Reviewer's report

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

Version: 1 Date: 27 May 2019

Reviewer: Cecile Pagan

Reviewer's report:

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 mentioned 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.

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.
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.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

No

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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