

## Peer Review File

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### Reviewer A

Comment 1: Surgical technique: A 5-cm horizontal incision was made 2 cm above the suprasternal notch along the neck crease. Dissection proceeded along the hyoid bone and infrahyoid muscles to expose the mandible, allowing clearance of submandibular lymph nodes. The sternocleidomastoid muscle was mobilized to expose the jugular vein, and lymph nodes surrounding the jugular vein were removed, extending from the mandible to the supraclavicular region. Whilst it may be possible to dissect lower levels using the incision in a small neck patient, in patients with a long neck it may be difficult to clear the nodes using the small incision. It is highly unlikely that a 5cm can be used in EVERY SINGLE patient to clear the lateral neck nodes where indicated.

Replay1: We appreciate your valuable feedback on our manuscript. Upon further review, we acknowledge that specifying a uniform 5 cm incision for all neck dissection patients is impractical. In clinical practice, the incision length is typically determined by the surgeon, taking into account factors such as the patient's neck anatomy, the size of the lesion, and the extent of lymph node metastasis. As a result, the incision length generally varies between 5 and 10 cm. Following your suggestion, we have revised the manuscript accordingly.

Changes in the text: we have modified our text as advised: **A 5-10cm horizontal incision was made 2 cm above the suprasternal notch along the neck crease. [page3 line82-83]**

Comment 2: The authors indicate that a diagnosis was confirmed via intraoperative rapid frozen section. Was frozen section used in all patients to diagnose PTC, or the nodes before deciding on neck dissection?

Reply2: Thank you for your academic inquiry. As stated on page 3, lines 81–87, for patients suspected of thyroid carcinoma, preoperative fine-needle aspiration biopsy (FNAB) and genetic testing were performed. If results were positive, surgical treatment was initiated. Intraoperative frozen section pathology provided a preliminary diagnosis of papillary thyroid carcinoma (PTC), while final confirmation relied on postoperative paraffin pathology, verifying that the carcinoma was confined to a single thyroid lobe. Ultrasound screening was used to identify suspicious lateral cervical lymph nodes on the affected side. Patients with ultrasound-positive findings underwent FNAB. For those with suspicious ultrasound findings who did not undergo FNAB, intraoperative sampling of suspected lymph nodes was performed before lateral neck dissection to assess metastasis. If metastasis was confirmed by intraoperative frozen pathology or if preoperative FNAB was positive, lateral neck dissection was performed. Final confirmation of lymph node metastasis was based on postoperative paraffin pathology. For bilateral central compartment lymph nodes, routine dissection was performed intraoperatively, followed by both intraoperative frozen section analysis and postoperative paraffin pathology for definitive assessment.

All patients included in this study met the criteria for this diagnostic protocol.

Comment 3: TG levels were classified into high and low groups based on this 73 ng/mL threshold. However, in table 3 Tg cut of 60 ng/ml was used. Kindly explain the discrepancy.

Reply3: We are so sorry for our careless mistakes. We established a Tg threshold of 73 ng/mL based on the cut-off value corresponding to the maximum Youden index, categorizing patients into high-Tg and low-Tg groups. However, during the preparation of Table 3, the threshold was incorrectly recorded as 60 ng/mL, causing ambiguity. 60 ng/mL is the reference value provided based on the test results of 95% of the healthy population. We sincerely apologize for this oversight.

Changes in the text: As suggested by the reviewer, we have corrected the “60ng/ml” into “73ng/ml” in table3. 【page7 Table3】

Comment 4: Lymph node posterior to RLN was found to be prognostic indicator of contralateral metastasis. It is difficult to comprehend how the nodes were evaluated as posterior to RLN. Were the nodes sampled in groups as anterior or posterior at time of surgery? Did the surgeons indicate this in operative notes? Nodes are usually sampled en bloc in central compartment dissection.

Reply4: Thank you for your professional inquiry. Anatomically, the esophagus is situated posterior to the trachea, with a space between the right recurrent laryngeal nerve (RLN), the prevertebral fascia, and the esophagus that contains lymphatic and adipose tissue. Consequently, lymph nodes are located deep to the right RLN. These lymph nodes, positioned posterior to the right RLN, are referred to as posterior recurrent laryngeal nerve lymph nodes (LN-prRLN). For a detailed definition, please refer to the following publication: Lallemand B, Reynaud C, Aloviseti C, et al. Updated Definition of Level VI Lymph Node Classification in the Neck. *Acta Otolaryngol*, 2007, 127(3):318-322.

In this study, surgeons excised lymph nodes from the posterior recurrent laryngeal nerve group and sent them for pathological evaluation to determine the presence of LN-prRLN metastasis. The status of LN-prRLN was documented in the surgical records for all enrolled patients.

Comment 5: Multifocal disease in 22% of patients and could explain contralateral metastasis. Ideally these patients need to be removed from the analysis if the authors aim was to predict contralateral metastasis in unilateral PTC.

Reply5: We apologize for any confusion caused by the lack of a clear and explicit definition of multifocality in the original manuscript. In our study, multifocality refers to the presence of two or more cancer foci within a single thyroid lobe. All 105 cases included were papillary thyroid carcinoma (PTC) confined to a single lobe, with no patients exhibiting cancer in both lobes. To improve clarity, we have added a detailed explanation of multifocality in the revised manuscript.

Changes in the text: We have revised the following content of the article:

Tumor multifocality specifically refers to the presence of two or more cancer foci

within the same thyroid lobe, not the contralateral lobe. All included patients were confirmed to have papillary thyroid carcinoma (PTC) confined to a single lobe (either left or right) 【page 5 line159-162】

Comment 6: The demographic data presented in the study subjects need to be moved to the results section.

Replay6: Thank you for your valuable suggestion. We have removed this baseline data from the Study Subjects section of the manuscript. Since the Results section already includes a table that provides a detailed explanation of the demographic data of the enrolled patients, we will no longer repeat this information in the text.

Changes in the text: We have removed the demographic data of the enrolled patients from the Study Subjects section.

Comment 7: What was the need to fast the patients for 8-12 hours?

Replay7: Thank you for your careful consideration. According to the Chinese guidelines for anesthesia in thyroid surgery, patients are routinely required to fast for 10-12 hours before surgery. For detailed information, please refer to Expert Consensus on Enhanced Recovery After Surgery (ERAS) in Thyroid Surgery (2018 Edition) 【page 28 line5】. However, the guidelines also mention that the fasting and fluid restriction duration should be appropriately adjusted based on the patient's specific condition and individual assessment. Therefore, in actual surgical practice, we ensure that patients adhere to fasting and fluid restriction protocols to reduce anesthesia-related risks.

Comment 8: Why was preoperative Tg measurement done on the day prior to surgery. Evidence that preoperative measurement of serum Tg impacts patient management or outcomes is not yet available.

Replay8: Thank you for your question. The serum thyroglobulin (Tg) test is performed alongside the blood biochemical test, typically on the morning of the second day after hospital admission while the patient is in a fasting state. Previous studies have shown that Tg may be related to lymph node metastasis. If Tg increases continuously after surgery, indicating residual cancer lesions, the preoperative benchmark value is of great significance.

Incorporation size of lymph node metastasis focus and pre-ablation stimulated Tg could more effectively predict clinical outcomes in differentiated thyroid cancer patients without distant metastases (doi: 10.3389/fendo.2023.1094339. eCollection 2023.) The literature supports this contention.

Comment 9: The authors mention in discussion that normal estrogen expression appears to exert a protective effect, potentially slowing cancer progression. Kindly provide evidence to support the argument.

Replay9: We sincerely appreciate the valuable comments. We have checked the literature carefully and added more reference into the Discussion part in the revised manuscript.

In the first cited reference, the author reported that the upregulation of estrogen receptor ER $\alpha$  is linked to the development of thyroid cancer, whereas ER $\beta$  may exhibit tumor-suppressive properties. Thus, the balance between ER $\alpha$  and ER $\beta$  appears to be a critical factor in thyroid cancer progression, supporting the argument presented in this paper.

In the second cited reference, Studies have shown that in ovarian and breast cancer, ER $\beta$  overexpression downregulates cyclin D1, thereby exerting an anti-proliferative effect. However, under conditions of low estrogen levels, this inhibitory effect of ER $\beta$  may be weakened, allowing the pro-cancer effects of ER $\alpha$  to dominate. This indirectly supports the argument of this paper.

Nevertheless, the precise mechanisms by which estrogen and its receptors influence PTC progression remain unclear. Further research is necessary to elucidate these mechanisms and validate their role in disease progression.

Changes in the text: In response to the reviewer's suggestion, we have reviewed and added two additional references to support this argument:

[16] Zhongqin Gong, Shucui Yang, et al. The Isoforms of Estrogen Receptor Alpha and Beta in Thyroid Cancer. *Front Oncol*, 2022 Jun 24;12:916804. doi: 10.3389/fonc.2022.916804. eCollection 2022.

[17] Jia M, Dahlman-Wright K, Gustafsson JÅ. Estrogen receptor alpha and beta in health and disease. *Best Pract Res Clin Endocrinol Metab*. 2015;29(4):557–68. doi: 10.1016/j.beem.2015.04.008.

【page 13 370-375】

Comment 10: In table 5, multiple needs to be changed as multifocal.

Reply 10: Thank you for your careful review. Since there is no Table 5 in the manuscript, we assume you were referring to Table 2 and have made the corresponding revisions accordingly.

Changes in the text: As suggested by the reviewer, we have corrected the “multiple” into “multifocal” in page 6 Table 2.

### **Reviewer B**

Comment 1: “Contralateral thyroid” is misspelled in Table 2 and should be “contralateral thyroid”

Reply 1: We sincerely thank the reviewer for careful reading.

Changes in the text: As suggested by the reviewer, we have corrected the “contralateral thyroid” into “contralateral thyroid” in page 6 Table 2.

### **Reviewer C**

Comment 1: Although only the factors extracted by the Lasso regression model are used in the nomogram, when considering its use in actual clinical practice, there may be advantages to use factors that can be diagnosed preoperatively.

In simple analysis, there is a significant difference in contralateral metastasis with tumor size, so is it possible to create a practical nomogram using such factor?

Reply 1: We greatly appreciate your professional review of our article. Based on your recommendations, we incorporated tumor size as a predictive factor, developed a second predictive model, and generated a corresponding nomogram. Comparison of the DCA curves for both models revealed that the Lasso model, constructed using three predictive factors selected through Lasso analysis, exhibited the highest clinical utility. Consequently, we have retained it as the final predictive model for this study. The specific revisions are as follows.

Changes in the text:

1、 We have revised the following content of the article:

A clinical predictive nomogram (Figure 2A) was developed using three variables selected through logistic regression, with contralateral central lymph node (Cont-CLNs) metastasis as the clinical outcome in patients with LLNM-PTC. This model was designated as the Lasso model. In univariate analysis, tumor size showed a significant association with contralateral lymph node metastasis, indicating potential clinical relevance. Considering its potential application in clinical practice, tumor size was incorporated as an additional variable, leading to the development of a second model, named the More model (Figure 2B).

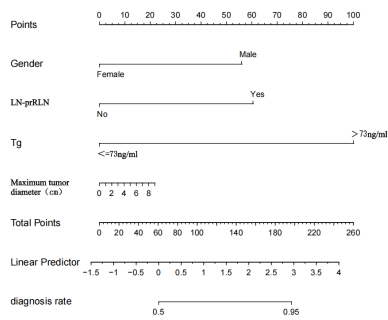
In both nomograms, the length of each variable's line segment reflects its relative contribution to the clinical outcome. The total score, calculated as the sum of individual variable scores, corresponds to a probability scale that estimates the risk of Cont-CLNs metastasis in LLNM-PTC patients. 【page7 line182-192】

2、 We have revised the following content of the article:

Decision curve analysis (DCA) demonstrated that the Lasso model consistently provided a higher net benefit across all threshold ranges compared to the More model and both no-intervention and full-intervention strategies. This suggests that the Lasso model is more effective for predicting contralateral central lymph node (Cont-CLNs) metastasis. Consequently, it was selected as the final clinical predictive model for this study (hereafter referred to as the predictive model) (Figure 3).

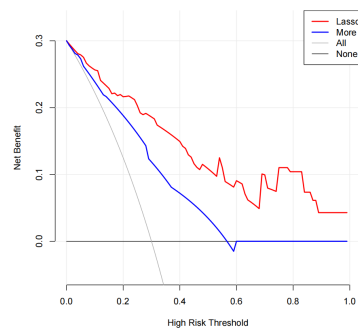
For internal validation, the Lasso model was assessed using bootstrap resampling with 1,000 iterations. The analysis yielded a calibrated AUC of 0.771 (95% CI: 0.6839–0.8573), indicating good predictive performance. The corresponding ROC and calibration curves were generated (Figure 4). 【page7 line194-203】

3、 We developed a new predictive model incorporating tumor size and constructed a corresponding nomogram:



(Figure A presents a nomogram developed using three predictive variables selected through LASSO. Figure B expands on this model by incorporating tumor size as an additional predictive factor. The length of each variable's line segment reflects its relative contribution to the clinical outcome. The total score, calculated as the sum of individual variable scores, corresponds to a probability scale that estimates the risk of Cont-CLNs metastasis in LLNM-PTC patients) 【page 9 line 223-226】

4、 We analyzed and constructed a new DCA curve and compared it with the DCA curve of the previous model:



(The red line (Lasso) represents the model established using the three predictor variables selected through Lasso screening. The blue line (More) represents the model built upon these three variables with the addition of tumor size as a predictor. The Lasso model consistently demonstrated significantly higher net benefits across all threshold ranges compared to the More model and no or full intervention.) 【page 9 line 232-235】

5、 We have swapped the order of Figures 3 and 4.

**Comment2:** In line 41, there is a spelling mistake in the “Introduction”.

Reply 1: We are really sorry for our careless mistakes. Thank you for your reminder.

Changes in the text: We have corrected the “induction” into “introduction” in page2 line 29.

### Reviewer D

**Comment 1:** Clear characterization of the population, 105 patients with tumors on the right and left and bilateral: this is not clear; are the metastases bilateral? are the nodules in the thyroid bilateral? I had difficulty understanding.

Replay1: We sincerely apologize for any inconvenience caused. As stated in the Inclusion Criteria section 【page 3, lines 82–86】 of the manuscript, all 105 enrolled patients had unilateral lobar papillary thyroid carcinoma (either left or right lobe). Postoperative pathology was used to confirm that all included cases were indeed unilateral lobar carcinoma. However, central lymph node metastasis may occur bilaterally, which is the primary focus of our study.

**Comment 2:**Described surgical technique: some imperfections such as mentioning the white line of the neck: it does not exist; taking care of the inferior laryngeal nerve in the lateral dissection and not mentioning the lymphatic ducts and the XI nerve: this is an aspect to be improved; mentioning lymph nodes posterior to the inferior laryngeal nerve and not describing them in the technique is also an omission.

Reply 2:We sincerely appreciate your valuable suggestions. In our manuscript, the term "white line of the neck" refers to the superficial layer of the deep cervical fascia at the midline. This region has a simple anatomical structure and provides an optimal approach for neck access. Given the absence of authoritative international literature defining this concept, and to prevent ambiguity, we have revised the term to "anterior midline of the neck" .

Secondly, based on your suggestions, we have added a description of the protection of the lymphatic vessels and the accessory nerve during the surgical procedure. Additionally, we have added a description of the dissection of the lymph nodes behind the recurrent laryngeal nerve in the surgical operation.

Changes in the text:we have re-written this part according to the suggestions.

(1)We have corrected the “ the white line of the neck:” into “the anterior midline of the neck” in page3 line85.

(2) with careful preservation of structures such as the recurrent laryngeal nerve, lymphatic ducts and the XI nerve was added. 【page3 line106】

(3)During the dissection of lymph nodes posterior to the recurrent laryngeal nerve, carefully separate the surrounding tissues using scissors or an ultrasonic scalpel to expose the lymph node area. Initiate the dissection from the posterior aspect of the recurrent laryngeal nerve, proceeding along its posterior trajectory. Employ bipolar electrocautery or an ultrasonic scalpel as necessary to minimize the risk of thermal injury. Once adequately mobilized, excise the lymph node group in its entirety was added. 【page3 line95-100】

**Comment 3:**Serum thyroglobulin: describing when and how they were collected also influences the result

Reply3: Thank you for your valuable feedback. As described in the manuscript on page 4, lines 131–134, we collected venous blood from patients on the morning of the second day after admission and sent it to our hospital’s laboratory for Tg measurement. To enhance clarity for readers, we have added the specific blood collection time in the manuscript.

Change in the text:We added (about 6:00am) in page3 line 119.

**Comment 4:** The tables need to be better presented, they are not self-explanatory: they need more texts to help the reader interpret them

Reply4: Your suggestions are highly valuable from an academic perspective. We have carefully considered your advice. Since each table already has a clear title and textual explanation of its content, we have added the statistical methods used to create the tables above them. Additionally, we have provided more detailed descriptions for the figures in the manuscript to help readers better understand the content of the images.

Changes in the text:

(1) Logistic regression was performed to analyze the clinical indicators of the study population. The dependent variable was contralateral central lymph node metastasis (0 = no metastasis, 1 = metastasis), while the independent variables comprised the clinical indicators presented in Table 1 was added 【page4 line142-145】

(2) Logistic regression analysis was performed using the pathological characteristics of the enrolled patients as the independent variables, with the same dependent variable as described above was added 【page5 line156-157】

(3) The right figure indicates that three predictor variables with non-zero coefficients were identified as the key factors influencing contralateral central lymph node metastasis in LLNM-PTC patients. These variables resulted in the smallest error and the best model fit. The predictor factors identified from the left figure, corresponding to the intersection with the  $\lambda = 3$  line, include gender, lymph node metastasis of the recurrent laryngeal nerve, and thyroglobulin levels was added 【page8 line 211-216】

(4) Figure A presents a nomogram developed using three predictive variables selected through LASSO. Figure B expands on this model by incorporating tumor size as an additional predictive factor. The length of each variable's line segment reflects its relative contribution to the clinical outcome. The total score, calculated as the sum of individual variable scores, corresponds to a probability scale that estimates the risk of Cont-CLNs metastasis in LLNM-PTC patients was added 【page9 line223-226】

(5) The red line (Lasso) represents the model established using the three predictor variables selected through Lasso screening. The blue line (More) represents the model built upon these three variables with the addition of tumor size as a predictor. The Lasso model consistently demonstrated significantly higher net benefits across all threshold ranges compared to the More model and no or full intervention was added 【page9 line232-235】

(6) The model's calibration AUC is 0.771, with a 95% confidence interval of 0.6839–0.8573, indicating good fit was added 【page10 line244-245】

**Comment 5:** The discussions need to focus on the results tables and methods

Reply5: Thank you for your academic advice. At your request, we have carefully revised the discussion section to focus more on the results of this article

Changes in the text: A total of 105 patients were enrolled in the study following strict inclusion and exclusion criteria. Clinical data were collected and analyzed using statistical software. The highest Youden index for Tg was 0.298, with a cutoff value of 73 ng/mL, yielding a specificity of 95.9%. Based on this threshold, patients were classified into high and low Tg level groups. Lasso regression was applied for



dimensionality reduction, identifying three key predictive factors. These factors were subsequently analyzed using multivariate logistic regression, all of which demonstrated statistical significance ( $P < 0.05$ ). A validated nomogram effectively visualizes disease risk probability, demonstrating substantial clinical applicability. The More model was developed by incorporating tumor size as a predictive factor. Decision curve analysis (DCA) was conducted to compare the two nomograms, showing that the Lasso model consistently provided higher net benefits across all threshold probabilities, establishing it as the final model. Internal validation via the Bootstrap method resulted in a calibration AUC of 0.771 (95% CI: 0.6839–0.8573).

【page10 line268】

**Comment 6:** Conclusions need to be presented as they are in the abstract

Reply6: We greatly appreciate your valuable suggestions. Following your advice, we have incorporated a conclusion section.

Changes in the text: A new Conclusion section was added. In summary, this study investigates risk factors for Contralateral Central Lymph Node Metastasis in Unilateral Papillary Thyroid Carcinoma patients with lateral lymph node metastasis and develops a clinical prediction model to assess these risks. The findings suggest that patients with risk factors such as metastasis to lymph nodes posterior to the recurrent laryngeal nerve, male gender, and elevated serum thyroglobulin levels may benefit from prophylactic Cont-CLN dissection.

【page11 line308】