Author's response to reviews

Title: The combination of four molecular markers improves thyroid cancer cytologic diagnosis and patient management

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Author's response to reviews: see over
Dear Editor,

Please find included the revised version of the manuscript entitled “The combination of four molecular markers improves thyroid cancer cytologic diagnosis and patient management” by Panebianco et al. in consideration for publication in *BMC Cancer, Translational Oncology Section* (MS:1840605813168730).

We would like to thank you and the referees for the thorough revision.
Please find below the point-by-point response to our Reviewers. For clarity's sake changes in the MS are highlighted in yellow. We also improved the style of written English using a native-English speaker recommended by BioMed Central.

We sincerely hope you will find the revised MS acceptable for *BMC Cancer*.

Kind Regards,

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Point-by-point response to specific Reviewer comments

Reviewer 2:

a) If a positive BRAF V600E mutations means papillary cancer why you do four molecular markers after this study?

The BRAF mutation, as we reported in our paper, has become a prognostic biomarker in thyroid cancer. However, even after BRAF V600E analysis, there will still be a certain percentage of malignant tumor that would remain indeterminate (tumors that do not display BRAF V600E mutation). The presence of BRAFV600E mutation assures the malignancy of the nodule, however its absence cannot support the benignity of thyroid nodules by itself. We propose the use of a four-gene model to be applied when the molecular analysis results negative for BRAF mutation. We used samples carrying BRAF V600E mutation to build our computational model as positive control and they resulted correctly classified as malignant. Thus, our proposed 4 gene model, as additional step to BRAF mutational analysis, increases the percentage of thyroid nodules correctly classified and helps to decrease the percentage of indeterminate lesions.

b) What are the results of four molecular markers other than papillary cancer such as follicular cancer, follicular adenoma or poor differentiated cancer

We thank the Reviewer for his/her comment. The FNA samples used in our study were histologically diagnosed as papillary thyroid carcinoma (PTC) or benign nodules (BN). We did not have samples with histological diagnosis different from those. We chose to collect PTC samples since they belong to the most common type of thyroid cancer.

Reviewer 3:

Discretionary Revisions

a) Most readers are not familiar with Bayesian Neural Networks and the discriminant analysis. Could authors describe it in detail?

We thank the Reviewer for his/her suggestion. We have described the Bayesian Neural Network and Discriminant Analysis in the Methods paragraph “Statistical Analyses”. We also have added references (n. 30-31-32-33-34-35) on Bayesian Neural Networks and discriminant analysis.
b) MicroRNA can be detected in sera of patients with cancer. It is better to provide the correlation of the expression of miR-222 and miR-146b between tumor tissue and patient’s sera.

In recent years, profiles of miRNAs in plasma and sera have been found to be altered in cancer [1, 2]. Some studies showed that certain serum and plasma miRNAs are up-regulated in Papillary Thyroid Carcinoma, for example Yu et al. [3] demonstrated a significant correlation between the serum expression of miR-222 and tumor tissue of patients with PTC; Lee et al. [4] showed that miRNA146b and miRNA222 were over-expressed in plasma from patients with PTC. On the other hand, it has been demonstrated that miRNA 222 and miRNA146b are altered sera/plasma markers not the only in patients with thyroid cancer, in-fact altered sera/plasma levels of the same miRNAs have been found also in patients affected by other cancer. For instance, a correlation between the level of miRNA 146b in the sera of patients with prostate cancer [5] and lung adenocarcinoma [6] has been found, moreover serum expression of miRNA222 was associated with hepatocellular carcinoma (HCC) [7] and breast cancer [8]. Furthermore the detection of miRNAs in the serum, as well as the other circulating fluid, has some limitations in term of specimen collection and processing, RNA extraction and amplification efficiency [9]. Therefore, even if sera analysis of miRNA222 and 146b levels could be less invasive as biomarker, the authors think that the detection levels of these miRNAs directly on FNA biopsy samples would resulted more specific. Since FNA contains only thyroid cells of the examined nodule, it is clinically more relevant and specific for thyroid cancer evaluation. Moreover, the molecular analysis of FNA cytology results more practical and easier at routine level.

Reviewer 4:

Did you compare the results of 4 gene model in the same patient using different cytology? I wonder the consistency of results in the same patient.

All patients included in our cases had one FNA sample of the lesion. The diagnostic prerequisites were not sufficient to repeat the FNA procedure. We did not repeat the exam for ethical reasons and to avoid any discomfort to the patient. We have included this in Methods: FNA samples section.
References


