Author’s response to reviews

Title: High normal TSH is associated with lower mannan-binding lectin in women of reproductive age

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Author’s response to reviews:

Dear Editors,

Thank you very much for giving us the opportunity to resubmit the paper entitled “High normal TSH is associated with lower mannan-binding lectin in women of childbearing age” (Ms. Ref. No.: BEND-D-19-00064R1).

According to the Editor and Reviewers’ remarks, which we found to be very helpful, we have introduced changes to the first version of the manuscript. The changes are specified below.

Editor Comments

1. Improve readability of result's section

   We have introduce some changes in the Results section to made it more readable (page 5, lines 21-23, 42-44).

2. Spell out all abbreviations at first mention (incl. TSH)

   We have spell out all the abbreviations at the first mention in the text

Reviewer 2
1. Does this study have strong statistical power? 95 subjects, only one third with TSH values higher than 2.5?

We agree with the Reviewer that the number of patients included in our study is relatively small (95), concerning statistical power. However, we would like to remind that our results published before (Karbownik-Lewinska et al., Horm Metab Res. 2017; 49: 321-326) were obtained when very similar number of patients was considered, namely n=99. Of great importance is the fact that statistical significance of particular results obtained in the current study was confirmed by different statistical tests. Although the sample size of 100, or according to other authors of 50, is usually recommended in statistics to perform regression analyses, most statisticians generally accept to use this test also in case of much lower sample size under special circumstances, especially when the results can be expected or when they are confirmed by other statistical methods. Of course, we intend to continue our research, but – at the same time – we are sure that the results presented in the current manuscript are of great importance and are worth publishing at this stage. Therefore, we believe that the results of our statistical analyses are reliable and that our explanation will be accepted by the Reviewer.

2. It is stated in Discussion "Similarly, we have observed in the present study that MBL level was not statistically associated with L-thyroxine treatment, which again underlines strong association between MBL level and thyroid function". Please state how many patients were on TSH supplementation therapy and if there was any difference on MBL levels between euthyroid on treatment vs. naïve subjects.

According to the Reviewer suggestion we added the required information:

In the Methods section (p. 4, lines 25-27) - “Nineteen (19) patients were on L-thyroxine replacement therapy (25-150μg daily) due to previously diagnosed hypothyroidism.”

In the Results section (p. 6, lines 3-8) - “Consistently, MBL level in patients on L-thyroxine replacement (n=19) did not differ from MBL level in patients not treated with L-thyroxine (n=76) (mean ± SEM; 1145.89±122.90 ng/ml vs. 1179.86±59.42 ng/ml, respectively; p=0.800.

3. Which is the effect of age on MBL circulating concentrations in normal conditions? You state about elderly subjects, but what are the data for women of childbearing age? Was this association expected? Please in the graph state differently those with TSH values higher than 2.5.

We would like to thank the Reviewer for stressing this very important issue. However, there are no published data on association between MBL level and age in women of childbearing age. Thus, our results are first published concerning this issue. At the same time they were expected on the basis what was observed in elderly and because immune system efficiency decreases naturally with age. As the Reviewer suggested, we marked differently patients with TSH>2.5 and TSH<2.5 in Figures 2 and 3.
4. Is your study group balanced for age and BMI? Please provide a table for results among groups, and describe in text the results of multivariate analysis.

Yes, our study group is balanced for age and BMI. We have already stated in the Methods section (page 4, lines 29-35): “The patients were divided into two groups, i.e. seventy (70) subjects with TSH < 2.5 mIU/l (Controls), and twenty five (25) subjects (26.3% of the whole sample examined) with TSH ≥2.5 mIU/l, which were well matched at baseline in terms of age and body mass index (BMI).” However, according to the Reviewers suggestion we added a table with comparison between subgroups concerning all linear variables (Table I). Additionally, from the regression analysis (Table II) can be concluded that our study group is balanced for age and BMI. Therefore, we added the adequate statement in the Results section (page 5, lines 56-59): “The results of the regression analysis confirmed that patients with TSH ≥2.5 mIU/l and TSH < 2.5 mIU/l were matched in terms of age and BMI.”

Sincerely yours,

Prof. Małgorzata Karbownik-Lewińska M.D., Ph.D.