue macrophages and correlated with the inflammatory lesion in CD. Those subjects with a more severe (Marsh grade 3) lesion had higher levels of CD163 than did those with a milder (Marsh grade 2, grade 1 or grade 0) lesion[53]. Further studies are needed.

There are three fatty acid binding proteins in the cytosol of the intestine: intestinal FABP (I-FABP), liver FABP (L-FABP), and ileal bile acid binding protein (I-BABP). These are present in increased amounts in the serum of persons with enterocyte damage from, for example, mesenteric thrombosis or necrotizing enteritis. Because I-FABP and L-FABP are found predominantly in the enterocytes in the upper portion of the jejunal villi, it is not surprising that their concentration is increased in the plasma of persons with CD. When measurements were made within one year of the introduction of a GFD, these initially increased I- and L-FABP levels fell to normal[54]. Together with following the patient's symptoms, quality of life, and celiac serology, assessing intestinal permeability may potentially prove to be a useful non-invasive test to follow the histological improvement of CD patients on a GFD.

The current standard for the assessment of adherence to a GFD in adult CD patients is largely based on a personal clinical evaluation. However, most serological assays appear to compare adequately in sensitivity and specificity to a thorough nutritional evaluation of the assessment of adherence to a GFD[55]. This is important, since only approximately 45%-80% of patients with CD adhere strictly to a GFD. This is thought to place them at increased risk

of developing metabolic bone disease, anemia, gastrointestinal symptoms, as well as impaired psychological well-being and quality of life.

MUCOSAL HISTOLOGY

Criteria for the diagnosis of CD include the initial demonstration of small bowel architectural changes including mucosal villous atrophy with crypt hyperplasia, along with increased intra-epithelial lymphocytosis. However, this may be a slowly developing process and the changes are not specific. In addition, some persons may suffer from CD symptoms before histological evidence can be documented. While some authors have suggested that an anti-tTG level can be defined which gives a positive predictive value of 100% for CD[56], it remains the standard of practice to always obtain a biopsy to determine if the histological changes of untreated CD are present before initiation of a GFD.

Some have noted that there may be significant differences between pathologists in mucosal biopsy interpretation[57]. The older Marsh classification, as modified by Oberhuber and colleagues, continues to be used by many pathologists. But for some, it may be considered complex because there are many different diagnostic categories. A simpler grading system has been proposed[57] based on three villous morphologies (A, non-atrophic; B1, atrophic, villous-ratio < 3:1; B2, atrophic, villi no longer detectable), and an intraepithelial lymphocyte count of > 25/100 enterocytes. Compared to the older classification system, this simpler classification schema was thought by the investigators to be superior.

However, the severity of villous atrophy based on histological analysis of biopsy specimens taken from the proximal intestine does not necessarily predict the severity of symptoms in CD for either children or adults. For example, when clinical symptoms of 18 CD patients with a good histological recovery were compared with 13 CD who had persistent small intestinal villous atrophy despite maintaining a GFD, symptoms could be absent despite the persistence of morphological abnormalities[58]. Other authors also noted the lack of association between the histological CD lesion and clinical manifestations[59]. Indeed, the lack of correlation between the degree of villous atrophy and symptoms was stressed in a further study of 499 CD patients, in which 44% had a classical presentation and 56% had atypical or silent CD[60]. These findings are not surprising, however, since the response to a GFD occurs initially in the most distal small bowel. Months to even years on a strict GFD may be needed before improvements in the proximal intestinal mucosa occur.

While duodenal biopsy represents the "gold standard" for the diagnosis of CD, capsule endoscopy (CE) has revealed that over a third of celiac patients have macroscopic mucosal changes extending beyond the duodenum, and in approximately 7%, the entire small bowel was involved[61]. As compared with duodenal biopsy for detecting changes in CD, the sensitivity of CE was reported to be 88%, specificity 91%, positive predictive value 97%, and negative predictive value 71%.

"Latent CD" is defined as abnormal celiac serology and a normal small bowel biopsy (Marsh stage 0). These so-called "latent CD" patients have an increased hazard (HR) ratio for death comparable to those with Marsh 1-2 and Marsh 3: 1.35; 95% CI, 1.14-1.58, median follow-up, 6.7 years; HR, 1.72; 95% CI, 1.64-1.79; median follow-up, 7.2 years; HR, 1.39; 95% CI, 1.33-1.45; median follow-up, 8.8 years, respectively[62]. This corresponded with excess mortality of 1.7 per 1000 person-years in "latent CD", 10.8 in Marsh 1-2, and 2.9 in Marsh 3 stage CD. This raises the possibility that it may be important to diagnose very early CD. However, this label of "latent CD" may differ from the original definition of latent CD (without serological studies) where abnormal small intestinal architectural changes were induced with a high gluten-containing diet (initially reported in dermatitis herpetiformis) and then normalized on a GFD.

Given that duodenal biopsy is still the "gold standard" for the diagnosis of CD, it is of interest to know that when only two duodenal biopsies are obtained, diagnosis of untreated CD is confirmed in 90%, however, increasing the number of biopsies to 3 increased detection to 95%, and to 4 biopsies, 100% respectively[63].

While some present with symptoms and a small bowel biopsy is done to exclude CD, others will present with positive serology and a duodenal biopsy is then obtained. Occasionally lesions may be patchy or detected only in the duodenum, resulting in potential for sampling error and a false-negative result. Confocal endomicroscopy (CEM) is a novel method that permits magnification *in vivo* of the gastrointestinal mucosa by up to 1000-fold. In persons with known CD, accuracy of CEM in diagnosing CD was reported to be excellent, with receiver operator characteristics under the curve of 0.946, sensitivity of 94%, and specificity of 92%[64]. CEM was also sensitive in the detection of histological changes following treatment with a GFD.

"Lymphocytic enteritis" (Marsh 1) may be associated with symptoms, yet serological markers of CD appeared to be of limited value in identifying these individuals. In 130 of 221 first-degree relatives of HLA-DQ2-positive patients with CD, relatives were positive also for HLA-DQ2, and 49% were Marsh 0, 25% Marsh 1, < 1% Marsh 2 and 10% Marsh 3. Only 17 of 221 relatives had positive serological markers for CD[65]. These authors argued that the higher number of symptomatic patients with lymphocytic enteritis (Marsh 1) supports HLA-DQ2 genotyping strategy followed by duodenal biopsy in relatives of CD patients. Further studies to confirm these observations are needed.

Anti-tTG levels have continued to be used in assessing initiation and maintenance of a GFD. It is believed that tTG levels might be followed to reduce the risk of complications and monitor histological changes in the upper small bowel[66].

CLINICAL PHENOTYPES

The ESPGHAN (European Society of Pediatric Gastro-

enterology, Hepatology and Nutrition) criteria distinguish between three different forms of CD so that classification might be more precise: the latent or *potential* form defined by the presence of anti-celiac antibodies; the silent form (*asymptomatic*) defined by the presence of anti-celiac antibodies and villous atrophy of the small intestine; and the symptomatic form defined by the presence of anti-celiac antibodies, villous atrophy and clinical symptoms.

The adult height of children with classical CD (e.g. symptomatic with diarrhea) is influenced by their compliance to a GFD. Children diagnosed with CD after 4 years of age show a slower and less complete catch-up growth. A delayed diagnosis of CD may be associated with a shorter adult height in men, but not in women[67].

While abdominal symptoms may respond quickly to a GFD, it may take up to a year or more after the introduction of a GFD for persons with CD to achieve normalization of their initially abnormal small bowel biopsy. Elderly patients respond more slowly than younger patients to a GFD.

"Gluten sensitivity" may be defined as symptoms, such as diarrhea, apparently induced by gluten-containing foods. These have been reported in the absence of changes in small intestinal histology. In persons with diarrhea-predominant irritable bowel syndrome (D-IBS), stool frequency and the gastrointestinal symptoms score return to normal values in 60% of D-IBS subjects who were positive for HLA-DQ2 and CD-associated serum IgG after six months on a GFD, compared to only 12% who were negative[68].

Among the complications of undiagnosed and, therefore, untreated CD are growth failure in children, infertility, anemia, osteoporosis, small intestinal non-Hodgkin lymphoma[69], and a 3.9-fold increased all-cause mortality rate[70]. Potentially, this may underscore the importance of diagnosing and treating even latent CD.

Celiac patients were reported to have a 5.4-fold higher risk of non-Hodgkin's lymphoma, but no increased risk of Hodgkin's or chronic lymphatic leukemia. A shared susceptibility amongst siblings is observed[69]. It remains controversial whether there is an increased risk of developing lymphoma in CD if the disease is asymptomatic[58,71].

There is a 5-fold increase in risk of lymphoproliferative malignancy in CD in comparison to the general population[72].

CLINICAL ASSOCIATIONS

The prevalence of autoimmune diseases (e.g. autoimmune thyroid disease) is increased in persons with CD, as compared with the healthy control population. Conversely, CD is increased in persons with autoimmune diseases. The cumulative risk of autoimmune disease in patients with CD is 8% at age 15, and 16% at age 30 years. Factors associated with an increased risk of autoimmune diseases associated with CD include a family history of autoimmune disorders, and a diagnosis of CD before the age of 36[73]. Once the diagnosis of CD has been made, patients who are adherent to a GFD have a 6% risk of developing autoimmune disease at 10 years, *vs* 16% in those who are not compliant with a GFD. Expressed differently, the incidence of autoimmune disease is 5.4 per thousand patient years during adherence to a GFD, *vs* 11.3 per thousand patient years during non-adherence.

Asymptomatic CD is also seen in children and adults with autoimmune hepatitis and autoimmune bile duct disease. CD may be associated with asymptomatic increases in transaminase values. Persons with autoimmune liver disease should be examined for possible CD. In persons with CD who have acute hepatitis, an autoimmune cause should be suspected[74].

More and more clinical associations have been suggested for CD. For example, 42% of CD patients had oral soft tissue lesions, as compared to only 2% of non-CD patients[75]. Recurrent aphthous stomatitis disappeared in 89% of patients after one year of a GFD.

Biopsy-defined CD has a 4-fold higher prevalence in those with irritable bowel syndrome[76]. Mental disorders, non-compliance with the GFD, active medical co-morbidities, and dissatisfaction with doctor/patient communication were associated with reduced CD Questionnaire scores[77].

Up to 10% of patients with CD have neurological symptoms ranging from polyneuropathy, epilepsy, myoclonus, multifocal leukoencephalopathy, dementia, chorea, migraine, memory/attention impairment and peripheral axonal and demyelinating neuropathies as well as acetylcholine-antibody positive myasthenia gravis[78]. Autoimmunity may act as a mechanism triggering neurological dysfunction[79], and anti-neuronal, anti-gliadin and tTG antibodies may contribute to neurological impairment through Apaf-1 activation with Bax and cytochrome C translocation, leading to impairment of mitochondrial-dependent apoptosis. There is no statistically significant association between CD and subsequent development of Parkinson's disease, Alzheimer's disease, hereditary ataxia, symptoms of ataxia, Huntington's disease, or spinal muscular atrophy[80].

Adult but not pediatric patients with CD have an increased risk of sepsis, particularly pneumococcus infection[81]. In CD there is an increased prevalence of splenic hypofunction[82].

Using community-based cohorts and a record-linkage database, the adjusted relative risk of cardiovascular disease in CD was 2.5 for new EMA positive *vs* EMA negative individuals[83]. This suggests that CD may be associated with an increased risk of cardiovascular outcome. There may also be an association between CD and eosinophilic esophagitis[84].

Aldolase B deficiency causes hereditary fructose intolerance, and this may be associated with CD[85]. In CD patients as compared with individuals with dyspepsia or healthy controls, serum ghrelin concentrations are higher, not correlated to the severity of duodenal histological lesions, and revert to normal during the institution of a GFD, despite persistent duodenal lymphocytic infiltration[86]. It is not clear if these alterations in ghrelin concentrations have any biological importance in CD.

In 18 CD patients, there was increased intestinal 5-HT-enterochromaffin cell numbers, higher peak plasma 5-HT levels and postprandial area under the curve of 5-HT levels after a high-carbohydrate meal, as well as increased platelet 5-HT stores[87]. The authors suggested that serotonin excess may mediate dyspeptic symptoms in untreated CD. Further evaluation is required.

A meta-analysis of serological or histological diagnosis of CD in unselected adults with dyspepsia showed that the numerically increased prevalence was not statistically significant[88]. Another systematic review and meta-analysis by the same group, examining 14 studies with 2,278 persons diagnosed with IBS, had an approximately 4% prevalence of CD. The OR for biopsy-proven CD in IBS cases *vs* controls was 4.34[89]. In children with CD, 40% had elevated transaminase values; of those with elevated transaminase values, 95% were cryptogenic and normalized on a GFD, and 5% had autoimmune hepatitis that required immunosuppression plus a GFD to normalize clinical and biochemical parameters[90].

TREATMENT

Gluten free diet

What is the definition of a GFD? Even "gluten-free" products may not be completely free of gluten. In 1998, the World Health Organization/Food and Agriculture Organizations Commission proposed that foods which are said to be "gluten-free" could not contain more than 200 ppm of gluten. Each individual with CD may have a unique threshold or tolerance to the amount of gluten in the diet. Daily gluten intake of less than 10 mg is unlikely to cause significant histological abnormalities in the intestine of patients with CD[91]. Patients adhering to a GFD report an improved health related quality of life.

A systematic review has revisited the complications and need for long term follow-up in CD[92]. A 16-question disease-specific symptom index has been validated in adults with CD[93]. Such an index might be used to monitor the response of a CD patient to a GFD.

The treatment of CD is the life-long use of a GFD. A primary goal in the care of patients with CD is to improve the quality of their lives, through a collaboration of the stakeholders[94]. Lanzini *et al*[95] assessed in 465 consecutive CD patients, the histological outcome after a GFD consumed for a median of 16 mo. While CD serology became negative in 83% of CD patients with Marsh Ⅲ lesions on a GFD, mucosal biopsy histology normalized in only 8%, improved except for increased intraepithelial lymphocytes in 65%, was unchanged in 26% and worsened in 1%. The authors concluded that "complete normalization of duodenal lesions is exceptionally rare in adult celiac patients despite adherence to GFD, symptoms disappearance and negative CD related serology".

Early diagnosis and treatment are important in CD, as some of the associated complications may be irreversible, unless the CD is treated[96]. Growth retardation, osteoporosis and abnormal dentition will remain permanent if not treated early. The prevalence of associated depression is up to 37%, similar to that of persons with other chronic conditions[97].

In children with CD, long-term consumption of oats may be well tolerated[98], although concern has been expressed regarding possible contamination of oats with other gluten-containing grains. Other investigators have demonstrated that transamidation of wheat flour inhibits the response to gliadin of intestinal T cells in CD[99]. It has always been assumed that patients with CD must remain on a GFD for life. However, up to 10% of CD patients diagnosed in childhood were reported to develop long-term latency of their CD when returning to a gluten-containing diet[100]. In patients who have been on a GFD and have no symptoms, even if there were small bowel CD-like histological abnormalities which remained, some had no evidence of clinical relapse even though they had been on a gluten-containing "normal" diet for more than 2 years.

Individuals with villous atrophy but no symptoms are said to have "silent" CD. Almost half of CD patients may clinically tolerate a gluten-containing diet, yet continue to have mucosal abnormalities. Indeed, approximately 10% of CD patients diagnosed in childhood may develop clinical tolerance to gluten. In the United Kingdom, about one-third of CD subjects are under no active follow-up[101].

Alternatives to a gluten free diet

There may be poor compliance with the GFD because it is difficult and gluten-free products are expensive. For these reasons, new approaches have been taken in the treatment of CD. Some of these include orally administered endopeptidase, antagonists to S100B protein, IL-15 blockers, elemental diets and transamidation of wheat flour. Lactobacilli added to sourdough for fermentation are able to break down the proline-/glutamine-rich gluten peptide. This may play a role in the future treatment of CD[102]. Supplementation of nutrients may become essential depending upon the severity of malnutrition. One double–blind placebo-controlled multicenter trial showed a significant improvement in general well being after 6 mo of supplementation with vitamin B[103].

Prolyl-endopeptidases are able to digest ingested gluten. Oral therapy with prolyl-endopeptidases, exogenous protease enzymes, represents a new approach to managing CD[104]. Bacterial prolyl-endopeptidase from *Flavobacterium meningosepticum* removes gluten toxicity by cleaving it into small fragments which lack T-cell stimulatory properties[105]. After prolonged exposure to high concentrations of bacterial prolyl-endopeptidase, the amount of immunostimulatory gliadin peptides reaching the local immune system in CD is decreased[106]. The prolyl-endoprotease from *Aspergillus niger* (AN-PEP) is a member of the serine peptidase family, and this degrades gluten peptides rapidly[107]. This AN-PEP is capable of accelerating the degradation of gluten in a gastrointestinal model that closely mimics *in-vivo* digestion[108]. The

pH optimum of the enzyme is compatible with that found in the stomach, and the enzyme is resistant to degradation by pepsin.

The gluten proteins may be purposely modified to abolish their capacity to stimulate the interferon gamma from CD4+ T-cells[109]. Another approach to the therapy of CD is the designing of non-toxic wheat, rye or barley based on their protein homology[110,111].

Other therapeutic approaches would include the binding of gluten to HLA-DQ2 or HLA-DQ8, or blocking the gluten-reactive T cells by immunotherapy (e.g. vaccination). For example, transamidation of wheat flour with a food-grade enzyme and an appropriate amine donor (microbial transglutaminase and lysine methyl ester) can be used to block the T-cell mediated gliadin activity[99]. Gluten contains many immunogenic peptides, but there may be weak varieties with a natural low number of T-cell-stimulatory epitopes[111].

Polymeric binders reduce the deleterious effects of gliadin on intestinal epithelium in cultured cells and transgenic mice[112]. These binders have a strong affinity for gliadin, inhibiting cytoskeleton disorganization and ultrastructural changes in intestinal epithelial cells. Their beneficial use in humans remains to be established.

Antigen-presenting cells include dendritic cells, macrophages and B-cells. A unique subset of dendritic cells appears to be responsible for local activation of gluten-reactive T-cells in the celiac lesion[113]. Enteric glial cells (EGC) release neurotropic factors and are activated by inflammatory insults. EGC-derived S100B protein released in astroglial cells is increased in the duodenum of patients with CD, with increased S100B messenger RNA and protein expression, increased iNOS protein expression, and increased nitrite production in treated CD[114]. This does not occur in those CD patients on a GFD, or in non-CD control subjects. Products derived to block EGC-derived S100B protein may have a therapeutic role.

IL-15 has proinflammatory and anti-apoptotic properties. IL-15 is over-expressed in the enterocytes and lamina propria mononuclear cells of untreated CD, where its level reportedly correlates with the degree of mucosal damage[115]. IL-15 also promotes IEL survival. Blocking IL-15 and suppressing uncontrolled IEL activation and survival has the potential to provide a new therapeutic approach to prevent tissue damage in CD. In the intestinal mucosa of CD patients, IL-15 impairs Smad3-dependant TGF-beta signaling in human T-lymphocytes downstream from Smad3 nuclear translocation[116]. There is upregulation of phosphor-c-jun. This provides further support to the suggestion of the potential therapeutic effect of blocking IL-15.

Intestinal permeability is increased in patients with CD, and is associated with alterations in tight junction proteins (e.g. zonulin). Addition of zonulin may prevent T-cell mediated stimulation in CD. In a double-blind randomized placebo-controlled study of milligram doses of AT-1001, an inhibitor of paracellular permeability derived from *Vibrio cholera*, prevented the expected increases in intestinal permeability in subjects with CD challenged with gluten[117]. AT-1001 use is also associated with a diminution in the anticipated rise of interferon-gamma levels.

REFRACTORY DISEASE

Recurrent symptoms sometimes develop in biopsy-proven CD patients on a GFD. The most common cause of non-responsive CD, which occurs in about 30% of CD patients, is non-adherence to a GFD. A Celiac Dietary Adherence Test (CDAT) consistency in a 7-item questionnaire was developed using a logistic regression, and validated against transglulaminase serology for the assessment of adherence to a GFD[118]. Usually, poor compliance to a GFD is thought to be responsible, although compliance may sometimes be difficult to establish. Intentional dietary indiscretion may be evident, but sometimes, there is limited awareness of gluten-containing substances. Gluten is ubiquitous, being documented in pill capsules and other materials, such as communion wafers.

Other causes, detailed elsewhere[119], may be responsible for recurrent symptoms even though the CD patient appears to be following a strict GFD. Some causes include associated primary or secondary pancreatic insufficiency, small intestinal bacterial overgrowth, collagenous sprue, or lymphocytic or collagenous colitis. Rarely, a complication may be responsible (e.g. lymphoma, carcinoma).

Sometimes, no initial response to a GFD ever occurs and symptoms persist. In these subjects, biopsies may be abnormal, but the gluten-dependent nature of the small bowel abnormalities was never documented. This has been labeled "unclassified sprue" or "sprue-like intestinal disease". Some of these persons may eventually prove to have a lymphoma. As many as approximately half of patients with CD on a GFD for more than two years may be able to tolerate a gluten challenge, even though they have mucosal abnormalities[100]. About 10% of CD patients diagnosed in childhood may develop temporary tolerance to gluten. However, because of the continuing mucosal abnormalities, they remain at risk of developing the complications of CD. Indeed, adolescents who do not adhere to a GFD have a lower quality of life[120].

Immunohistochemical labeling has helped to define an abnormal prognostic profile of intra-epithelial lymphocytes in the small bowel mucosa, so-called "refractory celiac disease, type 2 (RCD 2)" characterized by an aberrant clonal IEL population with loss of IEL antigens. About half of RCD 2 patients develop an enteropathy-associated T-cell lymphoma (EATL) within 5 years, and a particular HLA-DQ subtype, DQ2, if homozygous, predisposes to RCD 2[121].

Complete duodenal mucosal recovery in CD may be limited and may require prolonged periods of a GFD. In one study, remission was seen in 65% or no histological improvement was seen in 26% of patients[95]. This might be anticipated, however, if only duodenal biopsies are being taken since the proximal small intestine is most severely affected and varying periods of time are required for recovery to be significant. An apparent failure to histologically respond to a GFD may only reflect that duo-

denal, rather than more distal small intestinal biopsies were repeated.

Refractory CD has been defined as persistent or recurrent villous atrophy with crypt hyperplasia and increased intraepithelial lymphocytes (IELs) despite a strict GFD for greater than 12 mo (or if severe persisting symptoms necessitate intervention independent of the duration of the GFD)[122]. In assessing the GFD response, the site of re-biopsy and duration on a strict GFD are crucial to the definition of refractory disease. In some, as suggested by this definition, the opportunity for re-evaluation is very limited because of rapid progression of the intestinal disease.

RCD is usually manifested by recurrence of symptoms and intestinal abnormalities, despite adherence to a GFD. RCD can be further defined from a prognostic perspective by the histological appearance of monoclonal or polyclonal intraepithelial lymphocytes (RCD type 2) *vs* normal lymphocytes (type 1)[123]. These changes and the development of an EATL were shown to be adverse factors in the prognosis of CD, particularly in the first two years after CD has been deemed to be refractory[124].

Refractory celiac disease (RCD) type 2 but not type 1 shortens the sufferer's life expectancy[123].

Corticosteroids may improve clinical symptoms in some patients with RCD. Unfortunately, the histological response to steroids has not been consistent[123]. Patients with RCD 1 may benefit from immunosuppressive therapy, whereas those with RCD 2 may respond to Cladribine or to stem cell transplantation. Treatment with cladribine and anti-CD-52 has been shown to be associated with histological improvement. Azathioprine and anti-tumor necrosis factor-α have shown only limited success. The development of an overt lymphoma within 8 wk of treatment was seen in 3 out of 4 of these patients, thereby preventing further use. Alternative strategies that have been suggested include stem cell transplantation to replace the abnormal intra-epithelial lymphocyte population, and the blocking of IL-15[125-129].

CONCLUSION

Celiac disease is being increasingly diagnosed because of the recognition that the disease may be present without significant intestinal symptoms, may be associated with other autoimmune disorders and may be suspected from serological screening. Definition of the disease includes an intestinal biopsy before treatment with a GFD along with documentation of a definitive GFD response. In some patients, this may necessitate further intestinal biopsy after a period on a GFD. Serological testing may be useful in providing additional evidence that CD is present and may be useful in some patients to assess GFD compliance. Recent studies focused on the genetic basis and pathogenesis of CD have emerged to improve understanding of the complex molecular alterations that occur with CD.

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