Peer Review File

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Reviewer A

In this study by Dr. Kim and colleagues, the authors retrospectively assess thyroid tumors with a preoperative cytological diagnosis of "Follicular Neoplasm or Suspicious for a Follicular Neoplasm" (Bethesda IV category) in terms of histopathological attributes, NRAS and BRAF mutations as well as clinical parameters. The study comprises 416 patients, and the authors report a rate of malignancy (RoM) of >50% - which is surprising. The study also suggest that nuclear atypia is associated to malignant disease in this Bethesda category. Although confirmatory in nature, the cohort size is impressive. I have several suggestions for the authors to consider:

1. The definition of nuclear atypia in this study was "nuclear enlargement, pallor, grooves", i.e. PTC-related nuclear changes. I would recommend the authors to use the term PTC-related nuclear changes throughout the manuscript, as nuclear atypia has different meanings for different tumor forms, including thyroid tumors (for example, follicular thyroid adenomas with bizarre nuclei et.c.)
Reply 1: We have changed “nuclear atypia” to “PTC-related nuclear changes” throughout the manuscript and clarified the meaning in the text.
Changes in the text: … nuclear (nuclear enlargement, chromatin clearing, nuclear grooves, and nuclear membrane irregularity). The nuclear atypia raised the possibility of PTC, but was not diagnostic of malignancy. The specimen showing architectural atypia and nuclear atypia concerning for PTC was interpreted as follicular neoplasm with PTC-related nuclear changes. (see Page 8, line 5-9)

2. The study reports PTC-related nuclear changes in 9.1% of Bethesda IV category tumors. As I am aware, cases that demonstrate the nuclear features
of papillary carcinoma are excluded from this category (please see The Bethesda System for Reporting Thyroid Cytopathology, Edmund S. Cibas and Syed Z. Ali. Am J Clin Pathol 2009. Could the authors comment on this, and make the manuscript more

lucid regarding their institutional definition of a Bethesda IV lesion?

Reply 2: The diagnostic category “follicular neoplasm/suspicious for a follicular neoplasm” can show some nuclear atypia: “either enlarged, variably sized nuclei, and prominent nucleoli or enlarged nuclei with nuclear contour irregularity and mild and/or focal chromatin clearing.” Nuclear atypia by itself is not diagnostic of malignancy or even neoplasia, as hyperplastic nodules and follicular adenomas can demonstrate nuclear enlargement and hyperchromasia. In the present study, when nuclear features concerning for PTC are present in the Bethesda category IV, we interpreted the FNA/CNB specimen as follicular neoplasm with nuclear atypia. The term “nuclear atypia” has been reworded as “PTC-related nuclear changes” according to the reviewer’s comment.

Changes in the text: The nuclear atypia raised the concern for PTC, but was not diagnostic of malignancy. The specimen showing architectural atypia and nuclear atypia concerning for PTC was interpreted as follicular neoplasm with PTC-related nuclear changes. (see Page 8, line 6-9)

3. Building on point 2 above, could the high RoM in this study in part be due to the fact that many of these tumors should have been identified as FV-PTCs (Bethesda V/VI) already at the level of cytology? If so, is the interpretation that cytological findings of "nuclear atypia" (i.e. PTC-related nuclear changes) correlates to malignancy in Bethesda IV tumors a relevant finding?

Reply 3: If architectural features of follicular neoplasm are present and nuclear features of PTC are not definitive, such specimens was diagnosed as follicular neoplasm with nuclear atypia (PTC-related nuclear changes). If the follicular cells show definitive nuclear features of PTC and if there are at least focal elements associated with classic PTC, we interpret the specimen as “malignant, PTC.” When both architectural features of a follicular neoplasm and nuclear features concerning for PTC are present, but not definitive, we interpret the specimen as “suspicious for malignancy, PTC.”

Regarding the risk of malignancy/NIFTP (ROM) in thyroid nodules with follicular neoplasm, our study showed a similar ROM (50.2%) to that seen in previous studies in Korea. Yoon et al. reported the ROM of 58% in the CNB group.

Reference: Yoon RG, et al. Thyroid 2014;24(11):1612-7. doi: 10.1089/thy.2014.0140.

4. I assume all FV-PTCs diagnosed on histopathology lacked the inclusion criteria needed for an NIFTP diagnosis (WHO 2017)?

Reply 4: The purpose of this study was to identify the clinical and cytology/pathologic features to predict malignancy in patients preoperatively diagnosed with follicular neoplasm. We included NIFTP within the extent of malignancy. So, 63 patients were identified by pathologic slide review again. Finally, 146 patients were diagnosed as malignancy, and 63 patients were diagnosed as NIFTP.

5. Please comment on the similar results in terms of RoM in the FNAB and core needle biopsy groups. Is this an expected finding?

Reply 5: In the present study, CNB was performed in 278 (66.8%) patients, but there was no significant difference from FNAC in the diagnostic rate of malignancy (51.4% vs. 48.6%, p=0.755).

Lee et al. also reported that follicular neoplasm was frequently diagnosed in CNB, but the rates of malignancy were equivalent to FNAC

Reference: Lee SH, Park GS, Jung SL, Kim M-H, Bae JS, Lim DJ, et al. Core-needle biopsy for the preoperative diagnosis of follicular neoplasm in thyroid nodule screening: a validation study. Pathology-Research and Practice. 2016;212(1):44-50.

6. In the overall introduction, the authors fail to recognize recent advances in the prognostication of thyroid tumors - including TERT promoter mutational screening programmes both on the pre- and postoperative level (PMID: 31042674 and PMID: 31561592). I suggest the authors discuss these papers and if possible, elaborate on whether or not this study would have benefited from TERT promoter mutational analyses.

Reply 6: Thank you for your kind comments. TERT promoter mutational screening test have not been yet used for prognosis of follicular neoplasm due to our hospital policy. However, we are preparing further studies using TERT promoter mutational analyses

7. Please put all significant P values in bold or marked out with asterisks for the readers (all tables).

Reply 7: All significant P values were changed in bold in all tables.

Reviewer B

Operation (thyroidectomy or lobectomy) is currently required for definitive diagnosis of follicular thyroid cancer. The diagnosis of malignancy depends on the demonstration of blood vassel and/or capsular invasion. A very current research task is to find diagnostic methods that would allow to established the diagnosis of FTC before surgery. It would reduce the rate of unnecessary thyroid surgery for patients with benign nodules and would allow to plane appropriate scope of surgery in patients with thyroid cancer.
The aim of this study was to identify the clinical and cytologic features to predict malignancy in patients preoperatively diagnosed with follicular neoplasm.
The study is interesting, however it has some weak points.
Critical points:
1. There is no information whether patients had a single thyroid nodule or multinodular goitre.
Reply 1: Some patients have a single thyroid nodule, and the others have multinodular goiter. Incidental thyroid carcinoma was discovered by surgical specimen in 15 patients.

2. Did the authors have a system to identify the location of the tumor subjected to a biopsy to ensure that they examine the same tumor during postoperative analysis?
Reply 2: In our hospital, the surgeons sent postoperative surgical report to pathologists. The surgeons write down tumor characteristics, such as tumor location and size in that report. The pathologists diagnose the tumors by checking the surgical report and preoperative US.

3. The smallest size of thyroid cancer in histological examination was 0,1cm – the question is whether biopsy of such a small lesion was performed?
Reply 3: In the processing of revision, we have identified some errors, especially very small cancers. So, we checked the pathologic slides again, and identified that 15 small cancers were incidental thyroid carcinomas accompanying main lesions. The changed part was modified in manuscript.

4. Moreover, incidentally found microcancers should be excluded from the analysis, and they can't be considered during calculating a risk of malignancy in FN tumors.

Reply 4: We identified 15 patients with incidentally found microcancers. So, those cancers was excluded from the malignancy/NIFTP group.

5. How the authors would explain the presence of BRAF V 600E mutation in benign thyroid tumors?

Reply 5: As I mentioned earlier, we have identified some errors in pathologic things. The presence of BRAF V600E mutation in benign thyroid tumors was one of mistake during analysis of our study. We checked again for BRAF V600E mutation. We identified that there was no BRAF V600E mutation in benign thyroid tumor. This is updated in Table 4 and 5.