A Serological Diagnosis of Celiac Disease: A Pilot Study Toward Changing Local Practice

by

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Abstract

Introduction: Celiac disease (CD) is the most common autoimmune disorder of the gastrointestinal tract. In 2012, the European Society for Pediatric Gastroenterology, Hepatology and Nutrition changed their guidelines for CD diagnosis to include a serological diagnosis for certain patients instead of an endoscopy followed by biopsy. The purpose of this study is: (1) to apply a modified serological protocol in a prospective manner, (2) to evaluate the use of non-invasive monitoring of mucosal damage and (3) conduct qualitative interviews to determine if diagnostic strategy affects outcome, on a gluten free diet (GFD).

Methods: Pediatric patients were given the option of a serological diagnosis if their anti-tissue transglutaminase (aTTG) level was ≥200 U/mL. Those that had an aTTG <200 U/mL were diagnosed by biopsy. In both groups, intestinal permeability and inflammation were assessed using standard non-invasive measurements of sugar probes in urine and fecal calprotectin. Parental phone interviews were also conducted in a subset of each diagnostic group.

Conclusions: Our enrollment rate and parent response in interviews demonstrate that, in our local center, parents and patients welcome a non-invasive diagnostic strategy. There were no adverse affects in regards to symptom improvement or adherence to the GFD in those diagnosed by serology. After 12 months of treatment on a gluten-free diet, all CD patients showed recovery in intestinal permeability and inflammation through non-invasive measurements.

Preface

This thesis is an original work by Seema Rajani. The research project, of which this thesis is a part of received Alberta Health Services Administrative Approval for Research (File 31662) and research ethics approval from the University Of Alberta Research Ethics Board, Serological diagnosis of Celiac Disease at the Stollery Children's Hospital: A Pilot Study Toward Changing Local Practice, No. Pro00034476, October 17, 2012.

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Chapter 1. Literature Review

1.1 What is Celiac Disease and Gluten?

Celiac disease (CD) is an autoimmune disorder that is triggered by the ingestion of gluten and causes damage to the small intestine. It is known to occur through a genetic predisposition, however, these genes are necessary but not sufficient to cause celiac disease(1). Therefore, while genes confer susceptibility, other environmental factors are associated with the risk of CD developing in an individual, including viral infections, duration of breastfeeding and timing of the introduction of gluten-containing complimentary foods(2-4).

Gluten is a protein composite that can be found in wheat, barley and rye(5). It gives bread its elasticity by acting as a binding agent and it helps bread and other baked goods rise. It is made up of gliadin and glutenin, of which gliadin has a specific amino acid sequence that triggers the onset of an autoimmune reaction. Due to its proline rich sequence, gluten is a poorly digested and absorbed peptide, even to those without CD(6). Evolutionarily, the move towards agriculture over hunting and gathering, left the human body little time to adapt to the consumption of gluten in the grains. Furthermore, the processes now used to hybridize or genetically modify wheat, have made gluten more difficult for the average human body to digest(7).

1.1.1 History

Aretaeus, a Greek physician, was the first to mention the ailment of Celiac disease, in first century AD. He described “coeliacs” as those that “when they ate, had food pass

through their stomach and nothing went into the body"(8, 9). In the 19th century, Mathew Baillie, linking symptoms with diet, thought that a diet of rice would help those suffering from diarrhea and malnutrition(7, 10). Samuel Gee furthered this idea when he noticed children eating mussels daily were relieved of symptoms until the mussel season was over, and then saw symptoms returned(8). Once the link was made between diet and relief of symptoms, the banana diet became the new therapy for those with celiac disease. This movement, led by Sidney Haas, was considered the treatment for celiac disease and involved excluding bread, crackers, potatoes and other cereals(11). The association between wheat and celiac disease was not truly realized until the 1952, when Willem Dicke pieced the idea together because of the bread shortage in the Netherlands during World War II(12). He noticed children had no symptoms until bread became available from Allied airdrops and made the link between wheat and rye with celiac disease. A couple of years later in 1954, he worked with Charlotte Anderson in England to identify gluten as the main culprit in wheat(7, 10). In 1960, the first intestinal biopsies were used to diagnose celiac disease and serological tests were introduced in 1997(7). Since then, awareness and research surrounding celiac disease has vastly increased.

1.1.2 Prevalence

Celiac disease was originally thought to be a rare disease mainly afflicting those of Caucasian ethnicity. In 2001, Fasano et al., based on screening data, estimated the prevalence worldwide as 1:266 but it is now known to have a prevalence of 1:100 worldwide(13, 14). This increase is due to screening tools becoming widely available, the increased screening of high-risk groups and CD becoming noticed worldwide.

The screening of high-risk groups is a major factor in the increase of CD diagnosis(15). These groups include relatives of those with CD, those with autoimmune disorders like Type 1 diabetes and individuals with Down Syndrome (DS)(16). Relatives of those diagnosed with CD are at high risk because of their genetic susceptibility. CD and other autoimmune disorders, including those with thyroid disease and Type 1 diabetes, have common genetic factors that respond to foreign proteins in the body, triggering immune responses(10). The risk of having one autoimmune disorder is associated with a higher risk for developing other autoimmune disorders. Therefore, those with thyroid diseases and Type 1 diabetes have been shown to have a higher prevalence of celiac disease(10). Individuals with Down syndrome (DS) are at high risk because they have a higher risk of developing autoimmune disorders(17). All of these high-risk groups have varying prevalence rates of celiac disease ranging from 10% for 1st degree relatives of those diagnosed with CD to 12% in patients with Down syndrome(18). Therefore, with the knowledge that these groups have a higher likelihood than that of the general population to have CD, increased screening for these groups is suggested(16, 19-21).

With the increased availability of screening tools and awareness, the idea that only Caucasians can have celiac disease is also being reconsidered. In India, CD was thought to be very rare but now is estimated to have a prevalence of 1:96, similar to that of the United States and Canada(22). This increase in patients diagnosed with CD of South Asian descent has been seen in our local Multidisciplinary Pediatric Celiac Clinic as well, where we found that 1/3 of our patients diagnosed with CD were of South Asian descent(23).

In a study done in our local clinic, it was noted that the patients of South Asian ethnicity had increased symptoms surrounding weight concerns, rather than gastrointestinal symptoms(23). These patients were also diagnosed with increased anti-tissue transglutaminase (aTTG) levels, a screening tool for CD, compared to Caucasian patients and took an increased time to normalize(23). As awareness for CD continues to grow, there is an increased need for the study of CD in different ethnic populations. These different populations could show variances in pathology and symptomology as to what has been seen in Caucasian patients.

1.1.3 Symptoms

Symptoms for CD can be variable and can present as a wide spectrum of issues including gastrointestinal, growth or neurological issues. This variability is one of main causes for misdiagnosis of CD(14). CD has been classified as classical, non-classical, asymptomatic, and potential depending on the type of symptoms exhibited(17).

Classical CD has symptoms of malabsorption, which are mainly gastrointestinal symptoms(18). This type of CD is widely recognized and most diagnosed. It can present as diarrhea, abdominal distension, failure to thrive, poor appetite and muscle wasting. The original picture of classical CD was described as young children with distended abdomens, flat buttocks and thin arms and legs(14). Although some of these attributes are still seen regularly in clinic, the clinical picture of CD is shifting.

Non-classical CD regards those not afflicted by malabsorption but extra-intestinal symptoms such as anemia, osteoporosis, neurological and dental problems(17). Previously, these cases would not be screened for CD, due to their lack of

gastrointestinal symptoms. However, as awareness of the wide spectrum of CD symptoms increases, there are more non-classical CD cases being diagnosed. A major factor in this increase is the screening of high-risk groups(16).

Celiac disease also has an asymptomatic form where a patient has no outward symptoms of CD, however is positive on serological screening. Also, upon endoscopy and biopsy investigation of the small intestine the patient has damage similar to symptomatic patients. Although these patients are initially seen as asymptomatic, some later become categorized as subclinical, because they noticed an improvement in their health and in their quality of life after commencing on a gluten-free diet (GFD)(17). This can be as subtle as increased energy or decreased irritability and moodiness, symptoms that went unnoticed when on the gluten-containing diet.

Potential CD is described as those patients with no symptoms, normal small intestinal mucosa but elevated CD serology, positive genetic factors or are in a high-risk group(17). These patients are said to have the potential to have CD and to damage their mucosa with continuation on a gluten-containing diet. These patients are not put on a gluten-free diet but may be continually followed-up with the potential of having a subsequent positive biopsy a few years later.

Cases of CD in regards to symptoms has been compared to an iceberg in that classical CD is the most widely recognized and diagnosed but is just the tip of the iceberg (**Figure 1-1**)(14). As we go under the waterline, there are more cases of non-classical and asymptomatic patients. These groups are under-diagnosed because of the lack of awareness around varied clinical presentations of CD and the few, if any, outward

symptoms seen by patients. Asymptomatic patients are still at risk for the same long-term complications as those with classical CD because they still have mucosal damage (14, 24-26). However, the problem is being able to find and diagnose these patients. In the last decade, awareness of these high-risk groups has increased and further screening has been undertaken in order to lower the waterline of diagnosis(13, 16). This awareness, in hand with the ease and inexpensive screening tools are allowing for more individuals to be diagnosed.

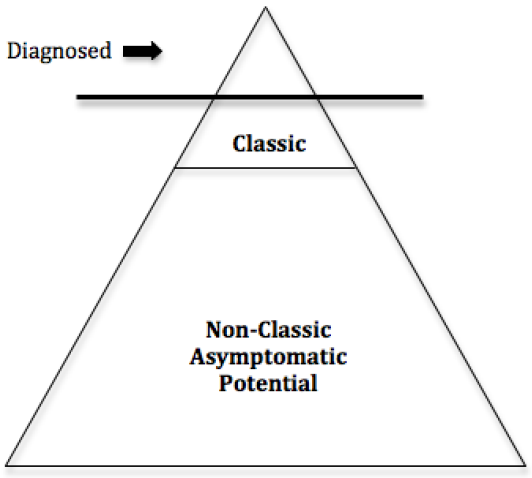

Figure 1-1. The celiac disease iceberg model adapted from Fasano et al.(14). Classic symptoms of CD, shown to be the tip of the iceberg, are those cases diagnosed the majority of the time. However, below the waterline, there are cases of CD being undiagnosed due to non-classic, asymptomatic and potential CD cases.

1.1.4 Gene Associations

The pathogenesis of CD includes the activation of the human leukocyte antigen (HLA) complex made from HLA genes. Two of these associated with CD are *HLA-DQA1* and

HLA-DQB1, which are both found on chromosome 6(1). The genes have a role in identifying and separating the body's proteins from foreign ones. *HLA-DQA1* and *HLA-DQB1* make proteins that form a complex called an antigen-binding DQαβ heterodimer. It attaches to the outside of cells and interacts with internal and foreign proteins(6). If it recognizes a foreign peptide, such as gliadin, it can trigger an immune response.

Celiac disease occurs when gene variants of the two HLA genes combine to form heterodimers DQ2 or DQ8. DQ2 can be found as two variants, DQ2.5 (DQA1*05:01, DQB1*02:01), which is the most common, and DQ2.2 (DQA1*02:01,DQB1*02:02). DQ8 is produced from HLA-DQA1*03, DQB1*03:02 (6).

DQ2 and DQ8 are present in 30% of the Caucasian population and in 90-95% of the celiac population(1). DQ2 and/or DQ8 are necessary but not sufficient for CD to occur. Although there have been patients that have a diagnosis in CD without the related alleles, these cases are said to be less than 0.05%(1, 27).

1.1.5 Immune Pathogenesis

In CD, gliadin is able to pass through tight junctions of the small intestine by forming a complex with secretory Immunoglobulin A (IgA), an antibody produced by epithelial cells(28). Upon entering the lamina propria, the neutrally charged glutamine in gliadin is converted into a negative glutamic acid by tissue transglutaminase (tTG), an enzyme found in intra- and extra-cellular tissues, including in the small intestine(5). HLA-DQ2 or HLA-DQ8 molecules have a high affinity for negative amino acids, therefore this change of sequence in gliadin allows it to form a complex with the HLA molecules in the mucosa of the small intestine(1). These complexes are antigen-presenting cells which

activate CD4+ helper T-cells that signal production of inflammatory cytokines, interferon-γ, interleukin-4 and tumor necrosis factor α(5). CD4+ T-cells also make antibodies against gliadin and tTG, known as anti-gliadin antibodies (AGA) and anti tissue transglutaminase antibodies (aTTG). The cytokines secreted by the CD4+ T-cells provide signals that lead to CD8+ cytotoxic lymphocyte cell activation(6). This activation can cause the destruction of epithelial cells and villous atrophy. The CD4+ T-cells have increased activity and infiltrate the lamina propria, while CD8+ T-cells infiltrate the epithelium(5). CD8+ T-cells and the release of cytokines and antibodies cause the gut to be in a constantly inflamed, which can lead to increased permeability or 'leakiness' of the gut(29).

1.1.6 Complications

Aside from the persistence of symptoms, untreated celiac disease can lead to a higher risk of long-term complications. These can be intestinal malignancies, autoimmune disorders, osteoporosis, liver disease and an increased mortality rate(30). These increased risks can be normalized when CD is treated and patients remain on a GFD.

CD has also been linked to infertility, recurrent pregnancy loss and infants with intrauterine growth restriction(18). It is estimated that those affected with CD have four times the risk of recurrent spontaneous abortions and pregnancy complications compared to the general population(31). Women with untreated CD can also have irregular menstrual cycles. Men can also be affected with infertility due to undiagnosed CD and similar with women affected by CD, may also have children with lower birth weights(18).

The duration of gluten exposure can put CD patients at risk for other autoimmune disorders(1, 32). A multicenter study showed autoimmune disorders have a seven-fold higher prevalence in CD patients than the general population(32). Type 1 diabetes is the most linked to CD, with 5-6% of individuals diagnosed with type 1 diabetes having CD(18). Patients with type 1 diabetes are usually screened for CD because it can present in asymptomatic form or symptoms can be confused as diabetic symptoms. Treatment of CD can help control diabetes as well as normalizing a patient's risks to other long-term complications(17).

Cancer is one of the highest risks for patients with celiac disease. In particular, patients with untreated or refractory CD have the greatest risk of developing non-Hodgkin lymphoma (NHL). NHL is a group of different types of cancer from the lymphatic system(14). A multicenter European study found the odds ratio for a patient with CD was 2.6 for NHL and associated mainly with small-bowel NHL and enteropathy-associated T-cell lymphoma(33). Another form of cancer that CD patients are at higher risk for is small intestine cancer such as adenocarcinomas. These, like NHL, are also rare in the general population occurring in about 3.7 out of 1 million people(34) . The percentage of risk for CD patients was thought to be nearly 80 fold in the 1970s but more recent studies suggest that the risk is now around 10 fold(25, 34).

Osteoporosis is a common cause of concern for patients with CD, given the disease affects the intestine's ability to absorb nutrients, such as calcium leading to low bone mass and deterioration of bone tissue(14). It is common in both adults and children with CD and is more severe in symptomatic patients than asymptomatic, although both

are at risk(35). The risk in osteoporosis is bone fractures that can cause pain and disability at an early age. The gluten-free diet allows for mucosal recovery, which in turn allows for calcium to be absorbed into the body. Although this can take a few months or years, mucosal recovery, especially in pediatric patients, diminishes the risk of osteoporosis(14).

Another concern for patients is dermatitis herpetiformis (DH), which is a condition where the skin can become red, itchy and blister. It can occur on the elbows, knees, buttocks, back, scalp, face and groin(18). It is associated with the HLA-DQ2 haplotype and it is estimated that 6.1% of patients with DH will have family members with CD(36). A gluten-free diet has been shown to control and even clear up lesions in those affected by DH(14, 36-38).

Due to all these complications, patients with untreated CD have an increased rate of mortality compared to the general population(16, 18, 34). It can take from 3-5 years on a strict gluten-free diet to reduce that risk down to the general population risk(16).

1.1.7 Treatment

The only treatment for CD is a gluten-free diet (GFD). This is the complete lifelong elimination of gluten from the diet. This removes the trigger for immune reaction and allows for mucosal recovery in the intestine. In children, a GFD can allow for complete remission of mucosal damage(39). A GFD is considered a large lifestyle change because gluten is quite prominent in our society today. It is found in bread, but also in anything with flour including cakes, cookies, bagels, pasta, sauces, sugars, chips and spices. In the last few years, a GFD has increased in popularity even in those not diagnosed with CD.

The public has become aware of the body's difficulties digesting gluten, and has been lobbying for companies to make more gluten friendly products. This has been an advantage for celiac patients, as it has expanded the market and availability of GF foods to a point where it has not been seen before in North America(40). This has been beneficial especially for children recently diagnosed with CD because they can eat the same types of foods as their peers without feeling different.

Studies have shown that the earlier the implementation of the GFD occurs, the easier it is for the patient to follow and become strict with the diet(41, 42). Therefore early diagnosis of CD in children gives them the best chance at maintaining a strict gluten-free diet, as they get older. Along with early implementation, there is a need for a good support system and education. Constant access to a dietitian, a physician and membership in community support groups is highly recommended for those that follow the GFD(14, 43).

The involvement of a dietitian is stressed because they are aware of nutritional deficiencies in a GFD and are trained in motivational support, which is much needed in lifestyle changes(44, 45). Gluten is abundant in North American lifestyle, and is a hidden ingredient in many popular household supplies. Total gluten elimination can lead to the reduction of good sources of iron, calcium, vitamin D and fiber as well as an increase in sugars one consumes(40). Fiber is very difficult to obtain and therefore a high-fiber diet is recommended when on a gluten-free diet(18). While the intestinal mucosa is recovering, the absorption of calcium and vitamin D can create problems leading to osteoporosis(35). Supplementation of vitamin D can help correct this.

Patients on the GFD need to be diligent in ensuring they are still getting foods with nutritional value. This can be difficult and can have affect the quality of life of patients(46).

The burden of a GFD for a patient with celiac disease is substantial, due to the strictness and longevity of the diet. This is not likely the popular perception or implementation of a GFD. For many CD patients, small indiscretions or cross-contamination can cause the return of symptoms. Even after several years, patients on the GFD still consider the diet to be a burden(47). The cost and availability of gluten-free foods are difficulties for those trying to follow the diet. Gluten-free foods are known to be more expensive than those that contain gluten. In a study done by Stevens et al., comparing 56 gluten-free products with their regular counter parts, they found, on average, gluten-free products cost 242% more(48). The increase in cost can make it especially hard to find a suitably nutritionally balanced meal while maintaining an affordable price range. A survey conducted by Zarkadas et al. showed 61% of participants found difficulties with the cost of commercially prepared GF food(49).

The burden of a GFD is especially apparent when dining out. The cost of gluten-free foods is increased when substituting items such as corn pasta, or gluten-free crust(50). 87% of respondents in the survey by Stevens et al, found limited choices at fast food restaurants while 77% found it limiting dining in restaurants(48). 64% were worried about the cook not being trained in making gluten-free meals and 34% found it difficult because restaurants could not provide information for gluten content on the menu items. This is emphasized in a study done by Whitaker et al., in the United Kingdom,

where 54% reported they enjoyed doing things such as dining out less often as they used to(47).

1.2 Diagnosis of Celiac Disease

1.2.1 Screening

The first screening test for CD was anti-gliadin antibodies (AGA), followed by anti-endomysial antibodies (EMA). However, the former, although inexpensive had low sensitivity and specificity, while the latter, with high specificity and sensitivity, was expensive and time consuming. Although, EMA is still used in clinical settings, the primary screening tool now is anti-tissue transglutaminase (aTTG). The aTTG has a high sensitivity (90-98%) and specificity (94-97%), is inexpensive and its automated analysis has a fast turn-around time(51). The sensitivity and specificity of current screening tools are summarized in **Table 1-1**. Current guidelines call for the use of both EMA and aTTG as screening tools for CD(19, 20).

Table 1-1. Sensitivity and specificity of serological screening tools(14).

	Sensitivity	Specificity
AGA-IgA	75-90%	82-95%
EMA	85-98%	97-100%
aTTG	90-98%	94-97%

1.2.2 Intestinal Biopsy

Once serological screening indicates a patient has celiac disease, histological confirmation through biopsy of the proximal small intestinal mucosa is considered the diagnostic gold standard for CD(19, 20). The mucosal damage is graded as a Marsh score in relation to the progression of the CD lesion in the proximal of the small intestine(52). The scores are based on a three level scale, where the lesion is either infiltrative, hyperplastic or destructive(53). **Figure 1-2** shows histopathology stains of Marsh scores 0-3 obtained in pediatric patients at the University Of Alberta. Marsh 0 is noted as a normal mucosa having long finger like projections known as villi, which enable the absorption of nutrients. Mucosa in Marsh 1 lesions still have notable villi, however there are signs of immune cells entering the intestinal lining(54). These immune cells as known as intraepithelial lymphocytes (IELs) and can cause damage to the small intestine due to release of cytokines causing the killing of cells(55). Marsh 2 grading shows some crypt hyperplasia, and increased IELs(54). Crypt hyperplasia occurs when the crypts of Lieberkühn, located in the epithelia, elongate due to increased influx of inflammatory cells(55). Marsh 3 shows destructive lesions, which have crypt hyperplasia, infiltrative IELs and villous atrophy(54). Due to the different levels of villous atrophy seen in CD patients, Marsh 3 has been further split into 3 subcategories which represent the different levels of villi flattening; 3a mild villous blunting, 3b truncated villi and 3c complete villous flattening(54).

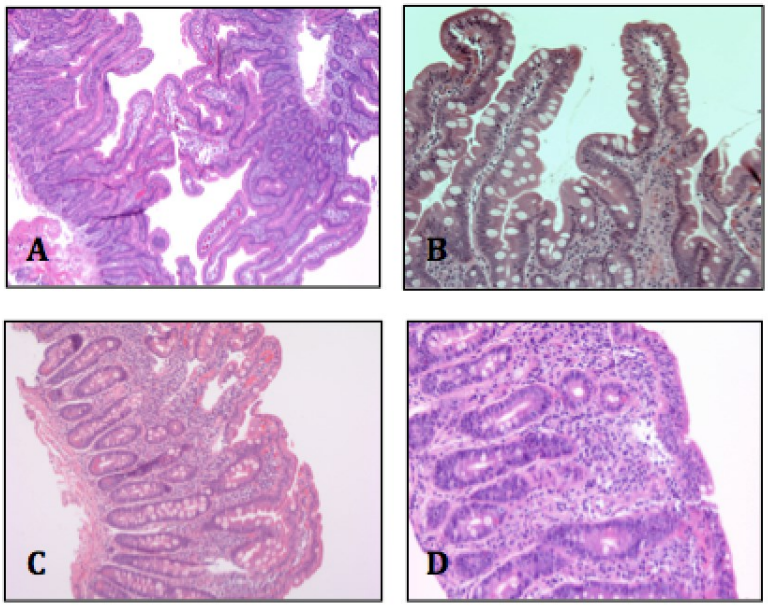

Figure 1-2. Histopathology stain of varying Marsh scores found in pediatric patients diagnosed at the University of Alberta Hospital. (a)Marsh 0, a normal intestine with healthy villi (b)Marsh 1 with infiltration of intestinal epithelial lymphocytes (IELs) in the epithelium with healthy villi. (c)Marsh 2 also with increased IELs and crypt hyperplasia (d)Marsh 3 with severely flattened villi and large crypts. Stains courtesy of Professor Consolato Sergi.

1.2.3 Genetic Testing

Genetics has recently been discussed as a diagnostic aid for CD. HLA haplotyping has been used as a clinical tool to differentiate those relatives of patients with CD at risk for potential CD. Those that do not have the genes related to celiac disease are said to be at low risk for CD(1). Studies show an almost exclusive association between CD and HLA DQ2 and DQ8, concluding that without those genes CD cannot occur(56). However, 30% of Caucasians have the HLA haplotypes associated with CD but do not have celiac disease, therefore genetic testing on its own is not a valid tool for diagnosis(57). Genetic testing is also more expensive than aTTG or EMA testing. Genetics can be used as a

determination of level of risk for CD, but there is some controversy regarding the alleles of CD and related risk(1, 27). Between the two haplotypes, DQ2 puts an individual at higher risk for CD than HLA-DQ8. HLA-DQ8 combined with DQ2 has a higher risk for CD than DQ8 alone. Individuals that are homozygous DQ2 are said to have the highest risk for CD(1, 27).

1.2.4 Controversies Over Intestinal Biopsy

The gold standard for CD diagnosis is a biopsy and confirmatory pathology(17, 19, 20). However, with the increase in sensitivity and specificity of serological tests, the necessity of this expensive and invasive procedure is being questioned. Some experts feel it is important to be able confirm intestinal damage through biopsy, especially in asymptomatic patients(58). The role of excluding other pathologies has been cited as an important reason not to skip the biopsy, however this pertains in particular to adult patients and very young patients(59). There is also some concern that aTTG serological tests are not sensitive or specific enough to fully diagnose a patient(58).

The counter argument reflects that an intestinal biopsy for the diagnosis of CD is not without flaws. Histological analysis can be subjective, and the damage can be left to interpretation depending on the experience of the technician(57, 60). Arguelles-Grande et al. found “modest” agreement between pathologists in different settings when diagnosing CD and that there was lower agreement with lower damage(61). Alarmingly, they also found that in over 40% of cases, pathologists graded a sample that had a score of Marsh 3a either as normal or less severe. This study also showed that CD was being under diagnosed by 20% in community settings.

The orientation and handling of a biopsy can affect its reading, leading to false negatives of CD(62). The biopsy needs to be the correct size, in good condition, and oriented correctly on the slide before it is embedded. 11% of samples were inadequately oriented in the study done by Arguelles-Grande et al., in which they also found that proper orientation before embedding the sample was not regular practice in most North American centers(61). Studies have been done to determine the correct instrument used to obtain the samples, forceps or suction, and the size of the forceps, trying to ensure the optimal reading of samples(63, 64). It was determined that forceps are used over suction, so that the proper size of biopsy can be taken and aid in the proper orientation and cutting of the sections.

The location and the number of biopsies taken can also cause a CD diagnosis to be missed. Damage from CD in the small intestine is patchy, allowing some areas to be damaged and some to be normal(57, 65-68). To combat this, it is recommended that when sampling, at least 4 samples be taken from different locations of the small bowel (67, 69). Pais et al. showed that taking two samples gave a 90% detection rate, while taking 4 samples increased the detection rate to 100%(67). The distal duodenum is recommended over proximal samples due to Brunner's glands which can affect the reading, however an increasing number of studies have also suggested the duodenal bulb as an important sampling location(62). Some studies have shown that patients can have atrophy solely in the bulb, therefore they consider the bulb the main site for sampling in order to diagnosis a patient with CD(66, 68, 70-72). Kurien et al. showed an increase of detection rates of 18% when they took bulb biopsies along with distal sampling(66). While Mangiavillano et al. determined that 10.6% of their patients would

have missed a diagnosis of CD if bulb biopsies were not obtained(66, 72). Bonamico et al. had two studies in which they showed 4.2% and 2.4% of their patients had solely bulb lesions indicating a diagnosis of CD(65, 68). Unfortunately, despite increased awareness of these issues an adequate number of biopsies or sampling of various locations is not always taken(73, 74).

1.2.5 Could Serology Replace Intestinal Biopsy in Children?

With the pitfalls of the biopsy and the increase in accuracy of serological tests, the question has been raised of a serological way to confirm CD in place of the biopsy. For children and their families anesthesia can be a cause of fear, worry and concern(75). As well, there is a small risk of bowel perforation(76, 77). The increase of serological screening will continue to uncover more patients requiring a confirmatory biopsy diagnosis making the cost and accessibility for serological testing much more feasible for smaller facilities.

In 2011, Mubarak et al. found that symptomatic patients in the Netherlands with aTTG levels ≥100 U/mL were all found to have celiac disease, concluding that ≥100 U/mL was a sufficient level to diagnose CD in patients without biopsy(78). This cut-off level was replicated in a study done by Barker et al., where 98% of their patients with ≥100 U/mL were diagnosed with CD(79). A study done in Edmonton, also showed retrospectively that patients ≥200 U/mL were all diagnosed with CD, implying that a high titre could diagnose patients with CD rather than having a biopsy(80). Another retrospective study, done in Italy, agreed with ESPGHAN guidelines, in showing that even a 7-fold cutoff level with EMA confirmation showed damage in the small intestine(81). In a

multicenter study, done in Italy, all patients diagnosed with CD having patchy mucosal damage or solely damage in the bulb were aTTG positive(68). In a study done by Donaldson et al., all patients that had an aTTG ≥100 had evidence of CD. Of those with an aTTG ≥100 U/mL, 99% had a lesion of at least Marsh 2, while 96% had a lesion of Marsh 3(82). In 2008, Hill et al. concluded that a biopsy was unnecessary in patients with a limit greater than 10 times the upper limit of normal, showing that the positive predictive value (PPV) for aTTG is 100% in those cases(83).

1.3 Diagnostic Algorithms and Guidelines

1.3.1 Consensus in Diagnosis

Since 1970 and until recently, the diagnostic criteria for celiac disease were similar in both Europe and North America. A diagnosis of CD required a small intestinal histology as well as symptom resolution on a GFD. The initial role of serological testing was as a screening tool and supported the need for a confirmatory diagnosis, in which case the gold standard was an intestinal biopsy.

1.3.2 North American Diagnostic Guidelines

The North American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) have maintained their views on the diagnosis of CD, and continue to recommend the gold standard to be a biopsy for those that are asymptomatic and symptomatic (**Figure 1-3**)(19). The only difference in diagnostic guidelines between asymptomatic and symptomatic patients is that if asymptomatic patients have a normal aTTG, they should be periodically tested, or have an HLA genetic test to rule out being at risk for CD. Where as with symptomatic patients, a normal aTTG could rule out CD.

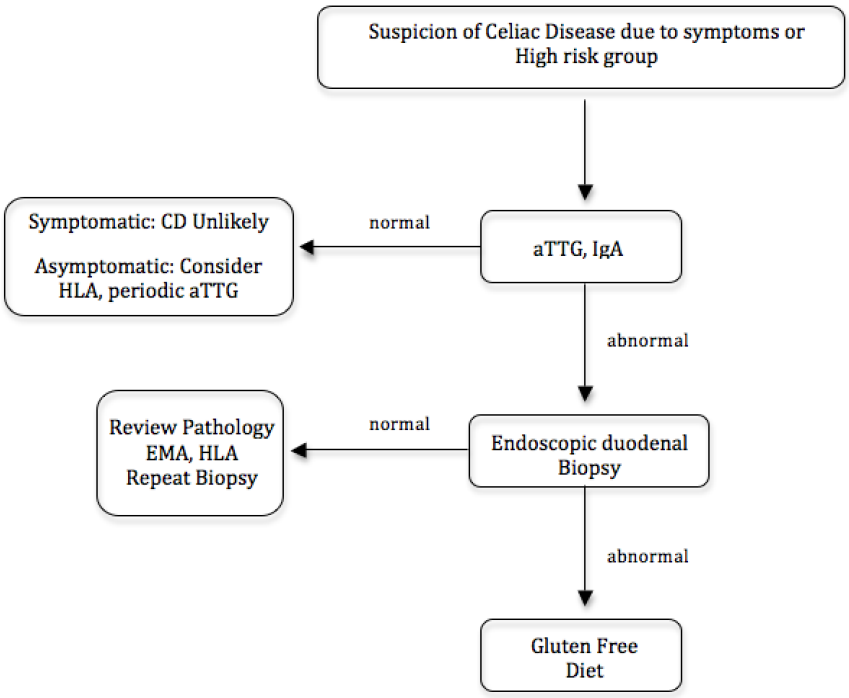

Figure 1-3. NASPGHAN guidelines for celiac disease diagnosis of asymptomatic and symptomatic patients adapted from Hill et al.(19).

1.3.3 European Diagnostic Guidelines

The European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) have recently updated their diagnostic guidelines (**Figure 1-4,1-5**)(20). Those pediatric patients that have an aTTG greater than 10 times the upper limit of normal, a positive anti-endomysial antibody (EMA) and are symptomatic are eligible for genetic testing to determine if they are at risk for CD, rather than a biopsy. Their high aTTG level and positive genetics would be enough to diagnose them as having CD. The Northern American Society of Pediatric Gastroenterology, Hepatology and Nutrition (NASPGHAN) have not implemented these rules, which are based on retrospective data.

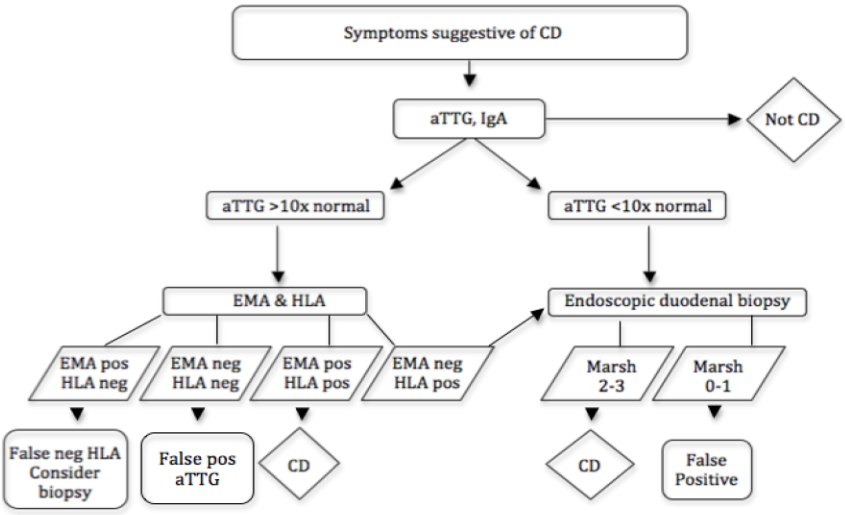

Figure 1-4. Current ESPGHAN guidelines for diagnosis of celiac disease in symptomatic patients adapted from Husby et al.(20).

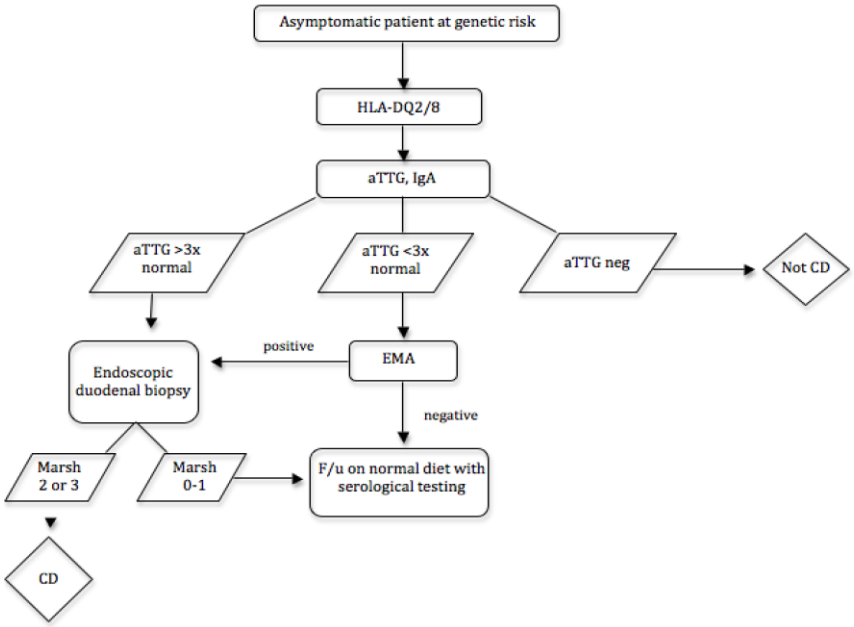

Figure 1-5. Current ESPGHAN guidelines for diagnosis of celiac disease in asymptomatic patients adapted from Husby et al.(20).

1.4 Patient and Caregiver Preferences

In 2011, ESPGHAN pediatric gastroenterologists were surveyed regarding current practices of the diagnosis of CD. In this study, 77% of gastroenterologists wanted the ESPGHAN guidelines changed and 44% wanted to omit the small bowel biopsy when diagnosing CD in specific cases(84). In a local study done in Edmonton, investigators showed that not every patient with an aTTG indicating a possible diagnosis for CD was being referred for an endoscopy. This could mean, patients or their family physicians were opting for a GFD without biopsy confirmation.

Parental preference between the two diagnosis options has not been studied. However, studies regarding parental feedback on the small bowel biopsy have been published, although they are few in number. In 2003, Swedish research done in two different centers, found that on average 37% of parents and about 25% of children worried about the biopsy(85). In this study, there were several patients that had previous experience with the biopsy procedure from having gone through it before. Our understanding about how patients and parents feel about the diagnostic process is significantly limited at this time, despite the forward movement in the medical community to consider serological diagnosis as an alternative to biopsy.

1.5 Monitoring Mucosal Healing Of Celiac Disease

Follow-up for patients diagnosed with CD is essential for success of treatment. Studies have shown that follow-up with a physician and dietitian helps enforce adherence to the gluten-free diet especially in children. Monitoring of CD requires assessment of celiac symptoms, as well as a measure of mucosal healing(19).

1.5.1 Role of Biopsies

Biopsies allow objective evidence of the damage caused by the ingestion of gluten. At the same time additional diagnoses may be determined, such as gastritis and eosinophilic esophagitis, which appear to have an increased risk in celiac disease patients (although the clinical significance of this finding in asymptomatic patients is not well understood)(86-91). Baseline histological findings from biopsies also allow for comparison to a second biopsy after being on gluten-free diet(58). Recent studies show that this is of relevance given that long term histological recovery does not seem to be observed in all patients(92). Both of these issues are particularly important for adults, where refractory celiac disease and early pre-malignant changes need to be diagnosed and managed with repeated biopsies(21, 58). However, in most pediatric clinical settings, a repeat biopsy after implementation of the GFD, is not a routine measure of mucosal recovery and usually only occurs if there is no clinical response to the GFD.

1.5.2 Role of Anti-tissue Transglutaminase

Serological testing is used in clinical settings to determine mucosal healing and to monitor adherence to the GFD. An elevated aTTG can occur in patients that have ingested gluten. The level of aTTG has also been used to measure mucosal damage in patients. Studies have shown that continually elevated aTTG levels link to the degree of mucosal damage in patients on a GFD(93-95). Mucosal healing shows through lowering of aTTG to normal levels, however this can take months or years(39). A study done by Bannister et al., demonstrated the effectiveness of serology to replace the need for a repeat biopsy(39). They concluded that use of aTTG combined with anti-deamidated gliadin peptide IgG (DGP) gave a negative predictive value of 98%.

1.5.3 Non-invasive Measurement of Mucosal Permeability

Inflammation and damage caused by immune cell epithelial infiltration can compromise the tight junctions between cells and cause the intestine to be permeable(96). Non-invasive measurement of intestinal permeability in CD has been achieved using orally administered sugar probes. The amount of probes excreted in the urine reflect changes occurring in the proximal intestine and sensitivity of this measurement is estimated to be 96-100%(97, 98).

Lactulose, a disaccharide, and mannitol, a monosaccharide, are large and small sugar probes respectively, and their size difference allows for transfer in the intestine through different pathways(99). Normally, mannitol is readily absorbed in the intestine and excreted in urine. However, mannitol excretion in celiac patients is decreased because of the loss of small tight junctions at the top of villi inhibiting mannitol uptake. Therefore, a decrease in mannitol serves as a reflection of villous atrophy in the small intestine(97, 100). Catassi et al. showed that the urine recovery percentage of mannitol in asymptomatic patients, was not decreased compared to controls, showing a smaller extent of damage than in symptomatic patients(97).

Lactulose is not readily absorbed in the intestine and is passively transported between cells. An increase in lactulose in urine shows an increase of "leakiness" between the cells, allowing lactulose to transfer out of the intestine(101). Pearson et al. showed a five-fold increase of the lactulose-to-mannitol ratio (L/M) in CD patients compared to controls(99). Hamilton et al. and Uil et al. were able to show levels of L/M recovering to normal in CD patients after treatment with a gluten-free diet(100, 102).

The measurement of sucrose permeability has also been used to determine proximal gastrointestinal damage(103). Unlike lactulose, sucrose gets broken down easily in the intestine. Therefore, an increase of intact sucrose absorption shows damage in the proximal intestinal epithelium(104). Lactulose takes longer to break down, showing permeability for the whole intestine(103). Smecuol et al. studied sucrose permeability in active celiac patients and GFD-treated CD patients. They showed increased sucrose levels in active CD patients, which returned to normal on a gluten-free diet(103). In another study, Smecuol et al. estimated sucrose permeability sensitivity to be 75% and specificity to be 91%(105). In their study, there were false positives showing increased sucrose permeability but these patients had other gastric lesions on endoscopy.

1.5.4 Non-invasive Measurement of Mucosal Inflammation

In the pathogenesis of CD, there is infiltration into the lamina propria and epithelium of the intestine by antibodies, cytokines and CD8+ cells. This immune response is the cause of inflammation in the small intestine. Fecal calprotectin (FC), a protein found in the stool, can be measured biochemically and has been shown as a marker for inflammation, especially in patients with inflammatory bowel disease (IBD)(106-108). Previous studies have shown that the concentration of FC is increased in patients with newly diagnosed CD and decreases after implementation of a GFD(109, 110). Ertekin et al. found increased histological severity correlated to increased calprotectin levels, in children with total-villous atrophy compared to partial(109). They also showed a decrease of FC in patients on a GFD. Balamtekin et al., found that higher levels of FC corresponded to patients with GI symptoms against those with non-GI symptoms(110). However, other studies have shown that this is not the case(111, 112). The most recent

report by Capone et al., failed to notice a correlation of FC levels with GI symptoms, histological severity and levels of aTTG in 50 newly diagnosed adult CD patients compared to controls(112).

1.6 Summary

Celiac disease is common with a prevalence of 1%(13). Research has allowed for serological tools to become increasingly accurate and inexpensive, and growing awareness of CD is uncovering more individuals affected by this disease. This raises the issue of the need of a cost effective, rapid diagnosis and the possibility of a less invasive method, especially for children. Taking this into consideration ESPGHAN changed their diagnostic guidelines to reflect the issues of a biopsy, but NASPGHAN has yet to change their stance(19, 20). With increased research regarding these new guidelines and prospective studies showing the advantage of such non-invasive methods, mounting evidence could encourage North American societies, like NASPGHAN, into changing current guidelines.

Chapter 2. Rationale, Aims & Hypothesis

2.1 Rationale

The purpose of this pilot study is to evaluate the effectiveness of serological diagnosis at a tertiary referral center for pediatric celiac disease: Stollery Children's Hospital Multidisciplinary Pediatric Celiac Clinic. A serological diagnosis is cost-effective and allows for a rapid and non-invasive route of diagnosis for children. At the local center, the wait time for a scope can be up to 4 months, which means a child will have to

remain on gluten during that time adding to their discomfort and affecting their quality of life. Serological diagnosis has a quick turn-around time of about two weeks. A serological diagnosis also offers children and parents a non-invasive route, as opposed to undergoing general anesthetic and a procedure that is not without risks. .

A key aim of this study is to modify ESPGHAN criteria to be appropriate to the local setting. These modified guidelines allow patients and their families a non-invasive method of diagnosis and reduces the wait time for a diagnosis allowing for faster treatment. According to researchers at the local center in Edmonton, one third of children in Edmonton with positive CD serological screen from their family physicians are not being referred to the celiac clinic for a confirmatory biopsy. Although the reason for these patients not receiving the biopsy is unknown, it could be due to either parental or physician preference. A number of these children have aTTG levels below the recommended serological diagnosis threshold stated by ESPGHAN. Not only are these children not receiving a confirmation of diagnosis but they are also missing the support and education shown to lead to greater success of adherence to the diet(19, 21, 45, 113). Our hopes in implementing a non-invasive diagnostic route for CD will allow those patients that want to opt out of the biopsy, another confirmatory diagnostic route using HLA genetic testing in combination with aTTG screening.

ESPGHAN guidelines currently call for an EMA serological test in addition to aTTG testing. Our study does not include this in our criteria, as an EMA test is no longer used as a diagnostic test at the University of Alberta laboratory. Previous studies have shown that EMA is consistently positive with aTTG levels ≥100 U/ml(79, 80, 114). A study

done by Brusca et al. also showed that all aTTG and EMA serological combinations in their study were equal to using aTTG alone, making the confirmatory EMA test redundant(93). In addition to this, studies have compared both tests and considered that aTTG is as reliable as EMA, if not better(94, 115). They also take into account disadvantages around the use of EMA, which are dependent on the observer as well as more time consuming(57). This was taken into account when designing our diagnostic criteria for this study.

The current serological diagnostic guidelines by ESPGHAN also consider only patients showing outward symptoms of CD(20). Our study offers this route to both asymptomatic and symptomatic patients. Asymptomatic patients are faced with the same risks of osteoporosis, infertility and increased mortality rates as symptomatic CD patients(24-26). Patients that are reported as asymptomatic also have to face the burden of a GFD. Although they show no outward symptoms of CD, they can have relief of extra-intestinal manifestations, such as fatigue and irritability, that they were not aware of previous to diagnosis(116). The internal damage and the burden of the diagnosis is the same for asymptomatic patients as symptomatic, therefore we did not find it reasonable to exclude them. Most asymptomatic patients seen at the clinic are screened because of family history of CD or Type 1 diabetes. Patients with diabetes will not be offered a serological diagnosis due to fluctuating aTTG levels and the possibility of normalization of aTTG levels even on a gluten-containing diet(117). They are also excluded from the study because of the use of sugar probes in our non-invasive measurements.

Monitoring CD is essential for effective treatment. All patients will be followed up by the dietitian after diagnosis and receive formal dietary counseling on the gluten-free diet. After approximately 6 months to a year after diagnosis the patients will be followed up in clinic, where blood work is reviewed and the importance of the gluten-free diet is reinforced.

In order to provide objective support for a serological diagnostic approach in addition to genetics and aTTG testing, at referral and one-year appointments, non-invasive methods are used to measure intestinal damage and recovery. Studies have shown these to be reliable methods of determining intestinal damage(97, 98, 105). Rather than a modification to ESPGHAN diagnostic criteria that might be utilized in the clinic, we want to demonstrate without biopsy, mucosal disease and healing in the first prospective study conducted utilizing serological diagnosis.

Finally, in order to understand patient and parent preferences for diagnosis, qualitative data will be generated through parent interviews. This enhances understanding of the quantitative findings in an explanatory way(118). Interviews will explore the perceptions of parents on the diagnostic process, and their child's adherence to the GFD. Interviews will provide parents with a platform to speak about their experience of the diagnostic process of CD and about their child's health on a GFD. Interview questions will explore parents' thoughts and feelings regarding their experience with the diagnosis of CD, the general invasive and non-invasive methods, and their child's adherence to the gluten-free diet. Through these parent interviews we will determine if the non-invasive serological route of diagnosis for celiac disease was accepted among

parents, how parents felt about the option for a non-invasive diagnostic test for CD and diagnosis without a biopsy had any effect on their child's adherence to the diet.

Based on the literature and our knowledge and understanding of the local setting, including availability of serological tests, we defined the following diagnostic approach as appropriate for our clinic and this pilot study (**Figure 2-1**). This approach allows those patients that have an aTTG ≥200 U/ml the option of a serological diagnosis. These patients would have a confirmatory aTTG test as well as an HLA haplotype test. If positive, the patient would be diagnosed as having celiac disease without histological confirmation, and would be asked to collect urine and stool samples for non-invasive monitoring of intestinal permeability and inflammation.

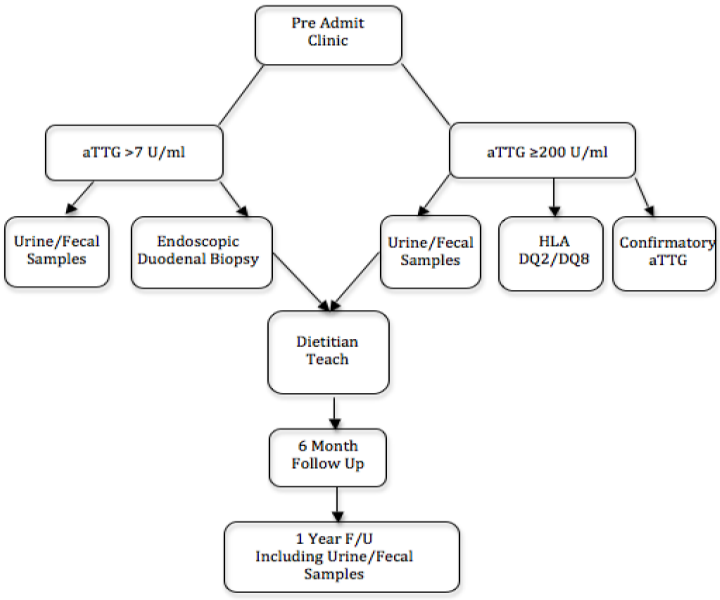

Figure 2-1. The pilot approach of a serological diagnosis in celiac disease in the Multidisciplinary Celiac Clinic at the Stollery Children's hospital.

2.2 Aims

1. Pilot a serological diagnostic strategy, based on modified ESPGHAN criteria at the Multidisciplinary Pediatric Celiac Clinic at the Stollery Children's Hospital

2. Objectively monitor symptom and mucosal improvement on a gluten-free diet using non-invasive methods in patients having serological and endoscopic diagnosis

3. Assess patient and parent preference for serological versus endoscopic diagnosis based on recruitment and using qualitative methodology

4. Assess how either diagnostic strategy may impact adherence to the gluten-free diet through self-report and parent interviews

2.3 Hypothesis

1. Patients with a serological diagnosis will demonstrate at least the same baseline abnormalities in permeability and inflammation as patients with an endoscopic diagnosis

2. Patients with a serological diagnosis will demonstrate similar improvement in aTTG levels and in permeability and inflammation as patients with an endoscopic diagnosis

3. Patients and parents will welcome serological diagnostic strategy as evidenced by ease of recruitment and positive feedback from parents through interviews

4. A serological diagnostic strategy will not adversely impact adherence to a gluten-free diet as evidenced by qualitative data and improvement in aTTG levels, permeability and inflammation

2.4 Expected Findings

When comparing fecal calprotectin, lactulose-to-mannitol ratio and sucrose in both diagnostic groups we expect to see a significant increase in all values for patients with CD when comparing them to control values. We also expect to see at least similar levels of increased permeability and inflammation between the two diagnostic groups. Serological comparison of aTTG levels in both groups at follow-up and their comparison of ≥7.0 U/mL, will show improvement in both groups and will be compared as a percentage at normal for each group. Acceptance of the non-invasive route of diagnosis will be shown through a high recruitment rate into our study in both groups as well as supported through qualitative data, which involved interviews from parents about their child's diagnosis of CD. These interviews will also explore the parent's perspective of their child's dietary adherence to the GFD, supported quantitatively through lowering aTTG levels, symptom improvement and self-report of adherence to the diet.

Chapter 3. Methods

3.1 Patient Population

Patients were recruited at the Stollery Children's Hospital's Multidisciplinary Pediatric Celiac Clinic after referral from their family doctor for abnormal aTTG screening (>7.0 U/mL). Consent and assent information sheets and forms are provided in Appendix A.

Patients were excluded if they had diabetes, language barriers, or had been off dietary gluten for an extended period of time prior to being seen in the clinic. Patients, between 3-17 years old, that had an initial aTTG level ≥ 200 U/mL were given the option for serological diagnosis (SD) that included a second aTTG screen, followed by an HLA haplotype test. If the genetics were positive and the second aTTG screen remained above ESPGHAN standards, they were diagnosed as having CD. The patients that were referred to the clinic with an aTTG level >7.0 U/mL were recruited as control patients and went for the endoscopy and intestinal biopsy (ED) and their marsh scores were recorded. Both groups were assessed using a standard initial contact form provided in Appendix B. This form includes information such as initial aTTG levels, referring symptoms, height, weight, and grading of severity of symptoms and well-being. Both groups were monitored for one year after diagnosis and commencing the GFD with a follow-up appointment within the first year of diagnosis.

In both groups, patients were asked to provide an overnight urine collection and a stool samples as measurements of mucosal damage and recovery. These samples were taken at time of diagnosis and one year after commencing on a gluten-free diet. Patients that came to the clinic on a gluten-free diet were asked to return onto a gluten-containing diet for 2-6 weeks before collecting the samples. Patients that were unable to return samples or were unavailable for follow-up were excluded from the study.

3.2 Serological Screen

The first aTTG screen was done by their family physician in order to be referred to the Multidisciplinary Pediatric Celiac Clinic. For those in the biopsy group, this initial aTTG

test was sufficient to allow for diagnosis by biopsy. For those that chose the non-biopsy route of diagnosis a second confirmatory aTTG test was done along with an HLA test. Patients were included in the study if their first aTTG was over 200, and the second was above ESPGHAN standards. This allowed for some variability between first and second test if there was already reduced gluten intake by patients. Patients were also included if they showed increased risk of celiac disease due to the presence of HLA-DQ2 or DQ8.

3.3 Histopathological Analysis

Patients that underwent biopsy were asked to fast 12 hours prior to the procedure and were only allowed to have clear liquids 6 hours before. They were put under general anesthetic and an esophagogastroduodenoscopy and biopsy was performed. Six samples of the distal duodenum and two samples of the duodenal bulb were obtained in each of the patients. Two pediatric pathologists reviewed all the samples and designated marsh scores to the damage. Those with Marsh scores of 1 to 3c were all included in the study, as they were diagnosed as having celiac disease.

3.4 Non-invasive Measurement of Mucosal Healing

Two methods implemented in our study were L/M ratio and sucrose excretions as determinates of intestinal permeability and measure of fecal calprotectin (FC) concentration as an indicator of active intestinal inflammation. Samples were collected from pediatric CD patients, in both groups, at time of diagnosis and after one year on a GFD. Intestinal permeability samples were also collected from healthy individuals and used as controls when comparing levels of sugar probes(119). The control for FC levels was the laboratory value of <50µg/g which has been established in other studies(120).

We aimed to compare both permeability and FC concentrations at diagnosis as celiac patients as a whole, as well as between the higher and lower level aTTG groups. When comparing sample measurements, groups were separated as intention-to-treat with all ≥200 U/ml aTTG levels in Group 1 and <200 U/ml aTTG levels in Group 2.

3.4.1 Assessment of Mucosal Permeability

Healthy controls were recruited as part of another study involving permeability testing for eosinophilic esophagitis(119). These controls were children from the local community that were screened to ensure they were asymptomatic with no gastrointestinal symptoms or family history of celiac disease.

Controls and patients were asked to fast for 2-4 hours after dinner, and then to empty their bladder, after which, they drank a sugar drink containing mannitol, lactulose and sucrose. The amount of sugar drink given was dependent on their weight. Patients that were <25 kg were given 2 bottles, 25-34kg had 3 bottles, 34-45kg had 4 bottles and >45kg had an adult sized bottle. A sugar-drink contained: 100g sucrose, 5g lactulose and 2g mannitol in 450mL of water. The bottles given were taken consecutively within 30 minutes. After consumption, they collected any urine expelled during the night as well as their first urine in the morning. Patients were instructed not to consume any alcohol, laxatives or anti-diarrheal medication 24 hours prior to their test. After collection, the container was returned and the total volume was recorded. 5mL aliquots of the urine were kept at -80 degrees Celsius until analysis. Analysis of lactulose-to-mannitol ratio and total sucrose was done by high-performance liquid chromatography

(HPLC), a method of separating and quantifying components in a liquid, and analyses were adjusted for urine weight and volume.

3.4.2 Assessment of Mucosal Inflammation

The patient's first morning stool was collected ensuring that it was not contaminated by urine or toilet water, by the patient or parent. Patients were given a commode as well as gloves and a sterile container that contained a scooper. Once collected it was kept frozen at -80 degrees Celsius until analyzed. Analysis was done through enzyme-linked immunosorbent assay (ELISA) tests. The ELISA kit is from Immunodiagnostik AG, Bensheim Germany. 15mg of stool was weighed out and put into a fecal extraction buffer. The sample was then centrifuged and the supernatant tested using an enzyme immunoassay specific for Calprotectin. The microplate wells were coated with a monoclonal anti-calprotectin antibody. A peroxidase conjugated second antibody and tetramethylbenzidine (TMB) as substrate was used to quantify the amount of calprotectin in the sample. The intensity of the color produced is proportional to the concentration. The amount per gram of stool was calculated from the measured concentration and the dilution factor of the extraction. In 2003, it was shown that the laboratory cutoff value for FC in children ages 4-17, could be the same as adults, which is below 50μg/g (120). This has become the laboratory normal value, and was therefore used as the control value in our study.

3.5 Adherence to the Gluten-free Diet

Adherence to the gluten-free diet was measured by a dietitian through an annual assessment form, standardized at the Multidisciplinary Pediatric Celiac Clinic, supplied

in Appendix C. This assessment involves patient and parent report about recent intentional and accidental gluten exposure, with a "yes" and "no" rating by the dietitian in regards to adherence to the GFD. Adherence was also assessed through symptom improvement, quantified by the physician using the same standardized form. The severity of symptoms was graded through parental and patient report and compared to initial symptoms at the first clinic appointment. Serological monitoring through normalization of aTTG levels and intestinal recovery shown through non-invasive measurements also supported adherence to the GFD. Qualitative data allowed insight into adherence of GFD including motivation to stay consistent with the GFD, symptom improvement and lifestyle.

3.6 Statistical Analysis

SPSS 22 was used to analyze statistical comparisons(121). Analysis was determined by data distribution. Independent sample t-tests were used to determine differences between normally distributed demographic variables (age, height, weight, gender), symptom improvement, adherence, aTTG decline from base to follow-up and percentage of aTTG normalized between each group. Non-parametric tests, Kruskal Wallace and Mann Whitney, were used to compare differences in aTTG, lactulose-to-mannitol ratios, total sucrose and fecal calprotectin between groups, given data skewing. Non-parametric Wilcoxon signed-rank test was used to compare FC values to control value <50 μg/g. Paired t-tests were used when comparing baseline and follow-up demographics (height and weight), while Wilcoxon related sample tests were used to compare aTTG, lactulose-to-mannitol ratio, sucrose and fecal calprotectin between time periods.

Power Calculation: A sample size of 60 per group would provide power approximating 80% to detect a 0.5SD difference in aTTG, and approximating 100% to detect a 1SD difference in aTTG, between baseline and one year on a GFD. It would also enable us to detect approximately a 15% difference in the proportion of patients having an abnormal aTTG between two diagnostic groups.

3.7 Qualitative Study

3.7.1 Qualitative Recruitment

The majority of patients were recruited through convenient sampling at their follow-up appointments in the Multidisciplinary Pediatric Celiac Clinic, where the interviewer explained the qualitative portion of the study. The interviewer also contacted some potential parent participants and explained the qualitative study when confirming their upcoming follow-up appointments by phone. All consenting parents were asked for a follow-up time in which the interview could be completed. The consent form for the qualitative portion is provided in Appendix D.

3.7.2 Qualitative Interviews

The child's initial symptoms, age, and route of diagnosis (biopsy or serological) were known before the interviews with the parents because their children had already enrolled in the quantitative portion of the study. The fact that I had met all of the parents at study enrollment and had been in contact with them throughout the year of diagnosis, all contributed to the parents' ease during the interview.

The interview guide is supplied in Appendix E. Questions regarding knowledge and feelings of the parents and children about the biopsy before the first clinic appointment

were added during the interview process along with questions regarding parent knowledge of their child's association of gluten with symptoms.

I conducted the interviews by telephone, recorded the interview on a digital recorder and uploaded it to a password-protected computer. I listened and re-listened to the each interview while transcribing them and then listened to the audio recording once again comparing it to the transcription. During the comparison between audio recording and transcription, I checked for accuracy and revised the transcription to remove any identifying information for confidentiality purposes.

Interviewing combined with the transcription and re-listening of interviews allowed the interviewer to be fully immersed and familiarized with the data(122, 123). Immersion in the data and background knowledge added to the rigor of this study because it allowed for investigator responsiveness to the data rather than strict adherence to the instructions and questions. Methodological coherence also added rigor in the sense that questions were added to the interview guide as the data demanded(124). Constant verification between transcription and audio recording was also an aspect of this study. Sampling from the parents of patients already treated for one-year on the gluten free diet allowed for inclusion of parents that were truly knowledgeable about the GFD and the study itself.

3.7.3 Qualitative Analysis

The analysis of qualitative data was done through thematic analysis using a predominately deductive approach that was driven by the specific research questions(122). Each parent's answer to the questions, listed in the interview guide,

were organized into the two groups of serological or biopsy diagnosis depending on their child's route of diagnosis. Comparisons were made within and between diagnostic groups for each question. An inductive approach to analysis was also used to identify themes that transcended the specific topics raised by the researcher through the interview guide. This interview-by-interview analysis was done to ensure that all thoughts and feelings were thematically analyzed.

Coding the data occurred while the interview was read after transcription, with the highlighting and noting of words, emotions and phrases. The data was tabled question by question with common themes for each group to allow visualization of the themes, both per group and for the question(122, 123). Themes were generated looking at common codes. These codes were looked at by focusing on each parent's response in the group (SD and ED) and then compared between the two groups. General themes of the questions and interviews as a whole were also compared and used to create two main themes. Responses were also tallied to minimize researcher bias in regards to thoughts of overhearing certain words, phrases or feelings. Results are presented for each question followed by a description of the overall themes identified inductively.

Chapter 4. Baseline Results

4.1 Enrollment

From January 2013 to June 2014, 168 eligible patients were seen in clinic, 118 of which consented to be part of the study; 53 for serological diagnosis (SD) and 65 for endoscopy and biopsy diagnosis (ED). Throughout the study 27 of these were excluded,

10 in the SD group and 17 in the ED group. Exclusions were due to 7 negative biopsies, 1 negative genetic test, and those patients that did not return samples or were lost to follow-up. As a result, a total of 91 patients remained in the study, 41 with a serological diagnosis and 50 with a biopsy diagnosis (**Figure 4-1**).

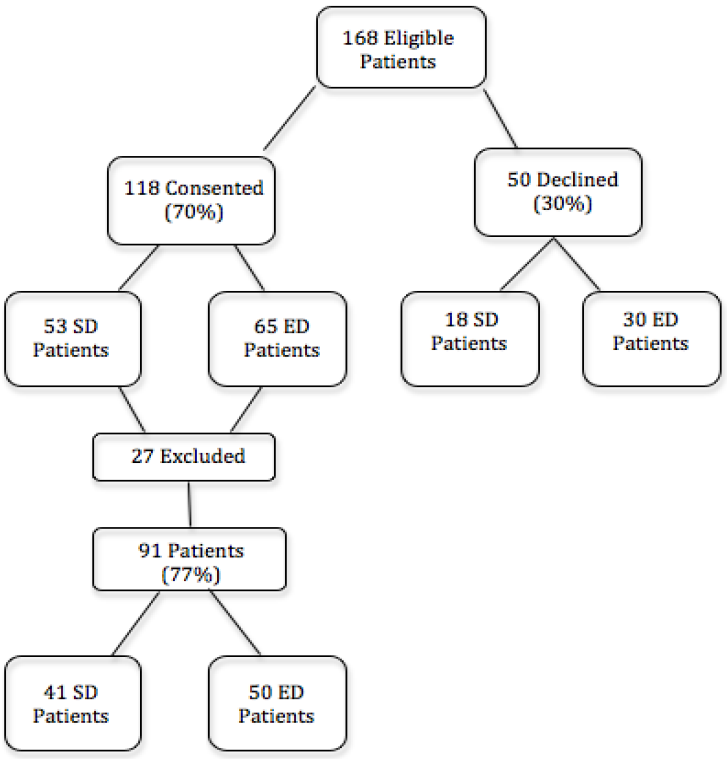

Figure 4-1. Enrollment into the pilot study from January 2013-June 2014.

4.2 Reasons for Declining the Study

There were 168 patients seen in the clinic that were eligible to join the study. Along with our ineligibility criteria of having co-morbid diagnosis, or diabetes there were some additional reasons patients were not enrolled that were situation specific. For example: if the physician thought the child and/or parents were already overwhelmed

with the process of the diagnosis; if there was a perceived language barrier that would impede follow-up for sample collections. We initially tried to enlist the help of a translator in clinic, but subsequently noted difficulties in regards to sample collections and the parents' inability to contact me with questions by phone after the clinic. Another exclusion was patients who had come to clinic already on a gluten-free diet for a number of months, and had decided against gluten re-challenge prior to the scope.

Of the 168 patients that were seen in the clinic, we saw 74 patients eligible for a SD, 92 eligible for a biopsy diagnosis and 2 patients with aTTG levels not recorded. 50 patients in total declined to be part of the study, 18 with aTTG levels over 200 U/ml, 30 under 200 U/ml and 2 patients with aTTG levels not recorded (**Figure 2-1**).

The most common reason parents declined the study, especially those being recruited to the biopsy group, was the additional burden of collecting samples on top of the diagnostic procedure. Many parents and children rejected the idea of collecting the urine and stool samples out-right because they did not want to collect biological samples and also due to difficulty of collecting samples; example, if the child was too young or had behavior issues.

The top reasons for parents who had children eligible for a non-biopsy route and deciding to go for a biopsy, were so that they could see the damage that the gluten had caused, they wanted to be sure of the diagnosis and they wanted to stick with "traditional methods." Parents were also concerned of other medical issues that could be missed with a simple blood test. One mom felt that her child would be more serious about the diagnosis if she had an invasive procedure. Another reason for choosing the

scope over the blood test was that for some families there was only a short wait between their initial appointment and their scope.

4.3 Demographics

There were 41 patients diagnosed serologically (SD) and 50 by biopsy (ED). Of the 50 that had a biopsy, 5 patients had an aTTG greater than 200 U/ml. Demographics are compared in **Table 4-1**. There were a total of 7 asymptomatic patients, 4 SD and 3 ED. These patients were referred because of family history and deemed asymptomatic, however after further investigation these patients ended up having other issues such as behavioral issues, iron deficiency, and back pain.

GI symptoms were the primary concern for both groups; 73% SD and 84% ED. 20% in the SD group and 30% of ED patients had concerns of growth. Behavioral issues such as irritability showed equally in both groups at 42%. Anemia and fatigue were also large concerns in the SD group (66%) and ED group (48%). Joint pain and headaches were similar in both groups with 39% of SD patients complaining of these issues and 38% in ED. 15% of SD patients and 6% of ED patients also had other concerns such as leg cramps or night sweats. In the SD group, 20 out of 39 patients compared to 21 out of 49 in the ED group had a family history of CD. 2 patients in the SD group and 1 patient in the ED group were missing this information.

There was no significant difference in age, gender ratio, height or weight at the initial clinic appointment in each group ($p>0.05$). There was also no difference in the number of asymptomatic and symptomatic patients seen in either group ($p>0.05$). However, there was a significant difference in aTTG between SD and ED groups ($p<0.001$).

Table 4-1. Comparison of baseline demographics between serological and biopsy diagnostic groups.

	Serological Diagnosis (n=41)	Biopsy Diagnosis (n=50)	p-value
Age (years)[1]	8.5 (3.5)	9.2 (3.5)	>0.05
Gender (M:F)	16:25	18:32	>0.05
aTTG (U/ml)[2]	600 (200-4100)	42 (7.8-2500)	<0.001
Height (cm)[1]	130.2 (22.2)	132.6 (20.7)	>0.05
Height (z-score)[1]	-0.063 (1.04)	0.05 (0.97)	>0.05
Weight (kg)[1]	31.7 (15.5)	32.9 (15.7)	>0.05
Weight (z-score)[1]	-0.04 (1.00)	0.03 (1.01)	>0.05
GI symptoms	73%	84%	>0.05
Growth Concerns	20%	30%	<0.001
Behavioral Symptoms	42%	42%	>0.05
Anemia/Fatigue	66%	48%	>0.05
CNS	39%	38%	>0.05
Other	15%	6%	>0.05
Family History	51%	43%	>0.05

[1]mean (standard deviation), [2]median (range)

4.4 Genetics and Histopathology

HLA and Marsh score frequencies of patients in the pilot study are shown in **Table 4.2** and **4.3**. Of the 41 patients that had a serological diagnosis, 6(14.6%) were homozygous

for DQ2, 28 (68.3%) had DQ2 with another allele, 2(4.9%) had DQ2 and DQ8, and 4(9.8%) had DQ8 with another allele. 1 (2.4%) patient was missing genetics.

Of the 50 patients that went for biopsies, 2(4%) had Marsh 1, 9(18%) had Marsh 2, 18(36%) had Marsh 3a, 9 (18%) had Marsh 3b and 12(24%) had Marsh 3c.

Table 4-2. HLA allele frequencies of serological diagnosis patients.

HLA alleles	Serological Diagnosis (n=40)
DQ2/DQ2	6 (14.6%)
DQ2/DQ8	2 (4.9%)
DQ2/DQX	28 (68.3%)
DQ8/DQX	4 (9.8%)

Table 2-3. Marsh scores of biopsy diagnosis patients.

Biopsy Marsh Score	Biopsy Diagnosis (n=50)
Marsh 1	2 (4%)
Marsh 2	9 (18%)
Marsh 3a	18 (36%)
Marsh 3b	9 (18%)
Marsh 3c	12 (24%)

4.5 Lactulose, Mannitol and Sucrose

67 (74%) celiac patients in the study collected urine samples that were analyzed; 42 patients in Group 1 (≥200 U/ml aTTG) and 25 in Group 2 (<200 U/ml). In addition to study patients, 26 control subjects, without CD or other GI complications were recruited through conjunction with another study(119). **Table 4-4** shows comparisons of L/M and sucrose between celiac groups and controls.

Using Kruskal-Wallis and Mann Whitney non-parametric tests, L/M and sucrose were significantly higher in CD patients as a whole (combining Group 1 and Group 2) compared to controls ($p<0.001$). L/M was also significantly higher when comparing each group separately (1 and 2) to controls ($p<0.001$). This was repeated in comparing sucrose values of each Group 1 and Group 2 to controls ($p<0.001$, $p<0.05$). In comparison between Group 1 and 2, L/M was significantly higher ($p<0.05$), while sucrose showed no significant difference ($p>0.05$).

Table 4-4. Comparisons of lactulose-to-mannitol ratio and sucrose for intestinal permeability between celiac groups and controls.

	Celiac Patients (n=67)	Group 1 (n=42)	Group 2 (n=25)	Controls (n=26)
L/M	0.041 (0.010-0.290)	0.049 (0.020-0.290)	0.033[1] (0.010-0.160)	0.022[2] (0.010-0.070)
Sucrose (mg/ml)[2]	0.31 (0.05-2.79)	0.34 (0.05-2.79)	0.28[3] (0.05-0.87)	0.099[4] (0.03-1.72)

All values shown as median(range). Significance calculated by Mann-Whitney ($p<0.05$)

[1]Group 1 and 2 were significantly different ($p<0.05$)

[24]All celiac groups were different than controls in lactulose:mannitol ($p<0.001$) and sucrose ($p<0.05$)

[3]Group 2 was not significantly different than Group 1 ($p>0.05$)

4.6 Fecal Calprotectin

70 (77%) patients in the study returned a stool sample for measurement of inflammation. Of these baseline samples, 67 have been analyzed so far and thus used in comparisons; 41 in Group 1 (≥200 U/ml aTTG) and 26 in group 2 (<200 U/ml aTTG). The value used for controls is the laboratory normal value of <50 µg/g. Comparisons are shown in **Table 4-5**.

The Mann Whitney non-parametric test showed FC was significantly higher in Group 1 than in Group 2 ($p>0.05$). Comparisons against the control value of 50µg/g were calculated using non One-sample Wilcoxon Signed Rank Test. FC was significantly

higher in celiac patients as a whole and in Group 1 compared to the control value ($p<0.001$). There was no significant difference ($p>0.05$) between Group 2 and the control value.

Table 4-5. Comparison of fecal calprotectin between celiac groups and laboratory normal control.

	Celiac Patients (n=67)	Group 1 (n=41)	Group 2 (n=26)	Laboratory Normal Control
Fecal Calprotectin (µg/g)	67.1 (4.9-3068)	81.6 (6-3068)	50[1] (4.9-1755)	<50[2]

Values shown as median (range). Significance determined by Mann-Whitney and one-sample Wilcoxon Signed Rank Test

[1]Group 2 is significantly different than Group 1 ($p<0.05$)

[2]Celiac patients and Group 1 are significantly different than control ($p<0.001$), Group 2 is not ($p>0.05$)

Chapter 5. Follow-up

5.1 Follow-up Clinics

Due to limitations in clinic follow-up space, it was not possible to see all patients at both their 6 and 12-month follow-up time points as planned. Therefore, only patients seen at 12-month follow-up were used in the final analysis (n=42), and for comparisons of aTTG only those that had 12-month blood work were used (n=40).

5.2 Follow-up Demographics

There were 16 SD and 26 ED patients seen at their 12-month follow-up appointments. **Table 5-1** shows comparisons of demographics between diagnostic groups. There were no significant differences in age and gender in regards to the two groups at follow-up and there was no significant time difference when patients of each group were being seen in the clinic ($p>0.05$). There were also no significant differences in height and weight between the two groups at 12 months ($p>0.05$). SD group had significantly higher aTTG levels than ED group at follow-up ($p<0.05$), however the SD group also had a significantly higher decrease in their aTTG values from baseline to 12-month follow-up ($p<0.001$).

Symptom improvement in the SD group was seen for all 14(100%) symptomatic patients, while the 2 asymptomatic patients reported no changes. In the ED group, 25 (96.2%) patients felt better on the gluten-free diet, while 1 (3.8%) patient's symptoms were still present. In the SD group, 100% of patients reported adherence to the GFD, while in the ED group 23 of the 26 patients (88.5%) reported adherence to the GFD.

Taking into account 40 patients (17 SD, 23 ED) with 12-month aTTG blood work, the two groups did not significantly differ in the number of patients whose aTTG levels had normalized (<7.0 U/ml) ($p>0.05$).

Table 5-1. Comparison of follow-up demographics at 12 months between serological and biopsy groups.

	Serological Diagnosis (n=16)	Biopsy Diagnosis (n=26)	p-value
Age (years)[1]	9 (3.2)	10 (3.2)	>0.05
Gender (M:F)	7:9	12:14	>0.05
aTTG (U/ml)[2]	13 (1.7-65)	3.8 (1-420)	<0.05
% aTTG Decline[2]	98.2 (93.3-99.8)	93.2(68.2-98.8)	<0.001
Height (cm)[1]	133.8 (19.9)	137.5 (19.1)	>0.05
Height (z-score)[1]	-0.121 (1.03)	0.07 (0.99)	>0.05
Weight (kg)[1]	34.1(15.2)	34.5 (15.9)	>0.05
Weight (z-score)[1]	-0.014 (0.98)	0.01 (1.03)	>0.05
Diagnosis to follow-up (months)[1]	12.3 (1.73)	11.5 (1.8)	>0.05
Symptom Improvement	100%	96.2%	>0.05
GFD Adherence	100%	88.5%	<0.05
aTTG <7U/ml	41.2%	73.9%	>0.05

[1]mean(standard deviation), [2]median(range)

5.3 Baseline to Follow-up Demographic Comparisons

Demographic comparisons from baseline to follow-up are show in **Table 5-2.** Both groups were significantly higher in height and weight from baseline to one-year follow-up ($p<0.001$). The aTTG levels were significantly lower for both groups between baseline and one year ($p<0.001$).

Table 5-2. Comparison from baseline to 12-month follow-up demographics by serological and biopsy groups.

	Serological Diagnosis[3]		**p-value**	**Biopsy Diagnosis**[4]		**p-value**
	Base	12-month		Base	12-month	
Height (cm)[1]	127.4 (21.0)	133.8 (19.9)	<0.001	129.5 (19.3)	137.5 (19)	<0.001
Height (z-score)[1]	-0.20 (0.98)	-0.12 (1.03)	<0.05	-0.09 (0.91)	0.03 (1.02)	<0.05
Weight (kg)[1]	29.6 (13.0)	34.1 (15.2)	<0.001	30.3 (14.5)	34.5 (15.9)	<0.001
Weight (z-score)[1]	-0.18 (0.83)	-0.01 (0.98)	<0.05	-0.13 (0.93)	0.01 (1.03)	<0.05
aTTG (U/ml)[2]	510 (230-4100)	11 (1.7-65)	<0.001	56 (7.8-170)	4 (1-420)	<0.001

[1]mean(standard deviation)

[2]median (range)

[3]Height, Weight N=16, aTTG N= 15

[4]Height N=24, Weight N=26, aTTG N= 21

5.4 Follow-up Lactulose, Mannitol and Sucrose

26 patients returned 12-month urine samples; 19 patients in Group 1 (≥200 U/ml), 7 in Group 2 (<200 U/ml). The same control values used at baseline were used for 12-month follow-up of controls. **Table 5-3** shows comparisons of samples at 12-month follow-up. Using Kruskal-Wallis and Mann Whitney non-parametric tests, L/M and sucrose were not significantly different in any of the comparisons made between celiac patients and the controls ($p>0.05$).

Table 5-3. Comparisons of 12-month follow-up lactulose-to-mannitol ratio and sucrose between celiac groups and controls**.**

	Celiac Patients (n=26)	**≥200 U/mL (n=19)**	**<200 U/mL (n=7)**	**Controls (n=26)**	**p-value**
L/M	0.0190 (0.010-0.320)	0.019 (0.010-0.320)	0.019 (0.010-0.030)	0.022 (0.010-0.070)	>0.05[1]
Sucrose (mg/ml)	0.112 (0.02-0.5)	0.111 (0.02-0.5)	0.142 (0.03-0.29)	0.099 (0.03-1.72)	>0.05[1]

Values shown as median(range).

[1]Comparisons of all groups through Mann-Whitney tests

5.5 Follow-up Fecal Calprotectin

32 patients have returned stool samples for follow-up fecal calprotectin analysis in the study. 21 of these patients are in Group 1 (≥200U/ml) and 11 in Group 2 (<200U/ml). Laboratory normal value of <50 µg/g was used once again as the control. **Table 5-4**

shows comparisons of 12-month FC levels in celiac groups compared to controls. Using the Mann Whitney non-parametric test, FC was significantly higher in Group 1 compared to Group 2 ($p>0.05$). Using One-sample Wilcoxon Signed Rank Test, FC was significantly lower in celiac patients as a whole compared to control ($p<0.001$). In comparison, Group 1 was not significantly different to control value ($p>0.05$) and while Group 2 was significantly lower than the control value ($p>0.05$).

Table 5-4. Comparisons of 12-month follow-up fecal calprotectin between celiac groups and control.

	Celiac Patients (n=32)	**≥200 U/mL (n=21)**	**<200 U/mL (n=11)**	**Laboratory Normal Control**
Fecal Calprotectin (µg/g)	21.9 (1.1-178.9)	31.8[1] (5.7-178.9)	10.2 (1.1-22.9)	<50[2]

Values shown as median (range). One sample Wilcoxon Signed Rank test was used

[1]Group 1 and Group 2 significantly different ($p<0.05$)

[2]Celiac patients ($p<0.001$) and Group 2 ($p<0.05$) significantly lower than controls, while Group 1 is not significantly different ($p>0.05$)

5.6 Baseline to Follow-up Samples Comparisons

There were 26 patients that returned both baseline and 12-month urine samples (19 in Group 1, 7 in Group 2) and 29 (20 in Group 1, 9 in Group 2) patients that returned baseline and 12-month stool samples. Comparisons of only those that brought in their 12-month samples with their baseline samples were made, using non-parametric paired Wilcoxon tests. **Table 5-5** shows comparisons of samples at baseline and 12

months in celiac patients as a whole, while **Table 5-6** shows comparisons within aTTG groups. There was a significant difference in all samples for lactulose-to-mannitol ratio, sucrose, and fecal calprotectin levels from baseline to 12 months ($p<0.001$).

Table 5-5. Lactulose-to-mannitol ratio, sucrose and fecal calprotectin comparisons from baseline to 12-month follow-up in celiac patients.

	Celiac Disease Patients			p-value
	N	Base	12-month	
Lactulose:Mannitol	26	0.040 (0.020-0.290)	0.019 (0.010-0.320)	<0.001
Sucrose (mg/g)	26	0.22 (0.07-2.79)	0.112 (0.02-0.5)	<0.001
Fecal Calprotectin (µg/g)	29	90.2 (21.2-3068)	22.9 (1.1-178.9)	<0.001

Values shown as median(range). Comparisons made using paired Wilcoxon test

Table 5-6. Lactulose-to-mannitol ratio (L/M), sucrose and fecal calprotectin (FC) comparison from baseline to 12-month follow-up of higher and lower level aTTG groups.

	≥200 U/mL[1]		p-value	<200 U/mL[2]		p-value
	Base	12-month		Base	12-month	
L/M	0.043 (0.020-0.290)	0.019 (0.010-0.320)	<0.05	0.029 (0.020-0.060)	0.019 (0.010-0.030)	<0.05
Sucrose (mg/g)	0.27 (0.08-2.79)	0.111 (0.02-0.5)	<0.001	0.19 (0.07-0.81)	0.142 (0.03-0.29)	>0.05
FC (µg/g)	101.7 (21.1-3068)	29.4 (5.7-178.9)	<0.01	62 (28.8-195.5)	11.7 (1.1-22.9)	<0.05

Values shown as median(range), Paired Wilcoxon tests used

[1]Lactulose:Mannitol, Sucrose N=19, Fecal calprotectin N=20

[2]Lactulose:Mannitol, Sucrose N=7, Fecal calprotectin N=9

Chapter 6. Qualitative Results

6.1 Participant Demographics

There were 21 participants in the interview portion of the study. All participants were mothers of children recently diagnosed with CD through the Multidisciplinary Celiac Disease Clinic at Stollery Children's Hospital. Parents were divided into groups depending on whether their child went through a biopsy route of diagnosis (ED) or a serological diagnosis (SD). In these two groups; 11 mothers had 12 children who

underwent a biopsy diagnosis, while 10 mothers had 11 children that went for a serological diagnosis. There were 2 parents who had 2 children in the study, but for each mother, both her children had the same route of diagnosis. The age range for the children in this study was 3-14 years old. The age range for the biopsy group was 4-14 years, while the age range for the serological group was 3 to 13 years. There was one more female (5 vs. 6) in the biopsy group compared to the serological diagnosis group, while both groups had 6 males. Gastrointestinal symptoms were the major concern in both groups and each group had two asymptomatic patients. Other common symptoms that were present were slow growth, irritability, fatigue or low energy and headaches. Seven of the 10 children in the SD group had a family history of CD, compared to 5 out of 11 in the ED group.

6.2 Diagnosis of Celiac Disease: Biopsy and Serological

6.2.1 Question 1: Parents' Thoughts on the Study

When the parents in the biopsy group heard about a non-invasive route of CD diagnosis they thought that it was "a great option for families" and especially appropriate for younger children. However, parents had their hesitations because it was still "in progress" and they "didn't know if it was a reliable way." Regardless of these hesitations, they hoped that their participation in the study would help further the research regarding the availability of non-invasive route in order to help future families with children undergoing diagnosis; "I'm glad. That's why I agreed to be part of the research. I think for a lot of kids [the biopsy] is probably the worst part of finding out

your diagnosis. It's one thing to take blood and it's another thing to start putting you under."

The parents in the serological diagnosis group recounted their strong positive emotions of elation, happiness, excitement and relief when they had been told about the study and the option for a non-invasive diagnostic test; "I was elated. Anytime you don't have to sedate a child for one reason or another for some kind of surgery is a bonus."

The parents in the serological group also mentioned the impact of family history of celiac disease, their child not wanting to have the biopsy, and avoiding the invasiveness of a biopsy as motivator for joining the study. There was one mom who said she wished she could have seen the damage, but was confident that a non-invasive route was best for her child and his anxiety; "I had really mixed emotions. I sort of wished we would have done the biopsy just to see if there was damage to the intestines." A common theme apparent in both groups that transcended the specific interview questions was option or choice; the choice of having a biopsy or a serological diagnosis; "I have friends who have CD or whose children have celiac disease and the one thing we were not looking forward to was the biopsy and just the procedure itself. So it was really nice to have that option of a non-invasive way of confirming."

6.2.2 Question 2: Experiences with the Biopsy and Genetic Test

After interviewing several parents, it became evident that an additional question was needed because when asked about their experience with the biopsy, parents mentioned prior knowledge of the biopsy before coming to the clinic due to researching or having a

family member that went through it. The added questions were: were you aware of the biopsy before coming to the first clinic appointment and were you prepared for it?

Responses to this question revealed, that in both groups, parents had researched the biopsy option before coming to the celiac clinic. Parents in both groups indicated that they were prepared for the biopsy, but the SD group expressed more concerns of worry, apprehension and stress when going to the clinic; "we had looked online and read a lot about 'oh its not that bad' because I realize its not a huge surgery or anything but it was still kind of stressful to put your kid through that if they don't need to be." Both groups of parents also felt that their child was "a bit nervous" or a "little bit scared of the procedure." Concern but preparedness was a theme in both groups, as well children's feelings of nervousness and anxiousness.

Although those parents whose children went for a biopsy felt nervous, anxious or scared when their child went for the procedure, they regarded the biopsy as a "fast procedure" and spoke highly of the staff being able to make themselves and their children feel comfortable; "It was good, obviously stressful because you worry about anything being done to your child but I mean the whole thing was good. There was the anesthesiologist that was fantastic and the nurses and everybody was really helpful and supportive."

In contrast, the parents in the SD group were "excited" about the genetic test thinking it was "cutting edge" and "more forward thinking." A parent remembered she felt "relieved, very happy and thankful to be apart of that study and not have to do that biopsy." Another mom explained the influence of finding out that her child was

genetically predisposed to CD; “I had read about the genetic component before hand and the fact that [my child] came back positive for it, made all of us sit back and think ‘hang on there, is there more of us? Could there be more of us?’ It was a positive thing.”

6.2.3 Question 3 & 4: Decision Making for a Serological Diagnosis

The majority of parents whose children had been diagnosed by the biopsy route stated that they would have considered a serological diagnosis if their child’s aTTG levels were high enough to warrant that route of diagnosis. Their reason for considering it was that it was an “easier option” than having a biopsy for which the child would have to “go under.” Parents expressed that if the serological diagnosis route was “tried and proven successful” and “if research showed that in most cases, even a small percentage that they found out if it was incorrect or whatever to me, I would rather them not have to go through the biopsy process then to go through it.” However, there was hesitancy in choosing that route due to the gluten-free diet being a lifelong change; “I would have been hesitant because it is a really big life change to stay celiac and I would have wanted to know 100% that this is what they had.” Parents felt that the biopsy provided them with an “absolute conclusion” giving them “concrete information” and “concrete proof.” One mother also said she wanted to know “how damaged [my child’s] stomach was.”

Parents, whose children underwent serological diagnosis expressed their main motivation was to “prevent [my child] from the operation.” This was due to the invasiveness of the biopsy procedure; “anytime they go into your body and invade it there is a chance of something to go horribly wrong” and the fact that they wanted their

child to avoid "going under." Another strong motivator for a couple of families was already knowing their child had CD through their family history and their child's symptoms; "We were fairly confident, given the family history, that yes [name of child] does have celiac disease so I really didn't see the need to put her through the process."

6.2.4 Question 5: Confidence in the Diagnosis

Both groups of parents were confident in the diagnostic route chosen for their child. The biopsy group found confidence in seeing the intestinal damage, and one parent felt like they doubted having the disease before the biopsy because of a lack of family history; "We didn't have a family history of it so I really struggled that this could even be possible. The biopsy was like no this is clear cut, 100%." Another mom took confidence from her family history; "I was pretty confident, just with our family history and knowing some of [child's] symptoms. I wasn't surprised by her diagnosis." The same underlying confidence of family history came through in the serological diagnosis group. CD already "being in the family" in addition to the blood tests gave them the confidence they needed in the diagnosis, making a biopsy an unneeded extra; "It didn't come out of the blue for me in the sense that we know its there in the background so I didn't feel I needed a biopsy to prove it." Family history of CD seemed to give parents in both groups confidence in their diagnosis as well as push parents with no family history of CD towards the biopsy.

6.3 Gluten-free Diet

6.3.1 Question 6: Improvement on the Gluten-free Diet

Parents in both groups provided numerous examples of their child's improvement on the gluten-free diet. They saw an increase in weight gain and energy and a decrease in the number of stomachaches, mood swings and headaches. Children were able to sleep through the night and take part in activities, all of which they were not able to do before because of their symptoms. Within each group it was common for parents to describe their child before and after the GFD as a "night and day difference" and a "completely different child."

6.3.2 Question 7: Following a Lifelong Gluten-free Diet

Adherence to a gluten-free diet is essential for healing of mucosal damage as well as symptom relief; therefore parents were asked if they thought their child would continue to follow a GFD for life. The longevity of their child's adherence to the GFD was a difficult question for parents to answer. There was hesitation in saying that their child would follow the GFD for life because their children were all quite young and parents had the majority of control over food intake. However, parents understood that during teen years when children are choosing foods for themselves adherence could be an issue; "I've read that in teenage years kids will kind of test the limits a little big more. So I don't know how my [child] will go through that stage, but as far as his understanding of it right now and his willingness to follow it right now, its great."

However, parents were quite confident with the increased availability of gluten-free products, the knowledge about long-term complications and education on making

gluten-free foods at home that their child would be well positioned to maintain the diet. Parents, in both groups, thought having family members with CD was helpful in that they were role models for their children and that CD was "always part of the conversation." They also described a gluten-free diet as an "easy trade off" for the children, in how much better they felt after changing their diet. Parents felt that symptom improvement, especially for those children that had extreme symptoms before going on the GFD would continue to be a strong motivator to maintain a GFD; "if [my child] does have gluten she knows immediately, she gets abdominal pain and is not very happy with life. So [my child] will never eat gluten again, if she can help it." A couple of parents also said how it was getting easier for their child as they continue to follow the diet and how starting a GFD at a young age was beneficial because it was "going to become normal." The common themes in both groups regarding maintaining a gluten-free diet were education and symptom improvement.

6.3.3 Question 8: Influence of Diagnostic Route on Adherence

Parents were also asked to consider if the diagnostic route, biopsy or serological, would influence their child's adherence to a gluten-free diet. Parents in the biopsy group felt that a biopsy would make a difference because it was a "little more serious" and would "eliminate any doubt"; "Having to actually go in and have that procedure done, I think it certainly hit home that no, this is pretty important." A couple of parents mentioned how their child was a "fact based person" so seeing evidence that there was damage was enough for them to accept the diet even at their young age. However, a few parents in this group said that it was more a "professional figure" or parent "explaining to [my child] that this is the case and the changes you need to make" and "educating [my child]

to what exactly gluten does to the body" that influenced adherence to the GFD, concluding that "the education side was more important than the seriousness of a blood test versus a biopsy." The return of symptoms when eating a gluten food could also be enough to make sure they stayed gluten-free and that the biopsy did not make "that much of a difference."

One child had been diagnosed serologically by their family physician a few months earlier and started a GFD. After realizing they were supposed to have a biopsy, they went back on gluten and came to our clinic. The parent in this case, explained that there was no change in adherence after the serological diagnosis and the biopsy diagnosis, and that "right off the bat he did 3 months of gluten-free. [My child] never ate anything he shouldn't. He said 'I have to switch my diet' and did." This child was 12 years old at time of diagnosis.

Parents in the SD group believed that family history of CD was a factor in their child's adherence to the diet. The fact that children had family members with CD made it easier making them feel like it was "nothing new." Also, the return of symptoms if something containing gluten was consumed was a big motivator for dietary adherence. Some children had such drastic improvements in their symptoms that they would not want to return to a gluten-containing diet; "The stomach pains that she had and the problems internally aren't something that I think she will ever forget. How much pain she was in all the time." Parents felt that a diagnosis whether from a biopsy or a blood test, is still a diagnosis and education around the disease was a more important factor; "We've done research with [my child], and he's done his own kind of science fair display board

telling what CD is…Education motivates everyone." One parent felt that their child was too young to associate a biopsy with adherence to the gluten-free diet, while a mother with an older child felt that a biopsy would actually give a negative association to the idea of CD and the child "might have resented celiac disease more knowing there was pain and fear associated with it."

In summary, there were mixed thoughts in regards to this question, parents in each group standing behind their own diagnosis. However, common themes of education and symptom improvement were evident once again.

6.4 Overall themes

6.4.1 Choice

The option of a non-invasive procedure against an invasive one was valued by both groups of parents because it afforded choice, a highly valued concept. Although, those in the biopsy group were ineligible for a serological diagnosis, they valued the idea of giving an option to families especially to those with young children. Most parents in this group would have considered a non-invasive route for their child if it were available to them, especially if it was a proven way of diagnosis. Parents who were eligible for the serological option were elated to have a choice in their child's diagnosis. Knowing their child and how they would react to the procedure, they were happy to choose the non-invasive route.

The theme of choice or option was evident in regards to their confidence in the diagnosis as well. They each had their own motivators in choosing their route of diagnosis, and those motivators were enough to give parents in both groups confidence

in their diagnosis. The option of entering into a non-invasive route, allowed parents to choose if they needed an invasive procedure or with their child's personality and their family history, it would be better to be diagnosed by a blood test.

6.4.2 Family History

Family history was a common theme in many of the parent's responses to the interview questions. The idea that "its in the family" gave some parents confidence in choosing the non-invasive route, and for those that went the biopsy route it gave support to the biopsy results. Family experience with the biopsy also contributed to their apprehension towards the procedure, as one mom mentioned that she had gone through it and did not want her child have to do the same; "my own experience was – my first one the anesthesia was a bit light and I was a little bit too aware of what was going on and I didn't want [my child] to have that experience because it would really upset them." Lack of family history also played a part in making those parents feel like they needed the biopsy to prove CD, while those that went the non-invasive route "knew [their child] had CD" from family history and were confident in the blood work. Family members with CD also played a role for a child's acceptance of the disease. Being surrounded by CD and having role models following the diet made it "not a big deal" and an easier transition for some children. These role models also made it easier for children to adhere to the diet.

6.5 Summary of Qualitative Findings

Themes of family history of celiac disease as well as the parental choice for route of diagnosis were apparent in the interviews. There was acceptance of a non-invasive

route for families and those that chose that option had common feelings of elation, happiness and relief for their children. The children in both groups responded well to a GFD, and long-term adherence was not something that parents thought their children would have difficulties with. Parents in both groups expressed education and symptom relief as strong motivators in choosing to follow the GFD, while those in the biopsy group also mentioned the seriousness of an invasive procedure as a motivator. Both groups were happy in the route they took for diagnosis and had their own personal reasons to choose the route they did, whether it was family history or their child's anxiety and well-being.

Chapter 7. Discussion

This pilot study was designed to determine if families attending the Multidisciplinary Pediatric Celiac Clinic at the Stollery Children's Hospital would welcome a non-invasive route of diagnosis and to determine if there were any adverse affects of diagnostic route on symptom or mucosal improvement. The addition of qualitative methods was used to enhance the quality of data as well as provide a voice to parents regarding a biopsy or serological route of diagnosis. Non-invasive methods were used as tools for measuring intestinal permeability and inflammation.

7.1 Overall Study Findings

Through a high enrollment rate and parent feedback in qualitative interviews, we concluded that families at our Multidisciplinary Pediatric Celiac Clinic welcome a non-invasive route of diagnosis. In interviews, parents explained joining the study to further

the research and help those families with children not wanting to go through the biopsy process. Although enrollment was highly successful, the inability to include patients due to a language barrier was a bias in enrollment because it excluded many ethnic families from the study.

At the time of study enrollment, there were very few differences between SD and ED groups in regards to their demographics. If anything, we might have expected to see an increased enrollment of younger patients in the SD group, because we thought a non-invasive test would be more favorable to parents with younger children. However, this was not the case as there was no difference in age between the two groups. There was also no difference in gender distribution, but in both groups there were more females diagnosed than males. This is common in CD with females outnumbering males approximately 2:1(1). As expected from the baseline results, there was no difference in age, gender, height or weight between diagnostic groups at 12-month follow-up.

The main difference between the SD and ED groups at enrollment was their aTTG levels, which was expected. There were 5 patients eligible for a serological diagnosis but decided to forgo that option and continue with the biopsy. These patients still wished to be part of the study and collected the samples, in addition to having a biopsy. The inclusion of these patients with aTTG ≥ 200 U/ml allowed for closer comparisons between the two groups, decreasing baseline aTTG levels as a factor of difference.

The aTTG levels between both groups were still significantly different after one year. Those in the ED group had a median of 3.8 U/ml while the SD group had a median of 13 U/ml. When comparing the number of patients at 12 months whose aTTG levels had

normalized to <7.0 U/ml, there was no significant difference between the two groups. Although at 12-month follow-up, over half of the SD group had not reached normal levels, it was noted that they had started with higher levels of aTTG than the majority of those in the ED group. This could mean they need more time to decrease to normal levels. A study by Hogen Esch et al. showed that only 80% of patients would be serologically negative for aTTG after 2 years on a gluten-free diet(125). However, the SD group had significantly higher rates of decline from baseline aTTG to follow-up than the ED group, showing that their aTTG levels were still getting dramatically better after being on the GFD. Guidelines suggest that the diagnosis of CD requires symptom improvement on a gluten-free diet(19, 20). Their rate of decline combined with symptom improvement is telling of the effect of the GFD, their evident adherence and of course the legitimacy of their CD diagnosis.

HLA testing was an important diagnostic tool in this study. DQ2 is the most common HLA type, occurring in over 90% of patients with CD and DQ8 occurring in approximately 10%(1). This was similar in our study population with 87.8% of patients having the DQ2 allele, whether it was in homozygous form or with another allele including DQ8. Close to 10% of those that did not have DQ2, had DQ8 with another allele(1). There was one patient in our study that was excluded due to being negative for DQ2 and DQ8. Upon biopsy, this patient was diagnosed as having CD. It is estimated that, less that 0.05% of CD patients are without HLA DQ2 and DQ8 alleles(1, 27). However, as ESPGHAN guidelines become more commonly practiced more cases of patients with rare genetics and diagnosed with CD could be uncovered.

At baseline, both groups had similar numbers of asymptomatic patients. There were very few truly asymptomatic patients in the study. Although some patients were screened because of family history and thought to be asymptomatic, in clinic they expressed concerns of fatigue, irritability and even mild abdominal pain. Therefore, sub-clinically showing symptoms of CD(17, 21). Patients rarely came to the clinic without secondary concerns. The majority complained of irritability, fatigue, joint pain and headaches in addition to the common GI and growth concerns.

At the 12-month appointment, the dietitian and the physician assessed adherence to the diet and symptom improvement through self-report by patients and their parents. The SD group had a 100% adherent rate, while the ED group had 88.5%, which was significantly different. This is an interesting finding given that clinicians have expressed concern over moving to a serological diagnosis on the basis that it might affect adherence to the diet. Through our qualitative interviews and follow-up dietitian report, our study shows that in our clinic a non-invasive serological diagnosis did not appear to have any negative effect on dietary adherence. In fact, it may have increased adherence and this might reflect a bias in self-selection for serological diagnosis. Parent knowledge of being in study investigating GFD adherence could have affected their motivation to make their child stay on the gluten-free diet. However, in our qualitative study we spoke to several parents in the SD group and they noted that the biggest factors in adherence for their child, was symptom improvement and education on the long-term affects of CD.

At follow-up, 100% of originally symptomatic patients in the SD group reported feeling better on the gluten-free diet, while 2 patients that were asymptomatic felt no different. 96.2% of symptomatic patients in the ED group felt better on a gluten-free diet, while the rest still had concerns with abdominal pain and growth. In qualitative interviews, parents thought that children feeling better on a gluten-free diet, especially those that had severe symptoms before, would be adherent to the diet because they didn't want those symptoms to return. Even at a young age, their children associated gluten with feeling bad and "didn't want to feel unwell." Parents described the change before and after the gluten-free diet as a "night and day" difference. Many parents commented not only on GI symptom and growth improvement but also energy and mood improvement, calling their child a "completely different child." They explained that since their children had noticed such improvements themselves, they would follow the diet.

The question of symptom improvement being a factor to stay adherent to the diet was reasoning behind a serological diagnosis being recommended for only symptomatic patients. Interviews in our study, show that symptoms are a large factor in making children to stay adherent to the diet. However, it was not the only factor. Education and family history of celiac disease were noted to be important as well. All asymptomatic patients had been screened for celiac disease prior to coming to the clinic because of their family history of CD, which has showed to be an important factor for adherence. It was reported by parents, that having relatives as role models for a child diagnosed with celiac disease would be a positive factor in adherence as well making the transition easier to a GFD because it "wasn't anything new."

In our interviews, we had one parent of an asymptomatic child explain that their child's motivation of staying adherent to the diet was education of CD. Education on long-term complications to a GFD is stressed in many guidelines when dealing with the follow-up of CD, in order to maintain adherence to the diet(19, 21, 113). Guidelines encourage dietitian involvement in following CD patients, so as to ensure sufficient nutritional requirements are being met, as well as to provide support for frustrations that can occur when following the GFD(19, 21, 45). Guidelines also suggest a physician following these patients closely, in order to ensure the long term complications are being understood and avoided(19, 113). In our interviews, parents had similar suggestions that a professional figure educating their child about the long-term complications of CD would make their child understand the seriousness of the diagnosis. A limitation in our study was not being able include more parents with asymptomatic children in our qualitative interviews.

7.2 Overall Sample Collection Findings

This study used non-invasive measurement tools as a way of demonstrating mucosal damage consistent with celiac disease. Measurements were taken in both the serological and endoscopic groups. 26 healthy children were also asked to collect samples as controls for intestinal permeability, while the laboratory normal for fecal calprotectin, 50µg/g was used for the control measurement of inflammation(119, 120). We compared patients according to their aTTG level on an intention-to-treat basis. Therefore, groups were divided into those given the option of a serological diagnosis (≥200 U/ml) against those with a biopsy diagnosis (<200 U/ml).

Increased ratios of lactulose-to-mannitol (L/M) and sucrose levels in urine show that the intestine is permeable(98, 103, 105). Our baseline samples showed significant increases in both L/M and sucrose between our celiac patients and our healthy controls, concluding that CD patients have more damage proximally and throughout their gut than controls. These findings were similar to studies such as Pearson et al., where they were able to show a five fold increase of L/M in CD patients compared to controls(99). We also found that the higher-level aTTG group had significantly increased L/M compared to the lower-level aTTG group, showing more damage of the intestine in its entirety. This difference between groups was not seen when comparing sucrose levels. This may reflect the location of bowel damage, as L/M allows for a view of damage in the whole gut as compared to sucrose, which shows proximal damage(101, 103). The differences in L/M and similarity in sucrose levels between the aTTG groups could imply that while both have significant proximal damage compared to controls, the higher-level aTTG group has higher damage causing permeability throughout their entire gut.

Fecal calprotectin was used in order to determine intestinal inflammation in celiac patients. Literature has shown that the laboratory normal value of FC for healthy children is <50μg/g, with higher levels requiring follow-up(120). This was used as our control value for comparisons. Our study showed that at baseline, celiac patients as a whole were significantly above 50μg/g. Comparisons between each group to the control value showed the higher-level aTTG group was significantly different and the lower-level aTTG group was not. These results infer that CD patients with higher levels of aTTG have increased mucosal inflammation in their intestine. Previous studies have

shown that increased levels of aTTG, have a higher probability of increased damage, as represented by higher Marsh scores(81, 82, 95). Furthermore, increased FC levels in patients with higher aTTG levels has also been shown in a study done by Ertekin et al.(109).

Follow-up sample collections for non-invasive monitoring were obtained again at the 12-month mark after diagnosis. 26 patients returned their urine samples for L/M and sucrose analysis. There were no significant differences in sugar probe levels when comparing all groups; celiac patients, over 200 U/ml and under 200 U/ml to the controls and to each other. This leads to the plausible conclusion that after one year on the gluten-free diet, the intestine has undergone significant mucosal healing and become less permeable. These results are similar to others studies done, measuring the effect of a GFD on permeability. Hamilton et al. and Uil et al. were both able to show CD patients' L/M levels returning to normal after treatment on a GFD(100, 102). Ukabam et al., showed normalization of permeability on a GFD matched the recovered histopathology of the jejunal mucosa(126). However, another study done by Cummins et al., showed that recovery in permeability occurs before histological mucosal recovery in the small intestine(127). In our study, this is plausible given the mild increased aTTG levels in conjunction with a lowered L/M.

Stool samples were also returned for analysis of fecal calprotectin. These results showed similar improvements as with the permeability tests. Celiac patients as a whole and the lower aTTG level group were significantly lower than 50 μg/g, which showed that they were in the normal range. The higher aTTG group showed no significant

difference between the 50 μg/g cut off showing that those levels had normalized as well. These results follow those of Ertekin et al. and Balamtekin et al. where they showed FC levels normalizing on a GFD in celiac patients(109, 110).

Finally, considering only those 42 patients that were seen both at baseline and at follow-up, there were significant increases in height and weight; and significant decreases in aTTG levels, showing a response to the GFD. At 1 year, CD patients and patients with higher-level aTTG had a significant decrease in L/M and sucrose, as well as a significant decrease in fecal calprotectin levels. This shows improvement in permeability and inflammation throughout the entire intestine. Although L/M was significantly different from baseline to 12-month in the ED group, sucrose permeability did not improve. However, at both time points sucrose permeability was not significantly different than normal controls. Therefore, although they didn't significantly improve, their levels were still within the normal range at baseline and at 12 months.

7.3 Limitations

Follow-up in this study was limited and the timing was not always consistent. It was difficult to see patients both at 6 months and at 12 months, due to the number of other celiac patients waiting to be seen. The follow-up window of one year was also a limitation as aTTG levels did not normalize in the majority of patients in the SD group by that time. Another limitation was the collection of samples from patients. The addition of samples to the protocol deterred many families from taking part in the study and un-returned samples were the top reason for exclusion of participants. This shows

a need for easier non-invasive tools in which mucosal damage can be measured. A limitation in our qualitative study was the small number of parents with asymptomatic children that were interviewed. This was limiting in the fact that we were unable to determine motivating factors for asymptomatic patients following the GFD.

7.4 Future Directions

An addition to this study could be following up those patients whose aTTG levels have not normalized after one year to see if and when they normalize. A hospital cost analysis showing the amount saved by eliminating the need for biopsies for patients over 200 U/mL would also strengthen this research. An interesting future direction for a qualitative study would be interviews with children diagnosed by both routes in order to determine if there was any affect of diagnostic route on their adherence to a GFD or view of CD.

7.5 Summary

This study has shown no adverse effects in introducing a non-invasive method of diagnosis of CD. Parents welcomed the change, and patients that underwent a serological diagnosis as opposed to a biopsy showed the same improvement in permeability and inflammation of intestine after one year on the GFD. The diagnostic process also showed no negative outcome towards a child's adherence to the GFD or symptom improvement. Increased baseline aTTG levels could mean higher damage to the intestine as seen in our non-invasive results, which could require more time to normalize on a gluten-free diet(82, 95). However, the significant rate of decrease in aTTG levels for the ≥200 U/ml group after one year of treatment as well as their

normalizing results for permeability and inflammation showed that they were responding to the GFD.

Celiac patients as a whole showed increased inflammation and permeability in their intestine though non-invasive tests. The higher aTTG group showed increased damage compared to the lower level of aTTG group in whole gut and proximal permeability. The subset of patients that brought in their samples at baseline and 12 months, showed significant improvement in aTTG, intestinal permeability and inflammation after being on the GFD.

Parents in both groups welcomed the idea of a non-invasive route of CD diagnosis for their children. However, each parent had their own personal reasons as to why they choose the diagnostic process, and individual reasons for confidence in the diagnosis, whichever route they take. Family history and their child's personality were factors affecting their choice. The idea of CD being "in the family" gave those parents the confidence that their child had CD, and hence confidence in forgoing the need for a biopsy result. Also, parents were aware if a biopsy would help or hinder their child. If parents knew their child was an anxious person they declined the biopsy. On the other hand, parents also knew if their child was a fact-based person that they would need to see the damage to follow the diet. Parents are aware of what their child needs to accept and stay adherent to the gluten-free diet.

A strong conclusion from this study, taking into account both qualitative and quantitative outcomes, is that a non-invasive route should be offered as a choice to patients with an aTTG ≥200 U/ml. This allows parents that freedom to choose what is

right for their family and the well-being of their child. This choice may also be a strong tool for engaging children and their parents in management of this disease that will continue to impact their lifestyle choices every day. Due to the positivity and significant results of this study, it is recommended that the Multidisciplinary Pediatric Celiac Clinic at the Stollery Children's Hospital offer parents of children with an aTTG level of ≥200 U/ml the option of a serological diagnosis for CD.

Works Cited

1. Megiorni F, Pizzuti A. HLA-DQA1 and HLA-DQB1 in Celiac disease predisposition: practical implications of the HLA molecular typing. Journal of biomedical science. 2012;19:88.

2. Pozo-Rubio T, Capilla A, Mujico JR, de Palma G, Marcos A, Sanz Y, et al. Influence of breastfeeding versus formula feeding on lymphocyte subsets in infants at risk of coeliac disease: the PROFICEL study. European journal of nutrition. 2013;52(2):637-46.

3. Stene LC, Honeyman MC, Hoffenberg EJ, Haas JE, Sokol RJ, Emery L, et al. Rotavirus infection frequency and risk of celiac disease autoimmunity in early childhood: a longitudinal study. The American journal of gastroenterology. 2006;101(10):2333-40.

4. Norris JM, Barriga K, Hoffenberg EJ, Taki I, Miao D, Haas JE, et al. Risk of Celiac Disease Autoimmunity and Timing of Gluten Introduction in the Diet of Infants at Increased Risk of Disease. JAMA. 2005;293(19):2343-51.

5. Qiao SW, Iversen R, Raki M, Sollid LM. The adaptive immune response in celiac disease. Seminars in immunopathology. 2012;34(4):523-40.

6. Sollid LM, Jabri B. Triggers and drivers of autoimmunity: lessons from coeliac disease. Nature reviews Immunology. 2013;13(4):294-302.

7. Guandalini S. Historical Perspective of Celiac Disease. 2008. In: Frontiers in Celiac Disease [Internet]. Farmington, CT, USA: Karger PublishersPediatric and Adolescent Medicine; [1-22].

8. Paveley W. From Aretaeus to Crosby- A History of Celiac Disease. British Medical Journal. 1988;297:1646-9.

9. Dowd B, Walker-Smith J. Samuel Gee, Aretaeus, and the Coeliac Affection. British Medical Journal. 1974;2:45-7.

10. Barker JM, Liu E. Celiac Disease: Pathophysiology, Clinical Manifestations, and Associated Autoimmune Conditions. Advances in Pediatrics. 2008;55(1):349-65.

11. Abel EK. The rise and fall of celiac disease in the United States. Journal of the history of medicine and allied sciences. 2010;65(1):81-105.

12. van Berge-Henegouwen GP, Mulder C. Pioneer in the gluten free diet: Willem-Karel Dicke 1905-1962, over 50 years of the gluten free diet. Gut. 1993;34:1473-5.

13. Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, et al. Prevalence of Celiac Disease in At-Risk and Not-At-Risk Groups in the United States. Arch Intern Med. 2003;163(3):286-92.

14. Fasano A, Catassi C. Current approaches to diagnosis and treatment of celiac disease: An evolving spectrum. Gastroenterology. 2001;120(3):636-51.

15. Rajani S, Huynh HQ, Turner JM. The changing frequency of celiac disease diagnosed at the Stollery Children's Hospital. Can J Gastroenterol. 2010;24(2):109-12.

16. Aggarwal S, Lebwohl B, Green PH. Screening for celiac disease in average-risk and high-risk populations. Ther Adv Gastroenterol. 2012;5(1):37-47.

17. Rostom A, Murray JA, Kagnoff MF. American Gastroenterological Association (AGA) Institute technical review on the diagnosis and management of celiac disease. Gastroenterology. 2006;131(6):1981-2002.

18. Bai J, Zeballos E, Fried M, Corazza GR, Schuppan D, M.J.G. F, et al. Celiac Disease. WGO-OMGE Practice Guidelines. World J Gastroenterol News. 2007;10:S1-S8.

19. Hill ID, Dirks MH, Liptak GS, Colletti RB, Fasano A. Guideline for the Diagnosis and Treatment of Celiac Disease in Children: Recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. Journal of pediatric gastroenterology and nutrition. 2005;40:19.

20. Husby S, Koletzko S, Korponay-Szabo IR, Mearin ML, Phillips A, Shamir R, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition guidelines for the diagnosis of coeliac disease. Journal of pediatric gastroenterology and nutrition. 2012;54(1):136-60.

21. Rubio-Tapia A, Hill ID, Kelly CP, Calderwood AH, Murray JA, American College of G. ACG clinical guidelines: diagnosis and management of celiac disease. The American journal of gastroenterology. 2013;108(5):656-76; quiz 77.

22. Makharia GK, Verma AK, Amarchand R, Bhatnagar S, Das P, Goswami A, et al. Prevalence of celiac disease in the northern part of India: a community based study. Journal of gastroenterology and hepatology. 2011;26(5):894-900.

23. Rajani S, Alzaben A, Shirton L, Persad R, Huynh HQ, Mager DR, et al. Exploring anthropometric and laboratory differences in children of varying ethnicities with celiac disease. Can J Gastroenterol. 2014;28(7):351-4.

24. Mustalahti K, Collin P, H. S, Salmi J, Maki M. Osteopenia in patients with clinically silent coeliac disease warrants screening. Lancet. 1999;354:744-5.

25. Catassi C, Fabiani E, Corrao G, Barbato M, De Renzo A, Carella A, et al. Risk of Non-Hodgkin Lymphoma in Celiac Disease. JAMA. 2002;287:1413-9.

26. Lebwohl B, Granath F, Ekbom A, Montgomery SM, Murray JA, Rubio-Tapia A, et al. Mucosal healing and mortality in coeliac disease. Alimentary pharmacology & therapeutics. 2013;37(3):332-9.

27. Piccini B, Vascotto M, Serracca L, Luddi A, Margollicci MA, Balestri P, et al. HLA-DQ typing in the diagnostic algorithm of celiac disease. Rev Esp Enferm Dig. 2012;104(5):248-54.

28. Mesin L, Sollid LM, Di Niro R. The intestinal B-cell response in celiac disease. Frontiers in immunology. 2012;3:313.

29. Meresse B, Ripoche J, Heyman M, Cerf-Bensussan N. Celiac disease: from oral tolerance to intestinal inflammation, autoimmunity and lymphomagenesis. Mucosal immunology. 2009;2(1):8-23.

30. Rubio-Tapia A, Kyle RA, Kaplan EL, Johnson DR, Page W, Erdtmann F, et al. Increased prevalence and mortality in undiagnosed celiac disease. Gastroenterology. 2009;137(1):88-93.

31. Tersigni C, Castellani R, de Waure C, Fattorossi A, De Spirito M, Gasbarrini A, et al. Celiac disease and reproductive disorders: meta-analysis of epidemiologic associations and potential pathogenic mechanisms. Human reproduction update. 2014;20(4):582-93.

32. Ventura A, Magazzu G, Greco L. Duration of Exposture to Gluten and Risk for Autoimmune Disorders in Patients with Celiac Disease. Gastroenterology. 1999;117:297-303.

33. Mearin ML, Catassi C, Brousse N, Brand R, Collin P, Fabiani E, et al. European multi-centre study on coeliac disease and non-Hodgkin lymphoma. European journal of gastroenterology & hepatology. 2006;18:187-94.

34. Rampertab SD, Forde KA, Green PH. Small bowel neoplasia in coeliac disease. Gut. 2003;52:1211-4.

35. Turner JM, Pellerin G, Mager D. Prevalence of Metabolic Bone Disease in Children with Celiac Disease is Independent of Symptoms at Diagnosis. Journal of pediatric gastroenterology and nutrition. 2009;49:589-93.

36. Cardones AR, Hall RP, 3rd. Pathophysiology of dermatitis herpetiformis: a model for cutaneous manifestations of gastrointestinal inflammation. Immunology and allergy clinics of North America. 2012;32(2):263-74, vi.

37. Fry L, Leonard JN, Swain F, Tucker WFG, Haffenden G, Ring N, et al. Long term follow-up of dermatitis herpetisformis with and without dietary gluten withdrawal. British Journal of Dermatology. 1982;107:631-40.

38. Leonard JN, Haffenden G, Tucker WFG, Unsworth J, Swain F, McMinn RM, et al. Gluten Challenge in Dermatitis Herpetiformis. New England Journal of Medicine. 1983;308(14):816-9.

39. Bannister EG, Cameron DJ, Ng J, Chow CW, Oliver MR, Alex G, et al. Can Celiac Serology Alone be Used as a Marker of Duodenal Mucosal Recovery in Children with Celiac Disease on a Gluten-Free Diet? The American journal of gastroenterology. 2014;109(9):1478-83.

40. Stephens G. Insights on the gluten-free market: once believed to be a passing fad, the gluten-free foods category has evolved to become a mainstream star. Nutraceuticals World. 2014;17(9):24-6.

41. Wagner G, Berger G, Sinnreich U, Grylli V, Schober E, Huber WD, et al. Quality of Life in Adolescents with Treated Coeliac Disease: Influence of Compliance and Age at Diagnosis. Journal of pediatric gastroenterology and nutrition. 2008;47:555-61.

42. Högberg L, Grodzinsky E, Stenhammar L. Better Dietary Compliance in Patients with Coeliac Disease Diagnosed in Early Childhood. Scandinavian journal of gastroenterology. 2003;38(7):751-4.

43. Mozer-Glassberg Y, Zevit N, Rosenbach Y, Hartman C, Morgenstern S, Shamir R. Follow-up of children with celiac disease - lost in translation? Digestion. 2011;83(4):283-7.

44. Martin S. Against the grain: An Overview of Celiac disease. J Am Acad Nurse Pract. 2008;20:243-50.

45. Stuckey C, Howdle P, Lowdon J. Symposium 1: Joint BAPEN and British Society of Gastroenterology symposium on 'coeliac disease: Basic and controversies' dietitians are better than clinicians in following up coeliac disease. Proc Nutr Soc. 2009;68:249-51.

46. Samasca G, Sur G, Lupan I, Deleanu D. Gluten-free diet and quality of life in celiac disease. Gastroenterology & Hepatology from Bed to Bench. 2014;7(3):139-43.

47. Whitaker JK, West J, Holmes GK, Logan RF. Patient perceptions of the burden of coeliac disease and its treatment in the UK. Alimentary pharmacology & therapeutics. 2009;29(10):1131-6.

48. Stevens L, Rashid M. Gluten-Free and Regular Foods:A Cost Comparison. Canadian Journal of Dietetic Practice and Research. 2008;69(3):147-50.

49. Zarkadas M, Dubois S, MacIsaac K, Cantin I, Rashid M, Roberts KC, et al. Living with coeliac disease and a gluten-free diet: a Canadian perspective. Journal of human nutrition and dietetics : the official journal of the British Dietetic Association. 2013;26(1):10-23.

50. Singh J, Whelan K. Limited availability and higher cost of gluten-free foods. Journal of human nutrition and dietetics : the official journal of the British Dietetic Association. 2011;24(5):479-86.

51. Rostom A, Dube C, Cranney A, Saloojee N, Sy R, Garritty C, et al. The Diagnostic Accuracy of Serologic Tests for Celiac Disease: A Systematic Review. Gastroenterology. 2005;128:S38-S46.

52. Marsh MN, Crowe PT. Morphology of the mucuosal lesion in gluten sensitivity. Bailliere's Clinical Gastroenterology. 1995;9(273-293).

53. Sollid LM. Molecular Basis of Celiac Disease. Annual review of immunology. 2000;18:53-81.

54. Oberhuber G, Granditsch G, Vogelsang H. The histopathology of coeliac disease: time for a standardized report scheme for pathologists. European journal of gastroenterology & hepatology. 1999;111(10).

55. Dickson BC, Streutker CJ, Chetty R. Coeliac disease: an update for pathologists. Journal of clinical pathology. 2006;59(10):1008-16.

56. Hadithi M, von Bloomberg ME, Crusius JBA, Bloemena E, Kostense PJ, Meijer JWR, et al. Accuracy of Serologic Tests and HLA-DQ Typing for Diagnosing Celiac Disease. Annals of Internal Medicine. 2007;147:294-302.

57. Gujral N, Freeman HJ, Thomson AB. Celiac disease: prevalence, diagnosis, pathogenesis and treatment. World journal of gastroenterology : WJG. 2012;18(42):6036-59.

58. Fernandez-Banares F, Alsina M, Modolell I, Andujar X, Piqueras M, Garcia-Puig R, et al. Are positive serum-IgA-tissue-transglutaminase antibodies enough to diagnose coeliac disease without a small bowel biopsy? Post-test probability of coeliac disease. Journal of Crohn's & colitis. 2012;6(8):861-6.

59. Newland CD, Bracken JM, Gorla K, Grandison N, Guandalini S. Multicenter Review of New ESPGHAN Guidelines for Celiac Disease: How Many Biopsies Can We Really Skip? Gastroenterology. 2013;144(5):S14.

60. Corazza GR, Villanacci V, Zambelli C, Milione M, Luinetti O, Vindigni C, et al. Comparison of the interobserver reproducibility with different histologic criteria used in celiac disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2007;5(7):838-43.

61. Arguelles-Grande C, Tennyson CA, Lewis SK, Green PH, Bhagat G. Variability in small bowel histopathology reporting between different pathology practice settings: impact on the diagnosis of coeliac disease. Journal of clinical pathology. 2012;65(3):242-7.

62. Shidrawi RG, Przemioslo R, Davies DR, Tighe MR, Ciclitira PJ. Pitfalls in diagnosing coeliac disease. Journal of clinical pathology. 1994;47:693-4.

63. Dandalides SM, Carey WD, Petras R, Achkar E. Endoscopic small bowel mucosal biopsy: a controlled trial evaluating forceps size and biopsy location in the diagnosis of normal and abnormal mucosal architecture. Gastrointestinal endoscopy. 1989;35:197-200.

64. Ladas SD, Tsamouri M, Kouvidou C, Raptis SA. Effect of forceps size and mode of orientation on endoscopic small bowel biopsy evaluation. Gastrointestinal endoscopy. 1994;40:51-5.

65. Bonamico M, Maraiani P, Thanasi E, Ferri M, Nenna R, Tiberti C, et al. Patchy Villous Atrophy of the Duodenum in Childhood Celiac Disease. Journal of pediatric gastroenterology and nutrition. 2004;38:204-7.

66. Kurien M, Evans KE, Hopper AD, Hale MF, Cross SS, Sanders DS. Duodenal bulb biopsies for diagnosing adult celiac disease: is there an optimal biopsy site? Gastrointestinal endoscopy. 2012;75(6):1190-6.

67. Pais WP, Duerksen DR, Pettigrew NM, Bernstein CN. How many duodenal biopsy specimens are required to make a diagnosis of celiac disease? Gastrointestinal endoscopy. 2008;67(7):1082-7.

68. Bonamico M, Thanasi E, Mariani P, Nenna R, Luparia RPL, Barbera C, et al. Duodenal Bulb Biopsies in Celiac Disease: A Multicenter Study. Journal of pediatric gastroenterology and nutrition. 2008;47:618-22.

69. Green PH. Celiac disease: how many biopsies for diagnosis? Gastrointestinal endoscopy. 2008;67(7):1088-90.

70. Caruso R, Marafini I, Del Vecchio Blanco G, Fina D, Paoluzi OA, Colantoni A, et al. Sampling of proximal and distal duodenal biopsies in the diagnosis and monitoring of

celiac disease. Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. 2014;46(4):323-9.

71. Levinson-Castiel R, Hartman C, Morgenstern S, Avitzur Y, Hirsch A, Rosenbach Y, et al. The role of duodenal bulb biopsy in the diagnosis of celiac disease in children. J Clin Gastroenterol. 2011;45:26-9.

72. Mangiavillano B, Masci E, Parma B, Barera G, Viaggi P, Albarello L, et al. Bulb biopsies for the diagnosis of celiac disease in pediatric patients. Gastrointestinal endoscopy. 2010;72(3):564-8.

73. Rostami-Nejad M, Villanacci V, Hogg-Kollars S, Volta U, Manenti S, Reza-Zali M, et al. Endoscopic and histological pitfalls in the diagnosis of celiac disease: A multicentre study assessing the current practice. Rev Esp Enferm Dig. 2013(105):326-33.

74. Lebwohl B, Kapel RC, Neugut AI, Green PH, Genta RM. Adherence to biopsy guidelines increases celiac disease diagnosis. Gastrointestinal endoscopy. 2011;74(1):103-9.

75. Amin MS, Harrison RL, Weinstein P. A qualitative look at parents' experience of their child's dental general anaesthesia. International Journal of Paediatric Dentistry. 2006;16:309-19.

76. Murray JA. The widening spectrum of celiac disease. Am J Clin Nutri. 1999;69(3):354-65.

77. Quine MA, McCloy BRF, Matthews HR. Prospective audit of perforation rates following upper gastrointestinal endoscopy in two regions of England. British Journal of Surgery. 1995;82:530-3.

78. Mubarak A, Wolters VM, Gerritsen SA, Gmelig-Meyling FH, Ten Kate FJ, Houwen RH. A biopsy is not always necessary to diagnose celiac disease. Journal of pediatric gastroenterology and nutrition. 2011;52(5):554-7.

79. Barker CC, Mitton C, Jevon G, Mock T. Can tissue transglutaminase antibody titers replace small-bowel biopsy to diagnose celiac disease in select pediatric populations? Pediatrics. 2005;115(5):1341-6.

80. Saginur M, AlRefaee F, Spady D, Girgis S, Huynh HQ, Prosser C, et al. Antitissue transglutaminase antibody determination versus upper endoscopic biosy diagnosis of paediatric celiac disease. Paediatr Child Health. 2013;18(5):246-50.

81. Alessio MG, Tonutti E, Brusca I, Radice A, Licini L, Sonzogni A, et al. Correlation between IgA tissue transglutaminase antibody ratio and histological finding in celiac disease. Journal of pediatric gastroenterology and nutrition. 2012;55(1):44-9.

82. Donaldson MR, Book LS, Leiferman KM, Zone JJ, Neuhausen SL. Strongly Positive Tissue Transglutaminase Antibodies are Associated With Marsh 3 Histopathology in Adult and Pediatric Celiac Disease. J Clin Gastroenterol. 2008;42:256-60.

83. Hill PG, Holmes GK. Coeliac disease: a biopsy is not always necessary for diagnosis. Alimentary pharmacology & therapeutics. 2008;27(7):572-7.

84. Ribes-Koninckx C, Mearin ML, Korponay-Szabo IR, Shamir R, Husby S, Ventura A, et al. Coeliac disease diagnosis: ESPGHAN 1990 criteria or need for a change? Results of a questionnaire. Journal of pediatric gastroenterology and nutrition. 2012;54(1):15-9.

85. Skoglösa J, Fälth-Magnusson K, Stenhammar L. Conscious or deep sedation: a questionnaire regarding the experience of parents, children and staff during small bowel biopsy. Acta Paediatrica. 2007;92(6):704-8.

86. Nenna R, Magliocca FM, Tiberti C, Mastrogiorgio G, Petrarca L, Mennini M, et al. Endoscopic and histological gastric lesions in children with celiac disease: mucosal involvement is not only confined to the duodenum. Journal of pediatric gastroenterology and nutrition. 2012;55(6):728-32.

87. Bhatti TR, Jatla M, Verma R, Bierly P, Russo PA, Ruchelli ED. Lymphocytic gastritis in pediatric celiac disease. Pediatric and developmental pathology : the official journal of the Society for Pediatric Pathology and the Paediatric Pathology Society. 2011;14(4):280-3.

88. Stewart MJ, Shaffer E, Urbanski SJ, Beck PL, Storr MA. The association between celiac diseas and eosinophilic esophagitis in children and adults. BMC gastroenterology. 2013;13:96.

89. Pellicano R, De Angelis C, Ribaldone DG, Fagoonee S, Astegiano M. 2013 update on celiac disease and eosinophilic esophagitis. Nutrients. 2013;5(9):3329-36.

90. Oderda G, Forni M, Morra I, Tavassoli K, Pellegrino P, Ansaldi N. Endoscopic and Histologic Findings in the Upper Gastrointestinal Tract of Children with Coeliac Disease. JPGN. 1993;16:172-7.

91. Thompson JS, Lebwohl B, Reilly NR, Talley NJ, Bhagat G, Green P. Increased Incidence of Eosinophilic Esophagitis in Children and Adult With Celiac Disease. J Clin Gastroenterol. 2012;46:e6-e11.

92. Wahab PJ, Meiger JWR, Mulder C. Histologic follow-up of people with celiac disease on a gluten free diet. Am J Clin Pathol. 2002;118:459-63.

93. Brusca I, Carroccio A, Tonutti E, Villalta D, Tozzoli R, Barrale M, et al. The old and new tests for celiac disease: which is the best test combination to diagnose celiac

disease in pediatric patients? Clinical chemistry and laboratory medicine : CCLM / FESCC. 2012;50(1):111-7.

94. Basso D, Guariso G, Fasolo M, Pittoni M, Schiavon S, Fogar P, et al. A New Indirect Chemiluminescent Immunoassay to Measure Anti–tissue Transglutaminase Antibodies. JPGN. 2006;43:613-8.

95. Donaldson MR, Firth SD, Wimpee H, Leiferman KM, Zone JJ, Horsley W, et al. Correlation of duodenal histology with tissue transglutaminase and endomysial antibody levels in pediatric celiac disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2007;5(5):567-73.

96. Uil JJ, van Elburg RM, Janssens PMW, Mulder CJJ, Heymans HSA. Sensitivity of a hyperosmolar or "low"-osmolar test solution for sugar absorption in recognizing small intestinal mucosal damage in coeliac disease. Digest Liver Dis. 2000;32:195-200.

97. Catassi C, Fabiani E, Ratsch IM, Bonucci A, Dotti M, Coppa GV, et al. Is the sugar intestinal permeability test a reliable investigation for coeliac disease screening? Gut. 1997;40:215-7.

98. Cobden I, Rothwell J, Axon ATR. Intestinal permeability and screening tests for coeliac disease. Gut. 1980;21:512-8.

99. Pearson AD, Eastham EJ, Laker MF, Craft AW, Nelson R. Intestinal permeability in children with Crohn's disease and coeliac disease. British Medical Journal. 1982;285:20-1.

100. Uil JJ, van Elburg RM, van Overbeek FM, Meyer JW, Mulder C, Heymans HSA. Follow-up of treated coeliac patients: sugar absorption test and intestinal biopsies compared. European journal of gastroenterology & hepatology. 1996;8(3):219-23.

101. Menzies IS, Laker MF, Pounder R, Bull J, Heyer S, Wheeler PG, et al. Abnormal intestinal permeability to sugars in villous atrophy. Lancet. 1979:1107-9.

102. Hamilton I, Cobden I, Rothwell J, Axon ATR. Intestinal Permeability in coeliac disease: the response to gluten withdrawal and single-dose gluten challenge. Gut. 1982;23:202-10.

103. Smecuol E, Bai J, Vazquez H, Kogan Z, Cabanne A, Niveloni S, et al. Gastrointestinal Permeability in Celiac Disease. Gastroenterology. 1997;112:1129-36.

104. Sutherland LR, Verhoef M, Wallace JL, Van Rosendaal G, Crutcher R, Meddings JB. A simple, non-invasive marker of gastric damage: sucrose permeability. Lancet. 1994;343(998-1000).

105. Smecuol E, Vazquez H, Sugai E, Niveloni S, Pedreira S, Cabanne A, et al. Sugar tests detect Celiac Disease among first-degree relatives. The American journal of gastroenterology. 1999;94(12):3547-52.

106. Aadland E, Fagerhol M. Faecal calprotectin: a marker of inflammation throughout the intestinal tract. European journal of gastroenterology & hepatology. 2002;14:823-5.

107. Berstad A, Arslan G, Folvik G. Relationship between Intestinal Permeability and Calprotectin Concentration in Gut Lavage Fluid. Scandinavian journal of gastroenterology. 2000;35:64-9.

108. Costa F, Mumolo MG, Bellini M, Romano MR, Ceccarelli L, Arpe P, et al. Role of faecal calprotectin as non-invasive marker of intestinal inflammation. Digestive and Liver Disease. 2003;35:642-7.

109. Ertekin V, Selimoglu MA, Turgut A, Bakan N. Fecal calprotectin concentration in celiac disease. J Clin Gastroenterol. 2010;44:544-6.

110. Balamtekin N, Demir H, Baysoy G, Uslu N, Orhan D, Akcoren Z, et al. Fecal calprotectin concentration is increased in children with celiac disease: relation with histopathological findings. Turk J Gastroenterol. 2012;23(5):503-8.

111. Montalto M, Santoro L, Curigliano V, D'Onofrio F, Cammarota G, Panunzi S, et al. Faecal calprotectin concentrations in untreated coeliac patients. Scandinavian journal of gastroenterology. 2007;42(8):957-61.

112. Capone P, Rispo A, Imperatore N, Caporaso N, Tortora R. Fecal calprotectin in coeliac disease. World journal of gastroenterology : WJG. 2014;20(2):611-2.

113. Herman ML, Rubio-Tapia A, Lahr BD, Larson JJ, Van Dyke CT, Murray JA. Patients with celiac disease are not followed up adequately. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2012;10(8):893-9 e1.

114. Basso D, Guariso G, Bozzato D, Rossi E, Pescarin M, Fogar P, et al. New screening tests enrich anti-transglutaminase results and support a highly sensitive two-test based strategy for celiac disease diagnosis. Clinica chimica acta; international journal of clinical chemistry. 2011;412(17-18):1662-7.

115. Collin P, Kaukinen K, Vogelsang H, Korponay-Szabo IR, Sommer R, Schreier E, et al. Antiendomysial and antihuman recombinant tissue transglutaminase antibodies in

the diagnosis of coeliac disease: a biopsy-proven European multicentre study. European journal of gastroenterology & hepatology. 2005;17(1):85-91.

116. Fabiani E, Catassi C, Villari A, Gismondi P, R. P, Ratsch IM, et al. Dietary compliance in screening-detected coeliac disease adolescents. Acta Paediatrica. 1996;412:65-7.

117. Waisbourd-Zinman O, Hojsak I, Rosenbach Y, Mozer-Glassberg Y, Shalitin S, Phillip M, et al. Spontaneous normalization of anti-tissue transglutaminase antibody levels is common in children with type 1 diabetes mellitus. Digestive diseases and sciences. 2012;57(5):1314-20.

118. Fetters MD, Curry LA, Creswell JW. Achieving integration in mixed methods designs-principles and practices. Health services research. 2013;48(6 Pt 2):2134-56.

119. Leung AJ, Persad S, Slae M, Abdelradi A, Kluthe C, Shirton L, et al. Intestinal and Gastric Permeability in Children With Eosinophilic Esophagitis and Reflux Esophagitis. Journal of pediatric gastroenterology and nutrition. 2014.

120. Fagerberg UL, Loof L, Merzoug RD, Hansson LO, Finkel Y. Fecal Calprotectin levels in healthy children studied with an improved assay. JPGN. 2003;37:468-72.

121. IBM Corp. Released 2013. IBM SPSS Statistics for Macintosh, Version 22.0. Armonk, NY: IBM Corp.

122. Braun V, Clarke V. Thematic analysis. 2012. In: APA handbook of research methods in psychology, Vol 2: Research designs: Quantitative, qualitative, neuropsychological, and biological [Internet]. Washington DC, US: American Psychological Association; [57-71].

123. Braun V, Clarke V. Using thematic analysis in psychology. Qualitative Research in Psychology. 2006;3:77-101.

124. Morse JM, Barrett M, Mayan M, Olson K, Spiers J. Verification Strategies for Establishing Reliability and Validity in Qualitative Research. International Journal of Qualitative Methods. 2002;1(2):2002.

125. Hogen Esch CE, Wolters VM, Gerritsen SA, Putter H, von Blomberg BM, van Hoogstraten IM, et al. Specific celiac disease antibodies in children on a gluten-free diet. Pediatrics. 2011;128(3):547-52.

126. Ukabam SO, Cooper BT. Small intestinal permeability as an indicator of jejunal mucosal recovery in patients with celiac sprue on a gluten-free diet. Journal of Clinical Gastroenterology. 1985;7(3):232-6.

127. Cummins AG, Thompson FM, Butler RN, Cassidy JC, Gillis D, Lorenzetti M, et al. Improvement in intestinal permeability precedes morphometric recovery of the small intestine in coeliac disease. Clinical Science. 2001;100:379-86.

Appendix

Appendix A. Consent and Assent Form

PARENT INFORMATION LETTER

Title of Project:	Serological Diagnosis of Celiac Disease at Stollery Children's Hospital: a Pilot Study Toward Changing Local Practice
Principal Investigators:	Dr Justine Turner, MBBS FRACP PhD
Co-Investigators:	Dr Rabin Persad, MBBS FRACP(C) Dr Hien Huynh, MBBS FRACP

Please take time to read this information sheet.

If you have any questions about this information please contact us to discuss at any time.

Celiac disease is a common autoimmune disease that affects the small intestine and may be complicated by poor absorption of nutrients, like iron and vitamins, so that it can adversely affect nutrition and growth. Celiac disease is most commonly diagnosed in children, usually after a positive blood screening test called the aTTG test. At Stollery Children's Hospital we have shown that most children that have an aTTG test greater than 200 IU/ml will have Celiac Disease.

To diagnose Celiac Disease in Canada it is recommended that all children with a positive screening test have a biopsy taken from the upper part of the intestine - during an upper endoscopy test under general anesthesia. The endoscopy test is a ***direct*** way of examining the intestine for inflammation or damage.

In 2012 in Europe it was decided that children that had a very elevated aTTG test and the right genes (those known to be associated with Celiac Disease) did not need to have a biopsy test but could be assumed to have Celiac Disease.

We are doing this study to compare the way we diagnose Celiac disease here (with a biopsy) with how it is now done in Europe (without a biopsy). If your child has an aTTG test ≥ 200 IU/ml and has the genes associated with celiac disease you will have the choice to not have the biopsy test done but to enter the study and your child will be followed over this year to see if he/she is improving on a gluten free diet (the life long dietary change that is the only treatment for celiac disease). Even if your child does not have an aTTG test ≥ 200 IU/ml you can help us with the study, as we can compare children that do have a biopsy to those children that do not have a biopsy diagnosis, to see if things are any different at the end of one year.

What will this involve?

There are two groups of children who will be in this study: children who have confirmed Celiac Disease diagnosed by the biopsy and children who have an aTTG result of 200IU/ml.

The study procedures are described below for each group:

1. If your child's aTTG is 200IU/ml and you would like to consider allowing your child to be in this study, your child will need to have a blood test to check if they have the genes associated with Celiac Disease (called HLA DQ2 or 8). If your child does have these genes (the blood test results will come back in about two weeks) then you have the choice to continue with the study.
2. If your child is confirmed to have celiac disease by aTTG and gene testing, as above, or by the biopsy and you consent to allow them to be in the study we will collect the information about your child that is included in their hospital chart over the first year your child attends the Celiac Clinic. This information includes symptoms they have, your family history of autoimmune diseases, aTTG test results and information about their diet. All this information is collected as a routine in the Celiac Disease Clinic.

For all children in the study, we will do an additional test that is not routine for children with Celiac Disease in our Clinic. This test requires a collection of stool on two occasions from your child; we would like to collect stool within the next two weeks after joining the study and in one year. We will provide a collection container for the stool and ask that you bring it to the hospital within 24 hours of collection. We will test the stool for fecal Calprotectin, which can tell us in an ***indirect*** way if there is inflammation in the intestine.

We will do one further test that is not routine in our Clinic. This is a test of how 'leaky' is the intestine – another indirect way of testing for damage. This test would be done in your home over one evening and night; now (*again within 2 weeks*) and in one year. The test requires your child to drink a sugary liquid and then to collect all

the urine they make after drinking the liquid for one night. The sugar-drink is completely safe, it contains known amounts of sucrose, lactulose and mannitol, which are all sugars that are absorbed in the intestine to different degrees. Your child would not be allowed to drink any alcohol or have laxatives or anti-diarrheal drugs for 24 hours prior to the test. After dinner, he/she would have to fast for 4 hours, other then drinking water, and then they would empty their bladder before drinking the sugar drink. After drinking the sugar drink, he/she will collect all the urine they pass overnight, and the first thing in the morning, into a special container(s) we will give you. We will ask you to bring us the container the next day for testing. We can test how well the sugars were absorbed by the intestine, by measuring what we find in the urine, to tell us if the intestine was leaky and absorbed sugars too easily.

How will it help?

Participation in this study will not be of direct benefit to those who participate. You may consider this study because you prefer that your child does not to have an endoscopy test under anesthesia, which is not risk free. We hope that the information we get from doing this study will help us determine if we can confidently diagnose children with Celiac Disease without going a biopsy.

What are the risks?

The risk in not having an upper endoscopy and biopsy test is that we may diagnose your child with Celiac Disease, because of the aTTG test, and we are wrong. Celiac disease requires a life long change in diet so you will want to be sure about this diagnosis. At this time we believe that the aTTG test $\geq$ 200 IU/ml is not seen in conditions other than celiac

disease and is a good diagnostic test. This is why they are using the test to diagnose Celiac Disease in Europe. However, we cannot guarantee that it is always 100% right.

Who will know?

During the study we will be collecting health data about your child. We will do everything we can to make sure that this data is kept private. No data relating to this study that includes your child's name will be released outside of the study doctor's office or published by the researchers.

The study doctor/study staff may need to look at your child's personal health records held at the study doctor's office, and/or kept by other health care providers that you may have seen in the past (i.e. your family doctor). Any personal health information that we get from these records will be only what is needed for the study.

During research studies it is important that the data we get is accurate. For this reason your child's health data, including their name, may be looked at by people from the University of Alberta's Research Ethics Board and their Auditors.

By signing this consent form you are saying it is okay for the study doctor/staff to collect, use and disclose information about you child from their personal health records as described above.

After the study is done, we will still need to securely store your child's health data that was collected as part of the study. At the University of Alberta, we keep data stored for 5 years after the end of the study.

If you decide to leave the study, we will not collect new health information about you child, but we will need to keep the data that we have already collected.

Voluntary Participation: Can you change your mind?

Being in this study is your choice. If you decide to be in the study, you can change your mind and stop being in the study at any time, and it will in no way affect the care or treatment that your child is entitled to

Even if you enter the study and then change your mind it is always possible to change your child's diet and to test them for Celiac Disease with the endoscopy and biopsy test at a later date. You would need to discuss the best time with your doctor, but this is not impossible.

What happens if my child is injured because of this research?

If your child becomes ill or injured as a result of being in this study, he/she will receive necessary medical treatment, at no additional cost to you. By signing this consent form you are not releasing the investigator(s), institution(s) and/or sponsor(s) from their legal and professional responsibilities.

Your Signature

We would like you to sign this form to show that you agree for your child to take part in the study. Your signature indicates that you have read the study information sheet, had the study explained to your full satisfaction and give your voluntary consent to participate. You will be free to withdraw this consent at any time and this will not impact on your child's care at Stollery Children's Hospital.

PARENT CONSENT FORM

Title of Project: Serological Diagnosis of Celiac Disease at Stollery Children's Hospital: a Pilot Study Toward Changing Local Practice

Principal Investigator(s): Dr. Justine Turner, MBBS FRACP PhD

Do you understand that your child has been asked to participate in a research study?

Y N

Have you read and received a copy of the attached Information Sheet? Y N

Do you understand the benefits and risks involved for your child in taking part in this research study? Y N

Have you had an opportunity to ask questions and discuss this study? Y N

Do you understand that you are free to withdraw your child from the study at any time, without having to give a reason and without affecting your child's future medical care?

Y N

Do you understand who will have access to your child's records, including personally identifiable health information? Y N

Do you want the investigator(s) to inform your child's family doctor or pediatrician that your child is participating in this research study? Y N

Doctor's name _____

Who explained this study to you?_____

_____

Child's Name _____

I agree for my child to take part in this study: YES ¨ NO ¨

Signature of Parent or Guardian _____ Date & Time _____

(Printed Name) _____

Signature of Parent or Guardian _____ Date & Time _____

(Printed Name) _____

Signature of Investigator or Designee _____ Date & Time _____

ASSENT FORM

Title of Project: Serological Diagnosis of Celiac Disease at Stollery Children's Hospital: a Pilot Study Toward Changing Local Practice

Principal Investigators: Justine Turner, MBBS FRACP PhD

Co-Investigators: Dr Rabin Persad, MBBS FRACP(C)

Dr Hien Huynh, MBBS FRACP

It is important that you read (or have read to you) all of the information on this form. This information will help you decide whether or not you want to participate in this study.

Please ask if there are words or information that you do not understand.

Purpose of this study

You have had a positive blood test that suggests you might have Celiac Disease, a disease that can damage the bowel, but can be fixed if you don't eat certain foods in your diet.

In Canada we diagnose Celiac disease with a biopsy test, done while you are asleep (called an anesthetic). In some parts of the world you don't need to have a biopsy test, they only look at results of a blood test to say if you have Celiac disease.

We want to look at what is the best way to tell if someone has Celiac disease so we will compare children who have a biopsy with those who don't. We will look at their health now and in one year.

What happens if you take part in this Study?

Once your parent or guardian has signed the consent form and you have agreed to participate in the study, you may need to have an additional blood test, to help us decide if you need to have the biopsy test or not. You can ask to have numbing cream before you have the blood test to reduce the small amount of discomfort from the needle poke in your arm.

Regardless of if you need to have a biopsy or not this study involves you doing the following:

What will you have to do?

1. We would like you to give us a sample of stool/poo on two occasions, collected into a special container. We will test this to see if you have signs of damage in the bowel now and in one year after you are on the special celiac diet.
2. We would like you to collect urine/pee for us over one night, into a special container, after drinking a sugary drink. This is also a test of damage to the bowel that we would like to do now and in one year after you are on the special diet. The sugar-drink is safe and tastes sweet. You would not be able to drink any alcohol or have certain medications for a day before doing this test. On the day of the test this is what you do: after dinner, you don't eat for 4 hours and can only drink water. Then you do a pee to empty your bladder. Then you drink the sugar drink. After drinking the sugar drink, you collect all the pee you pass that

night and until the first thing in the morning.

3. Finally if you do this study we will collect some information about you from your hospital chart, no one else will be able to know this information. Only your doctors and nurses at the hospital and the study investigators.

Will this help me?

Doing this study won't really help you, although you might prefer not to have the biopsy test.

Is it safe?

We think that the diagnosis of celiac disease can be made safely, without the biopsy test. However, some doctors believe that the very best way to be sure you have Celiac Disease is to have a biopsy test.

Can I quit this study?

You don't have to take part in the study at all, and you can quit at any time. No one will be mad at you if you decide you don't want to do this, or if you decide to stop part way through. You should tell the doctor or nurse that you want to quit.

Who will know?

No one except your parents and the doctor will know you're taking part in the study unless you want to tell them. Your name and your chart won't be seen by anyone except the doctors, research study employees and nurses during the study.

Your signature

We would like you to sign this form to show that you agree to take part.

Your mom or dad or guardian will be asked to sign another form agreeing for you to take part in the study.

Do you have more questions?

You can ask your parent or guardian about anything you don't understand.

You can also talk to Dr's Turner, Huynh or Persad. Their phone number is 780 267 5570.

You can also talk to the University of Alberta Research Ethics Office at 780-492-2615. This office has no connection with the study researchers.

I agree to take part in the study:

Signature of research participant: _____ Date:_____

Signature of Principal Investigator:_____ Date:_____

Appendix B. Celiac Initial Appointment/First Contact Form

CELIAC First Contact Patient label

Date: _____ Age: _____

Assessment completed by: _____ (print name & sign)

Referring Doctor: _____ Date of referral: _____

Presenting Symptoms: None

Gastrointestinal

Growth concerns

Dermatitis herpetiformis

Other specify _____

Current Symptoms:	Normal	Reduced	Poor	Very Poor	Terrible
Wellness	0	1	2	3	4
Activity	0	1	2	3	4
Appetite	0	1	2	3	4

Gastrointestinal Symptoms:	None	Mild/Infrequent	Moderate/weekly	Severe/daily
Abdominal Pain	0	1	2	3

Bloating/Gas	0	1	2	3
Diarrhea	0	1	2	3
Constipation	0	1	2	3

#Stools: _____ Nocturnal diarrhea: Yes No

Nausea: Yes No Vomiting: Yes No

Weight Loss: Yes No Amount: _____

Other growth concerns: _____

Fatigue: Yes No Iron Deficiency: Yes No Not checked

Irritability: Yes No

Headache: Yes No

Joint Pain: Yes No Which: _____

Above symptoms are triggered by gluten exposure: Yes No

Above symptoms are improved by gluten withdrawal: Yes No

Other details: _____

Screening: Family History Diabetes Down Syndrome Other _____

Family Members Affected: _____

Biopsy proven: Yes No Comment: _____

Other autoimmune family history: Type 1 Diabetes Hypothyroidism RA Other: _____

Patient screening Blood work: Date_____ ATTG_____

EMA_____

IgA_____

Dietary History:

On gluten currently: Yes No

Trialed off gluten: Yes No Details: _____

Age of introduction gluten: _____

Breast fed: Yes No Duration: _____

Food allergies: Yes No Details: _____

Past Medical History:

Gestation at delivery

Colic during infancy: Yes No Details: _____

Other Concerns_____

Medications / Vitamins: _____ Gluten Free: Yes No

_____ Gluten Free: Yes No

_____ Gluten Free: Yes No

Self-Reported Ethnicity:

Country where child born _____

Country where mother born _____ Ethnicity (Caucasian/Asian /Indian/Other)

Country where father born _____ Ethnicity (Caucasian/ Asian Indian/Other)

Examination:

Height _____ _____ %ile

Weight _____ _____ %ile

BMI _____ _____ %ile

Anemia Clubbing Jaundice Oedema Muscle Wasting

Tanner Stage: Breast _____ Pubic Hair_____ Male Genitalia _____

Abdomen: Distention Tenderness Masses _____

Cardiovascular system: Normal Abnormal Comment _____

Respiratory system: Normal Abnormal Comment _____

Neurological system: Normal Abnormal Comment _____

Dermatological system: Normal Abnormal Comment _____

Other: _____

Same day case completed: Yes No Details if no: _____

Appendix C. Celiac Annual Visit Form

CELIAC CLINIC Annual Visit Patient label

Date: _____ Age: _____

Health Assessment: Completed by: _____ (print name & sign)

Height _____ _____ %ile Change_____

Weight _____ _____ %ile Change_____

BMI _____ _____ %ile Change_____

Blood work: Date_____ Ferritin_____
ATTG_____ Vitamin D_____
TSH_____

Bone Density: Date_____ Spine Z score_____

Medications / Vitamins: _____ Gluten Free: Yes No
_____ Gluten Free: Yes No
_____ Gluten Free: Yes No
_____ Gluten Free: Yes No

General Symptoms:	Normal	Reduced	Poor	Very Poor	Terrible
Wellness	0	1	2	3	4
Activity	0	1	2	3	4
Appetite	0	1	2	3	4

Weight Loss: Yes No Amount: _____

Fatigue: Yes No

Irritability: Yes No

Joint Pain: Yes No Which: _____

Polydipsia: Yes No

Headache: Yes No

Cold intolerance: Yes No

Gastrointestinal Symptoms:	None	Mild	Moderate	Severe
Abdominal Pain	0	1	2	3
Bloating/Gas	0	1	2	3
Diarrhea	0	1	2	3
Constipation	0	1	2	3

Vomiting: Yes No

Above symptoms triggered by gluten exposure: Yes No

Other health concerns triggered by gluten exposure: Yes No

Specify_____

Concurrent Health Concerns_____

Other Concerns_____

Overall health improved on gluten free diet: Yes No

Dietary Assessment: Completed by: _____ (print name & sign)

Parent Patient (circle if applicable) managing with GFD: Yes No

Parent Label reading (Not reading / Starting / Proficient)

Patient Label reading (Not reading / Starting / Proficient)

Fibre Intake: Acceptable / Concerns _____

Milk Intake: Acceptable / Concerns _____

Iron Intake: Acceptable / Concerns _____

Comments/concerns: _____

In the last 6-12 months taken oats Yes No Planned

In the last 6-12 months exposed to gluten: Yes No

Purposefully Accidentally

Compliance with GFD_____

Further teaching required: Yes No (Routine 12 months)

Plan / Recommendations:

Follow up: 12 months Other_____

Lab Request Given: Yes No

Bone age / Bone Density Request: Yes No Date_____

Consult Physician: Yes No

Reason for concern: 1__ failure to grow

2__ unresolved symptoms

3__ noncompliance

4__ other _____

Appendix D. Qualitative Interview Consent Form

Information Letter: Phone Interviews

Purpose of this study:

This purpose of this part of the study is to find out parents' views of their child's diagnosis of celiac disease. We are especially interested in views o the non-invasive choice of diagnosing being offered in Europe. We would also like to explore the patients' commitment to the gluten free diet through parental report.

What we learn in this study will help us understand the feelings and experiences about the diagnosis through parents of children with celiac disease. This will help our clinic to be of better support to parents and families.

What will happen:

We would like to talk with mothers and fathers about the diagnosis of their child's Celiac Disease. Your interview will take place one-on-one with a researcher over the phone. The interview will last approximately 1 hour. We will record the interview so it can be typed out afterwards. Your identity and the content of the interview will be kept strictly confidential by the researchers.

Benefits & risks:

There are no direct benefits for you or your child for being in this study. However, you will have a chance to tell your story. Other parents of children with celiac disease will benefit from what we learn from you.

The only risk to you is being uncomfortable about what you tell us. We understand that some of the questions we ask you are about emotional experiences in your lives. You may choose not to answer a question, and you may end the interview at any time. You may ask the interviewer to turn off the recorder so you can "just talk" with the interviewer, knowing that your discussion will not be used as study data..

At the interview, we will give you the names and phone numbers of individuals within Alberta Health Services who can talk to you if you feel upset or worried. These individuals have no connection with the research study so you may speak freely with them about your experience in this study.

Confidentiality:

We will keep everything you say confidential except when professional codes of ethics or the law require reporting and your right to confidentiality and privacy cannot be upheld. Doctors and nurses involved in your child's care do not have access to your interview. We will remove your name and any identifying information from the typed out interview. All paper files will be kept inside locked cabinets inside a locked office. All electronic files will be kept on a password-protected server at the University of Alberta. Any files with identifying information (e.g., your name and address) will be kept in a separate location from your interview responses. We will keep the information you provide for at least seven years after we finish the study. The final report may contain your actual words but nothing will identify you. We will not use your name in any presentations or publications of the study results. We might look at the information

gathered for this study again in the future to help answer other study questions. If so, the ethics board will review the study to ensure we use the information ethically.

It's your choice:

You have the right to refuse to answer any questions. You can stop an interview at any time. You may request that the recorder be turned off at any time. If there was anything that you would like removed from the digital recording, we will be glad to do that as long as you ask before we analyze the interview. In the unlikely event that an illegal or unethical act is recorded, we will not be able to erase the recording and will be obligated to report such occurrences. You are free at any time to withdraw from the study. Your decision to participate in this study will in no way affect the care that you or your child receives. We would be happy to give you a report of the findings after the study is complete if you ask for it.

> If you have any concerns about any aspect of this study, you may contact the University of Alberta Research Ethics Office at 780-492-2615. This office has no affiliation with study investigators.

Parent Consent Form

Project Title: Serological Diagnosis of Celiac Disease at Stollery Children's Hospital: a Pilot Study Toward Changing Local Practice

Principal Investigators: Dr Justine Turner, MBBS FRACP PhD

Co-Investigators: Dr Rabin Persad, MBBS FRACP(C)

Dr Hien Huynh, MBBS FRACP

Dr Gwen Rempel, RN PhD

Graduate Student: Seema Rajani

Do you understand that you have been asked to be in a research study?	Yes	No
Have you read and been given a copy of the attached Information Letter?	Yes	No
Do you understand the benefits and risks involved in taking part in this research study?	Yes	No
Have you had a chance to ask questions and discuss this study?	Yes	No
Do you understand that you are free to refuse to take part in or withdraw from the study at any time? You do not have to give a reason and it will not affect you or your child's care.	Yes	No
Has the issue of confidentiality been explained to you?	Yes	No
If you have taken part in previous research with Dr Turner, do you understand that interview and questionnaire data that you provided may be used in analysis for this study?	Yes	No
Do you understand that the interview you give for this study may be used in future studies?	Yes	No
Do you understand who will have access to the data?	Yes	No
Would you like a report of the research findings when the study is done?	Yes	No
Would you be willing to be contacted about being part of related studies in the future?	Yes	No

This study was explained to me by: _____

I agree to take part in this study.

_____ _____

Signature of Research Participant Printed Name Date

_____ _____

Signature of Witness (if available) Printed Name Date

I believe that the person signing this form understands what is involved in the study and voluntarily agrees to participate.

_____ _____

Signature of Researcher Printed Name Date

Appendix E. Interview Guide

Biopsy Group:

1. What were your thoughts when you were first told about this study?
2. How did you feel about the experience you had when your child was going for biopsy by endoscopy?
    a. *Were you aware of the biopsy before coming to the first clinic appointment? Were you prepared for it?*
3. If you had been given the option for your child to NOT have a biopsy, but to be diagnosed by blood tests alone, would you have considered this?
4. If no, what would have been your concerns?
5. How confident were you when your child was diagnosed as having celiac disease with a biopsy?
6. Is your child doing better on a gluten-free diet (GFD)? Any examples of changes in your child since on a GFD?
    a. *Do they attribute these changes to eliminating gluten?*
7. How confident are you that your child is going to follow a GFD for life? Tell me more about this.
8. Do you think having had a diagnosis made by biopsy makes/will make a difference to your child choosing to follow a GFD for life?
9. Do you have any other comments? Anything else you would like to tell me about having a child diagnosed CD by biopsy

Serological Group:

1. What were your thoughts when you were first told about this study?
    a. *Were you aware of the biopsy before coming to the first clinic appointment? Were you prepared for it?*
2. What were you thinking and feeling when you heard about the possibility of a genetic test to diagnose your child's suspected CD? How did you feel about the genetic test?
3. What was your thought process when making the decision between your child not having a biopsy or having a biopsy to diagnose celiac disease?
4. What were your main reasons for choosing for your child to not have a biopsy?
5. How confident did you feel when your child was diagnosed with celiac disease by a blood test without a biopsy?
6. Is your child doing better on a GFD? Any examples of changes in your child since on a GFD?
    a. *Do they attribute these changes to eliminating gluten?*
7. How confident are you that your child is going to follow a GFD for life? Tell me more about this.
8. Do you think that the procedure for diagnosis – biopsy or no biopsy - would make a difference to your child choosing to follow a GFD for life?
9. Do you have any other comments? Anything else you would like to tell me about having a child diagnosed CD by genetics?