Gluten enteropathy: current views on diagnosis and treatment

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SUMMARY
Gluten enteropathy, widely known as celiac sprue, is an “old” disease as its basic clinical and pathogenetic features where established 50 years ago. Recently mounting new data have come to light concerning multiple aspects of this disorder. The previously rare clinical entity that presented with a severe chronic diarrheal syndrome following the consumption of certain cereals has changed to a common autoimmune related disease with various manifestations, genetic background and new modes of diagnosis and possible treatment. The contemporary gastroenterologist needs to be aware of all the new “tips and tricks” which will guide his thoughts in suspecting celiac disease and be able to recognize it promptly and reliably. This is clearly important as gluten enteropathy proves itself to be a treatable disease but with insidious and devastating complications if left untreated. In this review an effort has been made to encompass all the recently acquired knowledge concerning the clinical presentation, serologic detection, endoscopic signs, histologic findings and treatment of celiac disease.

Keywords: celiac, gluten, treatment, diagnosis, serology, histology, endoscopic markers, refractory sprue

INTRODUCTION
Gluten-sensitive enteropathy also known as celiac disease (CD) or celiac sprue is characterized by an aberrant reaction of the small intestine upon the presence of wheat proteins and other homologous peptides found in cereals. The interplay of genetic predisposition with environmental factors like gluten and perhaps bacteria, using the immune system as the intermediary to cause autoimmune reactions, results in the disruption of the normal intestinal epithelial function that provokes the disease.

The gluten protein is the essential trigger for the initiation of the cascade of reactions that leads to enteropathy. Deprivation of gluten from the diet leads to cessation of symptoms and partial restoration of the histological damage while reinstitution of gluten causes relapse. While gluten was the major discovery for the pathogenesis of the disorder it seems that its presence is not enough by itself to produce the clinical manifestations. Some HLA class II molecules have been found to be strongly correlated with the occurrence of the disease. Constituents of the innate and acquired immune system like antigen-presenting cells, B and T-lymphocytes are disorganized causing structural damage by inducing autoreactive responses.

A retrieval of recent studies written in English over the last five years (from 2000-2005) has been conducted in Medline to investigate the latest reports on pathogenesis, diagnosis and treatment of celiac disease.

PATHOGENETIC MECHANISMS
A fraction of gluten called prolamin is the main provoking agent. Prolamines are protein fractions with homologous sequences rich in proline (>15%) and glutamine (>30%). They are divided into α, β, γ and ω gliadins. As gluten enters the gastrointestinal tract it is subjected to digestion by gastric and pancreatic enzymes. It has been found that some gliadin peptides (31-49, 56-89 sequences of α2 gliadin) are resistant to degradation by peptic enzymes in patients with active celiac disease. This ability delivers adequate amounts of toxic and immunostimulatory peptides of prolamin at the villous of the enterocytes. The transport system of the intestinal wall of the afflicted seems to be defected. Studies have shown increased permeability of the intestinal mucosa.
by the paracellular route via increased zonulin production, a substance that deregulates intercellular tight junctions. More recently the transcellular route has also been found defective, a dysfunction that permits the transportation and processing of the offensive peptides more rapidly and in greater quantities.

Tissue transglutaminase (tTG) is the enzyme that is responsible for the increased antigenicity of the gliadin peptides. This calcium-dependent enzyme, produced mainly by epithelial cells, fibroblasts and monocytes, reacts preferably with glutamyl donor substrates thus creating covalent glutamyl-lysine bonds primarily between collagen molecules and other proteins of the extracellular matrix. In stress conditions and inflammation, the production of tTG is enhanced in order to assist in blood clotting, wound healing and restoration of the connective tissue. Gliadin peptides, which are rich in glutamyl residues are an appropriate substrate for tTG. Upon linking, tTG succeeds to deamidate the glutamine of its substrate into glutamic acid, which possesses negative charges with the ability to be incorporated into positively charged MHC class II receptor molecules on the membrane of dendritic cells and B-lymphocytes in the lamina propria.

HLA DQ2/DQ8 are strongly associated with celiac disease (95% of CD patients are DQ2 positive and the remaining 5% demonstrate DQ8). They have high specificity for presenting exogenous antigens and seem to easily bind to deamidated gliadin peptides that have negative charges on their amino acid residues, a property which is appropriate for fitting in their groove. The presented antigenic bulk is in the form of complexes between gliadin fractions with or without the participation of tTG. These complexes produce neoepitopes that stimulate the activation of naïve CD4+ T-lymphocytes upon their contact with the HLA molecules. More recently MHC class I genes have also been detected to be independently associated with the pathogenetic processes of the disease. The MIC gene family is expressed by enterocytes under stress and recognized by γδTCR, CD8+ intraepithelial T-lymphocytes, which then cause mucosal damage. A polymorphic gene, the MICA-A5.1 is expressed mostly in atypical CD patients and in contrast with HLA DQ2/8 is believed to confer protection by limiting the action of cytotoxic T-lymphocytes.

Many of the aforementioned actions are facilitated by the existence of proline residues in the peptidic chains of gliadins. Proline enhances the specificity of the tTG, confers resistance against the proteolytic peptic enzymes of the gut lumen and finally conserves a specific protein conformation that accommodates the binding into the HLA molecular groove.

The CD4+ T-lymphocytes encounter the antigenic gliadin and if they acquire the appropriate receptors become activated and subsequently present a Th2 response by releasing proinflammatory cytokines in further stimulating B lymphocytes to produce autoreactive antibodies against tTG, EMA and gliadin. A Th1 response is also elicited from CD4+ T-lymphocytes, which includes the release of INF-γ and TNF that enhance the action of cytotoxic T-lymphocytes and the MMPs (matrix metalloproteinases) respectively causing damage to the enterocytes and intestinal barrier.

This schematic approach to the evolution of disease process is far more complicated than previously thought. Apart from the three already known immunostimulatory gliadin epitopes (glia-α2, α9, γ1), newly recognized antigenic sequences have been added to the continuously extended spectrum of toxic peptides. There are now 11 stimulatory peptides known to induce a T-cell response, some of which belong to the other fraction of gluten, namely glutenin. The analysis of their aminoacid sequences showed that homology exists between glutenin-derived epitopes and antigenic peptides from the gliadin fractions and that stimulated T-cells is possible to react against both glutenin and gliadin epitopes through cross reactivity mechanisms. Furthermore, deamidation is not a necessary process for the stimulation of T-lymphocytes as non-deamidated gluten peptides can induce immunogenic reactions. As disease progresses and more epithelium is damaged, larger quantities of tTG are becoming available and then deamidation takes the leading part into the whole process. It is even possible that deamidation of gluten peptides have a negative impact in their immunostimulatory capacity rendering them unable to induce a T-cell activation. Apart from the wheat gluten there are other cereal grains that induce the disease like rye, barley and to a lesser extent oats. Similar prolamines to gliadin are hordeins in barley, secalins in rye and avenins in oats. These prolamines have been shown to acquire homologue immunostimulatory sequences with glia-α2 and glia-α9 epitopes and to provoke cross-reactivity with stimulated T-cells in vitro and in vivo.

The conundrum of the immune response in celiac disease becomes more difficult to interpret because of the existence of toxic but non-immunodominant epitopes like the 31-49 sequence of α-gliadin, which is detrimental for the intestinal mucosa but is not recognized by the gluten-restricted T-cell receptors. On the other hand not all individuals with HLA DQ2/8 in their genetic profile
are susceptible to CD. These findings support the notion that there is more to the pathogenesis of the disease than just the activation of the adaptive immune system. It has been shown that parts of the innate immune system participate in the cascade of immunological reactions that take place during the epithelial injury. Damaged epithelial cells produce interleukin (IL)-15 which sensitizes cytotoxic T-lymphocytes to become more potent and express natural killer receptors against MHC class I molecules on the surface of dendritic cells while in parallel produce IFN-γ that perpetuates the disastrous cycle in the epithelial layer.

Increased number of bacteria has been detected in the small intestinal mucosa of children with celiac disease. The mucus layer and glycocalyx of the mucosa has a different composition in celiac patients than in controls, as a result of goblet and Paneth cell metaplasia in accordance with higher amounts of INF-γ produced by intraepithelial lymphocytes. It can be postulated that the altered properties of the glycocalyx enhance the adherence of bacteria on the intestinal mucosa. The presence of bacteria may affect the balance of the immune system by stressing the CD4+ T lymphocytes to be in an activated state and thus more sensitized to exogenous antigens like gliadin. In the same way, bacteria could stimulate epithelial cells to produce MIC molecules, which attract CD8+ T-lymphocytes against the enterocytes. Epithelial cells could also be the source of release of transglutaminase that takes part in the deamidation of gliadin capable of stimulating T lymphocytes. Candida albicans could serve as a model for the justification of the aforementioned concepts. This yeast has a virulence factor (hyphal wall protein 1, HWP1) that contains similar amino-sequences with α- and γ-gliadins and a part of its (hyphal wall protein 1, HWP1) that contains similar amino-sequences with α- and γ-gliadins and a part of its chain serves as a substrate for tissue transglutaminase. The yeast adheres to the intestinal epithelium using HWP1 and epithelial transglutaminase. By cross reaction, mechanisms could therefore stimulate intraepithelial T-lymphocytes in genetically predisposed persons and could also act as a hapten through its formed complexes with transglutaminase in order to lead to the production of anti-tTG. Intestinal infection and gut permeability are thought to be factors associated with progression to CD.

**CLINICAL PRESENTATION**

Much progress has been made towards the understanding of the true nature and course of celiac disease. It is now recognized as one of the more frequent autoimmune diseases with a prevalence of 1:100-300 and incidence of 2-13/100,000/year in the Western World. A strong genetic predisposition has been revealed as either HLA DQ2 or HLA DQ8 haplotype have been found in all patients with CD and the concordance of the clinical evolution in monozygotic twins reaches 70% and 10-20% in first-degree relatives. As the prevalence of CD increases in association with more efficient tests to detect the disease in an earlier phase, the classical clinical picture has been changed and enriched with more atypical and extraintestinal symptoms. The acceptance of the histological evidence of total villous atrophy as the gold standard for the verification of the disease has changed. Now even more subtle pathologic alterations, as increased intraepithelial lymphocytes, have been accepted as part of the pathogenetic process.

Celiac disease affects the small intestine, mainly the duodenum and jejunum but with possible spreading even in the ileum. The female to male ratio has been estimated at 2:1. The disease can be detected in any age. The clinical presentation depends on duration of disease, age, the spreading of histological damage and the presence of extraintestinal manifestations. Factors that precipitate the onset of symptoms could be infection, pregnancy or gastrointestinal surgery.

The main clinical syndrome of diarrhea and malabsorption is not encountered frequently for two reasons. Primarily the usual localization of the histological damage in the upper small intestine leaves the ileum to compensate for the non-absorbed luminal content. Secondly, the detection of the disease is achieved earlier and the clinical deterioration is prevented. In the past diarrhea was present in 85% of patients and the lag time between initiation of symptoms and diagnosis was 11 years. Recently the frequency of presenting diarrhea has declined 43%. The bowel frequency rarely exceeds 8 times per day while even constipation can be seen in 10% of the cases. Symptoms of malabsorption are more prevalent in men. Pain is reported with a variable frequency from 5%-34%. It has been estimated that 3.3%-11.4% of patients diagnosed with irritable bowel syndrome (IBS) based on Rome II criteria have underdiagnosed celiac disease verified by histology and antibody testing. Because celiac pathology is 7 times more common in IBS patients, cost effectiveness analysis of testing for celiac disease in predominant diarrhea-IBS patients showed that the cost is acceptable in order to prevent the possible complications of an unmasked gluten enteropathy. If the prevalence of celiac disease is at least 1% in the general population, which is the actual estimated prevalence in Western countries, testing for celiac sprue before initiation of empirical IBS therapy...
would identify a significant number of celiac patients that present with symptoms resembling irritable bowel syndrome (75% of celiac patients present with such symptoms). Nowadays, the most common presenting symptoms are nonspecific, including dyspepsia, neurologic manifestations, arthritis, anemia, osteoporosis and infertility. About 50% of patients have no gastrointestinal predominant symptoms.

Children usually become symptomatic in the first six months after birth (>50%). Diarrhea, abdominal distention, vomiting, anorexia, weight loss and failure to thrive characterize their clinical condition. Later, patients present with short stature, iron deficiency anemia, rickets, late menarche and dental enamel defects.

The most common hematologic and biochemical abnormalities (Table 1) are mainly the result of the malabsorption of nutrients and vitamins from the proximal small intestine or the picture of hyposplenism in the blood films from older patients.

There are two basic discriminating clinical types namely the “classical” type and the “silent”. In the classical type, the patient exhibits both histological damage and symptoms and is also positive for celiac-specific autoantibodies. The predominant symptom is diarrhea but there are also other symptoms as a result of various degrees of malabsorption (Table 2). The silent type includes those patients with abnormal histology but without symptoms or atypical presentations (Table 3). The group of patients that constitutes the “latent” type shows no histological evidence of villous atrophy or crypt hyperplasia and no symptoms but demonstrate positive autoantibodies to endomysium. These patients have been found to possess increased numbers of γ/δ intraepithelial T lymphocytes, which is characteristic of celiac disease pathology and increased mitotic activity of the enterocytes in their intestinal biopsies. Furthermore these patients gradually develop symptoms.

There are other clinical conditions that develop with high frequency in celiac patients (Table 4). The majority of these associated conditions is autoimmune in nature and affects a wide range of body organs like skin, lungs, gut, kidney, liver and vessels. This variable insult is indicative of a generalized stimulation of the immune system, which in many cases produces autoreactive antibodies against a variable set of different body antigens. The most common immunoreactive disorders that evolve with celiac sprue and also regress after gluten free diet (GFD) are dermatitis herpetiformis, diabetes mellitus type 1 and autoimmune thyroiditis. These autoimmune phenomena are increased with prolonged gluten expo-

### Table 1. Hematologic and biochemical abnormalities in celiac patients

<table>
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<tr>
<th>Abnormality</th>
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<tr>
<td>Iron, folate, B12 deficiency</td>
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<tr>
<td>Hypocalcemia</td>
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<td>Prolonged prothrombin time</td>
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<td>Hypoalbunemia</td>
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<td>Hyposplenism (Howell-Jolly bodies, Heinz bodies, anacanthocytes, thrombocytosis)</td>
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<td>Hypertransaminasemia</td>
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<td>Increased alkaline phosphatase</td>
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<td>Increased erythrocyte sedimentation rate</td>
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### Table 2. Symptoms and signs at presentation of celiac disease

<table>
<thead>
<tr>
<th>Symptom</th>
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<tr>
<td>Diarrhea</td>
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<tr>
<td>Anorexia</td>
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<tr>
<td>Vomiting</td>
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<td>Abdominal distention</td>
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<td>Abdominal pain</td>
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<td>Weight loss</td>
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<tr>
<td>Glossitis</td>
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<tr>
<td>Bleeding diathesis</td>
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<tr>
<td>Osteomalacia</td>
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<td>Anemia (15% prevalence in CD)</td>
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</table>

### Table 3. Atypical presentations of celiac disease

<table>
<thead>
<tr>
<th>Condition</th>
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<td>Ataxia</td>
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<tr>
<td>Epilepsy</td>
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<td>Peripheral neuropathy</td>
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<tr>
<td>Depression, Schizophrenia</td>
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<tr>
<td>Aphthous stomatitis</td>
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<tr>
<td>Dermatitis herpetiformis</td>
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<tr>
<td>Alopecia</td>
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<tr>
<td>Psoriasis</td>
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<tr>
<td>Finger clubbing</td>
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<tr>
<td>Hypo/hyperthyroidism (6-8% prevalence in CD)</td>
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<tr>
<td>Pericarditis</td>
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<tr>
<td>Dilated cardiomyopathy</td>
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<tr>
<td>Infertility</td>
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<td>Spontaneous abortions</td>
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<td>Lassitude</td>
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<tr>
<td>Osteoporosis (3.4%-7% prevalence in CD)</td>
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<tr>
<td>Iron deficiency anemia (8-14% prevalence in CD)</td>
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<tr>
<td>Hepatitis-hypertransaminasemia (10% prevalence in CD)</td>
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</table>
tion and is estimated to be 8.2/1000 patients/year. The excess in death has been observed in symptomatic patients and in relation with the severity of their symptoms. In the first 3 years after diagnosis mortality has been found to be even more pronounced. Death risk is raised (four fold) if the interim between diagnosis and initiation of symptoms is 10 years or more. More recent studies have estimated lower mortality ratios (standardized mortality rate 1.3) but still in excess at the time closer (first year) to the time of diagnosis.

SEROLOGIC MARKERS

One of the most useful tools for the exploration of the celiac iceberg are the serologic tests which are based on the determination of a panel of autoantibodies against gliadin, endomysium and tissue transglutaminase. Anti-gliadin antibodies (AGA) are detected by ELISA method and anti-endomysial (EMA) by indirect immunofluorescence on tissue sections from monkey esophagus or human umbilical cord. Tissue transglutaminase (tTG) is the enzyme that forms the antigenic target of the previously reported antibodies against endomysium. Antibodies against guinea pig liver or human recombinant tissue transglutaminase can be detected using ELISA, radioimmunoassay or dot blot assays.

Celiac disease has proved to be one of the most insidious pathologic processes with a variety of atypical manifestations, which prevent the prompt diagnosis and therapy. It is estimated that the diagnosed versus undiagnosed cases of celiac sprue is 1:7. The serologic tests can be used in the following circumstances: a) select the patients that based on clinical suspicion are candidates for intestinal biopsy, b) screening patients at high risk of developing the disease, c) in cases of equivocal histological findings in an individual without the typical presentation, d) autoantibody determination is also warranted in the follow up of patients under GFD in order to monitor adherence.

The diagnostic accuracy of the serologic measurements is generally satisfactory but depends on several factors. Anti-gliadin antibodies have moderate sensitivity and specificity, anti-endomysial have the greatest specificity that reaches the most to 100%, as for the anti-tTG, they considered the most sensitive (>95%). Determination of the endomysial autoantibodies is cumbersome, time consuming, costly and observer dependent as well as restricted by the use of primates’ tissue (monkey’s esophagus). Another issue of confusion is the different cut-off values being used by various laboratories.
Lack of standardization and referral of their cut-off limit to local population are considered drawbacks of several commercial kits. Many clinical trials with the aim to evaluate the diagnostic potency of the tests include highly preselected patients or groups with high prevalence of the disease. The positive and negative predictive values of the serologic tests demonstrate a wide variation because they are dependent on the prevalence of the disease in the population.

The sensitivity and specificity of the serological parameters are shown in Tables 5 and 6. Anti-gliadin antibodies cannot be used for screening of patients with suspected celiac disease because of the low sensitivity. On the other hand false positive results can arise from other inflammatory conditions of the gut like parasitic infections, Crohn’s disease, eosinophilic enteritis, food allergies and tropical sprue. Raised antibodies against gliadin can also be found in IgA nephropathy. IgA AGA seems to be more sensitive and specific than IgG in the adult population. While IgA AGA show no substantial difference in their diagnostic accuracy between adults and children, IgG AGA show greater sensitivity in children.

Antiendomysial antibodies are more sensitive and specific than AGA. Their specificity reaches 100% and their positive predictive value is 96-100%. As a conclusion, a positive IgA EMA practically designates a patient with celiac disease. The few false positive results can be attributed to the presence of autoantibodies against smooth muscle that sometimes make difficult the interpretation of the fluorescence pattern of the test. IgA EMA show less specificity in the paediatric population and they demonstrate a low sensitivity in children less than 2 years of age. The titer of EMA correlates with the degree of villous atrophy so patients with partial or subtotal atrophy may test negative for these antibodies. The frequency of positive EMA is also related to the clinical severity of the disease as EMA are discovered more often in the classical form (96%) than in the silent (71%).

Anti-tTG antibodies with human recombinant substrates are more sensitive than EMA and AGA and therefore more suitable for screening purposes. Although sensitivity of rh-tTG antibodies is higher than EMA, the latter are more specific. Similar to the antiendomysial antibodies, anti-tTG have demonstrated a lower sensitivity (<85%) in patients with milder histological damage (<Marsh III). The more recently commercialized method using human recombinant substrate for the quantification of anti-tTG antibodies has proved to be more accurate (sensitive and specific) for the diagnosis of celiac sprue than the previously used method based on guinea pig liver substrates. Gp-based autoantibodies produce false positive measurements in other diseases like Crohn’s disease, food intolerance, diabetes or chronic hepatitis. This may be the result of contamination of the substrate with other antigenic substances which become the source of antibody production due to cross reaction or the result of the existing 7% differences in the antigenic epitopes of tissue transglutaminase between guinea pig and human species. Generally there are no age-relat-

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Table 5. Sensitivity, specificity, positive and negative predictive values of the serologic tests in adult celiac patients

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
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<tbody>
<tr>
<td>AGA</td>
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<tr>
<td>IgA</td>
<td>55-100%</td>
<td>82-100%</td>
<td>45-92</td>
<td>64-100</td>
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<td></td>
<td>(80%)</td>
<td>(80%)</td>
<td>89</td>
<td>79-97</td>
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<td>IgG</td>
<td>57-78%</td>
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<td></td>
<td>(&lt;80%)</td>
<td>(80%)</td>
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<td>Mo-es</td>
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<tr>
<td>IgA</td>
<td>86-100%</td>
<td>98-100%</td>
<td>96-100</td>
<td>83-99</td>
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<td></td>
<td>(95%)</td>
<td>(100%)</td>
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<tr>
<td>IgG</td>
<td>39-83%</td>
<td>80-98%</td>
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<tr>
<td></td>
<td>(95%)</td>
<td>(100%)</td>
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<tr>
<td>EMA</td>
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<tr>
<td>Mo-es</td>
<td>86-100%</td>
<td>98-100%</td>
<td>44-76</td>
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<tr>
<td></td>
<td>(90%)</td>
<td>(100%)</td>
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<tr>
<td>IgA</td>
<td>77-100%</td>
<td>91-100%</td>
<td>44-76</td>
<td>99-100</td>
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<tr>
<td></td>
<td>(90%)</td>
<td>(95%)</td>
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<tr>
<td>IgG</td>
<td>23-62%</td>
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<td>99-100</td>
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<tr>
<td></td>
<td>(98%)</td>
<td>(98%)</td>
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<tr>
<td>Uc</td>
<td>77-100%</td>
<td>91-100%</td>
<td>80-97</td>
<td>87-100</td>
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<td></td>
<td>(98%)</td>
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<td>EMA</td>
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<td>Uc</td>
<td>77-100%</td>
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<td>(98%)</td>
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<tr>
<td>Gp-tTG</td>
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<tr>
<td>Rh</td>
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<tr>
<td>Mo-es: monkey esophagus, Uc: umbilical cord, Gp: ginea pig, Rh: human recombinant, PPV: positive predictive value, NPV: negative predictive value</td>
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Table 6. Sensitivity & specificity of serologic tests in children

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<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
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<tbody>
<tr>
<td></td>
<td>IgA</td>
<td>IgG</td>
</tr>
<tr>
<td></td>
<td>Range (%)</td>
<td>(pooled estimate %)</td>
</tr>
<tr>
<td>AGA</td>
<td>52-100%</td>
<td>71-100%</td>
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<tr>
<td>(&gt;80%)</td>
<td>(80-90%)</td>
<td>(80-90%)</td>
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<tr>
<td>Mo-es</td>
<td>88-100%</td>
<td>90-100%</td>
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<tr>
<td>EMA</td>
<td>88-100%</td>
<td>90-100%</td>
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<tr>
<td>Uc</td>
<td>88-100%</td>
<td>90-100%</td>
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<tr>
<td>EMA</td>
<td>88-100%</td>
<td>90-100%</td>
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<tr>
<td>Gp-tTG</td>
<td>90-100%</td>
<td>94-100%</td>
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<tr>
<td>(93%)</td>
<td>(96%)</td>
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</tr>
<tr>
<td>Rh-tTG</td>
<td>90-100%</td>
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<td>(96%)</td>
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ed differences for the IgA anti-tTG referring to their diagnostic ability.

New methods have been developed to measure other autoantibodies present in CD. By immunofluorescence based on intestinal epithelial cells it is now feasible to analyse IgA antibodies against the enterocyte cytoskeleton actin filaments (IgA-AAA). The results of this test are correlated with the presence of villous atrophy with sensitivity, specificity, positive and negative predictive value of 83.9%, 95.1%, 97.8% and 69.2% respectively.35

There are 2%-3% of patients with celiac disease who present with IgA deficiency. This prevalence is 10-15fold more frequent in celiac sprue than in the general population. Common serologic investigation with IgA autoantibodies usually ends in negative results. In this situation IgG class antibodies, either AGA, EMA or anti-tTG can be measured. The specificity of the IgG EMA and anti-tTG scores higher than the same class of AGA and studies show that 98.7% of celiac IgA deficient patients are picked up by the first two assays.26

Because of the superior sensitivity of the rh-tTG antibody assay, many clinicians recommend the use of this test for initial selection of patients needing to proceed to a biopsy. When this test is negative it can be assumed that the patient is not suffering from CD although the concept of the use of a single test to rule out the disease is questionable.8,16 If rh-tTG antibodies are positive then a confirmation is requested using anti-EMA, which establishes with great certainty the validity of the result because of its high positive predictive value.27,29 In one third of CD patients there is no concordance between EMA and anti-tTG antibodies meaning that there are cases in which one test is positive and the other is negative. There is also a group of patients negative for the IgA class of both of the above assays demonstrating only IgG class antibodies without being IgA deficient. For these reasons it is proposed that a combination of IgA EMA and anti-tTG is necessary to screen for eligible patients with the additional measurement of either total IgA or an IgG-based test, preferably IgG EMA or IgG rh-antiTG,26 to count for the less common situations of IgA deficient or negative IgA patients.27,37 When there is no concordance between IgA EMA and anti-tTG and/or intestinal biopsy is not conclusive for the diagnosis, HLA determination may be of assistance as it has a very high negative predictive value.9,29 In any case, even if serology is negative and there is high clinical suspicion it is recommended that intestinal biopsy be performed, as it constitutes the “gold standard” for celiac disease recognition.16,35 In patients with apparently “normal histology” even though they are clinically and serologically compatible with CD, as for example in the patchy distribution of the histological damage, it is possible that the recently developed assay of IgA-AAA be of value as it turns to be positive in 95% of patients with total and subtotal villous atrophy.35

ENDOSCOPIC MARKERS

Serologic markers are useful but not absolutely necessary in the diagnosis of celiac disease. The more reliable method for detection is the duodenal biopsy taken during endoscopy of the upper gastrointestinal tract. Patients suffering from CD have commonly undergone
more than one endoscopic procedure because as many as 40% of them report frequent symptoms from the upper gut. However, these endoscopic attempts do not end up in a definite diagnosis either because biopsies of the duodenum are not considered necessary or because the atypical manifestations of CD do not alert the endoscopist to the probable existence of this condition. Consequently, upper endoscopy seems to play a vital role in the unraveling of the diagnostic conundrum by recognition of macroscopically suspicious mucosal lesions and above all by acquisition of material for microscopical analysis. There is an effort that relies upon the discovery of characteristic endoscopic signs, which would lead to a more vigilant inspection of the duodenal mucosa for the revelation of celiac disease. This is particularly important in the case of “silent” or atypical presentation, which is a very common manifestation of the disease in 30% of patients. Another difficult point in the diagnostic approach is the patchy distribution of mucosal damage, which can result in false negative histological analysis. There is also a subgroup of patients with negative anti-EMA and/or anti-tTG antibodies in whom there is a strong clinical suspicion and would be exceptionally helpful if intestinal biopsies could be supported by a less blinded endoscopical approach.

For these reasons the recognition of certain endoscopic markers has been a very challenging quest. Reduction or loss of Kerkring’s folds, scalloped duodenal folds, mosaic pattern, micronodular or granular appearance of the mucosa as well as the visualization of submucosal vessels are validated as endoscopic signs associated with celiac disease. These markers are considered the macroscopical expression of various degrees of villous atrophy and have been accepted as predictors of celiac pathogenetic alterations. A recent study has proposed a specific pattern of erosions found in the second part of the duodenum, to be added to the list of endoscopic markers. The erosions should be multiple and superficial and usually are accompanied by other mucosal alterations like scalloped or reduced height of duodenal folds. The range of sensitivity, specificity, positive and negative predictive values of the endoscopic abnormalities as accessed by various studies are shown in table 7.

There are pitfalls in the interpretation of these markers because their assessment is subjective as a result of lack of standardization (height of folds) and their presence is not unique for CD because they can be seen in other enteropathies like intestinal infections or food allergies. Their prevalence is also dependent upon the age and grade of histological damage of the patients involved. As a consequence, the sensitivity of these features in detecting CD, scores relatively low in relation to specificity which is substantially high, rendering their high positive predictive value a measure dependent upon the prevalence of the disease in the population under study. If the prevalence is high, a positive endoscopic marker can reliably confirm the existence of villous atrophy. The great variability in the reported diagnostic accuracy of the endoscopic signs can be attributed to the differences in the design of the studies. In those trials, which include a preselected group of patients with CD or with strong evidence for the disease, the sensitivity of the endoscopic features is exceptionally high (sensitivity 85-94%). In those trials in which patients consecutively underwent open access endoscopy for reasons other than suspected CD, like dyspepsia, the sensitivity is disappointingly low (sensitivity 50%). The most sensitive and reproducible, endoscopic signs have been found to be the scalloped folds and mosaic pattern with sensitivities of 89% and 86% respectively.

The presence and severity of the endoscopic abnormalities are strongly associated with the grade of histological damage. Total or subtotal villous atrophy is correlated with the loss of the duodenal folds or a mosaic-like mucosa while partial villous atrophy or hyperplastic crypts (Marsh type IIIa and II) are associated with scalloped folds or micronodularity. According to Niveloni A et al, all celiac patients with false negative endoscopic markers had type II histologic lesions. In the same manner, more abnormalities are seen in the classical form of the disease than in the “silent” or atypical form. Advanced age is also correlated with more prominently dam-

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<th>Table 7</th>
<th>Diagnostic accuracy of endoscopic markers</th>
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<tr>
<td></td>
<td>Sensitivity</td>
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<tr>
<td>Loss-reduction of duodenal folds</td>
<td>44-94%</td>
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<tr>
<td>Scalloped duodenal folds</td>
<td>66-86%</td>
</tr>
<tr>
<td>Mosaic pattern</td>
<td>26-89%</td>
</tr>
<tr>
<td>Micronodular mucosa</td>
<td>57.7%</td>
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<tr>
<td>Vasculature</td>
<td>5%</td>
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aged mucosa implying that more extended exposure to gluten is the source of more severely affected mucosa. The same conclusions derive from pediatric patients with celiac disease 39 in whom the mosaic pattern and scalloped folds predominate in the endoscopic picture. Loss of duodenal folds are not detected in children less than 5 years of age while an increasing number of them develop a reduction in the height of Kerkring’s folds with advancing age.

New technological resources have been employed for the amelioration of the diagnostic capability of endoscopic inspection. Chromoendoscopy, with use of methylene blue dye, although improves the visualization of characteristic endoscopic patterns does not seem to add more to the diagnostic potential than the eye of an experienced endoscopist during conventional endoscopy. In cases of total villous atrophy both in children and adults, chromoendoscopy is no better than a trained endoscopist.39,41 Kiesslich R.et al showed that use of chromoendoscopy with indigo carmine for patients with various duodenal abnormalities revealed significantly more lesions in the duodenal bulb than conventional endoscopy. Nevertheless, there was not a statistically significant difference between the two methods for the detection of lesions in the second part of the duodenum while all cases of total villous atrophy could also be traced by plain upper Endoscopy.40 The “immersion technique” includes the instillation of 100ml of water into the descending duodenum during upper endoscopy for better inspection of intestinal villi. This procedure was validated in high risk patients and the results showed an increase in the sensitivity and specificity in comparison with the standard endoscopic examination (sensitivity 85% and 80%, specificity 99% and 87%, respectively).32 High resolution magnification endoscopy proves to be very efficient in the detection of total or partial villous atrophy in patients with high probability of celiac disease.46 The sensitivity, specificity, PPV and NPV for any villous abnormality are 95%, 99%, 95% and 99% respectively. The magnification option is costly and needs more expertise but could be very useful for obtaining targeted biopsies in cases of partial and patchy villous atrophy or for clarification of the villous structure when dealing with low risk patients. A combination of all the aforementioned techniques (high magnification immersion chromoscopic duodenoscopy using indigo carmine) was reported to be very efficient for the demonstration of patchy villous atrophy and vascular pattern of the villi.47

Finally it seems prudent to rely upon the endoscopic findings when the pretest probability is high and this refers to patients at risk or with strong clinical suspicion. Detection of endoscopic alterations in patients who undergo endoscopy of the upper gastrointestinal tract for various indications or low suspicion of celiac pathology is expected to confer a positive result in a ratio of 1 per 194 endoscopic procedures40 or according to another trial to detect celiac disease with a frequency of 0.5-5.3%.39

**DUODENAL BIOPSY & HISTOLOGICAL FINDINGS**

Although there has been significant progress in the detection of celiac disease with the discovery of serologic and endoscopic markers, the fundamental method for definite diagnosis still remains the intestinal biopsy. The “golden rule” requires the identification of some degree of villous atrophy in a collection of at least three or four biopsy specimens13,43 obtained from the distal part of the duodenum. The commencement of GFD must be followed by clinical and histological remission. The pathological analysis of the affected mucosa is based on a continuum of successive microscopic lesions. This starts from an increase in the intraepithelial lymphocytes (>40IEL/100enterocytes) followed by crypt hyperplasia and ending in destruction of normal villous architecture. This scheme represents the histological analysis based on Marsh criteria and revised by Oberhuber (Eur J Gastroenterol Hepatol, 1999). According to these criteria there is a schematic division of villous atrophy from mild (villous/crypt height ratio 3:1) to moderate (v/c ratio 2:1) and to severe type (v/c ratio 1:1 or less).

Duodenal biopsies should be considered when there are clinical symptoms suggestive of CD, a positive serologic test or when specific endoscopic abnormalities are present during upper endoscopy in a patient with symptoms that could be included in the list of atypical manifestations. However biopsies should be taken whenever there is clinical suspicion even in the absence of characteristic serologic or endoscopic markers due to the variety of symptoms and increased prevalence of the disease in the Western World. After the institution of GFD it is recommended that an intestinal biopsy be taken after 6-12 months for the evaluation of histological improvement, which further supports the diagnosis and adherence to the therapeutic protocol.

Total villous atrophy is more frequent in the distal part of the duodenum and in the proximal jejunum. Based on these findings, biopsies are better obtained from as far as possible during endoscopy. Nevertheless, because at least 85% of patients have some degree of villous at-
trophy throughout the duodenum and 50% of the affected individuals have the same degree of villous atrophy at all sites,\(^4^6\) it is accepted that biopsies could be taken from the descending part of the duodenum.\(^3^3\) Although the evaluation of specimens originating from the duodenal bulb is difficult to be interpreted by the pathologist due to confusion with normally blunted villous architecture over Brunner’s gland, there are endoscopists who support the notion that biopsies should also be obtained from this site. The same histologic lesions are seen both in the bulb and the descending duodenum \(^4^8\) but there are times when only the bulb is affected.\(^4^9\) Based on the official recommendation for the acquisition of four biopsy specimens, the duodenal bulb must be included in order to increase the diagnostic yield.

It would be possible to limit the number of biopsies to only one specimen from any site in the duodenum in those patients with classic symptomatology, positive serology (tTG and/or EMA) and genetic susceptibility (HLA DQ2 or DQ8 positive).\(^4^8\) Other investigators go further and propose that intestinal biopsies are not necessary for the confirmation of diagnosis in cases of patients with the classic clinical presentation or with celiac-associated autoimmune diseases and serial positive serologic tests of tTG and subsequently EMA because of the high post-test probability of more than 99% being affected.\(^5^0\)

There is the growing opinion that routine duodenal biopsies should be taken whenever a patient is referred for an upper gastrointestinal Endoscopy.\(^5^1\) Iron deficiency anemia patients are found to be suffering from CD with a frequency of 2-3% and it is recommended that a duodenal biopsy should be taken for detection of possible celiac disease whenever there are no obvious or common causes to justify this condition during endoscopy. Patients with insulin-dependent diabetes mellitus have a 6% prevalence of celiac disease and 50% of celiac patients complain of dyspepsia, 30% of vomiting, 14% of non-cardiac chest pain and one third of abdominal pain.\(^3^8,^4^3\) Many individuals proceed because of these manifestations to a diagnostic upper endoscopy. The endoscopist who is aware of the atypical or even silent form of the disease should search for endoscopic markers. If such markers exist, he should not hesitate to take at least two biopsies, one from the bulb and the other from the second part of the duodenum and ask for the proper orientation of the specimens and vigilance of the pathologist. In the case where no endoscopic signs are present, the judgment of the endoscopist will solve the dilemma as to whether duodenal biopsies should be undertaken based primarily on the local prevalence of CD and his scientific awareness.

After the initiation of GFD, the serologic tests become negative commonly after 6 to 12 months but still are not sensitive markers of histologic remission or even gluten exposure. The evaluation of histologic recovery can only be made reliably by intestinal biopsy. However, it should be kept in mind that complete histologic restoration to normal is not expected. In one study only one fourth of patients showed normal endoscopic appearance and histology without villous atrophy during a gluten-free diet for a mean of 4 years. When IEL were stained, none of the patients had normal counts but all showed some reduction towards normal, which was considered an acceptable improvement.\(^5^2\)

The increased number of IEL is considered the first histological evidence of intestinal damage in celiac pathogenesis (Marsh type I lesion). The majority of these lymphocytes are T-cells with αβ+ TCR and their immunophenotype consists of CD3+, CD8+ antigens. About 20-30% of IEL have membrane γδ receptors in comparison with 10% in non celiac controls.\(^5^3\) Besides the routine examination of biopsy specimens by hematoxylin-eosin staining, it is sometimes necessary to use more advanced techniques like immunostaining or flow cytometry for the detection of specific subgroups of IELs. This is useful in cases when celiac disease is suspected but serology is negative, specimens are not well orientated or basic histological evaluation is equivocal. The same applies for patients with patchy lesions, those subjected to a gluten-restricted diet or in subclinical cases of CD. Under these circumstances the evaluation of IELs will confer additional information and will resolve much of our diagnostic dilemmas derived from the common lack of villous atrophy in those patients. Increased IELs (>25 IEL/100 enterocytes) found in the terminal ileum even during colonoscopy are associated with duodenal villous atrophy with a sensitivity of 60% and specificity of 100% and should prompt the endoscopist to search for possible celiac pathology with duodenal biopsies.\(^5^4\) The problem is that increased CD3+ intraepithelial lymphocytes between villi in a mucosa with normal villous architecture can also be found in other inflammatory conditions like Crohn’s disease, microscopic colitis and bacterial overgrowth as well as in autoimmune diseases like rheumatoid arthritis and psoriasis and even after NSAID ingestion.\(^5^5\) The mere count of IELs is not specific for CD and one method to overcome this problem is the evaluation of γδ+ lymphocytes, a method which has sensitivity, specificity, PPV and NPV of 93%, 88%, 95% and 85% re-
spectively. This subset of immune cells remains in ex-
cess in cases of asymptomatic patients or even in sub-
clinical cases with positive serology but with normal ap-
pearing mucosa including total IEL count. It is also re-
mains increased after gluten free diet when the mucosa 
has presented with complete remission by conventional 
histology. Some authorities have proposed the use of flow 
cytometry for counting γδ+ and also NK-like subset of 
IELs in the diagnostic algorithm besides serologic tests 
for the initial screening of patients with suspected CD 
because of the high negative predictive value which 
reaches 95%. Although the determination of γδ+ TCR 
subset is fairly sensitive and specific for CD, the prepa-
ration of biopsy samples requires the handle of frozen 
sections. A new method based on counting IELs on the 
villous tips promises to be a reliable and easily performed 
diagnostic approach. The process consists of measuring 
IELs per 20 enterocytes on top of five randomly selected 
villi under light microscope. For early stage celiac dis-
case, that means normal villous architecture and atypi-
cal or asymptomatic presentation, this method has sen-
sitivity and specificity of 84% and 88% respectively with 
a cut-off value of approximately 4-5 IEL/20entero-
cytes.

Finally the best approach to a low risk patient with 
probable celiac disease is tip villous IEL counts accom-
panied by serologic tests and if the results are still equiv-
ocal or further evidence is needed, then HLA haplotype 
determination or γδ+ cells identification, could be used.

**TREATMENT**

The mainstay of therapy in celiac disease is the strict 
adherence to a gluten-free diet.

The patient should avoid the consumption of any food 
containing wheat, rye, barley, triticale (wheat-rye hybrid), 
kamut and spelt. The grains, which are non-toxic are 
listed in Table 8. The efficiency of this stringent dietetic 
protocol is questioned because of patients’ non-compli-
ance either factitious or inadvertent, lack of adequate 
control on manufacture of gluten-free foods resulting in 
contamination, increased cost and nutritional deficits 
provoked by the exclusion of various food products. In a 
long term follow-up of 28 years duration in a cohort of 
young celiac patients (mean age 35 years) the adheren-
ce was noticed for patients detected with celiac dis-
 ease through screening. Patients less than 4 years of age 
have shown better compliance than older children with 
rates of 80% and 36% respectively. Unfortunately, com-
pliance with GFD tends to be lower in adulthood than in 
childhood because of weakening of the systematic fol-
low up. Another serious cause of quitting the diet is the 
higher rate of gastrointestinal symptoms reported main-
ly by women after 8-12 years under treatment. This may 
be the result of different amounts of gluten contained in 
the consumed foods. In a study of gluten challenge for 1 
month, the use of two different doses showed that the 
higher dose (0.5g/kg/d) was responsible for more severe 
intestinal inflammation and relapse in relation to the 
lower dose (0.2g/kg/d).

Gluten-free products set by the Codex Alimentari-
ous allow a maximum of 0.05 nitrogen per 100g dry, 
rye-starch containing food. This cut-off means that a 
gluten-free diet probably includes 40-60mg of gluten/ 100g 
dry product which further attribute 20-30 mg of gliadin. 
A recent trial which included adults and children patients 
on GFD for 2 years, either natural or wheat-starch based, 
suggested that the maximum quantity of gluten in glu-
ten-free products could be set with safety at 100ppm 
(=mg/kg). Based on the latter concentration, if a patient 
consumes 300gr of wheat flour then the amount of daily 
ingestion of gluten is approximately 30 mg and as the 
majority of patients with good adherence do not exceed 
100g of flour daily, then the quantity of gluten falls to

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<td>Potato</td>
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<td>Corn</td>
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<td>Rice</td>
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<td>Nuts</td>
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<td>Amaranth</td>
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<td>Buckwheat</td>
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<td>Legumes</td>
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<td>Millet</td>
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<td>Quinoa</td>
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<td>Soy</td>
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<td>Peas</td>
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<td>Fruits</td>
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<td>Plain meats</td>
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10mg. At this level of gluten intake, mucosal recovery can be achieved and quality of life can be protected and maintained at the same level as in the general population.65

Wheat-starch based gluten-free products can increase the palatability of these foods as they are well tolerated and less stimulating in relation to natural GFD. The effect of wheat starch was evaluated in a prospective and randomized study after daily intake of 82gr (mean) per 1 year by untreated celiac patients and found to have similar remission rates in histology, gastrointestinal symptoms, serology and biochemical markers with standard GFD. A novel Elisa kit is now available for the quantification of gluten and related prolamines like secalins and hordeins but not avenins in various products. The antibodies used in the test do not cross react with glutenin. The sensitivity of this kit seems to be 78% for gluten and 39% for rye and barley prolamines and can detect only as 3.2ppm of gluten.66 A more recently developed method is able to detect T-cell stimulatory epitopes of α2/9 and γ1-gliadin based on a competition antibody assay. The advantage relies on the ability to trace not only intact proteins but also protein fragments of gliadin containing aminoacids less than those required for the stimulation of gluten-restricted T-cells. This method can measure as little as 5 ppm of gluten or 2.5ppm of gliadin and because of cross reactivity can also detect homologues sequences not only in barley, rye and triticale but also in oats.67

There has been a lot of discussion about the possible implications from the inclusion of oats in the standard GFD. Because of the lower proportion of their protein fraction and the only 10% amount of proline in avenins, oats are considered less harmful than the other gluten containing grains.7 Their higher concentration of fiber is sometimes useful to overcome the constipation induced by the standard GFD. Lastly oats are one of a list of grains (like amaranth, buckwheat, legumes and quinoa) that contain increased amounts of vitamins and trace elements. The conclusion that can be drawn from randomized studies with follow-up from 15 to 57 years and daily ingestion of oats from 15-50gr is that oats can be used in the diet because they maintain quality of life, sustain acceptable adherence (71%) and have the same impact in serology and villous architecture as standard GFD. This proposal cannot apply to all patients, as oats seem to induce gastrointestinal disturbances and intolerance in some of them and particularly in children of small age (<2 years). This age group has shown to possess high titers of anti-avenin antibodies in relation to normal population.71 Antibody production can be related to the increased number of intraepithelial lymphocytes found after consumption of adequate amount of oats (50gr). Some patients not only are sensitive in the presence of oats but there is a chance that can manifest even villous atrophy.72 These reactions are probably the result of contamination of oat products with wheat during processing and manufacturing and the outcome of a cross reactivity phenomenon between gliadin-activated T-cells and avenin prolamines.

There is a need for surveillance after the institution of GFD. Patients that are non-compliant present in higher proportion with anemia, osteoporosis and osteopenia63,73 and of course they are subjected to increased risk of malignancies.25 Antigliadin antibodies (IgA and to a lesser degree IgG) start to diminish after 3-6 months of the restriction diet. Anti-EMA and anti-tTG antibodies need commonly 6-12 months to become negative. However, serology has not been universally accepted as a reliable marker of histologic recovery or dietary compliance.74,75 Sensitivity and specificity for IgA tTG and EMA for prediction of total villous atrophy during GFD are 60%, 90% and 73%, 91% respectively.76 Also in patients with insulin-dependent diabetes mellitus and autoimmune diseases of the liver and thyroid, EMA and tTG antibodies give false positive results. Dietetic history taken by a dietician during an interview is found to be a better correlate with histological damage and adherence to the diet.77 The official recommendations from the American Gastroenterological Association call upon a repeat biopsy 3-4 months after of the institution of GFD. It seems more appropriate to obtain a follow-up biopsy at 6-12 months bearing in mind that histologic recovery may not be detectable before this of time point. For some researchers complete histologic recovery is feasible78 but for others even 56% of patients can demonstrate intestinal lesions of any kind even after 7 years on a strict diet.77

The nutritional status of the patients must also be evaluated by measuring iron, B12, B6, folic acid, albumin, magnesium, calcium and zinc. It is very common even after 10 years on GFD for patients to be suffering from deficiencies of various vitamins and nutritional elements.79 The patients should also perform a complete blood count, measurement of bone density by dual energy x-ray absorptiometry, counting alkaline phosphatase and parathyroid hormone and generally be assessed for complications like autoimmune diseases or neurological disorders. Assessment must be annual and lifelong.13 It is also proper to propose serological tests to the first-degree relatives of the probands. Patients with depletions of iron and folic acid should be subjected to vitamin re-
placement. When osteoporosis is found, patients and particularly women in the post-menopause age, should be supplemented with oral intake of calcium. After the institution of GFD, it is expected that clinical improvement would take place approximately in the first two weeks of abstinence in the majority of patients (about 70%). Histologic recovery can be delayed as much as two years under GFD.80 Patients who are extremely ill at presentation with severe diarrhea, weight loss, dehydration and hypoproteinemia can be supported by the short-term use of steroids, intravenous fluid administration and nutritional supplements. It is important to exclude any medications, which might be the source of obscure gluten exposure. If the patient exhibits histologic improvement without symptomatic relief, other causes of intestinal dysfunction must be sought. Disorders that commonly coexist with celiac disease and provoke diarrhea or dyspeptic symptomatology include pancreatic insufficiency and disaccharidase deficiency, lactose intolerance, bacterial overgrowth and food allergies (soya).9,81,82 Microscopic colitis may be present in 25% of patients with CD while 10-15% of patients may be also affected by distal ulcerative colitis.13 If there is no clinical or histological response, the diagnostic evaluation should first consider the continuous inadvertent or factitious gluten consumption and then try to reveal its sources with the collaboration of an experienced dietician. If a detailed interview of dietary compliance can safely exclude any food transgressions then the possibility of more serious complications, like refractory sprue, ulcerative jejunitis, enteropathy-associated T-cell lymphoma and less devastating like autoimmune enteropathy and chronic giardiasis, must be examined.

Finally, besides the standard therapy based on diet, there are also new and more sophisticated methods under evaluation, in order to broaden the therapeutic alternatives and to achieve the resolution of gliadin toxicity more efficiently.5 The concepts of detoxification of gluten products, the degradation of gluten-epitopes by enzymes and vaccinotherapy are some of the innovations, which derive from the new knowledge of pathogenetic mechanisms. The artificial mutagenesis of immunostimulatory gliadin peptides by substitutions in their aminoacids sequences can render them inactive to provoke inflammation. The use of bacterial peptidases to reduce the antigenic load in the intestinal lumen by causing digestion of gluten peptides seems promising. The construction of peptide analogues of specific gliadin epitopes can be administered in order to achieve occupation of the HLA binding sites and confer immune tolerance. Antibodies against IL-15 could provide a useful anti-inflammatory drug as IL-15 is considered responsible for the stimulation of intraepithelial T-lymphocytes and the initiation of the inflammatory cascade.

Refractory sprue

Refractory sprue (RS) is a diagnosis of exclusion.80 It comprises patients with a clinical presentation of celiac disease and histologic abnormalities of various degrees of villous atrophy, who display resistance to the gluten-free diet. This non-responsiveness is obvious either from the beginning of the dietetic manipulations (primarily) or after a period of temporary symptomatic relief and histologic restoration, which is followed by a recrudescence of clinical manifestations. This definition presupposes a strict adherence to gluten-exclusion diet and a reasonable time interval of approximately one year at most during which histological remission is expected. Refractory sprue has a prevalence of 7-8% among celiac patients. It is considered a complication of celiac disease with poor prognosis due to the development of severe malabsorption, jejunal ulcers and intestinal lymphomas of T-cell origin.

There are some distinguishing histologic features between RS and uncomplicated celiac disease. Normal individuals and responsive celiac patients possess a population of intraepithelial lymphocytes, which in the majority (70%) are T-cells that exhibit the phenotype CD3+ (surface), CD8+. In normal subjects, 80-85% of IELs carry the αβ T-cell receptor (TCR) and 10-15% express the γδ TCR, the latter proportion is increased at 25-30% in celiac patients. Immunohistochemistry and PCR analysis have revealed that IELs in refractory sprue have an abnormal phenotype which consists of CD3α- (cytoplasmic positive), CD8-, CD4-, CD103+ markers, lack TCR receptors and demonstrate TCR γ gene rearrangements with clonal expansion. The same monoclonal lymphocytes have been detected in the gastric and colonic mucosa in 64% and 55% of patients suffering from RS respectively. The discovery of the abnormal T-cells in other parts of the gut besides the small intestine as well as in the blood stream suggests that refractory sprue is a wide spreading disease that affects many parts of the gastrointestinal tract.83 This aberrant monoclonal population of IELs has been detected in approximately 75% of patients with a diagnosis of RS. This subset of patients (type II) has a dismal prognosis, is resistant to immunosuppressive and steroidal therapies and commonly progress to overt enteropathy-associated T-cell lymphoma (EATL).84 The rest of the patients exhibit phenotypically normal, polyclonal IELs and constitute a population (type I) with favorable response to immunosuppre-
tials and long-term survival. In an open label study using prednisolone 40mg as a starting dose for 6 weeks followed by tapering and concomitantly azathioprine 2mg/kg for 1 year, clinical and histological response was noted at the end of the study in 80% of type I patients with RS in comparison to 37.5% of type II patients. Total remission was achieved in 40% and 12% of type I patients and II respectively. Nevertheless almost all type II patients died within 34 months from the beginning of therapy, the majority of them because of EATL. Therefore it is imperative to distinguish these two groups of patients for better management, as immunosuppressive therapy is able to produce deleterious effects on type II subset because of opportunistic infections or provocation of a cryptic to overt lymphoma. The relative risk for any cancer is 2-3 fold in celiac patients and 30-40 fold for EATL in comparison with the normal population. Patients with RS are at even higher risk for developing T-cell intestinal lymphomas and especially those, which show the aberrant immunophenotype (type II) to the point that many investigators have considered them as a precursor of malignant progression. Under this notion, some suggest that the best therapeutic approach is chemotherapy for such patients. One of the treatment options that have been used for RS patients is cyclosporin. In an open label study cyclosporine was titrated to provide safe serum levels between 100-200ng/ml and was administered for 2 months. Two thirds of the patients demonstrated histological improvement and 38% achieved a total histologic recovery. In a case report, infliximab was able to sustain favorable symptomatic and histologic outcomes for 18 months with the concomitant use of azathioprine (19). Disappointing were the results of the use of IL-10 in the dose of 8mg/kg for 3 months in a small number of patients with RS. There were noted side effects, rare histologic or symptomatic remission and total recurrence after withdrawal of the drug.

The EATL is a non-Hodgkin lymphoma (NHL), which arises predominantly in the jejunum but also in the ileum, stomach, colon and lymph nodes. Its presentation follows the diagnosis of celiac disease usually by 5-10 years but in 50% of the cases the detection of the malignancy can be made while the disease is silent or at the same time as celiac sprue is diagnosed. Lymphoma could be suspected in a patient with deteriorating clinical course with resistant and devastating diarrhea, weight loss, abdominal pain or low-grade fever in spite of a strict gluten-free diet or in patients presenting at some point with perforation, obstruction or haemorrhage. EATL is derived from an abnormal population of TCR α/β intraepithelial lymphocytes with monoclonal characteris-

tics and phenotype marked by CD3c+, CD4-, CD8-, and CD30+. They are also stained with TIA-1 (T-cell intracellular antigen) antibody against cytotoxic, azurophil granules in their cytoplasm. The marker CD30 has been found in patients with RS, who subsequently developed EATL, therefore has been suggested by some investigators as a screening tool for selecting patients at high risk for cryptic or overt lymphoma. The aforementioned aberrant phenotype is not constant but may present with variations like CD3- or CD8+ or CD30-. The diagnostic methods that are available for the delineation of the malignant aberration are immunohistochemistry, molecular studies of clonality and flow cytometry, the latter considered more sensitive for detection of the abnormal phenotype. Recent studies have questioned the previously estimated prevalence and type of lymphoma associated with celiac disease. They have reported a lower risk of NHL at a level below 5 fold and a lower incidence of EATL, estimated as one third of all NHL cases in celiac patients.

ALTERNATIVE DIAGNOSTIC MODALITIES

Several imaging examinations of the small bowel have been tested for the evaluation of patients with celiac disease. Their main purpose is to identify complicating lesions like jejunal ulcers, lymphomas and tumors, as duodenal biopsy remains the gold standard for the diagnosis of celiac disease. One quarter of celiac patients do not demonstrate radiological signs but in experienced hands many of the imaging capabilities have shown respectable accuracy in detecting characteristic intestinal abnormalities.

In a study of adult patients with untreated celiac pathology, enteroclysis have demonstrated high specificity of 100% and adequate sensitivity of 78% if a combination of at least three radiologic signs are present. The related features included: dilatation, fold thickening, flocculation and decrease of jejunal folds or increase of ileal folds. Certain ultrasonographic findings have been also correlated with the existence of celiac lesions. Dilated loops with increased fluid content and prominent folds of the small bowel as well as increased peristalsis and mesenteric lymphadenomegaly have a diagnostic accuracy that extends from 60 to 100%. The administration of anechoic contrast agents during ultrasound examination and the calculation of Doppler resistance index of the superior mesenteric artery can also be indicative of the histologic degree of damage.
Computed tomography (CT) of the abdomen can contribute to the diagnostic effort by exhibiting certain intestinal features. In a recent study including celiac patients under CT evaluation, the majority of them revealed abnormalities of the intestinal folds (82%), intestinal dilatation (75%) and intraluminal fluid excess (64%). Multidetector row helical CT enteroclysis showed high sensitivity and specificity (100%, 95% respectively) for defining lesions in patients with suspected small bowel diseases and especially for identifying small bowel lymphoma in individuals with probable refractory sprue.

MRI of the small bowel using the HASTE technique and assisted by the administration of oral contrast agents like Polyethylene Glycol Solution has shown a sensitivity of approximately 71% in identifying abnormalities suggesting celiac disease: dilated loops, thickening of mucosal folds, lymphadenopathy, splenic atrophy and malignancy.

Capsule endoscopy in a small study group of 10 celiac patients and equal number of controls have succeeded to demonstrate 100% specificity and 70% sensitivity in the recognition of villous atrophy assessed by four independent investigators. In the same study capsule endoscopy achieved a higher sensitivity than conventional endoscopy (70% vs 60%) for the localization of mucosal abnormalities like scalloped folds, mocaism or micronodularity. Another advantage of this modality is the capability of examining the entire small bowel in order to clarify the extent of the diseased intestine.

Push enteroscopy could be helpful in cases where the duodenal biopsies are equivocal due to patchiness of the jejunal mucosa.

Breath test, based on measurement of hydrogen on exhaled air after sorbitol digestion, seem to be more sensitive than serologic testing by AGA, EMA and t-TG antibodies for screening of first-degree relatives of celiac patients. These results were obvious particularly for the individuals with slight histological damage, characterized as Marsh I-IIIa. For example the compared sensitivity of serological tests and H2 sorbitol breath test for patients with Marsh IIIa lesions was <50% and >90% respectively.

Easy to handle laboratory kits for measurement of both IgA and IgG t-TG antibodies holds many promises for a reliable evaluation tool in mass screening or in the practitioners’ office for case finding. The dot blot assay can be performed quickly (in 20 minutes) taking only a drop of whole blood and showed a high sensitivity and specificity of 100% and 98% respectively compared to ELISA rh t-TG and EMA immunofluorescence. The one-step immunochromatographic assay can detect in less than 10 minutes the existence of IgA and IgG anti t-TG in the serum with a sensitivity and specificity estimated above 90%. These two tests could be used for the selection of patients who would proceed to a duodenal biopsy such as those at high risk like first-degree relatives and autoimmune disorders related to celiac disease. The same rule could apply to more complex testing such as genetic HLA DQ2 & DQ8 as more than 95% of celiac patients demonstrate these alleles conferring a genetic predisposition and about 3% of those positive for DQ2 will eventually develop celiac sprue.

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