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Title: Clinical and Immunologic Features of Ultra-short Celiac Disease

Short title: Assessing ultra-short celiac disease

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Abbreviations

CHAID – Chi-Squared Automatic Interaction Detector

D1 – Duodenal bulb

D2 – Second part of the duodenum

DXA - Dual-energy X-ray Absorbitometry

EMA – Endomysial antibody

HLA – Human Leucocyte Antigen

IEL – Intra-epithelial lymphocyte

tTG – Tissue transglutaminase antibody

USCD – Ultra-short celiac disease

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Author contributions – PDM recruited patients analysed the data and wrote the original manuscript, MK and KE recruited patients. ER collected follow up data. SSC analysed and reported duodenal biopsy specimens and aided with statistical analysis, PV analysed and reported duodenal biopsy specimens, MH recruited patients, JAM aided with data interpretation and edited the manuscript, DSS conceived the study, recruited patients and edited the manuscript. All authors had access to the study data and approved the final manuscript.

Abstract:

Background & Aims: The clinical effects of gluten-sensitive enteropathy with villous atrophy limited to the duodenal bulb (D1) have not been delineated in adults with celiac disease. We investigated the sensitivity of D1 biopsy analysis in detection of celiac disease, the number and sites of biopsies required to detect ultra-short celiac disease (USCD, villous atrophy limited to D1), and the clinical phenotype of USCD.

Methods: We performed a prospective study of 1378 patients (mean age, 50.3 years; 62% female) who underwent endoscopy at a tertiary medical center in the United Kingdom from 2008 through 2014; routine duodenal biopsies were collected from D1 and D2. Quadrantic D1 biopsies were collected from 171 consecutive patients with a high suspicion of celiac disease (mean age 46.5 years; 64% female). Clinical data from patients diagnosed with USCD, based on biopsy analysis, were compared with those from patients with conventional celiac disease (villous atrophy beyond D1) and individuals without celiac disease (controls). Numbers of intraepithelial lymphocytes (IELs) and immune phenotypes were compared between D1 vs D2 in patients with celiac disease.

Results: Of the 1378 patients assessed, 268 (19.4%) were diagnosed with celiac disease; 9.7% of these patients had villous atrophy confined to D1 (USCD, *P*<.0001). Collection of a single additional biopsy from any D1 site increased the sensitivity of celiac disease detection by 9.3%–10.8% (*P*<.0001). Patients with USCD were younger (*P*=0.03), had lower titers of tissue transglutaminase antibody (*P*=.001), and less frequently presented with diarrhea (*P*=0.001) than patients with conventional celiac disease. Higher proportions of patients with conventional celiac disease had ferritin deficiency (*P*=.007) or folate deficiency

(P=0.003) than of patients with USCD or controls. Patients with celiac disease had median 50 IELs/100 enterocytes in D1 and a median 48 IELs/100 enterocytes (*P*=.7) in D2. The phenotype of IELs from patients with D1 celiac disease was indistinguishable from those of patients with D2 celiac disease.

Conclusions: Collection of a single additional biopsy from any site in the D1 intestine increases the sensitivity of detection for celiac disease. Patients with USCD may have early-stage or limited celiac disease, with a mild clinical phenotype and infrequent nutritional deficiencies.

KEY WORDS: malabsorption; celiac disease histology; case finding; tTg

Introduction

Internationally the prevalence of celiac disease is now recognised to be 0.2-1.0%.[1-3] However, despite increased awareness, celiac disease remains under-diagnosed. A recent UK study demonstrated that only 1 in 4 patients with celiac disease are currently diagnosed.[4] Cohorts from the international literature have reported significant delays in diagnosis ranging from 4 to 13 years.[5, 6] Furthermore 5-13.6% of patients with newly diagnosed celiac disease have had a prior endoscopy where the chance to diagnose celiac disease was missed.[5, 7] In many patients no consideration was given to duodenal biopsy however 41% of patients in a recent US study had a non-diagnostic duodenal biopsy.[7] Historically small bowel biopsies were taken from the jejunum with a Crosby capsule to diagnose celiac disease.[8] However with the advent of fibre-optic endoscopy biopsies from the distal duodenum were shown to be as accurate as jejunal biopsies for recognising celiac disease.[8] The gluten load is highest in the proximal GI tract and the duodenal bulb (D1) would seem a logical place to identify signs of celiac disease. However D1 had been avoided as a possible biopsy site due to concerns over the difficulty in interpretation due to the potential presence of Brunner's glands, gastric metaplasia, peptic duodenitis and presumed reduced villous height.[9] Prospective data from a heterogeneous group of small studies has suggested that interpretation of D1 biopsies is possible and may be the only site of villous atrophy in newly diagnosed celiac disease, (ultra-short celiac disease, (USCD)) (Table 1).[8-13] D1 biopsy however is not yet fully accepted for several reasons. Firstly, the majority of these studies are based on small cohorts with inadequate control groups used. The number of adult cases of USCD described from these endoscopy based studies totals only 25. Thus the international experience of this condition is very limited. Data published globally have been

geared around establishing if a duodenal bulb biopsy should be taken and the potential for increased diagnostic yield. It has not been established if D1 biopsy is necessary for all indications. Secondly, the ideal number and site for D1 biopsy in new cases has not been fully established. This has only been evaluated in a single small cohort of 28 patients where it was suggested that biopsies from the 9 O'clock and 12 O'clock positions may be optimal.[11] Thirdly, it is not clear if the histology of D1 in celiac disease is equivalent to D2. Indeed a recent study has suggested that the normal count of intraepithelial lymphocytes (IELs) in healthy patients may be lower in D1 than D2.[14] To date there are no data assessing histological phenotype in D1 in celiac disease. Finally, crucially it has not been established if USCD represents the same clinical phenotype and has the same implications as more extensive, conventional celiac disease including long-term outcomes.

We aimed therefore, to establish the prevalence of USCD, in the largest patient cohort reported internationally, in the context of a routine duodenal biopsy strategy in all-comers to open-access diagnostic gastroscopy. We aimed to assess the ideal number and site of bulb biopsy for detection of USCD. Furthermore, we aimed to establish the clinical and histological phenotype of USCD.

Methods

Patients were prospectively recruited from a single teaching hospital in Sheffield, UK, between 2008 and 2014. Consecutive patients attending a single research endoscopy list, where routine duodenal biopsy is employed, were recruited in a significant expansion of our previous study of 376 patients.[12] Patients attending include those with suspected celiac disease but also include general and open access referrals for all upper GI symptoms. All

recruited patients received quadrantic biopsies from the second part of the duodenum. At least one further biopsy sample was taken from D1 in a separate formalin pot.

Within this cohort, in an expansion of our previously reported study [11], consecutive patients with a high clinical suspicion of celiac disease had standardised quadrantic biopsies taken from D1 in addition to the distal duodenum to identify the ideal number and site for D1 biopsy. In these patients the different topographic areas from which the quadrantic bulb biopsy specimens were taken correlating with a clock face. Specimens were taken from the 12 O'clock, 3 O'clock, 6 O'clock and 9 O'clock positions within D1, with the patient positioned in the left lateral position. This ensured biopsies were aimed at targeted areas rather than specifically to areas of obvious mucosal abnormality and allowed consistency of sampling between patients. Biopsy specimens from D1 were carefully inserted into a biopsy cassette with numbered compartments, reflecting specific D1 biopsy sites, before being fixed in formalin at the time of endoscopy. Each of the bulb specimens was placed in a separate paraffin wax block. All 4 of the biopsies from the distal duodenum were orientated and embedded in a single block of paraffin wax by the pathology department. Standard 3µm thick sections were taken from all samples and stained with hemotoxylin and eosin. Distal D2 biopsy specimens were analysed prior to bulb biopsy specimens. Each of the biopsy specimens was graded according to the modified Marsh criteria, to identify the presence and severity of villous atrophy. The Marsh criteria were applied consistently between the bulb and distal duodenum. We have 6 gastrointestinal pathologists who all routinely initially reported duodenal biopsies that were undertaken during the course of the study. Validation was then performed by 2 of these pathologists independently reviewing both USCD and conventional celiac disease cases. All patients enrolled received concurrent standard celiac

serology tests, as well as immunoglobulins, taken on the day of their examination. IgA tissue transglutaminase (tTG) was measured by using a single enzyme-linked immunosorbent assay (Aesku Diagnostics, Wendelsheim, Germany). A tTG titer of greater than 15 U/mL was used to define a positive test as per the manufacturer's instructions. IgA endomysial antibody (EMA) was detected by immunofluorescence on primate oesophagus sections (Binding Site, Birmingham, UK). Total immunoglobulins were measured on a Behring BN2 nephelometer (Dade Behring, Marburg, Germany). Patients were excluded from the study if they had any standard contraindications to endoscopic biopsy, if they had active gastrointestinal bleeding, if a suspected carcinoma was observed during the examination, or if they were pregnant.

Patients were defined as having celiac disease if they had the combination of positive antibodies (EMA or tTG) and evidence of increased IELs, crypt hyperplasia and villous atrophy, (Marsh 3a–3c) in any of their biopsy specimens. For cases of villous atrophy in which the patient had negative celiac serology, supporting evidence of celiac disease was sought including, family history, exclusion of other causes of villous atrophy, gluten challenge, repeat biopsy and/or response to a gluten free diet. All patients with villous atrophy confined to D1 had their HLA status checked with HLA DQ2 or DQ8 required for diagnosis.

To ascertain the phenotype and consequences of USCD patients were split into 4 groups based on histology findings. Group 1 was comprised of the patients diagnosed with USCD and they were compared to patients with conventional celiac disease (Group 2) and the remaining patients who were not diagnosed with celiac disease (Group 3). Presenting symptoms and serology taken at the time of endoscopy were compared across the whole cohort (Groups 1,2

and 3). A fourth group (Group 4) was identified to compare baseline hematology, biochemistry, bone profile and hematinics taken prior to commencing gluten free diet. These patients were age and sex matched controls identified from within Group 3 who had negative EMA and tTG and normal duodenal histology. Baseline bone densitometry using Dual-energy X-ray Absorptiometry (DXA) was also compared between groups 1 and 2. Osteopenia was defined as a T-score of -1.5 to -2.4 and osteoporosis as a T-score of less than -2.5. Blinded IEL counts were performed in 25 consecutive patients with celiac disease and compared to age and sex matched controls with negative serology. Counts were made manually using Image-J software (National Institutes of Health) on sections stained with hemotoxylin and eosin. Median IEL counts per 100 enterocytes were calculated from 5 representative villi using the previously validated method of Walker and colleagues.[15] In cases with villous atrophy IELs were counted for 50 enterocytes starting mid-way between 2 crypts in 5 separate sites. Immunohistochemistry staining for CD3, CD4 and CD8 was used in patients with villous atrophy in both D1 and D2 to assess for any phenotypic differences.

Follow up

All patients diagnosed with USCD and conventional celiac disease received specialist dietetic advice on the gluten free diet. Subsequently patients were invited to attend for repeat clinical evaluation 9-18 months following institution of a gluten free diet. Bloods for repeat serology, hematology, biochemistry and hematinics were taken. Dietary adherence was estimated using a previously validated 5 point questionnaire.[16] Patients graded any change in symptoms since institution of a gluten free diet using a likert scale graded from -10 to +10. On this scale 0 represented no improvement in symptoms -10 representing a significant deterioration in symptoms and +10 a significant improvement in symptoms.

Statistical analysis

The sensitivity for duodenal biopsy sites were compared using a McNemar test for correlated proportions. Univariate analysis of categorical presenting characteristics between USCD, conventional celiac disease and control patients was performed using Chi square. Multivariate analysis, correcting for age, gender and concurrent presenting symptoms, was performed using binary and multinomial logistic regression. Chi-squared Automatic Interaction Detector (CHAID) decision tree analysis was used to identify groups likely to be diagnosed with USCD or conventional celiac disease. The CHAID decision tree model uses multiple Chi-square tests corrected for multiple comparisons (Bonferroni) to identify the most significant independent variable. Once this has been identified the model splits subjects into groups based on this variable and further analysis is carried out in each of the new groups. A p value <0.05 was required for splitting of nodes and we chose an unlimited tree depth. The Kruskal-Wallis test was used for comparing non-parametric continuous values and ANOVA was used for parametric data to analyse the consequences of USCD and conventional celiac disease compared to controls. Absolute values were compared using a Chi-square. Follow up data were compared using a paired Wilcoxon sign-rank test for non-parametric data and a paired students t-test for parametric data. Analysis was undertaken using SPSS 21.0 (IBM). All p values provided are 2 sided.

Ethical considerations

Ethical approval of the study was sought and gained from the local National Health Service Research and Ethics Committee under the study number STH15416. All patients consented

to their procedures in accordance with the UK Joint Advisory Group for endoscopy guidelines.

Results

Patients

In total 1378 patients (mean age 50.3, 62% female) were consecutively recruited. Dyspepsia and diarrhea were the most frequent reasons for referral for endoscopy in 328 (27.6%) and 221 (16.0%) patients respectively. A full list of presenting complaints is shown in table 2. Of 1378 patients, 423 (30.7%) had a positive serological test taken at the time of endoscopy and 154 patients (11.1%) had previous equivocal duodenal histology (seronegative villous atrophy or raised IELs). This represents a referral bias as we are a centre with a specialist interest in celiac disease.

In total 268 patients (19.4%) were newly diagnosed with celiac disease. Multivariate analysis revealed a diagnosis of celiac disease was associated with younger age (P<0.0001), anemia (AOR 1.71, P=0.02), a family history of celiac disease (AOR 2.89, P<0.0001), lethargy (AOR 3.67, P<0.0001) and osteoporosis (AOR 6.14, P<0.0001). Celiac disease was not associated with reflux (AOR 0.10, p<0.0001) and non-specific dyspepsia (AOR 0.23 P<0.0001). Of 154 patients referred with previously abnormal duodenal histology 17 (11.0%) were ultimately diagnosed with celiac disease.

Is there an increased diagnostic yield with D1 sampling?

Of the 268 patients diagnosed with celiac disease 26 had villous atrophy confined to D1 and were diagnosed with USCD. All of these patients had an HLA type compatible with celiac disease (19 DQ2 heterozygous; 5 DQ2 homozygous; 2 DQ8 heterozygous). By taking an

additional D1 biopsy, therefore, the diagnostic yield was increased by 9.7% compared to standard D2 biopsy (*P*<0.0001). Of the 26 patients with villous atrophy confined to D1 7 (26.9%) had entirely normal D2 biopsies whilst 18 (69.2%) had Marsh 1 changes and a single patient (3.8%) had Marsh 2 changes in D2.

Non-celiac abnormalities were seen in 80 (5.8%) D1 biopsies. The most common histological abnormality other than celiac disease was peptic duodenitis which was seen in 4.1% of D1 samples compared to 1.4% of D2 samples (*P*<0.0001). Brunner's glands interfering with interpretation was rare, but was seen more commonly in D1 (1.2%) compared to D2 (0.3%) samples (*P*=0.002). In total 24 patients (9.0%) were diagnosed with seronegative celiac disease. Importantly seronegative celiac disease was not more common in USCD (2/26, 7.7%) compared to conventional (22/242, 9.1%) (*P*=1.0). In the 2 cases of seronegative USCD one had a previously raised tTG and was HLA DQ2 heterozygous and the other patient presented with ataxia, had repeat biopsy confirming villous atrophy and was HLA DQ2 homozygous. A diagnosis of seronegative celiac disease was only made in all cases after confirming an appropriate HLA type and a thorough re-examination looking for alternative causes of villous atrophy.

Is there an optimal site for targeted D1 biopsy sampling?

In total 171 patients (mean age 46.5, 64% female) recruited underwent quadrantic D1 biopsy. Of these, 65 patients (38%) were diagnosed with celiac disease and the remaining 106 (62%) patients served as controls. Villous atrophy was seen in D1 in 62 (95.4%) patients and D1 was the only site of villous atrophy in 7 patients (10.8%). Of the 62 patients with villous atrophy in D1, 6 (9.7%) had at least one D1 sample that showed no evidence of villous atrophy and in 23

(37.1%) there was a difference between the Marsh grades. A D1 biopsy taken from the 3 O'clock position in addition to standard D2 biopsy resulted in 100% sensitivity. However the addition of biopsies from any of the other topographical areas each only resulted in missing of a single case. There was no significant difference between the sites however the addition of a D1 biopsy from any site was superior to a distal duodenal biopsy alone, increasing the diagnostic yield by 9.3-10.8% (*P*<0.0001). The sensitivity of quadrantic D1 biopsy without D2 biopsy was 95.4% which was not statistically superior to D2 biopsy alone (*P*=0.34).

Are there any consequences to Ultra-short Celiac Disease?

In Group 1, 26 patients (mean age 37.3, 73% female) were diagnosed with USCD. Group 2 comprised of 242 patients (mean age 42.0, 66% female) with conventional celiac disease. Group 3 comprised of the remaining 1,110 patients who did not have celiac disease. Group 4 included 136 controls (mean age 42.3, 66% female) with negative EMA and tTG and normal duodenal histology to compare hematology and biochemistry values. Patients in diagnosed with celiac disease (Groups 1 and 2) were significantly younger than those without celiac disease (Group 3, *P*<0.0001). Interestingly, patients with USCD (Group 1) were younger than those diagnosed with conventional celiac disease (Group 2) (mean age 37.3 vs. 42.0, AOR 0.97 (0.94 – 0.998), *P*=0.03). On univariate analysis (table 2) patients with both USCD (Group 1) and conventional disease (Group 2) were less likely than controls (Group 3) to present with reflux (*P*<0.0001) or dyspepsia (*P*<0.0001) and more likely to have a family history of celiac disease (*P*<0.0001). Only 3.8% of USCD patients had diarrhea which was significantly lower than 24.1% of conventional celiac patients (*P*<0.0001). Furthermore on CHAID decision tree analysis (Bonferroni method) with unlimited tree depth to identify USCD amongst celiac patients the absence of diarrhea was the single discriminating factor (Adj *P*=0.019) (not

shown). On multivariate analysis (table 3) conventional celiac patients but not USCD patients were more likely than controls to present with anemia, diarrhea, a family history of celiac disease, lethargy, and osteoporosis. CHAID analysis reveals the most significant diagnostic yield, at 49.3%, in our cohort was for patients with a family history of celiac disease presenting without dyspeptic symptoms. The full decision tree for diagnosing all patients with celiac disease based on symptomatic presentation is shown in figure 1.

Patients with USCD (Group 1) had lower tTG titers (*P*=0.001) but had equal rates of tTG positivity (*P*=0.57) compared to conventional celiac disease (Group 2). The prevalence of ferritin deficiency was higher in conventional celiac disease (Group 2, 31.5%) than USCD (Group 1, 13.6%) and controls (Group 4, 16.7%) (*P*=0.007). The prevalence of folate deficiency was higher in conventional celiac disease (Group 2, 18.0%) than USCD (Group 1, 3.8%) and controls (Group 4, 6.3%) (*P*=0.003) There was no difference in bone densitometry findings between Groups 1 and 2. On multinomial logistic regression analysis tTG levels were confirmed to be lower in USCD (Group 1, AOR 0.91 (0.84 - 0.98) *P*=0.019) and folate levels were higher (AOR 1.17 (1.03 - 1.35) *P*=0.018) compared to conventional celiac disease (Group 2). A summary of the blood and DXA findings is shown in tables 4 and 5.

Median IEL counts in patients without celiac disease were lower in D1 (11) than D2 (16) (*P*=0.002) however all median IEL counts were below the currently agreed cut off of 25 IELs/100 enterocytes.[15] In celiac disease the median IEL count in D1 was 50 and in D2 was 48 (*P*=0.71*)*. IELs in D1 were immunophenotypically indistinguishable to those in D2 in celiac patients.

Follow up

Follow up data were available for 24/26 patients with USCD. Two patients were lost to follow up, despite repeated attempts to contact them, following their initial clinic visit and dietetic support. Patients with USCD (Group 1) demonstrated a significant decrease in their tTG (P=0.006) after a median of 16 months on a gluten free diet. A statistically significant increase in corrected calcium was also identified (P=0.03). There was no significant change in the other hematology or biochemistry parameters assessed. This is in contrast to patients with conventional celiac disease (Group 2) who, after a median of 15 months on gluten free diet, demonstrated a significant increase in folate (P=0.001), ferritin (P<0.0001) and B12 (P<0.0001) levels as well as a significant reduction in their tTG titers (P<0.0001). There was no significant difference in any follow up blood values between patients with USCD (Group 1) and conventional disease (Group 2). A full analysis of the follow up bloods is shown in table 6.

On analysis of symptoms, the majority of patients with USCD disease noted an improvement with only a single patient complaining of deterioration in their symptoms (score -1). There was no significant difference between the median symptom improvement scores between USCD and conventional celiac disease patients on a gluten free diet (median score +6 for both groups p=0.67). Interestingly rates of adherence based on the Biagi adherence score were marginally lower in the USCD patients (Group 1) compared to conventional disease (Group 2) (median 3 vs 4, P=0.02). This may be a representation of the more significant initial symptoms seen in the patients with conventional celiac disease.

Discussion

We have shown a significant increase in the diagnostic yield for celiac disease when a single D1 biopsy is taken from any site. Critically this is the first study to assess the clinical implications and presentation of USCD in adult patients. Our findings would support a

hypothesis that patients with USCD represent early celiac disease with a milder clinical phenotype presenting at a younger age with more infrequent nutritional deficiencies.

There is a growing body of evidence suggesting that D1 may be the only site of villous atrophy in patients with newly diagnosed celiac disease.[8-12] However the practice of D1 biopsy has not been universally accepted. Indeed, in a recent audit of non-specialist hospitals in the UK, a D1 biopsy was performed in only 18/914 (2.0%) of patients undergoing duodenal biopsy to diagnose celiac disease and only in 10% of patients in a recent US study.[17, 18] There may be several reasons for the poor uptake in D1 biopsy which we have been able to address in this study. Firstly the numbers of patients have been very small with only 25 patients described frequently in highly celiac enriched populations with few if any control patients. In our study 1378 patients were prospectively recruited, more than twice the combined number of patients in previous adult studies. Furthermore all patients had routine duodenal biopsy performed reducing potential ascertainment bias within our cohort and increasing the applicability to the general population. Secondly there remain concerns that interpretation may be impeded by the presence of non-celiac abnormalities such as peptic duodenitis or gastric heterotopia. However in our cohort non-celiac abnormalities were relatively rare and were rarely considered to interfere with interpretation of duodenal biopsies. There are also concerns around the cost and time burden of additional duodenal biopsy. In our cohort only one biopsy was required from any site in D1 to significantly increase the sensitivity of duodenal biopsy. This has important implications as adopting a D1 biopsy policy becomes more feasible if no specific area is required to be targeted. This may be in contrast to the only previous study into targeted bulb biopsy that suggested a 2 biopsy strategy from both the 9 o'clock or 12 o'clock positions in conjunction with quadrantic D2

biopsies may be optimal. In this small cohort this ensured that the most severe lesion was detected in 100% of cases. However, in our present study, only a 4 biopsy strategy ensured that the most severe lesion was seen in 100% of cases. In our opinion, although identifying the most severe lesion may be ideal, identification evidence of villous atrophy of any grade may be the most important diagnostic criterion. Further study may be required to identify the significance of severity of D1 atrophy.

Finally none of the studies performed in adult patients have analysed the presentation of patients with USCD. Only a single pediatric study has assessed clinical characteristics.[19] In a retrospective study of 101 children with newly diagnosed celiac disease, no differences in the mode of presentation USCD (n=10) and conventional disease could be elicited however the study was likely to be underpowered to detect significant differences. Furthermore no studies have attempted to assess the clinical impact of USCD. In our cohort patients with appeared to have a less severe clinical phenotype with less frequent diarrhea and fewer micronutrient deficiencies. This may be commensurate with the minimal loss of absorptive capacity associated with only a short segment of villous atrophy. Interestingly, however, patients with USCD were diagnosed at a younger age and had lower tTG titres suggesting that celiac disease may have been identified at an earlier stage. This may have resulted in fewer nutritional deficiencies from a shorter lead time to diagnosis. Furthermore, this may have other significant implications for patients allowing earlier intervention with a gluten free diet. This may ameliorate symptoms and reduce the burden of unnecessary investigations that can be associated with a missed celiac diagnosis.[7] Furthermore it may also reduce the potential for long term sequelae of celiac disease such as lymphoma or

osteoporosis. However, further long term follow up studies of patients with USCD are required to truly assess the long term implications of diagnosis.

If it is true that histological changes of celiac disease are confined to D1 it may be difficult to account for any symptoms or deficiencies in the USCD group. There may be several reasons for this apparent paradox. Firstly our patient population may also have influenced our results. This was a study of patients attending endoscopy for investigation of symptoms or other known associations with celiac disease so patients were inherently more likely to have symptoms. It is conceivable that if we had conducted a screening study a diagnosis of USCD may have been associated with a higher proportion of asymptomatic patients.

Secondly it is possible that in some of the USCD patients the diagnosis may have been missed in the biopsies taken from D2. This is certainly feasible given that celiac disease is recognized to be patchy. However the patients in our study all had adequate D2 biopsy (mean = 4.5) as recommended by international guidelines. Despite this, it is conceivable that the diagnosis could have been made by more extensive D2 biopsy or deeper duodenal or jejunal biopsy. However, in a previous study to identify the best site for D2 biopsy, even when 8 biopsies were routinely taken in distinct topographic areas in the proximal and distal D2 100% sensitivity for villous atrophy could only be achieved when a duodenal bulb biopsy was included.[8] Studies of deeper duodenal or jejunal biopsy are fairly limited. A 2014 study of 41 pediatric patients showed 1 (2.4%) had villous atrophy in the jejunum only.[20] A historical study from our center of 31 patients with a previously non-diagnostic duodenal biopsy found that 3 of the 5 patients who had celiac disease confirmed had disease limited to the jejunum, however no duodenal bulb biopsies were taken in any of these patients.[21] Furthermore the gluten load is known to be highest in the proximal small bowel and there is

evidence that the small bowel heals distally to proximally on a gluten free diet.[22, 23] Some of the patients USCD group had Marsh 1 and 2 changes in more distal biopsies. These patients could be considered to belong to the celiac disease spectrum however these changes are non-specific. Indeed other prospective studies have subsequently confirmed celiac disease in only 16% of unselected patients [24] and 43.3% of patients with HLA DQ2 or DQ8.[25] Furthermore much of the evidence for the diagnosis of celiac disease based on Marsh 1 changes alone is based on a single study of just 23 EMA positive patients.[26] This cohort of patients demonstrated significant improvement in symptoms and antibody titers on a gluten free diet. Importantly the bulb was not biopsied in this study and had it been then it is conceivable the USCD may have been present in these patients. Furthermore this study has not had the impact of changing clinical practice because clinicians wish to see the presence of villous atrophy before committing a patient to a lifelong gluten free diet. As a result patients with a Marsh 1 lesion in D2 frequently undergo re-evaluation with prolonged gluten challenge and further invasive procedures to confirm or refute a diagnosis of celiac disease. It could be argued that by taking a duodenal bulb biopsy this could be avoided in a significant number of patients if villous atrophy is confirmed in the duodenal bulb. Importantly in our cohort 7/26 (26.9%) patients had completely normal D2 biopsies, and a further 4/26 (15.4%) patients had a negative EMA. If a duodenal bulb biopsy had not been performed then a diagnosis of celiac disease could have been missed in 11/26 (42.3%) of our cohort. Finally, another possibility is that symptoms and reduced bone mineral density in the USCD group are as a result of chronic inflammation or co-existent disease. This hypothesis is further strengthened by the fact that there was a significantly increased rate of autoimmune disease (1 psoriasis, 2 autoimmune thyroid disease, and 1 sjögrens) seen in the USCD group compared to the conventional disease group.

One concern over the diagnosis of USCD is whether or not a strict gluten free diet is required for these patients. Some of these concerns appear to be validated by our present study as there is evidence of a milder clinical phenotype associated with fewer micronutrient deficiencies. However our follow up data have shown that the majority of patients have symptomatic improvement on a gluten free diet. Furthermore there is a significant drop in the tTG titer on institution of a gluten free diet. This would appear to suggest that the immune cascade has been switched off. Our hypothesis is that as patients are diagnosed significantly younger than those with conventional disease that this may present a unique opportunity to prevent further micronutrient deficiency in this select group of patients. Although there is no current evidence for this in USCD there is some supporting evidence from patients with Marsh 1 and 2 enteropathy. Patients with a positive EMA and Marsh 1 and 2 changes, from a Finnish cohort, were randomised to gluten free or gluten containing arms of the study. Histology significantly deteriorated in all patients who remained on a gluten containing diet.[26] Perhaps USCD represents the next step in the development of a more extensive enteropathy. Further study may identify which patients require long term follow up for now however USCD and conventional celiac disease patients should be treated as part of the spectrum of the same disease and receive standard follow up.

There are some potential limitations to our study. Firstly prevalence of celiac disease in our cohort was higher than might be expected for a routine endoscopy list. This was as a result of a referral bias to a centre with a specialist interest in celiac disease. This may have resulted in an overestimate of the prevalence of celiac disease in some patient groups however rates of celiac disease in different patient cohorts are similar to previous case

finding studies reported in the literature.[27-29] Furthermore this increased prevalence did allow us to accrue significant numbers of patients with USCD to allow for effective comparison of patient phenotypes.

In conclusion, a single additional D1 biopsy from any site increases the diagnostic yield. A diagnosis of USCD may represent early celiac disease with a mild clinical phenotype. The addition of a D1 biopsy to diagnose celiac disease may reduce the known delay in diagnosis that many patients with celiac disease experience. This may allow earlier institution of a gluten free diet and potentially prevent nutritional deficiencies and reduce the symptomatic burden of celiac disease.

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Table 1: A summary of the available studies into duodenal bulb biopsies for diagnosing celiac disease

Year	Country	Adults / Pediatrics	Number of patients	Number of celiac disease (%)	Number of USCD (%)
2001 [10]	Austria	Adults	51	21 (41.2%)	2 (9.5%)
2004 [30]	Italy	Pediatrics	95	95 (100%)	4 (4.2%)
2005 [31]	Italy	Adults	1	1 (100%)	1 (100%)
2008 [8]	UK	Adults	56	56 (100%)	1 (1.8%)
2008 [32]	Italy	Pediatrics	1013	665 (65.6%)	16 (2.4%)
2009 [33]	Canada	Pediatrics	35	29 (81.6%)	3 (11.4%)
2010 [19]	USA	Pediatrics	198	198 (100%)	10 (5.1%)
2010 [34]	Italy	Pediatrics	47	42 (89.4%)	5 (11.9%)
2010 [9]	USA	Adults	80	40 (50%)	5 (12.5%)
2011 [35]	Israel	Pediatrics	87	87 (100%)	6 (7.0%)
2011 [12]	UK	Adults	376	126 (33.5%)	11 (9.0%)
2012 [11]	UK	Adults	77	28 (36.4%)	5 (17.9%)
2013 [36]	Australia	Pediatrics	101	101 (100%)	8 (7.92%)
2014 [13]	Italy	Adults	42	25 (59.5%)	0 (0%)

Table 2: Univariate analysis of proportion of patients in each group with different presenting characteristics (percentages with different superscript letters are significantly different at a P<0.01 level)

Presenting characteristic	Total % (n=1378)	USCD % (n=26)	Conventional celiac disease % (n=242)	Controls % (n=1110)	P
Female Gender	62.2	73.1	65.6	61.2	0.231
Abdominal pain	13.2	3.8	12.0	13.7	0.287
Alternating bowel habit	2.6	11.5[a]	3.3[b]	2.3[b]	**0.010**
Anemia	12.5	19.2[a,b]	18.7[b]	11.0[a]	**0.003**
Autoimmunity	4.4	15.4[a]	6.2[a,b]	3.8[b]	**0.006**
B12/folate/vitamin D deficiency	4.9	7.7	7.5	4.2	0.084

Bloating	7.8	7.7	10.4	7.2	0.249
Diarrhoea	16.0	3.8[a]	24.1[b]	14.6[a]	**<0.0001**
Dyspepsia	27.6	7.7[a]	5.0[a]	32.9[b]	**<0.0001**
Family History	5.6	11.5[a]	14.5[a]	3.5[b]	**<0.0001**
Irritable Bowel Syndrome	6.5	3.8	8.3	6.1	0.396
Lethargy	5.7	7.7[a,b]	16.6[b]	3.2[a]	**<0.0001**
Nausea/Vomiting	5.6	0.0[a,b]	1.7[b]	6.6[a]	**0.005**
Neurological symptoms	8.5	11.5	4.6	9.3	0.051
Osteoporosis	1.4	3.8[a,b]	3.7[b]	0.8[a]	**0.001**
Previous abnormal histology	11.1	30.8[a]	7.1[b]	11.4[c]	**0.001**
Reflux	12.2	0.0[a]	1.7[a]	14.8[b]	**<0.0001**
Weight Loss	5.6	3.8	7.5	9.5	0.385

Table 3: Multinomial logistic regression of presenting characteristics

	Ultra Short Celiac Disease (n=26)				**Conventional celiac disease (n=242)**	
	Compared to controls (n=1110)		**Compared to conventional celiac disease**		**Compared to controls (n=1110)**	
Characteristic	**AOR**	**P**	**AOR**	**p**	**AOR**	**P**
Age	**0.94 (0.91 - 0.97)**	**<0.0001**	**0.97 (0.94 – 0.998)**	**0.03**	**0.97 (0.96 - 0.98)**	**<0.0001**
Female Gender	1.40 (0.54 - 3.61)	0.49	1.38 (0.52 – 3.65)	0.52	1.01 (0.72 - 1.42)	0.93
Abdominal pain	**0.09 (0.01 - 0.76)**	**0.03**	0.14 (0.02 – 1.29)	0.08	**0.61 (0.38 - 0.99)**	**0.05**
Alternating bowel habit	4.44 (0.93 - 21.32)	0.06	4.34 (0.82 – 23.00)	0.09	1.02 (0.40 - 2.59)	0.96
Anaemia	1.10 (0.32 - 3.74)	0.88	0.62 (0.18 – 2.18)	0.46	**1.77 (1.11 - 2.82)**	**0.02**

Autoimmunity	2.31 (0.63 - 8.50)	0.21	1.75 (0.44 – 6.92)	0.42	1.32 (0.67 - 2.58)	0.42
B12 /folate /vitamin D deficiency	0.77 (0.16 - 3.76)	0.75	0.72 (0.14 – 3.65)	0.69	1.07 (0.57 - 2.02)	0.82
Bloating	0.74 (0.15 - 3.61)	0.71	0.68 (0.14 – 3.71)	0.64	1.09 (0.64 - 1.87)	0.75
Diarrhoea	0.20 (0.02 - 1.66)	0.14	0.13 (0.02 – 1.10)	0.06	**1.53 (1.00 - 2.33)**	**0.05**
Dyspepsia	0.32 (0.06 - 1.62)	0.17	1.68 (0.30 – 9.49)	0.56	**0.18 (0.10 - 0.36)**	**<0.0001**
Family History	1.98 (0.50 - 7.91)	0.33	0.69 (0.17 – 2.75)	0.60	**2.86 (1.67 - 4.91)**	**<0.0001**
IBS	0.15 (0.02 - 1.36)	0.09	0.23 (0.02 – 2.20)	0.20	0.64 (0.35 - 1.17)	0.15
Lethargy	1.32 (0.27 - 6.50)	0.73	0.35 (0.07 – 1.73)	0.20	**3.74 (2.19 - 6.37)**	**<0.0001**
Nausea/Vomiting	NS	NS	Ns	NS	**0.34 (0.12 -0.99)**	**0.05**
Neurological symptoms	0.93 (0.22 - 3.92)	0.93	1.86 (0.40 – 8.73)	0.43	0.50 (0.25 - 1.01)	0.06
Osteoporosis	5.26 (0.50 - 54.91)	0.17	0.91 (0.09 – 9.62)	0.93	**5.81 (2.09 - 16.15)**	**0.001**
Previous abnormal histology	**4.33 (1.63 - 11.47)**	**0.003**	**8.67 (2.98 – 25.25)**	**<0.0001**	**0.50 (0.29 - 0.87)**	**0.01**
Reflux	ns	Ns	Ns	0.98	**0.28 (0.10 - 0.81)**	**0.02**
Weight Loss	0.39 (0.05 - 3.11)	0.37	0.46 (0.05 – 3.74)	0.60	0.87 (0.48 - 1.55)	0.63

Table 4: Analysis of absolute blood and DXA values in ultra-short celiac disease compared to conventional celiac disease and controls

	USCD (n=26)		Conventional CD (n=242)		Controls (n=136)			**Multivariate analysis** USCD compared to conventional CD	
	Mean	Median	Mean	Median	Mean	Median	p	AOR	p
tTG xULN (All patients n=1378)	6.8	4.8 [a]	12.9	20 [b]	0.96	0.27 [c]	**<0.0001**	**0.91 (0.84 - 0.98)**	**0.02**
Hemoglobin	13.8 [a]	14	13.6 [a]	13.8	13.9 [a]	13.8	0.43	1.10 (0.56 - 2.15)	0.88
MCV	87.4 [a]	88.4	88.4 [a]	88.6	89.8 [b]	90.2	**0.03**	0.95 (0.84 - 1.07)	0.38
B12	353.8	349.5 [a]	406.9	364 [a]	390.6	356 [a]	0.53	1.00 (0.99 - 1.00)	0.09
Ferritin	63.9	45.5 [a,b]	61.2	34 [b]	66.5	50 [a]	**0.03**	1.00 (0.99 - 1.01)	0.87

Folate	10.2	9.6 [a,b]	8.3	7.2 [b]	10.2	8.6 [a]	**0.002**	**1.17 (1.03 - 1.35)**	**0.02**
Calcium	2.3	2.3 [a]	2.31	2.31 [a]	2.3	2.29 [a]	0.84	7.33 (0.02 - 2446.7)	0.50
ALT	30.9	17 [a]	27.3	21 [a]	22.7	18 [a]	0.05	1.01 (0.99 - 1.03)	0.56
T-Score Spine	-0.72 [a]	-0.8	-0.7 [a]	-0.7	na	na	0.74	0.65 (0.296 - 1.46)	0.29
T-Score Hip	-0.32	-0.1 [a]	-0.38	-0.3 [a]	na	na	0.99	1.36 (0.51 - 3.64)	0.54

Table 5: Comparison of relative blood and DXA values in ultra-short celiac disease compared to conventional disease and controls (figures with different superscript letters are significant at a *P*<0.05 level)

	Percentage abnormality			
	USCD	CCD	Control	P
Anemia	8.0	9.0	5.2	0.38
Microcytosis	12.0	7.3	4.6	0.24
Low B12	0.0	5.8	3.9	0.50
Low Ferritin	13.6[a,b]	31.5[b]	16.7[a]	**0.007**
Low Folate	3.8[a,b]	18.0[b]	6.3[a]	**0.003**
Hypocalcemia	4.8	8.3	7.3	0.95
Elevated ALT	16.0	17.0	12.9	0.54
Osteopenia or worse	47.4	44.6	Na	1.0
Osteoporosis	10.5	13.5	Na	0.76

Table 6: Comparison of paired median blood values before and after a gluten free diet in Ultra-Short and Conventional Celiac Disease

	USCD			Conventional Celiac Disease		
	Pre-GFD	Post-GFD	p	Pre-GFD	Post-GFD	p
tTG xULN	**4.97**	**0.87**	**0.006**	**20.0**	**0.87**	**<0.0001**
Hemoglobin	13.70	13.60	0.72	13.70	13.70	0.76
MCV	87.30	88.20	0.88	89.20	90.20	0.13
Calcium	**2.26**	**2.36**	**0.03**	2.32	2.32	0.35
ALT	18.00	17.00	0.9	**22.00**	**20.00**	**0.004**
B12	315.5	295.50	0.14	**357**	**417.00**	**<0.0001**
Ferritin	41.0	34.00	0.05	**31.5**	**50.50**	**<0.0001**
Folate	9.55	8.50	0.79	**7.70**	**9.00**	**0.001**

Figure 1: CHAID decision tree for the diagnosis of celiac disease based on symptomatic presentation

Mooney PD, Kurien M, Evans KE, Rosario E, Cross SS, Vergani P, Hadjivassiliou M, Murray JA, Sanders DS. Clinical and Immunologic Features of Ultra-short Celiac Disease