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CLINICAL PHENOTYPE AND MORTALITY IN PATIENTS WITH IDIOPATHIC SMALL BOWEL VILLOUS ATROPHY: A DUAL CENTRE INTERNATIONAL STUDY

Running head: small bowel idiopathic villous atrophy

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ABSTRACT

Objective. Causes of small-bowel villous atrophy (VA) include coeliac disease (CD), its complications and other rare non-coeliac enteropathies. However, also forms of VA of unknown etiology may exist. We defined them idiopathic VA (IVA). To retrospectively classify the largest cohort of IVA patients and compare their natural history with CD.

Methods. Notes of 76 IVA patients attending two tertiary centres between Jan-2000 and Mar-2019 were retrospectively reviewed. CD, its complications and all the known causes of VA were excluded in all them. Persistence of VA during follow-up and lymphoproliferative features (gamma-TCR monoclonality and/or aberrant intraepithelial lymphocytes on duodenal biopsies and/or past history of extra-intestinal lymphoproliferative disorders) were used to retrospectively classify IVA, as follows. GROUP 1: IVA with spontaneous histological recovery (50 patients). GROUP 2: persistent IVA without lymphoproliferative features (14 patients). GROUP 3: persistent IVA with lymphoproliferative features (12 patients). Survival was compared between IVA groups and 1114 coeliac patients diagnosed between Jan-2000 and Dec-2017. HLA was compared between IVA patients and appropriate ethnicity-matched coeliac patients and healthy controls.

Results. 5-year survival was 96% in IVA group 1, 100% in IVA group 2, 27% in IVA group 3 and 97% in CD. On a multivariate analysis hypoalbuminemia (< 3.5 g/dL, p=0.002) and age at diagnosis (p=0.04) predicted mortality in IVA. Group 2 showed association with HLA DQB1*0301 and DQB1*06.

Conclusions. IVA consists of three groups of enteropathies with distinctive clinical phenotypes and prognosis. Mortality in IVA is higher than in CD and mainly due to lymphoproliferative conditions necessitating more aggressive therapies.

KEYWORDS: villous atrophy; mortality; HLA; non-coeliac enteropathies
INTRODUCTION

Coeliac disease (CD) is a chronic gluten-dependent enteropathy characterised by varying degrees of villous atrophy (VA) and a higher mortality than in the general population, mainly due to its complications [1-4]. Although small-bowel VA is due to CD and its complications in the vast majority of cases, these are not the only causes of VA and other non-coeliac enteropathies must be thoroughly investigated [5-11]. This is the case for autoimmune enteropathy (AE) [12,13], enteropathy associated with common variable immunodeficiency (CVID) [14,15], medication-related enteropathies [16-21], infections [22-26], small-bowel bacterial overgrowth [27], some lymphoproliferative disorders primarily affecting the small bowel [28], Crohn’s disease [29], tropical sprue [30] and collagenous sprue [31]. Some contemporary reports suggest that overall mortality in non-coeliac enteropathies with VA is higher than in CD [5,8,10]. However, these papers do not compare mortality in each subtype of non-coeliac enteropathy to CD [8,10] and it is likely that long-term outcomes are slightly different within the heterogeneous group of non-coeliac enteropathies [5-10, 12-16, 32]

Finally, in some instances, no definitive etiology for small bowel VA can be precisely identified [5-10, 33-36]. Even though patients with VA of unknown origin were described in small case series [5-10, 33-36], their clinical phenotypes, HLA typing, histology, natural history and therapeutic management have never been investigated systematically so far. For the purpose of the present paper, we would like to refer to these rare and still obscure enteropathies as forms of idiopathic villous atrophy (IVA).

By reporting the largest cohort of patients with small bowel IVA, the aims of this study are the following. Firstly, to retrospectively describe the clinical and histopathological phenotypes of patients with IVA (clinico-pathological study) and define their natural history and mortality in comparison to CD (follow-up and mortality study). Secondly, to study the HLA genetic profile of
patients with IVA and compare it with patients affected by CD and healthy controls (genetic study), as to understand whether, similarly to CD that develops in HLA DQ2/DQ8 individuals [1,2], a specific HLA genetic background could predispose to the development of these conditions.

**PATIENTS AND METHODS**

The study group included patients affected by small bowel IVA who attended two tertiary centres (Pavia, Italy and Sheffield, UK) between January 2000 and March 2019.

*Diagnostic criteria for IVA*

Patients with evidence of frank VA on correctly oriented biopsies (≥ Marsh 3a/Corazza-Villanacci grade B) taken from the second duodenal portion and in whom no specific cause for their VA was identified despite thorough investigations were enrolled. For patients referred to our centres, previous histology was carefully reviewed by expert histopathologists and only those with confirmed VA were included. In all these patients, CD and its complications as well as all the known causes of serology negative VA were excluded. More specifically, all the patients tested repeatedly negative to IgA endomysial antibodies (EmA), IgA tissue transglutaminase antibodies (TTG) and IgA gliadin antibodies. None had evidence of clinical and histological response to a gluten-free diet (GFD) and nobody had a family history of CD or a personal history of dermatitis herpetiformis. Enterocyte antibodies were negative and serum immunoglobulin levels normal, thus ruling out AE and CVID respectively. None of the patients were taking drugs known to be responsible for VA, namely ARBs, NSAIDS, methotrexate, mycophenolate [5,6,17-22]. Drug history was carefully reassessed particularly in those patients diagnosed before 2012, as to exclude a contributing role of olmesartan therapy [6]. *Giardia* specific stools antigens and other parasitic stool tests, tuberculosis quantiferon, HIV testing, small bowel aspirate/glucose H2-breath test,
Whipple’s PCR on duodenal biopsy/PAS staining on duodenal slides, *H. Pylori* were all negative. Traditional histology (ie. *Hematoxylin and eosin staining, Masson’s trichrome stain*) guided the exclusion of Crohn’s disease, collagenous sprue, eosinophilic enteritis, lymphoproliferative disorders and malignancies primarily involving the small bowel, including those known to be complications of CD. None of the patients had predisposing factors for small intestinal bacterial overgrowth [5]. All patients in this study were asked to continue on a gluten containing diet while these investigations were carried out [5,7].

**Criteria for the classification of IVA into clinical categories**

On the basis of our clinical experience and the previous literature describing some forms of IVA with spontaneous resolution [5,7,9,35,36] and others with persistence of VA [5-10,33,34], we set up criteria to retrospectively classify IVA patients into three groups (Figure 1). Patients in whom resolution of VA occurred spontaneously without any intervention (GFD, immunosuppressants) were classified into group 1. Conversely, patients displaying persistent VA unresponsive to at least 12 months of a GFD or immunosuppressants were furtherly divided according to a combination of findings raising the suspicion of lymphoproliferative features. These criteria included evidence of persistent gamma T-cell receptor (TCR) monoclonality on duodenal biopsies and/or presence of aberrant intraepithelial lymphocytes assessed by immunohistochemistry and/or flow cytometry, and/or past medical history of extraintestinal onco-haematological disorders. Patients in group 2 did not meet any of the above mentioned criteria. Conversely, patients in group 3 displayed persistent gamma TCR monoclonality and/or had an aberrant phenotype of intraepithelial lymphocytes.

**Data collection**
For the purpose of the clinico-pathological study baseline demographics, clinical and histological features of patients affected by IVA were retrospectively collected.

For the follow-up and mortality study data on clinical and histological response to therapies, onset of complications (type and date of complication), date of last follow-up in clinic/death, cause of death (when available) were recorded for IVA patients until March 2019. Histological response was defined as an improvement in the degree of VA on follow-up duodenal biopsy. Clinical response was defined as resolution of symptoms.

Survival was compared between IVA patients and 1114 coeliac patients (775 F, mean age at diagnosis 42±16 years) diagnosed in the two centres between January 2000 and December 2017 and followed-up until March 2019 (cumulative follow-up: 8758.75 person/years). All the coeliac patients in the control group were diagnosed on the basis of VA and positive IgA EmA/TTG [1-2]. During follow-up, 25 of these coeliac patients (13F, mean age at diagnosis of CD 56±9 years) developed a complication (7 type 1 refractory CD, 4 type 2 refractory CD, 4 enteropathy associated T-cell lymphoma, 5 abdominal B-cell lymphomas, 2 small bowel carcinoma and 3 oesophageal cancer). A further survival analysis was made between this group of 25 patients with complicated CD and IVA patients. Diagnosis of the complications of CD was made as previously described [1-4,37].

Finally, for the genetic study the HLA heterodimer frequencies of patients with IVA were compared to those of ethnicity-matched adult coeliac patients and healthy controls, in the same fashion we adopted in a previous study [38]. The control groups comprised 355 patients affected by uncomplicated CD (169 Italian- 122 females, mean age 31±14 years; 187 British – 130 females, mean age 44±17 years), 44 patients affected by complicated/refractory CD (27 Italian- 17 females, mean age 50±12 years; 17 British – 12 females, mean age 49±10) and 424 healthy controls (224 Italian - 104 females, mean age 46±9 years; 200 British - 92 females, mean age 50.3±5.57). The
HLA typing of healthy controls was obtained from the registry of Stem Cells Donors of Pavia, as previously described [38] and from British Bone Marrow Registry. We note that for the group of British patients affected by refractory CD only the HLA DQB1 profile was available. We calculated the frequencies of HLA molecules encoding the heterodimers HLA-DQ2.5 (DQA1*05 DQB1*02), HLA-DQ2.2 (DQA1*02 DQB1*02), HLA-DQ7.3 (DQA1*03 DQB1*03:01), HLA-DQ7.5 (DQA1*05 DQB1*03:01) and the DQB chain encoding for HLA-DQ5 (DQB1*05), HLA-DQ6 (DQB1*06), HLA-DQ8 (DQB1*03:02) and HLA-DQ9 (DQB1*03:03).

**Histology**

Severity of VA was graded according to Marsh-Oberhuber classification [39] on hematoxilin and eosin stained slides from second duodenal portion. Traditional immunohistochemistry (IHC) for CD3 and CD8 lymphocyte markers was performed on formalin-fixed, paraffin-embedded duodenal specimens.

Molecular analysis for gamma-TCR gene rearrangement was performed on DNA extracted from formalin-fixed paraffin embedded duodenal specimens by means of polymerase chain reaction, in accordance to standard Euroclonality/BIOMED-2 protocol.

Aberrant intraepithelial lymphocytes, the hallmark of type 2 refractory coeliac disease, were identified as >50% of CD3+CD8- intraepithelial lymphocytes on traditional IHC or >20% CD3-CD8-CD103+CD7+ cytoplasmatic CD3+ intraepithelial T-lymphocytes by means of flow cytometry (FC) [37].

**Coeliac serology**

IgA/IgG EmA and IgA/IgG enterocyte antibodies were detected by means of indirect immunofluorescence on monkey oesophagus/jejunum slides (INOVA Diagnostics, San Diego, USA
was used for the Italian patients. The Binding Site, Birmingham, UK was used for the British patients). IgA/IgG TTG and deamidated gliadin antibodies were tested by using ELISA kits (EliA Celikey IgA and Celikey IgG, EliA Gliadin DP IgA and EliA Gliadin DP IgG; Phadi AB, Uppsala, Sweden were used for the Italian patients. Aesku Diagnostics, Wendelsheims, Germany was used for the British patients). We specify that class IgG coeliac antibodies were tested only in case of total IgA deficiency.

**HLA typing**

Italian patients and controls were typed for HLA class II genomic polymorphisms at the high-resolution level by means of sequences specific primers- polymerase chain reaction (PCR-SSP) and/or polymerase chain reaction utilising sequence-specific primary oligonucleotides (PCR-SSO) [40,41]. DNA was extracted from peripheral blood samples using the Wizard genomic DNA Purification kit (Maxwell 16®, Promega Instrument; Madison, WI, USA) according to the manufacturer’s protocol. The polymorphism of the HLA-DQA1 and DQB1 genes was analyzed using commercial kits (Olerup SSP AB®, Stockholm, Sweden), and One Lambda Inc.; Canoga Park, CA, USA). The amplified products were visualized on 2% agarose gel, stained with 0.5 mg/mL of ethidium bromide, using the E-Gel precast Agarose Electrophoresis System (Invitrogen Life Technologies®, PA4 9RF Paisley, UK).

For British patients the HLA typing was obtained by means of a sequence-specific oligonucleotide polymerase chain reaction technique (LIFECODES HLA SSO typing – RAPID, IMMUCOR ® Georgia, USA). Genomic DNA was extracted from samples of peripheral blood using a DNA purification kit in combination with an automated DNA extraction platform (MagNA Pure Compact DNA Nucleic Acid Isolation kit and a MagNA Pure Compact instrument Roche Basel, Switzerland). For BBMR the automated DNA extraction platform used was Qiasymphony (QIAGEN®, Venlo, Netherlands).
Results were analysed using a Luminex ® 100 analyser (Luminex® Corporation Texas, USA) and MATCH IT! analysis software (IMMUCOR ® Georgia, USA).

**Statistical analysis**

Categorical variables were described as count and percentages; quantitative variables as mean and standard deviation if normally distributed (Shapiro-Wilks test), otherwise as median and percentiles. Clinical, laboratory and histopathological features were compared between IVA groups using one-way analysis of variance for normally distributed quantitative variables followed by Scheffè corrected 2x2 post-hoc comparisons. Categorical data were compared between the IVA and control groups by means of $\chi^2$ test and Fisher’s exact test followed by Bonferoni corrected 2x2 post-hoc comparisons.

HLA heterodimers frequencies were compared between the IVA and control groups by means of $\chi^2$ test and Fisher’s exact test. For the genetic study, a correspondence analysis, which represents an explorative multivariate statistical technique, was used to provide a graphical interpretation in a bidimensional graph of the relationship between the clinical groups (three IVA groups vs. coeliac patients and healthy controls) and the genetic variants [38].

Overall survival was described through a Kaplan-Meier curve. Univariate and multivariate (including factors significantly associated with survival at univariate analysis) Cox models were fitted to study long-term mortality. Results are expressed as hazard ratio (HR) and reported with 95% CI.

P-value <.05 was considered statistically significant. All tests were two-sided. The data analysis was performed with the STATA statistical package (release 15.1, 2017, Stata Corporation, College Station, Texas, USA).
**Ethics**

All the patients gave an informed consent at time of duodenal biopsy, both for clinical and research purposes. After verifying the good quality of the data, they were all irreversibly anonymised. The study was approved by the Ethics committee of the Fondazione IRCCS Policlinico San Matteo, Pavia, Italy according to the 1975 declaration of Helsinki (6th revision, 2008). Similarly, the study protocol was approved by the Yorkshire and Humber Research Ethics committee and registered with the local research and development department of Sheffield Teaching Hospital NHS Foundation Trust (REC reference 14/YH/1216).

**RESULTS**

Seventy-six patients affected by IVA were collected over a period of 17 years. Their baseline demographics, clinical characteristics, laboratory results and radiological findings are shown in Table 1. The most common presenting symptoms were weight loss, diarrhoea and dyspepsia. Weight loss was more frequent among patients with persistent VA, being significantly associated to group 3 (p=.01). Prevalence of dyspepsia was higher in group 1 (p=.003). Anaemia and hypoalbuminemia (< 3.5 g/dL) were more common in group 3 (p<.001). Family history of autoimmune disorders was more common in group 2 (p=.01).

**Follow-up and mortality**

Complete spontaneous histological recovery occurred in 47/50 patients in group 1 (Table 2) after a median of 10 months [IQR 5-14 months], whereas the remaining three patients declined follow-up biopsy given their persistent well-being. Four patients spontaneously started on a GFD for a few months after diagnosis of IVA, but then resumed and all were on a normal diet at time of histological follow-up. In group 2 (table 3), histological and clinical response to
immunosuppressant/biologics occurred in 5/14 patients. More precisely, two responded to azathioprine, one to open-capsule budesonide treatment and two to biologics (patients 1, 6, 12-14). In other two patients (n° 2 on azathioprine and n° 8 on budesonide), despite clinical response had occurred, histological recovery was slow. Conversely, in group 3 (table 4), 10 out of 12 patients had persistence of VA on follow-up biopsy and one refused follow-up gastroscopy. Complications were found in one patient in group 2 (patients 14, Table 3) and nine patients in group 3 (p<0.001). Median time from diagnosis of VA to development of complications in group 3 IVA was 13 months, IQR 3.5-26.5.

Eleven out of 76 patients (14.5%) died (4 in group 1 IVA for causes unrelated to the enteropathy, after a median time since diagnosis of 4.5 years; 7 patients in group 3 IVA, mainly because of a lymphoproliferative malignancy after a median of 15 months, IQR 9.5-40.5). Patients in group 2 were all alive after a median follow-up of 64 months, IQR 52-114. In the coeliac cohort 66/1114 patients (5.92%) died. Causes of death in this group included complicated CD in 13/66 (19.6%), cardiovascular causes in 9/66 (13.6%), other cancers in 8/66 (12.1%, including extra-intestinal epithelial malignancies), pneumonia in 8/66 (12.1%). In 13 patients the cause of death was unknown and in the remaining 15 the causes were heterogeneous. We specify that the 8 coeliac patients deceased because of cancers were not affected by a complicated or refractory form of CD.

Figure 2 shows Kaplan-Meier estimated survival in IVA and coeliac patients. Mortality in IVA was higher than in CD (Fig 2A, p<.001), but with great differences within the three IVA groups (Fig 2B). Overall 5-year survival was 96% in IVA group 1, 100% in IVA group 2, 27% in IVA group 3 and 97% in the coeliac cohort. There was no difference in long-term survival between IVA group 1 and CD (p=.22). Patients in group 3 IVA showed the poorest prognosis, even when compared to the 25 coeliac patients that developed a complicated form of CD (Fig 2C, p<.001).
On univariate analysis age at diagnosis (HR 1.05, 95%CI 10.1-1.09, p=.009), anaemia (HR 7.8, 95%CI 2.18-27.80, p=.002), hypoalbuminemia (HR 16.2, 95%CI 4.68-56.58, p<.001) and HLA DQ2.2 (HR 5.7, 95% CI 1.74-18.9, p=.004) were statistically significant predictors of mortality in IVA patients, whereas dyspepsia was a protective factor (HR 0.12, 95%CI 0.015-0.96, p=.046). Gender (HR 0.79, 95% CI 0.24-2.62, p=.707), diarrhoea (HR 1.11, 95% CI 0.32-3.77, p=.877), weight loss (HR 1.78, 95% CI 0.47-6.74, p=.393) and HLA DQ2.5 (HR 1.49, 95% CI 0.39-5.69, p=.560) were not significantly associated to increased mortality. On multivariate analysis only age at diagnosis (HR 1.04, 95%CI 1.00-1.07, p=.03) and hypoalbuminaemia (HR 10.7, 95%CI 2.32-50.00, p=.002) significantly predicted mortality.

**Histopathological and molecular features**

As shown in Table 1 partial VA was the histological hallmark of group 1 (p<.001), while no significant differences were found for intraepithelial lymphocytosis and crypt hyperplasia. Histopathological findings for group 1 and group 2 are specified in Table 2 and Table 3. Briefly, in group 1 histology was undistinguishable from untreated CD in the vast majority of patients and only in 6/50 histological features were in keeping with peptic duodenitis [24] (Table 2). In group 2 a coeliac-like pattern, characterized by crypt hyperplasia and intraepithelial lymphocytosis with normal CD3+ CD8+ phenotype, was evident in 11 patients (nº 1-11, table 3). Only in one patient (nº 4 in Table 3) histology revealed a subepithelial band of collagen, though criteria for collagenous sprue were not met [31]. A peptic duodenitis-like pattern characterised by expansion of the lamina propria by a mixed but predominantly mononuclear inflammatory infiltrate and neutrophilic cryptitis was found in 3 patients (nº 12-14). Interestingly, two of them had a diagnosis of ulcerative colitis. All patients in group 2 showed regular phenotype of intraepithelia lymphocytes assessed by FC or IHC and/or polyclonal gamma-TCR both at time of diagnosis and during follow-up.
Table 4 summarises the heterogeneous histological features of group 3. An aberrant histology and/or persistent monoclonality for gamma-TCR were found in patients 1-6. Patient 7 refused follow-up, so we were not able to assess persistence or resolution of gamma-TCR monoclonality. Histology undistinguishable from conventional CD was found in patients 6 and 8-12. In patients 9 and 10 a band of subepithelial collagen was found. They both had a history of chemotherapy for extraintestinal lymphoproliferative disorders. However, histological findings were neither sufficient to confirm a diagnosis of collagenous sprue [31], nor a possible causative effect of chemotherapy.

**Genetic study**

Comparison of the HLA heterodimers frequencies between IVA groups, healthy controls and coeliac patients is shown in Table 5. Of note, in group 2 HLA-DQ7.3 was carried by 33% of patients and HLA-DQ6 by nearly 60% (p<.001). In group 3, HLA-DQ5 molecules were carried by 41.65% of patients and HLA-DQ2 by half of them. Group 1 IVA was genetically heterogeneous, with HLA-DQ2.5 (35%) and HLA-DQ6 (45%), being the most frequent molecules. As expected, HLA-DQ2 molecules were more commonly expressed by coeliac patients. HLA-DQ8 was poorly expressed by group 3 IVA and patients with complicated CD.

A graphical visualization of the genetic HLA diversity between the three IVA groups, coeliac patients and healthy controls is shown by the correspondence analysis on heterodimers in Figure 3. The analysis confirms that group 2 IVA is HLA genetically different from coeliac patients, being associated with HLA-DQ7.3 and HLA-DQ6 molecules. On the contrary, group 1 and 3 IVA, despite being distinct from coeliac patients, are characterized by a greater HLA genetic heterogeneity.

**DISCUSSION**
In this study we have described the largest cohort of patients with small bowel IVA and we have provided a clinical-based classification of these enteropathies into three groups with distinctive clinical features, HLA and natural history. We have given the most extensive description about long-term outcomes in IVA, showing for the first time that mortality in IVA is overall higher than in CD, though remarkably different between IVA subgroups. More specifically, IVA patients with lymphoproliferative features (group 3) are burdened by the poorest prognosis and are mainly responsible for the higher mortality found in IVA than in CD. Age and hypoalbuminaemia at time of diagnosis of VA are important predictors of mortality in IVA and may be helpful to stratify patients warranting a more aggressive treatment and close follow-up. Our results on IVA add to some previous studies suggesting that overall mortality in non-coeliac enteropathies with VA is higher than in CD [5,8,10,32].

The clinical features of patients with self-limited IVA (group 1) were already described in the past [5,7,9,35,36]. The novel finding of our study is that mortality in these patients is not higher than in CD and it is mainly due to causes unrelated to the enteropathy. This is in line with a less severe and transient enteropathy, maybe triggered by viral infectious agents, as previously hypothesised [5,7,9,35,36].

Another strength of our study is the identification of diagnostic criteria for a new form of chronic non-coeliac enteropathy characterised by persistent VA, absence of any lymphoproliferative stigmata, specific HLA typing and long-term survival (ie. IVA group 2). Although similar patients were described in the past [5-10,33,34,42], we have now provided a more detailed description of their clinical and molecular phenotype. The first mandatory diagnostic criteria are the presence of a regular phenotype of intraepithelial lymphocytes assessed by IHC and/or FC and the absence of monoclonality for gamma-TCR both at time of diagnosis and during follow-up, thus excluding type 2 refractory CD [37]. Secondly, the HLA genetic background is dominated by HLA DQ7.3 and DQ6
molecules that are not listed among those conferring risk for developing CD [43]. Although it might be said that patients with similar features are affected by seronegative CD or refractory CD, we believe that HLA genetic background, the histological and molecular phenotypes, the absence of any sensitivity to gluten and the long-term survival are key to define a distinct clinical entity. Despite the quite high response rate to immunosuppressants and the high prevalence of family history for autoimmune disorders may support the hypothesis of an immune-driven chronic enteropathy, we think this is not sufficient to confirm a diagnosis of AE, given the negative results for enterocyte antibodies. While forms of seronegative AE do exist and are well defined in children, their existence in adults needs to be furtherly elucidated [5,12,13,42].

Interestingly, two main histological patterns were identifiable in group 2: a coeliac-like pattern in 78% of the patients and a peptic duodenitis-like pattern in the remaining 22%. All the patients with peptic duodenitis-like histology responded histologically to immunusuppressant and/or biologics. Two were concomitantly diagnosed with ulcerative colitis. Similar small bowel histology was previously described in patients with ulcerative colitis [44]. On the contrary, fewer patients with a coeliac-like pattern had histological response to traditional immunosuppressants.

Group 3 is burdened by a very high mortality that is mainly responsible for the poorer outcome we found in IVA than in CD. Identification of these patients was made on the basis of persistent monoclonality for gamma-TCR and a previous history of extra-intestinal lymphoproliferative disorders. However, this group is the most heterogeneous in terms of histological features, with some points that are worth discussing.

First, the specificity of gamma-TCR monoclonality analysis has recently been questioned, with transient monoclonal gamma-TCR described also in coeliac patients with poor compliance to a GFD, [45, 46] and even in patients with duodenal lymphocytosis associated to H. Pylori [47]. However, in our patients in group 3 monoclonality was persistent on follow-up biopsies in five out
of six patients (one refused follow-up). Most importantly, three of them developed lymphoproliferative complications and 50% of them died (patients 1-6, table 4). This would enable us to identify within group 3 a subgroup of patient with IVA, persistent monoclonality for gamma-TCR and high risk of lymphoproliferation.

The second criterion we adopted is previous history of extra-intestinal lymphoproliferative disorders. Four patients had a history of lymphoproliferative disorders pre-dating the diagnosis of VA (patients 7-10, table 4). Two of them received chemotherapy some years before and one was on treatment with idelalisib at time of diagnosis of IVA. Hence, a possible role of these medications in promoting a certain damage to the small bowel, although very remote, cannot be entirely excluded. Interestingly, forms of enterocolitis possibly related to idelalisib have been described [48].

Last but not least, two patients in this group (n° 11 and 12 in Table 4) were very difficult to be classified. From one side they did not show any clear molecular lymphoproliferative features at diagnosis, so they would have matched with group 2. However, the clinical suspicion had always been that of a lymphoma in both them. During follow-up one of them deteriorated and died of angioimmunoblastic T-cell lymphoma and the other died of oesophageal cancer within less than four years from diagnosis.

Finally, although CD and its complications were thoroughly excluded at time of diagnosis of VA, we noticed that some patients in group 3 died because of conditions that could be considered complications of CD. HLA DQ2 molecules were found in half of the patients in group 3, so we wonder whether they were seronegative coeliac patients that escaped the diagnosis before and then complicated afterwards.

**Proposals for clinical management of idiopathic villous atrophy and future perspectives**
Partial villous atrophy, absence of suspicious features for lymphoproliferation, and normal laboratory tests at diagnosis identify patients with a self-limited form of VA (group 1). These patients should not be prescribed a GFD and the most appropriate management may be a “watch and wait” strategy for at least six months, before assessing for histological recovery. This would avoid misdiagnoses of seronegative CD, particularly in those carrying HLA DQ2 or DQ8 molecules.

On the contrary, age at diagnosis and hypoalbuminaemia are important predictors to identify since the time of diagnosis patients with persistent IVA that should warrant aggressive treatments and close follow-up. In patients with persistent VA of unknown etiology we suggest to always characterise the phenotype of intraepithelial lymphocytes by means of IHC and/or FC and to test for gamma-TCR clonality, since in patients with negative results for these tests prognosis is excellent (ie. Group 2 IVA).

In conclusion, we think this study can represent a first step in the description of rare enteropathies with VA of unknown origin. Proposals for future research may include the investigation of the molecular pathways involved in IVA, a further discrimination of enteropathies displaying lymphoproliferative features and the evaluation of the therapeutic potential of regimens alternative to traditional immunosuppressants. Depending on the nature of the enteropathy, these could include autologous and allogeneic haemopoietic cellular therapies [49-51], both as rescue therapy for patients refractory to conventional immunosuppressants or as first line therapy for those with predominantly genetic or other features suggesting poor outcomes.
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LEGENDS FOR TABLES

Table 1. Baseline characteristics of patients with idiopathic villous atrophy
IVA: idiopathic villous atrophy; PVA: partial villous atrophy; SVA: subtotal villous atrophy; TVA: total villous atrophy; IELs: intraepithelial lymphocytes; CH: cript hyperplasia; SD: standard deviation; VCE: video capsule endoscopy; CT: computed tomography

Table 2. Histological features and outcomes in patients belonging to group 1
CPD: chronic peptic duodenitis; f-up: follow-up

Table 3. Histological features and outcomes in patients belonging to group 2
LPD: lymphoproliferative disorders; CT: computed tomography; NA: not assessed; SB: small-bowel; UC: ulcerative colitis; GFD: gluten-free diet; UJI: ulcerative jejunitis; 6-MPU: 6 mercaptopurine; AZA: azathioprine; mo: months since diagnosis

Table 4. Histological features and outcomes in patients belonging to group 3
LPD: lymphoproliferative disorders; CT: computed tomography; NA: not assessed; SB: small-bowel; TCL: T-cell lymphoma; BCL: B-cell lymphoma; NHL: non Hodgkin lymphoma; GFD: gluten-free diet; TI: terminal ileum; UJI: ulcerative jejunitis; diagn: diagnosis; f-up: follow-up; LN: lymph nodes; AZA: azathioprine; MSC: mesenchymal stem cells; resp.: response

Table 5. HLA heterodimers distribution in patients with idiopathic villous atrophy, coeliac disease and healthy controls.
HC: healthy controls; IVA: idiopathic villous atrophy; CCD: complicated coeliac disease; UCD: uncomplicated coeliac disease
LEGENDS FOR FIGURES

Figure 1. Criteria to classify idiopathic villous atrophy into clinical categories.
VA: villous atrophy, IVA: idiopathic villous atrophy

Figure 2. Kaplan Meier estimated survival curves for idiopathic villous atrophy groups compared to coeliac disease.
IVA: idiopathic villous atrophy; CD: coeliac disease

Figure 3. Genetic heterogeneity of idiopathic villous atrophy patients compared to coeliac patients and healthy controls. The correspondence analysis shows that the nearer the dots, the stronger the association. Black squares indicate the group of patients. Blue dots indicate HLA heterodimers (HLA-DQ2.5 encoded by DQA1*05 DQB1*02, HLA-DQ2.2 encoded by DQA1*02 DQB1*02, HLA-DQ7.3 encoded by DQA1*03 DQB1*03:01, HLA-DQ7.5 encoded by DQA1*05 DQB1*03:01) and green dots indicate DQB chains. CD: coeliac disease; IVA: idiopathic villous atrophy; HC: healthy controls
Figure 1.

Is there resolution of VA without any intervention?

- **YES**
  - Idiopathic VA GROUP 1
    - Transient IVA
    - Likely post-infective

- **NO**
  - Are there any lymphoproliferative features?
    - **NO**
      - Idiopathic VA GROUP 2
        - Non-lymphoproliferative IVA
        - Likely immune-driven
    - **YES**
      - GROUP 3
        - Cluster of onco-haematological disorders with IVA
Figure 3
|                      | TOTAL IVA N=76 | Group 1 IVA N=50 | Group 2 IVA N=14 | Group 3 IVA N=12 | Overall p-value | Group 1 vs Group 2 p-value | Group 1 vs Group 3 p-value | Group 2 vs Group 3 p-value |
|----------------------|----------------|-----------------|-----------------|-----------------|----------------|---------------------------|---------------------------|---------------------------|
| **DEMOGRAPHICS**     |                |                 |                 |                 |                |                           |                           |                           |
| Mean age±SD          | 48.5±17.7      | 49.2±18.7       | 43.3±13.8       | 51.9±13.8       | 0.431          | -                         | -                         | -                         |
| Females, n°          | 38             | 26              | 7               | 5               | 0.813          | -                         | -                         | -                         |
| Caucasian ethnicity  | 70             | 46              | 13              | 11              | 0.933          | -                         | -                         | -                         |
| **CLINICAL FEATURES, LABORATORY AND RADIOLOGICAL FINDINGS AT DIAGNOSIS** | | | | | | | | |
| Diarrhoea            | 46             | 27              | 9               | 10              | 0.166          | -                         | -                         | -                         |
| Weight loss          | 47             | 25              | 11              | 11              | 0.01           | 0.05                      | 0.009                     | 0.356                     |
| Abdominal pain       | 22             | 13              | 4               | 5               | 0.561          | -                         | -                         | -                         |
| Reflux               | 14             | 11              | 3               | 0               | 0.200          | -                         | -                         | -                         |
| Dyspepsia            | 29             | 26              | 2               | 1               | 0.003          | 0.01                      | 0.006                     | 0.636                     |
| Vomiting             | 20             | 15              | 2               | 3               | 0.495          | -                         | -                         | -                         |
| Anaemia              | 21             | 6               | 5               | 10              | <0.001         | 0.03                      | <0.001                    | 0.01                      |
| Hypoalbuminemia (<3.5g/dl) | 12             | 0               | 4               | 8               | <0.001         | <0.001                    | <0.001                    | 0.05                      |
| Low folate           | 10             | 1               | 4               | 5               | <0.001         | 0.001                     | <0.001                    | 0.484                     |
| Low B12              | 19             | 8               | 5               | 6               | 0.03           | 0.105                     | 0.01                      | 0.462                     |
| Low ferritin         | 17             | 6               | 9               | 2               | <0.001         | <0.001                    | 0.665                     | 0.014                     |
| Pathological VCE findings | 25             | 5               | 13              | 7               | <0.001         | <0.001                    | <0.001                    | 0.45                      |
| Pathological CT findings | 7              | 0               | 1               | 6               | 0.001          | 0.237                     | 0.001                     | 0.016                     |
| **PAST MEDICAL HISTORY** |                |                 |                 |                 |                |                           |                           |                           |
| Autoimmunity         | 14             | 8               | 2               | 4               | 0.345          | -                         | -                         | -                         |
| Family history of autoimmune diseases | 2             | 0               | 2               | 0               | 0.01           | 0.007                     | -                         | -                         |
| Travel endemics      | 8              | 6               | 2               | 0               | 0.420          | -                         | -                         | -                         |
| Gastroenteritis      | 12             | 11              | 0               | 1               | 0.101          | -                         | -                         | -                         |
| **DUODENAL HISTOLOGY AT DIAGNOSIS** |                |                 |                 |                 |                |                           |                           |                           |
| Histology_Marsh 3a   | 53             | 49              | 0               | 4               | <0.001         | -                         | -                         | -                         |
| Histology_Marsh 3b +3c | 23           | 1               | 14              | 8               | <0.001         | <0.001                    | <0.001                    | 0.019                     |
| Histology_IELs       | 69             | 44              | 13              | 12              | 0.416          | -                         | -                         | -                         |
| Histology_CH         | 60             | 36              | 12              | 12              | 0.08           | -                         | -                         | -                         |
| Pt N° | Age/sex | histology diagnosis | Other histological features | follow-up histology (months from diagnosis) | Outcome (months from diagnosis) |
|-------|---------|---------------------|----------------------------|---------------------------------------------|---------------------------------|
| 1     | 18/M    | Marsh 3a            | CPD                        | f-up refused                                | Alive (27)                     |
| 2     | 48/M    | Marsh 3a            | CPD                        | Marsh 0 (105)                               | Alive (107)                    |
| 3     | 40/M    | Marsh 3a            | Coeliac-like               | Marsh 3c (7)                                | Alive (98)                     |
| 4     | 28/F    | Marsh 3a            | Coeliac-like               | Marsh 0 (4)                                 | Alive (24)                     |
| 5     | 18/M    | Marsh 3a            | Coeliac-like               | Marsh 1, CPD (48)                           | Alive (73)                     |
| 6     | 58/M    | Marsh 3a            | Coeliac-like               | Marsh 1 (11)                                | Alive (77)                     |
| 7     | 49/F    | Marsh 3a            | Coeliac-like               | Marsh 1 (11)                                | Alive (81)                     |
| 8     | 40/M    | Marsh 3a            | Coeliac-like               | Marsh 1 (3)                                 | Alive (147)                    |
| 9     | 30/M    | Marsh 3a            | Coeliac-like               | Marsh 1 (4)                                 | Alive (156)                    |
| 10    | 73/F    | Marsh 3a            | Coeliac-like               | Marsh 1 (54)                                | Alive (68)                     |
| 11    | 58/F    | Marsh 3a            | Coeliac-like               | Marsh 1 (6)                                 | Alive (146)                    |
| 12    | 80/F    | Marsh 3a            | Coeliac-like               | Marsh 1 (12)                                | Dead (105)                     |
| 13    | 85/F    | Marsh 3a            | Coeliac-like               | Marsh 1 (5)                                 | Dead (14)                      |
| 14    | 28/M    | Marsh 3a            | Coeliac-like               | Marsh 1 (7)                                 | Alive (67)                     |
| 15    | 73/F    | Marsh 3a            | Coeliac-like               | Marsh 1 (2)                                 | Alive (60)                     |
| 16    | 45/M    | Marsh 3a            | CPD                        | Marsh 1 (14)                                | Alive (60)                     |
| 17    | 67/M    | Marsh 3a            | Coeliac-like               | Marsh 0 (28)                                | Alive (80)                     |
| 18    | 71/F    | Marsh 3a            | Coeliac-like               | Marsh 1 (10)                                | Dead (38)                      |
| 19    | 59/M    | Marsh 3a            | Coeliac-like               | Marsh 3a (18), then lost f-up               | Alive (105)                    |
| 20    | 27/F    | Marsh 3a            | Coeliac-like               | Marsh 3b (2)                                | Alive (111)                    |
| 21    | 78/F    | Marsh 3a            | Coeliac-like               | Marsh 1, CPD (6)                            | Dead (69)                      |
| 22    | 68/M    | Marsh 3a            | Coeliac-like               | Marsh 0 (5)                                 | Alive (129)                    |
| 23    | 30/F    | Marsh 3a            | Coeliac-like               | Marsh 1 (15)                                | Alive (91)                     |
| 24    | 31/F    | Marsh 3a            | Coeliac-like               | Marsh 0 (5)                                 | Alive (99)                     |
| 25    | 65/F    | Marsh 3a            | Coeliac-like               | Marsh 1 (14)                                | Alive (88)                     |
| 26    | 42/F    | Marsh 3a            | Coeliac-like               | Marsh 0 (20)                                | Alive (94)                     |
| 27    | 66/M    | Marsh 3a            | Coeliac-like               | Marsh 3a (5)                                | Alive (115)                    |
| 28    | 59/M    | Marsh 3a            | Coeliac-like               | Marsh 1a (5)                                | Alive (114)                    |
| 29    | 51/M    | Marsh 3a            | CPD                        | Marsh 1 (6)                                 | Alive (68)                     |
| 30    | 48/M    | Marsh 3a            | Coeliac-like               | Marsh 1 (3)                                 | Alive (123)                    |
| 31    | 59/F    | Marsh 3a            | Coeliac-like               | Marsh 0 (14)                                | Alive (165)                    |
| 32    | 46/M    | Marsh 3a            | Coeliac-like               | Marsh 1 (1)                                 | Alive (145)                    |
| 33    | 56/M    | Marsh 3a            | Coeliac-like               | Marsh 0 (5)                                 | Alive (81)                     |
| 34    | 37/F    | Marsh 3b            | Coeliac-like               | Marsh 0 (4)                                 | Alive (82)                     |
| 35    | 62/M    | Marsh 3a            | Coeliac-like               | Marsh 0 (9)                                 | Alive (118)                    |
| 36    | 23/F    | Marsh 3a            | Coeliac-like               | Marsh 0 (8)                                 | Alive (22)                     |
| 37    | 64/F    | Marsh 3a            | Coeliac-like               | Marsh 1 (6)                                 | Alive (67)                     |
| 38    | 62/F    | Marsh 3a            | Coeliac-like               | Marsh 3a (5)                                | Alive (97)                     |
| 39    | 69/F    | Marsh 3a            | Coeliac-like               | Marsh 1 (5)                                 | Alive (23)                     |
| 40    | 66/F    | Marsh 3a            | Coeliac-like               | Marsh 1 (3)                                 | Alive (67)                     |
| 41    | 18/F    | Marsh 3a            | Coeliac-like               | Marsh 0 (15)                                | Alive (179)                    |
| 42    | 43/F    | Marsh 3a            | Coeliac-like               | f-up refused                                | Alive (63)                     |
| 43    | 53/M    | Marsh 3a            | CPD                        | Marsh 0 (11)                                | Alive (22)                     |
| 44    | 21/M    | Marsh 3a            | Coeliac-like               | Marsh 0 (7)                                 | Alive (19)                     |
| 45    | 48/F    | Marsh 3a            | Coeliac-like               | Marsh 1 (23)                                | Alive (33)                     |
| 46    | 29/M    | Marsh 3a            | CPD                        | Marsh 1 (4)                                 | Alive (57)                     |
| 47    | 64/M    | Marsh 3a            | Coeliac-like               | Marsh 1 (14)                                | Alive (26)                     |
| 48    | 61/F    | Marsh 3a            | Coeliac-like               | Marsh 0 (9)                                 | Alive (61)                     |
| 49    | 31/F    | Marsh 3a            | Coeliac-like               | Marsh 0 (13)                                | Alive (68)                     |
| 50    | 18/F    | Marsh 3a            | Coeliac-like               | Marsh 0 (13)                                | Alive (49)                     |
| Pt No | Age /sex | HLA | History LPD | Abdomen CT | Histology diagnosis | Other histological features | Treatment | Clinical resp. | Histology f-up | VCE | Colonoscopy | Complications | Outcome (mo.) |
|-------|----------|-----|-------------|------------|---------------------|-----------------------------|-----------|----------------|---------------|-----|-------------|---------------|----------------|
| 1     | 29/M     | DQ5/DQ7.3 | -            | normal     | Marsh 3c            | Poly-clonal                | Coeliac-like pattern       | GFD Prednisone+ Budesonide, AZA | AZA | Marsh 2     | Mosaic mid SB | normal      | Alive (145)   |
| 2     | 25/M     | DQ6/DQ7.5 | -            | normal     | Marsh 3c            | Poly-clonal                | Coeliac-like pattern       | GFD Prednisone+ Budesonide, AZA | AZA | Marsh 3a/3b | Mosaic mid SB | normal      | Alive (186)   |
| 3     | 56/F     | DQ6/DQ7.3 | -            | normal     | Marsh 3b            | Poly-clonal                | Coeliac-like pattern       | GFD Budesonide                | -   | Marsh 3b    | Aftous ulcerations and mosaic mid SB | normal      | Alive (104)   |
| 4     | 64/F     | DQ6/DQ8  | -            | normal     | Marsh 3b            | Poly-clonal                | Coeliac-like pattern       | GFD Budesonide                | Budesonide | Marsh 3c    | Mosaic proximal SB | normal      | Alive (41)    |
| 5     | 47/M     | DQ6/DQ7.3 | -            | NA         | Marsh 3c            | Poly-clonal                | Coeliac-like pattern       | GFD Budesonide, 6-MPU         | 6-MPU | Marsh 3c    | Mosaic proximal SB | normal      | Alive (58)    |
| 6     | 64/F     | DQ2.5/DQ6 | -            | normal     | Marsh 3c            | Poly-clonal                | Coeliac-like pattern       | GFD, Budesonide, Budesonide   | Budesonide | Marsh 2/3a | Mosaic mid SB | normal      | Alive (78)    |
| 7     | 36/F     | DQ2.5/DQ6 | -            | normal     | Marsh 3b            | Poly-clonal                | Coeliac-like pattern       | GFD, Budesonide, AZA          | -   | Marsh 3c    | Mosaic proximal SB | normal      | Alive (64)    |
| 8     | 56/F     | DQ2.2/DQ5 | -            | NA         | Marsh 3c            | Poly-clonal                | Coeliac-like pattern       | GFD, Budesonide, Budesonide   | Budesonide | Marsh 3a    | Mosaic mid SB | NA          | Alive (50)    |
| 9     | 29/F     | DQ2.5 HETERO | -           | NA         | Marsh 3b            | Poly-clonal                | Coeliac-like pattern       | GFD                        | -   | Marsh 3b    | Mosaic proximal SB | NA          | Alive (134)   |
| 10    | 33/M     | DQ6/DQ9  | -            | Mesenteric adenopathy | Marsh 3b | Poly-clonal                | Coeliac-like pattern       | AZA Budesonide                | -   | Marsh 3c    | Atrophic SB | NA          | Alive (56)    |
| 11    | 57/M     | DQ2.5/DQ2.5 | -           | NA         | Marsh 3b            | Poly-clonal                | Coeliac-like pattern       | GFD, Budesonide               | -   | Marsh 3b    | Mosaic proximal SB | normal      | Alive (29)    |
| 12    | 44/F     | DQ7.3/DQ7.5 | -           | normal     | Marsh 3b            | Poly-clonal                | Peptic duodenitis-like pattern | Prednisone+ AZA               | AZA | Marsh 1    | NA          | UC          | Alive (52)    |
| 13    | 33/M     | DQ7.5/DQ7.5 | -           | normal     | Marsh 3c            | Poly-clonal                | Peptic duodenitis-like pattern | Prednisone, AZA, Vedolizumab | Vedolizumab | Marsh 1    | Atrophic SB | UC          | Alive (70)    |
| 14    | 34/M     | DQ5/DQ9  | -            | normal     | Marsh 3c            | Poly-clonal                | Peptic duodenitis-like pattern | Prednisone+ Adalimumab       | Adalimumab | Marsh 1    | Ulcers and erosions mid SB | normal      | Alive (47)    |
| Pt N° | Age/sex | HLA | History LPD | Abdomen CT | Histology diag. | yTCR | Other histological features | Treatment | Clinical Resp. | Histology f-up | VCE | Colonoscopy | complication | Outcome (mo.) |
|-------|---------|-----|-------------|------------|----------------|------|-----------------------------|-----------|---------------|----------------|-----|-------------|-------------|---------------|
| 1     | 71/M    | DQ2.2/DQ5 | -           | Enlarged LN, thickened ileal loops | Marsh 3c | Monoclonal | Aberrant CD8+CD30+ T-cell intraepithelial infiltrate | GFD + Prednisone | No | Marsh 3c | NA | normal | Peripheral CD8+ CD30-TCL | Dead (61) |
| 2     | 38/M    | DQ2.2/DQ2.2 | -           | NA | Marsh 3c | Monoclonal | Aberrant CD8+CD30+ T-cell intraepithelial infiltrate | GFD+ Prednisone+ single Infliximab infusion | No | Marsh 3c | NA | Previous history of Crohn’s | Peripheral CD8+ CD30-TCL | Dead (10) |
| 3     | 57/M    | DQ2.5/DQ2.5 | -           | UJI | Marsh 3c | Monoclonal | Aberrant CD3+CD8- CD2-CD5- T-cell intraepithelial lymphocytes | GFD+ Prednisone+ budesonide+ 1 infusion MSC | No | Marsh 3c | UJI | normal | UJI | Dead (11) |
| 4     | 37/F    | DQ7.5/DQ7.5 | -           | normal | Marsh 3c | Monoclonal | Aberrant CD3+CD8- CD2-CD5- T-cell intraepithelial lymphocytes | GFD+steroids+ chemotherapy then MSC transplant | No | Marsh 3c | Extensive SB mosaic | normal | - | Alive (25) |
| 5     | 60/F    | DQ5/DQ5 | -           | Multiple enlarged LN | Marsh 3c | Monoclonal | No increased IELs, increased numbers of lymphocytes and plasma cells in the lamina propria | GFD+ etoposide+ dexametasone | Partial | Marsh 3a | extensive UJI | normal | HLH+UJI | Alive (42) |
| 6     | 25/M    | DQ6/DQ9 | -           | normal | Marsh 3c | Monoclonal | Coeliac-like | GFD+AZA+ budesonide | partial | refused | Extensive SB mosaic | normal | - | Alive (28) |
| 7     | 45/F    | DQ7.5/DQ7.3 | -           | Indolent T lymphoblastic prolifereation Ti | Marsh 3a | Monoclonal | Chronic CD3+CD8+ CD4+ TdT+/- T-cell infiltrate | GFD+AZA+ budesonide | No | Marsh 3a | NA | Ulcers Ti | T-cell lymphoblastic lymphoma | Dead (9) |
| 8     | 50/M    | DQ2.5/DQ5 | CLL         | Multiple Enlarged LN | Marsh 3b | Polyclonal | Coeliac-like | GFD+idelalisib+ budesonide | Yes | Marsh 3b | Extensive SB mosaic | indolent BCL | indolent BCL colon | Alive (12) |
| 9     | 44/M    | DQ7.3/DQ6 | NHL Pulmonary fibrosis | Marsh 3a | Polyclonal | Coeliac-like Full thickness biopsy: band of collagen | AZA+prednisolone | No | Marsh 1 | NA | Oedematous colon | idiopathic sclerosing mesenteritis | Dead (9) |
| 10    | 70/F    | DQ2.5/DQ5 | Large BCL | normal | Marsh 3a | Polyclonal | Coeliac-like subepithelial collagen | GFD+budesonide | No | Marsh 3a | Extensive SB mosaic | normal | - | Alive (40) |
| 11    | 39/F    | DQ2.2/DQ2.2 | -           | multiple enlarged LN | Marsh 3a | Polyclonal | Coeliac-like | GFD+budesonide | No | Marsh 3a | NA | normal | Angioimmunoblastic TCL | Dead (36) |
| 12    | 87/M    | DQ5/DQ6 | -           | NA | Marsh 3c | NA | Coeliac-like | GFD+ Prednisolone+AZA | No | Marsh 3b | Mosaic proximal SB | normal | Oesophageal cancer | Dead (44) |
| HLA    | HC       | GROUP 1 IVA | GROUP 2 IVA | GROUP 3 IVA | CCD    | UCD    | p-value |
|--------|----------|-------------|-------------|-------------|--------|--------|---------|
|        |          | DQ2 ALL     | DQ2.2       | DQ2.5       | DQ5    | DQ6    | DQ7 ALL |
|        |          | 122/424 (28.77%) | 67/424 (15.80%) | 55/424 (12.97%) | 158/424 (37.26%) | 150/424 (35.38%) | 150/424 (35.38%) |
|        |          | 19/40 (47.50%)  | 5/40 (12.50%)  | 14/40 (35.00%)  | 11/40 (27.50%)  | 18/40 (45.00%)  | 12/40 (30.00%)  |
|        |          | 3/12 (25.00%)  | 1/12 (8.33%)   | 2/12 (16.67%)   | 3/12 (25.00%)   | 3/12 (25.00%)   | 6/12 (50.00%)   |
|        |          | 6/12 (50.00%)  | 3/12 (25.00%)  | 3/12 (25.00%)  | 5/12 (41.67%)  | 3/12 (25.00%)  | 44/44 (100%)    |
|        |          | 44/44 (100%)  | 15/44 (34.09%) | 29/44 (65.91%) | 6/44 (13.64%)  | 3/44 (6.82%)   | 44/355 (12.39%) |
|        |          | 337/355 (94.93%) | 156/355 (43.94%) | 181/355 (50.99%) | 44/355 (12.39%) | 65/355 (18.30%) | 63/355 (17.75%) |
|        |          | <0.001       | <0.001       | <0.001       | <0.001       | <0.001       | <0.001       |
|        |          | DQ7 ALL      | DQ7.3       | DQ7.5       | DQ8    | DQ9    |         |
|        |          | 150/424 (35.38%) | 1/424 (0.24%)  | 149/424 (35.14%) | 75/424 (17.69%) | 34/424 (8.02%) |
|        |          | 12/40 (30.00%) | 5/40 (12.50%) | 8/40 (20.00%) | 7/40 (17.50%) | 3/40 (7.50%)  |          |
|        |          | 6/12 (50.00%) | 4/12 (33.33%) | 3/12 (25.00%) | 1/12 (8.33%) | 2/12 (16.67%) |          |
|        |          | 3/12 (25.00%) | 2/12 (16.67%) | 2/12 (16.67%) | 0/12 (0%)   | 1/12 (8.33%)  |          |
|        |          | 7/44 (15.91%) | 44/44 (100%)  | 7/44 (15.91%) | 1/44 (2.27%) | 0/44 (0%)    |          |
|        |          | 63/355 (17.75%) | 44/355 (12.39%) | 63/355 (17.74%) | 5/355 (1.40%) |
|        |          | <0.001       | 0.025        | <0.001       |         |        |         |