

Peer Review File

Article information: <http://dx.doi.org/10.21037/gs-20-618>.

Reviewer A

1. The manuscript reads well, however the main conclusions are confusing.

It is not clear how the authors achieved some of the conclusions, as both the methods and results sections need to be expanded to provide further details.

Reply: Thank you for your comments. We have made extensive changes to our manuscript to make it easier to read and understand.

2. The authors mention that 34.5% of patients displayed multifocal disease- did these cases tend to show more lymph node mets and/or ETE than cases that were unifocal?

Reply: In our study, the ETE rate of unifocal was 46.6% (69/148), and that of multifocal was 51.3% (40/78); the lymph node metastasis rate of unifocal was 34.5% (51/148), and that of multifocal lesion was 32.1% (25/78). Chi square analysis showed that no significant difference was observed between unifocal and multifocal lesion in ETE and lymph node metastasis.

Changes in the text: This part is not included in the manuscript.

3. What were the cytomorphologic characteristics of the additional tumors in these patients, since they could have been different characteristics and the mets or

ETE could have been secondary to the other tumor. If that is the case, then the authors results are skewed- it would then not be accurate to determine that cytomorphic features of the tumor are the determining factor in the ETE or LN mets, since those could have potentially arisen secondary to the other tumors. I am not sure how authors can resolve this unless there was an evaluation of all tumors in the cases of multifocal disease. Additionally, it seems like there may have been many additional confounding factors.

Reply: This is really an important but neglected part in our previous manuscript. In our hospital, fine-needle aspirations (FNAs) were collected by surgeons or sonographers via ultrasound guidance, and ultrasound evaluation was carried out before puncture. In the case of multiple suspicious malignant nodules, if the suspicious malignant nodules were distributed in different gland lobes, the most suspicious nodule in each lobe was selected for puncture separately. If multiple suspicious malignant nodules were located in the same gland and the degree of malignancy was similar, the operator selected the nodule with the largest diameter, the nodule with extracapsular invasion or the nodule with invasion into the surrounding structures such as strap muscles, trachea, larynx, vasculature, esophagus, and/or recurrent laryngeal nerve.

It is really true as reviewer pointed that it may not be accurate to determine the cytomorphic features of tumors in the cases of multifocal disease, however, this issue is unavoidable for FNA specimen. Although the punctured nodule may not have exhibited the same characteristics as all nodules, it was indeed the most representative

since detailed ultrasound evaluation was carried out before puncture. The punctured nodule was the most malignant and aggressive nodule and its morphological characteristics were also most likely related to the clinicopathological parameters such as ETE or LN mets (We have added the above to the materials and discussion section of the new version).

Changes in the text: The above contents have been added to the page 8, line 8-12 and page 14, line 1-4.

4. Additionally, surgical pathology cases would need to be examined as a gold standard for comparison with the cytology cases.

Reply: We are very sorry that we have not made this issue clear, and the clinicopathological parameters in our study, such as tumor size, tumor focality, extracapsular invasion and lymph node metastases were all based on the surgical pathology.

Changes in the text: We explain this in the materials section of the new version in page 10, line 11-12.

5. It is unclear why the authors only included T1 cases.

Reply: The aggressiveness of pT1 PTC is a clinical concern. Compared to pT1 PTC, the treatment course for pT2 and above is clear, which is often surgical treatment or combined radiotherapy and chemotherapy [*Haugen B, Alexander E, Bible K, et al: 2015 American thyroid association management guidelines for adult patients with*

thyroid nodules and differentiated thyroid cancer. Thyroid. 2015.0020.]. For patients with pT1a PTC (≤ 1 cm), the decision to perform surgery or follow-up is controversial [Miyachi A, Ito Y, Oda H. *Insights into the management of papillary microcarcinoma of the thyroid. Thyroid 2018;28:23-31.*, Ito Y, Miyachi A, Oda H, et al. *Revisiting low-risk thyroid papillary microcarcinomas resected without observation: was immediate surgery necessary? World J Surg 2016;40:523-528.*], and it is necessary to conduct a comprehensive analysis based on the risk assessment and the wishes of the patient, but effective risk assessment methods are still lacking. Clinically, among patients with pT1a PTC at the same risk, some patients have extracapsular invasion (ECI) and lymph node metastasis (LNM) at an early stage, while some maintain growth in the glands for a long duration. On the other hand, although T1b patients undergo routine surgical resection, whether to perform preventive lymph node dissection for patients with a negative preoperative lymph node examination is questionable [Viola D, Materazzi G, Valerio L, et al. *Prophylactic central compartment lymph node dissection in papillary thyroid carcinoma: clinical implications derived from the first prospective randomized controlled single institution study. J Clin Endocrinol Metab 2015;100:1316-1324.*]. In some T1b patients with a negative preoperative lymph node examination, LNM is found in postoperative paraffin sections or LNM is found within a short period of time after surgery. These phenomena prompted us to consider whether it is possible to obtain evidence from cytomorphology to distinguish between

aggressive and indolent cases and provide a reference for patient follow-up or surgery
(We have added the above to the discussion section of the new version).

Changes in the text: The above contents have been added to the page 13, line 2-10.

6. The authors would need a stronger explanation for inclusion and exclusion criteria.

Reply: We are very sorry for the inaccurate expression, and we have re-written the inclusion and exclusion criteria. The inclusion criteria were as follows: (1) The patient underwent preoperative FNAC and surgery at Peking University Cancer Hospital between January 2018 and December 2019; (2) The histological pathology of the patient indicated pT1 PTC classified by AJCC 8th edition staging system, with tumor 2cm or less in the greatest dimension and minimal microscopic invasion. The exclusion criteria included FNAC smears comprising only a liquid-based smear.

Changes in the text: The above contents have been added to the page 8, line 3-6.

7. Additionally, can the authors obtain molecular data on the cases in the study in order to provide additional information in assessment of cytomorphologic features and their connection with more or less aggressive disease.

Reply: The suggestion is very nice and that's what we want to study in the next phases. As we all know, not all cases have enough materials for molecular detection, especially small focal PTCs, So far, we do not have enough data for this analysis.

Changes in the text: This part is not included in the manuscript.

8. Please expand on the cytological features to give a more detailed description in the methods section (page 7). It is not clear whether cellular adhesiveness means that cells had no cytoplasmic borders or whether it just means that they were groups as opposed to single cells.

Reply: We are sorry for the oversimplification of the methods section, and the detailed descriptions of the cytological features are provided in the Table 1 and the accessory notes. Cellular adhesiveness means the degree of aggregation between cells, and it just means that they were groups as opposed to single cells. And cellular adhesiveness was divided into 3 levels, that is predominantly the small sheet-like arrangement (score 0), predominantly the isolated pattern or solid clusters (score 2) and few discohesive single cells (score 1). Our study showed that the cytological feature of predominantly the isolated pattern or solid clusters indicated high aggressiveness.

Changes in the text: We added some description of cytological features in page 10, line 4-8 and Table 1.

9. The authors might consider breaking down the last sentence on page 10 (line 18-21) into smaller parts as this sentence is long and confusing.

Reply: We have re-written the last sentence on page 10.

Changes in the text: The original sentence is 'More cases with a predominant cytology of the isolated pattern or solid clusters had ETE than those with a

predominant cytomorphology of the small sheet-like arrangement (OR= 5.248), while there was no significant difference between cases with few isolated patterns or solid clusters and those that were predominantly of the small sheet-like arrangement.’, and it is replaced by the following sentence- ‘extracapsular invasion was found more often in patients with an adhesiveness score of 2 (predominantly the isolated pattern or solid clusters) than in those with an adhesiveness score of 0 (predominantly the small sheet-like arrangement) ($p=0.002$, OR=5.248). However, no significant difference was observed between patients with an adhesiveness score of 1 (few isolated patterns or solid clusters) and those with a score of 0’ (page 14, line 10-12).

10. On page 11 lines 1-4, the authors mention that "the results were easy to understand because the decrease in cell adhesion will lead to an increase in invasiveness"- can the authors please explain why and how a decrease in cell adhesion would lead to an increase in invasiveness; this may not actually be "easy" to understand. The authors also state that "a cytomorphology that is predominantly solid clusters may indicate high proliferative activity"- can the authors explain why solid clusters would indicate high proliferative activity? how did the authors come to this conclusion?

Reply: The results were along with some molecular research that the growth patterns of solitary and collective maybe manifestations of invasion [*Nagai T, Ishikawa T, Minami Y, et al. Tactics of cancer invasion: solitary and collective invasion. J Biochem 2020; 167(4):347-355.*].

In daily clinical practice, such a phenomenon also exists, in cells that show a predominantly isolated pattern, similar to poorly differentiated adenocarcinoma of the digestive tract, diffuse infiltration is often observed. The adhesion and restraint between the cells seems to be lost, and the cells become capable of invading their surroundings. Cytomorphology results exhibiting predominantly solid clusters may indicate high proliferative activity, the number of cells in solid clusters was greater than that in sheet-like arrangement slices in certain spaces, which might indicate that cells with predominantly solid clusters have high proliferative activity.

Changes in the text: We added some sentences in page 14, line 12 and page 15, line 1.

11. Page 11, lines 7-16 are detailed results that belong in the results section, and are too detailed of just results for a discussion section.

Reply: We have moved the detailed results to the results section.

Changes in the text: We moved the part to page 12, line 6-10.

12. Page 12, line 3- the authors should rephrase "can predict" to a statement that is not as strong/definitive, since there may be a certain level of contribution towards a prediction, the feature itself does not predict lymph node metastasis.

Reply: It is really true as reviewer suggested, and we are very sorry for our incorrect writing.

Changes in the text: The expression of "can predict" was replaced by "might predict" (page 15, line 9)

13. Page 12 line 19- what do the authors mean by "strong diagnostic significance"? do they mean no statistically significant difference? (which is the proper phraseology in statistics).

Reply: We are sorry for the inappropriate wording, we have rewritten this part.

Changes in the text: We have rewritten this part in page 16, line 5-7 ('For some other cytomorphologic features, such as intranuclear pseudoinclusions, nuclear size, nuclear pleomorphism and nuclear membrane regularity, although we did not confirm their significance for aggressiveness in the multivariate analysis, they also showed statistically significant differences in the univariate analysis').

14. Page 13 line 18- "it is not known whether...related to histological subtypes"- can the authors pull the surgical pathology cases for review and comparison?

Reply: The suggestion is very good, and if we could pull the surgical pathology cases for review and comparison, we might obtain more information about cytomorphologic feature. Due to the limited time and energy, in this study, we just analyzed nine cytological morphological features and assessed their associations with clinicopathological parameters to identify the cytomorphological features with high aggressiveness for clinical reference, and in the next study, we might evaluate the

relationship between several meaningful cytological morphological features and histological subtypes.

Changes in the text: This part is not included in the manuscript.

15. Tables 2 and 3 should include the sample size (n) and percent for each score, and each. There is not enough annotation/labeling in the tables, and they are confusing.

Reply: We have revised and simplified tables 2 and 3 based on your and other reviewers' comments.

Changes in the text: It can be seen in Tables 2 and 3.

Reviewer B

This study retrospectively reviewed the 226 cytology specimens and correlated nine cytomorphologic features with clinicopathologic parameters. Cellular adhesiveness and cytoplasmic amount were independently associate with extrathyroidal extension or lymph node metastasis. Even though the methodological approaches presented appear to be solid, there are some major issues that need to be addressed in detail.

1. This paper is generally described in colloquial style, making it difficult to read.

Please try to describe it in literary style as possible.

Reply: We had carefully revised the paper and polished it with AJE company, it was

edited for proper English language, grammar, punctuation, spelling, and overall style by one or more of the highly qualified native English-speaking editors at AJE.

2. In Title, this study did not evaluate prognosis, so the word “prognostic” should be removed.

Reply: Thank you for your suggestion. We checked similar mistakes in the article and corrected them.

Changes in the text: The title of the manuscript changed to ‘Cytomorphologic features as predictors of aggressiveness in patients with pT1 papillary thyroid carcinoma: a retrospective study of associations with clinicopathological parameters in 226 fine-needle aspirates’ (page 1, line 2-3).

3. Abstract, in the sentence of “... were strong predictors of some clinicopathological parameters ...”, please clarify the meaning “some”.

Reply: We have added some clinicopathological parameters to clarify it.

Changes in the text: We added some clinicopathological parameters in page 4, line 12 (‘Univariate analysis showed that cellularity, intranuclear pseudoinclusions, cellular adhesiveness, nuclear size, and nuclear pleomorphism were strong predictors of some clinicopathological parameters such as extracapsular invasion (ECI) and lymph node metastasis (LNM)’).

4. In page 4, lines 15 - 17, examples for “highly aggressive clinical behaviors” were described as higher rates of extrathyroidal extension (ETE), multifocality, nodal and distant metastasis, recurrence, and resistance to radioactive iodine therapy. But not all these parameters predict highly aggressive clinical behavior. Please clarify the meaning of aggressive behavior.

Reply: We are very sorry for our inaccurate expression of “highly aggressive clinical behaviors”. What we understand to be aggressive clinical behaviors is that the tumor invaded the surrounding tissue.

Changes in the text: The clinical manifestation of “resistance to radioactive iodine therapy” was removed.

5. In page 4, line 20, “while some are the classic or follicular subtype” please clarify “some”

Reply: We have rewritten this part.

Changes in the text: - We have rewritten this in page 6, line 4-7(‘Some aggressive PTCs are recognized by the World Health Organization (WHO) as biologically aggressive variants, including tall cell, columnar cell, solid, hobnail, diffuse sclerosing, and diffuse follicular variants, while some non-aggressive subtypes (classic or follicular subtype) can also manifest highly aggressive clinical behaviors.’).

6. In material section, please clarify the definition of pT1 PTC. If pT1 was classified by 8th AJCC staging manual, extrathyroidal extension only includes minimal microscopic invasion that has no prognostic implication. Gross extrathyroidal extension only is related with prognosis.

Reply: We have clarified the definition of pT1 PTC in revised version.

Changes in the text: We have added a part to the material section in page 8, line 4-5 (The histological pathology of the patient indicated pT1 PTC classified by AJCC 8th edition staging system, with tumor 2cm or less in the greatest dimension and minimal microscopic invasion).

In our revised version, extrathyroid invasion has been changed to extracapsular invasion. Although extracapsular invasion of thyroid cancer has no prognostic significance, it is still considered as an aggressive indicator in our research, a well-defined tumor capsule is a particularly favorable prognostic indicator [*Haddad R, Bischoff L, Busaidy N, et al. NCCN clinical practice guidelines in thyroid carcinoma. Version 2. 2019*], we have added this to page 14, line 6-8.

7. How was the metastatic status of lymph node confirmed? Did all patients undergo lymph node dissection along with thyroidectomy?

Reply: In our study, the clinicopathological parameters were all based on postoperative histopathological reports, including the metastatic status of lymph node. And all patients underwent lymph node dissection along with thyroidectomy.

Changes in the text: We have added a part to the material section in page 10, line 11-12 ('Surgical pathology is the gold standard, and the clinicopathological parameters of tumor size, tumor focality, ECI and LNM in our study were based on the surgical pathology.')

8. In page 8, line 7. versions that were produced by SPSS Inc. before the IBM acquisition (Versions 18 and earlier) would be given an origin or publisher of SPSS Inc. in Chicago. Versions that were released after the acquisition would be given an origin or publisher of IBM Corp. in Armonk, NY.

Reply: Thank you for your correction. We have revised it according to your suggestion.

Changes in the text: We have modified our text as advised -'Data were analyzed with the Statistical Package for the Social Sciences, version 19.0 (IBM Corp., Armonk, NY, USA).' (page 11, line 3-4)

9. Using TBSRTC the numerical code alone should not be used without the term of diagnostic category.

Reply: There is indeed an error here. We have revised it and the word "diagnostic category" was added before the numerical code.

Changes in the text: 'Cytological diagnosis included 18 (8.0%) cases of TBSRTC V and 208 (92.0%) 19 cases of TBSRTC VI.' have been rephrased as 'The cytological diagnosis indicated 18 (8.0%) DC V cases and 208 (92.0%) DC VI cases.'(page 11, line

10).

10. Please clarify the definition of cellular adhesiveness. This study used two different methods for preparing cytology. As liquid based cytology often makes the FNA specimen to have single isolated cells, the score of cellular adhesiveness will be higher in liquid based cytology compared to conventional smears.

Reply: It's a very professional question, and we are very sorry for our obscure writing. In our series, 141 of the 226 samples included only conventional smears, and the remaining 85 samples included conventional smears and liquid-based smears.

Conventional smears were used to examine the architectural features (cellularity and cellular adhesiveness), background components (lymphocytes and multinucleated giant cells) and several nuclei and cytoplasm features (nuclear size, presence of intranuclear pseudoinclusions and the amount of cytoplasm), and liquid-based smears were used to evaluate the nuclear pleomorphism and nuclear membrane regularity.

Changes in the text: We have added the above to the materials and methods section (page 9, line 2-3, page 10, line 1-3).

11. All odds ratios should show their 95% confidence interval.

Reply: We have added the 95% confidence interval in table 3.

Changes in the text: It can be seen in Tables 3.

12. Tables 2 and 3 are too complex and cumbersome.

Reply: We have simplified and modified tables 2 and 3.

Changes in the text: It can be seen in Tables 2 and 3、

13. Regarding the nine cytomorphologic features, nuclear enlargement is the minimal requirement criterion for the diagnosis of PTC. However, 119 of 226 cases had the nuclear size score of 0. Please discuss this issue in detail.

Reply: We agree with the idea that “nuclear enlargement is the minimal requirement criterion for the diagnosis of PTC”, while there are no relevant guidelines for the degree of nuclear enlargement to diagnose PTC, including the WHO Classification of Tumours of Endocrine Organs. And in our study, the “large nuclei” was defined as the nuclei was at least three times the size of a granulocyte to show that it’s likely to be highly invasive, and the nuclear size score of 0 indicated the absence of the “large nuclei”.

Changes in the text: We have written these in Table 1 and the accessory notes.

Reviewer C

This is an interesting study correlating some cytologic features of small PTCs with their clinical features (eg, ETE and Lymph node metastasis). The paper could benefit from several improvements and there are also some issues:

1) Thorough English editing is required. Many sentences are wordy, confusing or requiring clarifications, and/or should be rephrased (eg, page 9, line 5-8 and page 11, line 3-4 among other)

Reply: We had carefully revised the paper and polished it with AJE company, it was edited for proper English language, grammar, punctuation, spelling, and overall style by one or more of the highly qualified native English-speaking editors at AJE.

Changes in the text: The sentences (**page 9, line 5-8:** ‘Multivariable logistic regression analysis further revealed that the incidence of extracellular thyroid invasion in cases with high cellular adhesiveness scores was significantly higher than that in cases with low cellular adhesiveness scores’) have been rephrased as ‘Multivariable logistic regression analysis (Table 3) further revealed that ECI was present in more patients with an adhesiveness score of 2 (predominantly the isolated pattern or solid clusters) than in those with an adhesiveness score of 0 (predominantly the small sheet-like arrangement) ($p=0.002$, OR=5.248), However, no significant difference was observed between patients with an adhesiveness score of 1 (few isolated patterns or solid clusters) and those with a score of 0.’ (page 12, line 3-6).

The sentences (page 11, line 3-4: ‘cytomorphology that is predominantly solid clusters may indicate high proliferative activity’) have been replaced by ‘The results were along with some molecular research that the growth patterns of solitary and collective maybe manifestations of invasion.’ (page 15, line 1).

2) The apparent adhesion of the cells may differ according to the type of cytologic preparation. Did the authors notice differences between the smears and liquid based preparations? This should be further clarified and discussed. Similarly, the

amount of cytoplasm may also vary across different cytologic preparations.

Reply: It's a very professional question, and we are very sorry for our obscure writing. In our series, 141 of the 226 samples included only conventional smears, and the remaining 85 samples included conventional smears and liquid-based smears. Conventional smears were used to examine the architectural features (cellularity and cellular adhesiveness), background components (lymphocytes and multinucleated giant cells) and several nuclei and cytoplasm features (nuclear size, presence of intranuclear pseudoinclusions and the amount of cytoplasm), and liquid-based smears were used to evaluate the nuclear pleomorphism and nuclear membrane regularity.

Changes in the text: We have added the above to the materials and methods section (page 9 line 2-3, page 10 line 1-3).

3) How was ETE defined? Microscopic only? Microscopic ETE is subjective and may not be associated with different prognosis/outcomes. This needs clarification.

Reply: In our revised version, ETE has been changed to extracapsular invasion (ECI), and the definition and significance of ECI was clarification.

Changes in the text: The definition of ECI was added (page10, line 12-13)- 'ECI is defined as minimal microscopic invasion, without invasion into the surrounding structures such as strap muscles, trachea, larynx, vasculature, esophagus, and/or recurrent laryngeal nerve.'

Although extracapsular invasion of thyroid cancer has no prognostic significance, it is still considered as an aggressive indicator in our research, a well-defined tumor

capsule is a particularly favorable prognostic indicator [*Haddad R, Bischoff L, Busaidy N, et al. NCCN clinical practice guidelines in thyroid carcinoma. Version 2. 2019*], we add this part into page 14, line 6-8.

4) page 5, line 6-7. FNA is very helpful to distinguish benign from malignant lesions but not so much to distinguish neoplastic from non neoplastic lesions. Please correct.

Reply: Thank you for your suggestion, and we have revised it.

Changes in the text: We have modified our text as advised (page 6, line 12-13)- ‘Fine-needle aspiration cytology (FNAC) has been widely used as the most effective preoperative evaluation tool to distinguish benign from malignant lesions.’

5) The significance of the cellular adhesion including adhesion molecules could be further discussed along with references to previous studies on this topic which would further support the result of this study.

Reply: Thank you for the suggestion, it is very constructive.

Changes in the text: We have added a part of this content to the discussion section (page 14, line 9-12)- ‘Further multivariable logistic regression analysis (Table 3) showed that cellular adhesiveness was a strong independent predictor of extracapsular invasion (ECI) and lymph node metastasis (LNM) , The results were along with some molecular research that the growth patterns of solitary and collective maybe

manifestations of invasion [*Nagai T, Ishikawa T, Minami Y, et al. Tactics of cancer invasion: solitary and collective invasion. J Biochem 2020; 167(4):347-355*].?