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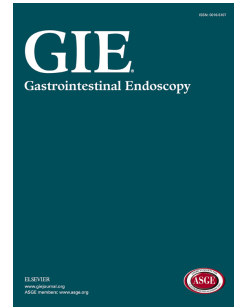
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The use of small-bowel capsule endoscopy in cases of equivocal celiac disease

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Abstract:**Background and aims:**

Seronegative villous atrophy (SNVA), raised intraepithelial lymphocytes (IELs) and crypt hyperplasia on duodenal histology can be secondary to celiac disease (CD) or other causes such as medications or infections. Our aims were to assess the role of small-bowel capsule endoscopy (SBCE) in these patients and to ascertain whether findings on SBCE at diagnosis can predict disease outcome.

Methods:

Patients (177) with SNVA, IELs +/-crypt hyperplasia on duodenal histology were studied. These patients all had an equivocal diagnosis of CD.

Results:

Overall, 56 (31.6%) patients had a positive SBCE. Most patients had disease affecting the proximal third of the small bowel (SB) (33, 58.9%). The diagnostic yield of SBCE was 40.0% (22 patients), 51.4% (18 patients), 27.0% (10 patients), and 14.0% (7 patients) in patients with an unknown cause for SNVA (SNVA-UO), patients with SNVA who responded to a gluten-free diet (SNVA-CD), SNVA-KNOWN CAUSE, patients with raised intraepithelial lymphocytes +/- crypt hyperplasia respectively. In SNVA-UO, SBCE at diagnosis was more likely to be positive in patients with persistent SNVA (10, 90.9%) and persistent SNVA with lymphoproliferative features (4, 80.4%) than patients with spontaneous resolution of SNVA (8, 20.5%) ($p=0.0001$). All patients in the SNVA-CD group who eventually developed adverse events had a positive SBCE ($p=0.022$). They also had more extensive SB disease than those without adverse events (50% vs 1% $p=0.002$). More-extensive SB disease on SBCE correlated with a higher SNVA-related mortality in patients with SNVA-UO and SNVA-CD ($p=0.019$). Severity of histology did not correlate with mortality ($p=0.793$).

Conclusions:

A positive SBCE at diagnosis predicts a worse outcome. More importantly, more extensive disease in these patients is associated with poor survival. Targeting patients with extensive disease at diagnosis with more aggressive therapy can help to improve prognosis.

Keywords: seronegative villous atrophy, seronegative celiac disease, small-bowel capsule endoscopy, mortality, survival;

Introduction:

Although the criterion standard for the diagnosis of celiac disease (CD) is duodenal histology, celiac serology and genetic studies can be negative in the presence of villous atrophy (VA) (1, 2). This has led to the introduction of another term: seronegative villous atrophy (SNVA).

In patients with suspected CD, SNVA on duodenal histology can be secondary to IgA deficiency or a low gluten intake at the time of diagnosis (3, 4). SNVA can also be secondary to drugs, infections and immune-mediated conditions (5). In some patients, the cause remains unknown (6). The most common causes for SNVA are as follows: CD

(SNVA-CD) (28%-31%), medication-related (6%-26%), infective (7%-27%), and idiopathic (14%-18%) (5, 7). Despite undergoing extensive investigations, determining the etiology of disease in most of these patients is challenging and requires further secondary investigations. The introduction of small-bowel capsule endoscopy (SBCE) has enabled us to study this condition further.

Similar to SNVA, milder changes on duodenal histology of raised intraepithelial lymphocytes (IELs) and/or crypt hyperplasia can occur secondary to immune-mediated conditions, medications, and infections (8, 9). These patients do not have enough changes on duodenal histology to support a diagnosis of CD as VA and positive CD serology are required features (10). Some of these patients will eventually have a confirmative diagnosis of CD (11). One explanation is that CD can affect the small bowel (SB) with varying degrees of severity (12). Some patients might be following a low-gluten-containing diet, and changes will only become evident after a gluten challenge.

As recommended by the European guidelines, SBCE is indicated in patients with equivocal CD (13). Up to 30% of patients with CD present with persisting signs and symptoms, which necessitates further investigations, including repeat duodenal histology and SBCE (14). SBCE can play a major role in patients with refractory celiac disease (RCD) in assessing disease extent and ruling out adverse events at diagnosis (15) and at follow-up (16).

Our aim was to assess the role of SBCE in patients with distinct causes for SNVA and Marsh 1 and 2 histology. Firstly, we determined whether SBCE can play a role at diagnosis in addition to conventional investigations. Secondly, we examined findings on SBCE to assess whether they can help predict outcomes.

Methodology:

Study design and participants:

One hundred seventy-seven patients with SNVA or raised intraepithelial lymphocytes (IELs) +/- crypt hyperplasia on duodenal histology were prospectively recruited over a 19-year period (2000 – 2019). They all had a SBCE after a histological diagnosis, total immunoglobulin A levels (IgA) and contemporary CD serology (IgA endomysial antibodies, EMA and IgA tissue transglutaminase antibodies anti-TTG). All patients had negative EMA and anti-TTG levels.

Patients with SNVA or raised IELs +/- crypt hyperplasia on duodenal histology referred to the SB unit for SBCE by their respective caring physician within the study period were included in this study. The caring physician, then determined the type of follow up (repeat duodenal histology and SBCE) these patients received.

A separate group of patients with Marsh 1 and 2 histology was included in this study as these patients form part of the group of patients with an equivocal diagnosis of CD (17). Absence of VA and negative CD serology in these patients can be explained by the patchy nature of CD in the SB, low gluten intake in the diet and incorrect orientation of the duodenal specimens (12, 18-20). They do not have enough histological changes (VA) to fulfill a diagnosis of CD and their CD serology is negative. However, they have changes (raised IELs or / and crypt hyperplasia) that could be found in patients with CD or in those who will eventually develop CD as shown in previous studies (21, 22). Our study provides a broader overview of patients with equivocal changes on histology that could potentially be related to CD by including patients with Marsh 1 and 2 histology.

Duodenal histology:

At least 2 biopsy specimens from the duodenal bulb (D1) and 4 specimens from the second part of the duodenum (D2) were taken. All duodenal biopsy specimens were fixed in buffered formalin and embedded in paraffin wax. Standard 3- μ m-thick sections at 3 levels were stained with hematoxylin and eosin. All histological samples were reviewed by 2 expert histopathologists. In the case of discrepancy, a third histopathologist was involved in the adjudication process. IELs was defined as >25 per 100 enterocytes (23). VA was identified according to the Marsh-Oberhuber criteria, using the most-severe lesion present: Marsh 1 (raised IELs), Marsh 2 (crypt hyperplasia), Marsh 3a (partial villous atrophy, PVA), Marsh 3b (subtotal villous atrophy, SVA), or Marsh 3c (total villous atrophy, TVA) (24).

Histological samples were also assessed for differences in immunohistochemistry based on CD3 pan-lymphocyte marker and specific CD8-T cytotoxic and CD4-T helper intraepithelial lymphocyte expression.

Celiac serology:

CD serology testing was IgA based. EMA were detected on immunofluorescence on primate oesophagus sections from The Binding Site (Birmingham, UK). Anti-TTG levels were assayed using ELISA kits (Aesku Diagnostics, Wendelsheim, Germany). Titres less than or equal to 15U/mL were considered normal.

Criteria for diagnosis of SNVA and categories of patients:

Criteria for the diagnosis of SNVA were similar to those used by previous researchers (5, 7) including:

1. Review of medical and surgical history and medication use.
2. Patients underwent the following investigations, including HLA-DDQ2 and DQ8 genetic studies, HIV serology, Tuberculosis quantiferon, antienterocyte and antiglobet cell antibodies, stools for giardia antigen and other bacteria.
3. The biopsy specimens were reviewed by expert gastrointestinal histopathologists to confirm the diagnosis.
4. If patients were on a gluten-free diet (GFD) at the time of presentation, they were asked to undergo a gastroduodenoscopy after a gluten-challenge of 10g/day for 6 weeks.
5. Further biopsy specimens were obtained to rule out *Helicobacter pylori*, Whipple's PCR, and SB aspirate to rule out infection.
6. In case of persistent symptoms or where the suspicion of Crohn's disease / microscopic colitis was high, an ileocolonoscopy was carried out.
7. SBCE at the time of diagnosis was carried out at the discretion of the physician but based on clinical need.

For the purpose of this paper, a histological response (as opposed to a clinical response) to a GFD was considered to be diagnostic of SNVA-CD (group 2). This is a stricter criteria than a clinical response to a GFD and eliminates the risk of diagnosing patients incorrectly as we know that in some patients, a clinical response to a GFD does not mirror a histological response (6).

Patients with no identifiable cause for SNVA (SNVA-UO) all had alternative causes for SNVA excluded. They were further subclassified into patients with (1) resolving /transient

SNVA, (2) persistent histological changes and no response to a GFD, or (3) persistent histology despite a trial of GFD and with lymphoproliferative disorders before or after the diagnosis of SNVA (figure 1).

Patients with SNVA-CD (group 2) had a histological response to a GFD. Other supporting features included raised IgG celiac serology, features such as dermatitis herpetiformis, a positive first-degree family history of CD and HLA genotype supportive of a diagnosis of SNVA-CD.

Patients in group 3 had a cause for VA in their medical history or by testing for infective and inflammatory conditions. Patients in group 4 had a diagnosis of Marsh grade 1 or 2 but no evidence of VA.

All of these patients underwent extensive investigations as outlined in the criteria above and in Figure 1.

Small-bowel capsule endoscopy:

Each patient was asked to stay on clear fluids for 24 hours before the SBCE and to drink 2 liters of Klean-Prep the day before the SBCE. All patients underwent SBCE using Pillcam SB 2 or 3 (Medtronic, Minneapolis, Minn, USA) (25, 26). SBCEs were reviewed by expert SBCE reviewers (>300 capsules each/ year). The expert reviewers of the capsule endoscopies were aware of the clinical picture including symptoms at presentation, findings on duodenal histology, serological results and findings on previous SBCEs. Findings such as fissuring of mucosa, scalloping of folds, mosaic pattern, nodularity, VA, and ulcers were recorded. Currently there is no validating scoring system for the diagnosis of CD on SBCE. Features associated with CD have been studied before. These include fissuring of mucosa, scalloping of folds, mosaic pattern, nodularity, VA and ulcers (27-29). Hence, we used similar assessment for our patients in this study at the time of reporting. A SBCE was considered to be positive (positive diagnostic yield, DY) if 3 or more features of CD mentioned above were present.

The distribution (proximal, mid, distal, diffuse SB) and extent of affected SB mucosa was also recorded.

In this study, extent of abnormal SB mucosa refers to SB mucosa with macroscopic features of CD.

Statistical analysis:

Statistical analysis was carried out using SPSS version 23 (IBM Corp. Released 2015. IBM SPSS Statistics for Mac, Version 23.0. Armonk, NY, USA: IBM Corp.). Frequencies and means were calculated to characterize each group. Nonparametric statistical tests were used namely, the Fishers exact test, and Kruskal–Wallis test Results were considered to be statistically significant if the p value was less than 0.05.

Ethical considerations:

The study protocol was approved by the Yorkshire and the Humber Research Ethics committee (IRAS 232382) and registered with the local research and development department of Sheffield Teaching Hospital NHS Foundation Trust under the registration number STH 19998. All images used in this study were deidentified. No additional consent was required for the study with the use of deidentified videos as assessed and approved formally by the Research Ethics Committee.

Results:

A total of 177 patients (107 females, 60.5%; mean age of 49.7+/-16.9 years) were included in this study. A total of 177 patients (107 females, 60.5%; mean age of 49.7+/-16.9 years) were included in this study. They underwent a SBCE within a median of 93 days from histological diagnosis. The median time between index SBCE and repeat histology was 125 days. Most patients only had proximal SB disease (33, 58.9%) (table 1)

Only 5 patients (2.8%) had incomplete visualisation of the SB on SBCE. One patient with diffuse features of SNVA on SBCE had a repeat complete SBCE that showed duodenitis only. This patient had SNVA-UO. Repeat duodenal histology showed persistent TVA. Two patients (1 had raised IELs on histology, another patient had SNVA-UO) did not have any features of SNVA on SBCE and they did not undergo a repeat SBCE. The patient with SNVA-UO and a normal SBCE had spontaneous resolution of SNVA confirmed on repeat duodenal histology. Two patients with incomplete SBCE had proximal SB ulcers. One patient was treated for *H pylori*. Another patient with a history of nonsteroidal anti-inflammatory drug (NSAID) use underwent repeat duodenal histology that was normal after stopping NSAIDs for 6 months.

There was no statistical difference in DY according to age at diagnosis (median age SBCE positive: 51 years, SBCE negative: 50 years; $p=0.804$), HLA status ($p=0.608$), ethnicity ($p=0.531$) and gender ($p=0.188$).

Group 1 (SNVA-UO):

Fifty-five (31.1%) had no identifiable cause for SNVA. The DY of SBCE was 40% (22) in this group of patients.

Thirty-nine patients (70.9%) had spontaneous resolution of SNVA on histology. Eleven (20.0%) patients had persistent SNVA. Five (9.1%) patients had persistent SNVA and developed lymphoproliferative features. SBCE was positive in 8 (20.5%) of those with spontaneous resolution of SNVA, 10 (90.9%) of patients with persistent SNVA and 4 (80.4%) patients with persistent SNVA who developed lymphoproliferative features ($p=0.0001$).

Eight patients ($n=11$; 72.7%) with persistent SNVA on histology received further treatment including steroids +/- immunosuppressants: Budesonide - 4 patients, budesonide and 6-mercaptopurine: - 1, prednisolone and azathioprine - 2, prednisolone and adalimumab - 1. One patient received vedolizumab for a diagnosis of ulcerative colitis. Six patients ($n=11$; 54.5%) underwent a repeat SBCE, which showed persistent changes on SBCE. Another patient ($n=11$; 9.0%) had a normal repeat SBCE. All 11 patients with persistent SNVA underwent repeat duodenal biopsies showing persistent VA.

In the group of patients with persistent SNVA and lymphoproliferative features, 3 patients had a history of chronic lymphocytic leukaemia, large B-cell lymphoma and non-Hodgkin's lymphoma respectively. Two of them received budesonide and prednisolone, azathioprine respectively. The same patients who received treatment also underwent a repeat SBCE that showed persistent changes of SNVA on SBCE. Another 2 patients developed angioimmunoblastic T-cell lymphoma and indolent mature B-Cell lymphoma after their diagnosis of SNVA.

Group 2 (SNVA – CD):

A total of 19.8% (35) of patients in this study had a histological response to a GFD and were therefore classified as having SNVA-CD. Five of these patients (14%) were IgA deficient. The DY of SBCE in this group of patients was 51.4% (n=35; 18). Seven patients developed adverse events: 3 patients developed RCD I, and 2 developed RCD 2. Two patients developed RCD 2 and ulcerative jejunoileitis. All had a positive SBCE at the time of diagnosis (p=0.022). They also had more extensive SB disease than those without adverse events (50% vs 1% p=0.002).

Group 3 (identifiable cause):

Thirty-seven patients (20.9%) had alternative causes for SNVA (Supplementary Table 1). The DY of SBCE in patients with identifiable causes for SNVA was 10 (27.0%). Three of these patients had SB ulcers secondary to *H pylori*, NSAIDs and Crohn's disease respectively. From those patients with a positive SBCE, who had repeat duodenal histology, only one patient had persistent SNVA.

Group 4 (raised IELs+/- crypt hyperplasia):

Fifty patients (28.2%) had raised IELs (Marsh class I). In addition, 5 of these patients (10.0%) also had crypt hyperplasia (Marsh grade 2). All patients had negative EMA and anti-TTG except for 4 patients who had a mean anti-TTG of 20 (U/mL).

On repeat (36 patients) duodenal histology, 26 patients (72.2%) had normal histology after a mean of 45 +/-SD 40.8 months. Seven patients (19.4%) had persistence of Marsh 1 and 2 disease, 1 patient (2.78%) had non-specific duodenitis on repeat histology. Two patients (5.56%) had Marsh grade 3 disease.

The DY of SBCE was only 14% (7 patients). One patient had proximal VA. Six patients had ulcers in their SB (4 distal, 1 mid, 1 diffuse). Two of these were eventually diagnosed with Crohn's disease. The others had underlying infective aetiologies including tuberculosis in 2 patients.

Three patients with a positive SBCE, had repeat duodenal histology that was normal. Four patients with a normal SBCE had repeat duodenal histology which was normal in 3 cases.

Causes for histological changes in group 4 can be found in Supplementary Table 2.

Comparison of groups:

There was no statistical difference in age at diagnosis across study groups (median age: group 1: 50, group 2: 52, group 3: 51, group 4: 48.5 years; p=0.704). The HLA status is shown in Supplementary Table 3 (p=0.0001). There was no statistical difference in gender across groups (p=0.178). Most patients were predominantly white (p=0.001, p=0.002) (Supplementary Tables 4 and 5).

Patients with SNVA-UO and those with SNVA-CD were more likely to have positive SBCEs. Those with raised IELs /crypt hyperplasia were more likely to have normal SBCEs (p=0.001) (table 2). Patients with SNVA-UO and SNVA-CD also had the most-extensive disease (p=0.018) (table 2).

Features of SNVA were similar in groups 1 (SNVA-UO) and 2 (SNVA-CD). Patients in groups 3 (SNVA KNOWN CAUSE) and 4 (raised IELs +/- crypt hyperplasia) more commonly had ulcers (table 3).

In patients with VA (groups 1–3), those with more severe histological pattern on duodenal biopsies were more likely to have a positive SBCE (PVA: 21, 26%, SVA: 12, 60%, TVA: 13, 59%, $p=0.001$). More extensive SB disease also corresponded to a more severe histological pattern (PVA 1%, SVA 15%, TVA 17%, $p=0.001$).

Eight (14.5%) patients within group 1 (SNVA-UO) had a positive second SBCE after a mean of 19 months (\pm SD 7.1). These patients had their immunosuppressive medications altered. A third SBCE was carried out in 3 patients in group 1 (SNVA-UO after a mean of 14 months (\pm 0.1)). All of them had a positive SBCE. All patients (5, 14.3%) who had a repeat SBCE within group 2 (SNVA-CD) had persistently positive findings ($p=0.643$). This resulted in further dietary review to ensure strict adherence to a GFD. Where this was confirmed and on reviewing histology, a diagnosis of RCD was made prompting commencement of steroids and immunosuppressants.

Two patients in group 3 (SNVA-KNOWN CAUSE) (5.41%) had a repeat SBCE. In one patient, the repeat SBCE was normal. In another patient, florid ulceration was present. This patient was diagnosed and started treatment for Crohn's disease. The first SBCE had also shown ulcers. Three patients (6.0%) in group 4 (raised IELs +/- crypt hyperplasia) had repeat SBCEs. One patient had distal SB ulcers and was treated for TB. This patient was not known to have TB before their investigations, and she was of Asian ethnicity. Another patient had mosaic pattern of the mucosa proximally and had been on regular NSAIDs. Another patient had a normal SBCE.

Overall, 11 patients (6.2%) passed away (all-cause mortality) by the end of the follow-up period. There was no statistical significant difference in mortality across groups (group 1: 3, 5.5%, group 2: 4, 11.4%, group 3: 3, 8.1%, group 4: 1, 2.0% $p=0.327$). However, when only deaths related to SNVA were considered, mortality was highest in the SNVA-CD group (3; 8.6%) secondary to RCD adverse events. One patient (1.8%) with SNVA-UO passed away from lymphoma ($p=0.048$). None of the patients in groups 3 and 4 died from CD-related causes. On constructing survival curves for patients with SNVA-UO and SNVA-CD (CD-related mortality), patients with extensive SB involvement had worse survival than those with normal or proximal involvement on SBCE ($p=0.019$) (figure 2). There was no correlation between survival and severity of duodenal histology ($p=0.793$) (figure 3).

Discussion:

This study describes the findings on SBCE in subgroups of patients with different causes for SNVA in the SB, and in this aspect it is the first of its kind. It highlights how SBCE at the time of diagnosis of SNVA can be important in the prediction of disease course and in the identification of features such as SB ulcers that need follow-up and could be consistent with other diagnoses such as Crohn's disease.

There are only 2 studies on the use of SBCE in patients with SNVA. In a study by Kurien et al,(30) patients with equivocal CD were compared with patients with nonresponsive CD. Patients with equivocal CD were further subdivided into 32 patients with SNVA and 30 with Marsh grade 1 or 2 histology. In those patients with SNVA, 28% had a positive SBCE that showed features of CD or Crohn's disease. This is slightly lower than the overall DY

reported in our study (group 1–3: 39.4%). In patients with Marsh 1 and 2, 1 patient was diagnosed with CD and another with SB Crohn's disease (30). This is much lower than the DY we reported for a similar group of patients (14%). In another study by Lujan-Sanchis et al,(31) 19 patients with SNVA-CD had a DY of 73.7%. This is higher than the DY in our study for group 2 (SNVA-CD 51.4%). One explanation for the difference in DY is the stricter diagnostic criteria that we used to diagnose SNVA-CD: histological response to a GFD.

The utility of SBCE in subgroups of patients with SNVA was further assessed in our study. Patients with SNVA-UO and SNVA-CD were most likely to have positive SBCEs and they were likely to have the most extensive SB involvement unlike patients with an identifiable cause for SNVA and those with raised IELs +/-crypt hyperplasia. Therefore, we can say that patients with SNVA who also have positive findings on SBCE are more likely to fall within one of these 2 groups of underlying etiology. The cause of SNVA/Marsh 1 and 2 histology is not always established at the time of initial investigations. Patients with a negative SBCE are more likely to have an underlying cause to explain the histological changes. It is important that this is ruled out by means of a thorough history and secondary investigations such as further duodenal biopsies for TB. In groups 3 and 4, SBCE was also helpful as it identified patients with SB ulcers. Patients with a diagnosis of tuberculosis, were not known to have the condition before investigations for SNVA, Marsh 1 and 2 histology. Those with changes secondary to NSAIDs use only admitted to their intake on further questioning. It has been suggested that patients with SB ulcers will benefit from a repeat SBCE after at least 6 to 8 months to ensure healing of the SB mucosa (32).

In this study we have established a correlation between extent of SB disease and survival in patients with SNVA-UO and SNVA-CD. However, we failed to establish a similar relationship with duodenal histology. These findings establish an additional role for SBCE in predicting disease outcome. In patients with SNVA-UO, a positive SBCE was associated with persistent positive histology at follow-up. This association highlights the fact that patients with macroscopic SB changes need to be targeted early with immunosuppressive therapy (7) due to the risk of persistent disease that in turn can be associated with risks of malabsorption and the development of lymphoproliferative disorders. Patients with SNVA-CD can develop adverse events such as RCD, ulcerative jejunoileitis, lymphoma and adenocarcinoma similar to patients with seropositive CD (33, 34). All patients who eventually developed adverse events in the SNVA-CD group had a positive SBCE and they also had more extensive disease than patients with uncomplicated SNVA-CD. These findings underline the important role SBCE can play in patients with SNVA-CD where the main treatment is a GFD. Patients with SNVA-CD with a positive SBCE can be monitored more closely due to the higher risk of developing adverse events.

Mortality in patients with SNVA has been compared to that in seropositive CD patients in 2 studies (English and Italian studies), both confirming the higher mortality in the former group. In one study the mortality in SNVA-CD, SNVA-non-CD, seropositive CD patients was 11.2%, 8.7% and 3.2% respectively (5). In another study, mortality was 6 deaths /100 person years in patients with SNVA, whereas only 0.2 deaths /100 person years were reported in seropositive CD patients (35). These findings tie in with other differences that distinguish SNVA-CD and seropositive CD. The former patients tend to be older and have a higher risk of adverse events (36). In our study, CD-related mortality was highest in the SNVA-CD group. This is secondary to RCD-related adverse events. We were also able to inversely correlate survival in patients with SNVA-UO and SNVA-CD with extent of

mucosal involvement on SBCE. This confirms the importance of regularly screening patients with SNVA-CD for adverse events and managing those with extensive disease aggressively.

A limitation of the study is the small cohort of patients included. However, other studies on patients with SNVA undergoing SBCEs have included an even smaller number of patients (up to 32 with SNVA, 30 Marsh I, II) (30, 31). Other limitations include the single center nature of this study and the broad time interval for data collection. Expert capsule reviewers were also not blinded to the clinical findings, serology and duodenal histology results. This might have resulted in over or under reporting of features of SNVA on SBCE. Additionally, a few patients did not have HLA assays, and some of them did not have a second duodenal biopsy. Not all patients with SNVA, raised IELs+/- crypt hyperplasia during the study period had a SBCE. In addition, only a few patients had a repeat SBCE and repeat duodenal histology as decided by the caring physician which resulted in very small numbers of patients with repeat investigations. A repeat SBCE is useful to reassess disease extent, assess response to therapy and in some cases (raised IELs+/- crypt hyperplasia) it can help to clarify the diagnosis. This made it difficult for us to draw any conclusions from features on repeat SBCEs.”

Conclusion:

SBCE can be useful in patients diagnosed with SNVA or Marsh 1 and 2 histology as it can identify features of CD or other conditions such as distal inflammation that can be associated with SB Crohn's disease. Unlike duodenal histology, SBCE can play an additional role in predicting disease outcome. In patients with SNVA-UO, positive findings on SBCE and more extensive disease can predict those with persistent SNVA who will require treatment with immunosuppressants. In SNVA-CD the same pattern is true for patients who eventually develop CD-related adverse events. In both SNVA-CD and SNVA-UO, more extensive disease on SBCE is indicative of a poorer prognosis. These patients need to be monitored more closely and alternative therapy such as steroids and immunosuppressants considered if adverse events develop. Although the number of patients with repeat SBCE in this study is small, re-evaluation of disease extent using SBCE can result in modification of therapy in patients with persistent or progressive changes.

Features on SBCE	N (%)
Fissuring	13 (7.3)
Scalloping	18 (10.2)
Nodularity	3 (1.7)
Mosaicism	20 (11.3)
Villous atrophy	23 (13.0)
Ulcers	16 (9.0)
Region	N (%)
Proximal	33 (58.9)
Proximal, mid	7 (12.5)
Proximal, distal	1 (1.79)
Mid	3 (5.36)
Mid, distal	1 (1.79)
Distal	6 (10.7)
Diffuse	5 (8.93)

Table 1: Features on capsule endoscopy in patients with raised IELs +/- crypt hyperplasia and SNVA;

	Findings of SNVA on SBCE ($P = .001$)		Mean percentage extent of disease ($P = .018$)* (%+/-SD)
	Absent N (%)	Present N (%)	
Group 1 (SNVA-UO)	33 (60.0)	22 (40.0)	10.2 (+/-26.5)
Group 2 (SNVA-CD)	17 (48.6)	18 (51.4)	8.41 (+/-20.3)
Group 3 (SNVA-KNOWN CAUSE)	27 (73.0)	10 (27.0)	5.49 (+/-22.9)
Group 4 (Raised IELs +/- crypt hyperplasia)	43 (86.0)	7 (14.0)	2.47 (+/-14.2)

Table 2: Positive SBCE / diagnostic yield and extent of disease on SBCE;

	Group 1 (SNVA-UO)	Group 2 (SNVA-CD)	Group 3 (SNVA KNOWN CAUSE)	Group 4 (Raised IELs)	P value
Fissuring	8 (14.5)	5 (14.3)	0 (0)	0 (0)	0.0001
Scalloping	11 (20.0)	6 (17.1)	1 (2.7)	0 (0)	0.0001
Nodularity	1 (1.8)	2 (5.7)	0 (0)	0 (0)	0.185
Mosaicism	10 (18.2)	10 (28.6)	0 (0)	0 (0)	0.0001
Villous atrophy	11 (20.0)	7 (20.0)	4 (10.8)	1 (2.0)	0.012
Ulcers	3 (5.7)	2 (5.7)	5 (13.9)	6 (12.0)	0.471

Table 3: Features of all patients on SBCE;

Supplementary tables:

Causes	N (%)
NSAIDs	5 (13.5)
H pylori	13 (35.1)
Giardia	6 (16.2)
Tuberculosis	3 (8.1)
Medications*	3 (8.1)
Infective **	4 (10.8)
Crohn's disease	1 (2.7)
Autoimmune	1 (2.7)
Radiation enteritis	1 (2.7)

Supplementary Table 1: Causes for SNVA in group 3;

* Aspirin: 1 patient; Angiotensin receptor blocker: 2 patients;

** Recent diagnosis of gastroenteritis: 2 patients; Positive duodenal aspirate (small intestinal bacterial overgrowth): 1 patient; hookworm infection: 1 patient;

Causes	N (%)
H pylori	6 (12)
Celiac disease	2 (4)
Tuberculosis	2 (4)
Medications*	6 (12)
Transient /Infective	10 (20)
Crohn's disease	3 (6)
Small intestinal bacterial overgrowth	2 (4)
Sarcoidosis**	1 (2)
Undiagnosed in	18 (36)

Supplementary Table 2: Causes of raised intraepithelial lymphocytes +/- crypt hyperplasia in group 4;

*aspirin: 3, NSAIDs: 3; **patient with an established history of gastric sarcoidosis

	DQ2 DQ8 heterozygous n(%)	DQ2 DQ8 homozygous n(%)	DQ2 heterozygous n(%)	DQ8 heterozygous n(%)	DQ2 homozygous n(%)	DQ8 homozygous n(%)	DQ2 DQ8 negative n(%)
Group 1 (SNVA- UO)	2 (3.6)	0 (0)	17 (30.9)	6 (10.9)	2 (3.6)	0 (0)	26 (47.3)
Group 2 (SNVA- CD)	4 (11.4)	0 (0)	10 (28.6)	2 (5.7)	15 (42.9)	1 (2.9)	3 (8.6)*
Group 3	1 (2.7)	0 (0)	13 (35.1)	2 (5.4)	0 (0)	0 (0)	16 (43.2)

(SNVA-KNOWN CAUSE)							
Group 4 (Raised IELs)	0 (0)	1 (2.0)	18 (36.0)	5 (10.0)	5 (10.0)	0 (0)	21 (42.0)

Supplementary Table 3: HLA status in patients with SNVA (p=0.0001);

*Two of the patients who were HLA-DQ2, DQ8 negative in the SNVA-CD group were HLA D Qa1*05 positive which is a genotype that is also compatible with CD.

	Ethnicity	
	Caucasian n(%)	Non-white n(%)
Group 1 - SNVA-UO	50 (90.9)	5 (9.1)
Group 2 - SNVA-CD	35 (100)	0 (0)
Group 3 - SNVA-KNOWN CAUSE	27 (73.0)	10 (27.0)
Group 4 - Raised IELs +/-crypt hyperplasia	39 (78.0)	11 (22.0)

Supplementary Table 4: Ethnicity across different groups; p=0.001

SNVA-UO: Seronegative villous atrophy of unknown cause; SNVA-CD: Seronegative villous atrophy secondary to celiac disease; IELs: intraepithelial lymphocytes.

	British Caucasian n(%)	African n(%)	Asian n(%)	Bangladeshi n(%)	Chinese n(%)	Irish n(%)	Pakistani n(%)	Spanish n(%)
Group 1 - SNVA-UO	50 (90.9)	1 (1.8)	2 (3.6)	0 (0)	1 (1.8)	0 (0)	1 (1.8)	0 (0)
Group 2 - SNVA-CD	35 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Group 3 - SNVA-KNOWN CAUSE	27 (73.0)	2 (5.4)	8 (21.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Group 4 - Raised IELs +/-crypt hyperplasia	38 (76.0)	1 (2.0)	3 (6.0)	2 (4.0)	0 (0)	1 (2.0)	4 (8.0)	1 (2.0)

Supplementary Table 5: Ethnicity across different groups; p=0.002

SNVA-UO: Seronegative villous atrophy of unknown cause; SNVA-CD: Seronegative villous atrophy secondary to celiac disease; IELs: intraepithelial lymphocytes.

Figure 1: Algorithm for the diagnosis and management of patients with seronegative villous atrophy or having duodenal histology of Marsh 1 and 2;

Figure 2: Mortality in group 1 (SNVA-UO) and 2 (SNVA-CD) from CD-related causes; (Normal: no features of SNVA on SBCE; proximal: features of SNVA in the proximal third of the small bowel; proximal and beyond or beyond proximal: features of SNVA that extend beyond the proximal small bowel or involve mid and distal small bowel;)

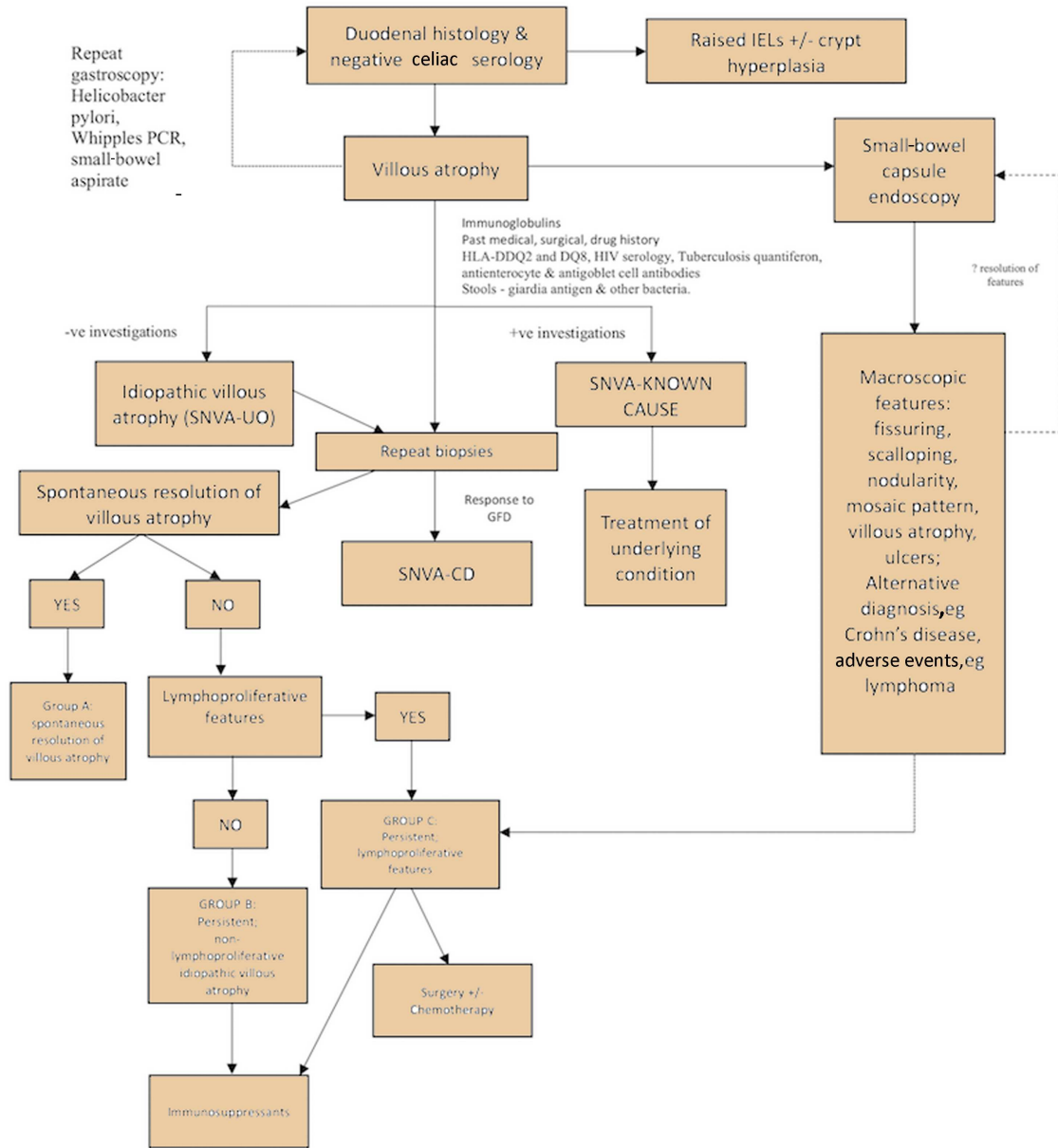
Figure 3: Mortality in group 1 (SNVA-UO) and 2 (SNVA-CD) patients from CD-related causes according to severity of duodenal histology; (PVA: partial villous atrophy, SVA: subtotal villous atrophy, TVA: total villous atrophy;)

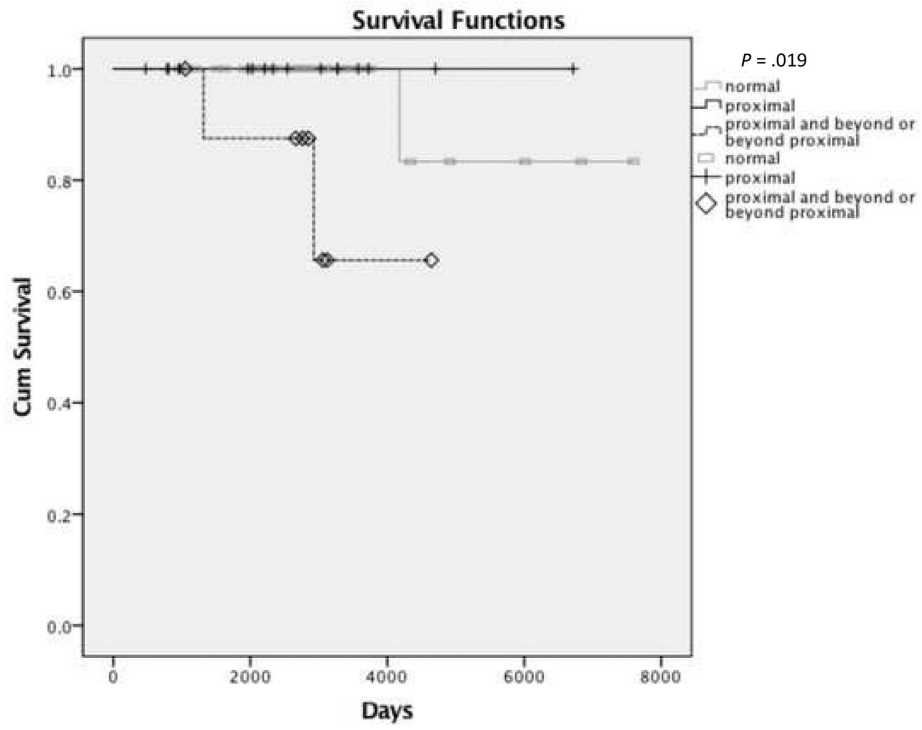
References:

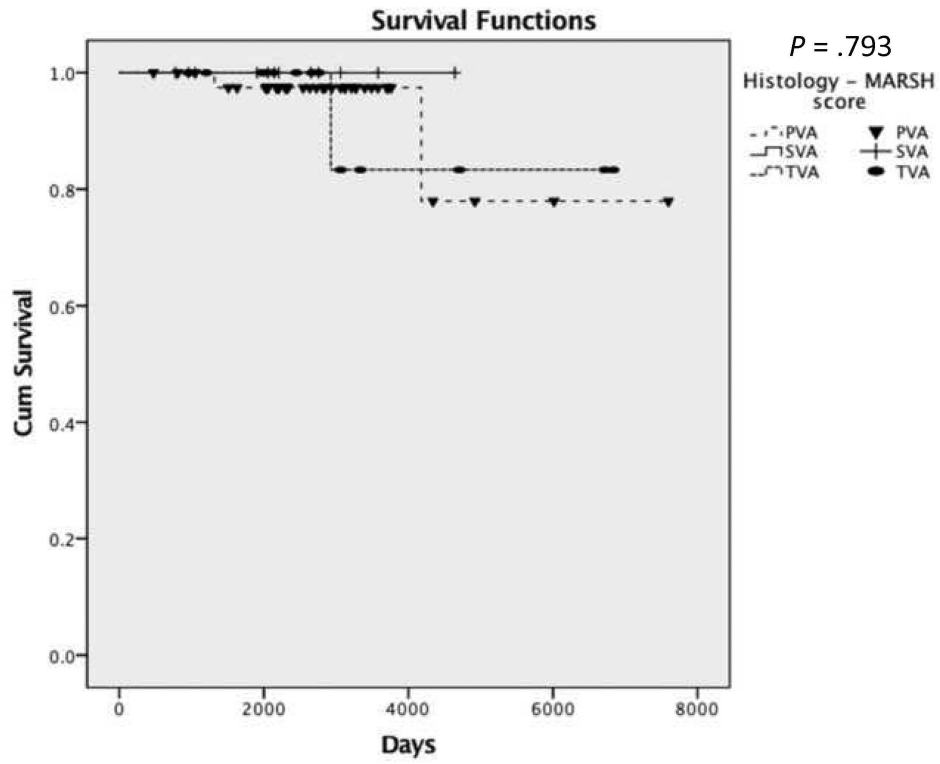
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Seronegative villous atrophy (SNVA)
Intraepithelial lymphocytes (IELs)
Celiac disease (CD)
Small bowel capsule endoscopy (SBCE)
Small bowel (SB)
Villous atrophy (VA)
Refractory celiac disease (RCD)
Duodenal bulb (D1)
Second part of the duodenum (D2)
Partial villous atrophy (PVA)
Subtotal villous atrophy (SVA)
Total villous atrophy (TVA)
Endomysial antibodies (EMA)
Iga tissue transglutaminase antibodies (TTG-igA)
Gluten free diet (GFD)
No identifiable cause for seronegative villous atrophy (SNVA-UO)