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A prospective study of the aetiology of lymphocytic duodenosis

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A prospective study of the aetiology of lymphocytic duodenosis

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We thank the reviewers for their comments, which we feel has improved this manuscript. We hope we have addressed the reviewers comments appropriately.

Reviewer: 1

1. Comments for Transmission to the Authors

The title is rather meaningless and the histological parameters should be defined from the outset – i.e. in the abstract – do you not mean lymphocytic duodenitis – normal architecture and intraepithelial lymphocytes >25/ 100 enterocytes?

If so then say so! – I would suggest you discuss this with the histopathologists who made the diagnosis and include them in this work and on the authorship

Comment 1a: We have now changed the title to the one suggested by the reviewer which we hope reflects this study more accurately. ‘A prospective study of the aetiology of lymphocytic duodenitis.’

Title page 1 top line ‘A prospective study of the aetiology of lymphocytic duodenitis.’

Comment 1b. Lymphocytic duodenitis was diagnosed exactly on the basis that the reviewer 1 suggested: lymphocytic duodenitis = normal architecture and intraepithelial lymphocytes >25/ 100 enterocytes. We clarified this with our 3 GI pathologists as suggested. We have also now stated that in our study group that lymphocytic duodenitis = normal architecture and intraepithelial lymphocytes >25/ 100 enterocytes.

Page 2 -1st paragraph, page 3 – 3rd paragraph : Lymphocytic duodenitis (LD) is defined by normal villous architecture and intraepithelial lymphocytes (IELs) >25 per 100 enterocytes

Comment 1c. With regards to the 3 GI pathologists who work with us – we have now acknowledged them in the manuscript.

Page 1: “We wish to acknowledge the help of Professor TJ Stephenson, Dr SS Cross and Dr A Dube (Department of GI Histopathology, Royal Hallamshire Hospital, UK) who were all involved in the reporting and review of these cases”.

2. Define the background -I’d hardly define a retrospective review of 124 patients as “case reports”, use the term previous retrospective studies... (see Vande Voort et al below).

Comment 2: We are sorry that we did not fully acknowledge Vande Voort et al study in the abstract and that the abstract could be misleading. For this reason we have now changed the abstract background from “case reports” to “previous retrospective studies” – page 2- 1st paragraph

3. Patients should be diagnosed with (not “labelled”)

Comment 3: We have now also changed the word labelled to diagnosed. Page 2, 1st paragraph

4. Simplify the aim, state the methods more clearly and the results and conclusions – along the lines of:

Lymphocytic duodenitis (LD) is defined by....

Patients should not be diagnosed with coeliac disease, solely by histology.

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5 Aim: A prospective study of the aetiology of lymphocytic duodenosis.
6

7 Method: One hundred patients with lymphocytic duodenosis were rigorously
8 investigated for coeliac disease and other known associations for LD by initial
9 investigations of coeliac serology, and exclusion of infection. Of 34 with no
10 explanation for LD, 29 underwent repeat duodenal biopsy following gluten challenge.
11

12 Results: Coeliac disease was present in 16% of patients with LD. In the absence of a
13 positive coeliac diagnosis, LD was most commonly associated with drugs (21%),
14 infection (19%), immune dysregulation (5%) inflammatory bowel disease (2%),
15 microscopic colitis (2%) and IgA deficiency (1%). Of 34 with no known associations,
16 18/34 had symptoms of irritable bowel syndrome and in 29 patients investigated with
17 repeat duodenal biopsy the IEL count returned to normal in 22.
18
19

20 Conclusions: In 66% of cases of LD, a known association can be found by further
21 investigation; importantly 16% will have coeliac disease. In those with no apparent
22 cause, there may be an association with IBS and the IEL count became normal on
23 repeat biopsy in 22%.
24
25

26
27 **Comment 4: We have now taken all the reviewer 1 comments on the structure and**
28 **content of this abstract and incorporated into our paper**
29

30
31 **“Abstract**

32 **Background: Lymphocytic duodenosis (LD) is defined by normal villous architecture**
33 **and intraepithelial lymphocytes (IELs) > 25 per 100 enterocytes. Such patients should**
34 **not be diagnosed with coeliac disease, solely by histology, as previous retrospective**
35 **studies have suggested other associations with LD.**
36

37
38 **Aim: A prospective study of the aetiology of LD.**
39

40 **Method: One hundred patients with LD were rigorously investigated for coeliac**
41 **disease and other known associations for LD by initial investigations of coeliac**
42 **serology, and exclusion of infection. Of 34 with no explanation for LD, 29 underwent**
43 **repeat duodenal biopsies following a gluten challenge.**
44

45
46 **Results: Coeliac disease was present in 16% of patients with LD. In the absence of a**
47 **positive coeliac diagnosis, LD was most commonly associated with drugs (21%),**
48 **infection (19%), immune dysregulation (4%) inflammatory bowel disease (2%),**
49 **microscopic colitis (2%), sarcoidosis (1%) and IgA deficiency (1%). Of 34 with no**
50 **known associations, 18/34 had symptoms of irritable bowel syndrome (IBS) and in**
51 **29/34 patients investigated with repeat duodenal biopsies the IEL count returned to**
52 **normal in 22.**
53

54
55 **Conclusions: In 66% of cases of LD, a known association can be found by further**
56 **investigations; importantly 16% will have coeliac disease. In those with no apparent**
57 **cause, there may be an association with IBS and the IEL count became normal on**
58 **repeat biopsy in 76%.”**
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See full abstract page 2

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5. Introduction

Define LD, role of IELs in a clear manner. – We have now defined this according to the previous reviewer 1 suggestions.

Comment 5 - “Lymphocytic duodenosis (LD) is defined by normal villous architecture and > 25 IELs per 100 enterocytes.^{4,6} Some investigators may call this Marsh grade 1 (if suspicious of coeliac disease). LD is found in approximately 2% - 3.8% of duodenal biopsies.⁶⁻⁸

Page 3, paragraph 3

6 The revised IEL counts are also better defined in these references, and Corazza’s classification of coeliac disease supersedes Marsh by incorporating the concept of 25 IELs - J. Clin. Pathol. 2005; 58; 573-574, recent work confirms this in a population study, Gastroenterology. 2010; 139:112-9

Comment 6- We have now incorporated both Corazza’s quoted 25/100 using the same reference as ours (ref 4) and added in the new gastroenterology reference (2%-3.8% of duodenal biopsies (ref 6- Gastroenterology. 2010; 139:112-9).

7. Retrospective studies: 124 patients Vande Voort JL, Murray JA, Lahr BD et al. Lymphocytic duodenosis and the spectrum of celiac disease. Am. J. Gastroenterol. 2009; 104:142-8 – this study looked at 124 patient.

Comment 7 - We have now addressed this in the text accordingly. “Vande Voort et al undertook a retrospective review of 124 patients with LD.¹⁹ The investigators described features which would help to discriminate patients with LD who had underlying coeliac disease from those who did not, for example the presence or absence of HLA DQ2 or DQ8. However they did not systematically investigate these patients for other causes of LD.” Page 3, paragraph 3

8a. The early recognition of celiac disease is clearly recommended in 2009 NICE guidelines <http://www.nice.org.uk/nicemedia/pdf/CG86FullGuideline.pdf>
This should be read and cited appropriately.

Comment 8a- We agree and have incorporated NICE into the revised manuscript – reference 27.

8b. HLA typing is expensive – and serology is the first line investigation. If this is negative and you still strongly suspect celiac disease HLA typing can help, but this is not a first line investigation. This should be stated.

The serology paragraph should precede this.

Comment 8b- We agree and have moved the serology paragraph so that it precedes the HLA paragraph. In addition we have stated that HLA is not a first line test: Please see pages 4 (2nd paragraph) running through to page 5

“Investigations to support the diagnosis of coeliac disease should involve testing for tissue transglutaminase antibody (tTG) and/or endomysial antibody (EMA) in the presence of a normal IgA level.²⁷ However, patients with LD with possible/potential

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coeliac disease may present with a negative EMA, as the prevalence of a positive EMA strongly correlates with the severity of mucosal damage.²⁸

For cases where the diagnosis is uncertain (such as in patients with LD) there may be a role for human leucocyte antigen (HLA) typing²⁷ - HLA DQ2 or DQ8 are closely linked with coeliac disease, occurring in up to 98% of cases,²⁹ but they are also present in 25% of the normal population.³⁰ An absence of these haplotypes can therefore be used as a negative predictive test. Approximately 50% of patients with LD have been shown to be negative for HLA DQ2 or DQ8, thus not belonging to the spectrum of coeliac disease.¹⁹ HLA typing is expensive and should be reserved for equivocal cases. If the serology is negative and you still strongly suspect coeliac disease HLA typing can help, but it is not a first line investigation.

A recent Finnish study has shown that patients with LD who have both a positive EMA and HLA to belong to the spectrum of coeliac disease. The investigators randomised these patients to either a gluten containing diet (n=10) or GFD (n=13), and found progressive clinical, biochemical and histological deterioration in all those randomised to a gluten containing diet. Furthermore these individuals improved all parameters when they were commenced on a GFD, after the initial study period.³¹

A gluten challenge can also be useful in causing further mucosal deterioration in those patients with potential coeliac disease in whom the initial small intestinal biopsies reveal only minor abnormalities.³² However, an initial trial response to a GFD is not as helpful, as up to 38% of patients with LD who have had a favourable response to a GFD are negative for the DQ2 or DQ8 haplotypes.¹⁹

9. Aim: was this not to develop a systematic approach to diagnose the underlying cause of LD and treat patients effectively?

Comment 9- We have now changed the aim to what has been suggested by reviewer 1
Previous: For these reasons the aim of our study was to develop a systematic and prospective approach towards investigating patients with raised IELs, thereby enabling a means to detect and be aware of the prevalence figures for the underlying causes.

New: For these reasons the aim of our study was to develop a systematic approach to diagnose the underlying cause of LD and treat patients effectively.

Page 5, last paragraph

10. Methods

Did you go back to the files at a time point to do this study or has it been ongoing from 2003? If you went back it is retrospective study surely?

Did you only have 100 cases of LD in seven years? or did you only recruit patients who agreed to participate in the study? And therefore what is the prevalence of this condition in your unit?

Comment 10- The senior author of this paper (David Sanders) was initially involved in a study where the investigators described that the prevalence of LD was 2% (ref 8- Hopper AD, Hadjivassiliou M et al, Senior Author Sanders DS. What is the role of serologic testing in coeliac disease? A prospective, biopsy-confirmed study with economic analysis. *Clin Gastroenterol Hepatol.* 2008 Mar;6(3):314-20.). Based on that initial clinical study – Prof Sanders then started to adopt a systematic approach to the investigation of these patients since 2003. Prof Sanders undertakes a gastroscopy list every week with 10 patients undergoing gastroscopy. Over a 7 year period performing 400 gastroscopies per year (40 weeks of practice x 10 gastroscopies) if 2% of these cases have LD (as previously reported then he has seen 2% of 2800 = 56

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3 cases of LD. More recently (in the last few years) other colleagues have also been
4 referring LD patients to him for further evaluation. We hope this accounts for the 100
5 patients prospectively recruited in this study. We did not have any patients who did
6 not want to take part in this study – however, there were some who had the initial
7 tests and then declined the 2nd gastroscopy as we have already mentioned in the
8 results section
9

10
11 11) As I understand the study was conducted in two stages:
12

13
14 Step 1 –The cause of LD available from history and investigations performed around
15 the time of the first endoscopy?
16

17 Step 2 – if no apparent cause, gluten challenge, investigation of GI infections
18 (including H. pylori) – for those who had not had Hp status established at time of
19 repeat endoscopy did you check that they had not taken eradication therapy in the
20 intervening period – and how long was this?
21

22 Comment 11 - We are sorry if this is confusing. After patients were recognised as
23 having LD (following their first endoscopy) we then questioned them for symptoms
24 and tried to establish if they had ever had H.Pylori testing (in primary care or
25 elsewhere). At the time of their second endoscopy (which was 6 weeks to 3 months
26 later) we would then take a CLO test for H.Pylori.
27

28 12) If the intervening period was some time from the original biopsy did you re-
29 evaluate symptoms at the time of rebiopsy? Yes we did. We have now incorporated
30 this additional information:
31

32 Comment 12 - ‘This involved a six week gluten challenge followed by repeat coeliac
33 serology, duodenal biopsies and small bowel aspirate for microscopy, culture and
34 sensitivity. If no previous H.pylori status was documented then patients were
35 questioned for symptoms at the time of second biopsy and a CLO test was also
36 performed.’
37

38 Page 6, line 13-16
39

40 13. Results

41 These are poorly presented and would be better served as follows
42

43 a) In 100 patients, an underlying cause for LD was found in 66%, principally drug
44 related (21%) and 16% had coeliac disease, of 5 cases of infection these were
45 And similar for immune dysregulation – is this not autoimmune disease and
46 separately sarcoidosis..... Of 22 /29 patients who were willing to undergo re-biopsy,
47 in the IEL was reduced to <25/ 100 enterocytes on the second biopsy, whilst 7 had a
48 persistently raised count with no apparent cause..... How many needed a two step
49 approach to find an underlying cause?
50

51 Comment 13 -We have now addressed all these issues:
52

53 “In order to find an underlying cause, 88/100 patients had to be investigated using the
54 two-step approach. In these 100 patients, an underlying cause for LD was found in
55 66%, principally drug related (21%), coeliac disease (16%) and H. Pylori (14%).
56 There were 5 cases of gastrointestinal infection: giardia, threadworms, campylobacter
57 and two cases of small bowel bacterial overgrowth. There were 4 cases of
58 autoimmune disease including systemic sclerosis, hypothyroidism, rheumatoid
59 arthritis and primary biliary cirrhosis. There was one case of sarcoidosis. Detailed
60 causes of LD are shown in figure 2.

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3 All cases of coeliac disease were positive for HLA DQ2 or DQ8, whereas 47/84 non-
4 coeliac cases carried the DQ2 or DQ8 haplotypes ($p = 0.0004$).

5
6 Despite an extensive work up, in 34 cases we were unable to identify a cause for the
7 raised IELs. Of these, 29 /34 patients were willing to undergo re-biopsy. In 22/29 the
8 IEL count was reduced to < 25 per 100 enterocytes on the second biopsy, whilst 7 had
9 a persistently raised count with no apparent cause. When questioning these 34
10 patients, 18 had gastrointestinal symptoms consistent with a diagnosis of irritable
11 bowel syndrome (IBS) using the ROME II Criteria.³⁴ Page 7 – results section
12
13

14 14) What was the range of IEL counts? – i.e did those who “normalised” have
15 26/27/28 and in all biopsies? to start with and were higher counts seen in coeliacs for
16 example – the count range should be stated and correlated with causes – were the
17 actual counts stated in reports or do you need to revisit this?

18
19 Comment 14: Unfortunately we have not performed quantitative analyses beyond
20 indentifying patients who have LD. Our GI pathologists comment on LD as
21 previously defined but do not give an absolute count. However, Kakar’s study (ref 9)
22 has previously shown no significant difference in IEL counts between coeliacs versus
23 non-coeliacs.
24
25

26 15) Therefore in 34%, no cause was apparent, although 18 in this group (18% overall)
27 had symptoms of IBS.

28
29 Comment 15– We agree with this and have now addressed this in the text: (results last
30 paragraph page 7)

31
32 “Despite an extensive work up, in 34 cases we were unable to identify a cause for the
33 raised IELs. Of these, 29/34 patients were willing to undergo re-biopsy. In 22/29 the
34 IEL count was reduced to <25 per 100 enterocytes on the second biopsy, whilst 7 had
35 a persistently raised count with no apparent cause. When questioning these 34
36 patients, 18 had gastrointestinal symptoms consistent with a diagnosis of irritable
37 bowel syndrome (IBS) using the ROME II Criteria.³⁴”
38

39 16) Discussion: Is the first sentence really true? It is not clear from the method how
40 the patients were recruited – please clarify this before making this statement.

41
42 Comment 16- Yes the first sentence is correct but we have clarified this in the
43 methods:

44
45 “The study was conducted between the periods February 2003 to February 2010 at the
46 Royal Hallamshire Hospital in Sheffield, a tertiary care centre for gastroenterology.
47 One hundred patients with LD (> 25 IELs per 100 enterocytes) were sequentially
48 identified and investigated to determine a cause for their raised IELs. All patients that
49 were identified agreed to participate. The study was registered with both South
50 Sheffield local ethics and audit committees.” – page 6, paragraph 1
51

52 17) In the discussion on no apparent cause the range of counts should be discussed,
53 IELs are dynamic and the idea of a post infective picture is good – did you explore
54 this clinically with these patients?

55
56 Comment 17 - Unfortunately we have not performed quantitative analyses beyond
57 indentifying patients who have LD. Our GI pathologists comment on LD as
58 previously defined but do not give an absolute count. However, Kakar’s study (ref 9)
59 has previously shown no significant difference in IEL counts between coeliacs versus
60 non-coeliacs. We think that this will be the next step in our work but unfortunately do
not have that data presently. For this reason we have not discussed this intimately.

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3 However we have mentioned some aspect of this in the discussion: (page 8 discussion
4 last 3 lines of the page)
5

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7 “The significance and association of IELs and IBS is contentious. Studies have shown
8 conflicting data, with reported IEL counts ranging from normal to slightly elevated.<sup>36-
9 44</sup>

10
11 18) The conclusion could include an algorithm for finding the underlying cause in LD
12 with an emphasis on doing coeliac serology and taking a comprehensive history. Is
13 HLA typing on all feasible?
14

15 Comment 18 : We have now included an algorithm as part of the method section and
16 addressed this in the conclusion. See algorithm page 10
17

18
19 “In conclusion, in 66% of cases of LD, a known association can be found by further
20 investigation; importantly 16% will have coeliac disease. HLA typing may have a role
21 in differentiating between coeliac and non-coeliac cases.²⁷ In those with no apparent
22 cause, there may be an association with IBS and the IEL count became normal on
23 repeat biopsy in 76%” – page 9, last paragraph
24

25
26 19) There are irritating grammatical errors throughout.

27 Comment 19- We apologise for this and hope that the revised manuscript has
28 corrected this problem
29

30
31 Reviewer: 2

32 Comments for Transmission to the Authors
33

34
35 Major points:

36 20) The authors address an important question, which has surprisingly not been
37 investigated prospectively until now. However, the study has several limitations and
38 mainly has to be rewritten to address some questions more precisely. Especially, the
39 point how long patients were followed, has to be addressed, if possible (further
40 outcome).
41

42
43 Comment 20 - Of the 100 patients investigated 74 were women. The age range was
44 from 16 to 83, with a median age of 47. The mean period of follow up was 18 months
45 (range 2-72). Added to the results section page 7, paragraph 1
46

47
48 21) How was CD defined? Further deterioration of Marsh status? Only together with
49 positive serology? Only, when Marsh status normalized after GFD? This has to be
50 clarified, actually at the beginning of the study. Has this been done? How was CED
51 defined (including data from capsule endoscopy)?
52

53
54 Comments 21: All patients at the end of step 1 where there was a suspicion of coeliac
55 disease or no clear cause, proceeded to step 2, involving a 6 week gluten challenge
56 with repeat coeliac serology and duodenal biopsies. “A diagnosis of coeliac disease
57 was made in those with positive serology (EMA or TTG), relevant symptoms and an
58 HLA pattern of DQ2 or DQ8. These patients also had to have either progression of
59 their LD to villous atrophy or a persistence of their LD. Finally in these individuals
60 we also ensured that they had a symptomatic response to the GFD.”

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3 This has been added to the method section page 6, 3rd paragraph
4

5
6 22) How was CED defined (including data from capsule endoscopy)?

7 Comment 22) We presume that CED is coeliac disease. We did not perform capsule
8 endoscopy on all our patients.
9

10
11 23) I miss the statistical methods. We are sorry that we had not incorporated this:

12 Comment 23 – “Statistical analyses of data were performed using SPSS. Differences
13 between the groups were assessed using Fisher’s Exact Test.” We have added this to
14 the methods section – page 6, final paragraph
15
16

17 Minor points:

18 Abstract:

19
20 24) I personally would omit the note that this is the first prospective trial in the
21 abstract, but actually would state that another method to categorize increased IELs
22 was investigation of concomitant medications, follow-up of patients including repeat
23 biopsy, investigation for IgA-deficiency, search for microscopic colitis and
24 concomitant gastrointestinal infections.
25

26 Comment 24 - The revised abstract now reads as- Aim: A prospective study of the
27 aetiology of lymphocytic duodenosis

28 This is in line with the 1st reviewer. We hope that this is acceptable to the second
29 reviewer and editorial board. We have made this decision because the study is
30 prospective but also because this was a major revision for the 1st reviewer but listed as
31 a minor point by the second reviewer.
32
33

34 25) I would suggest to use the pharmacological name of aspirin. At least in some
35 countries “aspirin“ is only used by certain companies.

36 Comment 25- We have now changed this accordingly using acetylsalicylic acid (ASA)
37
38

39 Introduction:

40 26) What is meant with antigenic challenges in contrast to antigenic stress? Please,
41 clarify. Please use past consequently (page 3, line 37; page 5, line 18)
42 and also in M and M.....
43

44 Comment 26 - We are sorry for this confusion and have now changed this in the text
45 - page 3, line 2
46

47 27) page 4, line 18: Where do we know that immune dysregulation is the reason for
48 increased IEL counts in thyroid .. disease? In the case of uncertainty omit this
49 speculation.
50

51 Comment 27- There are 2 previous studies (ref 9,13) which have used the term
52 immune dysregulation and described patients in a similar way. Nevertheless we agree
53 that this is speculative and have stated this in the text
54

55 “However, conditions such as thyroid disease, rheumatoid arthritis, psoriasis and
56 connective tissue disorders can cause raised duodenal IEL counts independently
57 through a process of immune dysregulation^{9,13}- this could be viewed as a speculative
58 opinion and further work is required to delineate this relationship.” – page 4, line 8
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“The concept of immune dysregulation causing LD has previously been noted and is poorly understood, possibly even speculative.^{9,13} Of the 4 cases in our study where LD was attributed to immune dysregulation, all went through step 2 to ensure that both coeliac disease and gastrointestinal infections were excluded.” – page 8, paragraph 3

28) Has CD been excluded completely in these cases? Yes we did exclude CD based on the definition of CD in our methods section

Comment 28- “A diagnosis of coeliac disease was made in those with positive serology (EMA or TTG), relevant symptoms and an HLA pattern of DQ2 or DQ8. These patients also had to have either progression of their LD to villous atrophy or a persistence of their LD. Finally in these individuals we also ensured that they had a symptomatic response to the GFD.” – page 6, 3rd paragraph

29) Dermatitis herpetiformis is not only frequently associated with CD, but almost in 100 % associated with CD.... (cite publication).

Comment 29- We have now changed this accordingly “Also dermatitis herpetiformis (DH), an itchy blistering rash, is almost always associated with coeliac disease,²² with up to 50% of patients demonstrating only subtle mucosal changes on duodenal biopsy.^{23,24}”

M and M

30) In case of H. pylori infection as causative agent or bacterial overgrowth: was IEL count tested after treatment of underlying cause and had the IEL count normalised? Was normalisation of IEL count after treatment of underlying cause prerequisite for diagnosing this as underlying cause? In what time interval was H. pylori eradication tested?

Comment 30: H.pylori has previously been shown to cause raised IELs with levels being shown to decrease (not necessarily normalise) post eradication - ref 17,18. We did not count pre and post eradication levels as this was not the aim of our study. Based on the previously available data we accepted H. Pylori as a cause for LD (ref 17,18). We undertook repeat assessments within 6-12/52 of patients following eradication therapy. In those patients who agreed to have a further biopsy – the LD normalised.

31) How was inflammatory bowel disease diagnosed? Please, state this (Crohn’s disease or ulcerative colitis). Was there a special endoscopic or histologic feature in the endoscopies/ biopsies of these two patients?

Comment 31 - UC was diagnosed on the basis of colonic biopsies which revealed mucosal inflammation and Crohn’s was base on the second duodenal biopsy revealing granulomatous changes.

Results page 7 lines 9-13.

32) How long was the follow-up of the patients without a specific cause (endoscopically and/ or clinically)?

Had the initially increased IELs already normalized after the six-weeks gluten-challenge?

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Comment 32: 22 of the patients had a normal IEL count after the gluten challenge. The patients without a cause found who have still not normalised their IEL counts (n=7) are under active follow up. The long term outcome is not yet known.

33. How was irritable bowel disease defined (special questionnaire)?

Comment 33 - We used the ROME II criteria and have added this- "When questioning these 34 patients, 18 had gastrointestinal symptoms consistent with a diagnosis of irritable bowel syndrome (IBS) using the ROME II Criteria.³⁴" Page 7 last line

34) Discussion: Overall the discussion is somewhat short and eg the hypothesis of immune dysregulation is not discussed at all as well as the CED patients .

Comment 34 – We have added a paragraph on immune dysregulation "The concept of immune dysregulation causing LD has previously been noted and is poorly understood, possibly even speculative.^{9,13} Of the 4 cases in our study where LD was attributed to immune dysregulation, all went through step 2 to ensure that both coeliac disease and gastrointestinal infections were excluded." – page 8, paragraph 3

35) Tables -In the line of „no causes found“, one should also state the number of patients in whom increased IELs were self-limiting.

Tables could be graphically improved.

Comment 35 :We have addressed the graphical images. Please see figures 1 & 2. In figure 2 we have added that 22/29 (76%) normalised their IEL counts on repeat biopsy

All changes in the manuscript have also been highlighted in red

A prospective study of the aetiology of lymphocytic duodenosis

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Contributors: IA helped design the study; recruit patients; collect, analyse and interpret data; and draft the article. KEE and ADH helped recruit patients; collect, analyse, interpret data; and review the final version of the manuscript. DMS helped collect data and review the final version of the manuscript. DSS conceived and designed the study; helped recruit patients; collect, analyse and interpret data; and review the final version of the manuscript.

Abstract

Background: Lymphocytic duodenosis (LD) is defined by normal villous architecture and intraepithelial lymphocytes (IELs) > 25 per 100 enterocytes. Such patients should not be diagnosed with coeliac disease, solely by histology, as previous retrospective studies have suggested other associations with LD.

Aim: A prospective study of the aetiology of LD.

Method: One hundred patients with LD were rigorously investigated for coeliac disease and other known associations for LD by initial investigations of coeliac serology, and exclusion of infection. Of 34 with no explanation for LD, 29 underwent repeat duodenal biopsies following a gluten challenge.

Results: Coeliac disease was present in 16% of patients with LD. In the absence of a positive coeliac diagnosis, LD was most commonly associated with drugs (21%), infection (19%), immune dysregulation (4%), inflammatory bowel disease (2%), microscopic colitis (2%), sarcoidosis (1%) and IgA deficiency (1%). Of 34 with no known associations, 18/34 had symptoms of irritable bowel syndrome (IBS) and in 29/34 patients investigated with repeat duodenal biopsies the IEL count returned to normal in 22.

Conclusions: In 66% of cases of LD, a known association can be found by further investigations; importantly 16% will have coeliac disease. In those with no apparent cause, there may be an association with IBS and the IEL count became normal on repeat biopsy in 76%.

Introduction

Duodenal intraepithelial lymphocytes (IELs) are involved in intestinal immune surveillance and activation, with levels increasing in response to **antigenic stress**.^{1,2}

The normal IEL count was initially established in 1971 to be < 40 IELs per 100 enterocytes. This figure was determined from jejunal biopsies using a Crosby or Watson capsule, with counts being performed on 7µm thick sections.³ However, more recently the normal IEL count has been revised to < 25 IELs per 100 enterocytes. This is through duodenal biopsies being obtained endoscopically with histology performed on sections cut at 3-4 µm.^{4,5}

Lymphocytic duodenosis (LD) is now defined by normal villous architecture and > 25 IELs per 100 enterocytes.^{4,6} Some investigators may call this Marsh grade 1 (if suspicious of coeliac disease). LD is found in approximately 2% - 3.8% of duodenal biopsies.⁶⁻⁸

A few retrospective studies have identified the causes of LD which include coeliac disease,^{9,10} gastrointestinal infections,^{11,12} immunological disorders,^{9,13} non-steroidal anti-inflammatory drugs (NSAIDs),^{9,14} inflammatory bowel disease (IBD),¹⁵ IgA deficiency¹⁶ and more recently H.pylori.^{17,18} **Vande Voort et al undertook a retrospective review of 124 patients with LD.¹⁹ The investigators described features which would help to discriminate patients with LD who had underlying coeliac disease from those who did not, for example the presence or absence of HLA DQ2 or DQ8. However, they did not systematically investigate these patients for other causes of LD.**

Over the last decade studies have focused mainly on evaluating patients with **LD** that belong to the spectrum of coeliac disease. Recognition of coeliac disease, even at this early stage is important, as these patients may have already developed symptoms and

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3 complications, such as anaemia and osteoporosis, which can improve on a gluten-free
4 diet (GFD).¹⁰ Equally, excluding coeliac disease will prevent inappropriate
5 prescription of a potentially expensive and socially inhibiting GFD, and instead
6 allows recognition and treatment of any other underlying cause.
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12 Methods of identifying those patients with LD that belong to the spectrum of coeliac
13 disease include revisiting the patient's history and investigations as well as
14 demonstrating histological deterioration on gluten. Enquiring specifically in the
15 history about autoimmune disorders, such as Graves disease, is important due to the
16 association with coeliac disease.^{20,21} However, conditions such as thyroid disease,
17 rheumatoid arthritis, psoriasis and connective tissue disorders can cause raised
18 duodenal IEL counts independently through a process of immune dysregulation^{9,13} -
19 this could be viewed as a speculative opinion and further work is required to delineate
20 this relationship. Also dermatitis herpetiformis (DH), an itchy blistering rash, is
21 almost always associated with coeliac disease,²² with up to 50% of patients
22 demonstrating only subtle mucosal changes on duodenal biopsy.^{23,24} Finally, in first
23 degree relatives of those with coeliac disease the risk of developing gluten-sensitive
24 enteropathy is 5-10%.^{25,26}
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44 Investigations to support the diagnosis of coeliac disease should involve testing for
45 tissue transglutaminase antibody (tTG) and/or endomysial antibody (EMA) in the
46 presence of a normal IgA level.²⁷ However, patients with LD with possible/potential
47 coeliac disease may present with a negative EMA, as the prevalence of a positive
48 EMA strongly correlates with the severity of mucosal damage.²⁸
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54 For cases where the diagnosis is uncertain (such as in patients with LD) there may be
55 a role for human leucocyte antigen (HLA) typing²⁷ - HLA DQ2 or DQ8 are closely
56 linked with coeliac disease, occurring in up to 98% of cases,²⁹ but they are also
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3 present in 25% of the normal population.³⁰ An absence of these haplotypes can
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5 therefore be used as a negative predictive test. Approximately 50% of patients with
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7 LD have been shown to be negative for HLA DQ2 or DQ8, thus not belonging to the
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9 spectrum of coeliac disease.¹⁹ HLA typing is expensive and should be reserved for
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11 equivocal cases. If the serology is negative and you still strongly suspect coeliac
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13 disease HLA typing can help, but it is not a first line investigation.
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17 A recent Finnish study has shown that patients with LD who have both a positive
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19 EMA and HLA to belong to the spectrum of coeliac disease. The investigators
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21 randomised these patients to either a gluten containing diet (n 10) or GFD (n 13), and
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23 found progressive clinical, biochemical and histological deterioration in all those
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25 randomised to a gluten containing diet. Furthermore these individuals improved all
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27 parameters when they were commenced on a GFD, after the initial study period.³¹
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31 A gluten challenge can also be useful in causing further mucosal deterioration in those
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33 patients with potential coeliac disease in whom the initial small intestinal biopsies
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35 reveal only minor abnormalities.³² However, an initial trial response to a GFD is not
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37 as helpful, as up to 38% of patients with LD who have had a favourable response to a
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39 GFD are negative for the DQ2 or DQ8 haplotypes.¹⁹
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43 Despite the numerous possible causes of LD it has been noted by other investigators
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45 that patients may be given a diagnosis of coeliac disease solely on the presence of
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47 duodenal intraepithelial lymphocytosis.³³ For these reasons the aim of our study was
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49 to develop a systematic approach to diagnose the underlying causes of LD and treat
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51 patients effectively.
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Methods

The study was conducted between the periods February 2003 to February 2010 at the Royal Hallamshire Hospital in Sheffield, a tertiary care centre for gastroenterology. One hundred patients with LD (> 25 IELs per 100 enterocytes) were sequentially identified and investigated to determine a cause for their raised IELs. All patients that were identified agreed to participate. The study was registered with both South Sheffield local ethics and audit committees.

Patients then underwent a 2-step process aiming to identify the underlying cause of their LD. Step 1 involved revisiting the patient's history and investigations for diagnostic clues (figure 1). In those where no cause was apparent/unclear or where coeliac disease was a possibility (including any patient with a positive HLA), patients then proceeded to step 2 (figure 1). This involved a six week gluten challenge followed by repeat coeliac serology, duodenal biopsies and small bowel aspirate for microscopy, culture and sensitivity. If no previous H.pylori status was documented then patients were questioned for symptoms at the time of their second biopsy and a CLO test was also performed.

A diagnosis of coeliac disease was made in those with positive serology (EMA or tTG), relevant symptoms and a HLA pattern of DQ2 or DQ8. These patients also had to have either progression of their LD to villous atrophy or a persistence of their LD. Finally, in these individuals we also ensured that they had a symptomatic response to the GFD.

Statistical analyses of data were performed using SPSS. Differences between the groups were assessed using Fisher's Exact Test.

Results

Of the 100 patients investigated 74 were women. The age range was from 16 to 83, with a median age of 47. The mean period of follow up was 18 months (range 2-72).

In order to find an underlying cause, 88/100 patients had to be investigated using the two-step approach. In these 100 patients, an underlying cause for LD was found in 66%, principally drug related (21%), coeliac disease (16%) and H. Pylori (14%).

There were 5 cases of gastrointestinal infection: giardia, threadworms, campylobacter and two cases of small bowel bacterial overgrowth. There were 4 cases of autoimmune disease including systemic sclerosis, hypothyroidism, rheumatoid arthritis and primary biliary cirrhosis. There was one case of sarcoidosis. There were 2 cases of inflammatory bowel disease. Ulcerative colitis was diagnosed on the basis of colonic biopsies which revealed mucosal inflammation and Crohn's was based on the second duodenal biopsy revealing granulomatous changes. Detailed causes of LD are shown in figure 2.

All cases of coeliac disease were positive for HLA DQ2 or DQ8, whereas 47/84 non-coeliac cases carried the DQ2 or DQ8 haplotypes, ($p = 0.0004$).

Despite an extensive work up, in 34 cases we were unable to identify a cause for the LD. Of these, 29/34 patients were willing to undergo re-biopsy. In 22/29 the IEL count was reduced to < 25 per 100 enterocytes on the second biopsy, whilst 7 had a persistently raised count with no apparent cause. When questioning these 34 patients, 18 had gastrointestinal symptoms consistent with a diagnosis of irritable bowel syndrome (IBS) using the ROME II Criteria.³⁴

Discussion

This is the first study to date investigating the causes of LD in a prospective and systematic manner.

In 66% of cases an identifiable cause was found with drugs (NSAIDs or acetylsalicylic acid [ASA]), coeliac disease and H.pylori being the three most common causes. We are aware that a possible limitation of this study is that despite there being evidence supporting ASA induced small bowel injury, there is no specific data regarding ASA and raised IELs. Nevertheless, a recent study administering low dose ASA over a short time period noted its use to be associated with increased occurrence of small intestinal mucosal damage.³⁵ This was determined with the use of video-capsule endoscopy, faecal calprotectin and intestinal permeability testing. These findings therefore suggest that ASA can induce small bowel inflammation and enteropathy and are supportive of our own observations.

The concept of immune dysregulation causing LD has previously been noted and is poorly understood, possibly even speculative.^{9,13} Of the 4 cases in our study where LD was attributed to immune dysregulation, all went through step 2 to ensure that both coeliac disease and gastrointestinal infections were excluded.

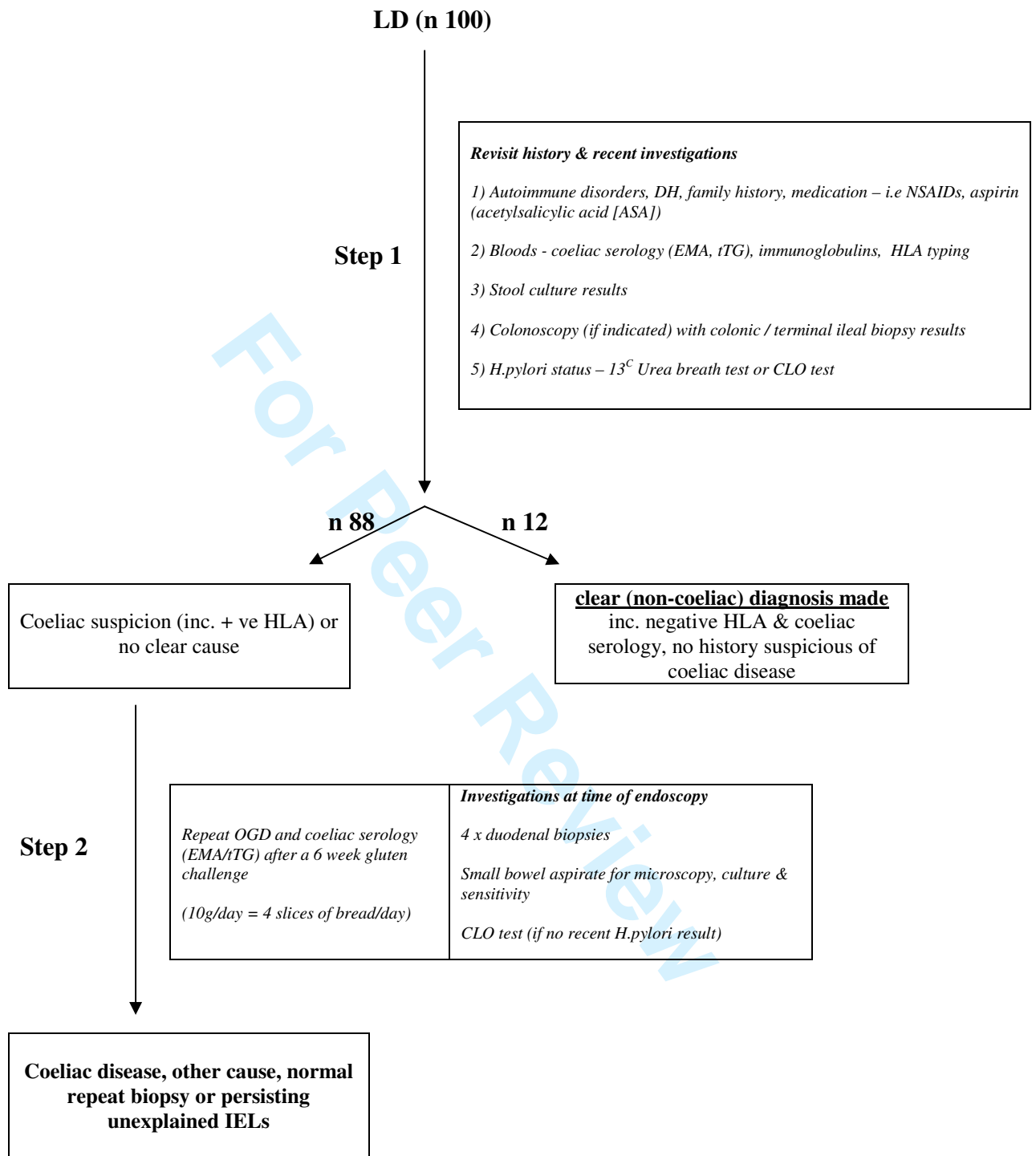
In 34% of cases a cause for LD was not found. However, the majority of these patients normalised their IEL counts on the 2nd set of duodenal biopsies. We speculate that this could be a post-infective picture (+/- IBS) and clinically would suggest reassurance in this group. In those with persistent LD the long term outcome is not yet clear and these patients remain under active follow-up. More than a half of the “no cause” found group had symptoms consistent with a diagnosis of IBS. The significance and association of IELs and IBS is contentious. Studies have shown conflicting data, with reported IEL counts ranging from normal to slightly elevated.³⁶⁻

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⁴⁴ Suggested hypotheses include intraepithelial lymphocytosis being a marker of a luminal factor that triggers a low grade inflammatory response or an immunological memory that persists after earlier antigenic provocations.⁴³ It has been postulated that these cells may play a role in releasing mediators that evoke enteric nervous system responses, excite sensory afferent pathways, and induce visceral hyperalgesia.⁴⁴

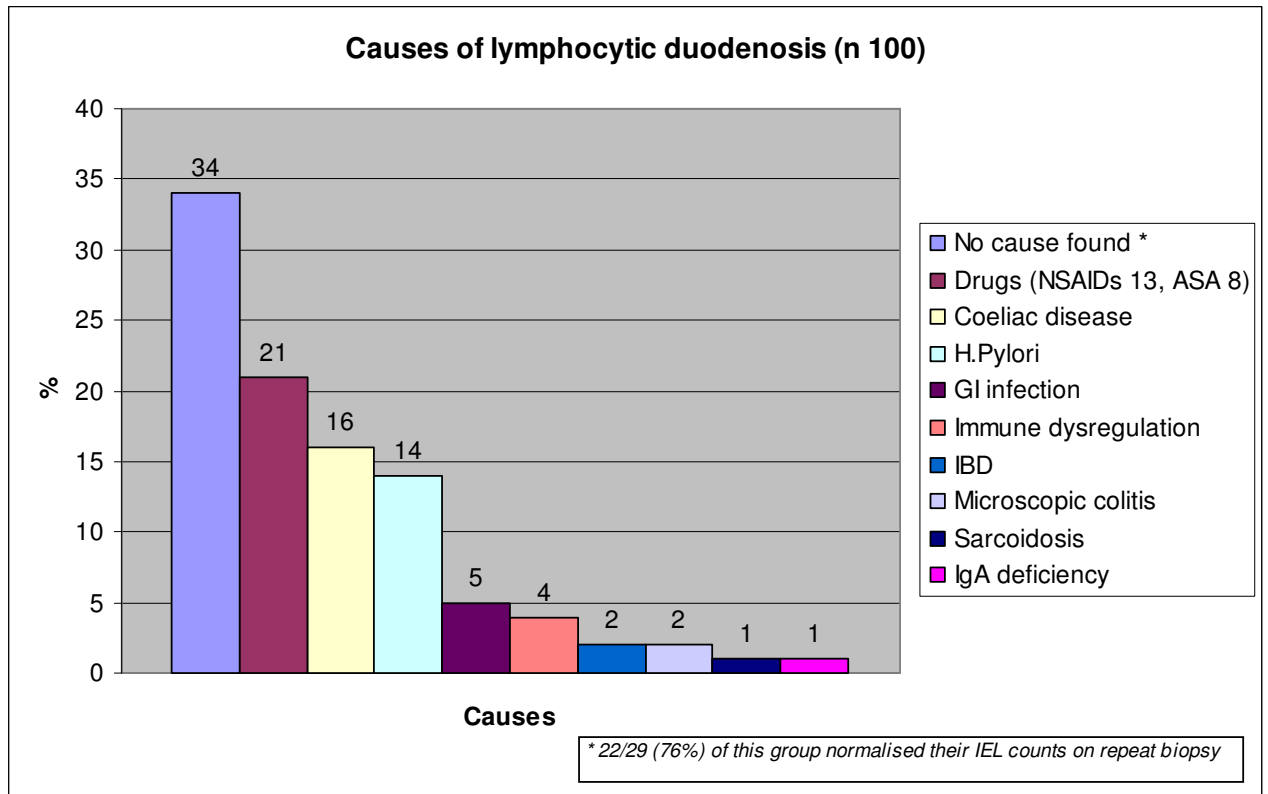
In conclusion, in 66% of cases of LD, a known association can be found by further investigations; importantly 16% will have coeliac disease. HLA typing may have a role in differentiating between coeliac and non-coeliac cases.²⁷ In those with no apparent cause, there may be an association with IBS and the IEL count became normal on repeat biopsy in 76%.

Figure 1: 2-step algorithm investigating patients with LD



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Figure 2: Causes of LD



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