Clinical utility of counting intraepithelial lymphocytes in celiac disease intestinal mucosa

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Summary

Background/aims: *Our aims were to establish the clinical utility of assessing the intraepithelial lymphocyte (IEL) density in intestinal biopsies from a large series of individuals and to determine the best threshold discriminating celiac disease (CD) patients and controls in two populations with different pre-test prevalence.* ***Methods:*** *We prospectively performed intestinal biopsy and CD-related serology in 349 subjects undergoing upper GI endoscopy. While 116 had symptoms suggestive of a small bowel disorder (high prevalence), 233 individuals were randomly selected from patients referred to endoscopy because upper GI symptoms (low prevalence). Diagnosis of CD was based on the concordance of classical histological features and a positive CD serology.* ***Results:*** *While 58 patients had a newly diagnosed CD (52 in the high and 6 in the low prevalence groups), 291 subjects did not meet diagnostic criteria of the disorder. Patients had a highly significant greater IEL density than controls (p<0.00001). Based on the ROC curve, a count of 22.8 IEL/ 100 epithelial cells had the highest performance for diagnosing CD in the overall population and for subjects in the high pre-test probability subgroup and 22.5% was the best cut-off for those diagnosed in the low risk population (area under the curves: 0.979, 0.979 and 0.993, respectively). An abnormal CD serology confirmed the diagnosis of CD in all the four patients with counts below 22.8%.* ***Conclusions:*** *Our study confirms that an IEL density of 22.8% is an adequate threshold to discriminate CD patients and controls in individuals irrespective of the prevalence of the disorder.*

Key words: *Intraepithelial lymphocytes, celiac disease, intestinal mucosa, Malabsorption, small bowel mucosa, serology.*

Utilidad clínica del recuento de linfocitos intraepiteliales en la mucosa intestinal para el diagnóstico de enfermedad celíaca

Resumen

Introducción: *El recuento elevado de linfocitos intraepiteliales (LIEs) es un rasgo destacado aunque inespecífico de la enteropatía de la enfermedad celíaca (EC). Un recuento mayor a 40 LIEs/100 células epiteliales ha sido considerado por mucho tiempo esencial para el diagnóstico. Sin embargo, estudios recientes con escaso número de muestras han cuestionado este valor de corte.* ***Objetivos:*** *Determinar el rango normal de LIEs en biopsias intestinales y establecer su capacidad diagnóstica de EC en dos poblaciones con diferente prevalencia.* ***Métodos:*** *Realizamos prospectivamente biopsias de duodeno distal y serología para EC en 349 pacientes consecutivos a quienes se les realizó una videoendoscopia digestiva alta. El grupo A consistió en 116 pacientes derivados a biopsia intestinal por síntomas sugestivos de malabsorción (considerados de alta prevalencia de EC) y el grupo B consistió en 233 pacientes randomizados entre quienes fueron derivados a endoscopía alta por síntomas gastrointestinales no sugestivos de EC (baja prevalencia de EC). El diagnóstico de EC se basó en criterios histológicos clásicos y serología positiva.* ***Resultados:*** *Cincuenta y ocho pacientes tuvieron EC*

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(52 en el grupo de alto riesgo y 6 en el de baja prevalencia) y 291 individuos no tuvieron criterios de la enfermedad. Los pacientes tuvieron una densidad de LIEs significativamente mayor que los controles (p<0.00001). Basado en las curvas ROC, el conteo de 22.8 LIEs/100 células epiteliales tuvo la mejor sensibilidad y especificidad para el diagnóstico de EC en la población general y entre los sujetos con alta probabilidad y 22.5% fue el mejor valor de corte para la población de bajo riesgo (áreas bajo las curvas: 0.979, 0.979 y 0.993, respectivamente). Todos aquellos pacientes celíacos con recuento de LIEs por debajo de 22% (n=4), tuvieron serología positiva para EC. El clásico valor de 40% tuvo una sensibilidad del 55%. ***Conclusiones:*** *Nuestro estudio confirma que una densidad de LIEs mayor a 22.8% constituye un adecuado umbral para discriminar EC y controles en individuos independientemente de la prevalencia de la enfermedad.*

The diagnosis of celiac disease (CD) is currently based on the finding of a characteristic small bowel mucosal inflammation and the demonstration of its gluten-dependence. The broad spectrum of gluten-dependent mucosal damage includes a variable degree of architectural villous changes, crypt hyperplasia, infiltration of the lamina propria by inflammatory cells (lymphocytes, macrophages and eosinophils) and the expansion of the intraepithelial lymphocyte (IEL) population.[1-2] Furthermore, some gluten sensitive patients can have a completely normal mucosa or only an IEL infiltration.[3-4] It has been shown that a raised IEL count is the first abnormality seen in treated CD patients after a gluten challenge [4] and could be the only histological feature in early stages of gluten sensitivity or in patients with minimal enteropathy.[5-6] Although diagnosis of CD should be based on firm evidences of gluten-dependence of the morphological findings, demonstration of intraepithelial lymphocytosis by pathologists has been considered a hallmark of CD. For more than 30 years, an IEL density greater then 40 IELs per 100 epithelial cells has been considered a threshold for diagnosing CD in duodenal or jejunal biopsies.[7-11] However, the clinical benefit of this cut-off has been recently questioned. Although the intraepithelial lymphocytosis is almost constant in CD patients with villous atrophy, the increased density in the context of a normal mucosal architecture could also be seen in other disorders. Thus, a recent study showed that only 10% of cases with high IEL density in otherwise normal mucosa had CD.[12] Very recent studies have suggested that a lower cut-off (between 20 and 25 IELs per 100 epithelial cells) seems to be a reliable alternative threshold to be consider ed as the upper limit of the normal IEL infiltration in duodenal biopsies.[13-15] Once again, the critical benefit of new thresholds and how patients with evidences of CD or gluten sensitivity fit in them remains uncertain. Interestingly, the rational use of a cut-off requires an understanding of the sensitivity and specificity of the threshold. In the case of the IEL density, no studies addressed this information and knowledge about its utility has been generated from studies conducted in a research setting. Moreover, there is a general consensus that the cut-off performance is less accurate in clinical settings where a significant number of patients with densities below the threshold are present. Furthermore, tests may have different performances according to the prevalence of the given disease in the study population.[16]

Our aims in this study were twofold: first to determine the IEL density in a large series of duodenal biopsies obtained from both, individuals with newly-diagnosed CD and those not having either CD or serological evidence of gluten sensitivity (GS) and, second, to establish the threshold that could better discriminate patients and controls in two populations with different pre-test probabilities.

Materials y methods

Subjects

From September 2004 to August 2005 a prospective study was performed aiming to detect CD in two different populations, individuals with a high pre-test probability of CD, group A, and subjects with a lower pre-test probability, group B.

Group A consisted in 116 consecutive individuals referred to upper GI endoscopy and intestinal biopsy by the Malabsorption and Small bowel Diseases Clinic of the Hospital de Gastroenterología due to the presence of clinical features suggesting a small bowel disorder. Group B consisted in 270 randomly selected subjects who were referred to endoscopy due to upper GI symptoms (dyspepsia, epigastric pain, gastro-esophageal reflux symptoms, etc.). All of them were interviewed before endoscopy giving detailed information about the purpose of the study and 233 gave written consent accepting intestinal biopsy and serology tests. Twenty-two subjects refused to participate in the study, nine were intole-

rant of the procedure and six had problems that made impossible to access the distal duodenum (esophageal and pyloric stenosis and gastric cancer). One of the 22 patients refusing to participate in the study had duodenoscopic markers indicative of enteropathy requiring intestinal biopsy. Although histology and serology confirmed CD, the patient was excluded from further analysis in the present study. In both populations we excluded subjects with prior diagnosis of CD or dermatitis herpetiformis, those on a gluten-free diet or having prior small bowel histology or a known CD serology. Epidemiological features of both populations are reported in table 1.

Celiac disease diagnosis was based on histological grounds (Marsh´s type II enteropathy or greater at the initial biopsy) and a concomitant positive CD-related serology. In order to avoid selection bias, the IEL count was not taken into account in the diagnostic criteria of CD. In the case of negative serology, the effect of a gluten-free diet was assessed at clinical and histological levels. Those cases with a positive CD serology but a non-atrophic biopsy (Marsh´s type O and I) were considered as GS. Clinical categorization of CD patients in classical, subclinical and silent was done according to former studies.[18,23]

Biopsies, histological procedures and quantitative morphometry

Biopsy samples were obtained from the distal duodenum by duodenoscopy following a standard protocol. At least 3 samples were obtained using conventional endoscopic forceps (open cup: 8mm). Samples were oriented carefully on paper, fixed in 10% formalin, embedded in paraffin wax and 4 mm sections were conventionally stained with H&E. Morphology and quantitative evaluation (intraepithelial lymphocyte count) were performed by independent experienced observers (A.C and Z. K.) who were unaware of the clinical and laboratory findings of subjects. Intestinal biopsies were categorized according to the modified Marsh´s classification in four types.[1,17] Type 0 is normal mucosa, type I is an infiltrative stage marked by a normal mucosa architecture in which the villous epithelium has intraepithelial lymphocytosis (> 30 IEL/ 100 epithelial cells). Type II shows the addition of enlarged crypts (hyperplasic stage) and Marsh III comprises a large spectrum of changes ranging from the minor villous atrophy to the severe villous atrophy (subcategorized as Marsh IIIa, IIIb and IIIc). Biopsies from a series of conflictive cases were assessed for counting IEL by a third experienced pathologist blinded to the former diagnosis and IEL count and also to clinical and biochemical findings in patients. Reassessed samples included all new CD patients, a series of normal samples and all those conflictive cases (e.g. new CD patients with a low density of IEL

Table 1. Demographic and clinical characteristics of celiac disease (CD) patients and controls. *Control subjects were grouped as A (high risk) and B (low risk) according to pre-test probabilities.*

	CD patients	Controls Group A	Controls Group B
Number of patients	58	64	227
Gender (female/male)	52/6	53/11	154/73
Mean age (range) (years)	36 (19-72)	45 (19-76)	37 (16-80)
Main presenting symptoms			
Inducing endoscopy (n of patients)			
Diarrhea		21	-
Weight loss		11	19
Chronic anemia		18	11
Epigastric pain		-	84
Gastroesophageal reflux		-	65
Dyspepsia		-	36
Portal Hipertension		-	9
Nausea-vomiting		-	3
Celiac disease familiarity		5	-
Thyroid disease		9	

and non-atrophic samples with increased counts).

Density was determined by counting IELs and epithelial cell nuclei in uninterrupted length of surface epithelium in three well-oriented, randomly selected areas of samples on H&E sections (x 400 magnification), At least 500 epithelial cells were necessary in order to establish a mean count. In non-atrophic biopsies, care was taken to count cells considering tips or bases of villi and they were reported as a cumulative mean and/or discriminated according localizations. Density was expressed as number of IEL per 100 epithelial cells (%IEL).

Laboratory determinations: Celiac disease-related serology

Serum samples were kept frozen until the assay. Determinations performed were: 1- IgA a-tTG (QUANTA Lite TM, h-tTG IgA, INOVA Diagnostic Inc.; San Diego, CA) by ELISA (cut-off provided by the manufacturer: 20 AU/mL); 2- a newly developed enzyme-linked immunosorbent assay (ELISA) to detect IgA and IgG antibodies reacting against a deamidated gliadin-derived polypeptides (IgA and IgG a-DGP) using a kit provided by the manufacturer for research use only (QUANTA Lite Gliadin IgA and IgG II– Inova Diagnostic Inc.; San Diego, CA) (cut-off provided by the manufacturer: 20 AU/mL);[18] 3- all patients with positive a-tTG antibodies were tested for IgA endomysial antibody by an immunofluorescence method using primate esophagus substrates (INOVA Diagnostics Inc.; San Diego; CA; USA) tested at 1:10 dilution; 4- conventional type IgA antigliadin antibodies (AGA) by ELISA using commercial kits (RADIM; Gliadina IgA EIA WELL; RADIM S.p.A. Rome, Italy) (cut-off values: 20AU/mL); 5- total serum IgA (radial immunodiffusion test; Diffu-Plate, Biocientífica S.A.; BA, Argentina).

Design of the study, ethical issues and statistical analysis

The design of the study was prospective based on the blinded analysis of histological features and serology in populations with some differences. While group A was based on the analysis of a series of clinically pre-selected consecutive patients attending a malabsorption clinic, group B was a randomly selected population of individuals attending an endoscopic unit by causes primarily not related with CD or malabsorption. The study was approved by the Research and Ethical Committees of the Gastroenterology Hospital. Data were analyzed using Statistix 7 for Windows Analytical Software (2000 Analytical Software, Tallahassee, FL, USA). According to data distribution, results are reported as mean ± SEM, or median and range as appropriate. Sensitivity, specificity, positive and negative predictive values and likelihood ratios were calculated using conventional formulas. Comparisons were performed using the Student's t test, Mann-Whitney U test, c2 or Fisher's exact test, as appropriate. The cut-off for normal IEL density was determined expressing the relationship between sensitivity and specificity of different cut-off values using the receiver operator characteristic (ROC) curve and the estimated area under the curve and 95% confidence interval (STATA 8.2; Stata Corp. College Station, TX. USA).

Results

Clinical data

Based on the combination of morphological analysis of biopsies, the CD-related serology and the clinical and histological outcome of patients with atrophy but negative serology, we detected 58 newly diagnosed CD patients (52 in the high and 6 in the low pre-test probability groups). All CD had al least one positive CD-specific serologic test. Twenty-eight other subjects had a positive test for one of the CD-related antibodies but a normal duodenal mucosa and were considered as GS cases. Finally, 263 subjects (50 from the high and 213 from the low pre-test probability subgroups) had no histological and/or serological criteria for diagnosis of CD or GS. Table 1 shows some demographic and clinical features of patients and controls (grouped according to the pre-test probability) and the prevalence of the main presenting symptom leading to clinical investigation.

Intraepithelial lymphocyte density determinations

Based on the traditional method of counting cell in the intestinal epithelial compartment of H&E stained sections, non-CD subjects had IEL counts ranging from 1.6 to 28.0 IEL/100 epithelial cells (table 2). No differences were detected comparing non-CD subjects in the group A (11.7±5.0) with those individuals in the high pre-test probability group (10.7±4.4; p=0.1818). Counts for patients with GS (n=28) were similar to those of non-CD controls (p=NS) (table 3). Furthermore, counts from villous tip of subjects

Table 2. Clinical data, biochemical and histopatological features of celiac disease (CD) patient and gluten sensitive (GS) individuals. *Categorization of patients is reported in the Methods section. BMI: body mass index. Histological features of intestinal mucosa are reported according to the Marsh's modified classification (1,17). Serology: a-tTG: anti-tissue transglutaminase antibodies; IgA and IgG a-DGP: antibodies reacting against gliadin-derived peptides.*

	CD patients	GS subjects
N of patients	58	28
Clinical characterization (n of patients)		
Classical CD	38	
Subclinical CD	18	
Silent CD	2	
BMI (g/cm2) mean ± SEM	19.9 ± 0.5	25.7 ± 0.9
Histological features (Marsh) (n of patients)		
Type 0	-	28
Type I	-	-
Type II	-	-
Type IIIa	3	-
Type IIIb	11	-
Type IIIc	43	-
Serology (n of patient positives)		
a-tTG	53	15
IgA a-DGP	56	13
IgG a-DGP	54	5

Table 3. *Intraepithelial lymphocyte (IEL) count in patients (CD), controls (non CD) grouped according to the precedence of subjects (with high –group A- and low –group B- pre-test probability) and subjects with serological evidences of gluten sensitivity (GS) but normal villous structure.*

IEL count %	non CD Group A	non CD Group B	CD patients	GS Subjects
Mean ± SD	11.7 ± 4.8	10.7 ± 4.8	42.0 ± 5.2	10.2 ± 4.6
Minimum	1.6	4.0	7.3	2.3
Maximum	28.0	23.3	88.0	23.0

with GS were similar to those of non-CD controls (11.0±0.9; range 2.3 to 23).

Patients with newly diagnosed CD had a significantly greater mean IEL count compared with controls and those with gluten sensitivity ($p<0.0000$ for both comparisons) (table 3). Although the range of counts for this population was very wide, the distribution of frequencies showed a concentration of cases with counts significantly higher than those for the control population (figure 1). Patients diagnosed in the group A had a similar mean IEL count to those diagnosed from the group B (data not shown).

Discriminative thresholds

Looking for a cut-off in the IEL count that could better discriminate CD patients from controls we employed the software based on the ROC curve estimation and the highest sum of sensitivity and specificity. Thus, the cut-off with best performance for the overall population corresponded to an IEL count of 22.8% which was 93.1% sensitive and 96.6 % specific (table 5). The ROC curve for the cut-off estimated an area under de curve of 0.979 (95% CI 0.952 to 1.007) (figure2). Using this cut-off, 13 of 289 (4.4%) non-CD patients had counts above it being 28% the highest IEL density determined. In contrast, four of 58 (6.9%) new CD patients had counts below the cut-off with 7.3% as the lowest count assessed. Table 4 shows some clinical aspects,

Figure 1. *Intraepithelial lymphocyte data distribution for celiac disease (CD) patients and non-CD controls represented in a box and whisker plots graphic (50% and 95% of data, respectively.*

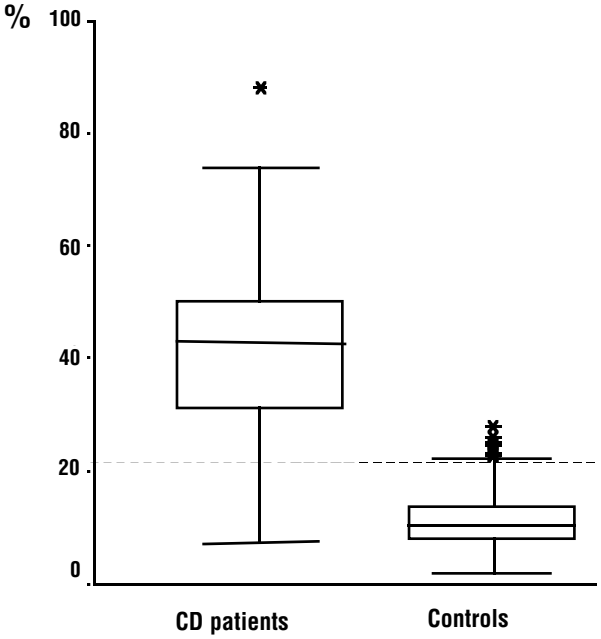
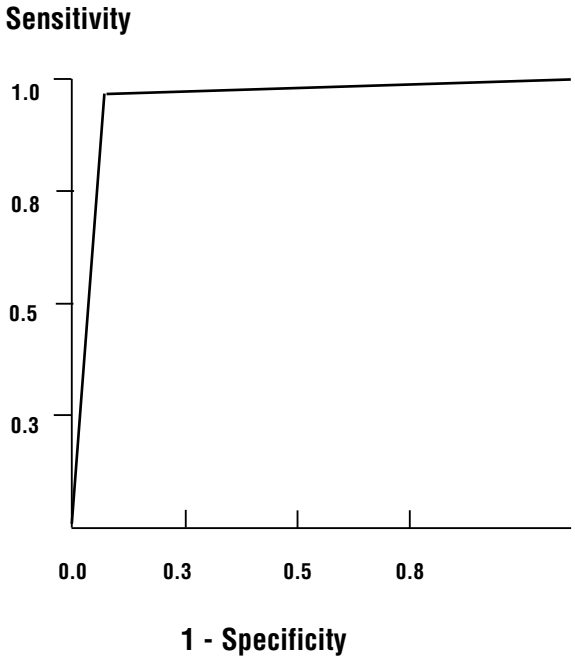


Figure 2. *ROC curve correlating sensitivity and specificity (1-specificity) for intraepithelial lymphocyte count at 22.8%. Area under the curve is 0.979.*



laboratory data and histological features of the four CD patients with counts below the cut-off. Interestingly, a duplicated blindly quantitative analysis of biopsies by a second pathologist gave almost similar results to those of the first assessment. The comparison of serology and the quantitative histology evidenced that all CD patients with discordant IEL counts had a congruent serology (e.g. positive specific antibodies in CD cases with counts below the 22.8% cut-off and a negative serology for controls with counts greater than the threshold). Furthermore, all four CD cases with low counts had a severe Marsh III enteropathy (table 4).

The analysis of patients and controls discriminated according to the level of pre-test prevalence shows that a density of 22.8% and 22.5% IEL are the best thresholds for high (area under the curve: 0.979, 95% CI 0.949-1.011) and low (0.993, 95% CI 0.980-1.005) pre-test probabilities, respectively. Table 5 shows the comparison of the statistical per-

Table 4. *Clinical aspects, laboratory data and histological features of four CD patients with counts below 22 IELs/100 epithelial cells. BMI: body mass index; Marsh type: modified Marsh´s classification (1,17); IEL: intraepithelial lymphocytes (IEL counts are expressed as reported by two independent blind reviewers).*

Patient	Age (years)	Gender	Time with symptoms before diagnosis (years)	BMI (g/cm2)	a-tTG IgA (AU/mL)	AGA-II IgA (AU/mL)	Marsh´s type	IEL count (%)	Serum albumin (g/L)
1	36	F	9	17.7	117	116	IIIb	19/15	4.1
2	19	F	4	15.6	59	54	IIIc	18.5/20	4.0
3	25	F	5	18.5	104	104	IIIb	19.5/22	3.3
4	56	F	12	22.3	57	110	IIIa	7.3/14.6	4.0

Table 5. *Statistical performance of the proposed cut-offs in the overall population and in subjects grouped in high and low pre-test probability. PPV: positive predictive value; NPV: negative predictive value; PLR: positive likelihood ratio; NLR: negative likelihood ratio.*

Parameter	Overall population	High pre-test probability	Low pre-test probability
Cut-off (%)	22.8	22.8	22.5
Sensitivity (%)	93.1	92.3	100
Specificity (%)	96.6	98.4	95.1
PPV (%)	81.8	97.9	35.3
NPV (%)	98.6	94.0	100
Diagnostic accuracy (%)	96.0	95.7	95.2
PLR	27.4	57.7	20.4
NLR	0.07	0.08	0.00
ROC curve (area under the curve)	0.979	0.980	0.993

formance (sensitivity, specificity, positive and negative predictive values and positive, diagnostic accuracy and positive and negative likelihood ratios) of tests in the overall population and in subjects discriminated according to the pre-test probability using the most appropriate cut-offs determined by the ROC curve. The table shows an excellent performance in any population considered. However, while the threshold exhibited an absolute sensitivity (100%) and high specificity in the low pre-test probability population, the positive predictive value was very low (35.3%) as consequence of the low number of new CD patients and a relatively high number of false positive cases in the subgroup. Interestingly, considering the performance of the 40% IEL density cut-off, sensitivity, specificity and positive and negative predictive values were: 55.2%, 100%, 100% and 91.8%, respectively.

Discussion

Locally resident IELs are primarily CD8 T-cells likely involved in the pathogenesis of CD. Recent evidence suggests that the combination of at least two different pathways may cause tissue injury in CD. An adaptive gluten-specific T-cell inflammation, on the one hand, and the innate immune response mediated by IELs, on the other. The role of intraepithelial lymphocytosis in CD pathogenesis seems to be related to epithelial cells, interleukin 15 and co-stimulatory molecules, which can induce direct tissue damage without the activation of lamina propria gluten-specific CD4 T-cells.[19] In the clinical context, the identification of a raised IEL density in association with additional features of enteropathy is considered a hallmark of CD and can be used as a valuable aid for the differential diagnosis of villous atrophy. Increased IEL density in otherwise normal small bowel histology has been associated with CD in two ways, as the earliest manifestation of gluten intake in treated CD patient, and as a mild histological evidence of CD. However, the gluten-dependence of intraepithelial lymphocytosis has been recently proved in only 10% of patients with normal villous architecture.[12] Therefore, most cases are related to several other disorders [19,20] such as: NSAIDs,[21] *Helicobacter pylori* infection,[22] bacterial overgrowth, giardiasis, tropical sprue,[10] etc. In the present study our goal was to determine the diagnostic relevance of counting IEL assessing a large series of consecutive individuals undergoing upper GI endoscopy in two different clinical setting based on the pre-test probability for CD.

Our study shows that IEL density in non-CD patients (including those with serological evidence of GS but not villous atrophy) ranged from 1.6 to 28 IELs per 100 epithelial cells. No differences in densities were observed comparing populations with high and low pre-test probability, or between non-CD subjects with those with GS but not mucosal atrophy. The study allowed us to identify 58 patients with newly diagnosed CD, 52 in the high and six in the low risk groups. Compared with control subjects, CD patients had a significantly increased IEL density ranging from 7.3% to 88%. Based on the ROC curve estimation and the best sensitivity and specificity for a given cut-off, a 22.8 IEL per 100 epithelial cells had the best statistical performance for both, the overall population and the two groups with different probabilities. Considering this threshold, four well-established CD patients (both belonging to the group A with a high risk for CD) had counts below the cut-off. All four cases had severe enteropathy (Marsh´s type III) and the presence of positive CD antibodies confirmed the diagnosis. By contrast, 13 of 289 non atrophic samples had IEL counts higher that the estimated cut-off (ranging from 23% to 28%). In all these cases, biopsies

showed a normal villous architecture and negative CD serology tests. These results are in agreement with those of recent studies on duodenal biopsies in control individuals.[13-15] All of them exhibit a coincident mean value for controls and established a very similar cut-off.

In our opinion the present study has several strengths. One of them is related to the prospective design of the study enrolling a large series of consecutive and randomized individuals underwent upper endoscopy and intestinal biopsy. As the performance of diagnostic methods is strongly influenced by the prevalence of a given disease, the design of the study was based on the collection of subjects from two populations with a different pre-test probability. Another strength of the study is given by the fact that the new threshold was estimated comparing determinations in newly diagnosed CD patients, subjects with GS but not enteropathy and in those without any evidence of CD. Finally, the diagnosis of CD was based on the concordance of morphological changes in the intestinal biopsy and a positive CD specific serology. However, diagnosis of CD did not comprise the IEL density, a fact that was considered essential in order to avoid a selection bias.

The most relevant finding of our study is that some CD patients may have active gluten induced enteropathy but normal IEL density. Although in our study such an event was rare (<7% of our cases), we must emphasize that this could be a potential confusing feature questioning CD diagnosis specially for patients with partial or total villous atrophy. The concordant presence of positive CD serology tests in all four cases was confirmatory of CD; however, this may not be the rule, especially in CD patients with mild architectural abnormalities, a situation often not associated with a positive CD-related serology.[17,20] Although a low IEL density in active CD with villous atrophy has been reported before, the magnitude of this observation has been underestimated. Very recently, Memeo et al[22] have reported that six of 38 (16%) newly diagnosed CD patients had IEL counts ranging from 20% to 30% (no information about the degree of severity of mucosal derangement was provided by the authors). Furthermore, we and others have shown a low IEL count in GS patients with otherwise normal villous architecture (e.g. Marsh´s type 0 in patients with dermatitis herpetiformis).[12,20,23] However, we must keep in mind the possibility of potential bias in our results as consequence of an underestimation of IEL counts. A sampling error due to a patchy distribution of lymphocytosis and an underestimation of the IEL counts resulting from the method used (based on H&E but not immunostaining) are likely the most important confounder factors. Some authors have suggested that differences between old studies and the more recent reports might be related to differences in IEL infiltration between jejunum and duodenum[24,25] However, comparative studies addressing these potential differences have not been conducted yet.

Our findings in the CD population are very similar to those from other recent studies where mean IEL counts are rounding, in general, 40% to 50%. Interestingly, using the former cut-off (40%) specificity would rise to 100% but sensitivity would drop to the non acceptable 55.2%. Although distribution of frequencies of IEL counts in patients and controls are clearly different (see figure 1), a substantial number of newly diagnosed CD patients have counts below 40%.

In conclusion, based on our present prospective observations and those from others, we suggest that the classical referred cut-off of 40% should not be used for diagnosis of CD at least in duodenal samples because a low sensitivity and negative predictive values. The 22.8% threshold was very helpful in order to discriminate patients and controls not only in the overall population but also in both, the high and low pre-test probability groups. However, despite sensitivity was optimum in the low pre-test prevalence population, the positive predictive value was low (32%) due to the number of false positive. Despite all these observations, a minor overlapping may still persists with patients having density values below the cut-off and controls with estimates above that value. Finally, the CD serology was a valuable aid to elucidate diagnosis in equivocal cases.

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