

## Plasma Cell-Free DNA to Differentiate Malignant from Benign Thyroid Nodules

**Background:** Molecular testing is increasingly used to identify malignancy in thyroid nodules (especially indeterminate category). Measurement of cell-free DNA (cfDNA) levels from plasma has been useful in diagnosis of cancers of other organs/tissues; herein we analyze cfDNA levels in patients with thyroid nodules to explore the possibility of establishing a cut-off for identification of malignancy.

**Methods:** Patients underwent ultrasonography (USG) and USG-guided fine needle aspiration as well as surgery, where indicated. cfDNA was extracted from plasma and quantified. In initial analysis (determination of cutoff), cfDNA levels were compared between Bethesda 2 and Bethesda 5&6 to establish a cutoff value that could differentiate malignant from benign nodules. In the subsequent analysis, the aforementioned cutoff was applied (validation of cut-off) to those with indeterminate nodules to check ability to predict malignancy.

**Results:** FNA (n = 119) yielded patients with Bethesda 2 (n = 69) Bethesda 5 & 6 (n = 13) who underwent histopathological confirmation. cfDNA levels in these 2 groups were  $22.85 \pm 1.27$  and  $96.20 \pm 8.31$  (ng/mL) respectively. A cfDNA cut-off of 67.9 ng/mL, with area under the curve of 0.992 (95% CI, 0.97-1.0) with 100% sensitivity and 93% specificity was established to identify malignant lesions. Indeterminate group (N=37) underwent surgery and using the previously identified cut-off for cfDNA, we were able to identify malignant lesions with a sensitivity of 100% and specificity of 92.3%. There was a very strong agreement between cfDNA-based classification with histopathology-based classification of benign and malignant nodules (Cohen's kappa 0.94;  $P < 0.001$ ).

**Conclusion:** Plasma cfDNA estimation could help differentiate malignant from benign thyroid nodules.

**Key Words:** thyroid nodule, cell-free DNA, indeterminate nodule, differentiated thyroid cancer



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