Challenges in Celiac Disease: Improving Diagnosis and Identifying Accurate Surrogate Endpoints to Assess Mucosal Recovery

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Not long ago celiac disease (CD) was described as a childhood illness characterized by presentation with gastrointestinal symptoms, irritability, and poor growth (1). Until just a few years ago it was thought that the disease was triggered at the time of introduction to dietary gluten. However, the development of a highly sensitive and specific diagnostic test for CD, the immunoglobulin A (IgA) tissue transglutaminase antibody (tTG) serology test, has allowed for non-invasive and cost-effective screening of at-risk individuals and groups (2). It is now clear that CD can develop at any age in individuals with a genetic predisposition who are ingesting gluten (3). Today, many patients present with extra-intestinal manifestations, are overweight at the time of diagnosis, or are asymptomatic and are screened as part of a high-risk group (4,5).

The prevalence of CD, like many other autoimmune diseases, is increasing. In adults the prevalence of CD has doubled every fifteen years since 1975 (3). Despite an improvement in clinically available serologic tests and increasing recognition of the heterogenous clinical presentation of CD, the majority of patients remain undiagnosed. Therefore our aim was to establish whether the prevalence of CD was increasing in children as it is in adults and to characterize the variable presentation of the disease. A retrospective review of patients in a pediatric practice, prospectively screening for CD, found that the prevalence of CD increased five fold over the five year screening period and when compared to the prevalence of CD in children in 2003 it had also increased (Leonard et al attached). Additionally the majority of patients diagnosed with CD were asymptomatic at presentation and found due to the screening program.

This data is in agreement with previous work that shows that compared to fifty years ago, patients with celiac disease now are presenting at a later age and their mucosal disease at presentation may be milder (4-8). Despite this, the guidelines and clinical practice of how we confirm whether CD has healed in children has not been revisited. Data from adult patients with CD suggests the persistence of enteropathy in more than 33% of patients on a GFD, irrespective of symptoms or positive serology (9,10). This persistent enteropathy in adults has been associated with an increased risk of lymphoma, low bone density, and fracture however there is no clear understanding of the risk of mortality (11-13). Therefore, our group aimed to evaluate whether tTG correlates with
mucosal damage at the time of a repeat endoscopy with duodenal biopsy in children with CD on a GFD. We found that nearly 20% of children on a gluten free diet did not achieve mucosal recovery and that the current serological measure to investigate mucosal healing, the serological test tTG, while accurate to diagnose CD is not accurate to assess mucosal recovery. Overall, this work suggests that improved screening protocols to diagnose CD in children are needed and that prospective studies which include objective measurements of dietary transgression are needed to establish whether the management criteria of CD in childhood should be reconsidered.

This work highlights that while great strides have been made improving serological screening tests to diagnose CD and in recognizing the heterogenous presentation of CD, it is still vastly under-diagnosed. Furthermore one in five children with CD treated with a GFD may have persistent enteropathy. Current guidelines which encourage the use of serological screening tests to assess mucosal recovery have not been validated and further research to identify accurate biomarkers of mucosal recovery are needed.


Screening for Celiac Disease In A Pediatric Primary Care Setting

Running Head: Screening for Celiac Disease

Article Category: Epidemiology

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Screening for Celiac Disease In A Pediatric Primary Care Setting

Abstract:

Celiac Disease (CD) is an autoimmune enteropathy in genetically predisposed individuals triggered by the ingestion of gluten. The prevalence in adults in the U.S. is increasing. Despite recognition of asymptomatic patients that benefit from screening and improved diagnostics, the majority of patients remain undiagnosed. The purpose of this study is to determine the prevalence of CD in at-risk and not at-risk pediatric patients in a primary care practice routinely screening for CD. The records of 2325 pediatric patients who underwent serological testing with IgA tissue transglutaminase (tTG) during a 5 year period were reviewed. Patients were categorized as at-risk or not-at-risk for CD. The prevalence of CD in at-risk patients was 1:26, the prevalence of CD in not-at-risk patients was 1:111. Our results suggest that the prevalence of CD in children approximates that of U.S. adults and that the true prevalence in children without known risk factors may be increasing.

Key Words: Adolescent, Autoantibodies/blood, Celiac Disease/Diagnosis, Celiac disease/epidemiology, Child, Diagnosis, Prevalence, Risk Factors, Screening, Transglutaminases/Immunology,
Screening for Celiac Disease In A Pediatric Primary Care Setting

Background:

Celiac disease (CD) is a chronic, inflammatory enteropathy caused by the ingestion of gluten in a genetically predisposed individual.\(^1\) The overall prevalence of CD in the United States has been reported as one in 133; those with a family history of CD are at a much greater risk.\(^2\) The prevalence of CD in adults has increased from a global prevalence of 0.03% in the 1970’s to current reports of 0.5% to 1.26% in Europe and the US.\(^2,3,4,5\) A large screening of a healthy population in the U.S. reports a frequency of 1:105 in adults without risk factors and 1:320 in children.\(^2\)

According to the North American Society For Gastroenterology, Hepatology, and Nutrition (NASPGHAN) guidelines, children presenting with persistent diarrhea, poor growth, gastrointestinal symptoms, short stature, delayed puberty, dental enamel defects and iron deficient anemia resistant to oral therapy should undergo screening for CD. Individuals with a first degree family member with CD and those with associated diseases such as type 1 diabetes mellitus (T1DM), Down syndrome, thyroiditis, Turner’s syndrome, or immunoglobulin A deficiency are suggested to undergo testing even if asymptomatic.\(^6\) Although guidelines from the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) have expanded the atypical symptoms for which testing is recommended, an estimated 33-67% of patients are asymptomatic at the time of diagnosis.\(^4,7\) Despite improved serological tests to diagnose CD and increased awareness of asymptomatic patients in high risk groups, the majority of individuals with CD are undiagnosed.

The evolution of the presentation of CD, increasing prevalence, and improvement in serological screening methods has led many to consider the utility and cost-effectiveness of screening programs in the United States(U.S).\(^8\) The purpose of this study was to determine the prevalence of CD in at-risk and not at-risk pediatric patients over a five year period in a primary care practice that has chosen to routinely screen their patients.
Methods:

Setting:

Serological testing for CD took place at a private practice consisting of two board-certified pediatricians with two office locations in the Greater Boston metropolitan area, Middlesex County, Massachusetts. Patients included in the study were referred over a five year period. Study procedures were approved by the Institutional Review Board at Massachusetts General Hospital.

Practice Population:

Patients and families seen in this practice are primarily residents of Essex and Middlesex County, Massachusetts. The practice cares for approximately 3103 patients under twenty-years of age. According to U.S. Census data from 2008-2013, the local population is predominately white (>80%). Nearly 90% of persons aged twenty-five years or older have completed high school or obtained a higher degree. The median household income in these counties is between $66,918 and $79,691.9 The practice population reflects this description.

Subjects:

Eligible subjects included patients between the ages of six months and twenty years who had serology testing for CD between January 2009 and May 2014. Patients were excluded from the study if they already carried a diagnosis of CD prior to 2009 except in the total practice prevalence calculation.

Patients were identified by review of the practice data which included all IgA tissue transglutaminase (tTG) serological tests run over the course of the study period. This list was cross referenced with data obtained by referral physician name and ICD-9 code for CD at Massachusetts General Hospital for Children (MGHfC).

Charts of patients identified were reviewed. All patients who underwent testing for CD were classified as at-risk or not at-risk according to available data. At-risk individuals were defined as children with a first-degree family member with CD, those who presented with CD-associated symptoms such as abdominal pain, diarrhea, constipation, or poor growth. Subjects with CD-associated disorders such as anemia, osteoporosis, alopecia, and autoimmune thyroid disease were included in the at-risk group. Finally patients younger than 9 years were also
included in the at-risk group as routine testing of patients occurred in patients older than 9 years of age. Patients classified as not-at-risk were asymptomatic at diagnosis, did not have any CD associated symptoms or conditions reported according to attainable records, and were older than 9 years of age.

Screening Protocol:

The time period for patient inclusion into the study was chosen based on the time during which the practice began routine screening for CD. This practice aims to screen adolescents, at the same time they are sending a lipid panel in accordance with the recommendation of the Expert Panel guidelines for cardiovascular health and risk reduction in childhood, commissioned by the National Heart, Lung, and Blood Institute. In approximately 2008 when this decision was made to begin screening, physicians screened patients that were at least 9 years of age and older at routine visits. Once a child was found to have abnormal celiac serology, their siblings were screened at the next visit if they were older than two years of age and parents were encouraged to undergo screening as well.

Serology:

Serum specimens for IgA tTG, and in certain cases anti-endomysial antibody (EMA) were sent to Quest Diagnostics for immunoassay. All tests sent for IgA tTG were reviewed; EMA testing was reviewed in selected cases. During the study period, the reference lab designated threshold for a normal IgA tTG value changed. Between 2009 and 2012 the value was 9 units per milliliter with equivocal values between 5-8 units per milliliter. After 2012, a positive value was designated as greater than 4 units per milliliter. EMA antibody testing was deemed negative if the titer was less than 1:5 per the reference lab.

Histology

Biopsies were obtained via upper endoscopy and specimens from the distal duodenum and bulb were studied. Histology reports of patients were reviewed by one of the authors of the study. Biopsy’s were scored using Marsh Criteria modified by Oberhuber (0=normal, I=increased intra-epithelial lymphocytes, II= increased intra-epithelial lymphocytes and crypt hyperplasia, III= increased intra-epithelial lymphocytes, crypt hyperplasia, and partial, sub-total, and total villous
atrophy; types IIIa, IIIb, and IIIc respectively.\textsuperscript{11,12} If biopsy revealed multiple Marsh scores, the most severe was used.

\textit{Diagnosis of CD}

Patients were referred to Pediatric Gastroenterology and Nutrition Unit at MGH/C for duodenal biopsy if they had an elevated or equivocal IgA tTG as defined by the reference lab and in one case an isolated elevated IgA EMA. Patients were diagnosed with CD if they had compatible serology and intestinal damage consistent with a score of Marsh III.

\textit{Statistical Analysis}

Descriptive statistics were used to evaluate IgA tTG performance and the means and standard deviation of age. The students T test was used to evaluate the age of presentation. The BMI did not approximate normal distribution and thus the median is reported. The Wilcoxon Rank Sum test was used to determine significance between groups comparing BMI at diagnosis.

\textbf{Results:}

A total of 2325 patients (51\% male) were tested for CD over the 5 year study period which represented approximately 75\% of the practice patients twenty years of age or younger. Since screening aims to occur after age nine, we expected that a certain number of individuals in the practice would not have been screened. The mean age of individuals tested for CD was 13.3 years with the range from 6 months to 20 years. Sensitivity and specificity of IgA tTG (Table 1) is in agreement with previous studies. Upon review, a total of 37 patients were diagnosed with CD during this time period. Ten patients who were diagnosed with CD prior to the study period were monitored via serology with IgA tTG during this time period. Only one of the 37 newly diagnosed patients did not undergo duodenal biopsy. This patient had a positive IgA tTG, positive IgA EMA and had a sibling diagnosed with CD with confirmatory biopsy on account of this practices’ screening process. For the purpose of this study, this patient was categorized having CD. At the end of the study period, the practice prevalence in patients under twenty years of age was 1.5\%. Patients diagnosed with CD were characterized as at-risk or not at-risk in order to better assess the prevalence of CD in these groups; their characteristics are shown in Table 2. The prevalence of patients at-risk for CD was 3.2\%. In this population, 70\% of patients classified as at-risk
presented with intestinal complaints such as abdominal pain, diarrhea, or constipation (Table 3). The prevalence of CD in children without known risk factors or symptoms was 1%. Patients with newly diagnosed CD were evaluated for a family history of autoimmune disease, including CD or other autoimmune disease, in first or second degree family members. This presence of a first or second degree family member with CD is known to increase a child’s risk of developing CD.\textsuperscript{2,13} In this study two of fourteen patients identified via case finding methods had a first degree family member with CD and both were asymptomatic. There were no known 2\textsuperscript{nd} degree family members with CD in patients in the at-risk group. In patients not at-risk with newly diagnosed CD, there were no individuals with a first degree member with CD, as that would have placed them into the at-risk group, however one siblings pair were diagnosed at the same time via screening and four of the twenty (20%) newly identified patients with CD had a second degree family member with known CD.

**Conclusion:**

The prevalence of CD in adults in the U.S. is approximately 1% and has increased 5 fold in the past thirty years.\textsuperscript{14} In this pediatric practice, the of prevalence of CD was 1.5%. The prevalence of CD in at-risk children was 3.2% and the prevalence in children not at-risk was 1%. We classified patients as at-risk according to the current NASPGHAN and ESPGHAN guidelines regarding who should be screened for CD based on presenting symptoms and family history as well as age since routine screening in the practice would not have occurred before age 9. Our findings are comparable to previous studies which place the prevalence of CD in children in the U.S. between 1.7\% and 3.2\%.\textsuperscript{15,2}

The presence of a first-degree family member with CD increases a child’s risk of developing CD to nearly 14 times that of the general pediatric population.\textsuperscript{2} In children with a second degree relative with CD their risk increases by 10 times that of the general pediatric population.\textsuperscript{2} As a secondary analysis we matched our risk groups to that from Fasano et al in 2003 which took into account patients with a second degree family member with CD.\textsuperscript{2} In our practice population, the prevalence of CD in at-risk children was 1:26 compared to the previously reported 1:25 in 2003 and 1:57 reported in 2000.\textsuperscript{2,15} The prevalence of CD in our cohort of
pediatric patients without a known risk factor for CD was 1:111 compared to 1:320 in 2003.\textsuperscript{2} Although exploratory in nature, this increase in likelihood of having CD despite a lack of signs, symptoms, or a family history of CD may be clinically meaningful and is suggestive of a potential increase in the prevalence of CD in the pediatric population. This increase is in accordance with previous research that showed the prevalence of CD has doubled every fifteen years for the past thirty years in adults.\textsuperscript{14}

In our cohort, 60\% of all newly diagnosed patients were asymptomatic which is consistent with recent data.\textsuperscript{4} At-risk children diagnosed with CD were significantly younger than patients identified by general screening, which is expected given that they had risk factors which prompted early testing. BMI was significantly lower in the at-risk patients which would also be expected since the majority of these children (86\%) had intestinal complaints. Current NASPGHAN guidelines suggest screening for CD in patients with first-degree relatives with CD. In this practice we may have identified an additional four individuals by expanding the family history intake to include a history of CD in second-degree family members. Therefore, expanding the screening guidelines to include this group may be useful. Additionally, by following NASPGHAN guidelines and testing first degree relatives once a diagnosis of CD is made, an additional four patients were found to have CD, two of which were parents of index cases.

Our results are limited by the study population which is restricted to a private practice location with residents from primarily two Massachusetts counties. However, the study with which we compared our findings obtained 87\% of their not-at-risk pediatric study population from schoolchildren in a total of four West Virginia counties while at-risk children were recruited at routine office visits and CD support group meetings. It is unclear how different these populations may be but certainly both are from a fairly localized region. Age and gender representation were similar between cohorts. Our population, given the large Caucasian representation may have been biased towards a higher prevalence of CD. However, sampling in both cohorts is comparable.

The practice prevalence of 1.5\% is the lower limit in this population given that 25\% of the practice has not been tested for CD. This is expected since a percentage of patients should not
have yet met the age criteria for routine testing. In addition, since CD can present at any age it is possible that patients tested in 2009 may have seroconverted by the end of the study. Due to the retrospective nature of this study, it is possible that the calculated prevalence of patients at-risk for celiac disease is exaggerated as the number of patients with a family history of CD or associated autoimmune disorders may have been underreported. This limitation highlights the increased prevalence of CD in our otherwise not-at-risk group, as an underrepresentation of the family history data would further increase the likelihood of CD in the not-at-risk group, which has already increased nearly 3 fold compared to 2003.

Routine screening for CD remains a controversial topic. Asymptomatic individuals with a positive EMA receive benefit from a gluten free diet (GFD) in terms of intestinal, serological, and symptomatic measures. However, compliance with a GFD in asymptomatic adolescents found via screening is poor. Although IgA tTG has been shown to be effective in screening for CD, CD can develop at any age. Thus, the timing and frequency of optimal serological screening is unknown. As the cost of HLA testing decreases, stratifying individuals by risk given their HLA haplotype may help to better identify this optimal screening time. Earlier identification of individuals at-risk through HLA testing, potentially at birth may allow for better identification, closer monitoring, and potentially improved compliance with the GFD once diagnosed in children at the highest risk. A recent study found that 38% of patients with a first degree family member with CD and homozygosity for HLA DQ2 may develop CD by age 10. IgA tTG is a relatively inexpensive test, its use may be maximized if high risk ages are known and screened. Combining celiac screening with other tests, in this case the lipid panel generally performed between age 10 and 12, may be a feasible way to screen high risk patients and minimize risks. Ongoing studies evaluating the natural history of CD, especially those based on haplotype, will contribute greatly to our understanding of this issue. Prospective studies evaluating the cost-effectiveness and adherence to a GFD in a screened positive pediatric population are needed.

This study suggests that the prevalence of CD in children approximates that of U.S. adults and that the true prevalence in children without known risk factors may be increasing. By strictly adhering to NASPGHAN and ESPGHAN guidelines on identifying at risk patients and additionally
screening healthy patients older than 9 years old, this practice increased the prevalence of CD in their practice by 5 fold. Following current screening guidelines and expanding them to include screening of second-degree family members with CD, resulted in diagnosing approximately only half of the individuals in this practice ultimately diagnosed with CD. While genetic screening for patients with a family history of CD may help to stratify patients known to have an increased risk, screening patients without a known history remains a challenge especially for asymptomatic patients. Physicians should keep CD at the top of their differential diagnosis for the wide variety of gastrointestinal symptoms, extra intestinal manifestations and well described CD-associated conditions. Obtaining a detailed family history paying particular attention to history of autoimmune disease and CD in first and second degree relatives may improve diagnostic rates.


Table 1. IgA tTG Performance

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>97.4%</td>
</tr>
<tr>
<td>Specificity</td>
<td>99.9%</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of Celiac Disease Cases Found According to Risk Status

<table>
<thead>
<tr>
<th></th>
<th>Case Finding (17)</th>
<th>Screening (20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>7 F (39%)</td>
<td>11 F (61%)</td>
</tr>
<tr>
<td>*Age (mean ± SD)</td>
<td>8.4 (6)</td>
<td>15 (3.2)</td>
</tr>
<tr>
<td>**BMI (median)</td>
<td>16.5</td>
<td>21.2</td>
</tr>
<tr>
<td>Prevalence</td>
<td>3.2%</td>
<td>1%</td>
</tr>
</tbody>
</table>

*Statistically significant p=0.0002
** Statistically significant p=0.019

Table 3. Symptoms reported by Children At-Risk for Celiac disease

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Cases (%)</th>
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<tbody>
<tr>
<td>Abdominal Pain, diarrhea, constipation</td>
<td>12 (70%)</td>
</tr>
<tr>
<td>Reflux alone</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>2 (12%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Alopecia</td>
<td>1 (6%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>17</strong></td>
</tr>
</tbody>
</table>
Value of IgA tTG in Predicting Mucosal Recovery in Children with Celiac Disease on a Gluten Free Diet
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Figures: 0
Tables: 5

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Abstract  Objective:  Our objective was to determine the rate of mucosal recovery in pediatric patients with celiac disease on a gluten free diet. We also sought to determine
whether IgA tissue transglutaminase (tTG) correlates with mucosal damage at the time of a repeat endoscopy with duodenal biopsy in these patients. **Methods:** We performed a retrospective chart review of one-hundred and four pediatric patients, under 21 years of age, with a diagnosis of celiac disease defined as Marsh 3 histology, and who underwent a repeat endoscopy with duodenal biopsy at least twelve months after initiating a gluten free diet. **Results:** We found that 19% of pediatric patients treated with a gluten free diet had persistent enteropathy. At the time of the repeat biopsy, tTG was elevated in 43% of cases with persistent enteropathy and 32% of cases in which there was mucosal recovery. Overall the positive predictive value of the autoantibody tissue transglutaminase was 25% and the negative predictive value was 83% in patients on a gluten free diet for a median of 2.4 years. **Conclusions:** Nearly one in five children with celiac disease in our population had persistent enteropathy despite maintaining a gluten free diet and IgA tTG was not an accurate marker of mucosal recovery. Neither the presence of symptoms nor positive serology were predictive of a patients histology at the time of repeat biopsy. These findings suggest a revisitation of monitoring and management criteria of celiac disease in childhood.

Keywords: coeliac, remission, serology, duodenal, pediatric

1. WHAT IS KNOWN
• Over 90% of pediatric patients with celiac disease achieve mucosal recovery after one year on a gluten free diet.

• Current guidelines recommend follow-up serology both to assess dietary adherence and for use as a surrogate marker of mucosal recovery

2. WHAT IS NEW

• 1 in 5 children with celiac disease may have persistent enteropathy despite adherence to a GFD

• IgA tTG may not be an accurate marker of mucosal recovery in these patients

• We did not identify characteristics predictive of persistent enteropathy in this population

Introduction:
Not long ago celiac disease (CD) was described as a childhood illness characterized by presentation with gastrointestinal symptoms, irritability, and poor growth (1). The development of a highly sensitive and specific diagnostic test for CD, the immunoglobulin A (IgA) tissue transglutaminase antibody (tTG) serology test, has allowed for minimally-invasive and cost-effective screening of at-risk individuals and groups (2). This has transformed our clinical appreciation of CD and how dietary adherence and mucosal recovery is monitored. Currently, children diagnosed with CD are followed in close collaboration with dietetic colleagues for counseling to educate and promote adherence to the gluten free diet (GFD). Growth and nutritional markers are monitored. In addition, current guidelines recommend follow-up serology both to assess dietary adherence and for use as a surrogate marker of mucosal recovery (3,4,5,6). In practice, antibody levels, usually the IgA tTG, are measured six months and then again one year after initiation of the GFD. When the level normalizes, it is presumed that mucosal recovery, attained through dietary adherence, has occurred. Adult patients with CD have similar follow-up with dietary counseling and the use of serologic markers. Studies evaluating mucosal healing in adults with CD on a GFD show that even after two years on a GFD, between one-third and two-thirds of patients have persistent mucosal damage, consistent with Marsh 3 criteria, (7,8) irrespective of IgA tTG levels. Recent data evaluating pediatric mucosal recovery is scarce, however, early data has suggested a more complete and faster healing time in children compared to adults (9). While not corroborated by evidence, anecdotally this has been explained by a decreased regenerative capacity of the adult intestine, resulting in slow and incomplete healing.
While current guidelines endorse and recommend the use of serology as a marker of dietary adherence and mucosal recovery, these serology tests have not been validated for this purpose. Our objective was to determine the rate of mucosal recovery in the pediatric population and to evaluate whether tTG correlates with mucosal damage at the time of a repeat endoscopy with duodenal biopsy in children with CD on a GFD.

**Methods:**

We performed a retrospective chart review at two hospitals and identified 104 pediatric patients who fulfilled our inclusion criteria described below. Study procedures were approved by the Partners Human Resource Committee (PHRC) and Institutional Review Boards (IRB) at MassGeneral Hospital for Children (MGH/C) and Boston Children’s Hospital (BCH).

Subjects: Data for patients seen between January 2012 and March 2015 were extracted from medical records at MGH/C. Patients were identified by ICD-9 code. Additional data were obtained for patients seen between January 2008 and December 2013 at BCH, identified using the BCH Celiac Database, a database of all children diagnosed with biopsy proven CD at that institution. 61 patients were identified at MGH/C and 43 patients were identified at BCH.

Inclusion Criteria: To be included in the study, patients were required to be 21 years or younger at the time of diagnosis of CD, must have had diagnostic endoscopy with Marsh 3 lesions, and have undergone a second endoscopy with duodenal biopsy at least twelve months after initiating a gluten free diet. All patients meeting these criteria were included in the study.
Setting: The Celiac Research Program at Harvard Medical School is a collaboration between Beth Israel Deaconess Medical Center, Boston Children’s Hospital, and MassGeneral Hospital for Children, all located in Boston, MA, USA. These three hospitals serve as quaternary care centers and referral centers for children and adults with CD and gluten-related disorders. Patients from Beth Israel Deaconess Medical Center were not included in this study.

Data collection:

Data extracted from medical records included predominant clinical symptoms, serology tests, and duodenal histology at the time of the diagnostic and repeat endoscopy. Serology tests reviewed included immunoglobulin A (IgA) level, IgA tissue transglutaminase (tTG), and anti-endomysial antibody (EMA) when available. Serological values collected within four months of the endoscopy were included in the analysis. We dichotomized serological values into positive/borderline or negative according to cut-off values defined by the laboratories which performed the tests. Mucosal changes were scored by more than one pathologist at each institution using the Marsh criteria as modified by Oberhuber (0 = normal; 1 = increased intraepithelial lymphocytes [>25/100 epithelial cells], normal crypts and villi; 2 = increased intraepithelial lymphocytes, normal villi, crypt hyperplasia; 3 = increased intraepithelial lymphocytes, villous atrophy, crypt hyperplasia) (10,11). If endoscopic evaluation revealed multiple Marsh scores, the most severe was used.

For analysis, subjects were evaluated by the presence or absence of symptoms, length of time on a gluten free diet, and categorized into two groups based on the primary indication for the repeat endoscopy. Group A was defined as subjects who had a repeat
endoscopy primarily due to clinical concern. Subjects were included in this group if they underwent a repeat endoscopy due to reporting new or persistent symptoms, reporting symptoms that prompted concern for a potentially new diagnosis, or positive serology despite following the GFD. Group B included subjects who did not have clinical symptoms or concerns that prompted the endoscopy but rather underwent a repeat endoscopy as routine follow-up for CD, eosinophilic esophagitis (EOE) or other endoscopic finding. The routine practice at one of the centers (MGH/C) is for patients older than twelve years of age with CD to undergo a repeat endoscopy with small intestinal biopsy after at least one year of treatment with the GFD to confirm mucosal healing.

Finally, we recorded whether subjects received any dietetic counseling, defined as a nutrition consultation with a Registered Dietician, during the interval between the initial endoscopy and the follow-up endoscopy. We also reviewed the physicians and dieticians notes commenting on the subjects adherence to the GFD at the clinic visit prior to the repeat endoscopy and scored adherence using criteria modified from Leffler et al: (1) Excellent = patient never eats gluten intentionally and/or has rare exposure, (2) Good = inadvertent exposure once per month, (3) poor = exposure 1-2 times per week, (4) noncompliant = not on a GFD, or (5) unable to assess GFD adherence from medical record (12).

Statistical Approach:

Categorical data are presented as frequency (percentage) and group comparisons made with either the Pearson chi-squared statistic or Fisher’s exact test when the expected cell count was <5. Continuous data are described as mean±SD if normally
distributed and median (interquartile range; IQR) otherwise. Most continuous outcomes were right-skewed and therefore group comparisons were made with the Wilcoxon rank-sum test. Two-group comparisons of normally distributed variables were evaluated by Student’s t-test. All tests of significance were two-sided with $\alpha = 0.05$, and all analysis performed with SAS (Cary, NC).

**Results**

*Subject Characteristics (Table 1)*

At the time of data collection 104 subjects were identified for inclusion into the study. Subjects were 10.7±5.0 years of age at diagnosis and 60% were female. Consistent with the literature, tTG performed well at diagnosis with 89% of subjects with a positive IgA tTG. Eleven subjects had a negative tTG at diagnosis. Two subjects with a normal IgA tTG were asymptomatic at diagnosis. One of these subjects was IgA deficient, homozygous for DQ2, and had a family history of celiac disease. This patient underwent endoscopy to evaluate a gastric lesion and was found to have duodenal villous blunting. The second asymptomatic subject had a normal IgA tTG but an elevated IgA EMA and a family history of CD which prompted the endoscopy. The other nine patients had gastrointestinal complaints which prompted an evaluation by a gastroenterologist. Six out of nine individuals had HLA testing which confirmed genetic susceptibility for CD. One of these six patients had an elevated IgA EMA in the presence of a normal IgA tTG and one patient was IgA deficient. Of the three patients that had gastrointestinal complaints, normal IgA tTG, and no HLA testing, one patient had an elevated IgA EMA, one patient was under two years of age, and all three had improvement on a gluten free diet with subsequent resolution of villous atrophy.
Overall, the majority of subjects presented with gastrointestinal complaints such as abdominal pain and constipation. Approximately 14% of subjects were asymptomatic at diagnosis and identified via routine screening of high-risk populations. Additionally, 12% of subjects in our population had a comorbid autoimmune disease such as type 1 diabetes or thyroiditis. In line with the inclusion criteria, all subjects had intestinal enteropathy consistent with a Marsh 3 lesion at diagnosis.

Patient Characteristics at Repeat Biopsy (Table 2)

At the time of the repeat endoscopy, subjects had been following a GFD for a median of 2.4 years (IQR 1.4-4.0) with a range of 1-12 years. The primary indications for repeat endoscopy were due to persistent symptoms (42%), routine follow-up for CD resolution (28%), and those reporting new gastrointestinal symptoms (27%). Twenty-four subjects (34%) had persistently elevated serology at the time of the repeat biopsy. The majority of subjects (92%) had excellent adherence to the GFD. One-hundred and two subjects (98%) saw a dietician. Only one subject had no record of seeing a dietician as the other subject had a sibling with CD and the family had previously seen a dietician. Thirty-five subjects (34%) were asymptomatic at the time of the repeat endoscopy. However, 19% exhibited persistent enteropathy consistent with a Marsh 3 lesion at the time of the repeat endoscopy.

Predictive value of tTG in identifying Marsh 3 histology at repeat biopsy (Table 3)

In practice, IgA tTG was a poor predictor of Marsh 3 histology at repeat biopsy as sensitivity was 43%, specificity was 68%, the positive predictive value (PPV) was 25%, and the negative predictive value (NPV) was 83%. The poor predictability of IgA tTG was consistent regardless of whether subjects reported symptoms at the time of the repeat
endoscopy, the duration of the GFD, or whether the second endoscopy was performed due to clinical concerns or routine follow-up. Of note, repeat serology within 4 months of the repeat biopsy was available for 71 of the subjects. Subjects with serology obtained greater than 4 months from the time of the repeat biopsy were not included in this section of the analysis.

*Concordance of IgA tTG with histology at repeat biopsy*

At the time of the follow-up biopsy, tTG was elevated in 43% of subjects with persistent enteropathy and 32% of subjects with mucosal healing. The overall concordance of IgA tTG with histology at the time of the repeat biopsy is shown in table 4. These numbers are based on the 71 subjects that had an IgA tTG measured within 4 months of the repeat endoscopy.

*Concordance of symptoms with histology at repeat scope (Table 5.)*

When examining the concordance between symptoms and mucosal recovery in the entire cohort (n=104) strictly by the presence or absence of symptoms at the time of the repeat endoscopy; the majority of patients with complaints that may be related to CD had mucosal recovery (84%) (Marsh 0-2) however 45% of patients with persistent enteropathy were asymptomatic.

*Comparison of select characteristics by Marsh severity at repeat endoscopy*

We found no significant characteristics predictive of persistent enteropathy. Sex, age at diagnosis, presence of a positive tTG within four months of the repeat endoscopy, and self-reported adherence to a GFD were not significantly different in subjects with persistent enteropathy compared to those in remission. Additionally, there was no significant difference in persistent enteropathy when comparing subjects maintaining a
GFD for more than two years compared to those maintaining a GFD for less than two years. Finally, there was no difference in Marsh severity between subjects that underwent a repeat endoscopy due to clinical concern compared to those who underwent a repeat endoscopy for routine disease screening.

**Discussion**

Historically, making the diagnosis of CD required three phases and three biopsies (13). The current protocol, which requires only one biopsy, relies upon the assumption that the vast majority of children with CD experience complete mucosal recovery after one year of a GFD. (14,15). These guidelines were drafted over 25 years ago. Over the past several decades there has been a shift to an older age at onset of CD and a milder enteropathy at diagnosis. These findings along with the transformation in clinical presentation from almost exclusively gastrointestinal symptoms to a more diverse, systemic presentation provide further evidence that pediatric CD has changed (16,17,18,19,20). Despite this, the need for a follow-up biopsy has not been revisited. Data from adult patients with CD suggest the persistence of enteropathy in more than 33% of patients on a GFD, irrespective of symptoms or positive serology (7,8). This persistent enteropathy in adults has been associated with an increased risk of lymphoma, low bone density, and fracture(21,22,23). To date there is no clear understanding of the risk of mortality. In children, the long-term consequences of suboptimal healing are also unclear. However, malabsorption and ongoing inflammation in children may have negative repercussions on physical and cognitive development.

In our population, 19% of pediatric patients on a GFD had persistent enteropathy despite treatment on a GFD for at least one year. This is similar to findings from
Ghazzawi et al. who found that 15% of pediatric patients with CD had persistent enteropathy on a GFD and higher than Bannister et al. and Vecsei et al. who reported persistent enteropathy in 5% and 9% of pediatric patients with CD on a GFD respectively (24,25,26). Characteristics including presence of symptoms at the time of the repeat endoscopy, persistently elevated tTG, and following the GFD for less than two years were not predictive of persistent enteropathy. Poor adherence, particularly in teenagers could be a potential reason for persistent enteropathy. However, in this group of patients who underwent repeat endoscopy, adherence to the GFD was evaluated by a dietician or gastroenterologist and 92% of subjects were found to have excellent adherence to the GFD. This high level of adherence may not reflect our general patient population however. Patients with symptoms or persistently elevated IgA TTG and clearly identified nonadherence/ongoing gluten exposure are unlikely to undergo repeat endoscopy as often as children without clear gluten exposure and would therefore not be included in this analysis.

Furthermore, IgA tTG was not an accurate measure of mucosal recovery in this population of pediatric patients with CD on a GFD. The NPV of IgA tTG was highest at 87% in patients treated with a GFD for more than two years however PPV was poor at 36%. Additionally, the NPV of tTG was not improved when correlated with the presence or absence of symptoms at the repeat endoscopy. Overall at the time of the follow-up biopsy, tTG was elevated in 43% of subjects with persistent enteropathy and 32% of subjects with mucosal healing. Specifically in subjects that were symptomatic at the time of the repeat endoscopy, 84% had mucosal recovery upon repeat biopsy. Only 55% of patients with persistent enteropathy at the time of the repeat endoscopy were
symptomatic. Therefore, in our population, neither the presence of symptoms nor a positive tTG could be relied upon as a measure of mucosal recovery in patients with CD on a GFD.

Despite the clinical practice and endorsement of using serological tests as markers of dietary adherence and mucosal recovery in pediatric patients with CD on a GFD, these serology tests have not been validated for this purpose. Our findings raise concerns about this monitoring approach. Identifying the frequency of persistent enteropathy in children is important to begin to understand term consequences that may be associated to it. Furthermore identifying minimally invasive accurate surrogate endpoints for patients is of utmost importance given to identify patients that may benefit from potential for new therapeutic agents that may serve as adjuvant medications to the GFD(27). Our work is in agreement with previous research that supports a lower accuracy of serological studies at the time of follow-up endoscopy compared to at diagnosis in patients with CD on a GFD. Bannister et. al evaluated 150 pediatric patients (mean age 7.5 years) diagnosed with CD who had been on a GFD for a mean of 1.4 years (25). They found that 5% of patients had persistent mucosal damage defined as Marsh 3 histology. In this study, IgA tTG and IgG deamidated gliadin peptides (DGP) were measured and used in combination at the time of the repeat endoscopy resulting in a sensitivity of 65% and specificity of 85%, a positive predictive value (PPV) of 22% and negative predictive value (NPV) of 98% (25). When the statistical approach was repeated with any equivocal values regarded as “positive” the NPV improved to 100% but the specificity dropped to 68%. While this combined approach resulted in a higher NPV, accuracy overall compared to diagnosis was sub-optimal. A separate study evaluated 53 children with CD (mean age 11.3 years)
who underwent repeat endoscopy after approximately 2.2 years on a GFD (26). This study found that 9% of patients had persistent mucosal damage defined as Marsh 3 histology at the time of follow-up. In this study, the sensitivity of IgA tTG was 83% and specificity was 87% (26). These studies provide further evidence that current serological tests used as surrogate endpoints for mucosal recovery need further investigation. IgA tTG in combination with IgG DGP may provide a more accurate measure of mucosal recovery at the time of the repeat endoscopy. The negative predictive value of these tests when used in combination may be quite powerful.

The utility of IgG DGP as a marker of mucosal disease in patients with CD on a GFD was corroborated in a recent comprehensive study evaluating twelve serological tests at the time of follow-up endoscopy in adult patients with CD on a GFD (28). This study used retrospective samples to examine 100 adult patients with CD who underwent a repeat biopsy after an average of 4.5 years on a GFD. In addition to finding that the manufacturer cutoff levels created for the diagnosis of CD are sub-optimal for patients with CD on a GFD, this study also showed that the IgG DGP correlated with residual intestinal damage as measured by a follow-up small intestinal biopsy in adult patients (28).

While we did not identify predictors of persistent mucosal damage in this pediatric population, Lebwohl et al. evaluated 7648 children and adults over an 8 year period in order to determine what factors may be predictive of persistent villous atrophy. In this study, 31% of patients had persistent villous atrophy at the time of the follow-up endoscopy which occurred at a median time of 1.3 years (29). Persistent villous blunting
was more common in older patients and males, and less common in patients with higher educational attainment (29).

The limitations of our study include that the IgA tTG assay was run in multiple laboratories due to the retrospective nature. While not ideal, all laboratories were CLIA certified. For the purposes of the study, IgA tTG was dichotomized as positive or negative according to the cut off levels supplied by the laboratory performing the test. Additionally, we included only serology tests run within 4 months of the repeat endoscopy which reduced our sample size to 71 subjects for part of the analysis. Since both Boston Children’s Hospital and MassGeneral Hospital for Children are quaternary care centers, the results may not be generalizable to other institutions. Studies have suggested that patients referred to specialized centers were three times more likely to have non-responsive celiac disease (NRCD) than those initially followed at the quaternary care center (30). Furthermore, the use of centralized pathology reading, standard biopsy procedures including standardized locations and numbers of biopsies, and evaluating the mucosa for villous height to crypt depth ratio would provide a more accurate measure of mucosal recovery. Finally, while all patients were advised about the GFD by a knowledgeable dietician, their adherence assessment was based on physician and/or dietician report at the time of the clinic visit in this study.

We found that 19% of pediatric patients with CD on a GFD may have persistent enteropathy. While the long term effects are not known, persistent enteropathy may predispose pediatric patients with CD to future complications and suboptimal growth. Additionally, IgA tTG, while accurate at diagnosis, is a poor predictor of persistent enteropathy in children with CD on a GFD irrespective of whether symptoms were
Identifying a regulatory endpoint to measure mucosal recovery in patients with CD is an exceedingly difficult task given the patchy nature of the disease and the location in the small intestine, allowing for a limited area to obtain the small intestinal biopsy with our available endoscopic methods. Current serological tests, while accurate to identify patients suspected to have or at risk for CD, have not been validated to assess adherence to the GFD or mucosal recovery in patients with CD on a GFD. New therapeutics are on the horizon. To assess the efficacy of these agents under development, accurate endpoints are necessary. In terms of clinical care, we need to understand the potential risk factors that predispose pediatric CD patients to persistent mucosal disease and identify an accurate marker of mucosal disease to help identify which children may benefit from future therapeutic agents. Our findings, show that nearly one in five children with CD may have persistent enteropathy despite maintaining a strict GFD (based on self-report and provider assessment) and that IgA tTG is not an accurate marker of mucosal healing. This suggests that prospective studies which include objective measurements of dietary transgression are needed to establish whether the management criteria of CD in childhood should be reconsidered.

References


**Table 1.** Subject characteristics at diagnosis of celiac disease (n=104).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex</td>
<td>62 (59.6%)</td>
</tr>
<tr>
<td>Age at diagnosis (y)</td>
<td>10.7 ± 5.0</td>
</tr>
<tr>
<td>Other autoimmune disorder</td>
<td>12 (11.5%)</td>
</tr>
<tr>
<td>Positive or borderline serology at diagnosis</td>
<td>92 (89.3%)</td>
</tr>
<tr>
<td>Symptoms at diagnosis</td>
<td>89 (85.6%)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>53 (59.6%)</td>
</tr>
<tr>
<td>Constipation</td>
<td>26 (29.2%)</td>
</tr>
<tr>
<td>Weight loss; poor weight gain</td>
<td>21 (23.6%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>20 (22.7%)</td>
</tr>
<tr>
<td>Nausea and/or vomiting</td>
<td>19 (21.4%)</td>
</tr>
<tr>
<td>Abdominal distention</td>
<td>17 (19.1%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>12 (13.5%)</td>
</tr>
<tr>
<td>Short stature; poor growth</td>
<td>10 (11.2%)</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>7 (7.9%)</td>
</tr>
<tr>
<td>Reflux</td>
<td>6 (6.7%)</td>
</tr>
<tr>
<td>Arthralgia; arthritis; joint problems</td>
<td>5 (5.7%)</td>
</tr>
<tr>
<td>Neurologic</td>
<td>5 (5.6%)</td>
</tr>
<tr>
<td>Gassiness</td>
<td>4 (4.5%)</td>
</tr>
<tr>
<td>Rash</td>
<td>4 (4.5%)</td>
</tr>
<tr>
<td>Marsh 3 at initial biopsy</td>
<td>104 (100%)</td>
</tr>
</tbody>
</table>

1Serology data from the time of diagnosis unavailable for 1/104 subjects.
2Symptoms ascertained from medical chart review.
Table 2. Subject characteristics at repeat endoscopy (n=104).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptomatic</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>69 (66.4%)</td>
</tr>
<tr>
<td>No</td>
<td>35 (33.6%)</td>
</tr>
<tr>
<td>Reason for repeat endoscopy</td>
<td></td>
</tr>
<tr>
<td>Persistent symptoms</td>
<td>44 (42.3%)</td>
</tr>
<tr>
<td>Routine follow-up for CD resolution</td>
<td>29 (27.9%)</td>
</tr>
<tr>
<td>New symptoms</td>
<td>28 (26.9%)</td>
</tr>
<tr>
<td>Elevated serology</td>
<td>16 (15.4%)</td>
</tr>
<tr>
<td>Esophagitis follow-up</td>
<td>15 (14.4%)</td>
</tr>
<tr>
<td>New/additional diagnosis suspected</td>
<td>10 (9.6%)</td>
</tr>
<tr>
<td>Other endoscopic finding follow-up</td>
<td>2 (1.9%)</td>
</tr>
<tr>
<td>Months from start of GFD to repeat scope</td>
<td></td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>29.0 (16.9, 48.1)</td>
</tr>
<tr>
<td>Range</td>
<td>12.0 – 144.0</td>
</tr>
<tr>
<td>Dietetic counseling</td>
<td>102 (98.1%)</td>
</tr>
<tr>
<td>Adherence to GFD</td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>95 (91.3%)</td>
</tr>
<tr>
<td>Good</td>
<td>5 (4.8%)</td>
</tr>
<tr>
<td>Fair</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Non-compliant</td>
<td>2 (1.9%)</td>
</tr>
<tr>
<td>Unable to assess from medical record</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Repeat serology positive or borderline*</td>
<td>24 (33.8%)</td>
</tr>
<tr>
<td>Marsh score at repeat biopsy</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>64 (61.5%)</td>
</tr>
<tr>
<td>1</td>
<td>14 (13.5%)</td>
</tr>
<tr>
<td>2</td>
<td>6 (5.8%)</td>
</tr>
<tr>
<td>3</td>
<td>20 (19.2%)</td>
</tr>
</tbody>
</table>

*Repeat serology was only included if it was measured within 4 months of the date of repeat endoscopy (N=71).
**Table 3.** Test performance of IgA tTG in predicting marsh 3 histology at repeat biopsy

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Se</th>
<th>Sp</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>71</td>
<td>0.43</td>
<td>0.68</td>
<td>0.25</td>
<td>0.83</td>
<td>0.63</td>
</tr>
<tr>
<td>Symptomatic at repeat biopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>49</td>
<td>0.25</td>
<td>0.73</td>
<td>0.15</td>
<td>0.83</td>
<td>0.65</td>
</tr>
<tr>
<td>No</td>
<td>22</td>
<td>0.67</td>
<td>0.56</td>
<td>0.36</td>
<td>0.82</td>
<td>0.59</td>
</tr>
<tr>
<td>Months on GFD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24</td>
<td>30</td>
<td>0.20</td>
<td>0.52</td>
<td>0.08</td>
<td>0.76</td>
<td>0.47</td>
</tr>
<tr>
<td>≥24</td>
<td>41</td>
<td>0.56</td>
<td>0.81</td>
<td>0.45</td>
<td>0.87</td>
<td>0.76</td>
</tr>
<tr>
<td>Primary reason for repeat biopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>¹Group A</td>
<td>49</td>
<td>0.57</td>
<td>0.62</td>
<td>0.20</td>
<td>0.90</td>
<td>0.61</td>
</tr>
<tr>
<td>²Group B</td>
<td>22</td>
<td>0.29</td>
<td>0.87</td>
<td>0.50</td>
<td>0.72</td>
<td>0.68</td>
</tr>
</tbody>
</table>

¹Group A includes subjects who had a repeat endoscopy secondary to a clinical concern including a report of new or persistent symptoms, symptoms prompting concern for a second diagnosis, or positive serology despite maintaining a GFD.

²Group B includes asymptomatic subjects who had a repeat endoscopy for routine follow-up of CD or EOE.

*Repeat serology was only included if it was measured within 4 months of the date of repeat endoscopy (N=71).

**Table 4.** Concordance of IgA tTG with histology at repeat biopsy

<table>
<thead>
<tr>
<th>Marsh Score</th>
<th>Frequency (%)</th>
<th>tTG Positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>31 (43.6)</td>
<td>11/31 (35.5)</td>
</tr>
<tr>
<td>1</td>
<td>11 (8.5)</td>
<td>6/11 (54.5)</td>
</tr>
<tr>
<td>2</td>
<td>55 (7.0)</td>
<td>1/5 (20)</td>
</tr>
<tr>
<td>3</td>
<td>14 (19.7)</td>
<td>6/14 (42.9)</td>
</tr>
</tbody>
</table>

*Repeat serology was only included if it was measured within 4 months of the date of repeat endoscopy (N=71).
**Table 5.** Concordance of symptoms with histology at repeat biopsy

<table>
<thead>
<tr>
<th>Marsh Score</th>
<th>Frequency (%)</th>
<th>Symptoms Present (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>64 (61.5)</td>
<td>43/64 (67.2%)</td>
</tr>
<tr>
<td>1</td>
<td>14 (13.5)</td>
<td>11/14 (78.6%)</td>
</tr>
<tr>
<td>2</td>
<td>6 (5.8)</td>
<td>4/6 (66.7%)</td>
</tr>
<tr>
<td>3</td>
<td>20 (19.2)</td>
<td>11/20 (55.0%)</td>
</tr>
</tbody>
</table>

*Reported for all patients (N=104).