

# Counting Intraepithelial Lymphocytes. Immunohistochemistry and Flow Cytometer are Necessary New Steps in the Diagnosis of Celiac Disease

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**Abstract** The simple quantitation of intraepithelial lymphocytes per 100 epithelial cells in small bowel has been a valuable addition to the study of the histopathology of celiac disease. However new technology is necessary to increase the accuracy and significance of the subtypes of intraepithelial lymphocytes in the differential diagnosis and the evaluation of gluten related disorders.

**Keywords:** *celiac disease, intraepithelial lymphocytes, enterocytes, small bowel*

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The number of intraepithelial lymphocytes (IEL) can be counted as Siriweera, Qi and Yong [1] have published recently in the International Journal of Celiac Disease, using a length of surface epithelium of an entire villus and/or the villous tip in formalin fixed, paraffin embedded, Hematoxylin and Eosin stained biopsies under the light microscope. The results are expressed as lymphocytes per 100 epithelial cells.

The first attempt to estimate the numbers of IEL in man was reported in 1971 [2]. The technique used was a differential count of the cell types within the villus epithelium, thus as stated above measuring the IEL count per 100 villus epithelial cells. Skinner and Whitehead recommended that the reference value should be taken as the muscularis mucosa. The number of IEL per length of muscularis mucosa would give a true measure of the number of IEL. According to them the epithelial layer is, however, severely altered in coeliac disease and consequently is itself a variable and should not be used in this way as a unit of measurement of abnormality of other parameters [3]. In fact, according to Marsh morphometric studies, Ferguson's technique overestimates IEL numbers by up to a factor of two, due to the loss of nearly 50% enterocyte nuclei in sectioned tissue [4,5].

Ferguson [6] had argued that the relevance of the IEL relates to the microenvironment in a single villus or in the villus epithelium. Further she added, that IEL counts per epithelial cell have the advantage that they can be carried out in virtually any routinely processed biopsy. In the 3<sup>rd</sup> International Symposium of Celiac disease held in the Netherlands in 1974 Ferguson presented evidence that IEL infiltration into the epithelium developed within 24 hours in all of five adults given a single challenge of 30 g gluten [7].

Therefore, according to Ferguson using this simple quantitation, a useful technique was provided to the

gastroenterologist. Ferguson confirmed in 1977 that this method has been widely applied in a variety of research projects in adults and children and added that it may be of value in routine diagnostic pathology [6].

Siriweera et al [1] found that the upper limit of normal IEL/100 epithelial cells (EC) was 7.78 and the mean IEL/100 EC in celiac disease is 20.93, which are much lower than the values reported in the literature but according to Ferguson [6]. Otto [8] found a mean value only 6.3 with standard deviation 1.7 lymphocytes per 100 epithelial cells for 87 adults whereas in other 12 published articles the mean values in 131 children and 82 adults was 15 and 25. Unfortunately we do not know the exact site from where the biopsy samples were taken and the variability may be also explained by the thickness of the section.

Although it is important to emphasize that the pathologic findings need to be correlated with the clinical, endoscopic and serologic findings in all the patients suspected of celiac disease [1,9]. It is estimated that only between 5 to 15% of patients with increased IELs have celiac disease [10].

However, immunological and histological research has pointed out that it is the subtyping of the IEL that is of paramount importance in the diagnosis and follows up of celiac disease. Rust et al [11,12] isolated intestinal T cells from small intestinal biopsies of patients 18 patients with celiac disease and 14 controls. The isolated T cells were cultured and analyzed for TcR phenotype and for the expression of CD4 and CD8. The percentage of TcR gamma delta T cells present in the bulk cultures obtained from the biopsies of the patients suffering from celiac disease ranged between 0 and 57% whereas in similar cultures from control biopsy specimens contained between 0 and 2% of TcR gamma delta T cells. Savilahti et al found a

constantly elevated population of gamma/delta + T cells in the epithelium of celiac patients [13] and Holm et al also in Finland suggested that routine jejunal histological studies should include gamma delta T-cell counts in order to detect earlier of celiac disease latency. [14].

Studies of small intestinal IEL by using flow cytometry has found that celiac patients had higher levels of T cell receptor gamma delta IEL than control patients including *H. pylori* patients and other enteropathies. Further, the density of CD3-/CD7+ IEL, an intraepithelial lymphocyte subset poorly characterized by immunohistochemical methods, was significantly lower in celiac patients than in the control group [15]. It has been found recently that this celiac immunophenotype persists regardless of the biopsy's anatomical location or the corresponding histological findings [16].

It can be argued that not all departments of gastroenterology and pathology are prepared to use single-cell suspensions from the epithelial layer of small intestinal biopsies but nowadays as the group of Dr. Garbiñe Roy from Madrid has recently concluded that diagnostic biopsy, when required, combined with IEL lymphogram analysis (a term introduced by her) provides specificity to the histological findings and avoids possible misinterpretation of the histological lesions. This technique is also mandatory in the diagnosis of refractory celiac disease but this important aspect is outside the focus of this editorial [16,17,18].

Lonardi et al have used a new commercially available anti-TCR $\gamma$  antibody on formalin-fixed paraffin embedded intestinal biopsies. Anti-CD3 and anti-TCR C $\gamma$ M1 (clone  $\gamma$ 3.20) from Thermo Scientific were applied by immunohistochemistry. TCR $\gamma$ + IELs can therefore be identified in this routinely fixed biopsies and their evaluation adds useful information for the diagnosis. They have correctly concluded that TCR $\gamma$  staining coupled with CD3 may represent an additional tool to recognize cases of latent/potential celiac disease when serology and clinical data are not conclusive or when the histological diagnosis remains equivocal [19].

In conclusion, the incorporation of Immunohistochemistry and/or flow cytometer to the study of IELs will help the accurate diagnosis of celiac disease.

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