Reviewer’s report

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

Version: 0 Date: 15 Apr 2019

Reviewer: Cecile Pagan

Reviewer's report:

This study addresses the use of cholestane-3b,5a,6b-triol (C-triol) and chitotriosidase for the diagnosis of Niemann-Pick type C (NP-C) disease in a context of neonatal cholestasis. Since the first report of oxysterols as screening biomarkers of NP-C in 2010, various studies have evaluated the performances of C-triol and/or 7-ketocholesterol for this indication. This report, focused on the context of neonatal cholestasis, is a replication of a previous study by Polo et al (2016), with partly similar conclusions. This strategy allowed to diagnose 1 patient with NP-C among 108 screened. Some false positive results were observed.

Comments:

1. The introduction contains imprecise or even erroneous statements and should be edited.

2. C-triol is measured by LC-MS/MS, adapting a published method. The reference range mentioned is higher than in the reference report (and higher than those of most published methods). The methodology used to obtain this reference range should be described (including at least number of negative and positive controls and choice of cut-off), and the distribution of controls should be illustrated. Furthermore, inter-run CV at the cut-off concentration should be mentioned and should trigger a re-test procedure in case of borderline results. Specifically, some patients display C-triol concentration just below the chosen cut-off and have not been further investigated, but it is unclear if the difference to the cut-off is analytically relevant in these cases (possibly not, considering the usual precision CV of LC-MS/MS methods).
3. A correlation between C-triol and cholesterol is reported for one group of patients. This correlation should be confirmed using a non-parametric correlation test, given the apparent non-gaussian distribution of both variable. Such correlation was addressed, and not found, in early reports (Porter et al, 2010). If demonstrated in this study, it should be extensively discussed, and the interest of a C-triol/cholesterol ratio should be evaluated.

4. A clinical description of the confirmed NP-C case is needed.

5. Biological workup of patients displaying increased C-triol and/or chitotriosidase was completed by other enzyme activities and by the study of a gene panel. It is unclear why these complementary investigations were only performed in positive patients and not in the whole cohort in a diagnostic setting. Among the reported findings, a heterozygous LARS variant is identified in one patient, but a single heterozygous variant is not relevant for the diagnosis of an autosomal recessive disorder. If this variant is mentioned, this should be clearly stated.

6. The authors conclude 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) must be explained. Furthermore, other biomarkers, i.e. lysosphingolipids and specific bile acids, have demonstrated good performances for the screening of NP-C and should at least be mentioned. Among those, lysosphingolipid profile is especially convenient in the context of neonatal cholestasis as it allows the simultaneous and specific screening of NP-C, NP-A/B and Gaucher diseases (Pettazzoni et al, 2017) : this should be discussed.
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

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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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