

Peer Review File

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Reviewer A

Comment 1:

The issue of selection bias is not fully addressed and this invalidates the conclusions of your study (more detailed explanation below).

Reply 1:

Thank you for the comments. Because this study was a retrospective study and the data stemmed from the Seer database, selection bias was inevitable. The limitations of selection bias also mentioned in the discussion part. For details, please check page 15, line 8-17. Propensity score matching analysis was adopted in this article to minimize the influence of selection bias on the results. Thus, our conclusion is validating. (more details as shown in reply 3&4).

Changes in the text: We added descriptions of the limitation (See page 16, line 281-290).

Comment 2:

Jin et al published a paper earlier this year (Otolaryngology Head and Neck Surgery 2021; 164(1): 97-103) based on a larger sample of 2046 patients with locoregional MTC taken from the SEER database. This paper has the same basic flaw with respect to selection bias.

Reply 2:

Thank you for the comments. The article : Otolaryngology Head and Neck Surgery 2021; 164(1): 97-103) adopted PSM analysis to reduce selection bias, but still many variables were not contained in the matching analysis, including age, ETE, multifocality, surgery approaches. Studies have shown the variables above were closely related to the prognosis of medullary thyroid cancer. However, , the patients who selected in our study had no distant metastases and underwent total thyroidectomy. Also, those variables were included in the PSM analysis. At the same time, the degree of lymph node metastasis involvement may lead bias in the results and supplement analysis based on expert opinions were performed. According to our results, there were no statistically significant differences in tumor pathological stage characteristics, surgical approaches, and tumor growth characteristics between the surgery with RT group and surgery only group. PSM analysis displayed the effects on

minimizing the influence of confounding factors on the results.

In summary, although selection bias is inevitable, an appropriate method (PSM) was chosen to match the two sets of variables to reduce the impact of bias on the results.

Changes in the text: Original text without modification.

Comment 3:

For T-stage, and the presence of extrathyroidal extension this should be sufficient, but for nodal status, TNM staging does not differentiate between extent of nodal involvement, all cases being classified N1. There are studies clearly showing that the risk of distant metastases (and therefore, ultimately, death) is strongly related to the number of nodes involved. It would follow that clinicians may be much more likely to recommend RT if there are 30 nodes involved than 3. This potential imbalance of the groups cannot be overcome using SEER data.

Reply 3:

Thank you for the comments. The number of lymph node metastases may be closely related to the risk of distant metastasis as well as results in radiotherapy and higher mortality. The manuscript does have insufficient analysis of the degree of lymph node metastasis involvement, so supplementary analysis is supported as following.

The number of lymph node metastases was analyzed while our supplementary analysis were dividing into two groups. One was In the surgery with RT group and the other one was surgery only group. Depending on some articles (eg. Medicine (Baltimore). 2019 Jan;98(1):e13884;), the number of lymph node metastases was classified into four subgroups: 0, 1-10, ≥10, NA (undefined number of lymph node metastases). Chi-square analysis of the number of lymph node metastases found that there was no statistical difference between the two groups in the after-PSM cohort (supplementary table 1). The univariate Cox regression analysis found that the number of lymph node metastasis was not a risk factor for inducing poor CSS (Supplementary table 2).

All in all, after the analysis, the variable of lymph node involvement was not statistically significant with poor CSS after propensity score matching.

Supplementary table 1 Chi-square analysis on the degree of lymph node metastasis involvement after propensity score matching

Variables	After matching		P
	Surgery only (n=74)	Surgery with radiotherapy (n=74)	
Lymph node group			0.417

NA	0(0.0%)	2(2.7%)
0	13(17.6%)	13(17.6%)
1-10	32(43.2%)	30(40.5%)
≥10	29(39.2%)	29(39.2%)

Supplementary table 2 Cox proportional hazard regression analysis for the degree of lymph node metastasis involvement associated with CSS

Variables	Univariate analysis	
	HR(95%CI)	P
Lymph node group		
NA	Reference	
0	0.161 (0.015-1.793)	0.138
1-10	0.170 (0.020-1.463)	0.107
≥10	0.305 (0.038-2.452)	0.264

Changes in the text: The results of variable analysis of the degree of lymph node involvement are detailed in supplementary table 1 and supplementary table 2.

Comment 4:

To some extent you should have been alerted to this by the lower survival among radiotherapy patients. You report 3-year cancer-specific survival as 85% in the RT group and 95% in the surgery only group. This higher death rate has to be due either to a higher rate of distant metastases in the RT group, direct toxicity of RT, or a combination of the two. There is no evidence of treatment-related mortality from RT in other areas of head and neck cancer, so this is unlikely to be a significant cause. There is a systematic review by Rowell (your ref 15) that addresses these issues in more detail, and in combining evidence from studies with an overall lower risk of bias, shows a reduction in risk of locoregional relapse with RT.

Reply 4:

Thank you for the comments. The subjects of this study were medullary thyroid carcinoma patients without distant metastasis. PSM analysis was recruited to minimize the influence of confounding factors on the prognosis. These factors included pathological stage, lymph node involvement, and tumor growth characteristics (ETE, multifocality), age, etc. . The manuscript carried out an in-depth discussion on that survival rate of radiotherapy patients was lower than the surgery

only group. Poor CSS in radiotherapy group may due to the potential toxicity of radiotherapy. Therefore, it is recommended that MTC patients without distant metastasis should fully weigh the potential benefits of radiotherapy and the possible toxicity risks.

Changes in the text: The original text has been explained. (See page 14-15, line 248-255)

Reviewer B

Comment 1:

Seventy-four patients received radiotherapy in the study. However, I would like to know the purpose of the radiotherapy. Were all patients received radiotherapy for adjuvant treatment to neck after thyroidectomy. I would like to know that there were how many days from thyroidectomy to the first day of the radiotherapy. Is it possible that there were some patients who were received radiotherapy for early local recurrence or early distant metastasis. I wonder that the patients who were received radiotherapy had poor CSS just because they had early recurrence.

Reply 1:

Thank you for the comments.

The Seer database specified neither why patients in the radiotherapy group suffering radiotherapy , nor the data information of "how many days from thyroidectomy to the first day of radiotherapy".

The impact of radiotherapy on the prognosis was focused by our research, because most of current research was limited to radiotherapy for reducing the recurrence rate of medullary thyroid cancer. However, sustaining radiotherapy on the survival rate of medullary thyroid cancer is still controversial. Radiotherapy is recommended for incomplete resection or local recurrence when combined with ATA guidelines. For high-risk patients, adjuvant radiotherapy should be considered, but the potential advantages and disadvantages of treatment should be weighed. Radiotherapy may be an effective adjunct to prevent local recurrence, and it may also provide long-lasting and continuous control of locally advanced or metastatic disease (or both) in certain MTC patients. Most studies focus on MTC patients who have advanced disease symptoms or distant metastases, and there are no studies on the survival rate of MTC patients without distant metastasis. Currently, research mainly focuses on MTC patients with advanced disease symptoms or distant metastases. Nevertheless, there are still few number of MTC patients without distant metastasis in the database or clinically receiving adjuvant radiotherapy. In this population, the influence of adjuvant radiotherapy on the prognosis is worth exploring.

Therefore, the population selected in this study were all patients with M0 stage (no distant metastasis). Propensity score matching (PSM) analysis was used for minimizing the influence of confounding factors on the results of study. There were no significant differences in confounding factors such as pathological stage, lymph node involvement, tumor growth characteristics (ETE, multifocality), and age between the surgery with RT group and the surgery group. The risk of tumor recurrence in the two groups seems similar. The results of the research indicated that the survival rate of the radiotherapy group was lower than that of the surgery group. For this phenomenon, we also conducted in-depth discussions. After minimizing the influence of selection bias on the research results, we believe that patients with radiotherapy may cause poor CSS due to the potential toxicity of radiotherapy.

Changes in the text: The original text has been explained. (See page 14-15, line 248-255)

Comment 2:

I would like to know information about surgical margin. I think the patients who are received R1 or R2 resection trend to received radiotherapy to neck or tumor bed. It is better authors include R0 resection or R1-2 resection into propensity score matching.

Reply 2:

Thank you for the comments.

Because information on the margins of thyroid surgery was not involved in the SEER database, therefore, in order to minimize the bias caused by surgery, all the subjects in this study were containing patients who were undergone total thyroidectomy. In future clinical research, we will comprehensively collect patients data to further understand the impact of surgical margins on the prognosis of medullary thyroid cancer. And in the discussion part of the manuscript, the limitation of lacking surgical margins information is supplemented.

Changes in the text: Please check page 16, line 281-290.

Comment 3:

Age, sex, ETE and chemotherapy were significant factors in univariate Cox regression. However, they included group (surgery only vs surgery with radiotherapy), Sex and chemotherapy in multivariate analysis. The group was not significant factor in univariate analysis. Why they included group in multivariate analysis. ETE was significant factor in univariate analysis, but they did not include ETE in multivariate analysis. I'd like to know how to select variables that they included in multivariate analysis.

Reply 3:

Thank you for the comments. normally variables with a P value <0.05 in the

multivariate Cox regression analysis is considered as statistically significant. In our study, in order to screen out the variables that affect CSS as much as possible, variables with a P value <0.1 in the univariate Cox regression analysis is considered as statistically significant. (Similar statistical methods are explained in some articles, for example. *Transpl Int.* 2017 Jan;30(1):6-10). Therefore, in univariate analysis, variables including group, age, gender, tumor size, ETE, and chemotherapy were considered to be related to CSS, with setting p value <0.1 . The statistically significant variables in the univariate COX analysis were included in the multivariate COX analysis, and the forward stepwise regression analysis method was adopted. The results showed that the four variables of radiotherapy, chemotherapy, age, and gender were closely related to the CSS of medullary thyroid carcinoma, with P value <0.05 finally.

Changes in the text: The original text has been explained. (See page 9, line 129-131 and page 10-11, line 152-163)