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# Proceedings

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# Digital Microscopy Versus Conventional Microscopy In Assessment Of Jejune Intraepithelial Lymphocytes

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### *Introduction/ Background*

Leukocytes infiltrates assessment in the lining epithelium of the digestive tract is a common request in current pathology practice both in terms of diagnosis and quantification of the therapeutic response. Frequently analyzed are lymphocytes positive for CD4 and CD8 in the context of gluten sensitive enteropathy. Microscopic quantification remains a time consuming and a laborious work. The possibility of human error puts at question the accuracy of data provided. Implementation of digital image acquisition techniques brings a relative comfort in delimitation the areas of interest, quantifying those areas and introduces digital algorithms that can determine the number of positive signals on the area of interest.

### *Aims*

Establishing quantitative differences between the data acquired using optical microscopy versus digital evaluation of intraepithelial leukocytes (CD3+, CD4+, CD8+).

### *Methods*

We analyzed paraffin embedded jejune segments fixed in buffered formalin (10%), taken from healthy patients (n= 15), monitoring the positive lymphocyte count and CD4 + CD8 + ratio. From each block, 4 sections of 3 µm thickness were taken. One section was stained with hematoxylin and eosin, and the other 3 sections were stained immunohistochemically using three steps-indirect streptavidin method for anti CD3 monoclonal antibodies, anti CD4 and CD8. The sections were referred to five independent pathologists in order to quantify positive leukocyte number in 100 enterocytes using optical microscopy and subsequent transmission microscopy images projected on high-definition monitors. Readings were performed on optical microscopes Leica DM750 objective 10X 40X eyepieces FN20. Image acquisition was made with 5-megapixel Leica microscope camera, ICC50 model, in direct transmission (stream) USB 2.0, monitors Dell 27 "HDMI IPS. For normalization and comparison of data, reporting was done in an area of 1 mm2. The variation between observers, for the two methods, were calculated (k).

### *Results*

Interobserver variability for CD3 in optical was estimated as slight agreement (k0.36) in traditional microscopy compared to substantial agreement for digital assessment (0.68). For CD4 and CD8 markers the differences were less significant, substantial agreement in both categories (CD4 0.72 for optical evaluation vs 0.78 in digital evaluation data and for CD8 0.65 vs 0.72 respectively). In our opinion the use of digital image acquisition techniques in quantification of intraepithelial leukocytes increases concordance degree among observers through diminishing the subjective factors and by choosing identical ROIs.