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Duodenal lymphogram as a complementary tool in the diagnosis of celiac disease in adults

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Duodenal lymphogram as a complementary tool in the diagnosis of celiac disease in adults

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ABSTRACT

Introduction: celiac disease (CD) patients have a specific pattern of lymphocytic infiltrate in the duodenal mucosa. Flow cytometry is a complementary tool for the diagnosis of CD, which allows the quantification and characterization of intraepithelial lymphocytes (IELs) by what is commonly called a lymphogram. Here we describe our experience with this technique in the diagnosis of CD in adult patients.

Methods: lymphograms from 157 patients performed in our center between 2009 and 2017 were retrospectively analyzed. Fourteen patients had a previous diagnosis of CD and followed a gluten-free diet (GFD), 21 had a new diagnosis of CD and the remaining were considered as non-celiac. The association of the lymphogram results (total IELs, CD3- lymphocytes and TcRγδ lymphocytes) with the CD diagnosis, compliance with the

GFD, time since diagnosis and IgA anti-TG2 titer were determined.

Results: the area under the ROC curve of TcRγδ lymphocytes for CD patients varied between 0.86 and 0.86. The percentage of TcRγδ lymphocytes in GFD-treated patients was lower; 12 (8.5) *vs* 20.5 (8.7), p = 0.0153. However, it remained high compared to non-CD; 12 (8.5) *vs* 6.7 (6), p = 0.135. The time since diagnosis and IgA anti-TG2 titer correlated with the lymphogram results. *Helicobacter pylori* infection and treatment with angiotensin receptor antagonist 2 (ARA2) were associated with differences in the lymphogram results in patients without CD.

Conclusions: the duodenal lymphogram is a reliable complementary tool in adults for the diagnosis of CD. However, compliance and duration of the GFD and other factors may condition its diagnostic capacity.

Keywords: Lymphogram. Flow cytometry. Celiac disease.

INTRODUCTION

The increase of intraepithelial lymphocytes (IELs) in the duodenal mucosa is considered as the most sensitive and early histopathological finding of coeliac disease (CD). This infiltrate can be recognized by studying duodenal biopsies with conventional stains such as hematoxylin-eosin, or with more precise techniques that allow the characterization of CD3$^+$ lymphocytes, such as immunohistochemistry or flow cytometry.

Flow cytometry is a technique that allows the quantification and characterization of IELs subsets in a quicker and more objective way than conventional immunohistochemistry. Under normal conditions, IELs represent 5-15% of the total isolated cells in the duodenal epithelium and more than 70% are CD3$^+$ T lymphocytes. Of these, the subset of TcRαβ CD8$^+$ T-cells constitute 80%, while TcRγδ lymphocytes account for 10-12%. CD3$^-$ lymphocytes form a heterogeneous group that mainly express natural killer (NK) cell markers (1). According to the IELs profile, the specific pattern of CD (2,3) is characterized by an increase in total IELs (both TcRαβ and TcRγδ) in the duodenal mucosa and an increase in TcRγδ lymphocytes and a marked reduction in CD3$^-$ lymphocytes, compared to the total IEL. In addition, the increase in TcRγδ cells

is maintained despite following a gluten-free diet (DSG) and the subsequent clinical and histological remission (3,4). In pediatric patients, flow cytometry has shown a high diagnostic sensitivity and specificity (94%) (4). In addition, it allows the diagnosis of CD in adult patients, including complicated cases (5,6), and can differentiate type 1 and type 2 refractory CD (7,8).

A retrospective analysis of the IELs found in the duodenal mucosa was performed by flow cytometry (lymphogram) since 2009 at the Hospital Clínico Universitario de Valladolid (Spain). This analysis was performed thanks to the collaboration with the Mucosal Immunology Laboratory from the Institute of Molecular Biology and Genetics (IBGM, Universidad de Valladolid-CSIC). The aim of this study was to communicate our experience and results with the technique in the diagnosis of CD over a period of almost eight years.

MATERIAL AND METHODS

Patients and samples

This study analyzed the results of 175 lymphograms performed from March 2009 to November 2017 in patients who underwent duodenal biopsies due to a suspicion or previous diagnosis of CD. Patients whose biopsies did not allow an adequate histological examination and the biopsies of patients on a GFD without a confirmed prior diagnosis of CD were excluded from the analysis. Thus, a total of 157 patients were included in the study.

The diagnosis of 21 new CD cases was established according Fasano's criteria (9). All patients had compatible symptoms and villus atrophy. Eighteen of the 21 patients had a positive serology at the time of diagnosis and CD risk genotypes in the remaining three patients with a diagnosis determined due to the recovery of the histological lesion following a GFD. Serology was considered as positive when the IgA anti-TG2 titer was > 10 IU/ml. Genetic risk was assessed based on recently published recommendations (10). Fourteen patients had a previous CD diagnosis, all had a biopsy showing villous atrophy and a positive serology at diagnosis. One hundred and twenty two of the 157 patients with a lymphogram were non-CD.

Depending on the indication for the gastroscopy, *Helicobacter pylori* infection was studied in 86 cases, either by a rapid urease test or via biopsies of the gastric mucosa of the antrum and body. Seventy-three belonged to the non-CD group of patients. Three biopsies of the second portion of the duodenum and one of the duodenal bulb were collected, which were routinely processed for the pathological examination. The flow cytometry lymphogram was performed using one or two biopsies taken in the second portion of the duodenum.

Intraepithelial lymphogram by flow cytometry

Biopsies for flow cytometry were maintained in ice-chilled PBS until they were received at the laboratory within an hour. Epithelial cells and IELs were separated from the submucosa in HBSS (Lonza, Switzerland) supplemented with 1 mM dithiothreitol (DTT, Sigma-Aldrich) and 1 mM ethylenediaminetetraacetic acid (EDTA, Sigma-Aldrich) for 45 minutes at 37 °C, with stirring. Epithelial cells and IEL were obtained from the supernatant after a ten-minute centrifugation at 400 g. Specific antibodies against CD45-PE-Cy7 (HI30), anti -CD3-APC (SP34-2) and anti-TcRγδ-FITC (B1) (all from BD, USA) were used. Cells were resuspended in cytometer staining buffer (PBS supplemented with 1 mM EDTA and 0.02% sodium azide) and incubated for 20 minutes at 4 °C in the dark with the antibodies. Cells were washed twice in staining buffer and immediately acquired in an FC500 cytometer and CXP analysis program (Beckman Coulter). The cells were immediately acquired in an FC500 cytometer and CXP analysis program (Beckman Coulter). The total IEL population was identified as CD45$^+$. Within this population, the expression of CD3 and TcRγδ (referred to isotype controls) was studied. In this way, the proportion of CD3$^-$ (CD45$^+$CD3$^-$) and the proportion of TcRγδ (CD45$^+$CD3$^+$TCRgd$^+$) were determined within the total IELs.

Clinical and demographic information was obtained from each patient including age at the time of taking the biopsies, gender, current or previous diagnosis of CD and the date of diagnosis. The possible association between these variables and the results from the lymphogram (percentage of total IELs, CD3$^+$ lymphocytes, CD3$^-$ lymphocytes, Tγδ lymphocytes and γδ/CD3$^-$ ratio) was studied.

In addition, the results of the lymphogram were compared with IgA anti-TG2 titer and time on a GFD at the time of the lymphogram in the group of 32 CD patients with positive serology at diagnosis. The correlation between the results of the lymphograms and the value of hemoglobin and peripheral blood lymphocytes (in 119 patients), serum iron (in 118 patients), serum vitamin B12 (in 97 patients), C-reactive protein (in 67 patients), the presence of *Helicobacter pylori* (in 76 patients) and treatment with angiotensin receptor antagonist 2 (ARA2) (followed by eight patients) were studied in the group of non-CD patients.

The ethical guidelines of the 1975 Declaration of Helsinki (revised in 1983) were followed when performing the study and the permission of the Ethics Committee of the Hospital Clínico de Valladolid was obtained.

Statistical methods

Categorical variables were expressed as absolute and relative frequencies and were compared using the Fisher's test. The Kolmogorov-Smirnov test confirmed that all continuous variables followed a non-parametric distribution and therefore, were expressed using the median and interquartile range and compared with the Mann-Whitney U test. The concordance between the two forms of diagnosis were calculated using the Kappa index. The correlation between two continuous variables was obtained using the Spearman's test. The area under the curve of the percentage of TcRγδ lymphocytes and the ratio of TcRγδ lymphocytes/CD3⁻ cells was calculated for the diagnosis of CD in all patients and also in patients who followed a GFD. The IBM SPSS Statistics 20 and GraphPad Prism 5 programs were used for statistical analysis and graphic design.

RESULTS

Patients with a previous diagnosis of CD

Of the total lymphograms performed, 14 corresponded to CD patients following a GFD since diagnosis, for a median of 85 months, and the interquartile range was 88.5. Of these, 78.6% were female and their median age was 57 years, with an interquartile range of 22. All had a positive serology (IgA anti-TG2 > 10 IU/ml) at diagnosis. At the

time of performing the lymphogram, four patients had a positive serology and ten had villous atrophy.

New patients diagnosed with CD

A new diagnosis of CD was determined in 21 patients. All were following a gluten containing diet (GCD), 71.4% were female, with a median age of 32.0 years and an interquartile range of 26. Eighteen had a positive serology with an IgA anti-TG2 value of 85.87 IU/ml and an interquartile range of 55. Recovery from villous atrophy was confirmed with the GFD in the three patients with a negative serology.

Non-CD patients

The group of non-CD patients included 122 patients, 63.1% were female, with a median age of 41 years and an interquartile range of 29. Serology analysis (IgA anti-TG2) was determined in 83 patients and was negative in all cases. Four patients had a severity score of Marsh 1, one patient was Marsh 2 and five patients were Marsh 3. One of the patients with villous atrophy was being treated with olmesartan, another suffered from non-Hodgkin lymphoma, a third patient was receiving treatment with NSAIDs and there were no clinical and histological changes after a GFD in the remaining two patients.

The lymphogram in the diagnosis of CD

Table 1 and figure 1 show the values of IELs, CD3$^-$ lymphocytes, TcRγδ lymphocytes and the TcRγδ/CD3$^-$ lymphocyte ratio in the three groups of patients. The area under the ROC curve of the TCRγδ lymphocyte value for CD diagnosis (21 CD patients out of 143 patients) was 0.83 (95% CI, 0.718-0.956) and the best cut-off point was 13. The index of Kappa between the new diagnosis of CD based on the Fasano's criteria and the percentage of TCRγδ lymphocytes > 13 was 0.474.

A more stringent analysis was performed with only the 18 patients with a positive serology, which were compared with 80 controls with a Marsh 0 biopsy and a negative serology at the time of lymphogram. The area under the curve (ROC) of the TCRγδ lymphocyte value for the diagnosis of CD was 0.868 and the best cut-off point was

14.5. The Kappa index based on these more stringent criteria was 0.579.

Influence of the GFD on the lymphogram

The influence of the GFD on the results of the lymphogram is shown by comparing the values of the lymphogram of the 35 CD patients according to the diet they were following (Table 1 and Fig. 1). The area under the ROC curve of TCRγδ lymphocytes for the diagnosis of CD was 0.78 (95% CI, 0.687-0.878) and 13.4 was the best cut-off point. This analysis included all patients, as well as the 14 CD patients who were already following a GFD. The Kappa index was 0.422. Furthermore, the results of the CD lymphogram with a positive serology at diagnosis correlated with the time on a GFD (IEL r = -0.136, p = 0.458; CD3⁻ r = 0.442, p = 0.011; TcRγδ r = -0.445, p = 0.011; TcRγδ/CD3 r = -0.549, p = 0.001) and with the IgA anti-TG2 titer at the time of the lymphogram (IEL r = 0.412, p = 0.019; CD3⁻ r = -0.402, p = 0.022; TcRγδ r = 0.400, p = 0.023; TcRγδ/CD3⁻ r = 0.447, p = 0.012) (Fig. 2). However, no statistically significant differences were observed between the lymphogram results from CD patients when patients with villous atrophy and those without histological lesion were compared: IEL 10.85 (8.77) *vs* 4.92 (1.81), p = 0,110; CD3⁻12.00 (19.50) *vs* 22.50 (20.25), p = 0.269; TcRγδ 16.60 (11.00) *vs* 13.50 (6.75), p = 0.334; ratio γδ/CD3⁻ 29 (5.26) *vs* 0.611 (1.83), p = 0.305.

Influence of other factors in non-celiac patients

The correlation between age, hemoglobin, serum iron, levels of vitamin B12, peripheral blood lymphocytes and C-reactive protein (CRP) with the values of the lymphogram was studied in non-CD patients. A statistically significant correlation was found between the values of IEL with hemoglobin (r = -0.336, p < 0.001) and serum iron (r = -0.239, p = 0.009), between TCRγδ lymphocytes and serum iron (r = -0.206, p = 0.026) and between the TCRγδ/CD3⁻ ratio and vitamin B12 levels (r = -0.256, p = 0.012). The percentage of CD3⁻ lymphocytes was lower in the group of non-CD patients with a *Helicobacter pylori* infection when 31 patients with *Helicobacter pylori* infection were compared with 42 patients without an infection (CD3⁻ 15.10 [11.00] *vs* 27, 00 [15.45], p = 0.007). Eight of the 117 non-CD patients received treatment with ARA2-type drugs

(one patient olmesartan, two candesartan, two telmisartan and three valsartan) at the time of the biopsy for the lymphogram. The patient undergoing treatment with olmesartan had villous atrophy. Patients receiving ARA2 had a higher number of TCRγδ lymphocytes compared to the remaining controls (12.00 [15.00] *vs* 6.10 [6.62], p = 0.045).

DISCUSSION

Although the results presented here are consistent with other series, the typical pattern of CD in our study in active patients is not as clear as in previously published studies. In these studies, the percentage of CD3⁻ is lower and that of TcRγδ lymphocytes is higher (CD3⁻: 3.1% and 4.1 and TcRγδ lymphocytes: 35.7% and 26.5%) (6,11). The area under the ROC curve for a CD diagnosis in patients under a gluten diet is 0.83 for TcRγδ lymphocytes and 0.87 for the TcRγδ/CD3⁻ ratio. The Kappa index of agreement with the diagnosis based on the Fasano criteria (9) was moderate in both cases. The values obtained in this study were improved when only patients with strict criteria for CD were included in the study, such as atrophy and positive serology *versus* Marsh 0 with negative serology. However, it should be taken into account that the lymphogram is really useful in those cases with a difficult diagnosis, i.e., villous atrophy with a negative serology (8). The number of patients included in our study prevented us from exploring this.

Our series only includes adult patients with a median age of 41 years and we think this could explain some of the differences between the results published by other studies (4,6). As with serology, the immunophenotype of the CD lymphogram in adults may not be as clear as in children (12,13). Other factors such as treatment with ARA2 may influence our results. In addition, we cannot rule out some variability in the methodology between the groups and, as in our case, by the changes in those responsible for performing the technique throughout these eight years.

Despite the persistent elevation of TcRγδ lymphocytes in CD patients who follow a GFD (4,11,13), we confirmed that all lymphogram results were modified in adult patients following this diet. The influence of the GFD is shown in the diagnostic capacity of the lymphogram, as there was a reduction in the area under the ROC curve and the Kappa

index of TcRγδ lymphocytes when CD patients on a GFD were included in the analysis. In any case, the percentage of TCR lymphocytes in CD patients on a GFD is higher than in non-CD patients, even though it is lower than in those following a gluten-containing diet. This constitutes an important diagnostic resource in patients on a GFD.

Given the diagnostic relevance of serology, a correlation between the IgA anti-TG2 titer and the proportion of TcRγδ lymphocytes in patients with CD and a positive serology at diagnosis was found. IgA anti-TG2 titers could be an indicator of compliance with the GFD and this is consistent with the conclusions from previous studies (12). In addition, we confirmed that the different parameters evaluated by flow cytometry correlated with time since the diagnosis was determined and therefore, with the duration of the GFD.

Some studies have confirmed the association between the degree of the histological lesion and the lymphogram, especially for TcRγδ lymphocytes (12). However, others have found a constant increase of TcRγδ lymphocytes in latent and potential forms of CD (4), and this does not change despite the degree of histological lesion (13). Our data are very limited by the small number of cases included. Thus, when lymphograms from CD patients with villus atrophy and those without histological lesion were compared, the differences were not statistically significant.

In non-CD patients, a weak negative correlation was observed between the total number of IELs and the hemoglobin and serum iron values. The association between *Helicobacter pylori* and duodenal lymphocytosis, together with other factors such as treatment with NSAIDs, inflammatory bowel disease or bacterial overgrowth are discussed (14,15). Despite the negative correlation between hemoglobin and serum iron with the IEL and the TcRγδ numbers and the described association between iron deficiency anemia and *Helicobacter pylori* infection, the percentage of IELs was not increased in those cases with infection, although these patients showed a reduction of the CD3⁻ fraction.

We also found that treatment with ARA2 was associated with an increase in TcRγδ lymphocytes. This is of great importance, as although olmesartan-induced CD-like enteropathy is not frequent, the differential diagnosis between this entity with CD is very important (16,17). In fact, some of these patients have been described as having a

typical lymphogram of CD (17).

Among the limitations of this study is the absence of serology results in 39 of the patients, all of which had a biopsy without histological lesions and were considered as non-CD. Furthermore, the lymphograms were performed during different periods of time by four of the authors of the study (DB, CE, EM, RP) and *H. pylori* infection was not studied in all patients. In addition, it is known that compliance with a GFD, both in children and adults, is poorer than expected. It has been shown that dietary questionnaires and serology are not accurate tools to assess this compliance (18). Compliance or non-compliance with the GFD could have been more firmly established by the detection of immunogenic gluten peptides in feces and not only by anamnesis or serology. However, this technique was not commercially available for the majority of cases of the time when the lymphograms were performed.

In conclusion, the usefulness of the duodenal lymphogram in the diagnosis of CD is clear in adult patients. However, factors such as compliance with the GFD, duration and other factors that can influence the lymphogram of non-CD patients and the proper standardization of the technique should be taken into account for its correct interpretation.

REFERENCES

1. Camarero C, León F, Sánchez L, et al. Age-related variation of intraepithelial lymphocytes subsets in normal human duodenal mucosa. Dig Dis Sci 2007;52(3):685-91. DOI: 10.1007/s10620-006-9176-3

2. Eiras P, Roldán E, Camarero C, et al. Flow cytometry description of a novel CD3-/CD7+ intraepithelial lymphocyte subset in human duodenal biopsies: potential diagnostic value in coeliac disease. Cytometry 1998;34(2):95-102. DOI: 10.1002/(SICI)1097-0320(19980415)34:2<95::AID-CYTO6>3.0.CO;2-B

3. Roy Ariño G. Análisis de los linfocitos intraepiteliales por citometría de flujo en la enfermedad celiaca. In: Arranz EGJ (ed.). Enfermedad celiaca. Introducción al conocimiento de la enfermedad celiaca. 3rd ed. Spain: Ergon; 2016. pp. 167-78.

4. Camarero C, Eiras P, Asensio A, et al. Intraepithelial lymphocytes and coeliac disease: permanent changes in CD3-/CD7+ and T cell receptor gammadelta subsets

studied by flow cytometry. Acta Paediatr 2000;89(3):285-90.

5. Valle J, Morgado JMT, Ruiz-Martín J, et al. Flow cytometry of duodenal intraepithelial lymphocytes improves diagnosis of celiac disease in difficult cases. United European Gastroenterol J 2017;5(6):819-26. DOI: 10.1177/2050640616682181

6. Fernández-Banares F, Carrasco A, García-Puig R, et al. Intestinal intraepithelial lymphocyte cytometric pattern is more accurate than subepithelial deposits of anti-tissue transglutaminase IgA for the diagnosis of celiac disease in lymphocytic enteritis. PloS One 2014;9(7):e101249. DOI: 10.1371/journal.pone.0101249

7. Leon F. Flow cytometry of intestinal intraepithelial lymphocytes in celiac disease. J Immunol Methods 2011;363(2):177-86. DOI: 10.1016/j.jim.2010.09.002

8. Grupo de Trabajo del Protocolo para el Diagnóstico Precoz de la Enfermedad Celiaca. Protocolo para el diagnóstico precoz de la enfermedad celiaca. Ministerio de Sanidad, Servicios Sociales e Igualdad. Servicio de Evaluación del Servicio Canario de la Salud (SESCS); 2018.

9. Catassi C, Fasano A. Celiac disease diagnosis: simple rules are better than complicated algorithms. Am J Med 2010;123(8):691-3. DOI: 10.1016/j.amjmed.2010.02.019

10. Núñez C, Garrote JA, Arranz E, et al. Recommendations to report and interpret HLA genetic findings in coeliac disease. Rev Esp Enferm Dig 2018;110(7):458-61.

11. Montalvillo E, Bernardo D, Martínez-Abad B, et al. Increased intraepithelial Vα24 invariant NKT cells in the celiac duodenum. Nutrients 2015;7(11):8960-76. DOI: 10.3390/nu7115444

12. Sánchez-Castañon M, Castro BG, Toca M, et al. Intraepithelial lymphocytes subsets in different forms of celiac disease. Autoimmunity Highlights 2016;7(1):14. DOI: 10.1007/s13317-016-0085-y

13. Calleja S, Vivas S, Santiuste M, et al. Dynamics of non-conventional intraepithelial lymphocytes-NK, NKT, and gammadelta T-in celiac disease: relationship with age, diet, and histopathology. Dig Dis Sci 2011;56(7):2042-9. DOI: 10.1007/s10620-010-1534-5

14. Shmidt E, Smyrk TC, Boswell CL, et al. Increasing duodenal intraepithelial lymphocytosis found at upper endoscopy: time trends and associations. Gastrointest

Endosc 2014;80(1):105-11. DOI: 10.1016/j.gie.2014.01.008

15.   Owen DR, Owen DA. Celiac disease and other causes of duodenitis. Arch Pathol Lab Med 2018;142(1):35-43. DOI: 10.5858/arpa.2016-0608-RA

16.   Sáez González E, Díaz Jaime FC, Del Val Antonana A. Clinical, laboratory, serological, and histological profile of sprue-like enteropathy associated with olmesartan use. Rev Esp Enferm Dig 2016;108(10):685-6. DOI: 10.17235/reed.2016.4340/2016

17.   Esteve M, Temino R, Carrasco A, et al. Potential coeliac disease markers and autoimmunity in olmesartan induced enteropathy: a population-based study. Dig Liver Dis 2016;48(2):154-61. DOI: 10.1016/j.dld.2015.09.014

18.   Comino I, Fernández-Banares F, Esteve M, et al. Fecal gluten peptides reveal limitations of serological tests and food questionnaires for monitoring gluten-free diet in celiac disease patients. Am J Gastroenterol 2016;111(10):1456-65. DOI: 10.1038/ajg.2016.439

Table 1. Lymphogram results in non-CD patients under a gluten containing diet (GCD), CD patients on a gluten free diet (GFD) and CD patients on a GCD. Median and interquartile ranges

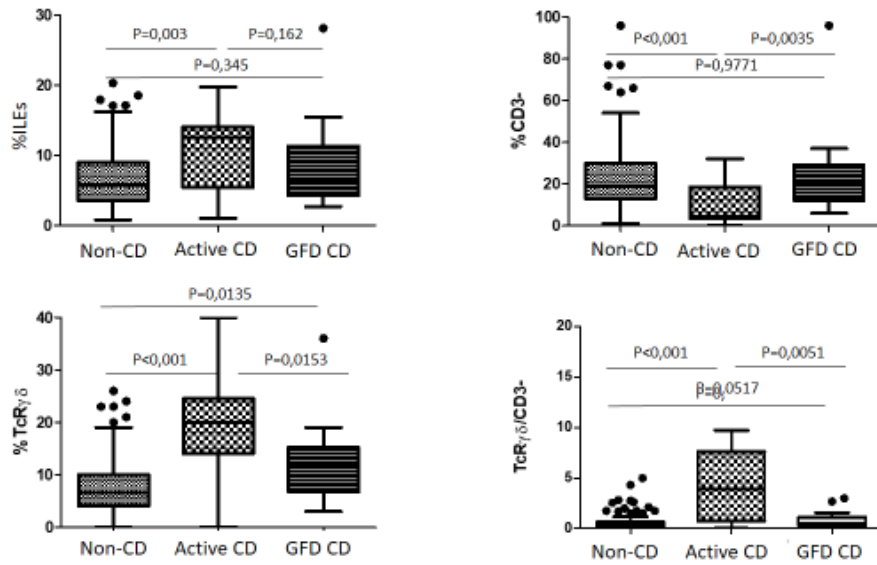|	n	IELs	CD3⁻	TcRγδ	TcRγδ/CD3⁻
Non-CD patients, GCD	122	5.69 (5.42)	19.00 (17.00)	6.70 (6.00)	0.30 (0.53)
CD patients , GFD	14	6.47 (7.05)	20.00 (17.25)	12.00 (8.50)	0.49 (0.88)
CD patients, GCD	21	12.55 (8.88)	5.1 (14.75)	20.50 (8.70)	3.96 (6.89)

Fig. 1. Lymphogram results in non-CD patients, active CD patients and GFD CD patients.
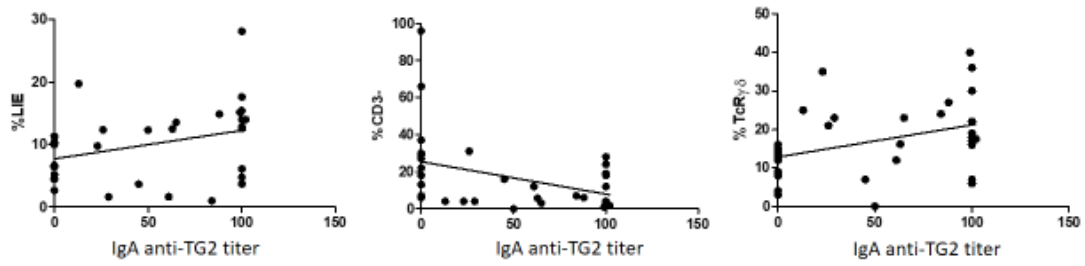Median and interquartile ranges. Mann-Whitney U test.

Fig. 2. Correlation of the lymphogram results and IgA anti-TG2 titer at the time of the lymphogram in 32 CD cases with a positive serology at the time of diagnosis. Spearman's test.