Author’s response to reviews

Title: Prevalence of Celiac Disease in Multiple Sclerosis

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Author’s response to reviews: see over
Dear Dr. Zivadinov,

Please find attached the article entitled "Prevalence of Celiac Disease in Multiple Sclerosis", which we had previously submitted on October 31st 2010, MS reference 1719936074672609, and which has been modified following the suggestions of the Publishing Committee and the two Reviewers.

We have implemented all the suggested changes into the Abstract; we have included the "Author's Contributions", and included an additional table (Table 5), which shows the clinical characteristics of the 8 patients with associated celiac disease, as well as two extra paragraphs which explain these findings in pp. 14 and 15.

All the additional changes have been highlighted in yellow to facilitate their identification. whereas the words or sentences we were suggested to remove are similarly highlighted in red.

Please find below our answers to the questions proposed by the two Reviewers addressed to each of them, including explanations on each and every issue.
RESPONSES TO REVIEWER 1

We appreciate your discerning and clarifying comments regarding our paper on the prevalence of CD in MS patients. Please find below our answers to your suggestions.

MAJOR CRITICISMS

1.a) It is extremely difficult, if not altogether impossible, to perform additional analyses in order to find which factors may have an impact on the risk of celiac disease development, since we only identified 8 patients with CD in a population of 72 MS subjects and no demographic, genetic, clinical, analytical nor serological differences were found between those 8 patients and the remaining 64. In this group of 72 MS patients, 39 of them were treated with beta-1b-interferon; the remaining 24 did not receive such treatment. Out of the 8 CD patients, 3 were treated with IFN-beta and 5 did not receive any treatment. It has been indeed described that interferon may precipitate the development of CD in patients with hepatitis C, but this happens when using alpha interferon, and we only employed beta interferon with our MS patients. One of the earliest studies on this topic confirms this point, as described in the literature (Cammarota G, Cuoco L, Cianci R, Pandolfi F, Gasbarrini G. Onset of coeliac disease during treatment with interferon for chronic hepatitis C. Lancet. 2000;356:1494-5). More recent studies have also corroborated this fact.

When analyzing the differences between the CD and non-CD groups, 56 potential variables may be of interest with respect to the Celiac / Non-Celiac group (8/64), but only 4 have a p value < 0.05. Assuming the null hypothesis (independence), if all of them were true, the probability of suffering 4 or more rejections would be 0.306 (the null hypothesis "being celiac does not have any impact" would not be discarded).
In any case, if we do not take into account p-value adjustment, the following statements are true:

1. Celiacs are younger by 10+ years (median; p < 0.05)
2. They have had MS for 11+ years (median; p < 0.05; probably as a consequence of the previous fact.
3. Whereas progression in non-celiacs is 50% (approximate value), in celiacs this figure increases to 87% (all but 1 show progression; p = 0.059)
4. All celiacs show SSEPs (+). This category is also predominant in non-celiacs, although not as much (68%; p < 0.05).
5. As for the DQ2, whereas non-celiacs are predominantly negative (75%), celiacs are mostly positive (68%) (p < 0.05).
6. Celiacs show significantly higher TSH values than non-celiacs (not significant).

1.b) These patients only received acute steroid treatment for short intervals in order to treat neurological exacerbations, and only once a year, approximately. Most of the 72 patients included in the study followed this treatment. The standard procedure involves the administration of an IV methyl-prednisone infusion (1 g / day) for a period of 5 days provided as outpatient care at a day hospital. It is difficult to justify this reduced the appearance of CD, since it was present in 11% of the treated patients.

1.c) We cannot think of other factors which may lead to the development of CD apart from a genetic predisposition which became evident due to the high frequency of celiac patients found among first-degree relatives: out of the 126 studied, 23 (32%) were celiac patients. This is a rather high figure, since in other family studies values are, normally, within the 10-30% range.

2) Our discussion is indeed very oriented towards physiopathology and the diagnosis of the celiac disease, as well as the description of clinical presentations in adults, which are frequently atypical, and their differences with respect to the
classical manifestations predominantly found in childhood. We also comment on the scarce sensitivity of the serological markers used (normally, type 2 transglutaminase) and the high negative predictive value of the known genetic markers. We think these discussions will be useful, since they are aimed mostly towards neurologists, who are often not used to diagnosing celiac patients; they would benefit from reviewing the changes and breakthroughs related to this disease which have taken place in the last decade.

Following the recommendations of the other reviewer, we have added Table 5, which contains additional clinical data on the celiac patients diagnosed in the present study.

**MINOR ISSUES**

1. Following your instructions, we have modified the second paragraph of the introduction, which now reads "MS predominantly...".
2. "Maternity" in Table 1, does indeed mean that they have had children.
3. In Table 1, the numbers in parenthesis were unclear. They have been corrected and clarified.
4. In the final sentence of page 11 we removed the statement about oats, because although it may contain minimal traces of gluten, it does not add to the study and may be confusing.
5. You mentioned there were a couple of paragraphs in pp. 12 and 13 discussing the physiopathology of celiac disease which could be deleted, but did not specify which ones. Thus, for the time being and if you find it appropriate, we are keeping the text as is because we feel it ties in nicely with the explanations about the difficulties of diagnosing CD in adults; besides, we have included additional information about the 8 celiac disease patients and their clinical and evolutionary features, and those paragraphs may help support these new comments.
RESPONSES TO REVIEWER 2

We appreciate your discerning and clarifying comments regarding our paper on the prevalence of CD in MS patients. Please find below our answers to your suggestions.

MAJOR CRITICISMS

1) We are very pleased to be able to provide you with more information regarding the 8 patients who exhibited an associated CD; we are confident these data would be useful to clarify the doubts and commentaries you mentioned:

1.a) The most frequent digestive symptoms were predominant constipation in 5 cases, and constipation with GERD in 3 cases. All cases were of moderate-acute intensity and thus it was necessary to follow a continuous treatment with omeprazole. Only one patient presented diarrhea with associated weight loss, and only two patients were totally asymptomatic from the digestive point of view.

1.b) The most common clinical presentation forms of MS were myelitis in 4 patients and encephalomyelitis (mixed) in the other 4 patients. No cases of ataxia were found in our series. MRI findings were similar to those of non-celiac patients. No treatment-related differences were present either, although it must be noted that 3 patients were treated with interferon-beta whereas the remaining 5 refused to take any pharmacological treatment and simply followed a gluten-free diet without additional medication.

1.c) The three patients treated with IFN beta b-1 did not exhibit a higher rate of autoimmune diseases or associated immunologic alterations. It is known that treatment with interferon may lead to the development of a CD or other autoimmune processes in patients with hepatitis C, but this is associated to the use of alpha-interferon, whereas our MS patients were treated with beta-interferon. One of the earliest studies on this topic confirms this point, as described in the

1.d) These patients with CD did not exhibit different auto-reactive antibodies compared to the rest of patients. Only two subjects had ANAs (+) at low titres (1/80 and 1/160, respectively).

1.e) After a follow-up period of about three years (average), the 8 patients showed improvements thanks to the gluten-free diet, both from the digestive (improvement or absence of symptomatology) and neurological (reduction of the annual rate of bouts and intensity) points of view. The overall health status also improved (stabilization or reduction of the EDSS score).

2) The family study was performed for all 72 patients with MS, not only for those with CD. A total of 127 first-degree relatives were assessed, and 23 patients with CD were found. This figure represents a prevalence of 32%, which is certainly high.

3) It is true that not all associated diseases are autoimmune diseases, but they appear somewhat frequently in patients with CD. Examples include ferropenic anemia and osteoporosis, produced due to issues with iron and calcium intestinal absorption, respectively. It is also common to find a higher prevalence of UTIs in patients with CD, especially women. Similarly, LFT's alterations are present in 10% of celiac patients (Bardella MT, Valenti L, Pagliari C, Peracchi M, Fare M, Fracanzani AL, Fargion S. Searching for coeliac disease in patients with non-alcoholic fatty liver disease. Dig Liver Dis. 2004;36:333-6). Many of these alterations can be corrected after a prolonged treatment with a gluten-free diet. For all these reasons, we have changed the title "autoimmune" for "associated diseases".

4) No assessment for the presence of infection by Helicobacter Pylori was performed in our series of 72 patients because we did not consider it relevant for our study. It is true this infection has been related to CD in patients with associated
ferropenic anemia, since active gastritis and duodenitis are frequently found, even with erosions. Similarly, lymphocytic enteritis lesions (Marsh I) were found in some patients. The 8 patients diagnosed with CD exhibited mild vellositary atrophy (Marsh III), which is a histological lesion not described as related to Hp infection. As for its relationship with MS, it seems rather unlikely; literature is scarce on the topic, and presents contradicting results. In fact, some studies even suggest a potential protective role in the Japanese population (Li W, Minohara M, Su JJ, Matsuoka T, Osoegawa M, Ishizu T, Kira J. Helicobacter pylori infection is a potential protective factor against conventional multiple sclerosis in the Japanese population. J. Neuroimmunol. 2007;184:227-31).

5) The first three tables describe the clinical characteristics of the 72 patients enrolled in the study, including demographic, clinical, radiological and exploratory data from the neurological point of view of MS. Table 4 describes the genetic, serological and histological features of the 8 patients with associated CD.

Following your comments, we have included an additional table (Table 5) in which presentation forms are described in more detail, both from the neurological and digestive points of view; it also shows whether patients were being treated with beta-interferon, the presence of ANAs, and we have also added two supplementary paragraphs to the discussion which clearly explain all these facts.

MINOR ISSUES

The English language has been reviewed and proofed by a native English Literature Professor.
Should any part of the text remain obscure or should you find any issue which would require additional clarifications, please let us know and we will do our best to explain them in a timely manner.

I look forward to hearing from you regarding the final decision on this paper.

Yours sincerely

Prof. Luis Rodrigo M.D.