Peer Review File

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Title: Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance: Asian vs non-Asian practice, and the Singapore Experience

Reviewer A

Very nice and detailed informative review collating all Asian and non-Asian studies pertaining to AUS/FLUS in thyroid cytopathology. It will be a highly cited article.

Reply: Thank you for your encouragement and positive comments!

Reviewer B

This manuscript by Dr. Ooi et al. is a well-written meta-analysis of AUS thyroid nodules in the literature and compared data among countries. The author correctly concluded that a higher incidence of AUS-Nuclear atypia coupled with a higher rate of PTC in resected AUS/FLUS nodules in the Asian cohorts. In contrast, AUS-Architectural atypia and follicular-patterned neoplasms featured more prominently in the non-Asian groups.

Major comments:

Comment 1: Please show your background data in this manuscript to characterize thyroid practice in Singapore. The first is the malignancy rate in all resected thyroid nodules; more than 60% of resected thyroid nodules were benign in most Western practice. In comparison, more than 50% of resected nodules were malignant in Asian practice. It means that more benign nodules undergo surgery (unnecessary overtreatment) in Western practice probably because of fears of malpractice litigation push clinicians for surgery with a low threshold for surgery. It is because diagnostic surgery and surgical treatments are only reliable methods to prevent missing malignancy. If benign thyroid lesions occupied more than 50% of surgically treated thyroid nodules in Singapore thyroid practice, your clinical doctors would follow Western clinical guidelines more strictly than other Asian clinicians.

The second is prevalence of NIFTP and UMP. If your prevalence of borderline thyroid tumors (NIFTP and UMP) occupied more than 5% of PTCs, your clinicians apply more diagnostic surgeries to clinically low suspicious thyroid nodules aggressively similar to the Western practice.

Reply:

- Overall ROM: We do agree that it would be very informative to know the ROM of all categories combined, of all resected cases, but unfortunately, we have very limited data on this, for only a single year. We have included this limited data in the Discussion and mentioned that the overall ROM is more aligned to non-Asian rates, thereby possibly reflecting more Westernized practices (page 25 - lines 612-617).

- Regarding NIFTP: Unfortunately, we do not have the formal incidence of NIFTP as this study was based on data collected up to 2014, prior to the adoption of this term. A review of existing data showed only 2 possible cases, which were insufficient to make a meaningful analysis. We have mentioned this in the Discussion, and also briefly elaborated on the impact of NIFTP on the ROMs of indeterminate nodules, highlighting the regional differences, based on the work of others (page 31 - lines 756-760).

Comment 2: The indeterminate cytological category was sub-classified into a lower-risk (AUS/FLUS) and a higher-risk (FN/SFN) indeterminate categories in the Western countries as recommended by the Bethesda reporting system (#4). In contrast, most pathologists in Asian countries use the AUS terminology differently, and the AUS category in Asian practice does not mean the low-risk indeterminate category and often showed a very high risk of malignancy (ROM). The indeterminate cytological category was traditionally sub-classified into follicular neoplasm (RAS-like tumors lineage with architectural atypia) and the other category, which accepts cases with PTC type nuclear atypia (BRAF-like tumors with nuclear atypia) in Asian countries, which was reported by Kakudo et al. (#105). As a consequence, a significant number of poor quality specimens with PTC-type nuclear atypia was classified in indeterminate (the other) category, which was often called AUS in Asian practice (AUS-N in this study) (while cases with RAS-like architectural abnormality were often excluded from the AUS and classified in FN/SFN category in Asia) when translating into the Bethesda system. It was correctly identified in this study on page 27, lines 5-6, and stated as the incidence of AUS-N appeared to be far higher amongst Asian series compared to non-Asian cohorts (70.3% vs 33.5%, $p<.001$). However, this Asian modification of the AUS category made the BRAF single gene test most efficiently identify PTCs in AUS nodules (extremely high ROMs and more PTCs on resection in AUS nodules) in Asian series, which were proved by several Korean studies (#25, #26, #32). Please note that significant numbers of cases with RAS mutations (cases with architectural atypia) are classified in AUS/FLUS category, and poor-quality specimens with PTC type nuclear changes were often classified as suspicious for malignancy category in Western practice (#23). It was also mentioned

on page 22 in this manuscript that cytologically classified as AUS/FLUS tends to be FVPTC or other follicular patterned cancers such as FTC (#81). This diagnostic criteria in the Bethesda system in the West requires multiple-gene panel tests on AUS nodules to rule-in or rule-out malignancy (both PTC and FTC), and made the BRAF single gene test not useful.

Reply:

- We have noted these points on the trend towards different inclusion criteria for AUS/FLUS between the two geographic regions, and have incorporated them into the Discussion. Briefly, we have discussed the higher AUS-N rates in Asian cohorts, which paralleled the higher incidence of PTC in surgically resected cases, and also possibly explained the different molecular approaches. (**Discussion:** page 26 - lines 644-656, page 29 - lines 696-709; and **Conclusion:** page 32 - lines 778-788).

- To highlight the point regarding follicular patterned lesions being included in AUS/FLUS in the West, we have added a line in the Discussion to explain the higher incidence of follicular neoplasms in resected nodules in non-Asian studies (page 29 - lines 718-722)

Comment 3: Please add your data on histological types of malignancy found in surgically treated AUS nodules. If there were significant numbers of FTCs in AUS nodules on resection in your Singapore practice, your diagnostic criteria of AUS nodules are more closely aligned to the Western diagnostic standards recommended by the Bethesda system. You can add one more evidence, and conclude that the data from Singapore appears more closely aligned to non-Asian trends, despite its geographical location in Southeast Asia and its predominantly Asian population.

Please note that the Asian cytopathologist tries to diagnose indeterminate nodules into two genetically distinct tumor lineages separately; RAS-like lineage (FTC, FV-PTC, and NIFTPs) in FN/SFN category and BRAF-like lineage (PTCs) either in AUS/FLUS, SM or M categories (#105). As most Asian patients cannot afford expensive multiple-gene panel tests, this reviewer believes this Asian diagnostic system, which does not require the costly multiple-gene panel tests, is much more cost-saving for triage patients for surgery or selects clinical follow-up (active surveillance). In the same time, the ROM of surgically treated AUS nodules is significantly higher than those of the Western practice with the aid of multi-gene molecular tests. This modification of AUS category decreases health care costs in thyroid nodule practice in Asian countries and ultimately prevents the overtreatment of patients with benign nodules and low-risk thyroid carcinomas. The Asian WG was established to

characterize Asian thyroid practice, in the same time to protect Asian patients with thyroid nodules from overtreatment, which was common in Western practice.

Reply:
- Follicular carcinoma (FC): We performed an analysis of the incidence of FC in Singapore, other Asian and Western countries. We found that the pooled incidence was similar in both cohorts, 1.9% ([95% CI, 0.8, 2.9) in Asian studies vs 1.6% [95% CI, 0.4, 2.7) in non-Asian studies; p <.76. Hence, we did not add this point in the results or discussion, as it would lengthen the manuscript significantly, while only adding limited value to the discussion. The discussion on follicular neoplasms has also already been made, which also serves to highlight the different practices of including follicular-patterned lesion in the AUS/FLUS category (see point 2 above).

- Regarding the genetically and cytologically distinct AUS/FLUS categorization in the Asian vs non-Asian practice, these points have been expanded on in relation to the incidence of AUS-N and usefulness of BRAF testing in Asian cohorts. They have been mentioned in the Discussion and Conclusion, highlighting the different practices in the application of TBSRTC, which then leads to different molecular practices (**Discussion:** page 26 - lines 644-656; page 29 - lines 696-709, lines 718-722; **Conclusion:** page 32 - lines 778-788).

Minor comments:

Comment 4: The abstract is a little bit too long. Sentences, such as, "This emphasizes the need for follow-up studies in individual practices, as there may still be considerable inter- and intraregional variations in disease prevalence and distribution," may be removed from the abstract and placed in the discussion section.

Reply: The abstract has been shorted from 427 to 357 words, and the sentence has been removed from the abstract and incorporated into the Discussion section (page 31 - lines 746-748)

Comment 5: Discussion is also lengthy and can be trimmed. This reviewer advises the author to modify some of discussions hopefully incorporating the above major comments.

Reply: The discussion and other parts of the manuscript have been trimmed, and, including the additional discussion points mentioned above, the total manuscript word count is 6010.