Peer Review File

Article information: http://dx.doi.org/10.21037/gs-20-920

Reviewer A

I would suggest the following revisions:

Comment 1: Please indicate clearly at the end of introduction section the hypothesis explored in this study.

Reply 1: We sincerely appreciate your input. In our present study, the hypothesis was that LN-prRLN metastasis might be predicted by the status of pre-laryngeal (Delphian) lymph nodes, pre-tracheal lymph nodes and para-tracheal lymph nodes ipsilateral to the tumor during carbon nanoparticles-guided intraoperative lymph nodes biopsy in cN0 thyroid carcinoma patients. When an intraoperative biopsy indicates no lymph node metastasis, the LNs-prRLN are less likely to metastasize. This would enable the avoidance of unnecessary LN-prRLN dissection during CLND. We have added these contents in the **Introduction** section of our revised manuscript.

Changes in the text:

Introduction, paragraph 3:

Our present study aimed to provide a reference for evaluating the necessity of LN-prRLN dissection by combining patient clinicopathologic characteristics and intraoperative lymph node biopsy. We hypothesized that the intraoperative lymph nodes biopsy might predict LN-prRLN metastasis in cN0 thyroid carcinoma patients and when an intraoperative biopsy indicates no lymph node metastasis, the LNs-prRLN are less likely to metastasize. Eventually, this would enable the avoidance of unnecessary LN-prRLN dissection during CLND.

Comment 2: Please add in the discussion issue of micrometastases of PTC and their clinical marginal significance. Please clarify how many patients with positive LNs posterior to the RLN had micro vs. macrometastases in your series.

Reply 2: Thank you very much for your constructive comments which could enrich our manuscript. In our series, postoperative pathologic examination results indicated that micro-metastases but no macro-metastases in lymph nodes were found in 5 cases, and micro-metastases in LN-prRLN were found in only 1 case. Only 2.65% of cN0 patients presented with micro-metastases after total CLND in postoperative pathologic examination. Due to the small number of cases, it is probably difficult to explain its clinical significance in our present study. However, for all of these 5 cases, there was no positive lymph nodes detected during intraoperative lymph node biopsy. Therefore, for the topic of our present study, it is possible that patients with no positive lymph

nodes during intraoperative biopsy might still represent a lower risk of LN-prRLN metastasis. We have added these contents in the **Discussion** part of our revised manuscript.

Changes in the text:

Discussion, paragraph 4:

With the development of personalized diagnosis and treatment concepts, personalized surgical volume and surgical approach are important in increasingly patient-centered decision-making. The ATA recommendation is to abandon prophylactic CLND in T1 or T2, noninvasive, and cN0 PTC patients. Patients with clinical N0 or ≤5 pathologic N1 micro-metastases (<0.2 cm in largest dimension) were classified as ATA low-risk group, which predicted a recurrence rate below 5% (26). However, according to the published literature, the incidence of lymph node metastasis in cN0 patients ranged from 25–82.3% (27), similar to the incidence (64.02%) in our series. Moreover, postoperative pathologic examination results indicated that micro-metastases but no macro-metastases in lymph nodes were found in 5 cases (2.65%), and micro-metastases in LN-prRLN were found in only 1 case. Therefore, in our clinical practice, prophylactic CLND is still routinely carried out due to the inconsistency between the clinical nodal staging and pathological staging. Because of the small number of cases, it is probably difficult to explain micro-metastasis clinical significance in our present study. However, for all of the 5 cases with micro-metastasis, there were no positive lymph nodes detected during intraoperative lymph node biopsy. Patients with no metastatic lymph nodes during intraoperative biopsy might still represent a low risk of LN-prRLN metastasis. In conclusion, the intraoperative lymph node biopsy method could provide reference for the risk of LN-prRLN metastasis to develop a personalized surgical volume.

Comment 3: Please put findings of your study in the context of most current ATA recommendation to abandon prophylactic CLN clearance and consider clinical nodal staging (based on imaging or palpation) as equivalent to pathological staging in cN0 patients (which canbe most beneficial for patients with micrometastases to the LNs). Underline in the discussion surgical volume and personalized approach as important tools available in more patient-centered decision-making.

Reply 3: We fully appreciate your concerns. In the most current ATA guideline, thyroidectomy without prophylactic central neck dissection is appropriate for small (T1 or T2), non-invasive, clinically node-negative PTC (cN0) and for most follicular cancers. Patients with clinical N0 or ≤5 pathologic N1 micro-metastases (<0.2 cm in largest dimension) was classified as ATA low risk group. However, the incidence of lymph node metastasis in cN0 patients was 64.02% (121/189) and macro-metastases were found in 95.9% of the patients with lymph node metastasis. The published studies

also showed the incidence of lymph node metastasis in cN0 patients ranged from 25%-82.3%(1,2) and contralateral central lymph node metastasis occurs in 3.88–30.63%(3). The metastatic lymph nodes eventually lead to recurrence and re-operation, and increase the incidence of surgical complications during re-operation and lower the qualities of life, which indicated that the clinical nodal staging (based on imaging or palpation) might be not fairly equivalent to pathological staging. Moreover, whether prophylactic lymph node dissection should be abandoned or not was not the major subject in our present study. In the clinical practice of PTC treatment in China, prophylactic CLND is widely carried out, and patients are more concerned with the thoroughness of treatment to avoid psychological burden. In Chinese guideline of DTC, prophylactic CLND was recommended under the proper protection of parathyroid and RLN. Therefore, we routinely performed prophylactic CLND in order to obtain more accurate tumor pathological stage in our center. But during the CLND, dissection of LN-prRLN can significantly increase the potential risk of RLN injury, lymphatic leakage, pleural injury as well as increase the operating time. Since prophylactic CLND is still routinely carried out in our center, whether central lymph node dissection can be simplified for specific patients and whether LN-prRLN dissection should be carried out under the risk of RLN injury is the main issue we discussed. In addition, we quite agree with your point that surgical volume and personalized approach is important in more patient-centered decision-making. Therefore, we proposed the intraoperative lymph node biopsy, which can provide reference for the personalized surgical volume. We also added these contents in **Discussion** section in our revised manuscript.

References

1. Sun W, Lan X, Zhang H, et al. Risk Factors for Central Lymph Node Metastasis in CN0 Papillary Thyroid Carcinoma: A Systematic Review and Meta-Analysis. PLoS One 2015;10:e0139021.

2. Chan AC, Lang BH, Wong KP. The pros and cons of routine central compartment neck dissection for clinically nodal negative (cN0) papillary thyroid cancer. Gland Surg 2013;2:186-95.

3. Sun W, Zheng B, Wang Z, et al. Meta-analysis of risk factors for CCLNM in patients with unilateral cN0 PTC. Endocr Connect 2020;9:387-95.

Changes in the text:

Discussion, paragraph 4:

With the development of personalized diagnosis and treatment concepts, personalized surgical volume and surgical approach are important in increasingly patient-centered decision-making. The ATA recommendation is to abandon prophylactic CLND in T1 or T2, noninvasive, and cN0 PTC patients. Patients with clinical N0 or ≤5 pathologic N1 micro-metastases (<0.2 cm in largest dimension) were classified as ATA low-risk group, which predicted a recurrence rate below 5% (26). However, according to the

published literature, the incidence of lymph node metastasis in cN0 patients ranged from 25–82.3% (27), similar to the incidence (64.02%) in our series. Moreover, postoperative pathologic examination results indicated that micro-metastases but no macro-metastases in lymph nodes were found in 5 cases, and micro-metastases in LN-prRLN were found in only 1 case. Therefore, in our clinical practice, prophylactic CLND is still routinely carried out due to the inconsistency between the clinical nodal staging and pathological staging. Because of the small number of cases, it is probably difficult to explain micro-metastasis clinical significance in our present study. However, for all of the 5 cases with micro-metastasis, there were no positive lymph nodes detected during intraoperative lymph node biopsy. Patients with no metastatic lymph nodes during intraoperative biopsy might still represent a low risk of LN-prRLN metastasis. In conclusion, the intraoperative lymph node biopsy method could provide reference for the risk of LN-prRLN metastasis to develop a personalized surgical volume.

Comment 4: Add to the list of limitations of this study that even soft oncological outcomes were not evaluated in this study (e.g. postoperative Thyroglobulin levels, nodal recurrence rate, disease free survival etc). These measures might provide additional arguments towards surcial approach proposed by you insted of active survellence of cN0 patients after the operation with dynamic risk stratification as a general rule.

Reply 4: This is a very important and valuable suggestion which really make our manuscript more accurate. As you mentioned, the follow-up indicators such as postoperative thyroglobulin levels, thyroid function, nodal recurrence rate, disease free survival were not evaluated in our present study. In order to clarify whether LN-prRLN dissection can be avoided, future randomized controlled trials to evaluate the oncological outcomes are necessary to further confirm our conclusions. We have added the content in **Discussion** and **Conclusion** section in our revised manuscript.

Changes in the text:

Discussion, paragraph 5:

Our present research was limited in that it was a single-center retrospective study, and the sample size was relatively small. Therefore, a multi-center study with a larger number of cases is necessary to confirm further the accuracy and feasibility of CN-guided intraoperative lymph node biopsy in predicting the status of LN-prRLN. Moreover, the follow-up indicators such as postoperative thyroglobulin levels, thyroid function, nodal recurrence rate, and disease-free survival were not evaluated in our present study. To clarify whether LN-prRLN dissection can be avoided in patients with no metastasis in intraoperative lymph node biopsy, future randomized controlled trials to evaluate the oncological outcomes are necessary to validate our conclusions.

Conclusion

The number of metastatic lymph nodes in CN-guided intraoperative lymph node biopsy, including pre-laryngeal (Delphian) lymph nodes, pre-tracheal lymph nodes, and para-tracheal lymph nodes ipsilateral to the tumor, were potential predictors of LN-prRLN metastasis in cN0 thyroid carcinoma patients. Patients with no metastatic lymph nodes during intraoperative biopsy might represent a low risk of LN-prRLN metastasis. However, for patients with cN0 and negative lymph nodes in an intraoperative biopsy, further randomized controlled trials are necessary to assess the prognostic outcomes and validate whether the dissection of LN-prRLN could be avoided during CLND.

Comments 5: Use of English should be improved throughout the entire manuscript.
Reply 5: We totally understand your concern. The revised manuscript has been checked by AME Editing Service. We hope the revised version will meet the Journal needs.

Reviewer B

The authors present a single-institution retrospective review of 189 right-sided prophylactic central lymph node dissection guided by intraoperative sentinel node dissection using carbon nanoparticles for PTC, in order to answer the question of whether information on microscopic nodal metastasis on sentinel node dissection of the prelaryngeal, pre- and paratracheal nodal stations would predict the presence of metastases in the deeper central neck posterior to the obliquely-running RLN on the right side. The justification for this study is that since extra dissection in this area risks injury to critical structures including the RLN, there would be potential benefit to spare the patient this dissection if possible. They found on multivariable regression analysis and ROC analysis that patients with positive metastases on sentinel node dissection and frozen section was highly specific and moderately accurate overall in predicting the presence of metastases posterior to the RLN with an AUC of 0.7 and a false negative rate of 6%.

Comment 1: Although the underlying concepts and assumptions are controversial - including the need for prophylactic CND in cN0 disease, the utility of SLND, use of carbon nanoparticles, the specific distinction of this more posterior area on the right side - the idea for the study is on its own interesting. and the authors do acknowledge the controversies. The main problem with the manuscript is linguistic and requires extensive English editing so that it is clear to read.
Reply 1: We totally understand your concern. The revised manuscript has been checked by AME Editing Service. We hope the revised version will meet the Journal needs.

Reviewer C

The authors retrospective review the metastatic status of lymph node posterior to right recurrent laryngeal nerve in 189 cases (LN-prRLN positive group, n=30; LN-prRLN negative group, n=159) that underwent carbon nanoparticles guided sentinel lymph node biopsy.

They found that the number of metastatic sentinel lymph nodes in intraoperative biopsy was an indicator of LN-prRLN metastasis in cN0 thyroid carcinoma patients, suggesting that the patients staging in cN0 with negative intraoperative lymph node status might no longer require them for LN-prRLN dissection during central lymph nodes dissection.

Overall, this study is well conducted and analyzed. The finding can be used as an intraoperative decision making to guide if LN-prRLN dissection is needed in individual patients to reduce the possible complication from unnecessary LN-prRLN dissection.

My comments are as follows:

Comment 1:

Surgical procedure

As an important part of the procedure, could the author describe more clearly how he performs carbon nanoparticles guided sentinel lymph node biopsy? For example, how many carbon nanoparticles are used? Where they injected with the nanoparticles? How long does it take from the injection of carbon nanoparticles to the presence of sentinel lymph node?

Reply 1: We sincerely appreciate this important concern. During the surgical procedure, After the thyroid gland had been completely exposed, CNs were injected subcapsularly into the capsule of the isthmus and thyroid lobes ipsilateral to the tumors. The concentration CN suspension was 25 mg/0.5 mL (LUMMY, Chongqing, China), and 0.05–0.1 mL of CNs were injected at each location. The black stained lymphatic vessels and lymph nodes in the perithyroidal region were observed after about 5 minutes. The black-stained pre-laryngeal (Delphian) lymph nodes, pre-tracheal lymph nodes, and para-tracheal lymph nodes were then resected for intraoperative frozen pathological examination. We have added these contents in the **Methods** part of our revised manuscript.

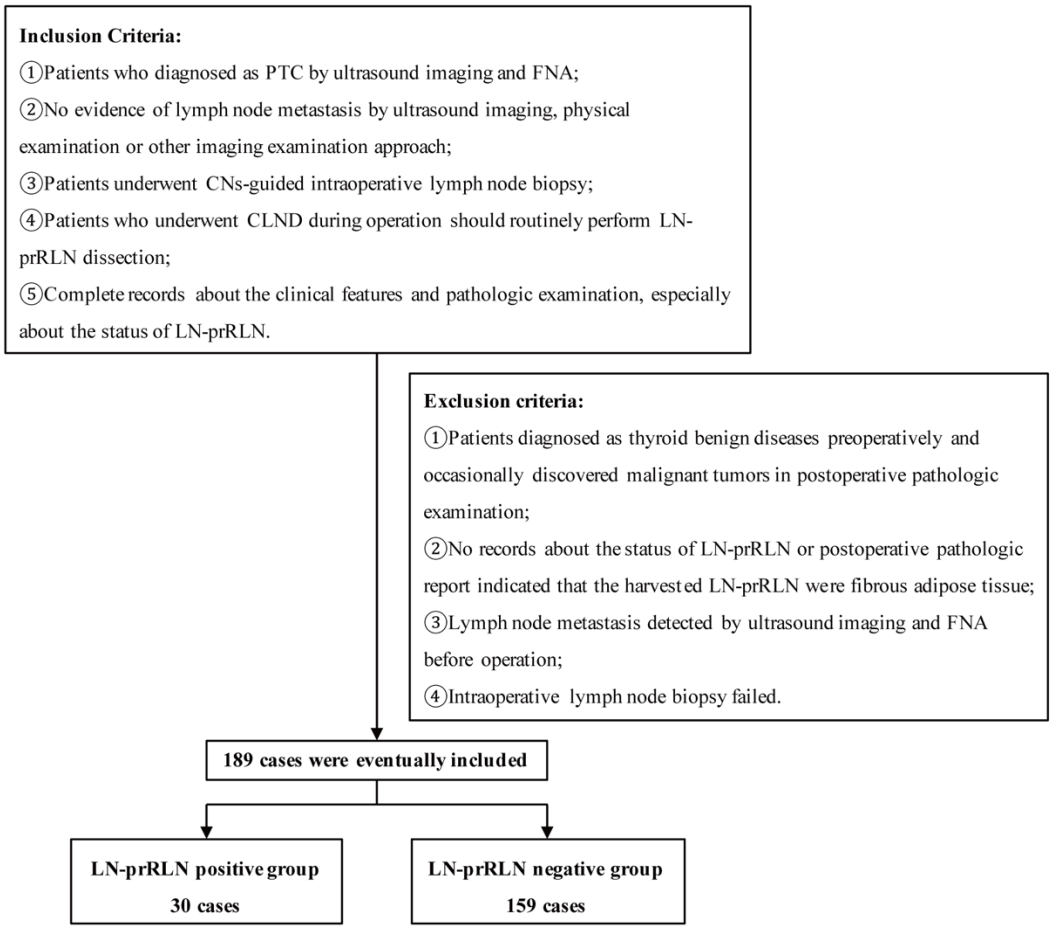
Changes in the text:

Methods, Surgical procedure:

To evaluate the lymph node status intraoperatively, we routinely performed the

intraoperative lymph node biopsy before the thyroid lobectomy. After the thyroid gland had been completely exposed, CNs were injected subcapsularly into the capsule of the isthmus and thyroid lobes ipsilateral to the tumors. The concentration CN suspension was 25 mg/0.5 mL (LUMMY, Chongqing, China), and 0.05–0.1 mL of CNs were injected at each location. The black stained lymphatic vessels and lymph nodes in the perithyroidal region were observed after about 5 minutes. The black-stained pre-laryngeal (Delphian) lymph nodes, pre-tracheal lymph nodes, and para-tracheal lymph nodes were then resected for intraoperative frozen pathological examination. Patients with unilateral lesions underwent thyroid lobectomy associated with ipsilateral CLND when the frozen pathological examination indicated no lymph node metastasis. Patients with bilateral lesions or a frozen pathological examination indicating lymph node metastasis underwent total thyroidectomy and bilateral CLND. When performing the right CLND, the right posterior laryngeal nerve lymph nodes were recorded and analyzed separately. All resected specimens were confirmed by pathological examination.

Comment 2: Figure 1
There is a mis-typing of LN-prRLN positive group: 159 cases. It will need to be corrected for the LN-prRLN negative group.
Reply 2: We are sorry for the mistake in our manuscript. We re-uploaded the modified image.
Changes in the text:
Figure 1



Comment 3: Table 1

Does the row "Number of metastatic lymph node" indicates the number of metastatic lymph node in total CNLD or the number of metastatic lymph node in sentinel lymph node?

Reply 3: We appreciate for your question and suggestion, and we apologize for the missing details in Table 1. The row "Number of metastatic lymph node" indicates the number of metastatic lymph node in total CNLD, and we re-uploaded the modified table in our revised manuscript.

Changes in the text:

Table 1

Table 1 Clinical characteristics in LN-prRLN positive and negative group [n(%)]

Variables	Status of LN-prRLN		Statistic	*p* Value
	Positive (n=30)	Negative (n=159)		
Age	35.5	44 (35,55)	-3.105	**0.002**

	(28.75,45.5)			
< 55	28 (93.3)	118 (74.2)	5.249	**0.022**
≥55	2 (6.7)	41 (25.8)		
Sex				
Male	16 (53.3)	58 (36.5)	3.010	0.083
Female	14 (46.7)	101 (63.5)		
Hashimoto's thyroiditis				
Yes	14 (46.7)	52 (32.7)	0.029	0.866
No	16 (53.3)	107 (67.3)		
The maximum diameter of tumor	1.0 (0.6, 1.5)	0.9 (0.6,1.2)	-1.007	0.314
T stage				
T1a	16 (53.3)	102 (64.2)	1.259	0.262
T1b	12 (40.0)	45 (28.3)	1.640	0.200
T2	2 (6.7)	7 (4.4)	0.285	0.593
T3	0 (0)	5 (3.1)	0.969	0.325
Number of metastatic lymph nodes in total CLND	5.5 (3,9)	1（0，4）	-5.039	**< 0.001**
RLN injury				
Yes	2 (6.7)	2 (1.3)	3.564	0.059
No	28 (93.3)	157 (98.7)		
Temporary hypoparathyroidism				
Yes	7 (23.3)	34 (21.4)		
No	23 (76.7)	125 (78.6)	0.056	0.812
Tumor Side				
Right	19 (63.3)	93 (58.5)	0.245	0.621
Left	2 (6.7)	18 (18)	0.578	0.447
Bilateral	9 (30.0)	48 (30.2)	0.000	0.984

Comment 4: Have the authors ever found the sentinel lymph node located at the LN-prRLN?

Reply 4: From an anatomical point of view, the LNs-prRLN are always in the deep region, so it is quite difficult to observe the LN-prRLN prior to thyroid lobectomy and right CLND. Therefore, there was no LN-prRLN resected during intraoperative lymph node biopsy, and we didn't observe the lymphatic drainage of LN-prRLN guided by CNs. In addition, the clinical significance of intraoperative lymph node biopsy is to

avoid unnecessary LN-prRLN dissection in order to reduce the risk of RLN. Therefore, due to the manipulation difficulties and the risk of RLN injury, it is not ideal choice for us to discover the sentinel lymph node located at the LN-prRLN.

Comment 5: Based on the finding of this study, what kind of surgical procedure do you recommend with patients who have right thyroid cancer, cN0 and negative sentinel lymph node? Would you do right unilateral CNLD but sparse LN-prRLN? or no further CNLD beyond just sentinel lymph node?

Reply 5: In our center, we routinely performed prophylactic central neck dissection in order to obtain more accurate tumor pathological stage, and then guide the follow-up treatment. According to the findings in our study, right unilateral CNLD but sparse LN-prRLN might be a better alternative for patients with cN0 and negative sentinel lymph node. However, further randomized controlled trials are necessary to assess the prognostic outcomes and further confirm whether the dissection of LN-prRLN could be avoided during CLND.