Author’s response to reviews

Title: The precursor for nerve growth factor (proNGF) is not a serum or biopsy-rinse biomarker for thyroid cancer diagnosis

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Version: 1 Date: 04 Aug 2019

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EDITOR COMMENTS:
The paper has been read by 2 independent reviewers and the Associate Editor. A number of significant concerns have been raised including the need to include a figure or a table. If all the reviewers' comments are addressed in full we can reconsider this manuscript for publication.

Response to Editor
We thank the Editor for their careful consideration. We have accepted all recommendations from the reviewers, and have responded in detail below. An additional table and figure have both been included as recommended.

REVIEWER REPORTS:
Adrian Warfield (Reviewer 1):
Point 1
This is a meritorious, well composed, logically presented manuscript with satisfactory syntax and few terminological foibles, which could easily be adjusted to house style. The methodology involves biochemical analysis for a novel growth factor measured on serum samples and needle rinse/washings collected following aspiration of thyroid lesions. As such, there is no conventional histomorphological or cytomorphological aspect for review. These authors’ claim, that this is potentially the first study to investigate the value of this growth factor as a serum or needle rinse biomarker for thyroid cancer diagnosis, is credible. Ergo, by default, this becomes the first paper to dismiss any such association. As a subordinate observandum, the authors do acknowledge putative relationship with hyperthyroidism and propose further investigation of this - it would, however, be primarily of aetio-pathogenetic interest and there is no suggestion that any such confirmed secondary association might prove to be of diagnostic benefit in the clinical setting. Thus, there would seem to be scant incentive for anyone to repeat this work and/or expand upon it. There is an intrinsic statistical component, which requires professional scrutiny. There are no images. Pending statistical validation, the decision to accept for publication here is substantially contingent upon editorial policy regarding publication of a null outcome, mindful of the risks of negative publication bias. Editorial judgement of how pivotal the findings might prove to the specialist readership, also to the wider scientific/medical community, triage with competing manuscripts and discretion over balance of journal content will all be material considerations, in line with the objectives of the Journal.

Response:
We thank Dr Warfield for his thorough review and agree with the points raised.

Point 2
Supplementation of the text by tabulated results and/or graphic illustrations ought to be considered. If done well, these almost always enhance the impact to a reader.

Response
We agree that the addition of tabulated results would enhance the paper and we have added additional material, in line with suggestions also made by Reviewer 2. An additional Figure has been added (new Figure 2B) and an additional table has been added (Additional Table 3).

Martin Read (Reviewer 2):
Point 1
In this study the authors have examined the value of proNGF as a serum and biopsy-rinse biomarker for thyroid cancer diagnosis. Overall, the manuscript is well-written, clear and of scientific importance to inform the wider research community of the value of utilising proNGF as a clinical biomarker in thyroid disease.

Response
We thank Dr Read for his careful review, and respond in detail to the comments below.

Point 2
The title reflects the main finding of the manuscript, i.e. that proNGF is not a clinical biomarker for thyroid cancer. However, the authors do show some positive results- i.e. serum proNGF may be a biomarker for hyperthyroidism and for follicular lesions in general. The impact of the
manuscript would be greater if the authors introduced some element of their positive findings into the title.

Response
We thank the reviewer for this suggestion, but we prefer not to change the title of the manuscript. Hyperthyroidism is only an incidental finding of our study, which was designed to investigate proNGF as a cancer biomarker. As a certain number of cases included in this cancer study happened to exhibit hyperthyroidism, we secondarily observed that they might be a relationship between proNGF and hyperthyroidism. As pointed by reviewer 1, at this stage the subordinate observation of a potential link between proNGF and hyperthyroidism is only putative and should constitute an incentive for other groups to repeat and/or expand on it. As such, we prefer not to highlight this relationship in the title.

Point 3
In Fig 2A it is not clear why there are error bars on the graph. The data appears to be finite percentage values derived from Table 2- i.e. 9/28 hyperthyroid cases (32%) v 19/176 euthyroid cases (11%). Please clarify.

Response
The error bars represent the standard error of the mean, however we acknowledge that this was not explained. We agree that the data in Figure 2A is sufficiently clear without presenting error bars, and these have been removed. We have also improved the Figure legend.

Point 4
The authors need to include a new figure (similar in design to Fig. 2B or 2C) comparing serum proNGF values stratified according to euthyroid versus hyperthyroid status. This new figure would be useful to reinforce the findings of Figure 2A/Table 2 showing that proNGF levels are higher in sera from patients who were hyperthyroid at the time of sampling.

Response
A new figure (now labelled Figure 2B) has been included addressing this point. The figure legend has also been amended.

Point 5
Serum proNGF data provided in Table 2 is a bit unclear as the median serum proNGF values (middle column) for hyperthyroid versus euthyroid status are both shown as zero but a p value of 0.002 is given. In fact the median serum proNGF values for all the different categories (i.e. malignancy, age, sex etc) are set to zero. Please state whether the middle column of data refers to the median value for serum proNGF and the value in brackets are the IQR. The authors should check the median proNGF values in Table 3 as well, which are all set to zero except for the follicular lesions (present) category. Further clarification is required.

Response
We can confirm that the data presented in Table 2 are correct. The table caption has been clarified to state that medians and interquartile ranges are presented. The newly presented Figure 2B will assist with interpretation of these data. As more than 50% of the samples were negative
for proNGF, the median value is correctly set at zero. However, samples from patients who were hyperthyroid at time of sampling were more likely to contain proNGF than patients who were euthyroid (demonstrated in new Figure 2B and in the outcome of the statistical tests). Therefore, even though the median value is still zero, the interquartile range demonstrates the different distribution of values, which is quantified using the non-parametric statistical test.

Point 6
The authors need to provide an additional table with individual patient characteristics (n=73) for cases with detectable proNGF from the biopsy rinse procedure (i.e. similar in design to additional table 2 for patients with detectable serum proNGF).

Response
Additional Table 3 has been included containing these data.