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POTENTIAL COELIAC DISEASE MARKERS AND AUTOIMMUNITY IN OLMESARTAN INDUCED ENTEROPATHY: A POPULATION-BASED STUDY

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Abreviations:
HUMT: Hospital Universitari Mutua de Terrassa, CD: Coeliac disease; IEL: intraepithelial lymphocyte, ARB: angiotensin II receptor blocker; anti-TG2: tissue transglutaminase antibodies; EmA: anti-endomisial antibodies; ANA: anti-Nuclear antibodies; ENA: extractable nuclear antigens. positive (ENA); VDR: vitamin D receptor.

Short running title: Incidence of Olmesartan induced enteropathy

Key words: Lymphocyte subpopulations; anti-TG2 IgA deposits; Lupus-like disease; Sprue-like.

ABSTRACT

Aims: 1) Assess the population-based incidence of severe olmesartan-associated enteropathy. 2) To describe patients of the Spanish registry, 2) Evaluate markers of potential celiac disease and associated autoimmunity.

Methods: Crude incidence rates in the area of Terrassa (Catalonia) were calculated. Clinical characteristics of patients in the Spanish registry were collected. Duodenal lymphocyte subpopulations and anti-TG2 IgA deposits were assessed in a subset of patients.

Results: Annual incidence rates (2011-2014) ranged from 0 to 22 cases per 104 treated patients. Twenty patients were included in the Spanish registry. Nineteen (95%) exhibited villous atrophy and 16 (80%) had severe enteropathy. Lupus-like disease occurred during olmesartan treatment in 3 patients. HLA-DQ2/DQ8 was positive in 64%. Markers of potential coeliac disease were present in 4 out of 8 patients (positive anti-TG2 deposits and/or increased CD3+gammadelta+ intraepithelial lymphocytes and reduced CD3-).

Histopathological changes and clinical manifestations including autoimmune disorders improved after olmesartan discontinuation but not after gluten-free diet, irrespective of the presence or not of coeliac markers.
Conclusions: Incidence of severe olmesartan-associated enteropathy was low. Autoimmune phenomena were present in a subset of cases and reversed after olmesartan removal. A genetic coeliac disease background and the presence of potential coeliac markers might uncover predisposing factors.

INTRODUCTION

In 2012 a new non-coeliac sprue-like enteropathy associated with olmesartan was characterized [1] Since then more than 100 cases have been reported,[2,3] including a cohort of 36 patients in a national survey in France.2 The clinical picture include severe diarrhea, frequently life-threatening, requiring hospitalization and treatment in intensive care units. The incidence of this enteropathy in olmesartan-treated patients and the whole spectrum of severity are unknown[4]. It is noteworthy that large phase III trials did not detect gastrointestinal adverse reactions in olmesartan-treated patients compared with those treated with placebo.[5,6] This discrepancy might be either the short duration of clinical trials, rendering them unable to detect a complication that occurs in medium- or long-term sustained treatment and/or the rarity of this severe entity.[4,5]

The mechanism underlying olmesartan-associated enteropathy is unknown but a high proportion of the reported patients had a genetic predisposition for coeliac disease (CD), ranging from 68 to 77%.[1,2] which is clearly higher than that described in the general population. It was speculated that the presence of HLA-DQ2 or HLA-DQ8 might increase the risk of immune-mediated damage.1 In addition, a number of patients in the French series had a past history of immune-mediated diseases.2 However, to our knowledge autoimmune phenomena associated with olmesartan administration have not been reported to date.

Olmesartan is an angiotensin II receptor blocker (ARB) widely used in the treatment of hypertension.[7] Apart from the known vascular effects, angiotensin II receptor AT (2) interferes with proinflammatory pathways including inhibition of cell growth, modulation of extracellular matrix, apoptosis and cellular differentiation.[8]

The aims of this study were to: 1) Determine the population-based incidence rate of olmesartan enteropathy in the area of Terrassa, Catalonia, Spain. 2) Describe the clinical picture of olmesartan-induced enteropathy and other immune disorders, and 3) Assess the genetic predisposition of CD and duodenal CD markers that could predispose to olmesartan damage.

Patients And Methods

Study setting for incidence calculation
The study period for estimating the annual population-based incidence of enteropathy associated with olmesartan was January 2011 to December 2014 and the setting was the catchment area of the Hospital Universitari Mutua Terrassa (HUMT). The hospital is located in north-eastern Spain (Catalonia), and it is of a mixed rural-urban type. On August 15, 2014, the population residing in this geographical area was 25[1,2]25 inhabitants (data obtained from the 2014 population census, ‘Institut d’Estadística de Catalunya’). In this area there is only one hospital, for both public and private practice, in which the only endoscopy unit and pathology departments in the area are located. The hospital offers universal coverage for primary and specialist services, with an established system for referral from primary to secondary care. There are some private practitioners in the area, but private gastroscopy and biopsies are performed at the hospital, and the same diagnostic protocol is followed to evaluate patients with chronic watery diarrhea in the outpatient clinics and during hospitalization. In fact, the Department of Gastroenterology of the hospital is considered a referral centre for chronic diarrheal diseases. This type of organization of the health provision has allowed us to perform epidemiological studies as we previously did in other digestive diseases. [9,10] Eight of the patients in the Spanish cohort were attended at the HUMT, but only 6 of them were resident in the catchment area of the hospital during the study period. The other 2 patients, residents of other areas, were excluded from calculation of incidence. To ensure that there were no lost cases of sprue-like enteropathy associated with olmesartan in the study area, the treatment and final diagnosis of all patients with a pathological diagnosis from the CD spectrum during the study period were reviewed.

Recruitment of the Spanish cohort of olmesartan-associated enteropathy

Cases of enteropathy associated with olmesartan were diagnosed at HUMT and presented to the meeting of the Spanish Gastroenterological Association. [11] Colleagues from six hospitals in Spain agreed to participate in a multicenter registry of cases diagnosed in their respective hospitals. Investigators were asked to contribute to an anonymous electronic database with clinical characteristics of the patients in the cohort. Patients were included if they met the following criteria: 1) chronic diarrhea or symptoms suggestive of enteropathy lasting for more than 4 weeks while treated with olmesartan, 2) histological abnormalities of the spectrum of CD, and 3) recovery after olmesartan withdrawal.

A total of 20 patients were included. Information collection was ongoing at the time of completion of this manuscript in February 2015. The institutional review board of the HUMT approved the study.

Clinical and laboratory data

The presence of the following symptoms was investigated: diarrhea, abdominal distension, abdominal pain, vomiting, and weight loss. The outcome after olmesartan discontinuation was recorded, as was the outcome after gluten-free diet and/or immunosuppressant therapy when administered. The presence of systemic and organ-specific autoimmune disorders was registered in relation to olmesartan (before, during and after withdrawal).
Routine biochemical and hematological profiling was performed to detect anemia, hypoalbuminemia, iron and other vitamin deficiencies (Vitamin D, folic acid and vitamin B12), renal failure, and electrolyte disorders (sodium, potassium, calcium, phosphate, and magnesium).

The clinical picture was considered to be severe if there were acid-base and electrolyte disorders, renal failure due to dehydration, and required hospital admission for disease control, as an indirect surrogate marker of severity.

The CD serological profile included: 1) IgA-tissue transglutaminase antibody (human anti-TG2) (or IgG anti-TG2 in IgA deficient patients) (ELISA detection kit EliaCelikeyTM, Phadia AB, Freiburg, Germany) and 2) IgA anti-endomisial antibodies (EmA) in patients with anti-TG2 values ranging from 2 to 8 U/mL (grey zone). The autoimmune serological profile included: anti-Nuclear antibodies (ANA) and IFI pattern, anti-dsDNA, and conventional extractable nuclear antigens if ANA positive (ENA; U1-RNP, Sm, SSA, SSB, CENP-B, Topo-I, and Jo-1).

Coeliac disease genetic markers

Genomic DNA from whole blood was purified using commercial QiampDNABlood Mini kit (Qiagen, Düsseldorf, Germany). A commercial reverse hybridization kit for the detection of CD heterodimers HLA-DQ2 (A1*0501/*0505, B1*0201/*0202) and HLA-DQ8 (A1*0301, B1*0302) was used (GenID, GMBH, Strasbourg, Germany). HLA-DQ2 haplotype is present in 18% of healthy controls and 93% of CD patients in our geographical area.[12] Positive coeliac genetics indicates the presence of HLA-DQ2, HLA-DQ8, or HLADQ2 and DQ8.

Histopathological assessment

Four endoscopic biopsies from the duodenum were processed using hematoxylin/eosin staining and CD3 immunophenotyping. Histopathological findings were staged according to the Marsh criteria, as revised by Rostami et al.[13] Lymphocytic enteritis (Marsh 1), recently named microscopic enteritis,[14] was defined as ≥25 or more intraepithelial lymphocyte (IEL) per 100 epithelial nuclei along with normal villous architecture.[15] Two antral mucosal samples were taken to assess gastric mucosal abnormalities in 10 of the 20 patients (50%). Colonic samples were taken in 13 of the 20 patients with normal mucosal appearance to rule out microscopic colitis (65%)[16] in the setting of chronic diarrhea assessment.

Flow cytometry and intestinal deposits of anti-TG2 IgA antibodies

Two additional duodenal biopsies were taken from 8 patients both for IEL subpopulation analysis by flow cytometry and to assess intestinal deposits of anti-TG2 IgA antibodies. A description of both techniques has recently been presented in detail.[17] The antibodies used to define the different IEL subsets were anti-CD45-APC (clone 2D1), anti-CD3-
PerCP (clone SK7), anti-CD103-FITC (clone Ber-ACT8), and anti-TCRγδ-PE (clone 11F2) (all from BD Biosciences, Franklin Lakes, NJ, USA). Cells were immediately analyzed on a standard 4-color FACSCalibur instrument (BD Biosciences, Franklin Lakes, NJ, USA). The complete CD cytometric pattern was defined as TCRγδ >8.5% and CD3− <10%, whereas the incomplete CD pattern was a selective increase of TCRγδ >8.5% based on previously established cut-off.[16] Fig. 1 shows the immunofluorescence (IF) positive and negative staining of intestinal deposits of anti-TG2 IgA antibodies. A positive IF staining of anti-TG2 IgA deposits and complete cytometric pattern are considered to have a high accuracy for CD diagnosis, even in mild forms of the disease.[17,18]

Statistical analysis

Data were expressed as percentages for qualitative variables and with median and range for quantitative variables. The Fisher exact test was used to compare the severity of duodenal lesion (complete atrophy—Marsh 3C—versus partial/subtotal atrophy—Marsh 3B and A) and the duration of olmesartan treatment (<18 months versus >18 months). To estimate cumulative incidence rates, incident cases (numerator of the rate) were those patients fulfilling the inclusion criteria described in this study. The date of incidence was that of the duodenal biopsy showing sprue-like enteropathy. The population exposed (denominator of the rate) were the inhabitants living in the catchment area during the study period and treated with olmesartan for >6 months. Active treatment with olmesartan and its duration among the inhabitants of the study area were collected from the computerized system for electronic prescription (SIRE, CatSalut, Health Department, Autonomous Government of Catalonia), which covers the whole population. The physician in charge inserted the trade name of the prescribed drug, the dose and duration of treatment. These data are available from all pharmacies, hospitals and primary care centers of the public health network in Catalonia. The information regarding olmesartan usage in the community has been provided by the pharmacy of the HUMT. To estimate annual incidence, the quotient was calculated between the new cases of enteropathy associated with olmesartan (incident cases) and the corresponding number of inhabitants exposed to olmesartan in each year of the study period. 95% confidence intervals (CIs) of cumulative incidence were computed.

RESULTS

Annual incidence rates (period 2011-2014) in the catchment area of Terrassa, Catalonia (Spain)

Between January 2011 and December 2014, 6 patients resident in the catchment area of the HUMT were attended due to severe enteropathy associated with olmesartan. The first case was admitted to the hospital with severe diarrhea and dehydration in May 2011. It was not until 2012, after the publication of Rubio-Tapia,1 that the patient was properly diagnosed. Annual incidence rates (period 2011-2014) are detailed in Table 1 Table 1. No other similar cases were found among residents in the study area that had not been treated with olmesartan.
Pathological and clinical findings of olmesartan-associated with enteropathy in the Spanish cohort

Twenty patients (12 females, 60%) from 6 Spanish public hospitals fulfilled the inclusion criteria. Nineteen had variable degrees of duodenal atrophy (9 cases Marsh 3C, 3 cases Marsh 3B, 7 cases Marsh 3A), and 1 patient showed a microscopic enteritis. The median age at inclusion was 73 years (range: 52-89 years). All of the patients had received olmesartan to treat arterial hypertension either alone (6 patients) or in combination with other antihypertensive drugs (olmesartan plus amlodipine in 5 patients, olmesartan plus hydrochlorothiazide in 7 patients, and olmesartan plus amlodipine and hydrochlorothiazide in 2 patients). All except 3 patients received a dosage of 40 mg per day with a median duration of 22 months (range: 5-40 months). There was no relationship between the duration of olmesartan administration and the severity of duodenal damage although a trend toward a higher proportion of patients with complete atrophy was observed in those receiving olmesartan for >18 months versus ≤18 months (78% versus 22%; p = 0.197). Time elapsed between olmesartan prescription and the development of first symptoms was a median of 3 months (range: 1-24 months).

Gastric mucosal biopsies were available for 10 of the 20 patients, with 3 of them showing increased intraepithelial lymphocytes fulfilling the diagnostic criteria of lymphocytic gastritis. Ten out of the 14 patients with multiple colonic biopsies of normal macroscopic mucosa showed the following microscopic abnormalities: 2 paucicellular colitis, 3 lymphocytic colitis, 1 collagenous colitis, 1 active focal colitis, and 3 chronic non-specific inflammation.

Sixteen patients (80%) had criteria of severe disease as previously defined and 14 of them (87.5%) required hospital admission. The most important life-threatening complications were renal failure (60%) and acid-base and electrolyte disorders, mainly hypokalemia (50%). All except the patient with microscopic enteritis had high volume chronic non-bloody diarrhea (95%), and the majority of them had significant weight loss (90%). The patient with microscopic enteritis had chronic relapsing diarrhea starting 6 months after olmesartan initiation. The diarrhea was accompanied by other gastrointestinal complaints: abdominal pain (42%), abdominal distension (16%), and vomiting (42%). The most important biochemical and hematological abnormalities were: iron-deficient anaemia (70%), selective iron deficiency (16%), hypoalbuminaemia (55%), hypocalcemia (16%), vitamin D deficiency (10.5%), and hypomagnesemia (16%).

Autoimmunity associated with olmesartan use

Four patients had previous autoimmune thyroidal diseases (3 autoimmune thyroiditis and 1 Graves-Basedow disease) and 3 patients developed immune-mediated conditions while they were treated with olmesartan, showing a complete recovery after olmesartan withdrawal. The most striking case considered to have an immune-mediated non-coeliac duodenal atrophy[18] is detailed in Fig. 2 and Table 2: case 1. The other 2
cases had arthralgia and positive high titre ANA. In one of them, this clinical picture was accompanied by fever and was relapsing, showing a cause-effect relationship with olmesartan administration and discontinuation. In the third case with positive ENA (anti-U1-RNP, and Sm), symptoms remitted with low-dose prednisone and olmesartan discontinuation.

Coeliac disease markers

All of the 20 patients had negative IgA anti-TG2 (values below 2 U/mL) and none of them was IgA deficient. EmA was performed in 4 patients, in spite of negative values of IgA anti-TG2; the results were negative.

HLA-DQ2 was positive in 45% and HLA-DQ8 in 20% of the cases. Two of these patients were positive for both HLA-DQ2 and HLA-DQ8.

In Table 2, a detailed description of the CD markers in 8 of the 20 patients at baseline and during the follow-up is provided. It is noteworthy that in spite of the positivity of highly specific CD markers, observed in half of these patients (patients 2, 4, 6 and 8), the duodenal lesion disappeared or ameliorated only after olmesartan discontinuation. The CD cytometric pattern remained relatively stable over time, as did the anti-TG2 deposits, irrespective of the intervention done.

Outcome

The physician in charge decided the therapeutic intervention. The decision generally relied on the presence or not of CD markers and evolved with time according to the increased awareness of the existence of olmesartan-associated enteropathy. Five patients were initially treated with gluten-free diet without response and afterwards olmesartan was discontinued. In six patients a gluten-free diet and olmesartan withdrawal were applied simultaneously, while in the remaining 9 patients olmesartan was removed while the patients continued on a gluten-containing diet. In all except 2 patients a complete clinical and histological response was demonstrated after olmesartan withdrawal. One was a 92-year-old man with a good clinical response to olmesartan discontinuation; however, histological recovery could not be demonstrated since he died due to an unrelated condition. In the other patient also showing a good clinical response, partial atrophy persisted 6 months after olmesartan withdrawal while taking candesartan. Though control biopsies were not done with a predetermined schedule, complete histological recovery occurred with a median of 6.5 months after olmesartan withdrawal (range: 3-12 months). In patients treated with gluten-free diet at the time of olmesartan discontinuation, the reintroduction of gluten did not have a negative impact on the outcome, irrespective of whether or not they had CD markers. In some patients in whom olmesartan was replaced by valsartan the duodenal mucosa, though improved, did not normalize until valsartan was also withdrawn (Table 2: patients 1, 3 and 4).

DISCUSSION
The particular setting of HUMT providing both private and public health services allowed us to learn the epidemiological characteristics of enteropathy associated with olmesartan in the period 2011-2014. Annual cumulative incidence rates showed that might affect up to 22 cases per 10,000 patients treated for at least 6 months. This low incidence may explain why this severe entity has not previously been detected, either in pivotal trials (2,232 diabetics treated for 3.2 years in ROAMAP study5) or in a cohort study in an endoscopic unit (2,088 patients taking Olmesartan and other antihypertensives[20]).

In all likelihood, the calculation of the incidence density instead of the cumulative incidence would have given us a more accurate idea of the speed with which the enteropathy appears among patients treated with olmesartan. However, treatments for hypertension are generally taken for years, and information about the precise duration of the treatment of each patient at the population level is not easy to obtain. This fact, together with the lack of strict control of drug adherence in patients with hypertension,[21] renders calculation of the annual cumulative incidence an acceptable approach.

The present study provides a registry of additional cases of olmesartan-associated enteropathy attended in several hospitals in Spain. Essentially, the clinical characteristics, are the same as those in previously described series.1,2,3 As in the case of the French series,2 we also found a patient with a milder clinical picture and probable lymphocytic enteropathy-associated with olmesartan. This fact together with the varying degrees of duodenal atrophy observed in the rest of the cases suggests that the spectrum of histopathological lesions is identical to that seen in CD. In addition, in a high proportion of patients in whom the rest of the gastrointestinal tract was biopsied, mucosal damage was also observed, ranging from lymphocytic gastritis to several forms of microscopic colitis, confirming that olmesartan may affect the entire gastrointestinal tract.

Three out of the 20 patients also had a simultaneous autoimmune disease mimicking lupus, suggesting that in some cases systemic involvement may occur. Though a cause-effect relationship between systemic manifestations and olmesartan administration was proved in only one case, in another patient the clinical picture was severe enough to rule out a deliberate rechallenge. These autoimmune phenomena are paradoxical since olmesartan could theoretically reverse autoimmune diseases through its effect on vitamin D receptor (VDR).[22] Olmesartan is an agonist of this receptor and it has been described how VDR dysfunction causes autoimmune diseases.

In the French series 10 out of 36 patients with olmesartan-associated enteropathy had a past history of autoimmune or inflammatory diseases, and 9 out of 11 patients had high titers of circulating ANA.2 We don't know of the existence of similar cases of LES-like disorders described in the literature regarding the administration of olmesartan with or without associated enteropathy. Their recognition is important because they may be also life-threatening.
Learning about the underlying mechanism of olmesartan-induced damage is of important pathophysiologic interest. It seems that it acts as a trigger and reproduces stereotyped patterns of both systemic autoimmune disorders and bowel damage. In this sense, a genetic predisposition to CD with a higher frequency than expected by chance in olmesartan-associated enteropathy has been reported, suggesting that there might be a shared pathophysiologic pathway.1 The percentage of HLA-DQ2 and DQ8 in our series (65%) is similar to that found in the French study (61%) but lower than that found in the Rubio Tapia et al study (81%). In fact, the risk of CD, mainly dependent on HLA-DQ2 status, is present in 18% of the general population in our area12 and was present in 45% of the patients in our study. The possibility of a bias toward a higher percentage of HLA-DQ2-positive patients being biopsied with possible CD in mind cannot be ruled out. However this seems unlikely in our study since the Rubio-Tapia et al study has served as a warning since 2012 for the detection of cases of this enteropathy irrespective of HLA-DQ2 status. Interestingly, half of the patients in whom CD markers of potential CD were looked for, showed either CD cytometric pattern (TCRγδ≥8.5%; CD3–≤10%), TG2 duodenal deposits, or both. Both CD markers have been found to be highly specific for CD diagnosis.[17,23] However, the clinical picture reversed and duodenal mucosa normalized only after olmesartan withdrawal, but not after gluten-free diet, in spite of persistence of CD markers over time in some cases. Thus, even when these CD markers are present, the diagnosis of olmesartan-induced enteropathy should prevail over the diagnosis of CD in patients taking this drug.

Though generally related to olmesartan, isolated cases related to other ARBs, such as irbesartan2 and valsartan[24], have also been reported. We did not find cases associated with other ARBs. However, the duodenal mucosa of 2 patients completely recovered only after valsartan removal and in other 2 cases clinical symptoms and the duodenal histology improved but did not normalize while they remained on valsartan or candesartan. Taking into account that the normalization of the duodenal mucosa occurred generally after 6 months of discontinuation, this fact suggests the possible existence of a class effect, though generally attenuated, induced by other ARBs. The reason why this severe enteropathy is almost exclusively related to olmesartan and rarely related to other ARBs, may be due to the existence of a differential affinity on receptors such as a VDR, PPAR and CCR2b, capable of modulate the immune system.[25]

Future studies should elucidate whether this enteropathy is the ‘tip of the iceberg’ of a wider clinicopathological condition spectrum including non-atrophic enteropathy, and also whether other ARBs are implicated.4 In addition, deeper knowledge of the underlying mechanism of this disease process may contribute to the understanding of this sprue-like damage as well as of systemic self-reactivity.

DECLARATION OF INTERESTS
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AUTHORSHIP STATEMENT:
Maria Esteve takes responsibility for the integrity of the work as a whole, from inception to published article.

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A Carrasco, A del Val, J Molina-Infante, Y Zabana, M Aceituno, J Ribes, and F Fernández-Bañares F critically reviewed the paper and provided valuable contributions.

M Esteve designed the research study and wrote the paper. All authors approved the final version of the manuscript.

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REFERENCES


FIGURE LEGENDS

Figure 1: Intestinal deposits of anti-TG2 IgA antibodies. Patient 1 of Table 1. Negative immunofluorescence (IF) staining before (A: Complete atrophy) and after olmesartan discontinuation (B: Normal mucosa). Patient 2 of Table 1. Positive immunofluorescence (IF) staining before (C: Complete atrophy) and after olmesartan discontinuation (D: Normal mucosa).

Figure 2: Clinical evolution of a 52-year-old man with essential hypertension treated with olmesartan. CD: Coeliac disease; GFD: Gluten-free diet; AZA: Azathioprine; aPLs: anti-phospholipid antibodies; aCLs: anti-cardiolipin antibodies, anti-β2GP(I): anti-β2-glycoprotein I antibodies.

DECLARATION OF INTERESTS

Declaration of interest: None.

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Table 1: Annual cumulative incidence rates of olmesartan-associated enteropathy in the catchment area of the Hospital Universitari Mutua Terrassa.

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases with</th>
<th>Patients treated</th>
<th>Incidence per 104</th>
<th>95% CI</th>
</tr>
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</table>


Table 2: Evolution of duodenal histopathology and potential coeliac disease markers (lymphocyte subpopulations and anti-TG2 deposits) according to the therapeutic intervention (olmesartan discontinuation, gluten-free diet, or both). When not specified, patients were taking a gluten-containing diet.

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>HLA-DQ2+ HLA-DQ8-</th>
<th>Baseline</th>
<th>Therapeutic intervention</th>
<th>Duodenal IEL lymphocyte subpopulations</th>
<th>Duodenal anti-TG2 deposits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Date)</td>
<td>(Jan 2013)</td>
<td>Olmesartan removal + gluten-free diet*</td>
<td>Selective CD3-reduction CD3+gd+ 5.0% CD3- 4.0%</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Duodenal histopathology</td>
<td>Marsh 3c</td>
<td>Valsartan removal + reintroduction of gluten-containing diet</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Dec 2013)</td>
<td>Marsh 3a</td>
<td>Normal pattern CD3+gd+ 3.7% CD3- 51.8%</td>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>(Dec 2013)</td>
<td>Marsh 0</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Patient 2</th>
<th>HLA-DQ2+ HLA-DQ8-</th>
<th>Baseline</th>
<th>(Date)</th>
<th>Duodenal histopathology</th>
<th>(Date)</th>
<th>Duodenal histopathology</th>
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<tr>
<td></td>
<td>(June 2013)</td>
<td>(Aug 2013)</td>
<td>(Mar 2014)</td>
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<tr>
<td></td>
<td>Marsh 3c</td>
<td>Marsh 1</td>
<td>Marsh 0</td>
<td></td>
<td></td>
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<tr>
<td>Patient</td>
<td>HLA-DQ2-HLA-DQ8-</td>
<td>Therapeutic intervention</td>
<td>Duodenal IEL subpopulations</td>
<td>Duodenal anti-TG2 deposits</td>
<td></td>
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</tr>
<tr>
<td>3</td>
<td></td>
<td>Olmesartan removal + gluten-free diet</td>
<td>Incomplete CD pattern CD3+gd+ 22.6% CD3- 47.7%</td>
<td>Positive +++</td>
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<tr>
<td></td>
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<td>Reintroduction of Gluten-containing diet</td>
<td>Incomplete CD pattern CD3+gd+ 12.7% CD3- 56.7%</td>
<td>Positive ++</td>
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<tr>
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<td></td>
<td></td>
<td>Incomplete CD pattern CD3+gd+ 35.3% CD3- 34.8%</td>
<td>Positive ++</td>
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<tr>
<td>3</td>
<td>HLA-DQ2-HLA-DQ8-</td>
<td>Olmesartan withdrawal Valsartan replacement</td>
<td>Normal pattern CD3+gd+ 2.1% CD3- 59.6%</td>
<td>Positive +++</td>
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<tr>
<td>4</td>
<td>HLA-DQ2-HLA-DQ8+</td>
<td>Olmesartan withdrawal Valsartan replacement</td>
<td>Normal pattern CD3+gd+ 1.5% CD3- 59.1%</td>
<td>Positive ++</td>
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<tr>
<td>5</td>
<td>HLA-DQ2-HLA-DQ8-</td>
<td>Olmesartan withdrawal</td>
<td>Normal pattern CD3+gd+ 1.2% CD3- 16.1%</td>
<td>Negative</td>
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</tbody>
</table>

**Patient 3**
- HLA-DQ2-HLA-DQ8-
- (Oct 2013) Duodenal histopathology: Marsh 3c
- (Jun 2014) Marsh 3a
- (Aug 2014) Marsh 0

**Patient 4**
- HLA-DQ2-HLA-DQ8+
- (Sept 2013) Duodenal histopathology: Marsh1 (53% IEL)
- (Jan 2014) Marsh1 (61% IEL)
- (May 2014) Marsh1 (31% IEL)

**Patient 5**
- HLA-DQ2-HLA-DQ8-
- (March 2014) Marsh 3a
- (Aug 2014) Marsh 0
<table>
<thead>
<tr>
<th>Patient 6 HLA-DQ2+ HLA-DQ8-</th>
<th>Duodenal anti-TG2 deposits</th>
<th>(Date) Duodenal histopathology</th>
<th>Therapeutic intervention</th>
<th>Duodenal IEL subpopulations</th>
<th>Duodenal anti-TG2 deposits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild positive +</td>
<td>(March 2014)</td>
<td>Baseline Olmesartan withdrawal</td>
<td>Normal pattern CD3+gd+ 3.7% CD3- 9.1%</td>
<td>Positive ++ Negative</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>(Jul 2014)</td>
<td></td>
<td>Normal pattern CD3+gd+ 3.6% CD3- 36.1%</td>
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<table>
<thead>
<tr>
<th>Patient 7 HLA-DQ2- HLA-DQ8-</th>
<th>Duodenal anti-TG2 deposits</th>
<th>(Date) Duodenal histopathology</th>
<th>Therapeutic intervention</th>
<th>Duodenal IEL subpopulations</th>
<th>Duodenal anti-TG2 deposits</th>
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<tbody>
<tr>
<td></td>
<td>(Oct 2014)</td>
<td>Marsh 3c</td>
<td>Olmesartan withdrawal</td>
<td>Normal pattern CD3+gd+ 1.0% CD3- 84.8%</td>
<td>Negative</td>
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<td>(January 2015)</td>
<td>Marsh 0</td>
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<table>
<thead>
<tr>
<th>Patient 8 HLA-DQ2- HLA-DQ8-</th>
<th>Duodenal anti-TG2 deposits</th>
<th>(Date) Duodenal histopathology</th>
<th>Therapeutic intervention</th>
<th>Duodenal IEL subpopulations</th>
<th>Duodenal anti-TG2 deposits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Aug 2013)</td>
<td>Marsh 3a</td>
<td>Gluten-free diet</td>
<td>Incomplete CD pattern CD3+gd+ 18.0 % CD3- 17.5%</td>
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<td>(Feb 2014)</td>
<td>Marsh3a</td>
<td>Reintroduction of Gluten-containing diet Olmesartan removal</td>
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<td>(Jul 2014)</td>
<td>Marsh 0</td>
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</table>

Histopathology according to Marsh classification revised by Rostami et al10,14 (Marsh 0: Normal mucosa; Marsh 1: Microscopic enteritis; Marsh 3a: Partial atrophy; Marsh 3b: Subtotal atrophy; Marsh 3c: Complete atrophy); IEL: Intraepithelial lymphocyte; Coeliac disease (CD) pattern: TCRgd>8.5% and CD3–<10%, incomplete CD pattern: selective TCRgd. Increase.

*Gluten-free diet for previous 12 months. Baseline biopsies of patient 1 were performed at another hospital and were available for histopathological analysis only (Marsh 3c).