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The Role of Serological Testing and HLA Genotyping in the Diagnosis of Celiac Disease in Slovak Cohort. Can Duodenal Biopsies be Omitted?

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Abstract Presented analysis focuses on usefulness of consecutive celiac disease antibodies testing anti-TG2 and EMA, and HLA-DQ2/DQ8 genotyping in symptomatic children and adolescents with suspected celiac disease refered to the latest ESPGHAN guidelines (2012), that permit confirmation of celiac disease without previous duodenal biopsy. A total of 258 children and adolescents (86 male and 172 girls), aged 2 to 18 years, were retrospectively examined performance of celiac disease testing according national guidelines from 2009 included duodenal sampling and the ESPGHAN nonbiopsy criteria in a pediatric population. In applying nonbiopsy criteria to our cohort, 33,3 % (86) of symptomatic children and adolescents with such high anti-TG2 titers and positive EMA could have been initially diagnosed without an intestinal biopsy. All of them presented with advanced intestinal atrophy Marsh 2-3. Part of the rational of the study was to determine sensitivity and specificity, positive and negative predictive values of the final laboratory tests and diagnostic accuracy of the combined tests for antibodies.

Keywords: *celiac disease (CD), ESPGHAN, anti-TG2, EMA, HLA-DQ2/DQ8*

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1. Introduction

The recent ESPGHAN (European Society of Paediatric Gastroenterology, Hepatology and Nutrition) guidelines for the diagnosis of celiac disease (CD) allow to avoid duodenal sampling in certain clinical situation. Nevertheless, regional specifications for the diagnosis of CD almost in Europe countries may differ. Currently, in Slovak Republic, small-bowel biopsy is still requested for determination of CD, while genetic testing is still omitted. The aim of study was to validate diagnostic yields of the tissue transglutaminase (anti-TG2), endomysial (EMA) antibody tests and the genotyping of HLA-DQ2/HLA-DQ8 for the ESPGHAN non-biopsy criteria in paediatric CD cohort [1].

2. Materials and Methods

We performed a retrospective analysis in a group of 258 CD patients (172 female, 86 males, age 2-18 years) with consecutive duodenal biopsy. Histology reports were graded according to the Marsh classification (Marsh 0-1 n=25; Marsh 2-3 n=233). Blood samples were tested for IgA, anti-tTG IgA (ELISA, Inova USA), EMA (indirect IF, Euroimmun) and HLA-DQ alleles associated with CD (microarray, Euroimmun) (Table 1).

3. Results

Overall, 154 out of 258 patients had antibody titres against anti-TG2 IgA tissue transglutaminase 10 times higher than the upper limit of normal (ULN), out of which only three patients had findings at a Marsh classification of 0 to 1, which represents a score of 1.2 %. In 114 symptomatic patients only 2.6 % had findings at a Marsh classification of 0 to 1. Sensitivity analysis in this test reached 66.1 %, specificity 76.9 % and area under the curve (AUC) 0.715.

The statistical data analysis showed that the patients who had antibody titres of anti-TG2 IgA more than 10 times higher than the ULN had their histological findings (at a Marsh classification of 2 to 3) more than 13 times greater than the patients with titres increased less than 10 times - 94.2% vs. 70.7%, chi2 = 26.16; P<0,001; OR=13.5.

In terms of endomysial antibodies (EMA), 178 out of 258 patients had positive test results, which represents almost 69.0 %. Only 9 patients from this group had a positive finding at a Marsh classification of 0 to 1, representing 5.0 %. In 127 symptomatic patients only 3.2 % had findings at a Marsh classification of 0 to 1. Sensitivity in this test reached 73.2 %, specificity 69.2 % and AUC 0.720.

The data analysis showed that the patients with positive EMA antibodies had nearly 5 times greater histological changes at a Marsh classification of 2 to 3 than the patients negative for EMA antibodies (90.8% vs. 70.4%, chi2 = 14.1; P<0.0002, OR = 4.7).

The results divided according to the titre of anti-TG2 antibodies, more or less than 10 times higher than the ULN; the positive or negative EMA tests are shown in Table 2.

The HLA-DQ antigens were examined in 258 patients with CD. The study was focused on positivity of HLA-DQ2, HLA-DQ2.2, HLA-DQ2.5, HLA-DQ2.2 plus 2.5, HLA-DQ8 and their combinations HLA-DQ2.2 plus HLA-DQ8 and HLA-DQ 2.5 plus HLA-DQ8, which are related to predisposition to CD. The results are presented in Table 3.

None of the associated DQ alleles were found in 41 patients. The frequency of all predisposing DQ alleles in the group of patients with CD was 84.1%. Up to 26.7% of the patients had a double amount of predisposing DQ-alleles (DQ2 plus DQ2, DQ2 plus DQ8), indicating the importance of the DQ gene in a predisposition for CD.

The number of symptomatic patients out of the whole group of 217 patients with a positive finding of HLA-DQ antigens was 155. The distribution of these patients is presented in Table 4. They had a finding at a Marsh classification of 0 to 1; two patients, representing 1.3 %, with this test's sensitivity 91.0 % , specificity 84.6 % and AUC 0.892. Data analysis showed that up to 91.2 % of the CD patients with a predisposing DQ allele had a significant histological finding at a Marsh classification of 2 to 3; only 51.2 % patients without these alleles had such a finding (chi2 = 56.23; P <0.0001). The extent of this relationship is expressed with OR = 18.5, which means that the risk of severe histological changes is about 18 times higher for the carriers of DQ-alleles.

In the next step, the symptomatic patients, HLA-DQ2 and/or DQ8 positive, were added to the group of symptomatic patients with highly positive serology. Thus, we have obtained the combined results suitable for the evaluation under the new 2012 ESPGHAN guidelines set out in Table 5.

The overview of the overall analysis procedure is shown in Figure 1, where the highlighted part refers to the results of serology and histological findings in the group of patients tested according to the 2012 ESPGHAN guidelines.

The evaluation of diagnostic methods was performed with the receiver operating characteristic (ROC) curve and the AUC value as an appropriate tool for the evaluation and optimisation of tests [2].

Figure 2 shows diagnostic accuracy of the combined testing in symptomatic patients with highly positive serology (tTG > as 10 times of the ULN and with a positive test for endomysial antibodies EMA) and in symptomatic patients with positive HLA-DQ2 and/or DQ8 test results. The evaluation of the test quality is considered as very good.

Table 1. Demographic data of symptomatic and asymptomatic patients with histologic Marsh grading

Histologic grading	Female		Male	
	n=172		n=86	
	Symptomatic n=122	Asymptomatic n=50	Symptomatic n=59	Asymptomatic n=27
Marsh 0-1	4	10	9	2
Marsh 2	2	5	2	2
Marsh 3a	27	11	14	6
Marsh 3b	44	11	19	12
Marsh 3c	45	13	15	5

Table 2. Distribution of histologic Marsh grading im symptomatic patients according consecutive TG2 and EMA and intestinal histological findings

Histologic grading	anti - TG2 IgA		EMA	
	>10x ULN	<10x ULN	positive	negative
Symptomatic patients	114	67	127	54
Marsh 0-1	3	10	4	9
Marsh 2	1	3	3	1
Marsh 3a	12	29	23	18
Marsh 3b	45	18	46	17
Marsh 3c	53	7	51	9

Table 3. Distribution of histologic Marsh grading and frequency of HLA antigens allels

Histologic grading	HLA DQ 2/DQ8 positive n=217						
	DQ2	DQ2.2	DQ2.5	DQ8	DQ2.2 +2,5	DQ2.2 +DQ8	DQ2.5 +DQ8
	41	50	50	18	42	10	6
Marsh 0-1	8	0	0	0	0	0	0
Marsh 2	3	3	2	2	1	0	0
Marsh 3a	9	7	12	2	5	3	2
Marsh 3b	8	22	20	8	18	3	1
Marsh 3c	13	18	16	6	18	4	3

Table 4. Distribution of histologic Marsh grading in symptomatic patients and HLA-DQ testing

Histologic grading	HLA DQ2/DQ8	
	positive	negative
Symptomatic patients	155	26
Marsh 0-1	4	10
Marsh 2	2	5
Marsh 3a	27	11
Marsh 3b	44	11
Marsh 3c	45	13

Table 5. Distribution of histologic finding in symptomatic patients according combination of high titers positivity of serological tests and genetic analys

Histologic grading	anti TG2 > 10x ULN + positive EMA + HLA DQ positive
Symptomatic patients	86
Marsh 0-1	0
Marsh 2	1
Marsh 3a	8
Marsh 3b	32
Marsh 3c	45

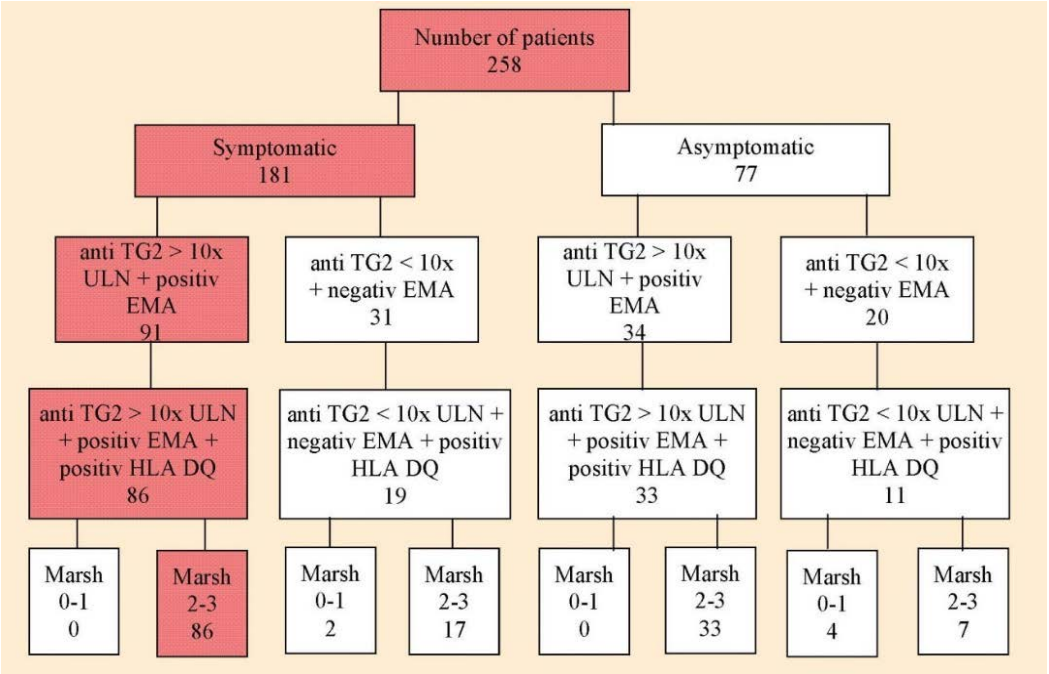

Figure 1. Serological results characteristic for coeliac disease and histological findings in the patient group

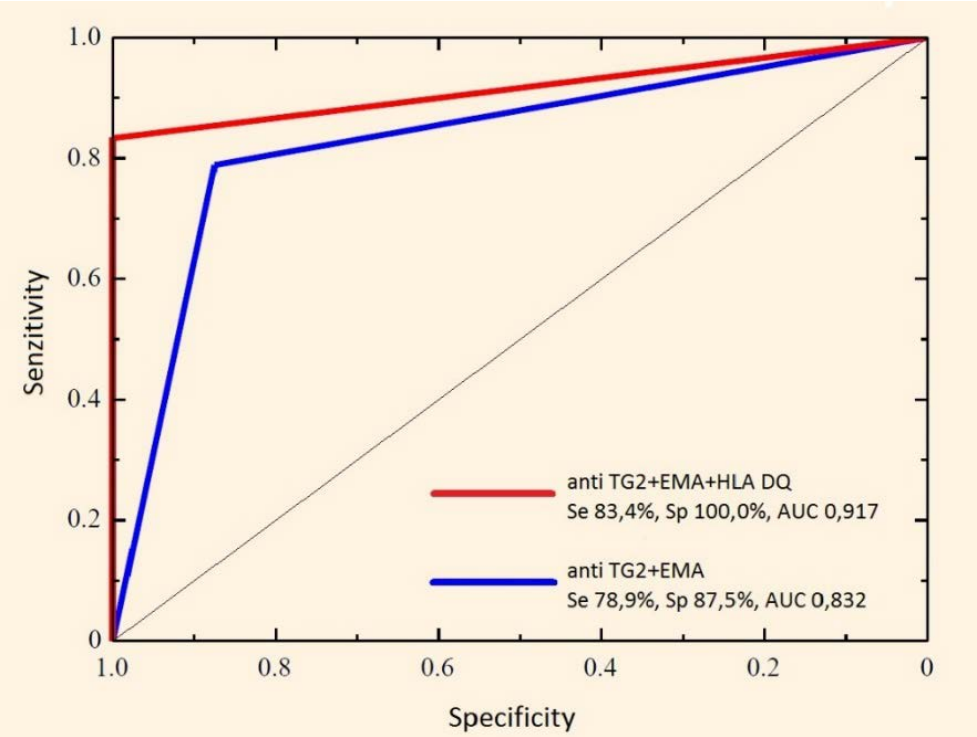

Figure 2. Diagnostic accuracy of the combined tests for antibodies to histological findings in the patient group

4. Discussion

Out of the whole group of 258 patients, 86 patients (33.3 %) met these criteria, with one patient having a histological finding at a Marsh classification of 2 and 85 patients at a Marsh classification of 3. The group of 86 patients is formed of the patients who meet the conditions under the 2012 ESPGHAN guidelines, i.e. their examination can be performed without an intestinal biopsy under the above stated 2012 ESPGHAN guidelines. Sensitivity in this group has reached 83.4 %, specificity 100 % and AUC 0.917. Stated results fully correspond with results of other works [2,3,4].

The significance of laboratory serological and genetic tests, carried out accurately, in a prescribed manner, is increasing, as their results can be used directly for making the diagnosis of CD without enterobiotic examinations [5]. Currently in Slovakia, guidelines for the diagnosis and pharmacotherapy of CD need to be harmonised with ESPGHAN in a way that would not alter the current system of prescribing gluten-free foods in Slovakia [6].

5. Conclusion

The study in Slovakia applied the 2012 ESPGHAN guidelines and showed that more than 33.0 % of children and adolescents meet the criteria for omitting enterobiotic examinations in making the diagnosis of CD. This number represents a very large group, up to a third of all patients with suspected CD. If, in making the diagnosis of CD, serological and genetic tests in the clinical practice in Slovakia have been until now considered as supportive and histological findings as crucial, the 2012 ESPGHAN guidelines bring to the process of making the diagnosis of CD a significant change.

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