

Peer Review File

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Reviewer A

Comment 1: "1. The patient had preoperative molecular testing for FNA and the results were negative. However, the size of the incidental PTC and MTC was very small. It is likely that the FNA might miss these small tumor foci and only included benign tumor cells resulting in the negative. It would be more informative for the readers if the authors can extract the DNA from the tumor foci only and submit for genetic testing including BRAF, TERT, RAS (for PTC) and RET mutations (for MTC)."

Reply 1: Thank you for your suggestion. Given patient's history and perioperative work up, the index of suspicion for hereditary medullary carcinoma is low. However, patient is considering to possibly undergo genetic counselling and germline testing as advised for patients with apparently sporadic MTC, including sequencing of exons 10, 11, and 13 through 16 of the RET gene.

We agree that molecular/genetic testing of respective FNA samples is a useful tool to anticipate aggressive tumor types preoperatively and therefore assist in planning the extent and timing of surgery. However, ThyGeNEXT oncogene panel and the ThyraMIR® did not reveal genetic alterations. The extent of surgery would not have been changed in this case, since we planned total thyroidectomy for an increasingly symptomatic MNG. Profiling of somatic tumor genetic alterations in this case of a nonaggressive papillary micro carcinoma would not have changed the postoperative management.

Changes in the text: No changes.

Comment 2: "2. Information for dilution and manufacturer of the immunostains used in this study should be provided."

Reply 2: Standard immunohistochemical protocols were followed.

Antibody	Clone	Dilution	Antigen retrieval	Company
CK19	RCK108	1:100	HIER EDTA Buffer	Dako Corp
HBME	HBME-1	1:50	HIER EDTA Buffer	Dako Corp
Galectin 3	9C4	1:100	HIER Citrate Buffer	Leica Microsystems
Ret Oncoprotein	3F8	1:40	HIER EDTA Buffer	Leica Microsystems

Changes in the text: Added "standard" before immunohistochemistry studies on Line 109.

Comment 3: "3. If possible, please clarify the family history of thyroid cancer in the mother. What type of thyroid cancer?"

Reply 3: Unfortunately, the patient does not remember which type of thyroid cancer and is unable to get this information.

Changes in the text: Added "an unknown type of" before thyroid cancer on Line 57.

Reviewer B

Comment 1: "1) Although it was described as a classic PTC in the main body of the manuscript, both images at low power (A) and high power (B) in Figure 2 show a discrete nodule composed of follicles exhibiting nuclear features of PTC. No apparent papillae, infiltrative border, or psammoma bodies are appreciated. From viewing images alone, NIFTP should be excluded. Authors need to describe and show histopathologic features of classic PTC (instead of NIFTP)."

Reply 1: Unfortunately, we do not have a replacement image available.

Changes in the text: No changes.

New comment: Just want to be sure that those tumors are indeed PTC (instead of NIFTP). One of the co-authors, a pathologist, should be able to re-review the case to clarify the issue and provide new images showing features of classic PTC.

New reply: The new submitted images show infiltrative neoplasm with well developed nuclear features of PTC, follicular variant. Currently there are no specific recommendations for classifying microcarcinoma with well developed nuclear features of PTC as NIFTPs.

Comment 2: "2) The margin and regional lymph nodes were not involved (line 72). Was there a neck dissection or were lymph nodes identified associated with the total thyroidectomy specimen? How many nodes were detected? The AJCC staging should be mpT1aN0 for PTCs and pT1aN0 for MTC."

Reply 2: The pathology report distance from invasive carcinoma from closest margin more than 10mm and no regional lymph nodes found or submitted. This is based on the total thyroidectomy specimen. We will update AJCC staging with the N0 recommendation.

Changes in the text: Changed "mpT1a" to "mpT1aN0" on line 68 and "pT1a" to "pT1aN0" on line 71. Added " based on the total thyroidectomy specimen" on line 73.

New comment: If no regional lymph nodes were found or submitted, "regional lymph node" should be deleted on line 72 and staging should be Nx (instead of N0) on lines 68 and 70.

New reply: Deleted "regional lymph nodes" on line 79. Staging changed from N0 to Nx on lines 73 and 78.

Comment 3: "3) As we all know, papillary microcarcinomas generally tend to have an excellent prognosis, although a small proportion of papillary microcarcinomas have aggressive clinical behavior, which may be related to tumor multifocality, superficial/subcapsular tumor location, lymph node involvement at presentation, BRAF V600E mutation, etc. Treatment options for papillary microcarcinomas may include active surveillance, surgical resection (either hemithyroidectomy or total thyroidectomy), with or without radioactive iodine therapy after surgery. However, medullary microcarcinomas have significant rates of poor prognostic features known to impact the survival of patients with MTC. These microcarcinomas are an important clinical entity that requires a comprehensive evaluation and surgical management. Therefore, the medullary carcinoma (even if it's a microcarcinoma) will dictate the clinical behavior of this synchronous tumor."

Reply 3: We agree with this statement in lines 120-122.

Changes in the text: No changes.

Comment 4: "4) Suggest replacing Figure 2D with an image at higher magnification as it's hard to appreciate the described histologic features of MTC "

Reply 4: Unfortunately, we do not have a replacement image available.

Changes in the text: No changes.

New comment: Unsure why a replacement image is not available. The pathologist should be able to help out.

New reply: The new submitted images show a well circumscribed nodule with centrally located nuclei, coarse chromatin and no nuclear features of PTC. These cells are positive for calcitonin, chromogranin and TTF1 supporting the diagnosis of medullary carcinoma. Medullary carcinoma of the thyroid gland can exhibit a wide range of morphological appearances and may not always show the classic plasmacytoid morphology with amyloid deposits. Therefore, immunohistochemical stains are crucial for making such diagnosis in the absence of classic morphology.

Comment 5: "5) Suggest replacing "cancer" with "carcinoma", especially in Table 1: Papillary thyroid cancer (PTC) and Medullary thyroid cancer (MTC) and using the terms "papillary

microcarcinoma" and "medullary microcarcinoma" (instead of micromedullary and micropapillary carcinoma, etc.) consistently and uniformly throughout the manuscript "

Reply 5: We agree, thank you for catching this.

Changes in the text: Changed instances of "micropapillary carcinoma" and "micromedullary carcinoma" to "papillary microcarcinoma" and "medullary microcarcinoma" respectively in lines 67 and 118. Changed "cancer" to "carcinoma" in table headers.

New comment: should change "cancer" to "carcinoma" in the entire manuscript

New reply: "Cancer" to "carcinoma" in the entire manuscript except in line 73, 112, and the references.

Comment 6: "6) On several occasions, abbreviations (such as IHC, TTF-1, CK7, etc. in the Figure 2 legend) are only being used once, consider deleting those "

Reply 6: We agree, thank you for catching this.

Changes in the text: Removed the abbreviations IHC, TTF-1, and CK7 from table 2.

Comment 7: "7) Add "FNA" between the patient's and biopsy (L114)"

Reply 7: We agree.

Changes in the text: Added "FNA" between the patient's and biopsy in line 115.