

Supplemental Information

1. VOTING ON BEST PRACTICE STATEMENTS AND GRADING THE STATEMENTS FOR QUALITY OF EVIDENCE

The group voted on statements and questions developed from each of the topic areas. Statements and questions were revised based on feedback provided from participants and further critical review of the available literature. Some of the statements were deleted by voting, and the content of these was condensed into comments pertaining to relevant statements that remained. Additional statements were added on matters that had not been addressed previously. All of the votes were anonymous. A 6-point scale was used: 1, agree strongly (A+); 2, agree moderately (A); 3, just agree (A-); 4, just disagree (D-); 5, disagree moderately (D); and 6, disagree strongly (D+). Agreement with the statement (the sum of voting for A+, A, or A-) by 70% of the voting members was defined a priori as consensus. The level of agreement in the final vote is provided for each statement, expressed as a percentage.

Quality of Data

Level A: Randomized controlled clinical trials validated in different populations

Level B: Retrospective cohort, exploratory cohort, outcomes research, case-control study

Level C: Case-series study or extrapolations from level B studies

Level D: Expert opinion without explicit critical appraisal

Grades of Evidence

Grades of evidence for each statement were based on the grading of the literature and were finally assigned by using the GRADE system of 2008¹³ as follows: 1. High: further research is unlikely to change our confidence in the estimate of effect. 2. Moderate: further research is likely to have an important influence on our confidence in the estimate of effect and may change the estimate. 3. Low: further research is very likely to have an important influence on our confidence in the estimate of effect and is likely to change the estimate. 4. Very low: any estimate of effect is very uncertain.

Strength of Recommendation

Factors that affect the strength of recommendations are also on the basis of the GRADE system utilizing the quality of the evidence, and uncertainty about patient-important outcomes and cost.

2. DETAILED BACKGROUND INFORMATION ON BONE HEALTH, HEMATOLOGIC PROBLEMS, ENDOCRINE-ASSOCIATED DISORDERS IN CD, THE LIVER IN CD, AND NUTRITIONAL PROBLEMS, TESTING AND MONITORING, AND CD

A. Bone Health

Mechanisms of Bone Injury

The mechanisms of bone injury of CD are multifactorial.¹⁴⁻¹⁶ These include the following: decreased

calcium and vitamin D intake, decreased absorption of nutrients, secondary hyperparathyroidism, and decreased level of physical activity.²⁶⁻³⁰ Avoidance of dairy products due to lactose intolerance contributes to inadequate intake of both vitamin D and calcium before and after diagnosis.²⁸⁻³⁰ The intestinal injury associated with CD triggers the release of several inflammatory mediators including interleukin-1, interleukin-6, interferon- γ , and tumor necrosis factor- α .^{14-16,31} These cytokines contribute to bone resorption that is much greater than bone formation and mineralization. Although controversial, some recent evidence suggests that autoantibodies to bone contribute directly to osteoporosis.¹⁴ In addition, children with CD and growth failure display decreased levels of growth hormone and IGF-1, 2 important growth factors for healthy bone.^{15,32}

Case-control studies demonstrate abnormalities of bone health in children of varying age with previously undiagnosed CD and varied clinical presentations.^{18,33} Several factors play a role in this. Abnormal calcium, phosphate, and alkaline phosphatase values are observed in a minority of children, especially in those with the "classic" picture of poor growth and malabsorption.^{21,23-25,27,28,30,33-35} With the advent of serologic testing, the classic presentation accounts for <20% of newly diagnosed children with CD.^{1,5,6} Increased levels of parathyroid hormone, as well as decreased levels of calcium and 25-OH vitamin D can be observed in both symptomatic and asymptomatic children and adolescents at the

time of diagnosis of CD, compared with controls.^{18,25,27–30,35} Although parathyroid hormone levels recover after 1 to 2 years of a GFD, vitamin D levels remain depressed in some but not all patients.^{20,24,27,29,30,35} This may be caused by the suboptimal intake of vitamin D and calcium while on a GFD, especially when dairy products are excluded.^{26,28–30} Inadequate intake of these nutrients is not limited to those on the GFD. Multiple studies demonstrate that European and North American children, especially adolescents, consume diets deficient in vitamin D and calcium.^{28,29,35,36} At the time of diagnosis, screening for calcium and vitamin D deficiency needs to be performed in geographic areas and patient populations where this may occur.

Bone Mineral Density

The importance of bone health in children with CD is underscored by the fact that adolescence represents the time of maximum bone mass accrual throughout life.³⁶ Peak bone mass is affected by diet, exercise, presence of disease, and medication use.^{36,37} Whole-body and lumbar-spine bone mineral densities (BMD) reliably assess bone mass in children and adolescents by comparison with age-matched controls by use of z scores. Variables that effect z scores include age, sex, height, skeletal age, and stage of puberty.^{36,38} A recent meta-analysis demonstrated that decreased BMD correlates with increased fracture risk in healthy children.³⁹ Thus, BMD can be used as an index of fracture risk in children and adolescents. Numerous longitudinal and case-control studies demonstrate a decreased BMD in symptomatic and asymptomatic children with newly diagnosed CD.^{18,19,21–25,40,41}

Fracture Risk

Multiple case-control and population studies demonstrate that the fracture risk for individuals with CD ranges

between 1.3 and 1.5 times the risk of the general population.⁴² A large Swedish population study demonstrated that this risk remained for 20 years after the diagnosis of CD.⁴³ However, this study did not control for adherence to a GFD. Case-control studies indicate that the risk of fracture is highest in individuals, including children with a classic presentation of CD, who go undiagnosed for prolonged periods.^{42,44} Fracture risk decreases to that of the control population with strict adherence to a GFD.⁴⁵ Longitudinal studies have not been conducted to evaluate fracture risk in adults strictly adherent to a GFD who were diagnosed with CD in childhood or adolescence.

Effect of the GFD

In children with CD, BMD recovers within 1 to 2 years of treatment with a GFD when the duration of symptoms before diagnosis is short and GFD compliance is excellent.^{17–21, 23–27} In adults diagnosed with CD, BMD improves but often does not normalize with a GFD.^{15,16} Studies differ on the extent of BMD recovery in adolescents with CD.^{17,19,20,22, 25,26,34} Prospective studies that evaluate recovery from disease and control for adherence to a GFD are required in adolescents with varying presentations of CD. The rate of BMD recovery appears to be most rapid in the first year of treatment with a GFD at all ages.²³ BMD does not recover when adherence to a GFD is poor and poor dietary adherence remains the major factor for increased fracture risk in patients with CD.^{15,20,22,25,28,30,45–47} BMD should be obtained in pediatric patients who have received GFD nutritional counseling but report ongoing noncompliance to a GFD for a period of 1 year.^{25,28,48} Children with 2 or more autoimmune diseases, in addition to CD, develop severe osteopenia that recovers incompletely on a GFD.⁴⁹ Bone density should be followed serially

until it normalizes in children with CD who present with symptoms or signs of poor bone health and in those with poor adherence to a GFD.^{28,33,48}

Calcium and Vitamin D Intake

When calcium and vitamin D deficiencies are identified, these should be treated until corrected. Instruction on age-appropriate intake of calcium and vitamin D should be provided during nutritional counseling at the time of diagnosis for all children and adolescents with CD. This may include recommendations for additional vitamin D supplementation that vary by geographic region depending on sunlight exposure.^{23,28,29} Unfortunately, no data exist to provide guidance on the optimal dose and duration of vitamin D supplements for children and adolescents with varying presentations of CD. In addition to diet, regular weight-bearing exercise should be encouraged because it contributes to the maximum accrual of bone mineral mass in children and adolescents.³⁶

B. HEMATOLOGIC PROBLEMS

Anemia

Iron Deficiency Anemia

Iron deficiency anemia (IDA) has been reported to occur in between 12% and 69% of newly diagnosed celiac cases.^{50–54} IDA is even found in asymptomatic individuals with CD. In 1 large series of patients with subclinical CD, IDA was diagnosed in almost half of the patients, with adults having a slightly higher incidence than children: 46% to 35%.⁵⁰ The pathogenesis of iron deficiency in CD seems to be straightforward since iron is

absorbed in the duodenum and proximal jejunum, the areas that are typically most affected in CD. The higher prevalence of anemia in Italian celiac patients with atrophic mucosa (Marsh Grade 3 histologic classification) compared with those with milder enteropathy (Marsh Grade 1 or 2) provides indirect support for this hypothesis.⁵⁵ The possible role of occult blood loss has also been proposed but appears doubtful since a large study in patients with CD and controls failed to document an increased prevalence of fecal occult blood loss in celiac patients.⁵⁶ In addition, another investigation employing⁵¹ Cr radiolabelled red blood cells proved that bleeding is uncommon in CD.⁵⁷

The treatment of IDA in CD is a GFD, supplemented with iron until stores have been restored. Once the morphology and absorptive functions of the intestinal mucosa have been restored by the GFD, hemoglobin levels progressively normalize over a period of up to 1 year, while it may take up to ~2 years to replete iron stores.⁵⁰⁻⁵²

Folate Deficiency

Another potential cause of anemia in children with newly diagnosed CD is folate deficiency.⁵⁸⁻⁶⁰ Folate is required for normal hematopoiesis, and it too, like iron, is primarily absorbed in the jejunum. Folate deficiency typically results in a macrocytic anemia but since a concomitant iron deficiency is common, this characteristic finding may not be present.⁵⁸ Because the direct assay of serum or red cell folate levels may lack of sensitivity, the use of serum and urine homocysteine levels is recommended to confirm the diagnosis since this metabolite is expected to increase in patients with folate deficiency.^{59,60} Folate deficiency can be a frequent finding in newly diagnosed CD patients although it does not always lead to clinically

significant anemia.⁶⁰⁻⁶² In patients with CD and folate deficiency, a GFD in conjunction with folate supplementation is advised.⁶⁰

Vitamin B₁₂ Deficiency

Although the main site of vitamin B₁₂ absorption is the ileum, a small proportion is also absorbed passively in the more proximal small intestine.^{60,63} Vitamin B₁₂ deficiency can be found in patients with CD, although the pathogenesis is unknown. Possible causes could include decreased gastric acid, bacterial overgrowth, autoimmune gastritis, or dysfunction of the ileum. It can be difficult to differentiate vitamin B₁₂ deficiency from folate deficiency, but in the former both methylmalonic acid and homocysteine will be elevated, whereas in folate deficiency, only homocysteine is elevated.⁶⁰ Vitamin B₁₂ deficiency should be considered in all patients with CD who have a megalocytic and/or macrocytic anemia.⁵⁸ The neurologic abnormalities that are described in vitamin B₁₂ deficiency are unlikely to occur in CD, as it would take a long time to deplete the body's large stores of cyanocobalamin.⁶⁰

Malabsorption of other nutrients may also contribute to the anemia seen in CD. Copper, vitamin B₆, pantothenic acid, and riboflavin have all been suggested as factors contributing to anemia in patients with CD.^{51,60}

Anemia of Chronic Disease

Finally, anemia of chronic disease (ACD) may occur in CD.⁶⁴ Harper et al⁵¹ reported that ~13% of anemic celiac patients had high serum ferritin levels and elevated erythrocyte sedimentation rate, supportive of the diagnosis of ACD. Similar findings were later confirmed by Bergamaschi et al⁶⁴ who described ACD in 17% of their adult celiac patients. However, no such data are available for the pediatric population.

Platelet Abnormalities

Thrombocytosis associated with CD appears to be more common than thrombocytopenia in patients with CD.⁶⁵⁻⁶⁷ The exact etiology is not known but may be a consequence of iron deficiency or, in some cases, functional hyposplenism.⁵¹ Inflammatory mediators may also play a role. The thrombocytosis typically resolves after institution of a GFD. Thrombocytopenia has rarely been reported in conjunction with CD and is thought to be autoimmune in nature, given that it has been reported in association with keratoconjunctivitis and choroidopathy.⁶⁸

Other Hematologic Abnormalities

Thromboembolic Problems

Thromboembolism and venous thrombosis have been reported rarely in adults but not children with CD.⁶⁵ Hyperhomocysteinemia due to folate, vitamin B₁₂, and vitamin B₆ deficiency can be seen in CD and may be related to the increased tendency to form clots.^{65,66} Other potential causes may include decreased levels of protein C and S (vitamin K-dependent anticoagulant proteins), elevated thrombin-activatable fibrinolysis inhibitor factor, thrombocytosis, and presence of an associated autoimmune disease.⁵¹ There may also be an environmental or genetic predisposition.

Coagulation Abnormalities

Abnormal coagulation, mostly due to prolonged prothrombin time, has also been associated with CD; 1 large study in adults reported a prevalence of 18.5%.⁶⁷ Affected patients may develop hematomas, hematuria, hemarthrosis, intestinal bleeding, retroperitoneal hemorrhage, intramuscular bleed, and intracranial bleed. The main etiology is vitamin K malabsorption, leading to a deficiency of vitamin K

dependent coagulation factors and prolongation of prothrombin time, international normalized ratio, and partial thromboplastin time. These abnormalities appear to be very uncommon in children.^{68,69}

IgA Deficiency

Selective IgA deficiency, the most common primary immunodeficiency, requires special mention in any discussion of hematologic abnormalities in patients with CD. The association between CD and IgA deficiency has been recognized for over 30 years; patients with IgA deficiency have a 10- to 20-fold increased risk of CD, and ~3% of children with CD are estimated to have IgA deficiency.⁷⁰ Although the majority of patients with IgA deficiency are clinically asymptomatic, these patients may develop anaphylactic reactions when transfused with blood containing IgA. Several genetic studies have linked this association to a shared haplotype.⁷⁰

Splenic Dysfunction

Both hyper- and (more commonly) hyposplenism have been described mostly, if not exclusively, in adults with CD.^{71,72} The causes appear to be multifactorial. The GFD has been reported to be effective in correcting hyposplenism.⁷²

Of interest, neither the guidelines from the NASPGHAN,⁶ nor the more recent ones by the ESPGHAN⁵ provide specific recommendations for investigations on any hematologic issues in newly diagnosed children or for follow-up.

C. Endocrine-associated Disorders in CD

Autoimmune Thyroid Disease

Thirty-four published reports were reviewed for this section. The vast majority of these reports are cross-sectional, with less

than half having concurrent data from control subjects. The data reported are limited by a number of methodological flaws, including no mention of the timing of the diagnoses and lack of the use of consistent definitions for thyroid disorders. Hypothyroidism is by far the most common form of autoimmune thyroid disease associated with CD. Excluding major outliers, the prevalence of autoimmune thyroiditis (having antithyroperoxidase or antithyroglobulin autoantibodies only) in individuals with CD ranges from 5% to 25% in children^{82–84} compared with 3% to 14% in the general population.^{85–88} Autoimmune clinical or subclinical thyroid disease is found in 6% to 12% of children with CD,^{84,87,89} compared with ~0.5% of children in the general population.⁸⁶ Even though the frequency of thyroid autoantibodies may be high, the predictive value is low, and such autoantibodies are likely to be predictive only of clinical thyroid disease over many years.^{84,86} Excluding preexisting thyroid disease, a cohort of 14 021 children with CD in the Swedish Registry revealed an increased risk of the development of hypothyroidism (hazard ratio [HR]: 6.0; confidence interval [CI] = 3.4–10.6), hyperthyroidism (HR: 4.8; CI = 2.5–9.4), and thyroiditis (HR: 4.7; CI = 2.1–10.2).⁹⁰

Autoimmune thyroid disease is also the most common autoimmune disorder associated with type 1 diabetes, occurring in 17% to 30% of patients with diabetes. The recommended test for initial screening of thyroid disease is thyrotropin in type 1 patients with diabetes.^{85–88} Because children with CD have a three- to sixfold higher risk for thyroid disease than do controls,^{85,87,90} thyrotropin alone would be the best test for screening, similar to recommendations from T1DM. No data exist on the optimal

timing of follow-up screening; periodic screening has been suggested.^{5,6}

T1DM

The co-occurrence of T1DM and CD has been reported in several studies, with 5% to 11% of individuals with CD expressing islet autoantibodies,^{82,91,92} compared with 0.3% to 2.3% in the general population.^{93–95} In children with a dual diagnosis of CD and T1DM, up to one-quarter already had either anti-tTG antibodies or previously diagnosed CD, before T1DM was diagnosed.⁹⁶ Moreover, in terms of the timing of the development of autoimmunity, a prospective study revealed that celiac antibodies appeared even earlier than islet autoantibodies, and that dual autoimmunity was associated with a younger age of onset than those with islet autoimmunity alone.⁹⁷

The best data available for the diagnosis of T1DM after onset of CD come from the Swedish registry, which excluded subjects with a previous diagnosis of type 1 diabetes before CD was diagnosed.⁹⁸ In this study, the risk of development of T1DM before age 20 was increased approximately twofold in patients with CD. The risk of developing T1DM within 5 years of the diagnosis of CD was 2.7, which is lower than the risk of T1DM patients developing CD.

D. The Liver in CD

Celiac Hepatitis

Celiac hepatitis is the most common presentation of liver disease in CD and is defined as an isolated elevation of ALT and AST.^{99–103} The elevation is usually <5 times the upper limit of normal and the ratio of AST/ALT is usually <1.^{99,100} A liver biopsy (which is rarely obtained) reveals mild or nonspecific histologic changes.^{99,100} The mechanisms underlying liver

injury are poorly understood in celiac hepatitis. One current hypothesis is that increased intestinal permeability to potentially harmful substances can have a direct toxic effect on the liver or initiate an immunologic reaction toward liver antigen.¹⁰³

Celiac hepatitis may be found in as many as 40% of adults and 60% of children at diagnosis; most patients are asymptomatic.¹⁰¹⁻¹⁰⁹ A GFD is the only treatment required. Liver function tests and liver histology usually normalize by 6 to 12 months on a GFD when follow-up testing is usually done. No further evaluation is necessary unless there is no response to a GFD.¹⁰¹⁻¹⁰⁹ If the LFTs do not normalize, or if they worsen while the patient is on a strictly GFD, further evaluation for diseases such as autoimmune hepatitis should be initiated.

Response to Hepatitis B Vaccine

The other liver problem related to CD is responsiveness to hepatitis B virus (HBV) vaccine. This vaccine became available in the early 1980s and most children in developed countries receive their first dose at birth and complete the series in the first year of life.¹¹⁰ Eighty-five to 90% of those vaccinated have a full response to the vaccine, defined as HBV antibody levels > 100 IU/mL. Persons with antibody levels between 10 and 100 IU/mL require an additional booster. Antibody levels <10 IU/mL are considered nonresponsive and require a second complete course of 3 vaccinations.¹¹⁰⁻¹¹²

An individual who does not develop protective antibodies after 2 complete series of vaccinations is considered nonresponsive.¹¹¹ This occurs in up to 15% of those vaccinated and can be associated with immunosuppression, renal dialysis, obesity, smoking, and alcohol abuse. Nonresponsiveness has also been associated with certain HLA types such as HLA B8, HLA-DR3-DQ2, SC01, and DR3, which are also

associated with autoimmune diseases such as CD and T1DM.¹¹²

The reported rate of unresponsiveness to HBV vaccine in celiac patients who received the vaccine while on a gluten-containing diet ranges from 32% to 70%.¹¹³⁻¹¹⁸ Studies that have looked at responsiveness in celiac patients vaccinated while on a GFD reveal a vaccine response rate similar to the general population. Ertem et al¹¹⁹ found only a 3.6% nonresponse rate to HBV vaccine in pediatric celiac patients vaccinated while on a GFD. Ertekin et al¹²⁰ studied 52 Turkish children and age-matched controls and found that patients who were compliant with a GFD (defined as a negative IgA tTG antibody) had a higher HBV response rate compared with those who were noncompliant with a GFD (positive IgA tTG antibody).¹²⁰ Other factors may also play a role in the response rate. A higher nonresponse rate to vaccination has also been reported in children who presented with failure to thrive and malabsorption as compared with more atypical presentations like constipation or abnormal liver function tests.

The public health impact of this nonresponsiveness is potentially a major problem. In 2003, Fasano et al⁹ published an epidemiological study of CD in the United States and estimated that there are more than 2 000 000 celiac patients in the United States alone. If 30% to 70% of patients with CD were unresponsive to HBV vaccine and are susceptible to HBV, the public health impact of this problem could be staggering in the United States and worldwide. Currently there are no published guidelines that support evaluating celiac patients for HBV vaccine unresponsiveness. However, the data available on unresponsiveness in those with untreated CD support such screening.

E. Nutritional Problems in CD

Anthropometric Impact

Nutritional status and growth at the time of CD presentation are often suboptimal in children and adolescents.¹²¹ Inflammation triggered by gluten can lead to maldigestion and malabsorption, resulting in poor weight gain and micronutrient deficiencies.¹²² CD can also impact linear growth alone without any associated gastrointestinal symptoms; the disease has been reported in up to one-third of children with isolated short stature.¹²³⁻¹²⁷ Suboptimal nutritional status in CD has been characterized by detailed analysis of body composition utilizing a combination of anthropomorphic measurements as well as dual-energy radiograph absorptiometry.^{23,128,129} Several studies have demonstrated that children with CD have significantly lower weight, height, fat mass, fat free mass, and bone mineral content, compared with age and sex-matched control groups.^{23,128,129}

However, the clinical manifestations of CD are diverse and the most common way that CD presents is changing.^{130,131} Perhaps reflecting similar trends in the general population, an increasing proportion of children are overnourished at the time of initial CD diagnosis. Recent studies have identified ~10% of children diagnosed with CD as overweight and ~5% as obese.^{132,133} After 1 year on a GFD, the majority of children who are undernourished will have restoration of body composition comparable to controls.^{23,129} Moreover, many children who are overnourished will have initial weight loss after the GFD is initiated, but movement toward ideal body weight is not universal and some children gain weight.^{132,133} Therefore, routine assessment of anthropometric measures—including height, weight, and nutritional proportionality

(BMI for those greater than 3 years old and weight-for-height ratio for those younger than 3 years old)—should be obtained for all children being evaluated for CD at the time of diagnosis and at follow-up visits to ensure that optimal growth is achieved.

Micronutrient Impact

In addition to impact on growth, the enteropathy characteristic of untreated CD may also influence micronutrient status. Several studies have revealed deficiency states for vitamin B₆, B₁₂, and folate,^{134,135} even in children with silent CD identified through screening.⁶² Vitamin D insufficiency and deficiency are highly prevalent in the general pediatric population,¹³⁶ and controversy exists as to whether children with CD are at increased risk. Some studies reveal a higher prevalence of vitamin D deficiency in patients with CD compared with controls,²⁷ whereas others have revealed no significant difference.^{137–139}

There is also a concern for trace element deficiencies in children with newly diagnosed CD, most notably for zinc and iron. Although serum zinc levels have been reported to be low in more than half of children at the time of CD presentation,^{139–142} these levels have improved within 4 weeks of starting a GFD, irrespective of zinc supplementation.¹⁴¹ Iron deficiency anemia is present in ~20% of children with newly diagnosed CD¹⁴³ and is an indication for CD screening.⁶ Although anemia usually improves with the use of a GFD plus iron supplementation, iron deficiency has been reported to persist in some children with CD.¹⁴⁴ Such children require evaluation by a hematologist. Selenium is another trace element that has been reported to be deficient in some children with CD compared with controls.¹⁴⁵ To date, there is insufficient evidence to support increased risk for

magnesium deficiencies in children with CD, especially if there are no malabsorptive symptoms present.¹⁴⁶

Unfortunately, the data on micronutrient deficiencies are limited by the small number of patients studied. Moreover, the clinical consequence of the deficiency, as well as response to GFD, have not been fully characterized. Because of the insufficient data, routine micronutrient screening in all patients with CD at the time of diagnosis or while on a GFD is not currently indicated. However, screening for micronutrient deficiencies should be considered if there is clinical evidence of a specific deficiency or in cases of malnutrition.

Medical Nutrition Therapy

MNT utilizing the GFD is essential for achieving optimal health and nutritional status for children diagnosed with CD. However, there are several challenges associated with the use of the GFD. The first challenge is adherence to the GFD, because gluten is present in many dietary staples, gluten-free labeling laws have not been universally agreed upon or mandated, and it is often difficult for children to follow a diet that requires them to be different from most of their peers.^{147,148} The second challenge is to ensure that the administered GFD is a healthy diet in terms of caloric and micronutrient intake. Commonly used gluten-free food choices can be associated with both macronutrient and micronutrient imbalances.^{149–151} For example, many gluten-free processed foods are higher in saturated fats than gluten-containing counterparts.¹⁵² Moreover, many gluten-free grains are often not enriched with vitamins and minerals, and therefore are lower in thiamin, riboflavin, niacin, folate, iron, and dietary fiber than gluten-containing grains.^{153,154}

Because of these challenges with the GFD, in terms of both adherence and

nutritional balance, several groups of experts have recommended that all children being evaluated and treated for CD should be followed by an experienced dietitian who is knowledgeable about the disease.^{5,6,155} The long-term clinical consequences of the GFD, potential nutritional deficiencies, and the utility of micronutrient supplements in children with CD have not been thoroughly investigated. To ensure that all nutritional needs are being met, the American Dietetic Association has recommended the use of an age-specific multivitamin in children with CD if the usual food intake reveals nutritional inadequacies that cannot be alleviated through improved eating habits.¹⁵⁵

F. Testing and Monitoring

CURRENT PRACTICE – INITIAL DIAGNOSIS

Serology

Measurement of IgA antibodies to tTG (anti-tTG) is recommended for initial testing, due to high sensitivity (94%), specificity (97%), and excellent standardization.¹⁵⁶ The IgA anti-endomysium (EMA) determination is nearly 100% specific of active CD, but should be used only as a confirmatory test since it is expensive and operator-dependent.¹⁵⁷ More recently, deamidated gliadin peptides (DGP) antibodies, particularly of the IgG class, have been introduced with a sensitivity and specificity comparable to IgA anti-tTG, but with a better performance in IgA-deficient subjects and in children younger than 3 years.¹⁵⁸ A recent study summarized the evidence from 2004 to 2009 on the performance of laboratory-based serological tests for diagnosing CD in children, using histology as the reference standard.¹⁵⁹ A total of 16 articles were included in a

meta-analysis, reporting on 3110 patients (1876 with CD, 1234 without CD). The results revealed that IgA-EmA and IgA-anti-tTG tests appear highly accurate to diagnose CD. IgG-anti-DGP tests may help in excluding CD. IgA-anti-gliadin antibody and IgA-DGP tests revealed inferior accuracy.^{4,159} Serology must always be interpreted in conjunction with clinical judgment because an estimated 10% of patients with CD have normal serology.¹⁶⁰

Serology in IgA Deficient Patients

IgA deficiency is the most common primary immunodeficiency in industrialized countries with a prevalence of ~1:600 (0.17%) in the general population.¹⁶¹ IgA deficiency appears to be a polygenic disorder, and several of the genes involved have recently been identified.¹⁶¹ The involvement of genes associated with autoimmunity may suggest that this condition in itself is an autoimmune disease.

The prevalence of IgA deficiency is significantly more common in patients with CD (1.29%) and other autoimmune diseases, like type 1 diabetes (0.9%).¹⁶² Because the landmark screening test for CD is based on IgA tTG antibodies, the chance exists that a small, yet not negligible portion of patients with CD, can test falsely negative to serological screening. Therefore, current guidelines recommend using the combination of tTG IgA antibodies plus total IgA as a cost-effective approach to capture patients with CD at screening.¹⁶³ However, IgG antibody tests are not as sensitive or specific to diagnose CD, and clinical judgment is required to decide when to use endoscopy in cases of IgA deficiency.

It has been recently reported that the tTG serum levels can predict IgA deficiency.¹⁶⁴ Using receiver operating characteristic curve

analysis, Shahnaz et al¹⁶⁸ identified a cutoff value for tTG IgA of ≥ 0.10 μmL to exclude IgA deficiency with a sensitivity of 0.92 and specificity of 0.84. Based on these results, the authors concluded that by using tTG IgA value quantitatively, only values < 0.10 μmL require total IgA measurements to rule out IgA deficiency and hence a potentially false-negative screening result. Until these potential alternative approaches are validated and replicated, the current recommendations remain to use the combination of tTG IgA plus total IgA as the first line of screening for CD.^{5,6,165}

Histology

A small intestinal biopsy is required in most patients with suspected CD for final diagnosis in the United States and Canada.⁶ The characteristic histological changes include more IELs (> 25 per 100 enterocytes), elongation of the crypts, and partial to total villous atrophy.¹⁶⁵ However, the biopsy, long considered the diagnostic gold standard, has been recently questioned as a reliable and conclusive test for every case.^{166,167} These reports identify patients with active CD who have mild or no evidence of enteropathy. Indeed, the wide variability of CD-related findings suggests that it is difficult to conceptualize the diagnostic process into rigid algorithms that do not always cover the clinical complexity of this disease.¹⁶⁸

Use of HLA Testing

The negative predictive value of HLA-DQ2 and DQ8 determination is very high, that is, the vast majority of subjects who are HLA-DQ2- and HLA-DQ8-negative will never develop CD. Therefore, testing for these genes in clinical practice is of great value to rule out CD, particularly in at-risk family members of patients with CD

and in subjects who have embraced a GFD without a proper diagnosis of CD.^{1,169}

The penetrance of the HLA-DQA1 and HLA-DQB1 loci in CD is extremely high compared with other autoimmune diseases. Approximately 90% to 95% of patients with CD carry DQ2.5 heterodimers, encoded by DQA1*05 and DQB1*02 alleles both in cis or in trans configuration, and DQ8 molecules, encoded by DQB1*03:02 generally in combination with DQA1*03 variant.^{169,170} Less frequently, CD occurs in individuals positive for the DQ2.x heterodimers (DQA1*05 and DQB1*02) and rarely (~2%) in patients negative for these DQ predisposing markers. HLA molecular typing for CD is, therefore, a genetic test with strong negative predictive value. However, given that the same genes are present in ~30% of the general population, its positive predictive value is extremely low. Nevertheless, it is an important tool able to discriminate individuals genetically susceptible to CD, especially in at-risk groups such as first-degree relatives (parents, siblings, and offspring) of patients, or in those patients who already embraced a GFD without being screened for CD.¹⁶⁹

MONITORING SUCCESSFUL COMPLIANCE TO THE GFD

Although the use of serology tests for CD diagnostic screening is well accepted, their usefulness for monitoring compliance to the GFD has not been fully proven. It has been recently reported that GFD treatment produced rapid and significant qualitative and quantitative changes in CD-related antibodies that may be useful for monitoring dietary compliance.^{171,172} Antibodies often return to normal in 6 to 12 months after starting a GFD. Nevertheless, more systematic studies are

necessary to specifically establish how sensitive these tests are in detecting inadvertent or voluntary ingestion of gluten traces by patients with CD.

First-degree relatives of patients with CD and children with trisomy 21, Turner syndrome, IgA deficiency, and autoimmune diseases, including T1DM, Hashimoto thyroiditis, autoimmune liver disease, Sjögren syndrome, and IgA nephropathy are all at increased risk for developing CD and should be screened by serology.^{5,6,163}

Current Guidelines

Based on these observations, the ESPGHAN has recently published guidance for the diagnosis of CD.⁵ However, there is some controversy regarding the advice on the use of stratifying levels of IgA anti-tTG antibodies test positivity in the absence of test standardization and the vagueness of the indication to test equivocal samples. Using repeat service audit, it was recently reported that the combination of anti-tTG antibodies followed by EMA is the best strategy for all degrees of mucosal abnormality using this test

combination.¹⁶⁹ Based on the same report, reliance upon immunoassay titer is not as effective, and cannot be applied consistently across populations in the absence of assay standardization.¹⁶⁹ Therefore, the authors recommended guidelines advocating that the use of tests should involve experts in laboratory diagnostics and external quality assurance to ensure that errors of generalization do not occur and that test performance is achievable in routine diagnostic use.¹⁶⁹ Because of concerns such as these, the proposed ESPGHAN guidelines have not yet been endorsed by the NASPGHAN.