Reviewer's report

Title: Vitamin B12 and folate levels in healthy Swiss senior citizens

Version: 3
Date: 19 November 2014

Reviewer: Johan Frederik Berg Arendt

Reviewer's report:

General consideration
This work assesses the biomarker levels of vitamin B12, folate and related markers in a large population of elderly in a region of Switzerland. While the work is based on a large and representative population, there are several major issues that warrant consideration. In particular, the comparison to so-called international reference intervals is a critical misunderstanding. This study is suitable to establish such reference intervals, but it is arbitrary to compare the results to intervals in other countries, measured on other biochemical platforms and with another extend of vitamin fortification.

Major Compulsory Revisions:
1. The writing is unnecessarily tangled and sophisticated to a degree that hampers the understanding of the work presented. Please re-write and shorten the entire text to improve comprehension and stringency.

2. The abstract is lacking coherence. The results and conclusion are not based on the methods outlined in the method section. See “Minor Essential revision” for more detailed issues.

3. Background, paragraph 2: the listing of morbidities makes little sense. Specify the relevant one’s with appropriate references, and omit the rest.

4. Background, paragraph 3: omit part about children, preferably the entire paragraph. Irrelevant to the study.

5. Background, paragraph 4: omit first four lines on absorption. Irrelevant to the study.

6. Background, paragraph 4: outline the extend of folate fortification in Switzerland. This is highly relevant for the study.

7. Background, paragraph 4: omit last sentence, if not, outline in detail.

8. Background, paragraph 6: the study of uromodulin as a marker for kidney function is irrelevant to this study.

9. Background, paragraph 7: the discussion on the use of reference intervals is well-taken. But it lacks understanding of the use of reference intervals, and the general issue that such intervals are difficult to compare across different
laboratories and different populations.

10. Methods, paragraph 1.: has the use of medicine in the study population been examined? Both metformin and PPIs have been described as B12-lowering drugs. Also, high dose vitamins as over-the-counter-drugs are widely used in several Western populations.

11. Methods, paragraph 3: the measurements of haematological parameters are not outlined. Neither is that of cystatin C.

12. Methods, paragraph 3: assay imprecision should be provided. That is crucial when interpreting the rather small numerical differences found in the study.

13. Methods, paragraph 4: what is meant by the “normalized concentrations”?

14. Methods, last paragraph in Statistics section: Again, applying reference intervals and cut-offs from other studies is quite controversial.

15. Results: Generally, the numerical differences are quite small. This is not dealt with in the Results and Discussion sections.

16. Figures 3 and 5 could be omitted without the loss of significant details of the results. That holoTC is the most contributing factor in the Fedosov equation is well-known; that kidney function decreases with age is also well-known.

17. Results, paragraph 3.1: holoTC is not depicted in figure 2. Figure 2 could be omitted without loss of information, since most of the results are given in Table 1. Alternatively, omit the box-plots and put letters and results from the correlation analyses in the multipanel figure.

18. Results, paragraph 3.1: How about correlation between eGFR and holoTC?

19. Results, paragraph 3.2: why was the two lower age groups combined?

20. Results, Table 1: why were there almost 50% missing in measurements of MMA among 60-69 year olds? And 25% among 70-79 year olds?

21. Discussion, paragraph 1: again the issue of reference intervals from other studies.

22. Discussion, paragraph 2: several analytical platforms show weaknesses towards different interactions. This has recently been outlined in the NEJM, Am J Clin Pathol and Clin Chem Lab Med.

23. Discussion, paragraph 3: again the issue of comparing results from completely different populations

24. Discussion paragraph 4: the unknown proportions of vegetarians, consumption of supplements and other drugs and the lack of information on fortification are major issues and need further discussion.

25: Discussion, paragraph 8: the assumption that no cognitive deficiency exist is
highly speculative

26: Discussion, paragraph 9: omit paragraph

27: References: In general, the number of references should be reduced, and a better choice of references is needed.

Minor Essential Revisions:
28. Abstract, Background: “… ill defined”? In Switzerland? In general? In healthy elderly? Please explain or omit

29. Abstract, Method: include the setting (Berne Canton, Switzerland) in the abstract

30. Abstract, Results: all biomarkers and statistical test mentioned in Result section in the abstract should be outline in the Methods section of the abstract, or else omitted. RBC folate and which test(s) the p-values are derived from are not outlined in the Method section.

31. Abstract, Conclusion: The same goes for the conclusions based on assessment of kidney function.

32. Background, paragraph 4: omit last sentence, if not, outline in detail.

33. Background, paragraph 6, line 3: “…produce succinic acid”. Add “and methionine”.

34. Method section: suggestion for new subheadings: 2. Materials and Methods; 2.1 Study population. 2.2 Biochemical measurements. 2.3 Statistics or Data Analysis

35. Results, paragraph 3.1: avoid the use of words such as “weak” and “strong”. Neutral terms, such as “high” or “low” are more appropriate

36. Results, paragraph 3.3, last sentence: not surprising that B12 and Hcy correlate with low coefficients

37. Discussion, paragraph 7: omit part about alcohol. High alcohol consumption would most often lead to high B12 levels.

38: Add letters to panels in figure 4

Level of interest: An article of importance in its field

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests