Reviewer's report

Title: Plasma 24S-hydroxycholesterol is increased in late onset Alzheimer's disease but not in vascular dementia

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Reviewer: valerio leoni

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The manuscript “Plasma 24S-hydroxycholesterol is increased in late onset Alzheimer's disease but not in vascular dementia” by Zuliani and co-workers contributes to add new insight into the field of brain cholesterol metabolism and neurodegenerative disease.

With a new and well validated LCMS technique they report here that plasma levels of 24OHC were significantly increased in early (?) LOAD patients and decreased in advanced Vascular Demented patients compared to matched controls. In patients with Cognitive impairment but no dementia (CIND) the levels of 24OHC were not reduced compared to controls.


Thus, a majority of scientific reports talks in favour of a reduction of plasma 24OHC as a consequence of neurodegeneration and loss of active neuronal cells able convert brain cholesterol into 24OHC and release into circulation. During an acute episode of demyelination a transient increased flux of 24OHC into the circulation could be hypothesised, according with the finding of increased concentrations of 24OHC in the circulation of mice with acute experimental autoimmune encephalomyelitis (Teunissen et al., 2007). No results in humans are supporting this hypothesis, yet.

However, the results presented are interesting and indeed will positively
contribute to the topic. It is likely that the turnover of brain cholesterol and thus the plasma levels of 24OHC change over the time along neurodegenerative diseases.

Major Compulsory Revisions

1. Zuliani and co-workers focused their attention on early stage of LOAD and more advanced stage of Vascular Demented patients. The plasma collection and analysis were performed in an appropriate way. However the evaluation of the degree of brain atrophy was based on CT instead of MRI scans, which is the method better validated for investigate brain atrophy in AD (Frisoni GB, et al. Nat Rev Neurol. 2010; 6:67-77). A sentence in the discussion should be added about the limit of the CT scans versus MRI.

2. The authors considered VD patients as a sort of late stage of brain atrophy compared to an early stage of LOAD, the patients investigated in the study. These two diseases are much different about pathogenesis mechanism and evolution. If the brain degree of atrophy was the major endpoint this should be better defined in the text. Also the data about the degree of brain atrophy in each group and a statistical evaluation is missing.

3. The definition of each the diagnostic group refers to international criteria for dementia and related diseases. However, CSF biomarkers are missing: the classification of patients with cognitive impairment seems to be problematic under this point of view.

Are CSF biomarkers Tau, Ptau and A#42 available? If yes they should be presented, if not, they it should be clearly stated in the text.

4. In table 2 and in results should be added and discussed the ANOVA or Kruskall Wallis comparison between CT scans in the 4 groups. Also the data should be reported in table 2.

5. Could CIND patients be considered MCI? It is not clear and this should be made more clear in the text.

6. Page 3 line 8: i suggest to change degraded with eliminated.

7. “it has been previously noted that an increase in 24S-OH-Chol production by CNS would be typical of several neurodegenerative disorders [24]”. This sentence is prone to several criticism. 24OHC was found increased in CSF form patients affected by CNS and neurodegenerative disease. However being 24OHC correlated with ApoE in CSF, it might be possible that higher amount of ApoE lipoproteins (due to a slower turnover, for example) is responsible for the increased CSF 24OHC observed. In plasma, 24OHC was found reduced in Multiple Sclerosis, AD, VD, Huntington Disease and Parkinson patients proportionally to the degree of brain atrophy assessed by MRI. Also animal models of neurodegenerative diseases reported decreased levels of 24OHC in the brain. Finally 24OHC was also found reduced in brain samples collected form patients with AD.
The conversion of cholesterol into 24OHC might be higher in early stage of AD, as the data of Zuliani are suggesting. The discussion should be reformulated stating the hypothesis that in an early stage of AD there is higher rate of cholesterol conversion into 24OHC.

8. Page 10, 3rd line by bottom: in a sample of older patients: I suggest to change as “in a sample of aging patients”. Otherwise older compared to who?

9. page 11 line 1: “the finding of elevated 24S-OH-Chol levels in plasma is consistent with an alteration of cholesterol homeostasis in the CNS of LOAD patients”. I wonder why REDUCED levels of 24OHC are not consistent with an impairment of brain cholesterol metabolism. I suggest to reformulate or remove this sentence.

10. pag. 11. “However, our data support the concept of plasma 24S-OH-Chol being derived from CNS in a stage of the disease where the rate of current neuronal degeneration is high, while the global atrophy of CNS is still not severe”. This sentence is not clearly formulated. I suggest to reformulate as: “Our data support the hypothesis that plasma levels of 24OHC are higher in an early stage of disease, when the rate of neurodegeneration is higher but the amount of cell loss and resulting brain atrophy still small”.

11. page 11: “The hypothesis might be indirectly supported by the finding of a trend toward higher 24SOH-Chol levels in CIND individuals, i.e. in a very early phase of neurodegeneration”: which trend? The levels of 24OHC in controls and CIND seems very similar.

12. “Unlike the study of Lutjohann et al. [11], but in good agreement with other following clinical observations [12; 13; 14]”: please remove good. Is there a bad agreement?

13. “Third, it has been previously noted that an increase in 24S-OH-Chol production by CNS would be typical of several neurodegenerative disorders [24]”. Bjorkhem and co-workers pointed out that the higher amount of CSF 24OHC might be a sign of local neurodegeneration, not that the neurodegeneration is higher in AD compared to VD. Also they pointed out that in case of neurodegenerative processes the 24OHC into CSF lipoproteins is increased compared to control individuals. This sentence is NOT supported by literature evidences and should be changed or removed.

In conclusion:

1. Is the question posed by the authors well defined?
   Yes but should be formulated better the hypothesis as suggested.

2. Are the methods appropriate and well described?
   Yes, but they used CT instead of MRI

3. Are the data sound? Yes
4. Does the manuscript adhere to the relevant standards for reporting and data deposition? Yes

5. Are the discussion and conclusions well balanced and adequately supported by the data?
Yes but too much emphasis is given to a lonely report and consistency about reduction of 24OHC in plasma as a consequence of neurodegeneration and atrophy were very underrated.

6. Are limitations of the work clearly stated? It should be improved

7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished? Yes

8. Do the title and abstract accurately convey what has been found? Yes

9. Is the writing acceptable? Yes

In conclusion I recommend the text for publication after few Major Compulsory Revisions

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests