

Peer Review File

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First External Peer Review

Reviewer A

This manuscript describes a retrospective evaluation of the intraoperative frozen analyses of the central lymph nodes to determine lobectomy or total thyroidectomy for cN0 PTC patients to avoid secondary surgery (completion thyroidectomy). I hope that the authors would keep the study and submit your follow-up article with long-term relapse-free survival.

1. Results, page 12, Line, 225-226. At the period of this study, the authors thought that patients with pN1a with a diameter of 5 mm would need completion surgery. So, the authors hoped to perform TT using the intraoperative frozen analyses to avoid two-stage surgery. According to Table 2, metastatic LN size in LTT was between 0.005 and 1.1 cm. However, the authors did not perform completion thyroidectomy for LTT patients with permanent pathological metastatic LN size > 5 mm. The authors should explain the inconsistency.

Response: We would like to thank Reviewer A for the time and effort in reviewing our manuscript and providing comments and suggestions, which have considerably helped us improve our manuscript. We have answered each of your points below and hope that our responses and revisions address all your comments.

We appreciate your comment on the critical point of the study. Unfortunately, there were numerical errors in the LTT (Less than total thyroidectomy) group of Table 2. We apologize for our mistakes. Using our own cut-off value of nodal size as 5 mm, the maximal diameter of metastatic lymph node (LN), which was reported in the frozen analyses, was 0.4 cm. However, larger metastatic LNs were found in 5 patients after permanent analyses: two patients have resected additional LNs after frozen analyses, which were found with larger metastatic LNs (maximum diameter 0.8 cm, 0.7 cm). In three patients, the reported maximum diameter of metastatic LN has been changed after the histopathologic examination (maximum diameter 0.5cm).

The LTT Cases with discrepant frozen and permanent results were relatively rare (2.1%), and these LNs were ultimately removed. Thus, close observation was planned for these patients based on the good prognosis of PTC after surgical removal. Following your comment, we corrected numerical errors in Table 2 and added descriptions of LTT patients with discrepant

frozen and permanent results in the Analyses of patients with discrepant frozen and permanent results of central LNs section of the Results, page 12, lines 224-228. We also commented on the close-observation plan for these patients in the Surgical outcomes section of the Results, page 13, lines 239-241. We mentioned this again with a reference, expecting a good prognosis after surgery in the Discussion, page 17, lines 326-329.

Added:

(a) Table 2

Characteristics	LTT	TT	P-value
Number of patients	243	47	
Frozen analyses			
Number of retrieved LNs (A)	3.9 ± 2.9 (0 - 19)	5.4 ± 3.3 (1 - 15)	0.002
Number of metastatic LNs (B)	0.7 ± 1.2 (0 - 6)	2.7 ± 2.7 (0 - 9)	<0.001
Metastatic LN size (max, c m)	0.1 ± 0.2 (0.005 - 0.4)	0.7 ± 0.3 (0.2 - 1.4)	<0.001
LN ratio (A/B)	0.2 ± 0.3 (0 - 1.0)	0.5 ± 0.4 (0.005 - 1.0)	<0.001
Permanent analyses			
Number of retrieved LNs	4.5 ± 3.5 (0 - 22)	6.3 ± 3.8 (1 - 18)	0.002
Number of metastatic LNs	0.8 ± 1.3 (0 - 7)	3.0 ± 2.8 (0 - 10)	<0.001
Metastatic LN size (max, c m)	0.3 ± 0.2 (0.005 - 0.8)	0.6 ± 0.3 (0.1 - 1.4)	<0.001
Extranodal extension, n (%)	8 (7.8)	9 (25.0)	0.007
LN ratio (A/B)	0.2 ± 0.3 (0 - 1.0)	0.5 ± 0.4 (0 - 1.0)	<0.001

(b) Analyses of patients with discrepant frozen and permanent results of central LNs section in the Results

Among 243 patients who had LTT, 5 (2.1%) patients had discrepant frozen and permanent results. Additional LNs were retrieved in two patients after frozen analyses, which were found with larger metastatic LNs (maximum diameter of 0.9 cm and 0.7 c). In three patients, the reported maximum diameter of metastatic LN has been changed through histopathologic

examination (maximum diameter of 0.5cm).

(c) Surgical outcomes section in the Results

Among the 47 patients who had TT, 5 (10.6%) showed transient postoperative hypocalcemia, and one (2.1%) patient experienced transient hoarseness. None of the patients experienced permanent complications from surgery. No additional surgery of completion TT was planned in any patients who had LTT. The close observation was planned for 5 patients with discrepant frozen and permanent results.

(d) Discussion

Clinically, the critical case would be the 5 patients who had LTT with larger metastatic LNs ($\geq 5\text{mm}$) diagnosed after permanent analyses. However, all metastatic LNs were ultimately removed, and PTC is well-known for a good prognosis after surgical resection (30). Thus, we also determined the close follow-up for these patients.

(e) Reference

30. Ulisse S, Baldini E, Lauro A, et al. Papillary Thyroid Cancer Prognosis: An Evolving Field. *Cancers* 2021;13:5567.

2. Results and Discussion. This kind of research requires the data and discussion of cost-effectiveness. It is better to show mean total operation time for LTT and TT, additional operation time compared with lobectomy without intraoperative frozen analyses, and mean cost for intraoperative frozen analyses.

Response: We appreciate your advice. We added the information of total operation time in Tables 1 and 2. The mean total operation time was longer in the TT group than LTT group (82.4 ± 22.0 vs 113.3 ± 26.5 minutes, $P < 0.001$). Approximately 30 more minutes were taken for the completion TT (total thyroidectomy). We commented on the above-mentioned findings in the Analyses of patients according to the surgical extent section in the Results, page 9, line 167-168.

In this study, frozen section analyses were performed for the central LNs in all patients, which cost 23 dollars (after health insurance) in our hospital. In our clinical settings, hemithyroidectomy with CCND (central compartment neck dissection) costs 711.9 dollars, and total thyroidectomy with CCND costs 1,077.06 dollars. (And there is no additional fee for elongated operation time in our country.) Thus, frozen analysis costs 2.1-3.2 % of the operation fee, which is assumed to be small and insignificant in this study. Moreover, evaluating the cost-effectiveness of frozen analyses requires comparative analyses between

the patients who received frozen analyses of the central LNs and those who did not. We should conduct further study on this topic in the future. According to your comment, we mentioned to clarify the ‘routine’ frozen section analyses of the central LNs in all patients in this study, in the Pathology section of the Materials and methods, page 8, lines 129-130. We also commented on the limited study settings to evaluate the cost-effectiveness of the frozen analyses in the Discussion, page 18, lines 345-346.

Added:

(a) Table 1

Characteristics	Value
Number of patients	290
Operation time (minutes)	87.4 ± 25.5 (40 - 180)

(b) Table 2

Characteristics	LTT	TT	P-value
Number of patients	243	47	
Operation time (minutes)	82.4 ± 22.0 (50 - 155)	113.3 ± 26.5 (55 - 180)	<0.001

(c) Analyses of patients according to the surgical extent section in the Results

We evaluated the patients according to the surgical extent as the LTT and TT groups (Table 2). Comparative analyses revealed that the mean tumor size was larger (0.8 ± 0.5 vs 1.4 ± 0.9 cm, $P < 0.001$), and fewer females (81.9% vs. 66.0%, $P < 0.001$) were involved in the TT group. The mean total operation time was longer in the TT group (82.4 ± 22.0 vs 113.3 ± 26.5 minutes, $P < 0.001$).

(d) Pathology section in the Materials and methods

Histopathological examination of the resected central LNs was performed using frozen and permanent analysis. Frozen section analyses were performed for the central LNs in all patients. For frozen analysis, two 3–4- μ m-thick cryosection samples were cut in the microtome and inspected within 30 min.

(e) the Discussion

This study had several limitations. First, this was a retrospective study in a single institution, including a certain time-period surgery with short-term follow-up. We acknowledge that the

long-term outcomes of larger subjects are essential to evaluate the relevance of surgical treatments. Therefore, the long-term outcomes of patients with respect to local recurrence or distant metastasis should be consistently investigated. Second, this study was based on our individual criteria for metastatic LN. Third, based on previous studies, the causal relationship between LN metastasis, tumor size, sex, and age was not investigated. Fourth, the other patient and tumor factors that would significantly impact disease prognoses, such as age, sex, tumor size, tumor multiplicity, etc., were not considered in determining surgical extent. **Fifth, assessing the cost-effectiveness of the frozen analyses was not feasible to evaluate in this study setting.**

Reviewer B

1. Title: I recommend using the running title as part of main title of the article: “Frozen section analysis of central lymph node in papillary thyroid cancer”

Response: We deeply appreciate your comment on the title, which should appropriately describe the critical points of this study. Following your recommendation, we revised the title of the manuscript to “Frozen section analysis of central lymph nodes in papillary thyroid cancer: The significance in determining the surgical extent.”

Added:

(a) Title

Frozen section analysis of central lymph nodes in papillary thyroid cancer: The significance in determining the surgical extent

2. The introduction is well presented and thus, no further adjustments are needed.

Response: Thanks for your compliment on the introduction of the manuscript. Thank you.

3. The materials and methods section including the statistical analysis section is well presented.

Response: We are grateful for your compliment on the materials and methods section of the manuscript. Thanks.

4. The result section including the attached tables and figures presented is well presented, although it is very extensive and provides too many tables with excessive data, it would be

convenient to reduce them

Response: Thank you for your advice. Following your comment, we have revised Table 4 into supplemental data, Table S1. We retained the related descriptions in the Analyses of patients according to the frozen and permanent results of central LNs section of the Results on page 11, lines 197-204.

Shown:

(a) Analyses of patients according to the frozen and permanent results of central LNs in the Results

Since the frozen and permanent results of central LNs were not identical, we assessed the patients with different frozen and permanent results and compared them with those with identical results. Different frozen and permanent results of the central LNs were observed in 79 patients, who tended to be younger (43.0 ± 11.3 vs 39.2 ± 8.2 , $P=0.002$).

In fact, more LNs were found, and additional resection was improvised after frozen analyses (4.0 ± 3.0 vs 6.3 ± 3.8 , $P<0.001$). More metastatic LNs (0.9 ± 1.7 vs 1.7 ± 2.1 , $P=0.005$) were identified in the permanent analyses in these patients. The N1a stage was also more frequent (43.7% vs. 60.3%, $P=0.013$) (**Supplemental Table S1**).

(b) Table S1

TABLE S1. Analyses of patients according to the frozen and permanent results of central LNs

Characteristics	F = P	F ≠ P	P-value
Number of patients	211	79	

5. The discussion and the conclusion sections were probably the most interesting to read. What needed to be added, depending on journal requirements of course, is a diary number for the ethical approval for this study. Highly recommended.

Response: Thank you for your comment. Fortunately, we have already mentioned the approval of this study by the Institutional Review Board of our institution, with the approval number (2021-11-005) in the Materials and methods, on page 8, Line 146. For your convenient review, we have changed the color of the approval number to red.

Shown:

(a) Materials and Methods

...The study was approved by the Institutional Review Board of CHA Ilsan Medical Center (2021-11-005), and individual consent for this retrospective analysis was waived.

General comments: Well written paper with valuable results.

Response: We would like to thank you for the time and effort in reviewing our manuscript and providing comments and suggestions, which have considerably helped us improve our manuscript. We have answered each of your points below and hope that our responses and revisions address all your comments

Reviewer C

This is an interesting manuscript on the subject of frozen section in central compartment dissection and resultant completion thyroidectomy. The subject is interesting and the data is also interesting. However, the manuscript needs considerable revision with strong English editing. I have certain questions which should be addressed in revising the manuscript.

#1, the title of the manuscript is confusing. There is a big difference between the title of the manuscript and the running title. The running title explains the philosophy of frozen section, but the original title of the manuscript is confusing.

Response: We deeply appreciate your comment on the title, which should appropriately describe the critical points of this study. Following your comment, we revised the title of the manuscript to “Frozen section analysis of central lymph nodes in papillary thyroid cancer: The significance in determining the surgical extent”.

Added:

(a) Title

Frozen section analysis of central lymph node metastasis in papillary thyroid cancer: The significance in determining the surgical extent

#2, the authors have good data on frozen section analysis of the central compartment lymph nodes and its use in determining the extent of thyroidectomy. However, I'm not sure they could get the lymph node ratio, size of the lymph node, and size of the metastatic disease in frozen section. The authors need to explain the feasibility of getting all this information on frozen section. They also need to explain how long it takes to get the frozen section and whether they do have a dedicated pathologist with expertise in thyroid and frozen sections. The follow-up is very short and the authors do need to discuss this. The authors have used the lymph node metastasis as a determinant for extent of thyroidectomy. However, they do need to explain their other primary tumor factors, which will also determine the extent of thyroidectomy.

Response: We deeply appreciate your comments. In our institution, frozen analyses results of the central LNs are reported as ‘the number of positive LNs excised to the total number of removed LNs’, with maximal diameter of the metastatic LN. Through the patients’ chart review, we have built the database, including the above-mentioned nodal information, and the LN ratio (LNR) was calculated subsequently. The estimated time to get the frozen results is within 30 minutes from the time that LNs have been sent, which have been described in the original manuscript on page 8, line 131. Three pathologists with expertise in thyroid diseases were involved in the frozen and permanent analyses. Following your comment, we added detailed descriptions on the frozen analyses report and the pathologists in the Pathology section of the Material and methods, page 8, lines 129-134.

Fortunately, the short-term follow-up of this study has been mentioned and explained as the first limitation of this study on page 17, lines 336-339. The other factors such as tumor size, male sex, and old age have been mentioned as the possible causal relationship with more LN metastasis. However, we have not considered them as the significant factors in determining surgical extent. As your advice, we added detailed descriptions of the other factors we have not considered in determining surgical extent as another limitation of this study in the Discussion, on page 18, lines 343-345.

Added:

(a) Pathology section in the Materials and methods

Histopathological examination of the resected central LNs was performed using frozen and permanent analysis. Frozen section analyses were performed for the central LNs in all patients. For frozen analysis, two 3–4- μ m-thick cryosection samples were cut in the microtome and inspected within 30 min. **The results were instantly reported in the number of metastatic LNs excised to the total number of removed LNs, with maximal diameter of the metastatic LN.** Permanent pathology was confirmed within two weeks after a thorough examination. Three pathologists **with expertise in thyroid diseases** were involved in the frozen and permanent analyses, as scheduled.

(b) Discussion

This study had several limitations. First, this was a retrospective study in a single institution, including a certain time-period surgery with short-term follow-up. We acknowledge that the long-term outcomes of larger subjects are essential to evaluate the relevance of surgical treatments. Therefore, the long-term outcomes of patients with respect to local recurrence or

distant metastasis should be consistently investigated. Second, this study was based on our individual criteria for metastatic LN. Third, based on previous studies, the causal relationship between LN metastasis, tumor size, sex, and age was not investigated. **Fourth, the other patient and tumor factors that would significantly impact disease prognoses, such as age, sex, tumor size, tumor multiplicity, etc., were not considered in determining surgical extent.**

#3, there is no information about Hashimoto's thyroiditis where we do find lymph node enlargement

Response: Thank you for your comment. Fortunately, the presence of Hashimoto's thyroiditis was included as one of the clinical features in Tables 1, 2, and 3. We have used the term 'lymphocytic thyroiditis' in the original manuscript. Per your comment, we have revised the term lymphocytic thyroiditis to "Hashimoto thyroiditis" in Tables 1, 2, and 3. And we described the pathologic diagnosis of Hashimoto thyroiditis in the Pathology section of the Material and methods, page 8, lines 139-142. We also commented on no significant differences in the presence of Hashimoto thyroiditis between LTT and TT groups in the Analyses of patients according to the surgical extent section of the Results, page 10, lines 178-179.

Added:

(a) Table 1

Characteristics	Value
Number of patients	290
Hashimoto thyroiditis, n (%)	151 (52.1)

(b) Table 2

Characteristics	LTT	TT	P-value
Number of patients	243	47	
Hashimoto thyroiditis, n (%)	126 (51.9)	25 (53.2)	0.866

(c) Table 3

Characteristics	LTT	TT	P-value
Number of patients	243	47	
Hashimoto thyroiditis, n (%)	117 (53.7)	34 (47.2)	0.342

(d) Pathology section in the Material and methods

Micrometastasis was defined as maximum metastatic focus size <0.2 cm, while ≥ 0.2 cm was defined as macrometastasis. The LN ratio (LNR) was defined as the ratio of the number of positive LNs excised to the total number removed. Papillary microcarcinoma (PMC) was diagnosed as PTC with a tumor diameter of <1 cm. Hashimoto thyroiditis was diagnosed with the typical histologic findings of extensive lymphocytic infiltrate with the germinal center formation with occasional tissue fibrosis and appearance of abundant Hürthle cells or oncocytes.

(e) Analyses of patients according to the surgical extent section in the Results

A significant difference was noted for all nodal characteristics. According to frozen analyses, the mean number of retrieved (3.9 ± 2.9 vs 5.4 ± 3.3 , $P=0.002$) and metastatic LNs (0.7 ± 1.2 vs 2.7 ± 2.7 , $P<0.001$) was larger in the TT group. Moreover, the LNR was higher (0.2 ± 0.3 vs 0.5 ± 0.4 , $P<0.001$) and the size of maximal metastatic LN was larger (0.2 ± 0.3 vs 0.7 ± 0.3 , $P<0.001$) in the TT group. Similar findings with more extranodal extension (ENE) (7.8% vs. 25.0%, $P=0.007$) were observed in the permanent analyses. This led to more N1a stages in the TT group (42.3% vs. 100%, $P<0.001$) (Table 2).

There were no significant differences in other tumor factors, such as extrathyroidal extension, tumor multiplicity, the presence of Hashimoto thyroiditis, and variants of PTC.

#4, are there any discrepancies between frozen section and permanent pathology?

Response: We appreciate your comment on the critical point of this study. Fortunately, we have already described in detail the patients with discrepant frozen and permanent results in the original manuscript, in ‘the Analyses of patients with discrepant frozen and permanent results of central LNs’ section of the Results, page 10-11, lines 203-221. We have also made a description with Figure 1. The majority of the patients (93.7%) had additional LN removal. The critical cases were the converted results of the LNs in five patients (6.3%). Moreover, during the revision, we specified the details about the 5 patients who had LTT with different frozen and permanent results, page 12, lines 224-228. Thank you for reminding us.

(a) Analyses of patients with discrepant frozen and permanent results of central LNs section in the Results

We further investigated the cause of this discrepancy in these patients. First, additional LN

retrieval was performed in 74 patients (93.7%). More negative LNs were excised in 49 (62.0%) patients, with an average of 2.2 ± 1.6 (range, 1–6). Meanwhile, the positive LNs were additionally resected in 25 (31.7%) patients, with a mean number of 2.2 ± 1.5 (range, 1–6). Among them, a larger metastatic LN was further identified during additional retrieval in nine patients. Mean differences in the maximal diameter of metastatic LNs between frozen and permanent analyses were measured 0.2 ± 0.1 cm (range, 0.1–0.5).

Lastly, the converted result of LNs in the permanent analyses, from negative to positive, was observed in five (6.3%) patients. The mean number of LNs with discrepant results was 1.2 ± 0.4 , and the mean diameter of these LNs was 0.04 ± 0.05 cm (range, 0–0.1). None of the positive LNs in the frozen analyses were confirmed to be negative in the final report.

Among 30 (38.0%) patients with more positive LNs in the final report, 10 (12.7%) patients turned N0 into the N1a stage in permanent analyses. These patients comprised eight (10.1%) patients with additional positive LN retrieval and two (2.5%) patients with converted LN results. Their frozen results were negative from malignancy, but the mean number of 1.1 ± 0.3 (range, 1–2) positive LNs were detected during the histopathologic examination. The mean size was reported 0.2 ± 0.1 cm (range, 0.1–0.3) (Figure 1).

Among 243 patients who had LTT, 5 (2.1%) patients had discrepant frozen and permanent results. Additional LNs were retrieved in two patients after frozen analyses, which were found with larger metastatic LNs (maximum diameter of 0.8 cm and 0.7 cm). In three patients, the reported maximum diameter of metastatic LN has been changed through histopathologic examination (maximum diameter of 0.5cm).

The manuscript is interesting, however, raises certain clinical questions which should be used in revising the manuscript.

Response: We would like to thank you for the time and effort in reviewing our manuscript and providing comments and suggestions, which have considerably helped us improve our manuscript. We have answered each of your points and hope that our responses and revisions address all your comments.

Second External Peer Review

Reviewer C

The authors have made appropriate changes in their manuscript, as per the recommendations of reviewers. The subject of study is quite interesting and I'm amazed that their pathologist can give so much information on frozen section. Conceptually it is a good approach. I would consider deleting Table 4, which does not add much to the manuscript and the information is available in the Results section. Also, the title was appropriately changed by the authors; however, I would consider changing "the significance in determining the surgical extent" to "the significance in determining the extent of surgery". I would leave this to the best judgment of the editors.

Response: We deeply appreciate your compliment on our revised manuscript, and further recommendations. Per your comment, we have deleted Table 4, and have revised the title of the manuscript to 'Frozen section analysis of central lymph nodes in papillary thyroid cancer: The significance in determining the extent of surgery. Thank you again for the considerate review.

Added:

(a) Diagnostic accuracy of frozen analyses of the central LN section in the Results
After excluding 74 patients with additional LN retrieval, the diagnostic accuracy of frozen analyses for central LNs was evaluated in 216 patients. The sensitivity, specificity, PPV, and NPV were 94.6% (88/93), 100% (123/123), 100% (88/88), and 96.1% (123/128), respectively. Statistical significance was reached, and the diagnostic agreement rate between the frozen analyses and the permanent pathologic report was very high (κ index=0.962). The likelihood ratio was 23.57 (P-value=0.000).

(b) Title

Frozen section analysis of central lymph nodes in papillary thyroid cancer:

The significance in determining **the extent of surgery**