Author’s response to reviews

Title: Increased serum IL-36β and IL-36γ levels in patients with neuromyelitis optica spectrum disorders: Association with disease activity

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Version: 1 Date: 03 Jul 2019

Author’s response to reviews:

Jul 03, 2019
BMC Neurology
Re: NURL-D-19-00068

Dear Dafne Solera:

Thank you for your letter of “Your submission to BMC Neurology - NURL-D-19-00068” regarding our manuscript. We appreciate the constructive comments from both editor and reviewers. We herein submit our revised manuscript in which we have addressed all the questions raised, made all corrections that have been recommended. The changes are highlighted
in the text of the revised manuscript. Below please find our point by point response to the reviewers’ comments.

Thank you for your reevaluation of our revised manuscript.

Sincerely yours,
Chun-Sheng Yang, MD, PhD
On behalf of all authors

Point by point response:

Denis Bernardi Bichuetti (Reviewer: 1)

Comments to the Author

1) Reviewer reports:

Denis Bernardi Bichuetti (Reviewer 1): I have reviewed with interest the work by Drs. Yang and Zhand et al, on the possible effects and association of IL36 and NMO.

With the current knowledge it is assumed than NMO is not only a demyelinating disease, but an inflammatory disease that sarges the blood brain barrier. Please correct the statement in the introduction.

Response: Thank you for your advice. We have corrected the statement in the introduction (page2, line 50).

Table 1 discloses that 30% of the patients presented a topographic lesion as "others", I recommend specifying since this is such a small sample. Also, reporting the imunosupressive agents and dosage is important, and I also recommend performing a correlation between time of imunosupression and IL36 dosage. At first, on might assume that levels are higher during an attack, but figure 3 shows that not all patients present with reduction on its levels, and adequate imunosupression might be driving this effect, and not time distance from the relapse. Furthermore, it would also be interesting to include a control group with another CNS autoimmune disease, so to state if the elevation of IL36 is exclusive fo NMOSD or a common finding in CNS autoimmune disease. If this is not feasible, it must at least be discussed.
Response: According to the recommendation, we have specified that 30% of the patients presented a topographic lesion as "others", and given the immunosuppressive agents and dosage. Please see Table 1.

Table 1 Demographic and clinical characteristics of NMOSD and HC

<table>
<thead>
<tr>
<th></th>
<th>NMOSD(50)</th>
<th>HC (30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, n (% female)</td>
<td>48(98%)</td>
<td>27(90%)</td>
<td>0.358</td>
</tr>
<tr>
<td>Age at sampling, years</td>
<td>46.46±15.04</td>
<td>51.30±12.71</td>
<td>0.144</td>
</tr>
<tr>
<td>Age at onset, years</td>
<td>41.9±15.71</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Follow-up duration, years</td>
<td>4.66±4.33</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Annualized relapse rate (ARR)</td>
<td>2.24±0.97</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>EDSS at nadir</td>
<td>3.98±2.12</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Presentation at sampling, n (%)</td>
<td>7(14%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Area postrema syndrome</td>
<td>2(4%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>26(52%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Brain stem syndrome</td>
<td>6(12%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Diencephalic clinical syndrome</td>
<td>3(6%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cerebral syndrome</td>
<td>6(12%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>length of newly identified spinal cord lesion (vertebral segments)</td>
<td>5(1, 15)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>AQP4-Ab, n (%)</td>
<td>28(56%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Imunosuppressive agents and dosage, n (%)</td>
<td>5(10%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Prednisonlone (12mg/d)</td>
<td>5(10%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Azathioprine (2mg/kg.d)</td>
<td>8(16%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Mycophenolate Mofetil(1.5g/d)</td>
<td>7(14%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Rituximab†</td>
<td>30(60%)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: NMOSD neuromyelitis optica spectrum disorders, HC healthy control, ARR annualized relapse rate, EDSS Kurtzke Expanded Disability Status Scale, ON optica neuritis, AM acute myelitis.

†: All patients were treated with rituximab (Biogen-Idec, Cambridge, MA, and Genentech, San Francisco, CA) 100 mg (equivalent of 50–59 mg/m2) IV, one infusion per week for 3 consecutive weeks. Continued dosage was dependent on the percentage of circulating CD19+ B-cell counts. Whenever it reached 1% of total lymphocyte population, rituximab 100 mg was reinfused.

The reviewer gave us an excellent advice that is performing a correlation between time of immunosuppression and IL-36 dosage. To follow this advice, we performed a Pearson
correlation test between time of immunosuppression and IL-36 dosage. There was no correlation between time of immunosuppression and IL-36β level either in acute or in the remission phase (r = 0.242, P = 0.183; r = -0.151, P = 0.409). Although there was correlation between time of immunosuppression and IL36-γ level in acute phase (r = 0.381, P = 0.031), no correlation was found between time of immunosuppression and IL36-γ level in remission phase (r = 0.117, P = 0.525) (page7, line 144 to 150). At this point of view, time of immunosuppression didn’t have influence on the significance of IL36-γ level between acute phase and remission phase. These verify the statistical result in the present study. In order to eliminate the influence of time of immunosuppression, we applied Paired-Samples T Test to compare IL-36 level between the the acute phase and remission phase in the manuscript. Thanks a lot.

As for including a control group with another CNS autoimmune disease, it is a comprehensive advice. This is a limitation of the present study. And it had been discussed in the revised manuscript (page 10, line 227 to 231). Thank you very much.

Although authors stated that they excluded MOG positive and other comorbidities immune diseases, only 56% of the sample is AQP4 positive. This is a slow number, unless tested under therapy, and I think it would be interesting to disclose when during disease course the antibody was tested.

Response: This is a profound question. 22 patients were AQP4-Ab negative in the present study. Among them, 16 were tested under immunosuppressive therapy which may influence the result. We will test AQP4-Ab if these patients suffer relapse again. Thanks a lot.

These are small complementary analysis, but I think they would ad value to the study. Furthermore, I think it is to early to implicate IL36 in NMO pathogens, even earlier to propose a therapeutic target. The current study only reports an association, we do not know if cause-relation or not, and I recommend reviewing these statements.

Response: Thank you for your comprehensive advice. To follow this recommendation, we made changes in the statements (page 9, line 191 to 194; page 11, line 235 to 237).

Reviewer: 2

Hongyu Zhou (Reviewer 2): This manuscript is almost ready for a final acceptance. My suggestions were marked in the manuscript.

a) “We did not include myelin oligodendrocyte glycoprotein immunoglobulin G (MOG-IgG)-positive patients because the pathophysiology of MOG-IgG-associated NMOSD is probably
different from that of AQP4-IgG-positive NMOSD.” If this concern is from the literature then reference it, as it relates to your statement.

Response: We have added reference number according to the reviewer’s advice (page 4, line 86). Thank you very much.

b) “The plasmids were donated by Professor Angela Vincent and Professor David Beeson from the Nuffield Department of Clinical Neurosciences, University of Oxford.” Not necessary to mention it.

Response: We have deleted it following the reviewer’s advice (page 5, line 108 to 109). Thanks a lot.

c) “Serum IL-36α, IL-36β and IL-36γ concentrations were evaluated using a human IL-36α, IL-36β and IL-36γ enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems).” Cite the country of origin of this kit in the parenthesis.

Response: We have added the country of origin of this kit (page 5, line 113). Thanks again.