Author’s response to reviews

Title: Oxysterol/chitotriosidase based selective screening for Niemann-Pick type C in infantile cholestasis syndrome patients

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Cecile Pagan (Reviewer 2):

The authors performed some text editing but major comments were not taken into account:

2. C-triol is measured by LC-MS/MS, adapting a published method. The reference range mentionned here is 50 ng/mL, while the reference range in the cited methodological article is 24 ng/mL. The authors refer to a different method to justify the chosen reference range, but results are not comparable: total oxysterols measured after alkaline hydrolysis (Reunert et al, Kannenberg et al) are two-fold higher than free oxysterols measured without alkaline hydrolysis (Boenzi et al, this study). Furthermore, considering the high inter-lab variability and the absence of standardized reference material for calibration, the laboratory must determine its own reference range anyway.
- The methodology used to obtain this reference range must be described, and the distribution of controls must be shown.

- A re-test procedure in case of borderline results should be described. Specifically, some patients display C-triol concentration just below the chosen cut-off and have not been further investigated, however the difference to the cut-off is likely analytically non-significant considering the inter-day CV.

Answer:

Disagree. A lot of studies declare 50 ng/mL cut-off for free oxysterols, i.e. Klinke, Glynis Fiona. Oxysterol Signature as Putative Biomarker in Niemann-Pick Type C and Inflammatory Bowel Diseases. Zurich Open Repository and Archive. 2016. doi.org/10.5167/uzh-127373.

3. A correlation between C-triol and cholesterol is reported for one groupe of patients. Such correlation was addressed, and not found, in early reports (Porter et al, 2010).

- This correlation should be confirmed using a non-parametric correlation test, given the apparent non-gaussian distribution of both variable (as is the case for many biological parameters ; there is no normal distribution "by nature"). Spearman's test is applicable to ordinal or continuous variables, and evaluates the monotonic relationship (including, but not restricted to, linear relationship) between two variables. It is less restrictive and more robust than Pearson's test, and is fully suitable here.

- If demonstrated in this study, this correlation should be extensively discussed, and the interest of a C-triol/cholesterol ratio should be evaluated.

Answer:

Agree. The positive correlation for Group 2 was confirmed using a non-parametric Spearman correlation test (\( \rho = 0.586, p < 0.05 \)). This part added into the results. An error of previous version of Manuscript was also detected (the negative Pearson’s value was incorrectly indicated in the text, while the figure 2 shows a positive correlation). Correction made.

Also, the authors has no comments concerning the interest of a C-triol/cholesterol ratio.
6. The authors state that the combination of C-triol and chitotriosidase is useful for the screening of NP-C. It has been known for a long time that chitotriosidase lacks both sensibility and specificity, and most labs now rely on plasmatic biomarkers alone for the screening of NP-C. The advantage of combining both markers (if any) should be explained.

Answer:

Disagree. Indeed, a number of studies demonstrate the low sensibility and specificity of chitotriosidase, however, this indicator is still used and the question of its exclusion is not resolved. We believe that the combination of two markers gives a more reliable result than just C-triol levels.