NEW STRATEGIES IN THE TREATMENT OF THYROID CARCINOMA

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NEW STRATEGIES IN THE TREATMENT OF THYROID CARCINOMA

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Editorial: New strategies in treatment of differentiated thyroid carcinoma

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Thyroid carcinoma, treatment strategies in thyroid cancer, thyroid cancer surgery, thyroid cancer diagnosis, thyroid cancer biomarkers, radionuclide I 131, thyroid cancer radiotherapy, thyroid cancer targeted therapy

Editorial on the Research Topic
New strategies in the treatment of thyroid carcinoma

In this Research Topic of manuscripts on *New Strategies In Treatment of Thyroid Carcinoma* (TC), edited by the journal Frontiers in Endocrinology, papers have been gathered that, from the perspective of basic sciences, can potentially contribute to provide issues for a better understanding of clinical problems.

Moreover, molecular biology and studies designed to improve the intrinsic behavior of papillary carcinoma are pivotal in the development of targeted therapies. The study by Yang el al. tries to ellucidate the role of estrogen receptors on the stimulation of cancer cells, through a significant decrease on the levels of endogenous PPARγ ligands PGJ2 and 15(S)-HETE. These findings open a venue to development of endogenous ligands PGJ2 and 15(S)-HETE, to treat papillary thyroid cancer.

The role of primary cilia alterations in differentiated TC and its absence in the anaplastic variety is described, as well as its relationship to the presence of the Aurore kinase protein, which is linked to disassembly of these cilia in TC. The article by Ma et al. describes these phenomenae, as well as the possibility of other biochemical pathways implicated with these organelles like Nek1 and Plk1, with the possibility of developing drugs which could restore the physiology of primary cilia of thyroid cells like U0126 and ganetespib.

TC has increased its incidence and prevalence during the last 20 years. This is secondary to a better understanding of its presentation, as well as of diagnostic studies performed at present, from which, different image tests like high definition ultrasound, CT scan and aspiration biopsy represent the most relevant ones.

On the other hand, a significant number of reports attest to efforts to improve the diagnosis potential of the aforementioned tests. In this e-book the report by Zhang et al., establishes the modification of plasma N-glycome alteration in Thyroid Carcinoma and

Benign Thyroid Neoplasms, which in the future could improve the diagnostic accuracy of ancillary tests as well as the detection of lymph node metastases and assist to design the surgical strategy of malignant lesions.

A report by Jin et al. in a large cohort of papillary carcinoma patients with Type II Diabetes Mellitus,establishes a glucose-lymphocyte ratio higher than 4.23 as well as other factors like age, tumor size and multifocality significant for the presence of central lymph node metastasis which allows discrimination of patients who could be spared a dissection of this specific compartment, with potential decrease of related complications. This reflects the present tendency to improve quality of life in TC patients already afflicted with other comorbidities. The establishment of such kind of nomograms will allow more personalized surgical strategies according to an accurate classification of risk factors (1).

In the same sense, the risk of lymph node and distant metastases in children 2-16 years of age is reported by Zeng et al. to be higher in the classic variant of papillary thyroid carcinoma, (CPTC); moreover, when associated to other risk factors like extrathyroid invasión would warrant a more aggresive treatment and closer followup than in those with ages>16 years, as well as in the presence of the follicular variant of papillary thyroid cancer.

Central lymph node metastasis (CLNM) are regarded as a predictor for local recurrence in patients with papillary thyroid carcinoma (PTC) but the role of prophylactic central lymph node dissection is controversial. Zeng et al. studied 1,054 consecutive PTC patients with the aim to identify the clinical factors associated with CLNM and develop a nomogram for making individualized clinical decisions. CLNM was determined in 31.4% (168/535) of non-Hashimoto's thyroiditis (HT) patients versus 39.2% (83/212) in HT patients (p=0.043). They concluded, that classical PTC patients with features like male gender, age<55 years old, tumor size>1cm, and Hashimoto's thyroiditis condition had a higher risk of CLNM.(7)

The paper by Pitalua-Cortes et al., evaluates 68Ga-PSMA-11, which is a novel tracer to detect recurrences either locorregional or metastatic in papillary thyroid carcinoma. Although a small series, with no clear definition of a gold standard, this molecular radiotracer has the potential to complement the I131 SpectCT, as well as to be of use for theragnostic application to select patients for possible therapy with Lu – PSMA–617, specifically regarding radioiodine resistant lesions.

Four papers concern different aspects related to the surgical technique used, in order to establish minimally invasive methods, the advantages of non-total thyroid resection, the attitude towards airway invasion, and the role of radiofrequency in the ablation of non- aggressive tumors. This last one alludes, consequently, to the avoidance of a surgical procedure.

At present, diagnosis of papillary microcarcinoma has increased, as already stated, and its treatment is still open to debate. However risk stratification and tuning of current systems regarding papillary thyroid cancer has increased, resulting in better design of surgical strategies in a way that patients with no lymph node metastases, multifocal tumors, and absent major extrathyroid extensión could be treated with a unilateral lobectomy as described by Zhao and Cui.

New techniques to prevent patients from neck scar and reduce complications with no inferiority results in treatment of thyroid papillary carcinoma have been introduced in recent times. The article by Rossi et al. provides a comprehensive review of the state of the art, where trans-oral endoscopic thyroidectomy with vestibular approach (TOETVA) appears as the most efficient one in terms of feasibility, expenses, and complication rate. Although requiring a learning curve experience and adequate selection of patients (carcinomas up to1-2 cm in diameter, with no lymph node metastasis, no multifocality, and with no extrathyroid extensión), along with adequate follow-up and determination of postoperative safe markers (thyroglobulin and antithyroglobulin antibody) levels, this technique is promising as long as these requirements are fullfilled, which will reduce the present oncologic concerns-including aggresive variants of TC (2, 3)- regarding safety and efficacy, which are still under discussion.

Most TC cases fall in the low to intermediate risk, however, major invasión to vascular and aerodigestive structures occurs in 7-13% of patients, and very often invasion to cricotracheal areas hinders larynx preservation. However, Piazza et al. have added perspective to endoluminal laryngotracheal invasion regarding the concept of circumpherential resection with end to end anastomosis including or not the cricoid cartilage: 25% and 75% of cases in their series, respectively. Additionally, the Shin III and Shin IV classification was present in 39 (26%), and in 62 (42%) procedures. The results regarding complication rates were 27% on the whole, with tracheostomy dependency of 4%. Limits for this technique were defined as 4 cm length in vertical extension and posterior cricoid invasion up to the aritenoids. Clearly, these procedures require extensive workup and expertise, as well as comparison with the inclusion of a total laryngectomy (4), with new investigations warranted on quality of life obtained by patients, since survival at 15 years follow-up reaches 30%.

The issue of termal energies to treat differentiated carcinoma of the thyroid is raised by Tufano et al. Radiofrequency ablation (RFA) seems, at present, the most efficient of the energies used so far. Although RFA is currently used mostly in patients with benign nodules, its indications in thyroid malignancy are in T1a and T1b tumors with no high risk factors, specifically in patients who are bad surgical candidates, refuse surgery or have a recurrent lesion in cases where previous surgery, and or radioioidine treatment make a rescue surgery technically hazardous and with potential to decrease the quality of life of the patient. Complications rates in malignancies are major (5.5%) and minor (10%) as reported in recent metaanalyses,

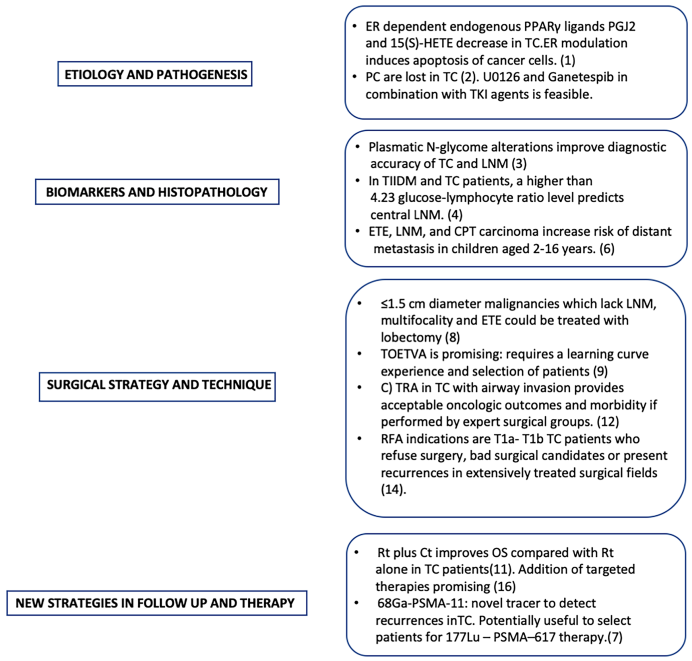

FIGURE 1
Pivotal Findings Regarding New Strategies In Treatment Of Thyroid Carcinoma: Er, Estrogen receptors; PC, Primary cilia, TC, Thyroid carcinoma; LNM, Lymph Node Metastasis; IIDM, Type II Diabetes Mellitus; ETE, Extrathyroid extension; CPT, Classic Papillary Thyroid; TOETVA, Transoral endoscopic thyroidectomy vestibular approach; (C)TRA Cricotracheal and tracheal resection with end to end anastomosis; RFA, Radiofrecuency Ablation; RT, radiotherapy; CT, Chemotherapy; OS, Overall Survival.

and slightly higher (10%% and 15%) in recurrent lesions. The use of these techniques, in thyroid carcinoma, warrants performance of well designed prospective clinical trials.

Finally, Zhou et al. highlight that radiotherapy plus chemotherapy contributes to prolonged overall survival and cancer specific survival compared with radiotherapy alone in anaplastic thyroid cancer patients, regardless of surgical resection and distant metastasis, reinforcing the new concepts explored by Wang and Zaffereo and their group on targeted agents (5). In the same venue to explore the role of radiotherapy in thyroid carcinoma, recent reports on the use of upfront radiation treatment followed by rescue surgery have appeared (6), which in combination with new targeted therapies could constitute a new approach for unresectable malignancies.

An exciting area for improvement of oncologic outcomes and quality of life of TC patients is under continuous development, where biology of tumor, better diagnostic image and biologic markers tools, non invasive treatment of malignancies, and evolving surgical techniques combined with targeted therapies, as well as judicious use of external radiotherapy, contribute to solve ancillary problems and to breach old barriers in the pursue of new strategies for treatment of differentiated thyroid cancer Figure 1.

Author contributions

Topic direction, analyses and criticism: JC, CS, AS. Manuscript design, writing, and drafting: JC, CS, AS, MO, DS. Manuscript review, analyses and administration: JC, CS, AS, MO, DS. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CASE REPORT
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Case Report and Review of Literature: Thyroid Metastases From Breast Carcinoma

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Rationale: The thyroid is a rare site for distant metastases from breast carcinoma. The incidence of thyroid metastases in fine needle aspiration biopsy (FNAB) was less than 0.2%.

Patient concerns: We report a case of 54-year-old woman with a history of breast carcinoma presented with diffuse scattered microcalcifications in thyroid and enlarged bilateral cervical lymph nodes detected on ultrasound (US). Physical examination of the patient revealed firm and enlarged thyroid lobes.

Diagnoses: FNAB and immunohistochemistry (IHC) of the thyroid lesion confirmed the thyroid metastases from breast cancer.

Interventions and Outcomes: Due to the comorbidities of breast carcinoma metastases to the right axillary, cervical lymph nodes and left chest wall, the patient received chemotherapy. After a follow-up of 19 months, the patient was alive without any new distant metastases.

Lessons: Our case highlights that thyroid metastases should be considered in a patient combined with thyroid lesions and a history of breast carcinoma. IHC played an important role in differentiating thyroid metastases from primary thyroid cancer.

Keywords: thyroid metastases, breast carcinoma, diagnosis, therapy, immunohistochemistry

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INTRODUCTION

Thyroid metastases from breast cancer are unusual, accounting for less than 0.2% of thyroid fine needle aspiration biopsy (FNAB) (1, 2). It is well known that the most frequent distant metastases of breast cancer are bone and visceral organs (3). Generally, the common origins of thyroid metastases seem to be breast cancer and lung cancer in autopsy cases (4). However, in clinical cases, the most common primary site is the kidney, followed by the breast (5). Recently, with the advent of advanced

Abbreviations: FNAB, fine needle aspiration biopsy; US, ultrasound; IHC, immunohistochemistry; GATA3, GATA-binding protein 3; HER2, human epidermal growth factor receptor 2; ER, estrogen receptor; PR, progesterone receptor; TTF1, thyroid transcription factor; TG, Thyroglobulin; GCDFP15, gross cystic disease fluid protein 15; CT, computed tomography; PAX8, paired box 8; HE, hematoxylin and eosin.

diagnostic methods such as FNAB and immunohistochemistry (IHC), thyroid metastases have been reported increasingly. Although FNAB can help evaluate benign and malignant thyroid lesions, it may be inefficient in differentiating metastatic thyroid lesion by FNAB.

Here, we presented a case of thyroid metastases from breast carcinoma diagnosed by FNAB and immunochemistry.

CASE REPORTS

A 54 year old woman presented to the West China Hospital, Sichuan University for evaluation of the thyroid nodule and enlarged bilateral cervical lymph nodes detected on ultrasound (US). She denied any symptoms related to the thyroid disease. She had a personal history of invasive ductal carcinoma in the left breast (ER negative, PR negative, GATA3 positive and HER2 positive). She underwent left mastectomy and axillary lymphadenectomy, followed by chemotherapy with epirubicin, cyclophosphamide, dexrazoxane, and trastuzumab. A core needle biopsy of right axillary adenopathy and mass in the left chest wall revealed metastatic breast carcinoma 2 years after surgery.

On physical examination of the thyroid, firm and enlarged thyroid lobes were identified. A 1 cm mass in the upper outer quadrant of the right breast and a 5 cm mass in the left chest wall were found. A 2 cm right axillary adenopathy were found. There were no palpable lymph nodes in the cervical region.

US showed heterogeneous enlargement and diffuse scattered microcalcifications in both lobes of the thyroid (**Figure 1**). A 0.7 cm hypoechogenic, well circumscribed nodule was also found in the left lobe of the thyroid. Right axillary and bilateral cervical lymph nodes were detected on US. The US results showed multiple lymph nodes in the lateral cervical region, the right was about 1.1 cm and the left was about 1 cm. Some of the dermal medulla was poorly demarcated, and dotted blood signals were seen inside the lymph modes. Thyroid function was normal.

FNAB of bilateral lobes of the thyroid (**Figure 2**) and cervical lymph nodes demonstrated malignant epithelial cells. Immunohistochemical staining of thyroid (**Figure 3**), which exhibited positive GATA3 and HER2, and negative TTF1, TG, GCDFP15, PR and ER, confirmed breast carcinoma metastases to the thyroid.

Computed tomography (CT) showed no evidence of metastatic lesion in the brain, lung, liver and bone. Given the comorbidities of right axillary adenopathy and left chest wall

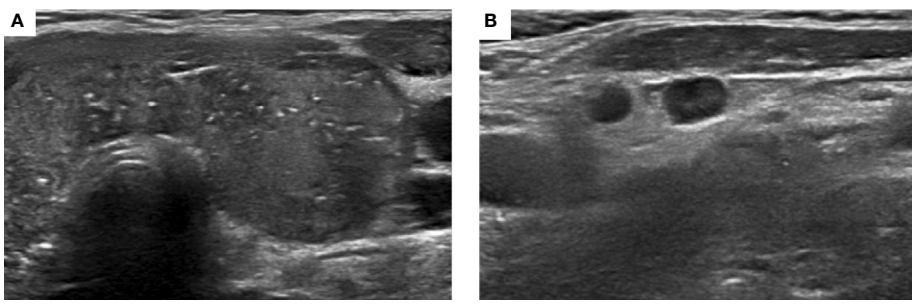

FIGURE 1 | Ultrasound of thyroid showed heterogeneous enlargement and diffuse scattered microcalcifications **(A)**, and enlarged cervical lymph nodes **(B)**.

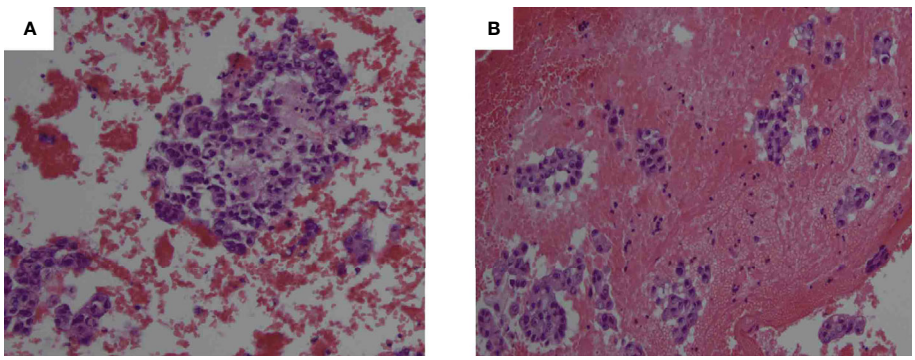

FIGURE 2 | Hematoxylin and eosin (HE) stained FNAB of the right thyroid lobe (**A**, 200× magnification) and left thyroid lobe (**B**, 200× magnification) revealed malignant epithelial cells.

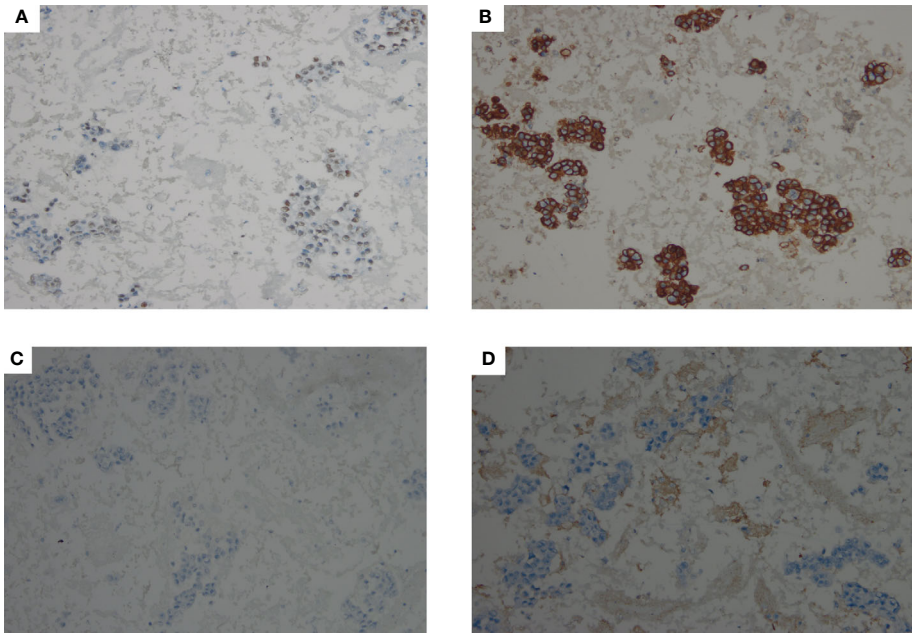

FIGURE 3 | IHC staining showed that malignant cells in thyroid were positive for GATA3 (**A**, 200× magnification) and HER2 (**B**, 200× magnification), and negative for TTF1 (**C**, 200× magnification), TG (**D**, 200× magnification).

metastases, the patient received chemotherapy. Because of financial difficulties, paclitaxel liposome and trastuzumab were administered in the patient. At the follow-up, the patient was alive at 19 months post-thyroid metastases diagnosis. Assessment by CT did not reveal any new distant metastases.

This study was approved by the ethics committee of the West China Hospital, Sichuan University, and written informed consent was provided by the patient for publication of this case publication.

DISCUSSION

To the best of our knowledge, the thyroid is an uncommon site of metastatic cancer. This may be attributed to the fast blood flow of thyroid, abundant oxygen and iodine (6). The incidence of thyroid metastases ranges from 1.25% to 24% in autopsy studies (7). But the incidence of thyroid metastases was lower in clinical series than autopsy studies (1, 8). It has been demonstrated that breast was the secondly frequent primary cancer site for thyroid metastases (3, 8). Currently, the etiology of thyroid metastases from primary carcinoma is not clarified. One study speculated that decreased oxygen and iodine resulted by local thyroid diseases (i.e., thyroiditis and goiter) may contribute to the genesis of thyroid metastases (9).

The clinical presentation of thyroid metastases from breast cancer were similar to primary thyroid cancer, such as no symptoms, palpable neck mass, thyroid lesions detected on imaging examination and compression symptoms. Some studies reported that patients with thyroid metastases from breast cancer were symptomless and presented malignant thyroid nodules by ultrasound (1, 3). Similar clinical manifestations were reported by Owens et al. (10) and Pensabene et al. (4). In our case, the patient also presented no symptoms related to the thyroid disease. Other studies showed patients with thyroid metastases from breast cancer demonstrated dysphagia and dyspnea (5–7, 11). In addition, the US features of thyroid metastases from breast cancer are not specific. Zhou et al. (1) reported that six patients showed heterogeneous echogenicity with scattered microcalcification, and two patients had hypoechoic solid nodule on US. Pensabene et al. (4) reported that one case showed enlarged thyroid with thyroid nodules on US. Similar thyroid nodule on US was reported by Magers et al. (2), Lacka et al. (6) and Owens et al. (10). Above US features also seen in our case and have been described previously. Therefore, there are no specific properties that clinically distinguish thyroid metastases from primary thyroid cancer.

Cytologic examination using FNAB is highly sensitive and specific in demonstrating a malignant thyroid nodule. But it is sometimes difficult to show the origin of the metastatic cancer (4). Owens et al. (10) reported that FNAB of thyroid metastases from breast cancer demonstrated malignant epithelial cells with enlarged nuclei and irregular nuclear contours, but in the absence of intranuclear grooves and pseudoinclusions. Magers et al. (2) found that metastatic thyroid cancer from breast cancer can cytologically and morphologically mimic primary thyroid cancer on FNAB. Additionally, metastatic breast cancer cells in the thyroid, which mimicked C cell hyperplasia and medullary thyroid carcinoma, were observed in the case of Ghias et al. (5). In our case, FNAB of thyroid showed malignant cells with enlarged nuclei.

It has been documented that immunohistochemical stains can play a critical role in differentiating between thyroid metastases and primary thyroid cancer (12). As noted, primary differentiated thyroid cancer commonly is TG, TTF-1 and PAX8 positive (12). Calcitonin, involved in the parafollicular cells, is associated with medullary thyroid carcinoma (13). In contrast, above immunohistochemical markers were negative for thyroid metastases (5, 14). On the other hand, considering the history of breast cancer, the immunoprofile of breast cancer such as ER, PR, HER2, GATA3, and GCDFP15, should be used to confirm the origin from breast cancer metastasis. Previous studies have demonstrated that GATA3 and GCDFP15 expression were positively associated with breast carcinoma and breast cancer metastasis (15, 16). GATA3 is superior to GCDFP15 in determining the breast origin (17, 18). Ghias et al. (5) reported that thyroid metastases cells were positive for ER, GATA3, and were negative for GCDFP, TTF-1, TG, and calcitonin. Similar result is also observed in our case. Bourcier et al. (19) showed thyroid metastases from the breast cancer was positive for ER, PR, and GATA3, but negative for HER2, TTF-1, PAX8, and calcitonin. In additon, GATA-3 (+), ER (+), PAX-8 (-), and TTF-1(-) in thyroid metastases cells were reported by Magers et al. (2).

For the treatment and prognosis of thyroid metastases from breast cancer, Surgery for thyroid metastases is debatable. Some authors stated that thyroidectomy seem to be effective for alleviating dysphagia and dysphonia resulted by large metastatic thyroid lesions (5, 20, 21). Thyroidectomy was recommended for isolated thyroid metastases from breast carcinomas (9). Other cases suggested that thyroidectomy has no efficacy in patients with thyroid metastases from breast cancer combined with multiple distant metastases and chemotherapies should be performed (1, 4). With the limitation of the small number of patient data, there is no definitive evidence to support surgery or chemotherapy or radiotherapy for thyroid metastases from breast cancer (4). In addition, several studies have suggested that the prognosis of thyroid metastases is poor (9). Zhou et al. (1) reported that two of eight patients with thyroid metastases from breast cancer died at less than 22 months post-thyroid metastases diagnosis. Kim et al. (3) reported one of five patients died at 26 months after diagnosis of thyroid metastases from breast cancer. Lacka et al. (6) reported that one patient with thyroid metastases from breast cancer had metastases in liver, and lung at 5 months after thyroidectomy, and died at postoperative 36 months. Metastases in bone at 32 months after hemithyroidectomy were observed in a patient with thyroid metastases from breast cancer who died at postoperative 45 months (4). The characteristics of thyroid metastases from breast cancer are presented in **Table 1**.

CONCLUSION

We reported the rare case of thyroid metastases from breast carcinoma, which highlights that thyroid metastases should be considered in a patient with thyroid lesions in combination with a history of breast carcinoma. FNAB and IHC may contribute to distinguish thyroid metastasis from primary thyroid cancer.

TABLE 1 | Reports of thyroid metastases from breast carcinoma.

Study	Year	No. of patients	Age (years)	Sex	Histology	Interval between Breast cancer and thyroid metastasis (months)	Others metastasis	Treatment	Survival time after diagnosis (months)
Kim et al. (3)	2005	5	34–55	F	Ductal carcinoma	18–85	Lung, neck LN	Chemotherapy	4–26
Owens et al. (10)	2005	1	64	F	NA	5	Liver, shoulder	NA	NA
Zhou et al. (1)	2019	8	43–69	F	Adenocarcinoma, Ductal carcinoma, Signet ring cell carcinoma,	6–82	Chest wall, Lung, Cervical and Mediastinal LN	Chemotherapy, total thyroidectomy, Right lobectomy	4–45
Egana et al. (11)	2012	1	83	F	Infiltrating lobular carcinoma	3	Live and bone	Left lobectomy	1
Lacka et al. (6)	2012	1	54	F	Ductal-lobular carcinoma	14	Live, lung	Total thyroidectomy	3
Bourcier et al. (19)	2018	1	54	F	Lobular carcinoma	NA	Cervical LN	Total thyroidectomy and cervical lymph node dissection, Endocrine therapy	NA
Pensabene et al. (4)	2018	1	64	F	Infiltrating lobular carcinoma	6	Bone	Left lobectomy	45
Ghias et al. (5)	2019	1	67	F	Ductal carcinoma	NA	Right cerebellopontine angle	Right lobectomy	NA
Present study	2021	1	54	F	Ductal carcinoma	22	Cervical LN, chest wall	Chemotherapy	NA

F, female; LN, lymph node; NA, Not available.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

This study was approved by the ethics committee of the West China Hospital, Sichuan University, and written informed consent was provided by the patient for publication of this case publication.

AUTHOR CONTRIBUTIONS

YW and SZ contributed equally to this work and are co-first authors. ZL, JZ, and TW designed the research. YW, SZ, BY, and PZ developed the literature search. YW and SZ drafted the article. All authors contributed to the article and approved the submitted version.

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REVIEW
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Recent Trends in Surgical Approach to Thyroid Cancer

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Over the past decade, the incidence of thyroid cancer has rapidly increased worldwide, and thyroid surgery has become one of the most common performed surgical procedure. Even though conventional open thyroidectomy remains the gold standard, this approach leaves a neck scar which could be worrying mainly for young women. The recent progress in surgical technology, as well as patient cosmetic requests, have led to the development of alternative access to the thyroid lodge. Thus, alternative techniques have been established in order to potentially provide a more appealing cosmetic result, both with a minimally-invasive cervical or remote-access approach. However, the introduction of these new techniques was initially approached with caution due to technical challenges, the introduction of new complications and, above all, skepticism about the oncologic effectiveness. Among several alternative approaches proposed, the minimally invasive video-assisted thyroidectomy and the robot-assisted transaxillary thyroidectomy became popular and obtained the favor of the scientific community. Moreover, the recent introduction of the trans-oral endoscopic thyroidectomy with vestibular approach, although the safety and the efficacy are still under discussion, deserves particular attention since it represents the only technique truly scarless and provides the best cometic result. The purpose of this article is to provide an overview of the current main alternative approaches for the treatment of thyroid cancer with particular focus on the oncological effectiveness of the procedures.

Keywords: thyroid cancer, RATT, MIVAT, TOETVA, minimally invasive

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INTRODUCTION

The incidence of thyroid cancer has markedly increased worldwide during the last decade, with thyroid surgery becoming one of the most common surgical procedures mainly due to an increasing use of neck ultrasonography and fine needle aspiration biopsy (FNAB). The exponential growth of these procedures in the last years have enabled to detect many more cases of tumors than in the past, and in particular more microcarcinomas. Although health screening programs leading to earlier detection surely play a role in the increasing identification of thyroid tumors, other environmental factors also may contribute (1).

The conventional open approach to thyroidectomy, initially proposed by Theodore Kocher in the late 1800s, leaves a neck scar that is associated with great concern, in particular for young women, who are very sensitive to cosmesis. This issue led to the introduction in the late 90s of alternative approaches to obtain a better cosmetic result or even avoid a visible neck scar (2–5).

Minimally invasive video-assisted thyroidectomy (MIVAT), which was originally described by Miccoli et al. (2) in the late 1990s, has proved to be a safe procedure that harbors potential advantages in cosmetic results and postoperative outcomes compared with the conventional procedure, including shorter scar length, better cosmesis, and reduced pain. Although descriptions of this procedure date back to more than 20 years, it still remains one of the favorite endoscopic techniques to remove the thyroid gland (6, 7).

Furthermore, during the past decade, an imposing number of different remote-access approaches have been described as a method of removing the thyroid gland while avoiding a neck scar. These techniques have been developed to potentially provide a cosmetically more appealing result for some patients and have often resulted as an expression of different habits and expectations of patients of different geographic regions and cultures. However, although initially surrounded by skepticism due to technical challenges, the introduction of new complications, and concerns about oncologic safety and cost, some of them have been approached progressively more widely by the community of endocrine surgeons. Anyhow, it is strictly recommended to adhere to selection criteria and to consider that these techniques should be approached by surgeons performing a high volume of thyroid surgery (8).

This review provides an overall evaluation of the main alternative approaches to conventional thyroidectomy (CT), such as minimally-invasive video-assisted thyroidectomy (MIVAT), robot-assisted trans-axillary thyroidectomy (RATT), and the transoral endoscopic thyroidectomy with vestibular approach (TOETVA), for the treatment of thyroid cancer, focusing on the oncologic safety and effectiveness of these procedures for this cohort of patients.

MINIMALLY INVASIVE VIDEO-ASSISTED THYROIDECTOMY (MIVAT)

Since its introduction in the late 1990s, MIVAT has been worldwide adopted thanks to its reproducibility and its comparable outcomes to the conventional open approach. This minimally invasive video-assisted technique permits surgeons to safely perform thyroidectomy and provides the benefits of the typical advantages proper of endoscopic surgery, including magnified vision, better cosmetic results, and reduced postoperative pain (9, 10).

Although initially introduced into clinical practice for the treatment of small benign thyroid nodules (2), the use of MIVAT for the treatment of thyroid cancer gained progressively more popularity, and several case series demonstrated its feasibility and safety even in this cohort of patients (11–13). All in all, ideal candidates for MIVAT are patients with an ultrasound-estimated thyroid volume not exceeding 25 ml with nodules smaller than 35 mm. On the other hand, absolute contraindications for MIVAT are large multinodular goiters, previous neck surgery or irradiation, locally invasive carcinoma, presence of lateral neck compartment lymph node metastasis. The presence of enlarged lymph nodes in the central compartment of the neck is not necessarily a contraindication since MIVAT proved to be fit even for VIth level lymphadenectomy, although we believe that it should be performed with caution and only in cases of incidentally intraoperative discovery of enlarged lymph nodes (14). Moreover, caution must be taken in case of small thyroid cancers when located very posteriorly because they could have an extracapsular infiltration: this situation could represent a reason for a prompt conversion to CT (7). Further, presence of thyroiditis, adverse anatomical aspects (short neck in obese patients) and hypervascularization of the thyroid gland, represent relative contraindications to MIVAT (14).

We investigated the oncologic completeness of MIVAT compared with CT in a prospective study of 33 patients: 16 underwent MIVAT and 17 underwent nearly total CT. No statistically significant differences were found in I-131 uptake and serum thyroglobulin levels, showing that the completeness obtained by MIVAT was comparable to the one by CT (15).

Furthermore, the excellent oncologic outcomes of this minimally invasive approach were confirmed in another study involving patients affected by differentiated thyroid carcinoma (DTC) with a median follow-up of 5 years (16). The study enrolled 221 patients: 171 underwent MIVAT and 50 underwent CT. At the time of remnant ablation, no differences in serum thyroglobulin, thyroid stimulating hormone (TSH) levels, or I-131 neck uptake were observed between the two groups. After a 5-year follow-up, the two groups were comparable in outcomes, with no thyroid cancer-related death or recurrence documented in either group. Finally, the cumulative dose of I-131 needed to definitively cure the thyroid cancer was the same regardless of the surgical approach, indirectly confirming that the two techniques are superimposable in oncologic completeness (16).

Our entire case series of DTC treated by means of MIVAT was evaluated in 2015 (9). In particular, 528 patients presenting with thyroid cancer were monitored for a median period of 7.5 years. The evaluation of thyroglobulin serum levels showed optimal results, as did the radioactive iodine dose required for completion and for recurrence in this cohort. Relapse was documented in 24 patients (4.5%); of these, 14 were treated surgically, and 10 were treated with a repeat administration of radioactive iodine. In the same period (2000–2009), 234 patients with a comparable stage disease underwent a CT. The cure rate in this control group was very similar: 80% of the patients were cured in the same follow-up interval (9). Although only 7.2% of patients in our case series did not receive I-131, it seems that according to the present standard guidelines, in most, if not all cases, radioactive iodine therapy would not be necessary after a total thyroidectomy performed *via* MIVAT when selection criteria are carefully followed (17).

Moreover, the 15 patients carrying a *RET* gene mutation who underwent a prophylactic thyroidectomy and central neck dissection *via* the MIVAT approach showed undetectable serum levels of calcitonin (9). Other authors have reported similar results (18).

Several institutions have assessed the efficacy of MIVAT in the treatment of thyroid cancer (19, 20). Del Rio et al. (19) performed a prospective study in 2015 to compare the oncologic outcomes in patients with DTC treated with MIVAT versus CT. Of 172 patients who were enrolled, 67 were treated with the minimally invasive technique and 105 with the open approach. After a mean follow-up of 5 years, the authors reported no statistically significant difference in oncologic efficacy; in particular, the two techniques were comparable for disease control after post-ablation scintigraphy and thyroglobulin levels (19). Accordingly, Lai et al. (20) evaluated the oncologic completeness of MIVAT in 16 patients with low- or intermediate-risk thyroid cancer, 6 of whom underwent incidentally (n = 5) or intentionally (n=1) central compartment neck dissection along with thyroidectomy. The radioiodine uptake and the radioiodine dose delivered in patients who underwent MIVAT were comparable to CT, and radioiodine ablation showed undetectable thyroglobulin levels (20).

Notably, Lombardi et al. (21) performed a comparative study with the goal to demonstrate the safety and feasibility of MIVAT and central neck dissection. They reported outcomes for 52 consecutive patients who were treated by means of the minimally invasive technique, and 52 patients who were treated by means of the conventional approach. They concluded that the two techniques were comparable in lymph nodes harvest, serum thyroglobulin off levothyroxine, postoperative ultrasound neck scan, and postoperative radioiodine uptake. The authors claimed that the endoscopic view allows an accurate exploration of the central compartment and enables identification of even slightly enlarged lymph nodes. Other authors also reported comparable oncologic results between MIVAT and CT with associated central neck dissection in patients affected by thyroid carcinoma (11, 22).

On one hand, we believe that video-assisted central neck dissection must be performed with caution and only in patients with intraoperative unexpected discovery of enlarged lymph nodes. On the other hand, this technique is appropriate for prophylactic central neck dissection in patients who are mutated RET carriers (9, 18).

Regarding complications, several studies dealing with MIVAT reported data comparable to CT, strengthening the idea that this is a safe technique (9). Indeed, although the narrow space and the few degrees of freedom, these outcomes can be achieved thanks to the magnified vision of the endoscope which allows an easy identification of parathyroid glands and recurrent laryngeal nerves (7).

The indications for MIVAT have been extended over the years from small benign nodules to low- and intermediate-risk thyroid cancer, showing a level of oncologic safety comparable to CT. Moreover, reduced pain and hospital stay, and increased patient satisfaction are the strengths of this approach. After a long debate, the initial reluctance has been swept away, and this technique has gained acceptance worldwide in the treatment of selected thyroid carcinoma. To obtain excellent results, strict adherence to criteria selection is required, especially at the beginning of the experience, and it is strongly recommended to perform MIVAT in high-volume centers by trained endoscopic endocrine surgeons. Unfortunately, MIVAT is a technique limited to a niche of patients due to the volume of the gland and the size of the nodule required to fit the selection criteria. Depending on the geographic area, only approximately 20% to 30% of patients may benefit of this approach (20).

ROBOT-ASSISTED TRANSAXILLARY THYROIDECTOMY (RATT)

The desire to avoid neck scarring after thyroid surgery has resulted in the development of endoscopic and robotic remote access techniques. The gasless transaxillary endoscopic thyroidectomy was proposed in November 2001 in South Korea at the Yonsei University College of Medicine, Seoul, to satisfy this necessity (23). However, endoscopic thyroidectomy showed several limitations, such as difficulty in instrument handling and restricted vision (24). The introduction of surgical robots was thought to overcome drawbacks of endoscopic surgery and to provide technical improvements, including magnified 3-dimensional (3D) vision, tremor-filtering systems, and additional degrees of freedom (25).

The approval of the da Vinci surgical system (Intuitive Surgical, Sunnyvale, CA, USA) by the United States Food and Drug Administration in 2000 made its use progressively more widespread (25). An important turning point in the development of the robot-assisted transaxillary approach was the description of the procedure using a single access that avoided the accessory sternal incision (26, 27). This less invasive procedure provides better cosmetic outcomes and improves patient comfort, arousing interest among the medical community (26).

The robot-assisted transaxillary thyroidectomy (RATT), popularized by Chung et al, who published their experience with 5000 cases in 2018, became widely used in countries in East Asia, although still under discussion in Europe and the Americas (25, 28). The negative connotation of having a horizontal neck scar, which is thought to denote death in Asian culture, may have played a role in the rapid spreading of this technique (29). Differences in body mass index and anthropometric characteristics and greater size of goiters and cancers, combined with the elevated costs of the procedure and the need of training, have hindered the diffusion of this approach in the United States and Europe (28). To date, RATT, although excellent results in feasibility, safety, and patient satisfaction are described, is limited to play a niche role in selected patients with appropriate pathology in high-volume centers (28).

Overall, indications for RATT varies among the centers, but nowadays substantially both benign pathologies and well-differentiated low risk thyroid carcinoma can be approached

with this technique. Guidelines recommended to limit RATT to patients affected by well-circumscribed nodule < 3 cm and with thyroid lobe < 5–6 cm in the largest dimension (8). Moreover, previous neck or breast surgery are usually considered contraindications, as well as neck radiotherapy, pacemaker implant, shoulder arthrosis, previous shoulder surgery, substernal extension and Grave's disease. Nonetheless, indications were progressively expanded as the experience increase and some Institutions performed RATT even in more advanced cases (25).

With regard of complications, several studies reported comparable outcomes between RATT and CT (30, 31). In particular, no statistically significant differences were reported in terms of classic complications (hypoparathyroidism and RLN palsy) rate (31). Moreover, the introduction of potential new complications, which was seen with great concern at the beginning of the experience, deserves a special mention. This group of unconventional complications includes: brachial plexus injury, axillary flap perforation, tracheal injury and surgical-track recurrence. Although patients should be informed of these additional risks, fortunately these complications are extremely rare and, concerning brachial plexus injury, almost always transient. Indeed, its incidence is reported up to 0.2% of patients, but it resulted permanent in 0.04% (32).

Several systematic reviews and meta-analysis reported oncologic outcomes that are equivalent to those of conventional thyroidectomy in terms of completeness and recurrence rate (31, 33).

Lee and colleagues (34) comparatively studied 94 patients who had undergone total thyroidectomy with central neck dissection. The patients were divided between those who underwent robotic (n = 43) and conventional (n = 51) approaches. The authors reported a similar number of retrieved lymph nodes, and no significant differences between the two groups were documented in stimulated thyroglobulin levels acquired during whole-body scans. Moreover, the ablation success rate was similar between the two approaches, and the follow-up ultrasound examination documented no abnormal findings in either group (34). The same authors, in a long-term follow-up evaluation, reported comparable outcomes between CT and RATT in anti-thyroglobulin antibodies, serum thyroglobulin, locoregional recurrence rate, and disease-free survival, claiming that RATT has superimposable impact to CT regarding oncologic completeness (35).

Once reliability of RATT for DTC was ascertained and the surgical skills increased over the time, indications progressively expanded to include more aggressive diseases. In 2018, Chung and colleagues (25) reported their experience with 4804 patients with thyroid cancer. First, it is worth mentioning that almost two-thirds of all of the operations consisted in less-than-total thyroidectomy and only one-third in bilateral total thyroidectomy. Moreover, this cohort of patients presented a medium tumor size of only 0.8 ± 0.6 cm. Anyway, as robotic experience increased, the authors were able to successfully treat even advanced cases, such as those with adjacent muscles invasion or perinodal infiltration. In particular, the authors enrolled 25 patients at T4a stage, and successful preservation of the invaded organs was obtained in 20 patients (25).

Concerning N stage, central neck lymph node metastases and lateral neck lymph node metastases were found in 1407 patients (29.3%) and 363 patients (7.6%), respectively, with a mean number of retrieved central and lateral lymph nodes of 6.3 ± 5.1 and 34.1 ± 17.5, respectively (25). The fine dissection allowed by the robotic system, along with the magnified 3D vision, enables an accurate lymph nodes removal with the number of harvest lymph nodes comparable to the number obtained with open surgery (28). Lee et al. (36) accordingly reported that RATT with modified radical neck dissection provided similar oncologic outcomes (including the results of radioactive iodine scans and postoperative serum thyroglobulin levels) and safety as conventional open procedures (36). Moreover, the robotic approach yielded better outcomes in quality of life and cosmesis (36).

Regarding the 1863 patients with thyroid cancer who underwent total thyroidectomy, therapeutic adjuvant radioactive iodine therapy was performed in 1460 (78.3%). Among these patients, diagnostic whole-body scans showed no abnormal uptake in 1380 patients (94.5%). Furthermore, the serum TSH-suppressed thyroglobulin level was less than 1 ng/mL in 1038 patients (55.7%) at 3 months after surgery. During the follow-up, tumor recurrence was detected by imaging and confirmed in 26 patients (0.5%) (25).

In 2018 we published our initial experience with 250 patients who underwent RATT (37). The final histologic examination reported carcinoma in 103 patients, with a mean diameter of 12.9 mm. According to the European consensus for thyroid cancer management, 26 patients were treated with low radioiodine (I-131) activities (1.1 GBq/30 mCi) for postsurgical thyroid remnant ablation. After 4 years of follow-up, all patients with a thyroid cancer diagnosis were free of disease, and those who underwent total thyroidectomy showed a mean value of 0.8 ± 1.4 ng/mL of TSH-suppressed serum thyroglobulin (37).

As reported in the studies published by the Korean group, we also progressively extended the indications of RATT with the increasing experience, especially for benign lesions. Nevertheless, a careful selection of patients is of paramount importance to achieve excellent results with this technique. To date, we still exclude patients with suspicious VIth level lymph nodes or T4 tumors (37).

Garstka et al. (38) published a comparative study including DTC patients who underwent robot-assisted transaxillary or conventional cervical approach with or without lymph node dissection. A total of 144 surgeries were included, 35 out of 144 were robotics. The Authors reported comparable outcomes in terms of mean tumor size, number of positive microscopic margins and number of lymph nodes removed when lymph node dissections were associated. No statistically significant difference in postoperative thyroglobulin levels was documented, with a comparable follow-up period, and no significant difference in recurrence rate was reported; in particular, no recurrence was reported in the robotic group (38).

Similarly, Noureldine et al. (39) reported their experience with a North America population of patients with thyroid cancer

(39). In their study, 35 patients underwent thyroidectomy by means of conventional approach, whereas 25 patients by means of robot-assisted transaxillary approach. They reported that no patient presented high uptake with post-operative I-131 whole body scan and that the mean serum thyroglobulin levels between the two groups were comparable. Moreover, the neck ultrasonography performed on all patients 1 to 3 months after the operation did not show any residual thyroid tissue or evidence of residual or recurrent disease. Although at the 2-year follow-up one patient in the robotic group required reoperation for recurrent disease in the central compartment, the authors concluded that excellent oncologic results can be achieved with RATT in selected patients affected by thyroid cancer (39).

Overall, RATT is feasible and oncologically safe in properly selected patients, and by avoiding a visible neck scar it is associated with excellent cosmesis. This approach proved safe even when applied to patients in the West, whose anthropometric parameters may vary considerably from the Asian population. We believe that in skilled hands, RATT can be considered a valid alternative to CT even in selected patients with thyroid cancer, especially those who have concerns about cosmetic outcome.

We hope that the advent of new medical device companies in the robotic surgical field, the development of new technologies, and the worldwide spread of the technique will gradually break down some limitations of the robotic system, such as the lack of haptic feedback, the long operative time, and the elevated costs, which, however, might be significantly reduced when the procedure is performed by experienced teams and through a limitation of disposable instruments (30, 40). It is worth to underline that at our institution we perform RATT using only 3 robotic arms, with the fourth kept folded: this reduces the length of the incision and the encumbrance of the instruments. Besides, this technique results in lowering the docking time and indirectly the robotic costs, which are further decreased by avoiding the use of the fourth arm. This is especially true with the use of da Vinci Si surgical system, with which the drape for the fourth arm is avoided, differently from the Xi version.

Finally, concerning the economic impact of the robotic procedure, it is important to take into consideration that RATT usually takes a shorter time compared with other robotic operations. This leads to the opportunity of covering empty spaces among the daily operating list and allows an improvement in the efficiency of the robotic operating room.

TRANSORAL ENDOSCOPIC THYROIDECTOMY WITH VESTIBULAR APPROACH (TOETVA)

The only technique that allows a scarless thyroidectomy is the transoral endoscopic thyroidectomy (TOET). Although various techniques for TOET are described, the most used is the TOET with vestibular approach (TOETVA) due to its surgical outcomes and low complication rate (41).

The first attempt of TOET was performed by Witzel et al. (42) using the sublingual route. Many other attempts were performed later on, but all of them were associated with a high complications rate. As result, TOET *via* the sublingual route is no longer performed in clinical practice (41). On the other hand, the first TOETVA was described by Richmon et al. (43). Since then, Anuwong and colleagues (41) refined the technique and performed more than 800 procedures in 2019.

This new natural orifice transluminal endoscopic surgery (NOTES) is performed by means of 3 small incisions (one on the midline for a 5- to 10-mm port and two laterals for 3- to 5-mm ports) in the lower lip's vestibule, resulting in a truly scarless thyroidectomy. The pre-mandibular space is first created with the help of the dilatator and followed by dissection under direct vision and CO_2 insufflation (44). Different from other endoscopic thyroid surgery techniques, TOETVA allows an excellent view of the surgical field and equal access to both sides of the central neck; nevertheless, the identification and dissection free of the recurrent laryngeal nerve is approached from top to bottom and may jeopardize the recurrent laryngeal nerve, which usually divides into several branches and therefore must be followed bottom up and not in the opposite direction (45).

Anuwong et al. reported that the eligibility criteria for TOETVA are the following: thyroid gland of a diameter not exceeding 10 cm, comprising either benign thyroid nodule, papillary microcarcinoma with no evidence of metastasis, follicular neoplasm, or well-controlled Graves' disease. Moreover, they reported that TOETVA can be done safely in patients who had previously undergone surgery or radiation at the chin and neck area (46).

Taking into consideration the limitations of TOETVA and the natural history of differentiated thyroid cancer, Wu et al. (47) reported that this natural orifice transluminal endoscopic approach can be safely performed in case of low-risk thyroid carcinoma up to 2 cm in diameter with adequate oncologic outcomes. Similarly, Anuwong et al. (48) did not consider patients with thyroid malignant tumors larger than 2 cm candidates for TOETVA because it is crucial to extract the tumor intact, and it cannot be morselized as is done with benign nodules.

Chai et al. (49) published in 2017 a retrospective study of 10 female patients who had undergone TOETVA due to papillary thyroid microcarcinoma. Only partial thyroidectomies were included (7 lobectomies and 3 isthmusectomies). The authors documented recurrent laryngeal nerve palsy in 2 patients, fully recovered in 3 months, and acceptable operative times. No oncologic follow-up data were reported (49).

In 2019, Luna-Ortiz et al. (50) performed a retrospective study of 46 patients with DTC who underwent TOETVA, reporting acceptable results. All patients were evaluated for postoperative serum thyroglobulin levels and anti-thyroglobulin antibodies at 4 weeks after surgery, and all exhibited values below 5 ng/dL. The authors claimed that TOETVA may be indicated in case of thyroid carcinoma that is not locally invasive and without lymph nodes involvement (50).

With regard of complications, some studies reported comparable outcomes to CT (46, 51), claiming that TOETVA can be safety performed in selected patients. Anyway, further studies will assess in the future the actual safety of the procedure and the real incidence of some new unconventional complications, such as mental nerve injury, flap perforation and bruising, which are to date rarely reported (46).

In summary, TOETVA is a new technique that provides the best cosmetic results considering that is totally scarless, with a short distance between the thyroid gland and the incisions. Although there are some reports of its feasibility in both benign and malignant lesions of the thyroid, long-term oncologic outcomes regarding thyroid cancer are still lacking. In our opinion, some concerns about this approach still persist, especially regarding the oncologic completeness and the technical feasibility. Besides, Anuwong himself stated that this approach should be used only for 1 to 2 cm thyroid carcinomas (44).

Anyway, since encouraging studies are reported in literature, larger case series with longer follow-up are required to understand the actual oncologic validity and safety of this approach.

CONCLUSIONS

The technological progress has led to the development of several alternative surgical techniques to the thyroid gland, either with a cervical or a remote-access approach, both robotic and endoscopic. Although most of them have been abandoned due to scarcity of quality outcomes, MIVAT and RATT have obtained the consensus from the scientific community. Nowadays, the feasibility and safety of MIVAT has gained widespread acceptance for both benign and malignant diseases, although a minority of patients are eligible for the technique.

Similarly, with the introduction of robotic systems in the surgical armamentarium, RATT progressively became more and more popular among surgeons, initially in Asia and successively in the West, with extension of the indications as the experience increased. To date, thyroid cancer is safely treated by means of RATT, and some case series reported appealing results even when central compartment and lateral neck compartment dissection are associated.

Finally, the recent introduction of TOETVA deserves a special mention because this is the only totally scarless technique to manage thyroid diseases. Notwithstanding, some technical and oncologic concerns about this approach persist, and further research with an adequate follow-up are mandatory to assess its safety, especially in case of malignancy where the integrity of the nodule is essential.

We believe that an accurate patient selection is of paramount importance when a nonconventional approach to the thyroid gland is planned. We strongly encourage that these procedures be centralized to high-volume centers with skilled endocrine endoscopic surgeons.

AUTHOR CONTRIBUTIONS

LR wrote the manuscript with the support of PM and GM and analyzed data collected from the literature. Moreover, PM and GM supervised the paper. SB reviewed the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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The Role of Primary Cilia in Thyroid Cancer: From Basic Research to Clinical Applications

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Primary cilia (PC) are microtubule-based organelles that are present on nearly all thyroid follicle cells and play an important role in physiological development and in maintaining the dynamic homeostasis of thyroid follicles. PC are generally lost in many thyroid cancers (TCs), and this loss has been linked to the malignant transformation of thyrocytes, which is regulated by PC-mediated signaling reciprocity between the stroma and cancer cells. Restoring PC on TC cells is a possible promising therapeutic strategy, and the therapeutic response and prognosis of TC are associated with the presence or absence of PC. This review mainly discusses the role of PC in the normal thyroid and TC as well as their potential clinical utility.

Keywords: primary cilium, thyroid cancer, cell cycle, ciliogenesis, therapeutic strategy

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INTRODUCTION

Primary cilia (PC) are solitary, nonmotile microtubule-based organelles that project from the cell surface and function similar to an antenna on the cell to sense and convey multiple extracellular signals (1). In thyroid follicular epithelial cells, PC protrude from the apical surface into the follicular luminal space, where they may sense the follicular luminal environment and transmit signals to follicular epithelial cells to maintain follicular homeostasis (2). Existing data have revealed that many signaling pathways important for development and disease, including the Hedgehog (Hh), Wnt, and platelet-derived growth factor (PDGF) pathways, are localized to PC (3). Therefore, a loss of PC is associated with the onset of malignancy in some human tumors.

Thyroid cancer (TC) is the most common endocrine cancer and has a rapidly increasing incidence but relatively stable mortality. The main histological subtypes of TC are papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), poorly differentiated thyroid cancer (PDTC), anaplastic thyroid cancer (ATC), and medullary thyroid cancer (MTC). The first four types originate from thyroid follicular epithelial cells, and MTC arises from thyroid parafollicular cells. PC is well preserved in PTC and FTC, and their frequency and length appear similar to those of normal thyroid follicles. Interestingly, defects in PC genesis have been observed in ATC (4). Additionally, oncogenic alterations, coupled to specific intracellular downstream signaling pathways, lead to the development of different subtypes of TC. PC as a mediator of these signaling pathways regulates TC development. Alteration in PC influences the communication

between TC cells and the tumor microenvironment, which in turn affected the therapeutic response and prognosis of TC.

In this review, we briefly describe the formation and structure of PC on thyroid follicular cells and explore the potential roles of PC in maintaining cellular homeostasis and promoting the progression of thyroid disease.

STRUCTURE OF PC IN THE NORMAL HUMAN THYROID GLAND

PC consist of the basal body, transition zone and axoneme. The basal body derived from the mother centriole of the centrosome that is composed of nine microtubule triplets (5). The axoneme is constructed from nine parallel microtubule doublets protruding from the mother centriole, which anchors the PC within the plasma membrane. The transition zone is a specialized ciliary domain that connects the basal body and axoneme backbone, which is localized toward the membrane in a Y-shaped arrangement known as the "ciliary gate." This gate separates proteins inside the PC from proteins in the cytoplasm and limits extracellular signal transduction.

In human thyroid follicular cells, PC are present in a ring-shaped 9 + 0 axonemal configuration, and the microtubules and diameters of PC steadily decrease toward the distal end of the cilia while becoming broader closer to the base of the cilium. Dynein arms and central pair microtubules are absent (6). Therefore, PC are non-motile structures. The length of PC ranges between 5.0 and 10.7 μm, and the mean length is 7.3 ± 1.2 μm (7). Almost all human thyroid follicular cells displays at least one PC that protrudes from the apical surface into the follicular lumen, and occasionally, the presence of two PC in a V-shaped distribution has been observed on adjacent cells. The PC of thyroid follicular cells taking advantage of their ideal localization coupled to specific intracellular downstream signaling pathways regulates thyroid development.

ABNORMAL LENGTH AND FREQUENCY OF PC IN TC

The frequency and length of PC often change when they respond to diverse stimuli from both inside and outside of thyrocytes. In TC, the distribution and frequency of PC are aberrantly changed, the number of thyrocytes exhibiting one or more cilia steadily decreases from ordinary PTC to FTC, and FTC, PDTC, and ATC usually lack cilia (8). However, conventional PTC and follicular variants of PTC display well-expressed PC, the length of PC is strikingly increased, and the frequency of PC seems to be unchanged compared with normal thyroid glands, only oncocytic variants of PTC have a decreased frequency and length of PC (9). More importantly, these variations of PC appear to be associated with the progression and prognosis of TC. Moreover, the pathogenicity of PC variations in the experimental animals of thyroid gland was also observed. A mouse model lacking PC showed normal folliculogenesis and hormonogenesis at ages of less than 7 weeks. After that, thyroid follicles became irregularly dilated and destroyed and displayed malignant properties (papillary or solid proliferative nodules), papillary or solid hyperplastic nodules were considered PDTC (2), and other types of TC were not observed.

In cell level, the frequency of PC is not different between human normal thyroid follicular cells and PTC cell lines, but the frequency of PC is significantly reduced in ATC cell lines (2). The loss of PC on ATC cells was associated with TC tumorigenesis and progression (10). However, a study from Junguee Lee et al. demonstrated that loss of PC in PTCs results in increased apoptosis, and it is associated with reduced tumor aggressiveness (4). In this context, the loss of PC appears to select TC cells with more malignant features. These discrepant conclusions require further confirmation due to the use of different research models.

In conclusion, some possible mechanisms for the association with PC abnormal changes and TC tumorigenesis have been proposed (**Figure 1**). So the loss of PC is at least partially associated with the tumorigenesis and progression of TC.

PC MEDIATE HH, MAPK, AND PI3K SIGNALING IN TC

Some signaling pathways related to the occurrence, development, invasion, and metastasis of TC are mediated by PC, and the receptors for these signaling pathways are often localized to PC. In the presence of Hh, Ptch1 is transported out of the cilium, and Smo is transported into the cilium, where it promotes the formation of the activator form of Gli. Gli protein levels increase in the cilium, and Gli proteins are then transported out of the cilium and into the nucleus, where they activate Hh target genes. In the absence of Hh, Ptch1 is localized to the ciliary membrane, Smo is excluded from the cilium, and Gli is converted to its repressor form (11). At an early phase of TC development, TC stroma secreted Hh ligand mediates tumor-stroma interaction and Hh pathway are aberrantly activated, which supports TC cell invasion, migration, and growth in non-adherent conditions (12). Additionally, PC provide a spatially diverse platform for mediating interaction between the stroma and cancer cells, so PC genesis defects may disturb this interaction mediating Hh pathway aberrant activation. These data show that the PC-mediated Hh pathway participates in TC tumorigenesis.

On the other hand, PC-mediated growth factor binding to RTKs triggers the MAPK and PI3K-AKT cascades to regulate TC cell proliferation, and elevated RTK activity also promotes *RET/RAS/BRAF* mutations (13, 14). Moreover, oncogenic RAS/BRAF/MEK pathway influences Hh pathway activation in TC cell, generating a ligand independent non-canonical mechanism of activation.

Since PC are the carrier of these signaling proteins, and they integrate Hh and RTK signaling crosstalk to coordinate thyroid hormone synthesis and development. Changes of PC directly affect these related pathways, which is associated with tumorigenesis and TC development.

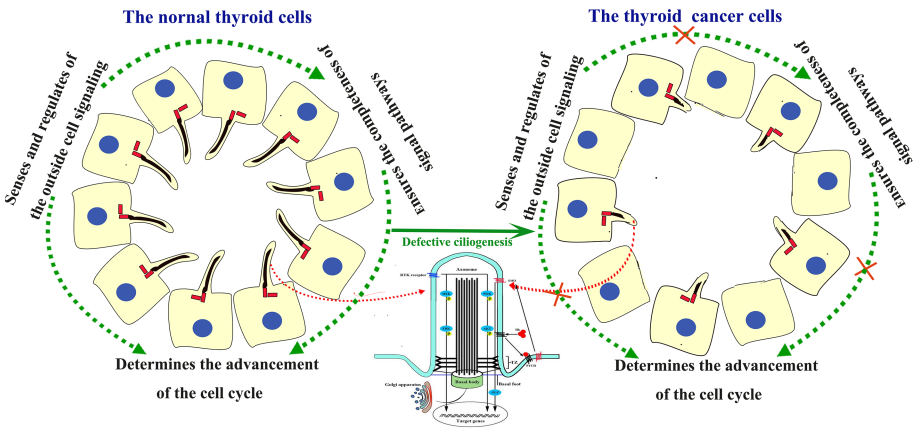

FIGURE 1 | Structure of the PC and signaling pathways involving cilia in the human thyroid gland and TC. In normal thyroid gland cells, the PC acts as the sensor and regulator of the outside cell signaling. In TC cells, the PC was absent. Absence of the PC block the signal from outside and neighbors, and disturb the signal pathways inside cells, thus lead to uncontrolled cell cycle process and tumorigenesis.

PC PROTRUSION AND RESORPTION AFFECT TC CELL FATE

PC protrusion and resorption are tightly coordinated with the cell cycle; in addition, the coupling of both ciliogenesis and the cell cycle depends on the centrosome, and the role of the centrosome shifts from cell division to ciliogenesis (15). Cells become quiescent, or proliferating cells enter G1 phase (16), the centrioles from the centrosome begin to migrate to the cell surface and subsequently form the basal body, and assembly of PC is initiated; the PC then extend from the basal body into the follicular lumen. In dividing cells, PC are resorbed before S phase or during G2 phase (17). Subsequently, the centrosome forms the mitotic spindle, and the cell enters mitosis. After mitosis, centrosomes are again available to assemble PC, either in G0 or in early G1 phase (18). Utrilla confirmed this process in thyroid cells (7). These findings suggest that ciliogenesis and the cell cycle appear to regulate each other *via* mutual crosstalk.

PC PROTEINS AFFECT TC PROGRESSION

PC proteins related to Aurora kinase A, NIMA-related kinases (Nek), Polo-like kinase (Plk1), and spermatogenesis-associated protein 4 (Spata4) have been identified as critical for the regulation of both cilia and the cell cycle. Aurora kinase A, which is localized to the centrosome and radial microtubules in PC (19), is highly or weakly expressed in TC tissues and cell lines, and this abnormal expression induces thyrocyte malignant transformation. Moreover, activation of Aurora kinase A in late G2 phase triggers spindle assembly and PC disassembly, and it becomes inactivated at the completion of mitosis. Overexpression of Aurora kinase A generally implies a poor prognosis and is a new molecular target in TC therapy (20). Nek, which is localized to PC and centrosomes, inhibits ciliogenesis and promotes spindle assembly, and it may contribute to the coordination between ciliogenesis and cell cycle progression (21). Overexpression of Nek 1 in classical and follicular variants of PTC has a high specificity and sensitivity and is often related to aggressiveness. Therefore, Nek 1 may contribute to the identification of malignant features during TC diagnosis (22). Plk1, which is localized to the transition zone of PC, rapidly evokes PC resorption and regulates cell cycle progression at the G2-M phase transition (23, 24). Plk1 expression is only occasionally observed in normal thyrocytes, but overexpression of Plk1 is more frequently detected in smaller PTCs, microcarcinomas, and incidental carcinomas. Overexpression of Plk1 may be an early event in PTC progression (25). Spata4 is a spermatogenesis-associated protein associated with thyroid hormone in fish (26), and spata4 knockdown leads to an arrest of cells in S phase and causes a decrease in the number of cilia in human retinal epithelial cells (27). The orderly regulation of these PC proteins between ciliogenesis and the cell cycle determines the survival of TC cells.

We focused on how PC proteins are linked to cell cycle progression and ciliogenesis in TC. Although the mechanisms by which TC cells lose their cilia are unknown, Aurora kinase A, Nek, Plk1, and Spata4 are associated with cilium disassembly and cell cycle regulation.

DRUGS WITH EFFECTS ON PC

The effects of drugs on PC in human cell lines and experimental animal models of TC have not been identified, but U0126 inhibits the elongation of cilia, ganetespib causes the loss of PC in experimental animal models of other diseases (28, 29).

Additionally, the administration of U0126 and ganetespib effectively inhibits the proliferation of different histological types of TC cells (30, 31). Evidence for the association of U0126 and ganetespib with PC should be investigated in TC.

Drugs targeting PC have been assessed in patients with TC, and the findings from clinical trials show that doxorubicin, paclitaxel and docetaxel have modest antitumor activity in patients with advanced, nonmedullary TC (32). Carboplatin is recommended as a treatment for ATC in clinical practice guidelines in oncology (33). The administration of these drugs for TC may provide a clinical benefit in adjuvant settings. Doxorubicin induces cilium formation in breast fibroblasts (34), paclitaxel causes cilium elongation in the quail oviduct (35), and docetaxel decreases cilia numbers in olfactory cells (36). In addition, carboplatin induces PC disassembly in sensory cells (37) and has been used to treat TC. Some RTK inhibitors have been recently approved for use in clinical practice, namely sorafenib and lenvatinib, have been approved for differentiated thyroid cancer (DTC) and PDTC, and vandetanib and cabozantinib have been approved for MTC (38), but their effect on PC has not been reported.

Anticancer drugs with documented effects on PC have been proposed. These drugs induce PC disassembly, restore ciliogenesis, lengthen PC, and prevent the accumulation of Smo in PC. They have been assessed in TC in preclinical and clinical studies, considering these factors will formulate rational therapeutic strategies for TC.

IS RESTORING CILIA A PROMISING THERAPEUTIC STRATEGY IN TC CELLS?

Although controversy exists regarding whether the length of PC is modified in PTC, the frequency of PC in TC cells is usually decreased. In addition, ATC and MTC exhibit loss of PC, and dysfunction of PC in TC cells is associated with tumorigenesis and malignancy. More importantly, in Hh pathway-dependent cancers, there is loss of PC as a mechanism of resistance to Smo inhibitors (39). Accordingly, restoring cilia may be a promising therapeutic strategy in TC (40).

There have been some pre-clinical examples of restoration of PC as a therapeutic target, such as restoration of PC by HDAC inhibitors in cholangiocarcinoma reduce cholangiocarcinoma cell growth (41–43). In addition, the use of HDAC inhibitors to treat TC has been well described, although they failed to trigger a major response against PTC, ATC, and MTC in clinical trials, HDAC inhibitors produced encouraging results in PTC, ATC, and MTC cell lines (44–46). Compounds (gefitinib and dexamethasone) that are able to restore cilia have been verified in cell lines of multiple human cancers (such as pancreatic, kidney, breast, and lung cancers) (47). Gefitinib shows limited effectiveness in patients with advanced TC (48), and dexamethasone exerts antiproliferative effects on a human MTC cell line (49).

It is hard to say that the restoring of cilia by those drugs was a primary effect or a secondary effect. However, several drugs with effects on PC have been tested in different human cell lines of TC, which indicate a possible application of these drugs in clinical studies. Future studies are required to evaluate the effects of these drugs on PC of TC.

CONCLUSIONS AND FUTURE PROSPECTS

Based on the findings described above, PC play a role in sustaining thyroid follicular cell polarity, differentiation, and proliferation and feature signaling pathways associated with TC. PC undergo cycles of assembly and disassembly that control TC cell survival, and PC loss in TC cells is usually linked to tumor aggressiveness in the clinic. PC in thyroid cells appear to function as tumor suppressors, and treatments that restore PC may be a potentially promising therapeutic strategy for TC. Preclinical and clinical studies will be required to test the roles of PC in the occurrence and progression of TC.

In the future, the relationship between ciliogenesis and pathological differentiation of TC tissue should receive increasing attention. Studies of the effects of potential oncogenic genetic mutations on PC formation may further reveal the mechanisms underlying TC pathogenesis, and powerful omics analyses have the potential to provide key insights into the role of PC in TC. Therefore, larger studies are required to assess whether the absence of PC drives TC transformation or is a secondary effect of tumorigenesis, which will be critical when considering ciliotherapy as a potential strategy.

AUTHOR CONTRIBUTIONS

C-XM, X-NM, Y-dL, and S-BF conceived the study and wrote the paper. All authors contributed to the article and approved the submitted version.

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Extent of Surgery and the Prognosis of Unilateral Papillary Thyroid Microcarcinoma

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It remains controversial whether patients with papillary thyroid microcarcinoma (PTMC) benefit from total thyroidectomy (TT) or thyroid lobectomy (TL). We aimed to investigate the impact of extent of surgery on the prognosis of patients with unilateral PTMC. Patients were obtained from the Surveillance, Epidemiology, and End Results database from 2004 to 2015. Cancer-specific survival (CSS) and overall survival (OS) were evaluated by Cox regression and Kaplan–Meier curves with propensity score matching. Of 31167 PTMC patients enrolled, 22.2% and 77.8% of which underwent TL and TT, respectively. Patients with TT were more likely to be younger, females, present tumors of multifocality, extrathyroidal extension, cervical lymph node metastasis (CLNM), distant metastasis, and receive radioactive iodine (RAI) compared with those receiving TL. The multivariate Cox regression model showed that TT was not associated with an improved CSS and OS compared with TL with hazard ratio (HR) and 95% confidence interval (CI) of 0.53 (0.25-1.12) and 0.86 (0.72-1.04), respectively. In addition, the Kaplan–Meier curves further confirmed the similar survival between TL and TT after propensity score matching. The subgroup analysis showed that TT was associated with better CSS for patients < 55 years, those with tumors of gross extrathyroidal extension, CLNM (N1b), and cases not receiving RAI with HR 95% CI of 0.13 (0.02-0.81), 0.12 (0.02-0.66), 0.11 (0.02-0.64) and 0.36 (0.13-0.90), respectively. TT predicted a trend of better OS for patients with N1b and distant metastasis after adjustment. In addition, TT was associated with better CSS than TL for patients with risk factors like N1b combined with gross extrathyroidal extension, and/or multifocality after matching. In conclusion, TL may be enough for low-risk PTMC patients. TT may improve the prognosis of unilateral PTMC patients with 2 or more risk clinicopathologic factors like CLNM, multifocality, extrathyroidal extension and a younger age compared with TL.

Keywords: papillary thyroid microcarcinoma, total thyroidectomy, lobectomy, prognosis, propensity score matching

INTRODUCTION

Papillary thyroid microcarcinoma (PTMC) is defined as a papillary thyroid carcinoma (PTC) ≤ 1.0 cm in diameter, which has been increasingly detected in recent decades across the world with the popularity of ultrasound and fine-needle aspiration cytology (1, 2). PTMC is generally an indolent disease with excellent prognosis (3). Recently, active surveillance has been recommended as an alternative approach for low-risk PTMC according to the American Thyroid Association (ATA) guidelines (4).

Thyroid lobectomy (TL) alone was sufficient for unifocal and intrathyroidal PTMC in the absence of clinically detectable cervical nodal metastasis (4). TL may be appropriate for PTMC patients when no evidence of extrathyroidal disease was found (5). However, The rate of pathological cervical lymph node metastasis (CLNM) was 48.0% for PTC (6), and 42.4% for PTMC when prophylactic central lymph node dissection was performed (7), which does pose a risk for local recurrence (8). Postoperative local lymph node recurrence was associated with reoperations and the consequently excess morbidity from reoperations (9).

Besides TL, total thyroidectomy (TT) is commonly performed on unilateral PTMC patients. TT was associated with more complications like hypocalcemia, recurrent laryngeal nerve injury, and high-dose hormone replacement throughout one's life. However, it remains unclear whether patients with unilateral PTMC benefit from TL or TT (10). We expect that PTMC patients with risk clinicopathologic features may benefit from more aggressive surgical treatment. However, it remains unclear due to the excellent prognosis of PTMC and limited qualified cases. In this study, we aim to compared the prognosis between patients receiving TT and TL with a large sample size.

PATIENTS AND METHODS

Ethics Statement

The patients were enrolled from the Surveillance, Epidemiology, and End Results (SEER) program (https://seer.cancer.gov/) from 2004 to 2015. This study was deemed exempt by the institutional review board approval for the deidentified patient information.

Study Population

Medical records were drawn using the International Classification of Diseases for Oncology code site C73.9. Histotype of PTC with values of 8050 (papillary carcinoma), 8260 (papillary adenocarcinoma), 8340 (papillary carcinoma, follicular variant), and 8341 (papillary microcarcinoma) were included. Values of 8050, 8260 and 8341 were classified as PTC, and 8340 for follicular variant PTC (FVPTC). The demographic, clinicopathologic characteristics, and treatment along with survival data were recorded. Race was categorized into white, black, and other (American Indian/AK Native, Asian/Pacific Islander). Extrathyroidal extension was divided into minimal extension and gross extension. Cervical lymph node metastasis was determined by derived AJCC N stage, 6th ed (2004+), which includes N0 (without nodal metastasis) and N1 (N1a, N1b, and N1). N1a means nodal metastasis to level VI (pretracheal, paratracheal, and prelaryngeal/Delphian lymph nodes). N1b represents nodal metastasis to unilateral, bilateral, or contralateral cervical or cervical or superior mediastinal lymph nodes. N1 (NOS) means regional nodal metastasis. Patients with multiple primary tumors, tumors in both sides of thyroid lobes, non-positive histology, age < 18 years, unknown or indefinite data of interest were excluded. Only patients with unilateral PTMC were included.

Statistical Analysis

Age and year of diagnosis were expressed as median (upper and lower quartile) for its skewed distribution and analyzed with Mann-Whitney *U* test. Category variables were presented as percentage and analyzed using chi-square test. The cancer specific survival (CSS) and overall survival (OS) were estimated by the Kaplan–Meier curves and compared by log-rank tests. The Cox proportional hazard model was established to estimate risk factors for CSS and OS with hazard ratio (HR) and a 95% confidence interval (CI). Propensity score matching (PSM) was performed using R software (ver. 3.3.3, http://www.r-project.org/) of package 'MatchIt'. One-to-one matching with a caliper of 0.1 was used to balance demographic, pathologic and treatment covariates between TL and TT (11). The matched variables included age, year of diagnosis, sex (male *vs.* female), multifocality (solitary and multiple nodules), extrathyroidal extension (no *vs.* yes), cervical lymph node metastasis (no *vs.* yes), distant metastasis (no *vs.* yes), and radioactive iodine (RAI). Subgroup analyses stratified by clinicopathologic characteristics were performed (12). All statistical differences were set at a two-sided p value < 0.05. The other data were analyzed by Stata software (Stata/MP ver. 14.2, StataCorp., College Station, TX), and GraphPad Prism (ver 7.0, GraphPad Software, Inc).

RESULTS

Patient Characteristics

The flow chart of selection was shown in **Supplementary Figure 1**. Finally, a total of 31167 patients with unilateral PTMC were enrolled, including 6929 (22.2%) undergoing TL and 24238 (77.8%) undergoing TT (**Table 1**). The following characteristics of patients were more likely to present with TT compared with TL: younger age, later year of diagnosis, female sex, tumors of multifocality, extrathyroidal extension (minimal and gross extension), CLNM (N1a, N1b and N1), distant metastasis, and treatment with RAI (**Table 1**).

Predictors for CSS and OS of Patients With PTMC

Results showed that increasing age, gross extrathyroidal extension, N1a, N1b, distant metastasis, treatment with RAI, and chemotherapy were associated with compromised CSS in PTMC patients with HR (95% CI) of 1.12 (1.09-1.14), 2.60 (1.22-5.56), 4.69 (1.92-11.5), 10.11 (4.64-22.03), 24.54 (11.17-53.89), 2.56 (1.25-5.25) and 14.10 (3.03-58.09) compared with the

TABLE 1 | The clinicopathologic features of PTMC patients treated with TL *versus* TT.

Variables	TL (*n* = 6929) *n* (%)	TT (*n* = 24238) *n* (%)	*P*-value
Age (year)	51 (41-61)	49 (40-58)	<0.001
<55	4179 (60.3)	15762 (65.0)	<0.001
≥55	2750 (39.7)	8476 (35.0)	
Year of diagnosis	2010 (2007-2013)	2011 (2008-2013)	<0.001
Sex			
Male	1322 (19.1)	4029 (16.6)	<0.001
Female	5607 (80.9)	20209 (83.4)	
Race			
White	5753 (83.0)	20316 (83.8)	0.221
Black	463 (6.7)	1591 (6.6)	
Other	713 (10.3)	2331 (9.6)	
Multifocality			
No	5705 (82.3)	14355 (59.2)	<0.001
Yes	1224 (17.7)	9883 (40.8)	
Extrathyroidal extension			
No	6805 (98.2)	22527 (92.9)	<0.001
Minimal extension	56 (0.8)	866 (3.6)	
Gross extension	68 (1.0)	845 (3.5)	
CLNM			
No	6814 (98.3)	20758 (85.6)	<0.001
N1a	70 (1.0)	1917 (7.9)	
N1b	22 (0.3)	1212 (5.0)	
N1 (NOS)	23 (0.3)	351 (1.4)	
Distant metastasis			
No	6923 (99.9)	24180 (99.8)	0.013
Yes	6 (0.1)	58 (0.2)	
Histotype			
PTC	4842 (69.9)	17171 (70.8)	0.121
FVPTC	2087 (30.1)	7067 (29.2)	
RAI			
No	6582 (95.0)	15931 (65.7)	<0.001
Yes	347 (5.0)	8307 (34.3)	
Chemotherapy			
No	6925 (99.9)	24215 (99.9)	0.354
Yes	4 (0.1)	23 (0.1)	

Age and year of diagnosis were expressed as median with interquartile, other variables were expressed as n (%). PTMC, papillary thyroid microcarcinoma; TL, Thyroid lobectomy; TT, total thyroidectomy; CLNM, cervical lymph node metastasis; PTC, papillary thyroid carcinoma; FVPTC, follicular variant papillary thyroid carcinoma; RAI, radioactive iodine.

corresponding counterparts in the multivariate Cox regression (**Table 2**). In addition, increasing age, male sex, black race, tumors of gross extrathyroidal extension, N1a, N1b, N1 (NOS), distant metastasis, and treatment with chemotherapy were associated with compromised OS in PTMC patients with HR (95% CI) of 1.10 (1.09-1.10), 1.68 (1.41-2.00), 1.99 (1.54-2.56), 1.67 (1.15-2.41), 1.29 (0.85-1.94), 2.40 (1.68-3.43), 6.19 (3.54-10.82), and 3.64 (1.12-11.80) relative to the corresponding groups after adjustment (**Table 2**).

In the univariate Cox regression analysis, TT was associated with improved OS compare with TL with HR (95% CI) of 0.74 (0.62-0.88) (**Supplementary Table 1**). In the multivariate Cox regression model, there was a trend toward a better prognosis in CSS and OS of TT over TL with HR (95% CI) of 0.53 (0.25-1.12) and 0.86 (0.72-1.04), respectively. However, the differences were not statistically different (**Table 2**).

Kaplan–Meier Curves Before and After PSM

Kaplan–Meier curves showed no differences in CSS between the TT and TL groups (**Figure 1A**). However, the median OS of TT was significantly longer than that of TL before matching (**Figure 1B**). After balancing the baseline characteristics between TL and TT, the differences between the two groups were significantly reduced (**Supplementary Figure 2**). The matched process yielded a total of 6929 paired cases. The differences in baseline covariates were well balanced after matching (**Supplementary Table 2**). However, there were no significant differences in CSS and OS between patients with TT and TL (**Figures 2A, B**).

We expected that patients with risk clinicopathologic characteristics may benefit from TT. Patients with tumors of multifocality can gain improved CSS from TT over TL (p = 0.049) after matching (**Figure 3A**). In addition, patients with tumors of extrathyroidal extension and CLNM showed marginally improved CSS from TT over TL (P = 0.050 and 0.054, respectively) (**Figures 3B, C**).

Subgroup Analysis by Multivariate Cox Regression Analysis

In consideration of the tread toward improved prognosis from TT, we further performed subgroup analysis to identify those who might benefit from TT over TL. Compared with TL, TT was associated with improved CSS for patients < 55 years, those with

TABLE 2 | The predictors for CSS and OS of PTMC patients by multivariate Cox regression.

Category	Cancer specific survival		Overall survival	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (year)	1.12 (1.09-1.14)	<0.001	1.10 (1.09-1.10)	<0.001
Diagnosis year	0.83 (0.74-0.92)	0.001	0.95 (0.92-0.98)	0.002
Sex				
Female	Ref	0.889	Ref	<0.001
Male	1.04 (0.57-1.91)		1.68 (1.41-2.00)	
Race				
White	Ref		Ref	
Black	0.96 (0.23-4.00)	0.954	1.99 (1.54-2.56)	<0.001
Other	0.87 (0.35-2.18)	0.762	0.71 (0.51-0.98)	0.038
Multifocality				
No	Ref	0.332	Ref	0.351
Yes	0.75 (0.42-1.34)		0.92 (0.77-1.10)	
Extrathyroidal extension				
No	Ref		Ref	
Minimal extension	1.71 (0.58-5.07)	0.334	0.98 (0.56-1.71)	0.930
Gross extension	2.60 (1.22-5.56)	0.014	1.67 (1.15-2.41)	0.006
CLNM				
No	Ref		Ref	
N1a	4.69 (1.92-11.5)	0.001	1.29 (0.85-1.94)	0.228
N1b	10.11 (4.64-22.03)	<0.001	2.40 (1.68-3.43)	<0.001
N1 (NOS)	2.54 (0.55-11.70)	0.231	2.17 (1.22-3.87)	0.009
Distant metastasis				
No	Ref	<0.001	Ref	<0.001
Yes	24.54 (11.17-53.89)		6.19 (3.54-10.82)	
Histotype				
PTC	Ref	0.736	Ref	0.127
FVPTC	0.90 (0.49-1.67)		1.14 (0.96-1.35)	
RAI				
No	Ref	0.011	Ref	0.116
Yes	2.56 (1.25-5.25)		0.83 (0.67-1.04)	
Chemotherapy				
No	Ref	0.001	Ref	0.031
Yes	14.10 (3.03-65.70)		3.64 (1.12-11.80)	
Surgery				
TL	Ref	0.095	Ref	0.116
TT	0.53 (0.25-1.12)		0.86 (0.72-1.04)	

PTMC, papillary thyroid microcarcinoma; HR (95% CI), hazard ratio (95% confidence interval); CLNM, cervical lymph node metastasis; PTC, papillary thyroid carcinoma; FVPTC, follicular variant papillary thyroid carcinoma; TL, thyroid lobectomy; TT, total thyroidectomy; RAI, radioactive iodine.

tumors of gross extrathyroidal extension, N1b, and those not receiving RAI with HR (95% CI) of 0.13 (0.02-0.81), 0.12 (0.02-0.66), 0.11 (0.02-0.64), 0.34 (0.13-0.90), respectively. In addition, TT was associated with marginally improved OS for patients with N1b,distant metastasis, and non-RAI with HR (95% CI) of 0.30 (0.08-1.08) and 0.06 (0.001-2.94), and 0.84 (0.70-1.02), respectively (**Table 3**).

The Prognosis of PTMC With Two or More Risk Clinicopathologic Factors

As shown above, PTMC patients with one risk factor gained survival benefit from TT. It's interesting to investigate the prognosis of patients with multiple risk factors. In the multivariate Cox regression model, TT can significantly improve CSS of patients with tumors of multifocality & gross extrathyroidal extension, multifocality & N1b, multifocality & extrathyroidal extension & CLNM, extrathyroidal extension & CLNM, or CLNM & age < 55 years compared with TL with HR (95% CI) of 0.13 (0.02-0.82), 0.04 (0.004-0.32), 0.08 (0.01-0.69), 0.13 (0.02-0.73), and 0.10 (0.01-0.82), respectively. Of patients with tumors of multifocality & N1b, TT was associated with improved OS compared with TL with HR (95% CI) of 0.18 (0.04-0.96) (**Table 4**).

Consistently, in the matched cohort, TT was associated with improved CSS of patients with tumors of multifocality & CLNM (**Figure 4A**), extrathyroidal extension & CLNM (**Figure 4B**), or multifocality & extrathyroidal extension & CLNM (**Figure 4C**) (Log-rank $P < 0.05$ for all).

DISCUSSION

In the present study, we investigated the extent of surgery and the prognosis of patient with unilateral PTMC. TT was not associated with improved CSS and OS compared with TL in the total population after PSM. However, TT predicted better CSS for patients < 55 years, those with tumors of gross extrathyroidal extension, N1b, and not receiving RAI. After balancing the covariates between the TL and TT groups, we found that TT can improve CSS for patients with tumors of

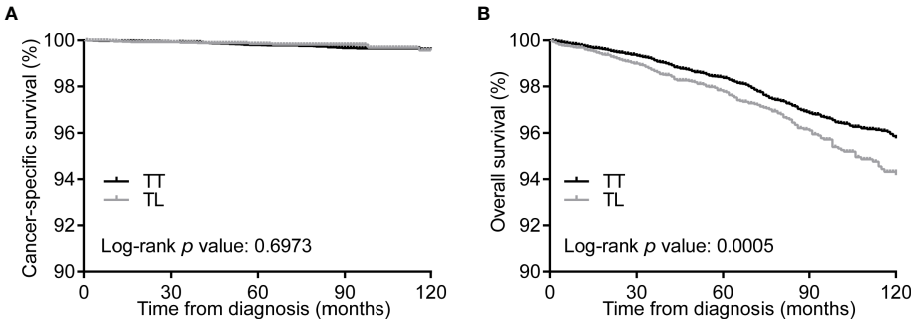

FIGURE 1 | Kaplan-Meier curves of CSS **(A)** and OS **(B)** of PTMC patients undergoing TT *versus* TL before propensity score matching. CSS, cancer specific survival; OS, overall survival; PTMC, papillary thyroid microcarcinoma; TT, total thyroidectomy; TL, thyroid lobectomy.

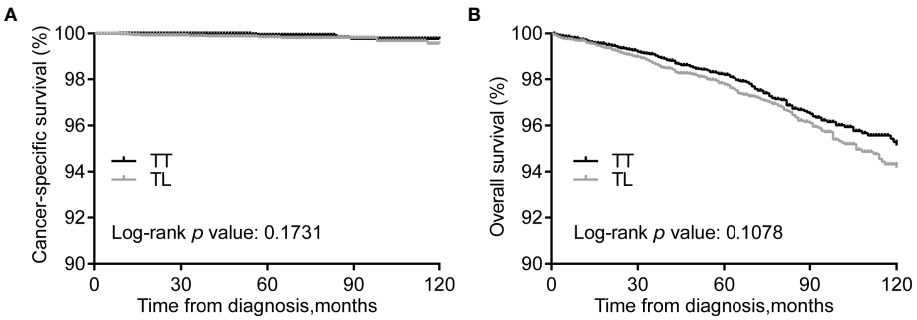

FIGURE 2 | Kaplan-Meier curves of CSS **(A)** and OS **(B)** of PTMC patients undergoing TT *versus* TL after propensity score matching. CSS, cancer specific survival; OS, overall survival; PTMC, papillary thyroid microcarcinoma; TT, total thyroidectomy; TL, thyroid lobectomy.

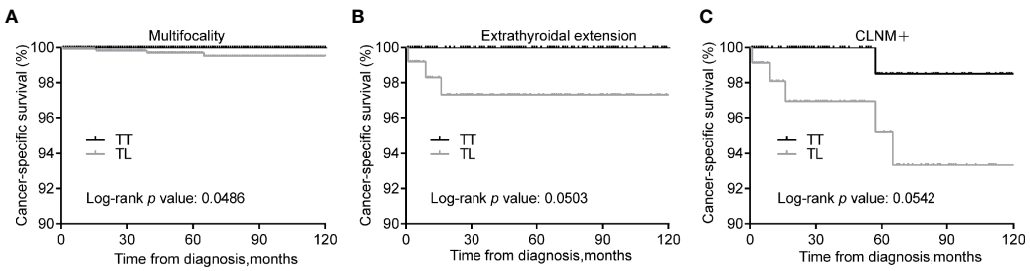

FIGURE 3 | Kaplan-Meier curves of CSS of PTMC underwent TT *versus* TL among patients with multifocality **(A)**, extrathyroidal extension **(B)**, and CLNM **(C)** after propensity score matching. CSS, cancer specific survival; PTMC, papillary thyroid microcarcinoma; TT, total thyroidectomy; TL, thyroid lobectomy; CLNM, cervical lymph node metastasis.

multifocality, extrathyroidal extension, and CLNM compared with TL. Importantly, we found that patient with multiple risk clinicopathologic factors like CLNM, extrathyroidal extension, and multifocality were more likely to benefit from TT over TL.

The optimal extent of surgery for PTMC has been controversial. Most of the single institutional studies and meta-analysis failed to discern any differences in prognosis of patients with PTMC underwent TT or TL, which might result from the indolent behavior of PTMC, the short-term follow-up duration, and the relatively small sample size (13–15). There may be a trend toward lower mortality rate of TT than TL. However, the limited number of mortality events prevented establishing a definitive correlation between the extent of surgery and prognosis of patients with PTMC (14).

Previous studies found that the recurrence and survival were not statistically different between PTMC patients undergoing TT and TL by the National Cancer Data Base (1985–1998) (16). However, some variables such as multifocality, extrathyroidal

TABLE 3 | The subgroup analysis of the prognosis of PTMC patients treated with TT *versus* TL by multivariate Cox regression.

Category	Cancer specific survival		Overall survival	
	HR (95% CI)	***P*-value**	**HR (95% CI)**	***P*-value**
Age < 55 years				
TL	Ref	0.028	Ref	0.327
TT	0.13 (0.02-0.81)		0.82 (0.56-1.22)	
Gross extrathyroidal extension				
TL	Ref	0.014	Ref	0.106
TT	0.12 (0.02-0.66)		0.42 (0.15-1.20)	
CLNM (N1b)				
TL	Ref	0.014	Ref	0.065
TT	0.11 (0.02-0.64)		0.30 (0.08-1.08)	
Distant metastasis				
TL	Ref	0.297	Ref	0.061
TT	0.12 (0.002-6.22)		0.06 (0.001-2.94)	
Non-RAI				
TL	Ref	0.030	Ref	0.077
TT	0.34 (0.13-0.90)		0.84 (0.70-1.02)	

The multivariate Cox regression was adjusted for all the other covariates.
PTMC, papillary thyroid microcarcinoma; TT, total thyroidectomy; TL, thyroid lobectomy; HR (95% CI), hazard ratio (95% confidence interval); CLNM, cervical lymph node metastasis; RAI, radiotherapy.

TABLE 4 | The prognosis of PTMC patients according to the extent of surgery by subgroup analysis stratified by clinicopathologic factors by multivariate Cox regression.

Category	Cancer specific survival		Overall survival	
	HR (95% CI)	***P*-value**	**HR (95% CI)**	***P*-value**
Multifocality & gross extrathyroidal extension				
TL (*n* = 30)	Ref	0.030	Ref	0.136
TT (*n* = 526)	0.13 (0.02-0.82)		0.36 (0.10-1.37)	
Multifocality & CLNM (N1b)				
TL (*n* = 13)	Ref	0.003	Ref	0.045
TT (*n* = 754)	0.04 (0.004-0.32)		0.18 (0.04-0.96)	
Multifocality & extrathyroidal extension & CLNM				
TL (*n* = 8)	Ref	0.022	Ref	0.100
TT (*n* = 521)	0.08 (0.01-0.69)		0.22 (0.04-1.34)	
Extrathyroidal extension & CLNM				
TL (*n* = 21)	Ref	0.021	Ref	0.061
TT (*n* = 734)	0.13 (0.02-0.73)		0.26 (0.06-1.06)	
Age < 55 years & CLNM				
TL (*n* = 88)	Ref	<0.001	Ref	0.189
TT (*n* = 2675)	0.10 (0.01-0.82)		0.39 (0.09-1.60)	

The multivariate Cox regression was adjusted for all the other covariates in each category.
PTMC, papillary thyroid microcarcinoma; TT, total thyroidectomy; TL, thyroid lobectomy; HR (95% CI), hazard ratio (95% confidence interval); CLNM, cervical lymph node metastasis.

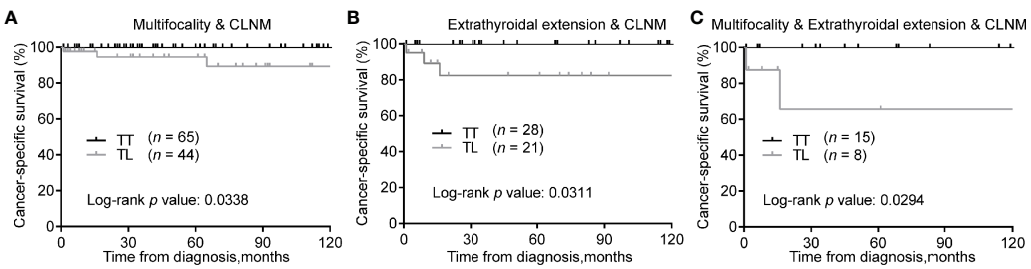

FIGURE 4 | Kaplan-Meier curves of CSS of PTMC underwent TT *versus* TL among patients with multifocality & CLNM **(A)**, extrathyroidal extension & CLNM **(B)**, and multifocality & extrathyroidal extension & CLNM **(C)** after propensity score matching. CSS, cancer specific survival; PTMC, papillary thyroid microcarcinoma; TT, total thyroidectomy; TL, thyroid lobectomy; CLNM, cervical lymph node metastasis.

extension, pathological type, chemotherapy and RAI were missing or incomplete, and subgroup analysis were not performed. Lee et al. did not find any significant differences in the risk of death and locoregional recurrence between TT and TL in a matched cohort with 506 paired PTMC patients from 1986 to 2006 (15). Some single institutional studies found that the recurrence rate of patients undergoing TT was similar with those undergoing TL (3, 15). However, a recent meta-analysis showed that TT was associated with lower recurrence rates than TL (14, 17). For PTMC of multifocality, TL may result in a higher rate of thyroid bed and lymph node recurrence than TT (18). The low recurrence rate of TT might result from a more radical resection of the contralateral thyroid lobe and cervical lymph nodes (5, 19), while transient and permanent hypoparathyroidism was higher for TT than TL (5).

We found that patients undergoing TT were more likely to be younger, and present with tumors of multifocality, extrathyroidal extension, CLNM, and distant metastasis. These features were associated with nodal metastasis, tumor recurrence, and unfavorable prognosis of patients (7, 8, 20). We found that patients undergoing TT showed a trend toward improved CSS and OS compared with patients receiving TL. Relative treatment effects may vary according to the heterogeneous study population, certain high-risk subsets may benefit most from the treatment (21). We thus expected that a subpopulation of PTMC patients may benefit from TT.

The subgroup analysis revealed that patients < 55 years, those with tumors of gross extrathyroidal extension had improved CSS from TT compared with TL. Younger age and extrathyroidal extension were risk factors for CLNM (7, 8). Of note, TT failed to improve the prognoses of patients with minimal extrathyroidal extension and N1a. For patients with N1b, TT significantly improved CSS of patients compared with TL. These findings highlighted the importance of detecting nodal metastasis in the lateral neck. The preferred hierarchy of treatment for PTC with distant metastasis includes TT, nodal dissection, postoperative RAI therapy, and thyrotropin inhibition therapy. As for refractory disease, kinase inhibitors were recommended (4). We found that patients with distant metastasis may benefit from TT over TL. However, the number of patients with distant metastasis was relatively small and the result needs to be validated in the following studies. The present study found that patients not receiving postoperative RAI might benefit from TT with improved CSS compared with TL. TT might facilitate to eradicate disease recurrence of the contralateral lobe, and potential metastatic lymph nodes, which was beneficial for those not receiving RAI.

We did not observe any differences in the prognosis between TT and TL for PTMC patient with multifocality in the multivariate model, which was consistent with a previous study (22). However, after PSM between TL and TT, TT showed improved CSS for multifocal tumor in unilateral PTMC patients compared with TL, while the OS was similar. TL may be a safe treatment approach for selected unilateral PTMC patients with multifocal and node-negative tumors (3), which was consistent with our results. The prognostic significance of multifocal tumors in PTC remains controversial (23). However, when tumors of multifocality together with CLNM, extrathyroidal extension, or both were presented, TT was associated with improved CSS compared with TL. Therefore, a more radical surgical treatment may be considered for tumors with more risk factors.

The study should be interpreted in consideration of several limitations. First, selection bias was inevitable even though we adjusted the covariates and performed PSM analysis. In addition, data like disease recurrence and thyroid-stimulating hormone inhibition were not available in the database. Additionally, the results of subgroup analysis should be interpreted with caution for the limited samples and events evaluated. Last but not least, the occult thyroid cancer in the contralateral thyroid lobe and the exact number of metastatic lymph nodes may also influence the outcome. The strengths of this study lie in the latest and largest samples to date, comprehensive variables adjusted, and subgroup analysis together with PSM analysis.

In conclusion, we for the first time investigated the association between the extent of thyroid surgery and prognosis of unilateral PTMC patients. The present results suggested that there was no statistical difference in prognosis between TL and TT for unilateral PTMC patients. TL is appropriate for unilateral PTMC without risk factors. However, PTMC patients with risk features such as a younger age, multifocality, gross extrathyroidal extension, and N1b may benefit from TT over TL, especially for those with multiple risk factors. These findings may have an impact on the treatment of unilateral PTMC. Large sample size and long-term follow-up studies are warranted to validate the present findings.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**/Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

HZ: conception, data acquisition. HZ and LC: data analysis and drafting the article. HZ and LC: revised it critically for important intellectual content. HZ: investigation, project administration, and supervision. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.655608/full#supplementary-material

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REVIEW
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Update of Radiofrequency Ablation for Treating Benign and Malignant Thyroid Nodules. The Future Is Now

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Thermal and chemical ablation are minimally invasive procedures that avoid removal of the thyroid gland and target symptomatic nodules directly. Internationally, Radiofrequency ablation (RFA) is among one of the most widely used thermal ablative techniques, and is gaining traction in North America. Surgery remains the standard of care for most thyroid cancer, and in the right clinical setting, Active Surveillance (AS) can be a reasonable option for low risk disease. Minimally invasive techniques have emerged as an alternative option for patients deemed high risk for surgery, or for those patients who wish to receive a more active treatment approach compared to AS. Herein, we review the literature on the safety and efficacy of RFA for treating benign non-functioning thyroid nodules, autonomously functioning thyroid nodules, primary small low risk thyroid cancer (namely papillary thyroid cancer) as well as recurrent thyroid cancer.

Keywords: autonomously functioning thyroid nodules, benign thyroid nodules, recurrent thyroid cancer, thermal ablation, radiofrequency ablation, primary thyroid cancer

INTRODUCTION

New technologies are available to safely ablate thyroid nodules without removal of the gland itself. Laser Ablation (LA) and Radiofrequency ablation (RFA) are the most widely used thermal ablative treatments for solid nodules. High-Intensity Focused Ultrasound (HIFU) as well as Microwave Ablation (MA) are two other modalities that fall under the umbrella of thermal ablative techniques

and are gaining traction as longer-term data emerges (1, 2). Although, chemical ablation *via* percutaneous ethanol injection is cheaper and requires less costly equipment, it is considered the treatment of choice for cystic nodules and tends to be less effective for solid nodules (3–5). Each minimally invasive technique has its subtle differences and nuances, but they all share ease of use, effectiveness and safety.

RFA is an outpatient image-guided thermal ablative procedure that is a potential alternative to surgery for treating symptomatic benign nodules (6–39). This approach eliminates the need for general anesthesia, incision, or removal of the thyroid gland, making it an attractive nonsurgical option. RFA has been offered in certain centers since 2000 for the treatment of primary and metastatic liver, lung, bone and kidney tumors and to ablate aberrant conduction pathways in the heart (40–45). Since the Korean guidelines for the ablation of the thyroid with RFA in 2009 and its revised versions in 2012 and in 2017, various international societies have established their own recommendations (17, 22, 36, 46–48). The American Head and Neck Society has initiated a global collaborative consensus guideline which will be published soon.

With the use of local anesthesia and/or sedation, the RFA probe is introduced into the midline of the anterior neck at the level of the isthmus (called the trans-isthmic approach) and the nodule is targeted using the "moving shot technique" where the operator moves the RFA needle back and forth in the nodule while visualizing the hyperechoic changes in the tissue during ultrasound guidance (18, 45, 48). The heat from the electrode tip causes tissue necrosis and fibrosis by introducing a high-frequency alternating current, which raises tissue temperatures to 60 to 100 degrees Celsius (19). Lidocaine injection can be used prior to ablation to anesthetize the thyroid capsule to hydro-dissect, which provides greater distance from the active RFA needle tip and vital surrounding structures and also provides a heat sink around the nodule to prevent injury to these structures. A solution of 5% dextrose (D5W) may be used to hydro-dissect and to create an aqueous barrier for areas that should remain sensate, such as the trachea, nerves or other vital structures.

Once the tissue is ablated, there are obvious sonographic features available to guide the operator in real-time. These include hyperechoic signals and 'micro-bubbling' as well as an increase in the generator impedance as the tissue stiffens, indicating coagulative necrosis (17, 23, 49). The operator approaches the tissue as "subunits" from the deepest to the most superficial portion of the nodule. Care is taken to avoid extending the probe beyond the posterior or lateral thyroid capsule to avoid injury to nearby structures, particularly within the "danger triangle" where the recurrent laryngeal nerve is tethered to the trachea close to the posterior-medial aspect of the thyroid (49). Immediate shrinkage of the nodule is appreciated as well as continued progressive shrinkage over a period of months to years. In benign nodules, the volume of the nodule is expected to typically decrease between 50-90%, which can vary due to both operator and tumor factors (7, 20, 21, 30, 34, 35).

Prior to RFA of a benign nodule, at least two benign biopsy results should be done (either FNA or core biopsy) (17, 22, 36, 46–48). For AFTN, the societies differ slightly in their recommendations, but generally one FNA result can suffice if the ultrasound features are consistent with benign ultrasound results (spongiform, cystic, encapsulated, no evidence of extrathyroidal extension or lymphadenopathy) (17, 22, 36, 46–48). If there are worrisome or suspicious sonographic features, but the biopsy result is benign, the FNA should be repeated. Furthermore, if the volume of the thyroid nodule is not completely delineated on ultrasound or doubt exists regarding the extent of the nodule, a CT scan of the neck can help to determine if and how much retrosternal extension is present and whether the patient is an appropriate candidate for RFA.

BENIGN NON-FUNCTIONING THYROID NODULES

Many reports have established the short-term efficacy (6-12 months) and safety of RFA for reducing the volume of benign nonfunctioning nodules (50-85%) (18, 20, 21, 26, 30, 50–52). Long-term data are starting to emerge with recommendations for the number of treatments required to maintain volume reduction of benign nodules. Lim et al. demonstrated up to 93.4% volume reduction rate at 4 years post RFA treatment for benign nonfunctioning nodules as well as improved local symptoms and cosmesis (6). In their series, only 5.6% of the nodules demonstrated regrowth, which tended to be at the peripheral margin (6).

Several papers have shown that RFA has the best reduction rate for smaller nodules (volume < 10 mL), with maintained success for up to 2 years (6, 9, 10, 28–31, 53). Deandra et al. found in their long-term series of 215 patients, that the best response was seen in nodules less than 10 mL, showing an 81% volume reduction rate at 5 years after one RFA procedure (31). Progressive shrinkage over time was noted through the five-year follow-up as well as maintenance of improved compressive symptoms and cosmesis (31). Similarly, in a retrospective multi-institutional trial, Bernardi et al. demonstrated in a series of 216 patients a long-lasting effect of a single RFA treatment with a median volume reduction rate of 77% after 5 years (7). Only 12% underwent a second treatment, and regrowth was noted in 20% of treated patients (7). As opposed to smaller nodules, larger benign nodules tend to require more than one treatment. The variability between the results in the literature can likely be attributed to the heterogeneity of nodule size treated across studies, the energy delivered to nodules, the technical expertise of the operator and the learning curve associated with achieving ablation of the nodule margin (54). These important studies provide a framework for counselling patients regarding the long-term effectiveness of RFA as well as the importance of discussing the potential need for retreatment to maintain the desired effect.

AUTONOMOUSLY FUNCTIONING THYROID NODULES

Benign autonomously functioning thyroid nodules (AFTN) tend to be more variable than for benign non-functioning nodules

likely due to the increased vascularity present in AFTNs and the increased possibility for leaving viable remnant tissue at the margin. The literature demonstrates that achieving euthyroidism post RFA is more consistent when the pretreatment volume of the AFTN is small and more homogeneous ultrasonographically. Cesareo et al. compared the reduction in medium sized nodules (>12 mL, n=14) versus smaller sized nodules (< 12 mL, n=15), and found that euthyroidism was achieved in 86% of small nodules versus 45% in medium size nodules (10). Similarly, Cappelli et al. report a volume reduction rate of 73% with TSH normalization in 94% of 17 patients treated with RFA with nodules of an average 7 mL (11). After one RFA session, Cervelli et al. demonstrated a volume reduction rate of 76.4% +/- 16.9% with a 91% (20/22 patients) TSH normalization at 12-month follow-up in 25 AFTNs that were homogenous in volume and of smaller pretreatment size (55). However, Cesareo et al. found only modest results (57%) of TSH normalization post RFA treatment in a systematic review covering 8 studies on 205 AFTNs (12).

When the volume of a nodule is reduced by > 80%, Cesareo et al. found a greater chance for thyroid function normalization and symptom resolution (10, 15). In a multicenter trial, Sung et al. demonstrated this concept with a significant reduction in nodule volume from a pretreatment mean nodule volume of 18.5 ± 30.1 mL to 4.5 ± 9.8 mL ($p < 0.001$) post RFA treatment in a series of 23 AFTNs (44 patients, 23 with AFTN and 21 with a pretoxic nodule) (13). This significant change in nodule volume and thus vascularity resulted in a significant improvement of triiodothyronine, free thyroxine, and thyrotropin in the final follow up (19.9 ± 12.6 months) (13). TSH levels normalized in 81.8% of the study patients without the development of hypothyroidism post RFA. These findings are congruent with those of Baek et al. in a series of 9 patients (4 AFTN and 5 pretoxic nodules) (14).

Even though several studies support targeting smaller AFTNs to ensure success, the correlation between pretreatment nodule size and treatment outcome of AFTNs is still controversial. A recent meta-analysis highlights the efficacy of RFA treatment on TSH normalization (71.2% of patients) and a volume reduction rate of 69.4% at a mean follow-up of 12.8 months. However, in a subgroup analysis, there was no significant difference in TSH normalization or volume reduction rate (VRR) between small or large nodules when divided into groups of 15mL, 18mL and 20 mL (56). Similarly, Bernardi et al. in their series of 30 patients found no significant correlation between baseline volume and treatment response after RFA (15). Thus, it seems that the rate of reduction of the nodule post RFA plays a more important role in the success of achieving euthyroidism rather than the pretreatment nodule size. However, smaller AFTNs seem to be overall most effectively treated and therefore maybe best for the novice operator to initiate RFA treatment. Due to the variability of results with AFTNs, the international guidelines are more cautious when recommending RFA as curative for AFTNs (17, 22, 36, 46, 47). Accumulating evidence from prospective studies will help in the pretreatment counseling of this patient population.

SURGERY *VERSUS* RADIOFREQUENCY ABLATION FOR BENIGN NODULES

For symptomatic benign thyroid nodules, an open thyroidectomy is the standard of care. However, surgery may not always be the best choice, particularly for older patients who are not ideal surgical candidates or for patients who do not wish to risk hypothyroidism, scarring, hoarseness or surgical recovery time especially for benign disease. In a meta-analysis comparing thermal ablation with conventional thyroidectomy, thermal ablation was safer and had significantly lower incidence of hoarseness, hypothyroidism and postoperative pain ($p<0.05$) (57). After thermal ablation, patients had significantly better postoperative cosmetic outcome and shorter hospitalization time compared with conventional thyroidectomy ($p<0.05$) (57). However, in terms of symptom improvement, both options were equally favorable, with no statistical difference between thermal ablation and conventional thyroidectomy ($p=0.58$), thus showing RFA as a promising treatment option (57).

Other trials, such as the one by Jin et al. found that thermal ablation is superior to conventional thyroidectomy for benign nodules in terms of patient satisfaction, post-operative quality of life, and shorter hospital stay (58). Although both options were found to have similar complication rates, thermal ablation can take longer to achieve the desired volume reduction than a definitive surgical approach. In a telephone survey of 126 patients treated with RFA and 84 treated with surgery for a single benign thyroid nodule, Bernardi et al. compared patient satisfaction (8). Overall, 94% of patients that underwent surgery were fully satisfied with the outcome and resolution of their nodule related symptoms or hyperthyroidism. For nonfunctioning thyroid nodules, RFA was as effective as surgery in patient satisfaction with resolution of nodule-related symptoms. On the other hand, for AFTN, surgery relieved 95.8% of patient's hyperthyroid symptoms whereas RFA was effective in 52.9% patients resulting in withdrawal of their antithyroid drugs. In terms of complications, however, RFA was superior to surgery and no cases of hypothyroidism were identified, whereas 37.5% of patients after surgery required thyroid replacement therapy (8).

Other studies have compared the complication rates of surgery and RFA for benign nodules and found a higher rate of complications in the surgical group compared to RFA (9). Che et al. compared complications after open surgery versus RFA and found that 71.5% versus 0% had hypothyroidism, 3% versus 0.5% had recurrent laryngeal nerve palsy, and 3% versus 0% had hypoparathyroidism (9). For the accurate calculation of post-surgical recurrent laryngeal nerve injury rates both the American Academy of Otolaryngology and the American Thyroid Association have recommended laryngeal exam postop given the known disparity between vocal cord paralysis and vocal symptoms (59, 60). When we compare surgery to RFA complications moving forward we will need to be steadfast in ensuring that laryngeal exam is preformed uniformly in both treatment groups.

One of the key benefits of RFA compared with surgery is the reduced chance of causing post-treatment hypothyroidism. Surgery involves removing the gland with the nodule and the normal

parenchyma, whereas RFA targets the nodule alone, leaving the normal tissue protected and preserving thyroid function (9). Whether RFA changes the tissue planes, making it more difficult to resect surgically at a later date remains to be determined.

Nevertheless, some limitations of RFA should be mentioned. RFA is not suitable for all types of thyroid nodules, particularly for large benign nodules when patients expect rapid results, substernal nodules, and deeply located nodules. Additionally, there are still some nodules with incomplete response and local regrowth in the follow-up period, which require repeat ablation or surgery, and some nodules which shrink slowly but fail to completely recede (61).

COMBINED THERMAL ABLATION AND RADIOACTIVE IODINE TREATMENT

The guidelines are more cautious when recommending treatment of large benign goiters, and more than one treatment is expected for larger sized nodules (17, 22, 36, 46, 47). The use of one treatment modality exclusively may only partially give a desired effect for benign large toxic nodules, however evidence for combining thermal ablation with radioactive iodine (RAI) has been promising. Even though surgery is considered the first line treatment, and current international guidelines do not endorse RFA as primary treatment for large toxic goiters, a combined approach may be an innovative safe solution for reducing the dose of RAI and rapidly controlling local symptoms of hyperthyroidism and compression without undergoing surgical removal of the gland (62). In a pilot study by Chianelli et al., combined LA therapy with RAI treatment induced faster and greater improvement of local and systemic symptoms compared to RAI alone (63). Korkusuz et al. found similar success with combined therapy using MA with RAI for Graves' disease and large toxic nodular goiters (64). A significant reduction in size was appreciated with restoration of euthyroidism and a reduced dose of RAI (64). Even though MA and LA have proven effective, RFA is more widely disseminated and is better studied to date (62).

Thermoablative procedures can be useful in older patients for providing relief at a rate that may be faster than RAI alone. There are limitations for each treatment modality, however when RAI and RFA are combined, the volume of the nodule is reduced more rapidly. Mader et al., used combined therapy (RAI and RFA) for large goiters which led to a significant reduction in thyroid volume ($p<0.05$) compared to the control group (RAI mono therapy) (62). However, by three months post treatment the volume reduction did not differ between the two groups ($p > 0.05$). All patients became euthyroid after treatment and no complications or discomfort were noted (62). With future work, combined therapy may limit the dose of RAI and provide fast relief in patients who refuse or have contraindications to surgery.

MALIGNANT THYROID NODULES

Recent studies have demonstrated the efficacy and safety of thermal ablation for low-risk papillary thyroid microcarcinoma (PTMC) (65–76) as well as for recurrent thyroid cancer where the risks of surgery outweigh the benefits or in patients who refuse repeat surgery (77–90). However, it should also be recognized that RFA for low-risk PTMC must be considered in the appropriate context, as many studies demonstrate excellent outcomes and minimal growth with simple active surveillance in this patient population. Preliminary work has not shown benefit for poorly differentiated aggressive tumors such as anaplastic carcinoma (69). For medullary thyroid cancer (MTC), surgery remains the treatment of choice. Few case reports have demonstrated RFA to be a safe and effective option for early MTC in patient's ineligible for surgery (91) or for patients with a regional recurrence after surgical resection of their MTC (92). However, the data is somewhat limited for MTC and remains controversial.

Careful evaluation of the desired nodule is required before ablation to ensure a successful outcome for the patient and to avoid delay for possible surgery. The main indications include: a) cytopathology confirmed papillary thyroid carcinoma (PTC) without evidence of aggressiveness b) single PTC without extrathyroidal extension c) no metastatic tumors at the time of treatment and d) ineligibility for surgery (78). The operator should note key features during evaluation, such as capsule invasion or lymph node metastases, and whether an aggressive variant of PTC is present. These features should prompt surgery instead of RFA. Currently, the Italian society does not recommend RFA for first line treatment of primary thyroid cancer, however emerging evidence has shown benefits, safety and efficacy for treatment of low-risk tumors (46).

RFA FOR PAPILLARY MICROCARCINOMA (T1AN0M0)

Detection of smaller thyroid cancers, namely PTMCs, is increasing in part because of increased medical imaging and accounts for 50% of papillary thyroid cancers (93). The World Health Organization (WHO) defines PTMC as a small papillary thyroid cancer or < 10 mm in greatest dimension (93). The American Joint Committee on Cancer (AJCC) TNM system classified T1 category (T1: ≤ 2 cm)into T1a: ≤ 1 cm and T1b: ≤ 2 cm (94). Although the 2015 American Thyroid Association guidelines (ATA) do not recommend biopsy of nodules less than 1 cm unless high risk features are present, these small indolent cancers are often incidentally detected (59). On one hand, while surgery is an option, it is an invasive approach for removal of such small indolent lesions that likely would otherwise not have caused significant morbidity. On the other end of the treatment spectrum, several landmark trials have shown no increase in mortality of patients undergoing active surveillance (AS) for small papillary thyroid cancers compared to those who underwent immediate surgery with 10 years of follow up (95, 96). Thus, the optimal management for PTMC is controversial. Some patients may be reluctant to undergo AS with a proven diagnosis of cancer, regardless of its indolence. The unnecessary anxiety of having a cancer diagnosis may make

some patients feel more comfortable actively treating their cancer without surgery or observation. Mounting evidence has established short term safety and efficacy of thermal ablation for PTMC (65–73). In addition to surgery and AS, thermal ablative techniques, such as RFA may bridge the gap in treatment options for patients wishing to have their PTMC managed in a minimally invasive way.

Several trials have demonstrated promising results for treating primary PTMC with RFA. Ding et al. used RFA to treat 38 PTMC in 37 patients with a low power setting of 20 W (65). All treated nodules achieved complete ablation, no complications occurred, and no hypothyroidism was encountered. After 12 months post treatment 37 of the 38 nodules were completely resolved with no evidence of nodule recurrence in 37 patients (65). Similarly, Zhang et al. demonstrated safety and efficacy in RFA-treated PTMCs over a 18-month follow-up (66). After treating the lesion(s) with 3-5W of power, a significant volume reduction rate was noted within the first 6 months follow up ($p<0.01$) but not after the 12 months follow up (66). Of the 98 nodules (92 patients), 10 resolved after six months, and 23 resolved in 12 months. All patients post RFA demonstrated no evidence of residual tumor on ultrasound or histological pathology after US guided biopsy (66). Again, no major complications were noted.

In a meta-analysis examining the efficacy and safety of all ablation techniques for PTMC, RFA showed the highest mean volume reduction rate (99.3%) compared to other thermal ablation techniques such as MA (95.3%) and LA (88.6%) ($p<0.001$) (75). Although significant heterogeneity between studies is noted, the pooled proportions of complete disappearance of PTMC was 57.6% (95% CI:35.4-79.8) and recurrence was 0.4% (95% CI:0-1.1) (75). Furthermore, the pooled proportions of overall and major complications for all thermal approaches were 3.2% (95% CI:1.1-5.2) and 0.7% (95% CI:0-1.5) demonstrating the safety of these techniques for PTMC (75).

A recent meta-analysis of 12 studies on the efficacy and safety of thermal ablation by RFA, MA or LA included 1,187 patients with 1,284 papillary microcarcinomas. All modalities induced reduction in nodule volume. MW achieved the highest standard mean deviation (-3.82; 95% CI -7.02; - 0.63) than RFA (-1.35; 95% CI -1.62; -1.09) and LA (-1.80; 95% CI -2.75; -0.85), but the difference was not statistically significant. Complete disappearance pooled proportion was 76.2%, 62.9% and 57.3% after RFA, MW and LA treatments, respectively. There was a lower proportion of recurrence after RFA (0.01%) than after MA (0.85%) and LA (1.87%). However, the differences were not statistically significant. The rates of complications observed were also low and similar between the compared techniques (97).

Zhang et al. compared RFA with surgery for patients with a low-risk PTMC, and followed them for five years (67). After 5-year follow-up, RFA was not found to be inferior to surgery with respect to oncological efficacy. Only one patient in the RFA group developed a new lesion (1 of 94) (1.1%) arising in the remaining ipsilateral lobe and none of the RFA group developed lymph node metastases (67). When compared with RFA, surgery took longer, had a longer hospitalization time, and was more costly ($p<0.001$). The surgical group had a lower thyroid-related quality of life, as well as more complications (2.5% recurrent laryngeal nerve palsy, 1.3% hypoparathyroidism) compared to neither of these complications in the RFA group ($p=0.095$) (67).

Yan et al. examined the long-term oncological efficacy of RFA in 414 PTMC patients after 42.15 ± 11.88 (range 24 – 69 months) months (76). After RFA, 366 out of 414 tumors (88.41%) completely disappeared, with a volume reduction rate of 98.81 ± 6.41% demonstrating long-term efficacy in this large cohort (76). The incidence of lymph node metastases post RFA was 0.97% (ipsilateral neck in three patients, and one in the central compartment), all of which underwent additional RFA with complete disappearance of the node during follow-up. Recurrent PTMC was found in 10 patients (2.42%); seven were in the contralateral lobe and three in the ipsilateral lobe, which were successfully treated with a second RFA procedure. The mean time of recurrent PTMC development was 27.60 ± 12.71 months (range, 6 – 48 months) (76). Similar to other trials, Yan et al. found no delayed or immediate complications.

As the field of minimally invasive techniques continue to grow, more long-term data will emerge and may substantiate the oncologic effectiveness of thermal ablation for primary microcarcinoma.

RFA FOR PAPILLARY THYROID CANCER (T1BN0M0)

Little evidence exists for thermal ablation of T1bN0M0 cancers, particularly with RFA, compared with T1aN0M0 PTC. The distinction between T1a and T1b is minimal, and the prognosis does not differ significantly between these two subdivisions as demonstrated by previous studies (98, 99). Xiao et al. compared surgery and RFA in patients with T1bN0M0 PTC (91 patients in each group) and found no significant difference between the two groups in local tumor progression and complications (74). In the RFA group, four patients had local tumor progression (4.4%, three persistent PTC and one develop lymph node metastases). Whereas in the surgery group, two patients (2.2%) developed lymph node metastases, and no new or persistent PTC was noted. When the complication rates were compared, the surgery group had four patients with permanent hypoparathyroidism (4.4%) after total thyroidectomy, meanwhile the RFA group developed no major complications, and only two patients experienced moderate pain (74). Even though the mean follow-up time was 25 months, the results are promising. In future studies comparison should be made between RFA treated patients and unilateral thyroid surgery as opposed to total thyroidectomy.

RFA FOR FOLLICULAR THYROID NEOPLASM

RFA treatment for follicular neoplasm is more controversial than PTC since surgical resection is required to exclude the presence

of vascular or capsular invasion to definitively diagnose whether the nodules is a follicular adenoma compared with a carcinoma. However, a recent 5-year study including 10 patients with follicular neoplasm < 2 cm in size found on thyroid biopsy reported that RFA is safe and effective in the short-term for such cases. Ha et al. demonstrate a significant reduction in the mean volume (99.5 ± 1.0%) of lesions, with eight ablated lesions (8/10, 80%) disappearing completely after single treatment on follow-up and no recurrences noted (range: 60-76 months) (100). Further large-scale trials are required to confirm effective oncological control, as it is unclear if sonographically invisible carcinomas still have metastatic potential.

RECURRENT THYROID CANCER

While the mortality rate for well-differentiated thyroid cancer tends to be favorable, the risk of recurrent PTC can range from 20 – 59% depending on patient and tumor risk factors (78). The mainstay of recurrent thyroid cancer is surgery, followed by RAI therapy and suppressive thyroid hormone replacement treatment, external beam radiotherapy and/or chemotherapy (59). Revision surgery for recurrent disease is not without its challenges due to scarring in the tissue bed, which can make identification and preservation of the RLN and parathyroid glands difficult. Various thermal ablative techniques (RFA, LA or MA) and chemical ablative approaches (ethanol ablation) can be used as an alternative method in patients deemed to be at high surgical risk, those in whom the surgeon deems non-operable or for those refusing to undergo repeat surgery. In each situation, a tailored and multi-disciplinary approach is imperative, as recurrent carcinomas can present in myriad presentations.

In patients treated with lobectomy, total thyroidectomy, and/or neck dissection as their primary surgery, Choi et al. examined patients with local recurrences and compared the efficacy and complication rate between the RFA group and repeat surgery group (77). After propensity score adjustment, the recurrence-free survival rates were comparable between the two groups (p=0.2), including the decrease in the mean serum thyroglobulin levels post treatment (RFA p=0.891 and surgery p=0.963) (77). Additionally, overall complications were noted more frequently in the surgery group, compared with the RFA group, particularly for hypocalcemia (surgery n=27; RFA, n=7;p<0.001) (77), which was similar to the findings of Kim et al. (78).

Various studies have looked at possible parameters to measure the efficacy after RFA for recurrent PTC. Most of these studies have looked at the short-term results (over 6 months), leaving the long-term outcomes still to be determined. Jeong et al. reviewed the literature (79) and found the mean reduction in tumor volume ranging from 50.9% to 8.4% (80–82); with complete disappearance noted in 25-94% of cancers (80, 81, 83–85); therapeutic success rates in 75% - 97% (80, 83, 86, 87); with symptom improvement observed in 64% of patients (82); and a decrease in serum thyroglobulin concentration in most patients signifying therapeutic success (80, 82–84). One study on seventy-three patients reported the mean volume reduction rate of recurrent tumors to be 98.4% ± 6.2%, and the complete disappearance rate 86.1% (78). Furthermore, two meta-analyses including one hundred and eighty-nine patients (87) and two hundred and seventy patients (88) with a local recurrence of their thyroid cancer found a significant decrease in volume and largest diameter of the tumor as well as thyroglobulin level before and after RFA treatment (87, 88).

Longer term data are beginning to emerge, with promising results for treating locally recurrent PTC with US-guided RFA. Chung et al. found after a mean follow up of 80 ± 17.3 months (range, 60-114 months), in a series of twenty-nine patients (forty-six recurrent PTC), that the tumor volume decreased significantly (p <0.001) with a mean volume reduction of 99.5% ± 2.9% and significant thyroglobulin decrease (p<0.001), and complete tumor disappearance of 91.3% by the final evaluation with no delayed complications noted (89).

When recurrences in the central compartment occur, invasion of surrounding structures such as the trachea, esophagus, RLN, and parathyroid glands are more likely, making reoperation in a previously operated tissue bed with a recurrence very challenging. Chung et al. reviewed a series of patients with RFA-treated recurrent thyroid cancer in the central compartment after total thyroidectomy. The mean volume reduction rate was 81.2% ± 55.7%, and complete disappearance of the tumor 72% after a mean follow-up of 47.0 ± 35.4 months (90). The lower rate of tumor disappearance for RFA-treated recurrences in the central compartment can likely be explained by the narrow working space and important neighboring structures. Treatment efficacy was significantly higher for tumors that were not in contact with the trachea, and unsurprisingly, lowest for tumors invading the trachea (90). Thus, the long-term efficacy of RFA treated recurrent papillary thyroid cancer is promising in the right clinical situation. As we anticipate future guidelines in North America, RFA treatment may be indicated for early localized disease to prevent progression into the neighboring vital structures in patients who are poor operative candidates or have other reasons to avoid surgery.

COMPLICATIONS OF RFA

Though multiple studies have demonstrated that thermal ablation is a safe technique, there are some associated complications which are mostly minor. In a meta-analysis of 12 studies including RFA treated benign nodules with follow up for more than three years, Cho et al. found an overall complication rate of 4.6% and a major complication rate of 1.3% in the RFA group (34). In a systematic review including 2786 nodules (24 studies including both benign and recurrent thyroid cancers) the overall RFA complication rate was 2.38%, with 1.35% for major complications (35). On subgroup analysis, the overall and major complication rate was higher for recurrent malignant nodules (10.98%) compared with benign nodules (2.11%) (35).

TABLE 1 | Complications following RFA of benign thyroid nodules [from a systematic review of 3409 patients by Wang et al. (101)].

Complications	N° cases
Pain and sensation of heat	281
Voice changes	32
Hematoma/hemorrhage	31
Vasovagal reactions	19
Nodule rupture	14
Horner syndrome	14
Increase in blood pressure	12
Nausea/vomiting	11
Fever	11
Cough	10
Skin burn	6
Recurrent nerve injury	4
Hypothyroidism	3
Needle track seeding	2
Thyroiditis and thyrotoxicosis	1
Brachial plexus injury	1
Pseudocystic transformation	1

The most common minor complication include pain with an incidence that ranges from 2.6% to 17.5% in a systematic review that included 32 studies with 3409 patients (101). In a systematic review by Chung et al. transient voice change was one of the common minor complications post-RFA, with an incidence of 0.94% (21/2245 for benign nodules) compared with 7.95%, 14/176 for recurrent cancer (35). Skin burns, hematoma and transient thyroiditis can occur but less frequently (34, 35, 102) (**Table 1**).

Major complications such as permanent recurrent laryngeal nerve injury, nodule rupture, hematoma requiring surgical drainage, Horner's syndrome or injury to the adjacent esophagus or trachea are rare (39, 103).

CONCLUSION

Given the accumulating international evidence, we anticipate that RFA will one day become routinely used for many benign and malignant conditions of the thyroid. We still require large prospective trials to be performed in diverse populations and with longer term data to substantiate oncologic efficacy. For the novice operator, comfort and ease results from a solid working knowledge of ultrasound guided FNA of the thyroid. Once this has been established, smaller benign nodules may be targeted first before treatment of larger or malignant nodules. Until more robust efficacy data is available for malignant thyroid nodules, RFA for malignant thyroid nodules should ideally be performed in a setting of prospective data analysis and/or clinical trials.

AUTHOR'S NOTE

This paper was written by members and invitees of the International Head and Neck Scientific Group (www.IHNSG.com).

AUTHOR CONTRIBUTIONS

Equal contributions were made by RT and PP-A for the writing of the initial manuscript. JR contributed to the manuscript edits and final review. Members and invitees of the International Head and Neck Scientific Group (www.IHNSG.com) made contributions to the writing and editing of subsequent drafts of the manuscript. All authors contributed to the article and approved the submitted version.

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Distinguishing Benign and Malignant Thyroid Nodules and Identifying Lymph Node Metastasis in Papillary Thyroid Cancer by Plasma *N*-Glycomics

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Background: Biomarkers are needed for patient stratification between benign thyroid nodules (BTN) and thyroid cancer (TC) and identifying metastasis in TC. Though plasma *N*-glycome profiling has shown potential in the discovery of biomarkers and can provide new insight into the mechanisms involved, little is known about it in TC and BTN. Besides, several studies have indicated associations between abnormal glycosylation and TC. Here, we aimed to explore plasma protein *N*-glycome of a TC cohort with regard to their applicability to serve as biomarkers.

Methods: Plasma protein *N*-glycomes of TC, BTN, and matched healthy controls (HC) were obtained using a robust quantitative strategy based on MALDI-TOF MS and included linkage-specific sialylation information.

Results: Plasma *N*-glycans were found to differ between BTN, TC, and HC in main glycosylation features, namely complexity, galactosylation, fucosylation, and sialylation. Four altered glycan traits, which were consecutively decreased in BTN and TC, and classification models based on them showed high potential as biomarkers for discrimination between BTN and TC ("moderately accurate" to "accurate"). Additionally, strong associations were found between plasma *N*-glycans and lymph node metastasis in TC, which added the accuracy of predicting metastasis before surgery to the existing method.

Conclusions: We comprehensively evaluated the plasma *N*-glycomic changes in patients with TC or BTN for the first time. We determined several *N*-glycan biomarkers, some of them have potential in the differential diagnosis of TC, and the others can help to

stratify TC patients to low or high risk of lymph node metastasis. The findings enhanced the understanding of TC.

Keywords: plasma protein *N*-glycome, sialylation, fucosylation, biomarker, mass spectrometry, lymph node metastasis

INTRODUCTION

Thyroid nodules (TN) are the most common thyroid disease and its incidence has been increasing worldwide in recent years. Studies revealed a prevalence of 2 to 6% with neck palpation, 19 to 35% with sensitive imaging devices (such as ultrasound diagnostic systems) and 8 to 65% in autopsy data (1, 2). Although around 90% of TN are benign, in 10% of cases TN predispose to thyroid cancer (TC). For the patients with malignant TN, most of them require timely thyroidectomy or other treatment such as central cervical lymph node resection. In some cases, hemithyroidectomy/active surveillance could be pursued. For the patients with benign thyroid nodules (BTN), a large proportion of them only needs standardized and regular follow-up, except for some special cases (e.g., nodular goiter) requiring surgery (3–5). Therefore, in order to facilitate clinical decision-making, it is tremendously important to preoperatively distinguish between benign and malignant TN. Clinically, the preoperative diagnosis of benign or malignant TN is not always straightforward and lacks a standard test. The routine examination procedures usually rely on a combination of ultrasound and fine-needle aspiration (FNA) cytology. FNA cytology is always chosen to evaluate the malignant risk when the TN are suspected as malignancy by ultrasound (6). Nevertheless, cytological uncertainty is present in 20 to 30% of FNA samples (7), which are classified as indeterminate thyroid nodules (ITN). Most patients with ITN are referred to surgery. Nevertheless, more than half of the ITN are identified as BTN by postsurgical pathology (8). Consequently, more than half of the patients with ITN underwent unnecessary surgeries, which brings psychological burden and an overload of medical expenditure for the patients and results in lifetime thyroxine supplementation. Besides, FNA cytology is an invasive method and patients with ITN have to suffer from both mental and physical trauma. Therefore, more precise and non-invasive molecular methods are urgently needed to preoperatively identify benign or malignant TN. In addition, it's reported that 30–80% of TC can occur cervical lymph node metastasis, which leads to a 10–42% increase in recurrence rate and a corresponding increase in patient mortality (9). How to indicate whether a patient has lymph node metastatic cancer before surgery is another key problem in the clinic of TC (9, 10). At present, clinicians often need to judge based on personal experience (10). Non-invasive diagnostic biomarkers for stratifying TC patients (low or high risk of metastasis) preoperatively are of great importance to surgical decision-making and reducing the long-term recurrence rate of TC.

Glycosylation is the most prevalent posttranslational modification of proteins that can greatly affect the structural and functional properties of the proteins (11, 12). The modification has effects in many biological processes such as protein secretion, degradation, transport to receptor interaction, and modulation of the immune response (12, 13). Furthermore, it has been reported that glycosylation is involved in the pathophysiology of various major diseases including cancer (14, 15). Protein glycomic signatures can dramatically change due to pathologic conditions (16, 17) and it has been revealed that aberrant glycosylation may be a result of initial carcinogenic transformation (18, 19). In addition, researchers found that altered glycosylation promoted cancer immune suppression and metastasis (20). Investigation of glycosylation profiles in the context of cancers may provide insight into the mechanisms regarding tumor progression and metastasis and help develop novel methods for the detection and prediction of specific cancer types. For the past few years, serological glycomic profiling provides a new approach for the discovery of non-invasive biomarkers. The total plasma protein *N*-glycome has been increasingly reported to have great potential as biomarkers in a multitude of diseases, especially cancer (15, 21–24). Several studies have indicated associations between abnormal glycosylation and TC (25–27), which exemplified a biomarker potential of the altered glycans. Though blood-based biomarker tests may offer a non-invasive and cost-effective way to detect or predict the disease, little is known about the plasma *N*-glycosylation profiles in BTN and TC.

In the present study, we evaluated the plasma *N*-glycome features of three subgroups including malignant and benign TN and HC. As the functions of sialylation depend on the linkage type, the here employed workflow applied linkage-specific sialic acid derivatization with discrimination between α2,3- and α2,6-linked sialic acids on the released *N*-glycans from plasma, followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis and automated data processing (28, 29). We sought to reveal differences in the plasma *N*-glycome of TC, BTN, and HC and discover non-invasive glycan markers for differential diagnosis of malignant and benign TN and preoperatively stratifying TC patients (low or high risk of metastasis), as well as provide insight into the possible involvement of plasma *N*-glycans in the early oncogenic events and metastasis of TC.

Abbreviations: AUC, area under the curve; BTN, benign thyroid nodules; CV, coefficient of variance; EMT, epithelial-to-mesenchymal transition; FNA, fine-needle aspiration; HC, healthy controls; HILIC-SPE, hydrophilic interaction liquid chromatography solid-phase extraction; ITN, indeterminate thyroid nodules; MALDI-TOF-MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; PTC, papillary thyroid carcinoma; ROC, receiver-operator-characteristics; SD, standard deviation; TC, thyroid cancer; TN, thyroid nodules.

MATERIAL AND METHODS

Study Population and Sample Collection

Plasma samples obtained from 75 patients diagnosed with TC, 25 patients diagnosed with BTN, and 50 HC were consecutively

collected between June 2019 and November 2020 from the Peking Union Medical College Hospital (Beijing, China). The three subgroups were age- and sex-matched as far as possible. HC were defined by medical doctors according to eligibility criteria and they should have no history of systematic diseases, have normal thyroid ultrasound, normal thyroid function, and biochemical parameters. Patients with BTN or TC were diagnosed on the basis of ultrasound and FNA and were confirmed by surgical histopathology. Ultrasound was performed by the same group in the present study. We used the American College of Radiology Thyroid Imaging Reporting and Data System (TI-RADS) for ultrasound features of the nodules. The ultrasound features and more detailed information on the cohort are presented in **Table 1**. All patients with TC were clinically classified as papillary thyroid carcinoma (PTC). We obtained approval from the regional ethics committee of the Peking Union Medical College Hospital and informed written consents from all participants were acquired.

Plasma *N*-Glycome Analysis and MS Data Processing

N-Glycans were enzymatically released from plasma glycoproteins according to a previously reported protocol (28). Briefly, 5 µl of plasma from each sample was denatured by adding 10 µl of 2% SDS and incubation for 10 min at 60°C. The glycan release step was performed by the addition of 10 µl of 2.5 × PBS containing 2% Nonidet P-40 and 1 U PNGase F, followed by incubation for 16 h at 37°C. During the derivatization procedure, sialic acid residues at the nonreducing ends of the glycan were derivatized to stable end-products (α2,3-linked sialic acids were lactonized and α2,6-linked were ethyl-esterified), allowing mass-based differentiation of sialic-acid linkage variants. Briefly, 1 µl of the released plasma was added into 20 µl of derivatization reagent (250 mM HOBt and 250 mM EDC in ethanol) and incubated at 37°C for 60 min. Thereafter, glycans were purified by in-house developed cotton-based hydrophilic interaction liquid chromatography solid-phase extraction (HILIC-SPE) micro-tips as previously described (28, 30) and glycans were finally eluted into MQ water. The samples were analyzed by MALDI-TOF-MS as previously described with minor modification (24). Briefly, 1 µl of the eluted samples was mixed with 1 µl of matrix (5 mg/ml sDHB in 50% ACN with 1 mM NaOH) on a MALDI target plate and dried by air for 2 h. The measurement of the derivatized glycans was performed on a Bruker rapifleXtreme MALDI-TOF mass spectrometer fitted with a Smartbeam-3D laser in reflectron positive mode and commanded by the proprietary software flexControl 4.0 (Bruker Daltonics). Instrument calibration was achieved using the Bruker Peptide Calibration Standard II. The measurements were recorded in the *m/z* window of 1,000–5,000 with 5k laser shots in a random walking pattern of 100 shots per raster spot at the frequency of 5,000 Hz.

Raw MS data from all samples was processed at once using the same parameters. They were baseline-corrected with the TopHat method and smoothed with Savitzky Golay algorithm by flexAnalysis software and.xy files were exported for further processing. The.xy files were re-calibrated with the in-house developed software MassyTools (29) (version 0.1.8.1.2) using a selection of well-known high-intensity glycan signals distributed across the detected m/z range (minimum five calibrants at S/N >9, **Supplementary Table S1**). Plasma *N*-glycan profiles were obtained from all 75 TC, 25 BTN, 50 HC, 12 quality control standard samples, and five blanks, of which 161 profiles passed our quality criteria during the re-calibration (blanks and one standard sample was excluded due to low intensity). For the cohort, 131 peaks were manually assigned to glycan compositions using the GlycoPeakfinder tool of Glycoworkbench as well as previously confirmed glycan compositions (28, 31). Using the composition list, the intensities for the putative glycan structures were extracted as background-corrected area from the raw data with the software MassyTools. Further curation of the extracted data was done in Microsoft Excel. After further curation (S/N >9, ppm error <20, and QC score <25%), 96 glycan compositions out of the 131 compositions remained for quantitative analysis (**Supplementary Table S2**). At last, the sum of glycan areas per spectrum was re-scaled to 1 to evaluate relative intensities. In order to combine the effects of single glycans sharing similar structures

TABLE 1 | Clinicopathological characteristics of all participants by subgroup.

	TC	BTN	HC
N	75	25	50
age, mean (range)	41.85 (24–58)	45.92 (18–67)	40.10 (25–61)
gender, male (%)	25 (33.33%)	9 (36.00%)	17 (34.00%)
without lymph node metastasis, n (%)	37 (49.33%)	\	\
with lymph node metastasis, n (%)	38 (50.67%)	\	\
pathological types	PTC	\	\
size of the nodules (cm)			
length (median, IQR)	1.25 (0.93–1.63)	2.35 (0.70–3.55)	\
width (median, IQR)	1.00 (0.80–1.43)	2.10 (0.62–3.35)	\
height (median, IQR)	0.80 (0.65–1.08)	1.70 (0.61–2.33)	\
blurred margins, n (%)	35 (46.67%)	6 (24.00%)	\
irregular margins, n (%)	35 (46.67%)	6 (24.00%)	\
TI-RADS category, n			
2–3	1	11	****
4	27	8	****
5	47	4	****

The data for size, margins, and TI-RADS is the ultrasound features. TC, thyroid cancer; BTN, benign thyroid nodules; HC, healthy controls; PTC, papillary thyroid carcinoma; TI-RADS, American College of Radiology Thyroid Imaging Reporting and Data System.

and to study the general glycosylation features, such as the number of antennae of complex type *N*-glycans (CA), the level of bisection (B), fucosylation (F), galactosylation (G) and sialylation (S), 91 derived traits were calculated from the 96 directly detected glycan traits on the basis of their common structural features (32–34) (**Supplementary Table S3**).

Experimental Design and Statistical Analysis

All 150 cohort samples (75 TC + 25 BTN + 50 HC) and 17 quality control samples consisting of five blanks (water) and 12 plasma standards were randomly distributed over two 96-well sample plates and prepared and analyzed as described above. After the removal of low-quality spectra during quality control steps, the cohort data consisted of 100 (75 + 25) cases and 50 controls. The calculations of derived glycan traits were performed in RStudio. Data quality of the cohort was assessed by the 12 standard plasma samples, which were randomly distributed in the two plates and calculating the average value, standard deviation (SD), and the coefficient of variance (CV) for all directly detected and derived glycan traits (**Supplementary Table S4**).

Direct and derived glycan traits were compared between subgroups (TC *vs.* BTN, TC *vs.* HC, and BTN *vs.* HC) using the nonparametric Mann–Whitney–Wilcoxon test since data was non-normally distributed. Multiple testing correction was used to adjust the significance threshold (P = 0.05/91—the number of derived glycan traits). The associations of glycosylation with lymph node metastasis (categorical variables) of TC were explored by logistic regression in RStudio. Derived glycan traits resulting in statistically significant p-values were further evaluated by receiver-operator-characteristics (ROC) test to assess their specificity and sensitivity in diagnosis and prediction using GraphPad Prism 8. The area under the curve (AUC) of ROC was used to assess the predictive accuracy of the glycan traits. In addition, predictive models were built by combining the altered derived glycan traits between cases and controls through logistic regression analysis in SPSS (version 23).

RESULTS

Data Reliability

The plasma *N*-glycome of patients with malignant or benign TN and matched HC (**Table 1**) were analyzed by MALDI-TOF-MS. Ninety-six of the detected glycan compositions passed our quality criteria for quantification (**Supplementary Table S2**), which were grouped into 91 derived glycan traits based on structural features of glycans including the number of antennae (CA), fucosylation (F), bisection (B), galactosylation (G), sialylation (S), and linkage-specific sialylation (**Figure 1** and **Supplementary Table S3**). As described previously, derived traits reflect the biosynthetic pathways of glycans and could facilitate interpretation of the results and biological effects (28, 32). Additionally, derived glycan traits seem to have better technical robustness compared to directly detected glycan traits (35), which was also confirmed in the present study (**Supplemental Table S4**). Average intensity, standard deviation (SD), and the relative SD (CV) from the technical replicates of 11 (one was kept out due to low quality) plasma standard samples that were randomly distributed in the plates and measured together with the cohort samples demonstrated overall method repeatability on direct- and derived-trait level (**Supplemental Table S4**). The average CV of top-20 directly detected glycan traits and all 91 derived glycan traits was 5.26 and 2.54%, respectively (**Supplemental Table S4**). Raw data files of directly detected and derived glycan traits for all the samples measured in the present study were provided (**Supplemental Table S5**).

Identification of Plasma *N*-Glycome Alteration in TC and BTN

Multiple directly detected glycan traits were found differentially expressed between HC, BTN, and TC. Typical annotated MALDI-TOF-MS spectra of plasma *N*-glycomes from HC, BTN and TC were depicted in **Figure 2**, demonstrating differences in peak patterns between the three groups. As derived glycan traits have better technical robustness and could facilitate interpretation of the results and biological effects, subsequently we mainly focused on the derived glycan traits.

Plasma *N*-glycome in TC and BTN showed changes in antennarity (A) of complex type glycans compared with HC. A decrease in the antennarity was found: tetra-antennary *N*-glycans within complex type (CA4) were decreased in TC and BTN profiles than in HC samples (**Table 2** and **Supplemental Table S6**), with a concomitant increase in monoantennary *N*-glycan species (CA1; **Supplemental Table S6**). In addition, TC and BTN patients showed lower levels of fucosylation than HC, especially for poly-fucosylation (CFa and A2Fa, difucosylation), diantennary and tetra-antennary species (A2Fa, A4F, A4L0F, and A2LF; **Table 2**). In addition to fucosylation differences, TC and BTN patients displayed a higher galactosylation of tetra-antennary glycans (A4G) compared to HC (**Table 2**), which was mainly due to the increase of galactosylation of non-fucosylated tetra-antennary glycan species (A4F0G; **Table 2**). In contrast, TC and BTN had a lower galactosylation of fucosylated sialylated diantennary glycans (A2FSG) compared with HC (**Table 2**). Altered sialylation was also found in TC and BTN compared to HC. Generally, sialylation of diantennary glycans was significantly decreased and sialylation of tetra-antennary species was significantly increased in TC and BTN compared with HC (**Table 2**). For example, sialylation per antenna within tetra-antennary glycans (A4S) was higher in subjects with TC or BTN than HC (**Table 2**), which was mainly driven by the increase of sialylation of non-fucosylated tetra-antennary glycans (A4F0S; **Table 2**). Moreover, sialylation per galactose within non-fucosylated tetra-antennary glycans (A4F0GS) was higher in TC and BTN compared to HC (**Table 2**), but in fucosylated glycans (A4FGS) it was lower in TC and BTN compared with HC (**Table 2**). With regard to sialylation linkages, the changes of α2,6- and α2,3- linked sialylation within tetra-antennary species (A4E, A4L, A4F0E, A4F0L, A4FGE) were in accordance with the results of A4S, A4F0S, and A4FGS (**Table 2**). However, α2,3-linked sialylation within diantennary glycans (A2L, A2FL) showed significant decreases in TC and BTN compared to HC (**Table 2**).

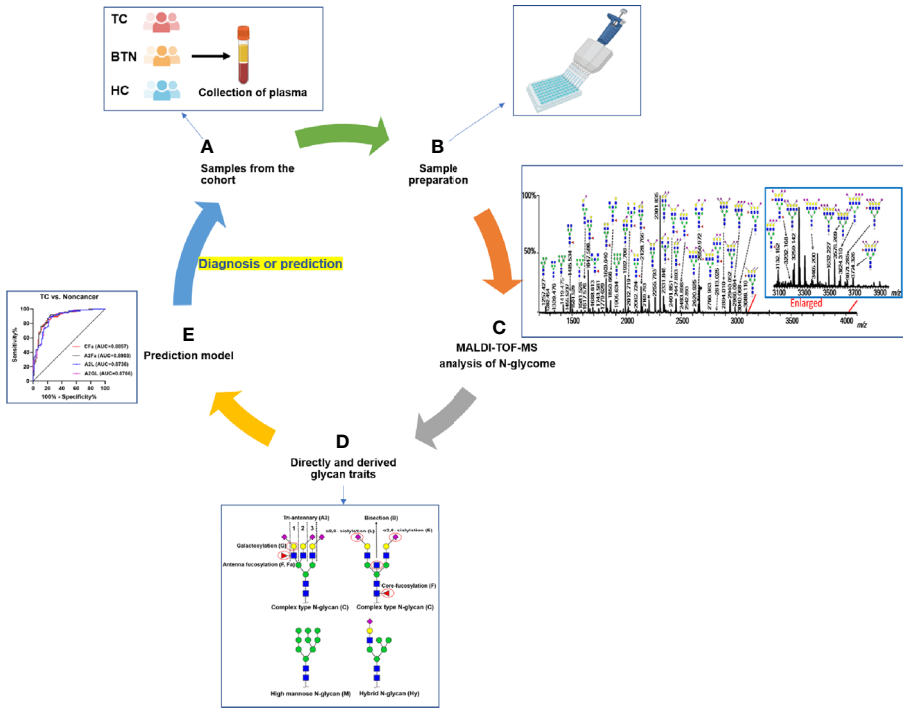

FIGURE 1 | Workflow of plasma *N*-glycome analysis of TC, BTN, and HC. **(A)** Collection of plasma samples from diseases and controls; **(B)** High-throughput sample preparation including glycan release, derivatization, and enrichment; **(C)** MALDI-TOF-MS analysis of plasma *N*-glycome and data preprocessing and annotation; **(D)** Calculation of derived glycan traits; **(E)** Construction of prediction models and ROC curve analysis to test their performance. TC, thyroid cancer; BTN, benign thyroid nodules; HC, healthy controls.

We did not find differences of bisection (B) between TN (TC + BTN) and HC (**Supplemental Table S6**).

Interestingly, BTN and TC showed very similar patterns of plasma glycans (**Table 2**). Most of the altered derived glycan traits mentioned above showed no difference between TC and BTN (**Table 2**), which means these traits were associated with TN (BTN + TC) and could be used to distinguish TN and HC, but could not differentiate between benign and malignant TN. Notably, four of these altered derived glycan traits, namely CFa, A2Fa, A2L, and A2GL, were significantly different among the three subgroups of TC, BTN, and HC (**Table 2** and **Figure 3**). Moreover, these four traits showed consecutive decreases from HC to BTN, and from BTN to TC (**Table 2** and **Figure 3**). This indicated that the four derived glycan traits might have potential as biomarkers for differential diagnosis of benign and malignant TN, as further investigated below.

Associations of Plasma *N*-Glycome With Lymph Node Metastasis in TC

The associations of plasma *N*-glycome with lymph node metastasis in TC were explored by logistic regression, for which only derived glycan traits that showed differences between TC patients with and without lymph node metastasis were included (**Supplemental Table S7**). Fucosylation within diantennary glycans with α2,3-linked sialic acid (A2LF) was found to be significantly positively associated with lymph node metastasis in TC ($P = 0.003826$; **Supplemental Table S7**). In contrast, α2,3-sialylation within non-fucosylated di- or tri-antennary glycans (A3F0L, A2F0L, and A2F0GL) was strongly negatively associated with lymph node metastasis ($P < 0.01$; **Supplemental Table S7**).

Performance of Plasma Glycan Traits in Identifying TC and BTN

ROC curves were assessed for the selected four derived glycan traits. The resulting ROC curve demonstrated the potential of CFa, A2Fa, A2L, and A2GL in identifying benign and malignant TN (**Figure 4**). According to our results, the AUCs of CFa, A2Fa, A2L, and A2GL were 0.7685, 0.7643, 0.7701, and 0.7861 when discriminating between malignant TN and benign TN (**Figure 4A**). Moreover, the AUCs of the four traits were 0.8144, 0.8328, 0.7688, and 0.7472 in differentiating BTN from HC (**Figure 4B**). Furthermore, the performance of the four traits was good with AUCs of 0.8857, 0.8903, 0.8736, and 0.8766 in the differential diagnosis of patients with TC and noncancer (BTN + HC) (**Figure 4C**). Finally, predictive models were built by logistic regression analysis in SPSS (version 23). Initially, the four derived traits of CFa, A2Fa, A2L, and A2GL were used for the models. Multiple combinations of these four traits were then evaluated with regard to predictive accuracy, resulting in the final models: For distinction of TC and BTN, the prediction model only included A2GL. For the distinction of BTN and HC, the prediction model was composed of CFa, A2Fa, and A2GL. For the distinction of TC and Noncancers, the prediction model only included A2Fa (**Figure 4**).

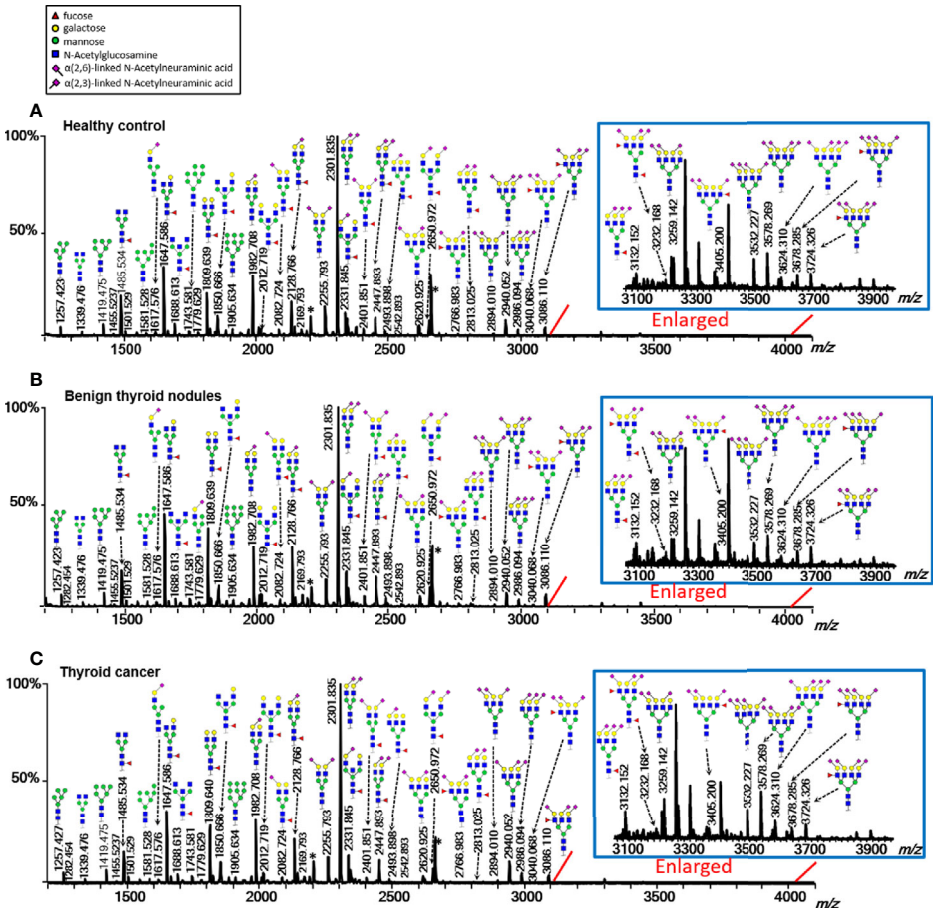

FIGURE 2 | Typical MALDI-TOF-MS spectra of plasma protein *N*-glycome for **(A)** healthy control, **(B)** benign thyroid nodules, and **(C)** thyroid cancer. Spectra were recorded in positive-ion reflectron mode on a Bruker rapifleXtreme mass spectrometer. Major *N*-glycan peaks were annotated and assigned to compositions and the presence of structural isomers cannot be excluded. The asterisk (*) are by-products.

Our results suggested that the prediction models including least number of traits achieved best performance in the differential diagnosis (**Figure 4**).

Performance of Plasma Glycan Traits in Stratifying TC to Low or High Risk of Lymph Node Metastasis

The existing method for predicting lymph node metastasis is ultrasound. Considering that A2LF, A3F0L, A2F0L, and A2F0GL showed strong associations with lymph node metastasis in TC, we attempted to build predictive models for identifying lymph node metastatic thyroid cancer based on these glycan traits. The performance of the established prediction models in predicting TC with or without lymph node metastasis was evaluated by ROC curves (**Figure 5**). Our results showed that the AUC value of ultrasound in predicting metastasis was 0.6170 (**Figure 5**), suggesting an "uninformative" test. The AUC value of the prediction model consisted of A2LF, A3F0L, A2F0L, and A2F0GL was 0.7148 (**Figure 5**), suggesting a "moderately accurate" diagnostic test. The performance was further improved when combing the four glycan traits with ultrasound with an AUC of 0.7645 (**Figure 5**).

DISCUSSION

TN are the most common thyroid disease and 10% of them are with a high risk of TC. Furthermore, TC is the most common endocrine tumor. Although the mortality rate has remained stable, the incidence rate of the TC has increased substantially (36). Deeper insight into the pathophysiology and screening of diagnostic and prognostic biomarkers is crucial for TC. Profiling of protein *N*-glycosylation with functional impact on the proteins has a high potential for advancing this. So far, aberrant glycosylation in TC has been reported in limited studies which mainly focused on glycosylation changes in cells, tissues, and plasma IgG (25–27). For example, employing MALDI-TOF(/TOF)-MS, researchers found four sialylated *N*-glycans and two high-mannose type *N*-glycans were significantly different between formalin-fixed paraffin-embedded tissues of PTC and adjacent normal tissues (26). In addition, Chen et al. detected aberrant serum IgG Fc glycosylation profiles in TC (27). Nevertheless, little is known regarding total plasma glycosylation changes in TC and BTN. Zhang et al. developed an integrated method for comprehensive *N*-glycoproteome profiling of human biofluids (37). By this

TABLE 2 | Differentially expressed derived glycan traits contributing to distinguishing between groups of patients with thyroid diseases and healthy controls.

Glycan traits	Descriptions	Median of TC	Median of BTN	Median of HC	P-value		
					TC *vs.* BTN	TC *vs.* HC	BTN *vs.* HC
	General						
CA4	tetraantennary species (A4) in complex glycans	0.0274	0.0267	0.0329	0.2217	**2.47E−12**	**3.35E−07**
	Fucosylation (F)						
A4F	in tetraantennary (A4)	0.3266	0.3164	0.4349	0.4911	**1.20E−12**	**1.48E−09**
A2LF	in diantennary (A2) with α2,3-sialylation	0.5864	0.6050	0.6525	0.2500	**2.22E−15**	**3.10E−06**
A4L0F	in tetraantennary (A4) without α2,3-sialylation	0.4688	0.4090	0.5692	0.0461	**5.69E−09**	**2.35E−06**
CFa	antenna-fucosylation in complex glycans	0.0339	0.0393	0.0464	**6.12E−05**	**1.31E−22**	**1.00E−05**
A2Fa	antenna-fucosylation in diantennary (A2)	0.0393	0.0446	0.0538	**8.00E−05**	**3.37E−24**	**2.93E−06**
	Galactosylation(G)						
A4G	in tetraantennary (A4)	0.8238	0.8530	0.7383	0.2248	**5.73E−14**	**2.09E−09**
A2FSG	in fucosylated sialylated diantennary (A2)	0.9682	0.9683	0.9713	0.6702	**5.08E−05**	6.88E−04
A4F0G	in non-fucosylated tetraantennary (A4)	0.6734	0.6836	0.5651	0.4911	**1.20E−12**	**1.48E−09**
	Sialylation (S)						
A4S	in tetraantennary (A4)	0.6162	0.6225	0.5305	0.8391	**2.89E−15**	**1.20E−09**
A4F0S	in non-fucosylated tetraantennary (A4)	0.4663	0.4647	0.3670	0.8020	**1.71E−14**	**4.44E−09**
A4FGS	per galactose in fucosylated tetraantennary (A4)	0.9527	0.9518	0.9638	0.2843	**2.42E−09**	**1.74E−04**
A4F0GS	per galactose in non-fucosylated tetraantennary (A4)	0.7029	0.6942	0.6397	0.0714	**7.19E−12**	**3.83E−04**
	α2,3-sialylation (L)						
A2L	in diantennary (A2)	0.0798	0.0879	0.0962	**5.53E−05**	**8.88E−16**	**1.59E−04**
A2FL	in fucosylated diantennary (A2)	0.1216	0.1331	0.1605	0.0040	**4.22E−14**	**6.93E−05**
A4L	in tetraantennary (A4)	0.2498	0.2538	0.1912	0.7351	**7.79E−14**	**4.15E−09**
A4F0L	in non-fucosylated tetraantennary (A4)	0.1955	0.1891	0.1419	0.7959	**2.44E−13**	**7.78E−08**
A2GL	per galactose in diantennary (A2)	0.0891	0.0984	0.1052	**1.95E−05**	**1.55E−15**	**5.15E−04**
A4FGL	per galactose in fucosylated tetraantennary (A4)	0.3545	0.3976	0.2826	0.0619	**1.69E−07**	**1.59E−05**
	α2,6-sialylation (E)						
A4E	in tetraantennary (A4)	0.3668	0.3634	0.3390	0.2989	**5.11E−15**	**6.86E−08**
A4FE	in fucosylated tetraantennary (A4)	0.0910	0.0914	0.1191	0.6936	**4.20E−11**	**7.12E−09**
A4F0E	in non-fucosylated tetraantennary (A4)	0.2773	0.2734	0.2245	0.7232	**5.53E−14**	**6.23E−09**
A4FGE	per galactose in fucosylated tetraantennary (A4)	0.5967	0.5510	0.6807	0.0631	**5.55E−08**	**1.59E−05**

Descriptions of the derived traits, median values of derived glycan traits in TC, BTN, and HC as well as p-values for the comparison by Mann–Whitney U test for the cohort are shown. The p values considered significant are below the significance threshold of 5.49E−4 (= p-value of 0.05 after multiple testing correction for 91 derived traits). The p-values highlighted indicated significance. Red and blue indicate the direction of changes up-regulation and down-regulation, respectively. Derived traits in gray shading showed the potential of distinguishing among the three groups of TC, BTN, and HC. TC, thyroid cancer; BTN, benign thyroid nodules; HC, healthy control. The subject of the derived traits calculation is represented by the last letter, e.g., galactosylation (G), and the group on which it is calculated by the preceding letters, e.g., fucosylated sialylated diantennary species (A2FS). This, for instance, translates A2FSG into the galactosylation per antenna within fucosylated sialylated diantennary glycans.

method, desialo-*N*-glycopeptides from the urine and plasma of HC, PTC, and PTC with Hashimoto's thyroiditis were analyzed. Finally, they identified 92 altered proteins and 134 intact *N*-glycopeptides from the plasma and urine samples of the three groups and revealed a novel indicator (ratio of fucosylated to nonfucosylated *N*-glycopeptide) contributing to clinical TC diagnostics (38). The published study by Zhang et al. mainly focused on glycopeptides (glycoproteomics) to obtain the glycosylation information in TC and controls and removed the sialic acid residues at the ends of the glycans when doing the analysis. In addition, the exiting study did not include BTN. In contrast, the present study focused on released glycans (glycomics) from the plasma of HC, BTN, and TC, including the linkage-specific sialic acids information.

The present study represents the first comprehensive analysis of the plasma *N*-glycome in TC and BTN. Importantly, several glycosylation features were found for the first time to differ between BTN, TC, and HC, namely complexity, galactosylation, fucosylation, and sialylation. Especially, with regard to sialylation, our approach included the discrimination of functionally disparate α2,3- and α2,6- linkages types. In addition, we found consecutive decreases of CFa (difucosylation), A2Fa (difucosylation), A2L (α2,3-sialylation in A2), and A2GL (α2,3-sialylation per galactose in A2) in BTN and TC compared with HC. These four derived glycan traits and prediction models based on them showed relatively good performance with "moderately accurate" to "accurate" AUC values, suggesting plasma *N*-glycome patterns may have potential as novel biomarkers for identifying TC and BTN assisting the existed diagnostic methods (such as ultrasound and FNA). Nevertheless, the sample size of BTN is not large enough in the present study. Moreover, testing, training, and validation samples are always needed during the discovery of cancer biomarkers (39). The results we obtained in the present study still need independent

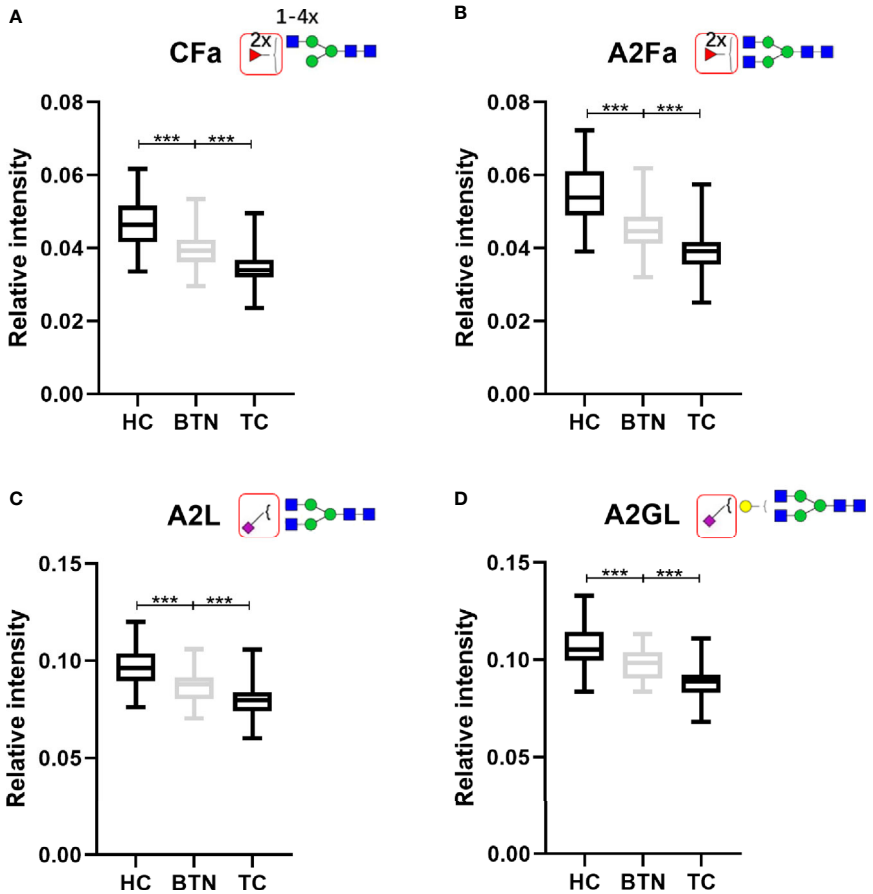

FIGURE 3 | The boxplots of derived glycan traits **(A)** CFa, **(B)** A2Fa, **(C)** A2L, and **(D)** A2GL, which showed consecutive changes in BTN and TC compared to HC. Numerical values indicate compositional limitations (i.e. 1-4 as the possible number of N-acetylhexosamines). Please see **Supplemental Table S3** for more detailed derived glycan trait descriptions. Glycan trait abbreviations: C, within complex; Fa, species with 2 fucoses (i.e. at least one antennary fucose); A2, diantennary; L, α2,3-linked sialylation; G, galactose. *** represents p-value < 0.001 (after multiple testing correction).

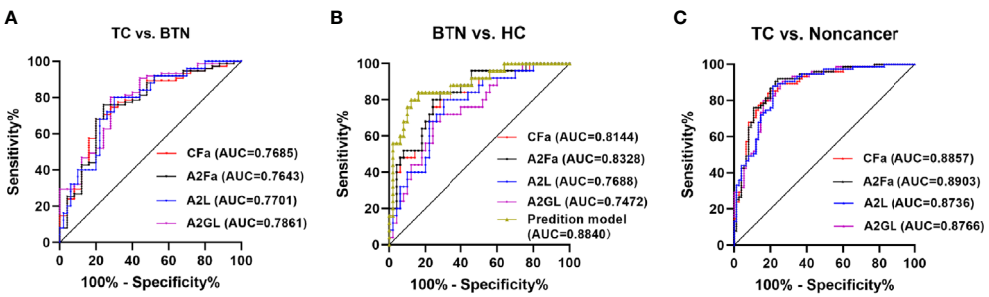

FIGURE 4 | ROC curve analysis for differential expressed derived glycan traits and prediction model based on them. **(A)** TC *vs*. BTN, **(B)** BTN *vs*. HC, **(C)** TC *vs*. Noncancer (BTN + HC). TC, thyroid cancer; BTN, benign thyroid nodules; HC, healthy controls.

validation in large cohorts, which is one of the limitations of this study. Interestingly, though many glycan traits were changed in BTN and TC compared to HC, BTN is very similar to TC in plasma *N*-glycome patterns (**Table 2**), which reminds us including benign diseases as disease control is very important during the discovery of cancer biomarkers.

Our investigation of dysregulation of *N*-glycan patterns in TC may point at pathophysiological processes involving multiple

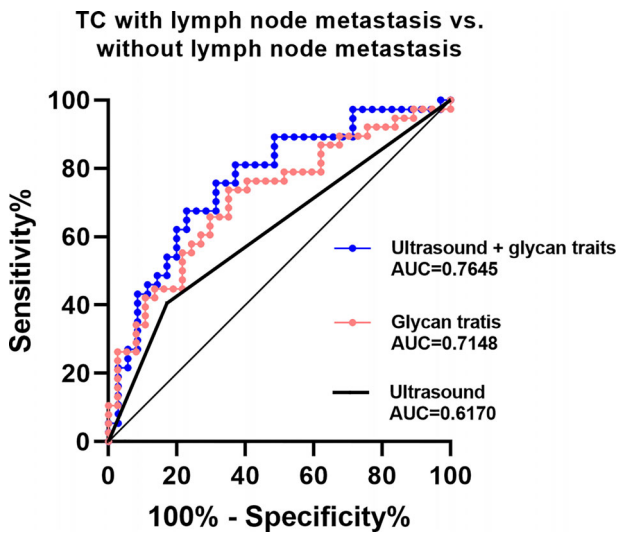

FIGURE 5 | ROC curve analysis for derived glycan traits which showed associations with lymph node metastasis.

proteins, as we discuss below. Fucosylation is one important mode of glycosylation in TC and is regulated by several kinds of fucosyltransferases. FUT3, 4, and 6 are responsible for antennary fucosylation (leading to the multiple fucoses). FUT4 was identified as an independent marker for PTC (40). In other types of cancer, FUT3 was involved in the proliferation, migration, tumorigenesis of pancreatic cancer cells (41). While, FUT5 and FUT6 were reported to be associated with the development of colorectal cancer (42). These results may help to explain part of the possible mechanism of dysregulated CFa (difucosylation) and A2Fa (difucosylation) in BTN and TC in this study. Nevertheless, the glycan traits containing fucosylation in the present study differ from what has been reported in other cancers, such as increased serum fucosylation (A2LF, A3LF, and A4LF) was observed in pancreatic cancer (43) and A3Fa were found increased in colorectal cancer (15). This indicated that alterations of plasma protein fucosylation might be cancer-specific, making plasma *N*-glycome patterns more promising as potential cancer-specific biomarkers. In addition, sialic acids are directly involved in the activation and modulation of the immune system, which depends on the linkage (44, 45). Our novel method enabled us to discriminate between the two types of linkage (α2,3 or α2,6) and get linkage-specific data of sialylation. We found that α2,3-sialylation within A2 was consecutively decreased in BTN and TC. These glycans may come from liver-produced acute phase proteins (32). Consistently, proteomic analysis revealed decreased levels of liver-derived glycoproteins (such as apolipoprotein A4, apolipoprotein C-I, apolipoprotein C-III, and alpha-1 antitrypsin) in PTC compared to BTN or HC (46–49). Besides, Arcinas et al. profiled secreted and cell surface glycoproteins of thyroid cancer cells using a glyco-capture method. Among the 397 proteins identified within the PTC cell line (TPC-1), 37 were identified as secreted glycoproteins, which may also contribute to the changed levels of *N*-glycans in the plasma of TC patients (50). Increased α2,3-linked sialylation in plasma has been supposed to be involved in the anti-inflammatory effects and has been reported in diseases such as IBD (51). Reduced α2,3-linked sialylation in TC, which is opposite to the status in other diseases, might reflect other processes that are not related to anti-inflammation and classical immune response. For example, hormones may be involved in the regulation of glycosylation in TC (26, 52). Since data on linkage-specific sialylation in diseases is scarce, the exact mechanisms of reduced α2,3-linked sialylation in TC need to be further studied. What's more, α2,6-sialylated glycans (H5N4E1, H5N4F1E1, H5N4F1L1E1, and H5N4F1E2) were found to increase in tissues of PTC (26), which were consistent with our results in plasma (**Table 2**). However, we did not find α2,6-sialyation differences between TC and BTN (**Table 2**).

In this study, we observed that A2LF, A3F0L, A2F0L, and A2F0GL were significantly associated with lymph node metastasis in TC and models constructed from the four glycan traits have high potential as predictive biomarkers. The combination of fucosylation with α 2,3-linked sialylation (LF) often suggests the terminal sialyl-Lewis X epitopes (32). The associations between sialyl-Lewis X on liver-derived proteins and metastasis have been reported in many types of cancer, such as breast cancer (53, 54), liver cancer (55), and renal cancer (56). Interestingly, the proteomic analysis showed that levels of liver-produced glycoproteins such as alpha-1-antitrypsin, which may be the origin of the glycan traits mentioned above, were associated with invasion and metastasis in PTC (57). Additionally, epithelial to mesenchymal transition (EMT), which is a key step in the metastatic process of cancer, is triggered by the secreted cytokine TGF-β (58), while fucosylation is important for the functions of TGFβ-R (25). The novel link of the lymph node metastasis of TC with fucosylation (A2LF) and α2,3-sialylation (A3F0L, A2F0L, and A2F0GL) was for the first time indicated in the present study, providing potential glycan biomarkers to stratify TC into low or high risk of lymph node metastasis.

The methodology used in the present study doesn't provide detailed information on the plasma protein origin of the glycan biomarkers. This limitation is well known in the field of glycobiology and can be addressed by protein-specific glycomic (glycoproteomic) analyses. However, it is still a big challenge in terms of sensitivity, throughput, and discrimination of glycan linkage information. In addition, measurement of thyroglobulin in washout fluid increases specificity and sensitivity of lymph node metastasis. However, the data for the thyroglobulin in washout fluid is not available for us. The results for the comparison between ultrasound and the *N*-glycan traits in the present study may not be comprehensive. In future studies, measurement of thyroglobulin in washout fluid from the needle used for lymph node aspiration cytology should be considered. On the other hand, studies in large validation cohorts and prospective investigations are still needed to validate our findings before the application of the biomarkers.

CONCLUSION

To our knowledge, this is the first study to identify plasma *N*-glycome in TC and BTN to date and included novel linkage-

specific sialylation information. Plasma glycosylation was proven to differ between BTN, TC and HC in main glycosylation features. We also revealed unreported associations between plasma glycan features and lymph node metastasis of TC. Several derived glycan traits and prediction models based on them showed high potential as biomarkers for differential diagnosis of BTN and TC and stratifying TC patients, which can function as a base for the development of blood-based tests. Future studies, preferably in a longitudinal and protein-specific manner, are warranted to assess the potential for early detection and surveillance based on the here reported plasma *N*-glycan features. Moreover, genetic studies including the expression of glycosyltransferases and glycosidases should improve insight into the mechanisms involved. Overall, this study enhanced the understanding of TC.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the regional ethics committee of the Peking Union Medical College Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

XX and ZZ conceived and initiated this study. ZZ performed the experiments and data analysis and interpreted the results with support of KR. ZZ, ZL and JW collected samples and clinical parameters. ZZ prepared the figures and tables, and wrote the original draft with support from KR and XX. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.692910/full#supplementary-material

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Differential Effects of Estrogen Receptor Alpha and Beta on Endogenous Ligands of Peroxisome Proliferator-Activated Receptor Gamma in Papillary Thyroid Cancer

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Purpose: The inhibition of estrogen receptor alpha (ERα) or the activation of ERβ can inhibit papillary thyroid cancer (PTC), but the precise mechanism is not known. We aimed to explore the role of ERα and ERβ on the production of endogenous peroxisome proliferator-activated receptor gamma (PPARγ) ligands in PTC.

Methods: 2 PTC cell lines, 32 pairs of PTC tissues and matched normal thyroid tissues were used in this study. The levels of endogenous PPARγ ligands 15(S)-hydroxyeicosatetraenoic acid (15(S)-HETE), 13-S-hydroxyoctadecadienoic acid (13(S)-HODE), and15-deoxy-Δ12,14-prostaglandin J2 (PGJ2) were measured by ELISA.

Results: The levels of PGJ2 and 15(S)-HETE were significantly reduced in PTC, but 13(S)-HODE was not changed. Activation of ERα or inhibition of ERβ significantly downregulated the production of PGJ2, 15(S)-HETE and 13(S)-HODE, whereas inhibition of ERα or activation of ERβ markedly upregulated the production of these three ligands. Application of endogenous PPARγ ligands inhibited growth, induced apoptosis of cancer cells, and promoted the efficacy of chemotherapy.

Conclusion: The levels of endogenous PPARγ ligands PGJ2 and 15(S)-HETE are significantly decreased in PTC. The inhibition of ERα or activation of ERβ can inhibit PTC by stimulating the production of endogenous PPARγ ligands to induce apoptosis in cancer cells.

Keywords: papillary thyroid cancer, peroxisome proliferator-activated receptor gamma, estrogen receptors, PGJ2, 15(S)-HETE

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INTRODUCTION

There is increasing evidence indicating that activation of peroxisome proliferator-activated receptor gamma (PPARγ) by its ligands can inhibit the growth of thyroid cancer, likely *via* multi-mechanisms including stimulation of the anti-tumor immune system, induction of cancer cell differentiation, increase of radioiodine uptake in thyroid cancer cells, cell cycle arrest, and promotion of apoptosis of cancer cells (1–12). However, the rationale for administration of PPARγ ligands to treat thyroid cancer is not clear as some studies have shown a reduction in PPARγ expression, yet others revealed normal PPARγ expression or the occurrence of PAX8-PPARγ which can inactivate rather than decreasing PPARγ in thyroid cancer (13–19).

Therefore, the defect in PPARγ pathway needs further investigation. Moreover, some publications have also challenged the safety of synthetic PPARγ ligands that are currently employed as anti-tumor agents in most studies. The administration of synthetic PPARγ ligands is now known to produce some significant side-effects including an increased risk of bladder cancer and cardiovascular diseases (3, 20, 21). These adverse effects have limited the therapeutic application of synthetic PPARγ ligands.

It is known that estrogen receptors (ERs) are involved in the development of thyroid cancer that is predominant in females. Estrogen executes its functions usually through its traditional receptors (ERα and ERβ). The activation of either ERα or ERβ appears to be associated with different outcomes (22). In cancers, ERα is positively associated with cell proliferation/growth. In contrast, ERβ negatively regulates cell growth. Tumors develop in ERβ-knockout mice but not in wild type mice (23). Although both normal and malignant thyroid tissues are known to express ERα and ERβ, the level of ERα appears to be more pronounced in malignant thyroid tissues and the ratio of ERβ to ERα is significantly higher in normal thyroid tissues when compared to malignant thyroid tissues (24–31). The increased level of ERα has been shown to stimulate the growth of thyroid tumor cells whereas the increased level of ERβ can suppress their growth (27–31).

Although both ERs and PPARγ belong to the family of nuclear receptor proteins and both can regulate thyrocyte proliferation and growth, there are very few studies on the relationship between ERs and PPARγ in cancer cells. This study therefore aimed to examine the impact of ERs on endogenous PPARγ ligands in papillary thyroid cancer (PTC), the most common form of thyroid cancers. Endogenous PPARγ ligands are *in vivo* metabolic products which are nontoxic at physiological concentrations. Unfortunately, studies have not been actively conducted to explore the therapeutic modulation of these natural endogenous ligands for possible treatment of cancers.

METHODS

Reagents

15(S)-hydroxyeicosatetraenoic acid (15(S)-HETE), 13-S-hydroxyoctadecadienoic acid (13(S)-HODE), 15-deoxy-Δ12,14-prostaglandin J2 (PGJ2), PGJ2 ELIS kits and 15(S)-HETE ELISA kits were purchased from Cayman Chemical (Ann Arbor, MI). 13(S)-HODE ELISA kits were from Enzo Life Sciences (Farmingdale, NY). 4,4',4"-(4-propyl-[1H]-pyrazole-1,3,5-triyl) trisphenol (PPT, ERα agonist), 2,3-bis(4-hydroxy-phenyl)-propionitrile (DPN, ERβ agonist), 1,3-bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy)phenol]-1H-pyrazole dihydrochloride (MPP, ERα antagonist), 4-[2-phenyl-5,7-bis (trifluoromethyl)pyrazolo[1,5-a]pyrimidin-3-yl]phenol (PHTPP, ERβ antagonist) and paclitaxel were obtained from Tocris (Bristol, UK).

Thyroid Tissue Samples

Papillary thyroid cancer (PTC) tissue samples of both tumor and non-tumor tissue from the same thyroid gland were collected from 32 patients including 6 males (35-58 years old) and 26 females (33-57 years old). All patients underwent routine thyroidectomy. All subjects provided written informed consent prior to specimen collection. Human Ethics approval (No. 2019.587) was obtained from the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee, and the study was performed in accordance with the 1964 Declaration of Helsinki.

Cell Cultures

Two human PTC cells (K1 and BCPAP) were used in this study. K1 cells were obtained from the European Collection of Authenticated Cell Cultures (ECACC) and BCPAP cells were kindly provided by Dr. Mingzhao Xing (Johns Hopkins University School of Medicine, Maryland). Both cell lines have been authenticated to be human papillary thyroid cancer cells (32). K1 and BCPAP cells were cultured in RPMI 1640 supplemented with 10% FBS at 37° in an atmosphere with 5% CO2 and were used for the experiments in their early passages (less than 25). In our early study, we have demonstrated that both K1 and BCPAP cells can express certain basic levels of ERα, ERβ and PPARγ proteins (33).

Cell Growth

The growth of cells was estimated by cell survival assay, which was determined by 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) protocol (33, 34).

Measurement of PGJ2, 15(S)-HETE and 13(S)-HODE

The levels of PGJ2, 15(S)-HETE and 13(S)-HODE were determined by ELISA kits and the assays were performed according to the instructions of the manufacturers. Briefly, for tissue samples, they were measured wet weight and then homogenized in 2 ml 1 x PBS (pH 7.4) using a homogenizer on ice. For cultured cell samples, cells were lysed by lysis buffer (10 mM Tris-HCl, pH 7.4, 400 mM NaCl, 1 mM EDTA and 1.0% SDS) and samples were centrifuged at 5000 rpm for 1 min at 4°C to obtain the supernatant. The tissue homogenates or the lysed cell samples were acidified by adding 2M HCl to pH 3.5, left at 4°C for 15 min. Samples were centrifuged at 2000 rpm for 20 min at 4°C. Samples were applied to these C18 reverse phase column and the columns were washed with 10 mL water followed by 10 mL

15% ethanol, and 10 mL hexane. The sample was eluted from column by addition of 10 ml ethyl acetate and then evaporated under a stream of nitrogen. 25 µl ethanol and 250 µl Assay Buffer were added to dry samples. A standard curve was generated by serial dilutions of the standard supplied in these kits. The levels of the ligands were calculated according to the standard curve. Concentrations of 15(S)-HETE, 13(S)-HODE and PGJ2 were calculated by 4 parameter logistic curve fitting program.

Analysis of Apoptosis

Cells were seeded in 6-well plates and incubated overnight to allow cells to attach to the plate. Terminal deoxynucleotidyl transferase Dutp nick end labeling (TUNEL) was conducted using an APO-DIRECT TUNEL assay kit (BD Biosciences, San Jose, CA). In brief, cells were suspended in 1% (w/v) paraformaldehyde in PBS, Ph7.4 at a concentration of 2×10^6 cells/ml after treatment. The cell suspension was then placed on ice for 60 min. After centrifuging cells for 5 min at 300 g, the supernatant was discarded. The cells were washed in 5 ml of PBS and the cell pellet was resuspended in PBS in a tube by gentle vortexing. The cells were then incubated in ice-cold 70% (v/v) ethanol overnight at -20°C prior to staining for apoptosis. Apoptosis was measured according to the protocol provided by the kit and the result was presented as folds of control conditions.

Statistical Analysis

Data were analyzed by student's t test or one-way ANOVA followed by the student's t test. All data were presented as means ± SD. The value was considered significant when $p<0.05$.

RESULTS

The Levels of PGJ2, 15(S)-HETE and 13(S)-HODE in PTC

The concentrations of PGJ2 and 15(S)-HETE were much lower in PTC tumor tissues than in the non-tumor tissues (**Figure 1**). The concentration of 13(S)-HODE was decreased in PTC tumor tissues compared with that in the non-tumor tissues but the difference did not reach a significant point ($p>0.05$, data not shown).

Impact of ER Modulation on the Levels of PGJ2, 15(S)-HETE and 13(S)-HODE in PTC Cells

In order to assess whether the production of endogenous PPARγ ligands, PGJ2, 15(S)-HETE and 13(S)-HODE, could be regulated by ERα and ERβ, PPT (ERα agonist), DPN (ERβ agonist), MPP (ERα antagonist) and PHTPP (ERβ antagonist) were employed in this study. These 4 agents have been well documented to modulate the activities of ERα and ERβ (35, 36). It was found that the activation of ERα by PPT markedly and dose-dependently inhibited the production of PGJ2 in both K1 and BCPAP cells. In contrast, the inactivation of ERα by MPP significantly and dose-dependently enhanced its production in both PTC cells (**Figure 2A**). Different from ERα activation, the activation of ERβ (by DPN) clearly increased the level of PGJ2 whereas the inactivation of ERβ by PHTPP decreased the PGJ2 production in both PTC cells (**Figures 2B, C**). Similar to PGJ2, the levels of 15(S)-HETE and 13(S)-HODE were regulated by these 4 ER modulators in both PTC cells. It appeared that their impact on 15(S)-HETE was more obvious than that on 13 (S)-HODE.

Impacts of the ER Modulation and Endogenous PPARγ Ligands on Cell Survival and Growth

The modulation of ERα and ERβ exerted opposite effects on PTC cell survival and growth (**Figure 3**). The activation of ERα (by PPT) or inactivation of ERβ (by PHTPP) dose-dependently increased the survival and growth in both K1 and BCPAP cells (**Figures 3A, D**) whereas activation of ERβ (by DPN) or inactivation of ERα (by MPP) significantly decreased the survival and growth in both K1 and BCPAP cells (**Figures 3B, C**). All three endogenous PPARγ ligands markedly reduced the survival and growth in both PTC cells and the effects of PGJ2 and 15 (S)-HETE were stronger than those of 13(S)-HODE (**Figure 3E**).

Impact of ER Modulation and Endogenous PPARγ Ligands on Apoptosis

PTC cells treated with the ERα agonist PPT or ERβ antagonist PHTPP barely affected the apoptosis compared with those without PPT or PHTPP treatment (control) (**Figure 4A**). However, both PPT and PHTPP significantly sensitized the

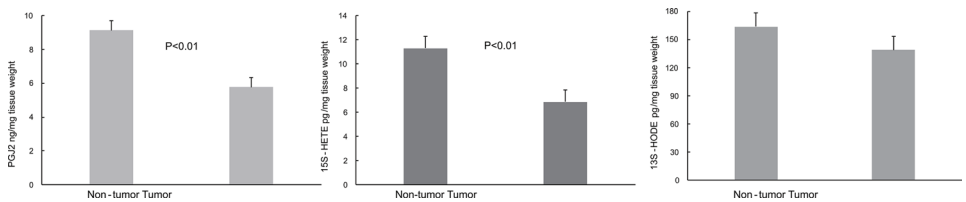

FIGURE 1 | The concentrations of PGJ2 and 15(S)-HETE in PTC. Thyroid tumor tissues and its matched non-tumor tissues were obtained from 32 patients. The concentrations of PGJ2 and 15(S)-HETE were measured using ELISA kits from Cayman Chemical (Ann Arbor, MI) and Enzo Life Sciences (Farmingdale, NY). The ELISA was performed according to the instructions of the manufacturers. The concentrations of these 2 ligands were expressed in ng or pg per mg wet weight of tissues.

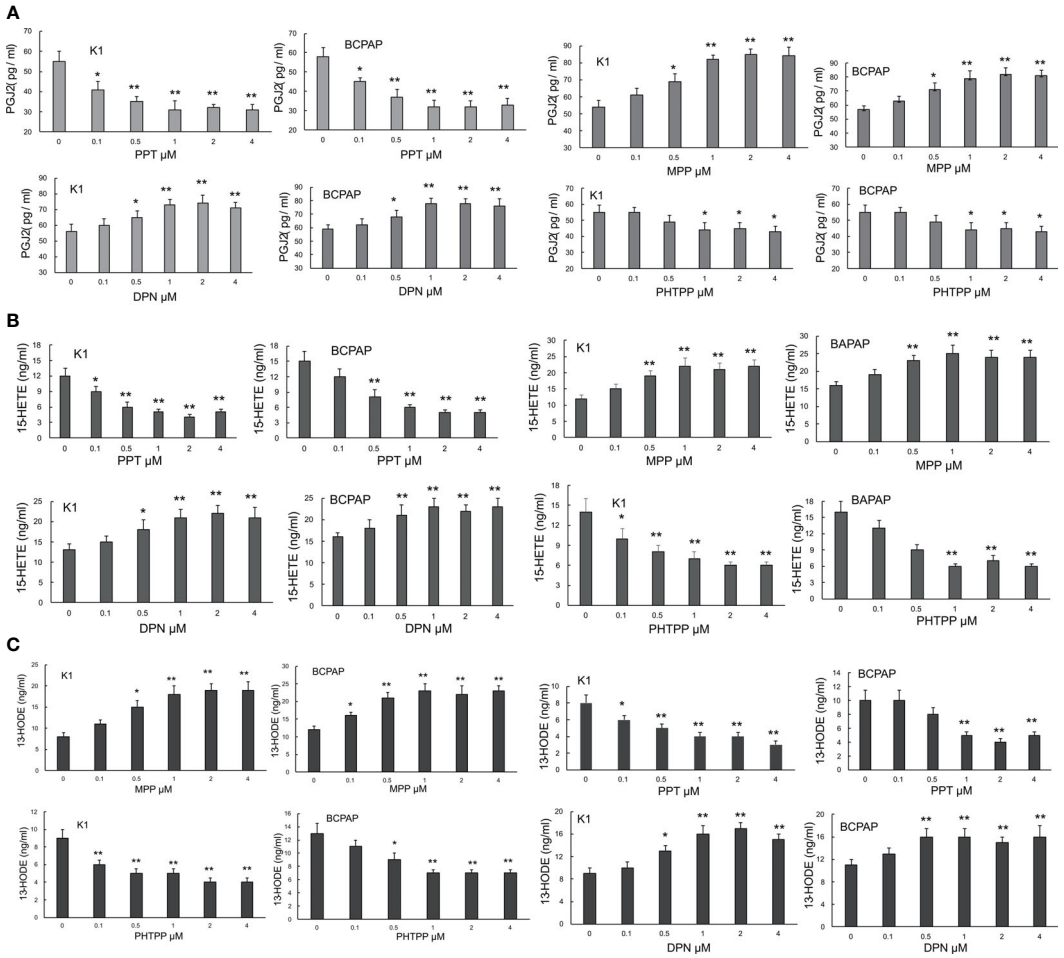

FIGURE 2 | The impact of ER modulation on the levels of PGJ2, 15(S)-HETE and 13(S)-HODE in PTC cells. K1 and BCPAP cells were respectively treated with PPT, MPP, DPN and PHTPP at the given concentrations (0, 0.1, 0.5, 1, 2, 4μM for all 4 modulators) for 48 hours. At the end of the treatment, the levels of PGJ2 **(A)**, 15(S)-HETE **(B)** and 13(S)-HODE **(C)** in cells were measured by ELISA kits (Cayman Chemical (Ann Arbor, MI, and Enzo Life Sciences, Farmingdale, NY). The ELISA was performed according to the instructions of the manufacturers. *$p < 0.05$, **$p < 0.01$, compared with the control (0 dose).

cells to apoptosis induced by paclitaxel, a chemotherapeutic agent that is commonly used in the treatment of thyroid cancer (37). The activation of ERβ (by DPN) or inactivation of ERα (by MPP) significantly stimulated apoptosis of PTC cells compared with the control, and both DPN and MPP further enhanced apoptosis induced by paclitaxel (**Figure 4A**). All three endogenous PPARγ ligands clearly induced apoptosis in both PTC cells and the effects of PGJ2 and 15(S)-HETE appeared to be stronger than that of 13(S)-HODE (**Figure 4B**). These three endogenous PPARγ ligands, especially 15(S)-HETE, also significantly enhanced the apoptosis induced by paclitaxel.

DISCUSSION

The results of this study have led to two novel findings. Firstly, the concentrations of endogenous PPARγ ligands, PGJ2 and 15 (S)-HETE (38–41), were significantly reduced in PTC, though the level of 13(S)-HODE was not different between tumor tissues and non-tumor tissues. Secondly, the activation of ERα negatively controlled the production of endogenous PPARγ ligands whereas the activation of ERβ positively regulated them. These two novel findings are significant in elucidating the roles of PPARγ and ERs in the growth and potential treatment of PTC.

The activation of PPARγ ligands is well known to cause the death of cancer cells *via* multiple channels such as activating the anti-tumor immune system, differentiating cancer cells, arresting cell cycle, promoting apoptosis and increasing radioiodine uptake (1–12). The rationale for the application PPARγ ligands to treat thyroid cancer is inconsistent or unclear. Some studies have indicated that the expression of PPARγ is reduced in thyroid cancer while others revealed the normal expression of PPARγ or the inactivation of PPARγ by the Pax-8-PPAR-γ fusion protein (PPFP) (14–19, 33). If the low expression of PPARγ is the major factor that causes the PPARγ system unable to function

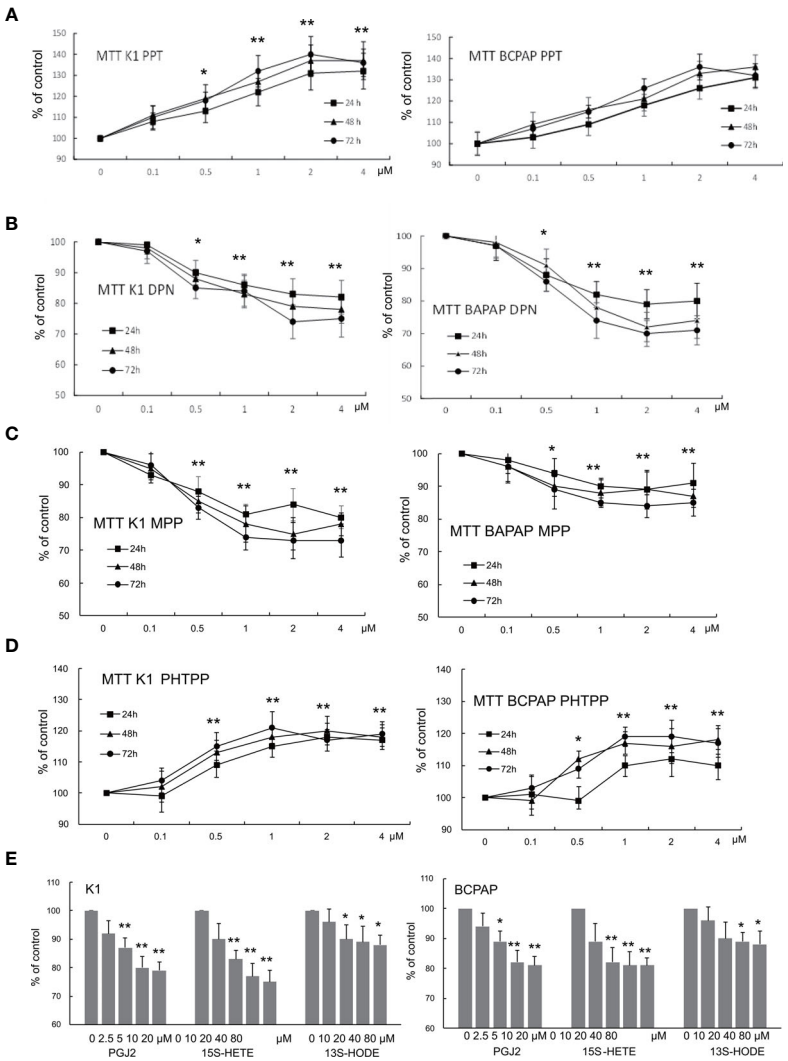

FIGURE 3 | The impact of ER modulation and endogenous PPARγ ligands on cell growth. K1 and BCPAP cells were respectively treated with PPT **(A)**, DPN **(B)**, MPP **(C)**, and PHTPP **(D)** at the given concentrations for 24, 48 and 72 hours. At the end of the treatment, cell survival was measured by MTT assay to estimate the cell growth and expressed as the percentage of control culture conditions (no treatment). To assess the effect of endogenous PPARγ ligands on cell growth, different doses of PGJ2, 15(S)-HETE and 13(S)-HODE, as indicated in the figure, were used to treat K1 and BCPAP for 48 hours **(E)**, and cell growth was determined by the survival assay as described above. The data were presented as the mean ± SD of 3 independent experiments with triplicate wells. *$p < 0.05$, **$p < 0.01$, compared with the control (0 dose).

normally, the proper treatment strategy should be to enhance the expression of PPARγ rather than the administration of PPARγ ligands. Thus, in such a situation, the administration of PPARγ ligands may not be an effective strategy to upregulate PPARγ functions. However, in practice, the induction of death in cancer cells is usually caused by the application of PPARγ ligands rather than by the upregulation of PPARγ itself. Therefore, the low expression of PPARγ itself is unlikely to be a key issue associated with the application of PPARγ ligands to treat thyroid cancer. Our study has demonstrated the decrease of endogenous PPARγ ligands, PGJ2, 15(S)-HETE and 13(S)-HODE in thyroid cancer. Insufficient PPARγ ligands can significantly downregulate the activity of PPARγ (38, 42), thus causing the PPARγ system unable to attack cancer cells. Accordingly, our findings have led to the discovery of a new pathway in which the activity of PPARγ is reduced by the low production of endogenous PPARγ ligands such as PGJ2, 15(S)-HETE and 13(S)-HODE. This new concept may well explain the rationale for the application of PPARγ ligands to treat thyroid cancer.

Earlier studies have demonstrated that the activation of ERα promotes the growth of PTC whereas the activation of ERβ inhibits the growth (27–30, 34). However, the responsible mechanism is not completely known. Our finding that the activation of ERα or inhibition of ERβ could significantly downregulate the production of endogenous PPARγ ligands,

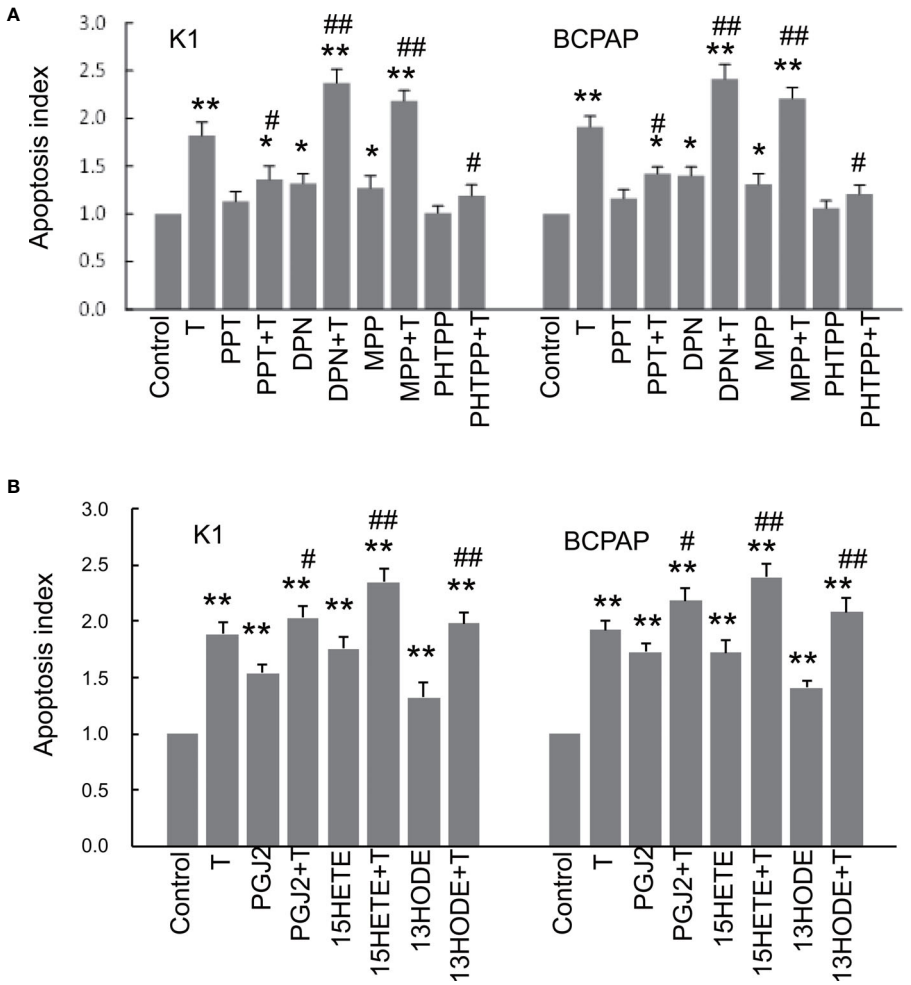

FIGURE 4 | The impact of ER modulation and endogenous PPARγ ligands on apoptosis. K1 and BCPAP cells were respectively treated with 4 different ER modulators (1 μM PPT, 1 μM DPN, 1 μM MPP, 1 μM PHTPP), 1 μM paclitaxel (T) or ER modulator plus T for 48 hours **(A)**. At the end of the treatment, apoptosis was measured by TUNEL assay kits (BD Biosciences, San Jose, CA). The apoptotic index was calculated as folds of the control condition (no treatment). To assess the effect of endogenous PPARγ ligands on apoptosis, cells were respectively treated paclitaxel (T) or PPARγ ligand plus T for 48 hours **(B)**, and apoptosis was measured as described above. The data were presented as the mean ± SD of 3 independent experiments with triplicate wells. *$p < 0.05$, **$p < 0.01$ compared with the control (0 dose); #$p < 0.05$, ##$p < 0.01$ compared with cells treated with T only.

PGJ2, 15(S)-HETE and 13(S)-HODE, whereas inhibition of ERα or activation of ERβ could markedly upregulate the production of these three endogenous PPARγ ligands in PTC, uncovering new signaling pathways through which ERα and ERβ differentially regulate the levels of endogenous PPARγ ligands. Extensive studies have shown that the activation of PPARγ by its ligands (either synthetic or endogenous) can inhibit the growth of thyroid cancer (1–12, 40, 41). In this study, we have confirmed that the application of PPARγ ligands, PGJ2, 15(S)-HETE and 13 (S)-HODE could inhibit the growth of PTC cells and promote apoptosis of tumor cells. Therefore, the inhibition of ERα or activation of ERβ may inhibit PTC by stimulating the production of endogenous PPARγ ligands to induce apoptosis in PTC cells. However, the upregulation of ERα or downregulation of ERβ may also promote the growth of PTC *via* decreasing the production of endogenous PPARγ ligands, which may also contribute to chemo-resistance. This novel concept is supported by a recent study which demonstrated that ERα signaling downregulates PPARγ to promote the progression of PTC (43). Nevertheless, we believe, this ER-regulated endogenous PPARγ ligand pathway should not be the sole pathway but one of channels for ERs to affect the growth of PTC or a certain subset of PTC.

The upregulation of endogenous PPARγ ligands such as PGJ2 and 15(S)-HETE appears to be a better strategy than the administration of a synthetic PPARγ ligand to inhibit thyroid cancer, at least in terms of side-effects. The administration of synthetic PPARγ ligands is associated with an increased risk of bladder cancer and other side effects (5, 20, 21). Endogenous PPARγ ligands are naturally produced *in vivo* and the cytotoxicity of these

endogenous ligands should be minimal. Therefore, the development of endogenous ligands PGJ2 and 15(S)-HETE to treat thyroid cancer should be particularly appealing.

In conclusion, we have demonstrated that the levels of endogenous PPARγ ligands PGJ2 and 15(S)-HETE are significantly decreased in PTC. Our data suggest that the inhibition of ERα or activation of ERβ may inhibit PTC by stimulating the production of endogenous PPARγ ligands to induce apoptosis in cancer cells. Conversely, the upregulation of ERα or downregulation of ERβ may lead to the low production of endogenous PPARγ ligands, causing resistance of cancer cells to chemotherapy.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

Human Ethics approval was obtained from the Joint Chinese University of Hong Kong-New Territories East Cluster Clinical Research Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

The experiments were designed by GC, SY, ZG, ZL, MT, and JC, and executed by SY, ZG, ZL, JD, and LX. The data analysis was conducted by GC, MT, SY, ZG, ZL, YZ, NT, JD, AV, CH, JC, and MW. Clinical samples and information were collected/provided by WW, AV, XZ, SQ, NT, and MW. The manuscript was written by SY, ZG, JC, ZL, MT, and GC with input from all of the other authors. All authors contributed to the article and approved the submitted version.

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Radiotherapy Plus Chemotherapy Leads to Prolonged Survival in Patients With Anaplastic Thyroid Cancer Compared With Radiotherapy Alone Regardless of Surgical Resection and Distant Metastasis: A Retrospective Population Study

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Background: Whether anaplastic thyroid cancer (ATC) patients benefit more from radiotherapy plus chemotherapy (RCT) than from radiotherapy alone (RT) was controversial. We aimed to investigate the effectiveness of RCT *versus* RT on ATC overall and within subgroups by surgical resection and distant metastasis in a large real-world cohort.

Methods: Patients with ATC diagnosed between 2004 and 2015 were identified from the Surveillance, Epidemiology, and End Results Program database. Inverse probability weighting (IPW) was performed to balance variables between the two groups. Multivariate Cox proportional hazard model and Fine-Gray compete-risk model were carried out to investigate prognostic factors relating to overall survival (OS) and cancer-specific survival (CSS). Subgroup analysis was carried out, and a forest plot was graphed.

Results: Of the 491 ATC patients, 321 (65.4%) were in the RCT group and 170 (34.6%) were in the RT group. The median OS was 4 months [interquartile range (IQR) 2–7] and 2 months (IQR 1–4) for patients in the RCT and RT groups, respectively. As indicated by the inverse probability weighting multivariate regression, RCT was associated with significantly improved OS (adjusted HR = 0.69, 95% CI = 0.56–0.85, $p < 0.001$) and CSS (adjusted subdistribution HR = 0.77, 95% CI = 0.61–0.96, $p = 0.018$). The prominent effect of RCT *versus* RT alone remains significant within each subgroup stratified by surgical resection and distant metastasis. Older age, single marital status, surgical

resection, distant metastasis, and tumor extension were significant prognostic factors of survival.

Conclusions: RCT contributes to prolonged OS and CSS compared with RT alone in ATC patients, regardless of surgical resection and distant metastasis. RCT should be preferentially applied to ATC patients.

Keywords: anaplastic thyroid cancer, chemotherapy, distant metastasis, radiotherapy, surgical resection, SEER database

INTRODUCTION

Thyroid cancer is a rare malignant tumor that accounts for about 2.9% of all site cancer cases in the USA, with more than 52 thousand newly diagnosed cases and nearly 2,200 new deaths yearly (1). Anaplastic thyroid cancer (ATC), which accounts for less than 2% of thyroid cancer but leads to more than 50% of the annual thyroid cancer-related mortality, remains one of the most aggressive and fatal tumors. ATC augments rapidly, invades neck mass and regional lymph nodes, with a median survival of 4 months and nearly 50% of newly diagnosed ATC patients having distant metastasis (2–4).

Even though novel immunotherapy and targeted therapy, such as pembrolizumab, bevacizumab, and sorafenib, have been administered with traditional therapy using surgical resection and radiotherapy with or without chemotherapy within clinical trials, the survival outcome of ATC patients remains disappointing (5, 6). Thus, the best treatment for ATC is still suggested as surgical resection combined with radiotherapy with or without chemotherapy (6–9). However, the effect of chemotherapy administered with RT was unascertained and variable in different studies focusing on different subsets of ATC patients. For example, some small retrospective studies failed to identify a significant benefit from radiotherapy plus chemotherapy (RCT) (10, 11), while other studies proposed the potential benefit of RCT (12). The inconsistency of the effect of chemotherapy may be due to the heterogeneity of ATC patients between different treatment groups because the heterogeneity biases the results of previous studies and is hard to control due to the extreme rareness of ATC. Moreover, because of the rareness of ATC, random control trials focusing on comparing RCT with radiotherapy alone (RT) are not feasible. Furthermore, no studies carrying out subgroup analysis by surgical resection and distant metastasis have been carried out.

Most previous studies have a small sample size with the heterogeneity of patients not well adjusted. Whether ATC patients could benefit more from RCT than from RT was unclear. Thus, a study comparing RCT with RT alone in a large representative cohort that comprises diverse subsets of ATC patients is of great importance. Therefore, we carried out a study using the inverse probability weighting technique to adjust heterogeneity of patients to investigate the efficacy of RCT *versus* RT on the ATC using the Surveillance, Epidemiology, and End Results (SEER) Program database of the National Cancer Institute.

MATERIALS AND METHODS

Data Source and Study Population

The SEER database was used to identify ATC patients diagnosed between 2004 and 2015 using the International Classification of Diseases for Oncology 3rd Edition (ICD-O-3). Patients with a primary site code of C73.9 and the ICD-O-3 histology codes of 8020–8035 were included. Patients were excluded according to the following criteria: (1) more than one malignant tumor; (2) without radiotherapy; and (3) no mass tumor found. A survival time of 0 months was recoded as 0.5 months to more accurately represent patients who died within 1 month of their diagnosis but did not reach the 1-month threshold (13).

As potential prognostic factors, year of diagnosis, race, age at diagnosis, gender, marital status, multifocality, lymph node invasion (American Joint Committee on Cancer (AJCC) N stage), distant metastasis (AJCC M stage), tumor size, surgery type, and tumor extension were derived from the corresponding fields of the SEER database (4, 14–16). The year of diagnosis was grouped into two intervals in the year 2010. The age at diagnosis was grouped into two intervals at age 65. Tumor extension codes, which indicates the continuous growth of the primary tumor, was merged into six groups, according to the definition of tumor extension codes (17): 100, 200, 300, and 400 as the group I, indicating tumors confined to the thyroid capsule; 450 and 480 as the group II, indicating minimal extra thyroid extension; 500, 520, 550, and 560 as the group III, indicating involving of parathyroid, recurrent laryngeal, vagus, cricoid cartilage, esophagus, larynx, sternocleidomastoid muscle, and trachea; 600, 620, 650, 700, 730, 800, and 810 as group IV, being equivalent to AJCC T4b stage and indicating involving of the thyroid cartilage, carotid artery (encased), jugular vein, and thyroid artery or vein, bone, skeletal muscle (other than the strap or sternocleidomastoid muscle), mediastinal tissues, and prevertebral fascia; 815 as group V, being equivalent to AJCC T4 NOS; and 999 as group unknown.

The detailed information about the RT protocols, chemotherapy agents, sequence of RT or RCT and surgery, immunotherapy, targeted therapy, the specific drugs and doses used are not available in the SEER database; thus, these factors could not be evaluated and controlled in this study.

Outcomes

Overall survival (OS) was the primary outcome. ATC-specific survival (CSS) was the secondary outcome, with death

attributable to reasons other than ATC being considered compete risk.

Statistical Analysis

The stabilized inverse probability weighting (SIPW) was applied to balance variables between groups (18). All the variables available in this study were included in logit regression models to calculate the probability of receiving RCT *versus* RT. SIPW weights were then calculated based on the precalculated logit models. We also calculated SIPW weights within each subgroup stratified by surgical resection and distant metastasis.

The Kaplan-Meier survival curves were plotted and compared by the Cox test due to application of IPW adjustment. Multivariate Cox proportional hazard regression models and Fine-Gray compete-risk models were applied to calculate the (subdistribution) hazard ratio [(s)HR] and their corresponding 95% confidence intervals (95% CIs). Univariate regression models were not performed. All the variables were included in a multivariate model without variable filtering because filtering variables from univariate regression basing on *p*-value is controversial.

In order to adjust for and minimize the potential immortal-time bias, the conditional landmark analysis was carried out at cutoffs of 1, 2, and 3 months because patients who were prescribed to receive RT or RCT need sufficient time after diagnosis to get the corresponding therapy (19, 20).

A two-tailed *p*-value of less than 0.05 was considered statistically significant. All the statistical processes were performed in the STATA 16.0 software (StataCorp, College Station, TX, USA).

RESULTS

Baseline Characteristics

The sample selection procedure is shown in **Figure 1**. Of the 491 patients available for the final analysis, 321 (65.4%) were in the RCT group and 170 (34.6%) were in the RT group. The median follow-up of patients in the RCT and RT groups was 5 (interquartile range (IQR) 3–12) and 3 months (IQR 1–6), respectively. The SIPW-adjusted median OS was 4 (IQR 2–7) and 2 months (IQR 1–4) for patients in the RCT and RT groups. The RCT group had more patients aged less than 65 (49.5% *vs*. 29.40%, $p < 0.001$). More patients were male in the RCT group than in the RT group (46.7% *vs*. 37.1%, $p = 0.04$). In the RCT group, there were more patients with solitary tumor (67.0% *vs*. 63.5%, $p = 0.008$) and more patients without distant metastasis (57.3% *vs*. 42.4%, $p = 0.007$). Less patients in the RCT group received no surgery (48.0% *vs*. 60.6%, $p = 0.024$) (**Table 1**).

Prognostic Factors Associated With Overall Survival and Cancer-Specific Survival

As shown in **Table 2**, based on the multivariate Cox model, patients who underwent RCT had significantly improved OS than those who underwent RT alone both before (unadjusted sHR = 0.69, 95% CI = 0.56–0.84, $p < 0.001$) and after the SIPW (adjusted HR = 0.69, 95% CI = 0.56–0.85, $p < 0.001$). After the SIPW, older age (adjusted HR = 1.31, 95% CI = 1.07–1.62, $p = 0.011$), single marital status (adjusted HR = 1.67, 95% CI = 1.21–2.30, $p = 0.002$), distant metastasis (adjusted HR = 1.87, 95% CI = 1.52–2.30, $p < 0.001$), and more aggressive tumor extension (reference: group I; group IV: adjusted HR = 1.64, 95% CI = 1.17–2.30, $p = 0.004$) were all significant negative prognostic factors of OS. While surgical resection (reference: No; Nonthyroidectomy: adjusted HR = 0.68, 95% CI = 0.53–0.89, $p = 0.004$; Thyroidectomy: adjusted HR = 0.51, 95% CI = 0.40–0.66, $p < 0.001$) was a significant positive prognostic factors of OS. Moreover, year of diagnosis, race, gender, multifocality, lymph nodes invasion, and tumor size were not significant prognostic factors of OS (p all >0.05).

Similarly, in the multivariate CR model, patients who underwent RCT had improved CSS both before (unadjusted

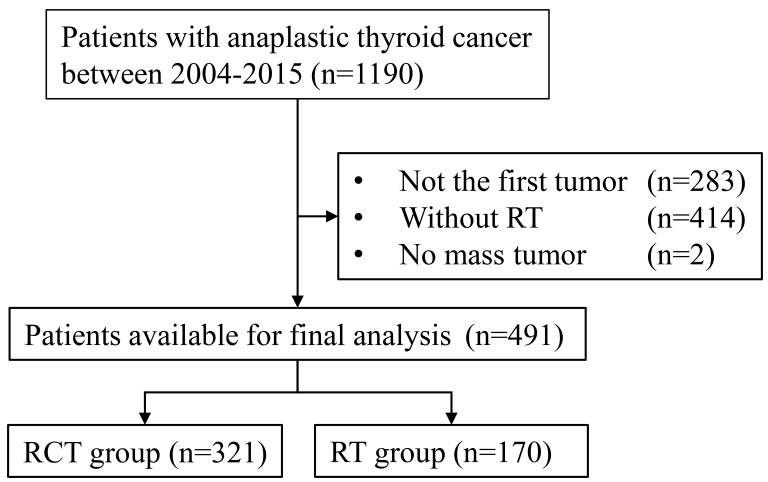

FIGURE 1 | Flowchart of the patient selection procedure. RCT, radiotherapy plus chemotherapy; RT, radiotherapy alone.

TABLE 1 | Baseline characteristics.

Characteristics	RT *N* (%)	RCT *N* (%)	*p*-value
Year of diagnosis			0.336
2004–2009	84 (49.4)	144 (44.9)	
2010–2015	86 (50.6)	177 (55.1)	
Race			
Black	14 (8.2)	22 (6.9)	0.245
White	135 (79.4)	273 (85.0)	
Other	21 (12.4)	26 (8.1)	
Age (year)			<0.001
<65	50 (29.4)	159 (49.5)	
≥65	120 (70.6)	162 (50.5)	
Gender			0.04
Female	107 (62.9)	171 (53.3)	
Male	63 (37.1)	150 (46.7)	
Marital Status			0.086
Married	94 (55.3)	200 (62.3)	
Single	15 (8.8)	40 (12.5)	
Divorced/separated/widowed	54 (31.8)	72 (22.4)	
Unknown	7 (4.1)	9 (2.8)	
Multifocality			0.008
Solitary	108 (63.5)	215 (67.0)	
Multifocal	21 (12.4)	61 (19.0)	
Unknown	41 (24.1)	45 (14.0)	
Lymph nodes invasion			0.595
N0	55 (32.4)	116 (36.1)	
N1NOS	17 (10.0)	26 (8.1)	
N1a	16 (9.4)	41 (12.8)	
N1b	59 (34.7)	102 (31.8)	
NX	23 (13.5)	36 (11.2)	
Distant metastasis			0.007
M0	72 (42.4)	184 (57.3)	
M1	87 (51.2)	121 (37.7)	
MX	11 (6.5)	16 (5.0)	
Tumor size (cm)			0.476
<2	4 (2.4)	3 (0.9)	
2–4	18 (10.6)	37 (11.5)	
≥4	114 (67.1)	227 (70.7)	
Unknown	34 (20.0)	54 (16.8)	
Surgery			0.024
No	103 (60.6)	154 (48.0)	
Nonthyroidectomy	26 (15.3)	57 (17.8)	
Thyroidectomy	41 (24.1)	110 (34.3)	
Tumor extension			0.188
I	26 (15.3)	44 (13.7)	
II	15 (8.8)	24 (7.5)	
III	42 (24.7)	74 (23.1)	
IV	66 (38.8)	157 (48.9)	
V	3 (1.8)	5 (1.6)	
VI	18 (10.6)	17 (5.3)	
Outcome			0.302
Alive	12 (7.1)	40 (12.5)	
Dead of other causes	7 (4.1)	10 (3.1)	
Dead attributable to ATC	148 (87.1)	266 (82.9)	
Dead of unknown cause	3 (1.8)	5 (1.6)	

RCT, radiotherapy plus chemotherapy; RT, radiotherapy alone; cm, centimeter; ATC, anaplastic thyroid cancer.

sHR = 0.75, 95% CI = 0.60–0.93, $p = 0.008$) and after the SIPW (adjusted sHR = 0.77, 95% CI = 0.61–0.96, $p = 0.018$) compared with those who underwent RT. After the SIPW, distant metastasis (adjusted sHR = 1.93, 95% CI = 1.54–2.41, $p < 0.001$), and more aggressive tumor extension (reference: group I; group IV: adjusted sHR = 1.73, 95% CI = 1.21–2.47, $p = 0.003$; group V: adjusted sHR = 1.78, 95% CI = 1.04–3.06, $p = 0.035$) were significant negative prognostic factors of CSS, while surgery (reference: No; Nonthyroidectomy: adjusted sHR = 0.66, 95% CI = 0.48–0.90, $p = 0.009$; thyroidectomy: adjusted sHR = 0.54, 95% CI = 0.42–0.70, $p < 0.001$) were significant positive prognostic factors of CSS. Furthermore, the

TABLE 2 | Multivariate Cox proportional hazard model of overall survival before and after inverse-probability weighting.

Characteristics	Origin cohort		IPW cohort	
	Unadjusted HR (95% CI)	*p*-value	Adjusted HR (95% CI)	*p*-value
Treatment				
RT	Reference		Reference	
RCT	0.69 (0.56–0.84)	<0.001	0.69 (0.56–0.85)	<0.001
Year of diagnosis				
2004–2009	Reference		Reference	
2010–2015	1.01 (0.84–1.21)	0.936	1.00 (0.82–1.21)	0.962
Race				
White	Reference		Reference	
Black	0.90 (0.67–1.21)	0.483	0.86 (0.64–1.17)	0.336
Other	1.11 (0.76–1.63)	0.575	1.11 (0.76–1.63)	0.578
Age (year)				
<65	Reference		Reference	
≥65	1.29 (1.06–1.57)	0.010	1.31 (1.07–1.62)	0.011
Gender				
Female	Reference		Reference	
Male	1.10 (0.92–1.32)	0.297	1.08 (0.89–1.32)	0.427
Marital status				
Married	Reference		Reference	
Single	1.56 (1.16–2.11)	0.003	1.67 (1.21–2.30)	0.002
Divorced/separated/widowed	1.15 (0.92–1.45)	0.228	1.16 (0.91–1.47)	0.238
Unknown	0.79 (0.45–1.38)	0.413	0.79 (0.45–1.38)	0.411
Multifocality				
Solitary	Reference		Reference	
Multifocal	0.80 (0.60–1.06)	0.118	0.79 (0.57–1.08)	0.143
Unknown	1.17 (0.93–1.46)	0.184	1.07 (0.85–1.35)	0.545
Lymph nodes invasion				
N0	Reference		Reference	
N1NOS	1.13 (0.84–1.52)	0.422	1.09 (0.79–1.49)	0.605
N1a	0.90 (0.65–1.24)	0.519	0.92 (0.66–1.27)	0.603
N1b	1.08 (0.86–1.37)	0.499	1.09 (0.84–1.40)	0.523
NX	0.86 (0.61–1.20)	0.375	0.82 (0.59–1.15)	0.248
Distant metastasis				
M0	Reference		Reference	
M1	1.88 (1.53–2.29)	<0.001	1.87 (1.52–2.30)	<0.001
MX	1.42 (0.93–2.17)	0.100	1.33 (0.83–2.13)	0.230
Tumor size (cm)				
<2	Reference		Reference	
2–4	0.77 (0.42–1.44)	0.418	0.78 (0.44–1.36)	0.379
≥4	0.94 (0.54–1.65)	0.830	0.95 (0.57–1.56)	0.835
Unknown	1.43 (0.78–2.64)	0.245	1.50 (0.87–2.58)	0.145
Surgery				
No	Reference		Reference	
Nonthyroidectomy	0.65 (0.51–0.83)	0.001	0.68 (0.53–0.89)	0.004
Thyroidectomy	0.52 (0.41–0.66)	<0.001	0.51 (0.40–0.66)	<0.001
Tumor extension				
I	Reference		Reference	
II	1.32 (0.87–2.02)	0.193	1.28 (0.81–2.01)	0.289
III	1.22 (0.88–1.70)	0.239	1.24 (0.87–1.78)	0.233
IV	1.60 (1.17–2.20)	0.003	1.64 (1.17–2.30)	0.004
V	1.42 (0.86–2.34)	0.169	1.39 (0.81–2.38)	0.236
VI	1.00 (0.64–1.58)	0.989	1.00 (0.61–1.62)	0.988

HR, hazard ratio; IPW, inverse-probability weighting; RCT, radiotherapy plus chemotherapy; RT, radiotherapy alone; cm, centimeter.

rest factors were also not significant prognostic factors of CSS (*p* all >0.05) (**Table 3**).

The SIPW adjusted Kaplan-Meier survival curves of CSS and OS are illustrated in **Figure 2**. Patients in the RCT group survived longer than those in the RT group, and the difference was statistically significant (Cox test *p* all <0.05).

Subgroup Analysis

To assess the effectiveness of RCT compared with that of RT within particular subsets of the ATC patients, we conducted SIPW-adjusted multivariate regressions for OS and CSS within each subgroup stratified by surgical resection and distant metastasis. The (s)HRs of RCT *versus* RT alone from

TABLE 3 | Multivariate Fine-Gray compete-risk model of cancer-specific survival before and after inverse-probability weighting.

Characteristics	Origin cohort		IPW cohort	
	Unadjusted sHR (95% CI)	*p*-value	Adjusted sHR (95% CI)	*p*-value
Treatment				
RT	Reference		Reference	
RCT	0.75 (0.60–0.93)	0.008	0.77 (0.61–0.96)	0.018
Year of diagnosis				
2004–2009	Reference		Reference	
2010–2015	0.91 (0.74–1.12)	0.368	0.88 (0.71–1.08)	0.22
Race				
White	Reference		Reference	
Black	0.91 (0.68–1.23)	0.558	0.91 (0.66–1.25)	0.559
Other	1.14 (0.79–1.64)	0.487	1.13 (0.77–1.66)	0.536
Age (year)				
<65	Reference		Reference	
≥65	1.13 (0.92–1.39)	0.237	1.15 (0.93–1.44)	0.204
Gender				
Female	Reference		Reference	
Male	0.90 (0.73–1.10)	0.297	0.86 (0.69–1.08)	0.207
Marital status				
Married	Reference		Reference	
Single	1.21 (0.85–1.72)	0.296	1.22 (0.82–1.82)	0.333
Divorced/separated/widowed	1.13 (0.89–1.43)	0.327	1.09 (0.86–1.40)	0.47
Unknown	0.78 (0.42–1.44)	0.427	0.71 (0.37–1.38)	0.317
Multifocality				
Solitary	Reference		Reference	
Multifocal	0.95 (0.73–1.24)	0.703	0.98 (0.72–1.32)	0.872
Unknown	1.22 (0.90–1.65)	0.208	1.16 (0.87–1.53)	0.311
Lymph nodes invasion				
N0	Reference		Reference	
N1NOS	1.09 (0.78–1.52)	0.619	1.06 (0.75–1.49)	0.759
N1a	0.85 (0.60–1.19)	0.333	0.86 (0.61–1.22)	0.408
N1b	0.88 (0.69–1.12)	0.292	0.82 (0.62–1.08)	0.15
NX	0.98 (0.71–1.36)	0.919	0.92 (0.67–1.26)	0.593
Distant metastasis				
M0	Reference		Reference	
M1	1.97 (1.60–2.44)	<0.001	1.93 (1.54–2.41)	<0.001
MX	1.39 (0.89–2.19)	0.151	1.31 (0.78–2.19)	0.306
Tumor size (cm)				
<2	Reference		Reference	
2–4	1.01 (0.53–1.95)	0.967	1.05 (0.58–1.93)	0.863
≥4	1.18 (0.64–2.16)	0.603	1.20 (0.68–2.10)	0.526
Unknown	1.48 (0.77–2.86)	0.241	1.56 (0.84–2.88)	0.156
Surgery				
No	Reference		Reference	
Nonthyroidectomy	0.67 (0.51–0.88)	0.003	0.66 (0.48–0.90)	0.009
Thyroidectomy	0.57 (0.46–0.73)	<0.001	0.54 (0.42–0.70)	<0.001
Tumor extension				
I	Reference		Reference	
II	1.52 (0.96–2.38)	0.072	1.47 (0.88–2.44)	0.141
III	1.34 (0.95–1.89)	0.098	1.34 (0.92–1.96)	0.126
IV	1.66 (1.19–2.31)	0.003	1.73 (1.21–2.47)	0.003
V	1.77 (1.08–2.90)	0.025	1.78 (1.04–3.06)	0.035
VI	1.15 (0.73–1.81)	0.555	1.12 (0.69–1.83)	0.642

sHR, subdistribution hazard ratio; IPW, inverse-probability weighting; RCT, radiotherapy plus chemotherapy; RT, radiotherapy alone; cm, centimeter.

multivariate regression within each subgroup are summarized in a forest plot (**Figure 3**). Moreover, the significant beneficial effects of RCT compared with RT alone on OS and CSS were present within all the subgroups, except for the surgical resection subgroup for CSS.

Landmark Analysis

In the landmark analysis, the beneficial effect of RCT remained persistent but lost statistical significance within most of the subgroups, except for the consistently significant efficacy of RCT within patients having no distant metastasis (**Table 4**).

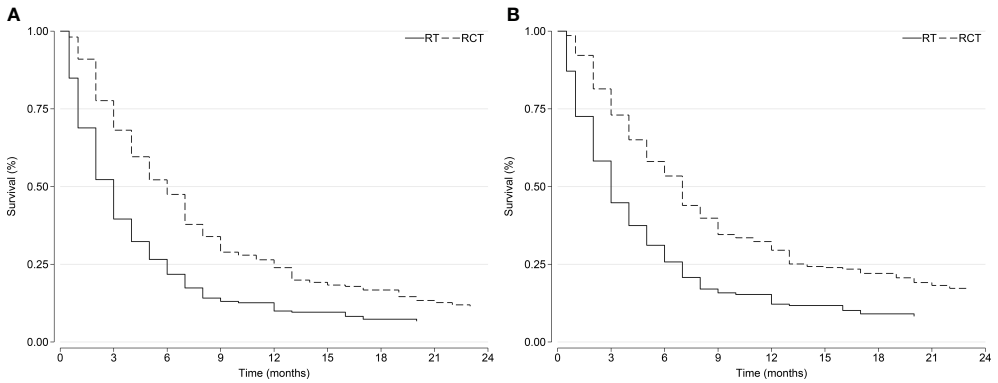

FIGURE 2 | Overall survival and cancer-specific survival curves after the inverse probability weighting: **(A)** overall survival and **(B)** cancer-specific survival. RCT, radiotherapy plus chemotherapy; RT, radiotherapy alone.

DISCUSSION

The critical findings of this study are that RCT leads to significantly prolonged OS and CSS in ATC patients compared with RT alone, regardless of surgical resection and distant metastasis. To our knowledge, this study is the first large-scale retrospective study comprehensively comparing RCT with RT alone within different subsets of ATC patients, controlling for some factors never adjusted in previous studies, such as multifocality and tumor extension. With SIPW adjustment and subgroup analysis carried out, this study obtains robust results about the superior effectiveness of RCT *versus* RT. Our study adds to the supportive evidence of the preferential applying of RCT in treating ATC patients. We investigated both the OS and CSS because OS has the least methodological issues, consistent with the AJCC publications. However, clinicians and patients are commonly more interested in CSS despite the potential obstacle in reliably determining the cause of death (21).

Overall, the median OS of patients undergoing RCT was twice that of those undergoing RT alone (4 *vs*. 2 months). IPW-adjusted multivariate regressions show the adjusted HR and sHR of RCT *versus* RT alone were 0.69 (95% CI = 0.56–0.85) and 0.77 (95% CI = 0.61–0.96) for OS and CSS, respectively. A similar positive effect of RCT *versus* RT alone was also present within each subgroup by surgical resection and distant metastasis. Notably, the superior role of RCT compared with RT in ATC with distant metastasis is especially promising and of great clinical importance.

The beneficial effect of chemotherapy was found in a study with 79 ATC patients and another study based on the National Cancer Database (12, 22). However, some other small studies failed to identify the positive role of chemotherapy in ATC

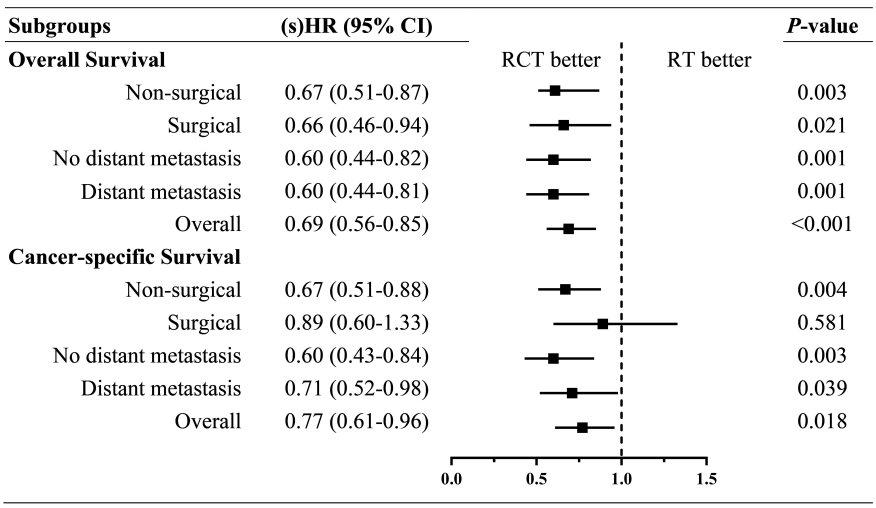

FIGURE 3 | Forest plot of (sub distribution) hazard ratios of RCT *versus* RT within each subgroup by surgical resection and distant metastasis. sHR, subdistribution hazard ratio; HR, hazard ratio.

TABLE 4 | Adjusted (subdistribution) hazard ratios of RCT *versus* RT overall and within each subgroup by surgical resection and distant metastasis after the inverse-probability weighting in the landmark analysis at cutoffs of 1, 2, and 3 months.

Subgroups	1 Month		2 Months		3 Months	
	N	Adjusted (s)HR (95% CI)	*N*	Adjusted (s)HR (95% CI)	*N*	Adjusted (s)HR (95% CI)
Overall survival						
Overall	455	0.77 (0.62–0.96)*	407	0.82 (0.64–1.04)	334	0.88 (0.66–1.16)
Nonsurgical	219	0.85 (0.64–1.13)	182	0.83 (0.60–1.15)	133	0.81 (0.52–1.28)
Surgical	232	0.67 (0.47–0.96)*	222	0.76 (0.52–1.10)	195	0.88 (0.58–1.34)
No distant metastasis	243	0.62 (0.45–0.85)*	230	0.66 (0.48–0.91)*	204	0.62 (0.44–0.88)*
Distant metastasis	188	0.77 (0.55–1.06)	157	0.80 (0.55–1.16)	100	1.10 (0.70–1.73)
Cancer-specific survival						
Overall	455	0.84 (0.67–1.07)	407	0.87 (0.67–1.13)	334	0.91 (0.68–1.22)
Nonsurgical	219	0.79 (0.59–1.06)	182	0.77 (0.55–1.08)	133	0.88 (0.54–1.43)
Surgical	232	0.91 (0.61–1.36)	222	0.92 (0.61–1.38)	195	0.85 (0.55–1.31)
No distant metastasis	243	0.58 (0.41–0.82)*	230	0.62 (0.44–0.87)*	204	0.59 (0.40–0.87)*
Distant metastasis	188	0.94 (0.67–1.33)	157	0.96 (0.64–1.43)	100	1.26 (0.77–2.06)

(s)HR, (subdistribution) hazard ratio; RCT, radiotherapy plus chemotherapy; RT, radiotherapy alone.
**p-value < 0.05.*

patients (10, 11). Given the limited sample size of the previous studies, multivariate regression carried out within subsets of ATC patients was unfeasible, such that imbalanced confounding factors may bias their results. A study showed that weekly paclitaxel administration results in significantly prolonged survival but not conventional chemotherapy using doxorubicin or cisplatin (23). However, a study including 100 ATC patients found that any chemotherapy regimen was associated with more prolonged survival (15).

Despite novel therapy, including new regimens and timely intense multimodal treatment, advancement in treating ATC has been very limited. Thus, there is a continuing need to develop more treatment patterns (24). Under the condition of limited treatments, the extended applying of traditional treatment is of great clinical significance. Previous studies have indicated the potential role of chemotherapy in ATC patients but have not comprehensively compared RCT with RT alone. Our study comprehensively compared RCT with RT and suggested the beneficial effect of RCT regardless of surgical resection and distant metastasis. However, confirmatory studies, phase II or possibly phase III studies, are still required and must be designed to define the role of RCT and RT in ATC patients. This is because after the landmark analysis to adjust for potential immortal time bias, the consistently beneficial effect of RCT relative to RT was only seen within ATC patients without distant metastasis. In contrast, the beneficial effects for other subgroups lost statistical significance slightly, although that might be led to by the smaller sample size after landmark analysis that sacrificed samples considerably. Moreover, It is worth mentioning that landmark analysis could also disregard the short-time survival benefit from RCT, particularly for this extremely aggressive cancer (20, 25).

Currently, the most frequently used chemotherapy agents include those against cell division machinery (taxane, paclitaxel, or docetaxel), against DNA repair pathways (anthracycline or doxorubicin), and against DNA structure (cisplatin or carboplatin) (15, 26). Moreover, the most recommended application of cytotoxic chemotherapy is taxane monotherapy in combination with anthracyclines or platin if necessary (26).

Consistent with previous studies, total thyroidectomy provides the best survival outcome compared with nonthyroidectomy and nonsurgical resection in this study (4, 14). An explanation for that is that more aggressive surgery types could contribute to better local control. Distant metastasis was an adverse prognostic factor of survival in our study, consistent with the previous studies (10, 16, 22). Moreover, we found that RCT produces improved survival for both distant metastatic ATC and nondistant metastatic ATC, highlighting the importance of RCT. In our study, the continuous growth of tumors was also explored and controlled. The results show that involving the thyroid cartilage, carotid artery (encased), jugular vein and thyroid artery or vein, bone, skeletal muscle (other than the strap or sternocleidomastoid muscle), mediastinal tissues, and prevertebral fascia was associated with worse survival. Clinicians should carefully consider the continuous growth of ATC.

Interestingly, we failed to find the association of lymph node invasion (AJCC N stage) with OS and CSS, just like a previous study (14). A possible explanation for that may lie in that the extremely short survival of ATC patients leads to the statistical insignificance of lymph node invasion for survival. Similarly, no association of tumor size with survival was found in our study, in line with a small study (16) but contrary to two previous studies presenting a negative correlation of tumor size with survival (14, 27). This discrepancy could be attributed to inadequate confounding factors controlled and small sample size.

At present, there have been no more efficient treatments developed for ATC patients, except for BRAF V600E-mutated ATC that could highly respond to dabrafenib plus trametinib and have a promising outcome (9, 26). A recent study suggested a most promising result of 1 year OS of 94% when applying dabrafenib plus trametinib to BRAF V600E-mutated ATC patients (2). Nevertheless, for ATC without BRAF V600E mutation, timely multimodal and multidisciplinary treatment

within high-volume expertise organizations is still the best treatment approach. Our study adds to the evidence of preferentially applying RCT *versus* RT to ATC patients regardless of surgical resection and distant metastasis.

This study has some limitations. (1) This study covered so long a period from 2004 to 2015 that missing factors may bias our findings. Although the year of diagnosis was divided into two intervals at the year 2010 and controlled in multivariate regression. (2) As the nature of the retrospective study, there might be missing confounders that may be important for analysis, which would lead to bias in our research. For example, we did not know the detailed location and the margin status after surgical resection, even though margin status was not associated with survival due to the extreme dismal prognosis of ATC (28). (3) Although variables between groups were balanced using SIPW, the unbalanced confounders of older age, multifocality, metastasis, and tumor extension might still affect the results of this study. Moreover, unmeasured confounding factors may still bias our results. (4) The detailed regimens and doses of chemotherapy were not available in the SEER database, so we could not further explore them. (5) The protocol of radiotherapy referring to dose, fraction size, frequency, and duration was not recorded by the SEER database; thus, the total dose of radiotherapy that could have a tremendous impact on survival was not available and controlled, although the so considered palliative volume was found to be superior to no radiation (22).

Although our study has some limitations, it is the first large retrospective study comprehensively investigating the superior effectiveness of RCT *versus* RT within subsets of ATC patients. Our study suggests the beneficial role of RCT for ATC. Our findings will give support to clinicians to preferentially perform RCT in ATC patients.

CONCLUSIONS

RCT results in significantly prolonged survival in ATC patients, regardless of surgical resection and distant metastasis. RCT should be preferentially performed in ATC patients. Further prospective trials with chemotherapy regimens and radiotherapy doses controlled are needed.

DATA AVAILABILITY STATEMENT

Publicly available datasets were analyzed in this study. These data can be found here: https://seer.cancer.gov/.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

WZ: Data curation, formal analyses, writing—original draft preparation, and writing—reviewing and editing. YY: Conceptualization, formal analyses, methodology, software, supervision, visualization, writing—original draft preparation, and writing—reviewing and editing. XZ: Conceptualization, data curation, formal analyses, supervision, writing—original draft preparation, and writing—reviewing and editing. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

An ethical review process was not required for this study because the data were obtained from the Surveillance, Epidemiology, and End Results (SEER) database, and we have signed the Data-use Agreement for the SEER 1975–2016 Research Data File.

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Tracheal and Cricotracheal Resection With End-to-End Anastomosis for Locally Advanced Thyroid Cancer: A Systematic Review of the Literature on 656 Patients

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Airway involvement by advanced thyroid carcinoma (TC) constitutes a negative prognosticator, besides being a critical clinical issue since it represents one of the most frequent causes of death in locally advanced disease. It is generally agreed that, for appropriate laryngo-tracheal patterns of invasion, (crico-)tracheal resection and primary anastomosis [(C)TRA] is the preferred surgical technique in this clinical scenario. However, the results of long-term outcomes of (C)TRA are scarce in the literature, due to the rarity of such cases. The relative paucity of data prompts careful review of the available relevant series in order to critically evaluate this surgical technique from the oncologic and functional points of view. A systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement on the PubMed, Scopus, and Web of Science databases. English-language surgical series published between January 1985 and August 2021, reporting data on ≥5 patients treated for TC infiltrating the airway by (C)TRA were included. Oncologic outcomes, mortality, complications, and tracheotomy-dependency rates were assessed. Pooled proportion estimates were elaborated for each end-point. Thirty-seven studies were included,

encompassing a total of 656 patients. Pooled risk of perioperative mortality was 2.0%. Surgical complications were reported in 27.0% of patients, with uni- or bilateral recurrent laryngeal nerve palsy being the most common. Permanent tracheotomy was required in 4.0% of patients. Oncologic outcomes varied among different series with 5- and 10-year overall survival rates ranging from 61% to 100% and 42.1% to 78.1%, respectively. Five- and 10-year disease specific survival rates ranged from 75.8% to 90% and 54.5% to 62.9%, respectively. Therefore, locally advanced TC with airway invasion treated with (C)TRA provides acceptable oncologic outcomes associated with a low permanent tracheotomy rate. The reported incidence of complications, however, indicates the need for judicious patient selection, meticulous surgical technique, and careful postoperative management.

Keywords: thyroid cancer, airway, surgery, tracheal resection, crico-tracheal resection

INTRODUCTION

Advanced resectable (T4a) thyroid cancer (TC) is a relatively uncommon clinical scenario, especially when dealing with differentiated tumors, being reported in just 5-15% of papillary carcinomas (1–4). This condition is associated with a significantly lower long-term survival rate compared to early-stage disease (1, 5, 6), particularly when the macroscopic extra-thyroidal extension involves more than one adjacent anatomical structure (7).

Aerodigestive tract invasion is more often seen in locally recurrent differentiated thyroid carcinoma (DTC) than at initial presentation. On the other hand, 60-70% of patients with such advanced neoplasms will have poorly differentiated or anaplastic carcinomas (8). The most frequently involved neighboring structures (after the strap muscles and recurrent laryngeal nerves [RLN]) are the upper trachea and laryngo-tracheal junction, due to their anatomic contiguity and relationship with the thyroid gland (9, 10), with a reported incidence of invasion of 0.4-0.7% of all TC (11). In descending order of frequency, the fourth and fifth most affected structures are the pharyngo-esophageal conduit and major vessels in the neck (8–10). The source of aerodigestive tract involvement is most frequently the primary tumor, while metastatic lymph nodes are responsible for less than 20% of cases (8).

Airway invasion by TC typically occurs in men (twice more frequently than in females), with a peak incidence in the sixth decade (11) and, usually, involves tumors larger than 3.7 cm (3, 12). Although rare, airway invasion has also been reported in the younger age group, considered to be in the "low-risk" prognostic category (13). Uncontrolled tumor progression in the airway represents one of the most frequent causes of death for TC, especially in the presence of unresectable tumors or loco-regional disease in which complete resection was not achieved (6, 14–16). Thus, in order to increase the chance of cure of these advanced neoplasms invading the airway, the first goal is to achieve a R0 resection within negative margins (5, 17, 18). However, due to the relative paucity of large series on this topic and in the absence of any prospective trials, the indications and comparative outcomes of different surgical techniques for airway management in advanced TC are still a matter of debate. There is general agreement that shaving the tumor off from the laryngo-tracheal axis is acceptable when the lesion involves only its external perichondrium (Shin I according to the classification by Shin et al.) (19), but there is no consensus on the best surgical technique for more extensive tumors (infiltrating the full-thickness of the cartilage [Shin II] or through it into the submucosa [Shin III] or the tracheal lumen [Shin IV]). Essentially, there are two different schools of thought: on one side, window resection with primary or secondary closure of the airway gap by soft tissue local flaps (20, 21) and, on the other, circumferential (crico-)tracheal resection with primary end-to-end anastomosis ([C]TRA) (22). Other groups have tried to design comprehensive, but somewhat cumbersome, algorithms in which both procedures can be performed according to the site, length, and width of airway involvement (8, 23). Head-to-head oncologic comparisons between these two surgical approaches are seriously limited by the low incidence of this condition, the heterogeneity of patients treated, and significant selection biases due to the retrospective nature of the studies. On the other hand, it is possible to objectively analyze postoperative morbidity, complication rates, and quality of life reported in the literature for each type of surgical technique.

The aim of this systematic review was to collect all the available English-language surgical series published between January 1985 and August 2021, reporting data on ≥5 patients treated for TC infiltrating the airway by (C)TRA, to better understand oncologic outcomes, complication rates, and airway-related quality of life.

MATERIALS AND METHODS

Article Collection

A systematic review of the literature was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (24). The search was simultaneously conducted on the PubMed, Scopus, and Web of Science online

databases, and updated to August 16, 2021. In order to retrieve all the publications dealing with (C)TRA for laryngo-tracheal involvement by TC the query string was composed as (tracheal resection) OR (tracheal involvement) AND (thyroid cancer) OR (thyroid neoplasm) OR (thyroid tumor). The search was conducted by two authors (C.P. and D.L.) who independently assessed the eligibility of the studies by screening article titles and abstracts, and then discussed their inclusion by reading the full-text of the selected works. Discrepancies were clarified by discussion between authors.

Eligibility Criteria

The Population/problem Intervention/exposure Comparison, Outcome, and Study design (PICOS) model was adopted for the review (25) (**Table 1**). The inclusion criteria were as follows: English language, publication from January 1, 1985 to the last day of online search (August 16, 2021), articles including data on (C)TRA for airway involvement by TC and reporting a case series of at least 5 patients. Exclusion criteria were: case reports, case series with less than 5 patients, papers purely describing results of surgical techniques different from (C)TRA (e.g., shaving, window resections, total laryngectomy) or that did not report sufficient data on outcomes and complications and focused on other related issues (e.g., radiological or clinical diagnosis, anesthetic issues, adjuvant treatments). Additionally, papers with duplicated or overlapping data from the same center were excluded, maintaining, when possible, the largest and more recent study among those available. Finally, a case series published by the first author (C.P.) (26), already included in this systematic review, was updated with data of patients treated from the time of the article publication (2016) to date, and their oncologic outcomes updated accordingly.

Quality Assessment

For each paper included in the systematic review, at the end of the selection process, evaluation of its quality was carried out following the Newcastle-Ottawa Scale (NOS) adapted for cross-sectional, cohort and case-control studies (27). The NOS was considered the evaluation method of choice, based on the recent literature (28, 29). The quality assessment was independently estimated by two different authors (D.L. and M.T.).

Data Collection and Statistical Analysis

Data on study design, number of patients, age, gender, diagnostic work-up, TC histology and degree of airway invasion, length and type of resection, perioperative mortality, surgical complications, rate of patients who remained tracheostomy-dependent after (C)TRA, and oncological outcomes were collected, and a specific database was built.

The primary outcome was proportion of patients who developed a complication, calculated as the number of patients with reported complications divided by the total number of patients treated by (C)TRA for TC. Secondary outcome was the proportion of tracheostomy-dependent patients, defined as the number of patients with long-term tracheostomy dependency divided by the total number of patients treated by (C)TRA.

Meta-analysis of proportions was conducted through a generalized linear mixed model based on logit transformation (30). Pooled analyses are presented as forest plots. For each study, proportions and relative 95% confidence interval (CI) are depicted as gray squares and horizontal lines, respectively. The weight of each study on the overall effect estimate is reported and represented by the square size. The pooled proportion estimate and relative 95% CI, depicted as a diamond, are reported at the bottom of the forest plot. Heterogeneity between studies was assessed with Higgins I^2 and τ^2 tests (31), defined as low if I^2<25%, moderate if between 25-50%, and substantial if >50% (32).

Publication bias was assessed through funnel plot assessment. Statistical analysis was performed with R (version 4.0.5, R foundation for Statistical Computing, Vienna, Austria). Statistical significance was defined as $p<0.05$.

RESULTS

Article Collection

The initial literature search yielded 1196 titles (525 records came from the PubMed database, 407 from Scopus, and 264 from Web of Science). Among these, 519 articles were excluded because present in two databases, and 147 due to publication in a language other than English. One article (33) was added from other sources, after being identified through the references of other manuscripts. Three-hundred-seventy-three articles were excluded after review of the title, and 48 by the abstract. From the remaining 110 full-text articles, 73 were excluded because they did not meet the eligibility criteria. Finally, 37 papers (3, 26, 33–67) were considered appropriate for the present systematic review (**Figure 1**, **Table 2**).

Quality Assessment

According to the NOS adapted for cross-sectional studies (range of the scale, 0-9), the scores ranged from 2 to 7 (median, 5). Detailed scores for each article are reported in **Table 3**. All included manuscripts were retrospective single institution cross-sectional studies, except for one (42), which was a retrospective bi-institutional case series.

Study Population, Perioperative Mortality, and Complications

Overall, 656 patients were included in the current systematic review. Gender of patients treated by (C)TRA was detailed in 18

TABLE 1 | PICOS model for the present systematic review.

P (population)	656 patients from 37 studies adhering to the inclusion criteria detailed in Materials and Methods
I (intervention)	(Crico-)tracheal resection and anastomosis for thyroid cancer invading the airway
C (comparator)	No comparison was intentionally performed with other surgical techniques and/or treatment modalities
O (outcomes)	Perioperative mortality, complication, postoperative tracheostomy-dependency rates, and oncologic outcomes
S (study design)	Systematic review

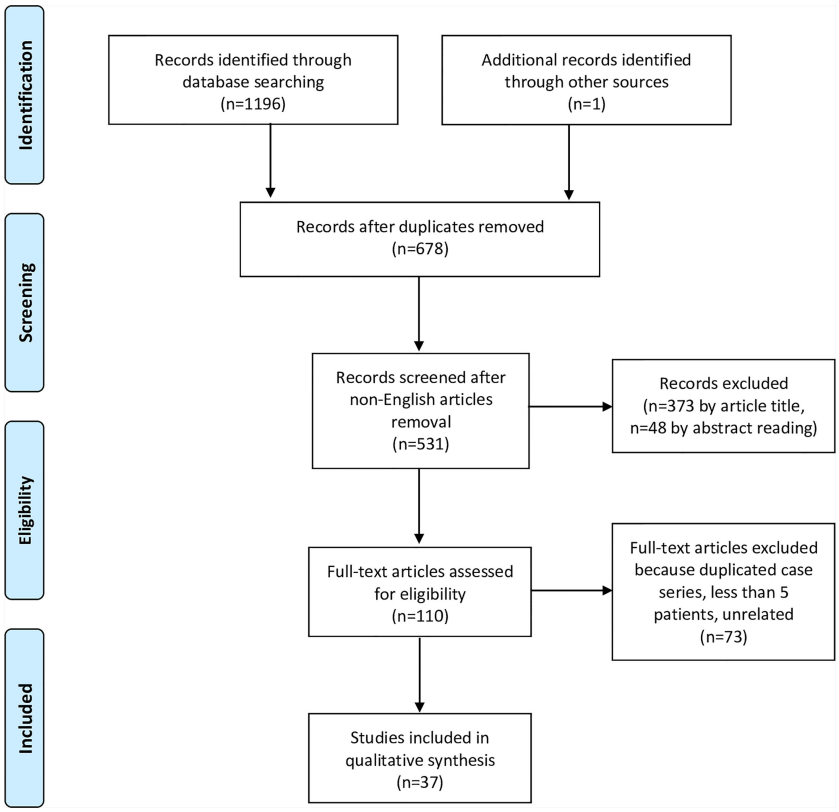

FIGURE 1 | Flowchart showing the study selection process according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.

TABLE 2 | Studies included in the systematic review of English-language, non-overlapping, surgical series including ≥5 patients treated by (C)TRA for TC invading the airway between January 1985 and August 2021 (No. of series=37, No. of patients=656). The table reports details about TC histotype, mortality, complications, tracheostomy-dependency, and oncologic outcomes.

Study no.	Author (Institution)	Year	No. of pts.	Histotypes	Perioperative mortality rate	Complication rate	Tracheostomy-dependency rate	Oncologic outcomes
1	Tovi et al. (33) (Soroka University, Beer-Sheva, Israel)	1985	5	DTC	0%	NA	0%	NA
2	Fujimoto et al. (34) (Tokyo Women's Medical College Hospital, Tokyo, Japan)	1986	6	DTC	0%	17% (permanent hypoparathyroidism x1)	0%	100% alive and well, 18-53 mos
3	Ishihara et al. (35) (Keyo University, Tokyo, Japan)	1991	60	Mixed	0%	47% (bilateral RLN palsy x21, temporary dysphagia x3, hypoparathyroidism x2, anastomotic stenosis x2, pharyngeal stenosis x1, vocal fold edema x1)	13% (permanent tracheotomy for bilateral RLN palsy x8)	10-yr OS 78.1% (in 34 R0 pts) 10-yr OS 22% (in 26 R1-R2 pts)
4	Maeda (36) (Kakawa Medical School, Kagawa, Japan)	1993	44	Mixed	0%	2% (bilateral RLN palsy x1)	NA	NA
5	Ozaki et al. (37) (Ito Hospital, Tokyo, Japan)	1995	21	Mixed	0%	5% (bilateral RLN palsy x1)	5% (permanent tracheotomy for bilateral RLN palsy x1)	76% alive and well, 17-121 mos 19% alive with distant

(Continued)

TABLE 2 | Continued

Study no.	Author (Institution)	Year	No. of pts.	Histotypes	Perioperative mortality rate	Complication rate	Tracheostomy-dependency rate	Oncologic outcomes
								metastases, 21-99 mos 5% dead for unrelated causes at 12 mos
6	Zannini et al. (38) (San Raffaele Hospital, University of Milan, Italy)	1996	8	DTC	0%	25% (anastomotic granulomas x2)	0%	50% alive and well, 11-109 mos 25% alive with distant metastases, 75-127 mos 25% dead for distant or regional disease, 26-32 mos
7	Musholt et al. (39) (Hannover University Medical School, Hannover, Germany)	1999	11	Mixed	9% (prolonged assisted ventilation with multiorgan failure x1)	18% (aspiration pneumonia x1, prolonged ICU treatment x1)	0%	64% alive and well, 3-43 mos 18% dead for local or distant disease, 8-25 mos 9% dead for unrelated causes, 42 mos
8	Yang et al. (40) (Veterans General Hospital – Taipei and National Yang-Ming University, Taipei, Taiwan)	2000	8	DTC	0%	12% (anastomotic leak x1)	0%	62% alive and well, 14-183 mos 38% alive with local, regional or distant disease, 39-71 mos
9	Koike et al. (41) (Noguchi Thyroid Clinic and Hospital Foundation, Oita, Japan)	2001	7	DTC	0%	NA	NA	100% alive and well, 15-22 mos
10	Kato et al. (42) (St. Marianna, Kawasaki and Yamagata University School of Medicine, Yamagata, Japan)	2003	18	Mixed	0%	5% (unilateral RLN palsy x1)	0%	NA
11	Nakao et al. (43) (Osaka Police Hospital, Osaka, Japan)	2004	40	DTC	7% (anastomotic dehiscence and fatal bleeding x2, anastomotic dehiscence and mediastinitis x1)	30% (anastomotic dehiscence x4, unilateral RLN palsy x3, pneumonia x3, bilateral RLN palsy x1, acute myocardial infarction x1)	7% (bilateral RLN palsy x3)	10-yr OS 67.7%
12	Tsai et al. (44) (National Cheng Kung University Hospital and Chi-Mei Hospital, Taiwan, China)	2005	16	DTC	6% (anastomotic dehiscence and fatal bleeding x1)	25% (anastomotic dehiscence x2, anastomotic granulomas x2)	0%	5-yr OS 88%
13	Wada et al. (45) (Yokohama City University Medical Center, Kanagawa, Japan)	2006	5	DTC	0%	20% (bilateral RLN palsy x1)	20% (bilateral RLN palsy x1)	5-yr DSS 83.9% 10-yr DSS 62.9%

(Continued)

TABLE 2 | Continued

Study no.	Author (Institution)	Year	No. of pts.	Histotypes	Perioperative mortality rate	Complication rate	Tracheostomy-dependency rate	Oncologic outcomes
14	Segal et al. (3) (Rabin Medical Center, Petah Tiqva, Israel)	2006	6	DTC	0%	NA	NA	5-yr OS 75%
15	Gaissert et al. (46) (Massachusetts General Hospital, Boston, Massachusetts)	2007	69	Mixed	1% (glottic edema with fatal respiratory insufficiency x1)	61% (temporary tracheostomy x13, other complications x7, bilateral RLN palsy x6, permanent hypoparathyroidism x5, aspiration x5, anastomotic dehiscence x3, dysphagia x3)	4% (bilateral RLN palsy x2, anastomotic dehiscence x1)	15-yr OS 26% (in DTC pts) 15-yr DFS 22% (in DTC pts)
16	Brauckhoff et al. (47) (University Hospital Halle, Saale, Germany)	2010	16	Mixed	6% (fatal anastomotic dehiscence x1)	31% (anastomotic dehiscence x2, esophageal fistula x2, other x1)	0%	5-yr DSS 85.1% 10-yr DSS 73.8%
17	Mutrie et al. (48) (Emory University School of Medicine, Atlanta, Georgia)	2011	9	Mixed	0%	NA	0%	5-yr OS 80%
18	Shadmehr et al. (49) (Shahid Beheshti University of Medical Sciences, Tehran, Iran)	2012	18	Mixed	4% (anastomotic dehiscence and fatal mediastinitis x1)	30% (anastomotic dehiscence x2, unilateral RLN palsy x2, permanent hypoparathyroidism x1, temporary tracheostomy x1)	0%	5-yr OS 61% (immediate resection group)* 5-yr OS 28% (delayed resection group)*
19	Ch'ng et al. (50) (Sidney Head and Neck Cancer Institute, Australia)	2012	6	Mixed	0%	0%	33%[§] (bilateral RLN palsy x2)	NA
20	Mossetti et al. (51) (Ospedale Molinette, University of Turin, Italy)	2013	8	Mixed	0%	62% (transient hypoparathyroidism x3, anastomotic leak x2, bleeding x1)	0%	12% DOD, 30 mos 63% AWD, 3-67 mos 25% NED, 10-25 mos
21	Morisod et al. (52) (University Hospital CHUV, Lausanne, Switzerland)	2014	6	Mixed	17% (massive anastomotic dehiscence with tracheo-innominate fistula x1)	50% (minor tracheal dehiscence x1, pneumonia x1, SIADH x1)	0%	17% DOC, perioperative death 17% DOD, 2 mos 66% NED, 6-41 mos
22	Lin et al. (53) (Memorial Hospital of Sun Yat-sen University, Guangzhou, China)	2014	19	DTC	5% (esophageal fistula x1)	26% (bilateral RLN palsy x2, esophageal fistula x2, anastomotic dehiscence x2, anastomotic stenosis x1)	16% (bilateral RLN palsy x1, anastomotic dehiscence x2)	5% DOC, 3 mos 10% AWD, 11-30 mos 85% NED, 2-55 mos
23	Hartl et al. (54) (Institute Gustave Roussy, Paris, France)	2014	23	Mixed	NA	NA	NA	5- and 10-yr OS 73% and 59%° 5- and 10-yr LC 83% (100% for R0 and 75% for R1)° 5- and 10-yr DSS 89% (95% for R0 and 84% for R1)°

(Continued)

TABLE 2 | Continued

Study no.	Author (Institution)	Year	No. of pts.	Histotypes	Perioperative mortality rate	Complication rate	Tracheostomy-dependency rate	Oncologic outcomes
24	Ranganath et al. (55) (Kidwai Memorial Institute of Oncology, Bengaluru, India)[£]	2015	10	Mixed	10% (chyle leak and septicaemia x1)	70% (hypoparathyroidism x7, aspiration x1)	0%	100% alive and well, 3-24 mos
25	Peng et al. (56) (The Second Xiangya Hospital of Central South University, Changsha, China)	2015	14	Mixed	0%	14% (anastomotic dehiscence x1, tracheomalacia x1)	7% (anastomotic dehiscence x1)	NA
26	Pappalardo et al. (57) (University of Insubria, Varese, Italy)	2016	7	Mixed	0%	0%	0%	100% NED, 18-108 mos
27	Kim et al. (58) (Seoul National University Hospital, Seoul, Republic of Korea)	2016	37	DTC	0%	NA	NA	5-yr DSS 90% 10-yr DSS 85%
28	Avenia et al. (59) (Santa Maria Hospital, University of Perugia, Italy)	2016	28	DTC	0%	32% (hypoparathyroidism x3, aspiration x2, dysphagia x1, anastomotic dehiscence x2, bilateral RLN palsy x1)	7% (bilateral RLN palsy x1, anastomotic dehiscence x1)	5-yr OS 70%*
29	Su et al. (60) (MD Anderson Cancer Institute. Houston, Texas)	2016	7	DTC	0%	NA	NA	NA
30	Wang et al. (61) (Memorial Sloan Kettering Cancer Center, New York, New York)	2016	7	DTC	0%	28% (temporary tracheotomy x2)	0%	NA
31	Piazza et al.[#] (26) (Spedali Civili, University of Brescia, Italy)	2016	33	Mixed	0%	28% (anastomotic dehiscence x3, unilateral RLN palsy x2, bilateral RLN palsy x1, bleeding x1, pulmonary embolism x1, pneumonia x1)	3% (bilateral RLN palsy x1)	5-yr OS (entire series) 63.4% 10-yr OS (entire series) 42.1% 5-yr OS DTC 81.8% 10-yr OS DTC 52.2% 5-yr OS non-DTC 12.5% 10-yr OS non-DTC 12.5% 5-yr DSS (entire series) 75.8% 10-yr DSS (entire series) 54.5% 5-yr DSS DTC 86.1% 10-yr DSS DTC 59.9% 5-yr DSS non-DTC 50% 5-yr DSS non-DTC 50%
32	Chen et al. (62) (Shandong Cancer Hospital, Jinan, China)	2017	21	DTC	0%	43% (temporary dysphagia x11, temporary hypoparathyroidism x9, air leak x5)	0%	5-yr OS 100% 5% DOC, 72 mos 5% DOD, 74 mos 28% AWD, 19-61 mos

(Continued)

TABLE 2 | Continued

Study no.	Author (Institution)	Year	No. of pts.	Histotypes	Perioperative mortality rate	Complication rate	Tracheostomy-dependency rate	Oncologic outcomes
33	Gupta et al. (63) (Basavatarakam Indo American Cancer Hospital & Research Institute, Hyderabad, India)	2020	11	DTC	0%	64% (temporary hypoparathyroidism x7, temporary tracheotomy x1)	0%	62% NED, 8-78 mos 81.2% OS (median follow-up 41 mos)
34	Chen et al. (64) (Sichuan Cancer Hospital, Chengdu, China)	2020	5	Mixed	0%	NA	NA	100% alive and well, 24-40 mos
35	Tiwari et al. (65) (Chennai Cancer Institute, Tamil Nadu, India)	2020	23	Mixed	0%	39% (air leak x5, bleeding x2, anastomotic dehiscence x1, aspiration x1)	0%	5-yr OS° 81.7% 10-yr OS° 47.8% 15-yr OS° 35.9%
36	Sharanappa et al. (66) (Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India)	2021	5	DTC	0%	20% (bleeding x1)	20% (bilateral RLN palsy x1)	5-yr OS 80%
37	Chakravarthy et al. (67) (Christian Medical College, Vellore, Tamil Nadu, India)	2021	19	Mixed	0%	36%° (temporary tracheotomy x1, temporary hypoparathyroidism x6, permanent hypoparathytoidism x1)	11% (bilateral RLN palsy x2)	13.6% DOD° 36.4% AWD° 13.6% NED°

*DTC, differentiated thyroid cancer; NA, not available; RLN, recurrent laryngeal nerve; OS, overall survival; DSS, disease specific survival; R0, microscopically free surgical margins; R1, microscopically involved surgical margins; R2, macroscopically involved surgical margins; NED, no evidence of disease; DOD, dead of disease; AWD, alive with disease; £includes partial data from overlapping series from Shenoy et al. (2012); *OS has been calculated without distinction between TRA/CTRA patients and laryngectomees; §in 2 pts. both RLN were intentionally sacrificed carrying to permanent tracheostomy; °data non distinguishing between TRA/CTRA procedures and other types of airway surgeries; #this series has been adjourned at August 2021.*

papers (26, 34, 35, 38, 40, 41, 43, 46, 50–53, 55, 57, 58, 62, 64, 66) for a total of 355 patients, of whom 59% were females.

Age of patients was reported in 12 manuscripts (26, 34, 39–41, 50–53, 57, 64, 66) for a total of 121 patients, with a mean of 60 years (range, 20-85).

Seventeen manuscripts reported data on (C)TRA for DTC alone (3, 33, 34, 38, 40, 41, 43–45, 53, 58–63, 66), while 20 for mixed histologies (26, 35–37, 39, 42, 46–52, 54–57, 64, 65, 67). Overall, the distribution of histopathological types in patients treated by (C)TRA was detailed for 376 of them (59%) and their frequency in descending order was as follows: papillary (79%), follicular (7%), poorly differentiated (5%), medullary (2%), Hürtle cell (2%), anaplastic (1%), follicular variant of papillary cancer (1%), metastasis to the thyroid gland from other organs (1%), and rare histotypes such as thyroid squamous cell carcinoma, giant cell carcinoma, and carcinoma with lymphoepithelioma-like pattern (2% all together) (26, 34, 35, 37–40, 42, 44–47, 49–53, 55, 57, 62, 64, 66).

Thirteen articles exclusively described the results of (C)TRA (26, 35, 37, 38, 40, 42, 48, 50–53, 55, 57), while 24 reported data about different treatment strategies also including (C)TRA (3, 33, 34, 36, 39, 41, 43–47, 49, 54, 56, 58–67).

Eight studies did not provide detailed information on the diagnostic work-up employed for detection and assessment of airway invasion by TC (3, 33, 35–37, 42, 45, 51). The remaining 29 manuscripts specified the diagnostic methods utilized (26, 34, 38–41, 43, 44, 46–50, 52–67). Expectedly, neck and chest x-ray for airway invasion assessment were rarely mentioned in studies published after 1996. In contrast, airway endoscopy (either flexible or rigid, under local or general anesthesia) was reported in 100% of the series, computed tomography (CT) in 93%, ultrasonography (US) in 45%, and magnetic resonance (MR) in 32%.

Distinction between types of resection (purely tracheal resection and anastomosis [TRA] or also involving part of the cricoid [CTRA] with consequent thyro-crico-tracheal anastomosis) was reported in 35 papers (3, 26, 33–47, 49–58, 60–67) for a total of 619 patients undergoing 466 (75%) TRA and 153 (25%) CTRA.

Length of resection was reported in 19 papers (26, 33, 34, 37, 38, 40, 42–44, 46, 49–53, 62, 64, 65, 67), usually as range and mean in centimeters or number of removed tracheal rings (for TRA), with associated portions of adjacent cricoid cartilage (for CTRA). In some instances, detailed tables allowed to exactly know the extent of (C)TRA for each patient. However, the length of (C)TRA for 350 patients ranged between 0.5 and 6 cm (mean, 2.5).

The Shin classification was explicitly used to quantify the depth of airway invasion by TC in 10 manuscripts (26, 37, 38, 40, 41, 44, 51, 57, 62, 67), for a total of 148 patients subdivided as

TABLE 3 | Quality assessment of papers included in the present systematic review (N=37).

Source	Selection				Comparability	Outcome			Total
	Representativeness of the exposed cohort	Selection of the non exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability based on design and analysis	Assesment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow up of cohorts	
Avenia et al. (59)	*	*	*			*			4
Brauckhoff et al. (47)	*	*	*		*	*	*	*	7
Chakravarthy et al. (67)	*		*			*	*	*	5
Chen et al. (62)	*	*	*		*	*	*	*	7
Chen et al. (64)			*			*			2
Ch'ng et al. (50)	*	*	*			*		*	5
Fujimoto et al. (34)	*	*	*		*	*		*	6
Gaissert et al. (46)	*	*	*		*	*	*	*	7
Gupta et al. (63)	*	*	*		*	*	*	*	7
Hartl et al. (54)	*	*	*			*	*	*	6
Ishihara et al. (35)	*		*			*	*	*	5
Kato et al. (42)	*		*			*			3
Kim et al. (58)	*	*	*			*	*	*	6
Koike et al. (41)	*	*	*			*			4
Lin et al. (53)	*		*			*	*	*	5
Maeda (36)	*		*			*			3
Morisod et al. (52)	*		*			*	*		4
Mossetti et al. (51)	*		*			*	*	*	5
Musholt et al. (39)	*	*	*		*	*		*	6
Mutrie et al. (48)	*		*			*	*	*	5
Nakao et al. (43)	*		*			*		*	4
Ozaki et al. (37)	*		*			*	*	*	5
Pappalardo et al. (57)	*		*			*	*	*	5
Peng et al. (56)	*		*			*	*	*	5
Piazza et al. (26)	*		*			*	*	*	5
Ranganath et al. (55)			*			*			2
Segal et al. (6)	*		*			*	*	*	5
Shadmehr et al. (49)	*	*	*		*	*	*	*	7
Sharanappa et al. (66)	*		*			*	*	*	5
Su et al. (60)	*		*			*	*	*	5

(Continued)

TABLE 3 | Continued

Source	Selection				Comparability	Outcome			Total
	Representativeness of the exposed cohort	Selection of the non exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability based on design and analysis	Assesment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow up of cohorts	
Tiwari et al. (65)	*		*			*	*	*	5
Tovi et al. (33)	*		*			*	*	*	5
Tsai et al. (44)	*	*	*		*	*	*	*	7
Wada et al. (45)	*	*	*			*	*	*	6
Wang et al. (61)	*	*	*			*	*	*	6
Yang et al. (40)	*		*			*	*	*	5
Zannini et al. (38)			*			*	*	*	4

follows: Shin I in 12 (8%) patients, Shin II in 35 (24%), Shin III in 39 (26%), and Shin IV in 62 (42%).

Data on perioperative mortality were provided in 36 articles (3, 26, 33–53, 55–67), for a total of 632 patients. The random effects model pooled risk of postoperative mortality was 2.0% (95% CI, 1.0-4.0%), with low heterogeneity. Forest and funnel plots are reported in **Figures 2A, B**.

Twenty-nine articles (26, 34–40, 42–47, 49–53, 55–57, 59, 61–63, 65–67), including 557 patients, provided data on the proportion of patients suffering from postoperative complications. Complications were mostly bilateral RLN palsy, anastomotic dehiscence, hypoparathyroidism, and pulmonary complications, which are listed in detail in **Table 2**. The overall summary estimate of the proportion of patients who developed any complication after (C)TRA for TC was 27.0% (95% CI, 20.0-36.0%) (**Figures 3A, B**). Heterogeneity was high (I^2 = 55.0%).

Data on long-term tracheotomy-dependency were reported in 30 studies (26, 33–35, 37–40, 42–53, 55–57, 59, 61–63, 65–67) for a total of 527 patients. The summary estimate of the proportion of patients remaining dependent on tracheotomy after (C)TRA was 4.0% (95%CI, 2.0-8.0%). Heterogeneity of studies was low (**Figures 4A, B**).

Oncological Outcomes and Adjuvant Treatments

Oncological outcomes details were available in 29 articles (3, 26, 34, 35, 37–41, 43–49, 51–55, 57–59, 62–66), as reported in **Table 2**. Seventeen studies (3, 26, 35, 43–49, 54, 58, 59, 62, 63,

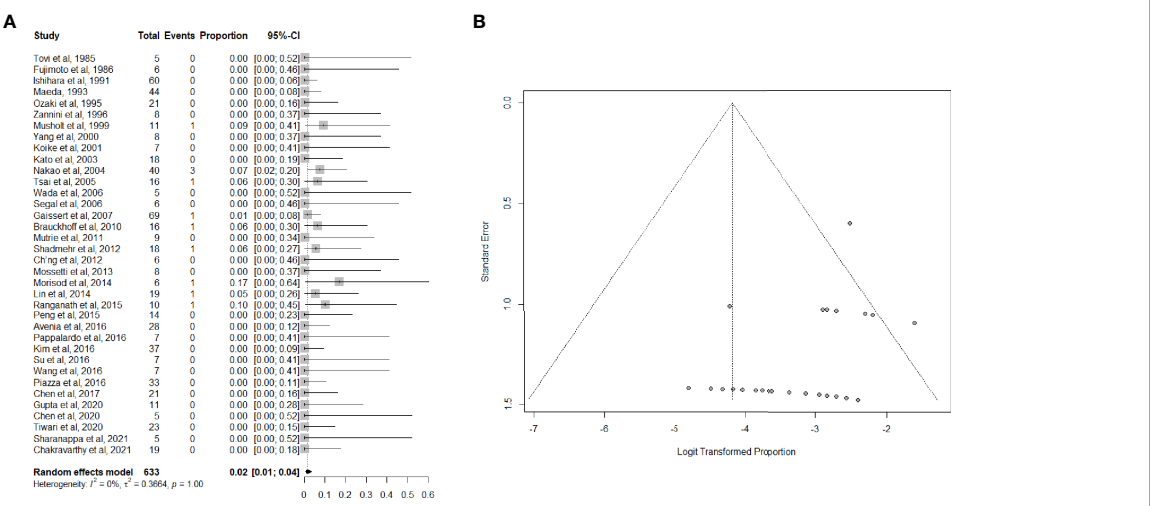

FIGURE 2 | **(A)** Forest plot showing the pooled analysis of postoperative mortality and **(B)** relative funnel plot.

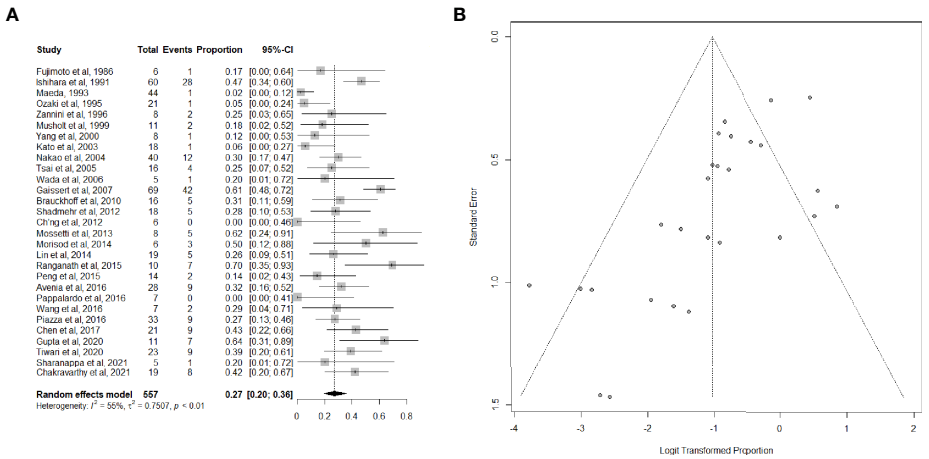

FIGURE 3 | **(A)** Forest plot showing the pooled analysis of postoperative overall complications and **(B)** relative funnel plot.

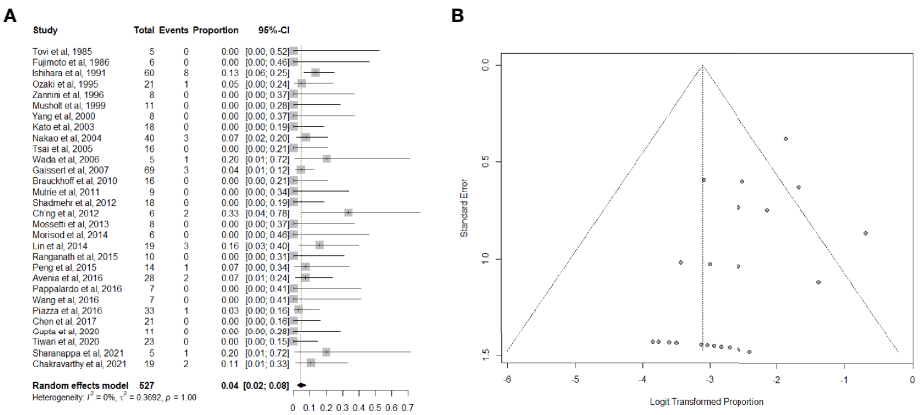

FIGURE 4 | **(A)** Forest plot showing the pooled analysis of tracheotomy-dependency and **(B)** relative funnel plot.

65, 66) provided survival estimates. Five-year overall survival (OS) rate, reported by 11 studies (3, 26, 44, 48, 49, 54, 59, 62, 63, 65, 66), ranged from 61% (49) to 100% (62), whereas only 5 papers (26, 35, 43, 54, 65) reported the 10-year OS rate [ranging between 78.1% (35) and 42.1% (26)], and two studies (46, 65) also provided the 15-year OS rate (26.0% and 35.9%, respectively). Five manuscripts (26, 45, 47, 54, 58) reported 5- and 10-year disease specific survival estimates, which were in the range of 75.8-90% and 54.5-62.9%, respectively.

Specific data on adjuvant radioactive iodine (RAI) were reported by 12 papers (26, 40, 44, 46, 51–54, 62, 63, 65, 66), while 8 (26, 44, 46, 51–53, 62, 66) contained details on the use of postoperative external beam radiotherapy (EBRT). Most patients (86%; 95%CI, 61-96%; I^2 = 22%) had been treated with adjuvant RAI, whereas indication to postoperative EBRT was far less common (11%; 95%CI, 4-30%; I^2 = 18%). No survival analysis according to adjuvant therapies was conducted by any of the included studies.

DISCUSSION

Mechanisms of Airway Invasion and Its Therapeutic Implications

When the cartilages or inter-cartilaginous ligaments are penetrated by neoplastic cells up to the level of submucosa, the TC spreads along the cartilaginous framework horizontally (following the inter-cartilaginous blood and lymphatic vessels) and vertically, before fungating into the airway lumen. As a consequence, the endoluminal real tumor extension is frequently more important than what can be seen from the outer surface of the organ (8, 19, 37). This, together with the uncertainty in the precise clinical assessment of the in-depth neoplastic extension within the cartilaginous framework, represents the most important pathological basis for justifying (C)TRA when dealing with tumors penetrating through the crico-tracheal axis, and the most evident limiting factor in supporting a

window resection (*per se* based on the evaluation of the neoplastic extent just at the level of the outer airway surface).

In appropriately selected cases (i.e., for short segment airway involvement not beyond the external perichondrium) and with carefully performed surgery, shaving achieves complete resection and offers local control rates as high as 95-100% (58, 61, 68, 69). On the other hand, shaving for TC with deeper airway involvement, where R0 resection cannot be achieved or confirmed by frozen sections of cartilaginous tissues and possible microscopic penetration of the tumor into the airway submucosa *via* lymphatics piercing the inter-cartilaginous spaces may remain undetected, local failure with dismal outcomes have been reported in the literature (70, 71). Moreover, while several series included in this systematic review related to patients treated primarily by (C)TRA reported 5-year survival figures >80% (34, 41, 44, 45, 47, 48, 55, 57, 58, 62–66), the complication and mortality rates are considerably higher and survival lower when the procedure is applied as a salvage operation for recurrence after more conservative initial surgery (72, 73).

The same holds true for window resections, which are advocated by some in case of deeper tumor infiltration into the airway (20, 21). Such a surgical procedure is limited in its width and length of resection due to constraints in terms of airway stability, especially if the surgeon attempts a primary closure of the tracheal defect. As a consequence, if pedicled or revascularized myofascial/myoperichondral or skin flaps for tracheo-cutaneous fistula closure (74) are not employed, an R0 resection with negative margins by such a technique is less probable than after (C)TRA.

In case of incidental intraoperative discovery of TC invading the airway deeper than its external perichondrium, with a shaving procedure likely resulting in an R2-R1 resection, the general consensus is to convert the procedure into (C)TRA if it can be safely performed by the surgeon during the same intervention, or abort the procedure and refer the patient to a tertiary center with adequate experience in airway management (22). Clearly, in the best-case scenario, such a therapeutic option should be anticipated by performing the appropriate preoperative diagnostic work-up (including airway endoscopy and detailed cross-sectional imaging studies), referring the patient preoperatively to another team if adequate surgical expertise for (C)TRA is not available.

Tumor Resectability and Indications for (C)TRA

Invasion of the prevertebral fascia, major cervico-mediastinal blood vessels and/or massive involvement of the thoracic trachea are situations which make TC unresectable, and are categorized as T4b (8, 75). On the other hand, the only contraindications to (C)TRA include: 1) cranio-caudal extent exceeding 5.5 cm (i.e. 11 tracheal rings or cricoid arch plus 9 tracheal rings) (76) even in young patients (while for older ones, shorter airway resections may already represent an issue, with 4 cm of length being demonstrated to significantly increase the incidence of anastomotic dehiscence) (36, 77, 78); 2) major full-thickness esophageal/hypopharyngeal involvement requiring more than shaving of the external muscular layer or limited full-thickness resection with primary closure; and 3) tumor reaching the glottic plane either anteriorly through the crico-thyroid membrane or posteriorly at the level of the crico-arytenoid joint(s) (26). All these factors should be adequately assessed in the preoperative setting, considering that the proximal and distal airway cuts should be performed one tracheal ring above and below the macroscopic invasion site as appreciated from the adventitial side and checked from inside at the level of the airway lumen, with confirmatory mucosal frozen sections as needed. Clearly, these are contraindications for (C)TRA, but such tumor extensions are amenable to more radical surgical procedures, such as pharyngo-laryngo-esophagectomy for extensive invasion of the larynx and/or esophagus. Similarly, more extensive tracheal invasion can be resected making a mediastinal tracheostomy. In such clinical scenarios, a balanced preoperative counseling that may guide patients along the difficult path of choosing between a better quality of life against a higher chance of perioperative complications should be taken into account.

Preoperative bilateral RLN palsy does not represent *per se* an absolute contraindication to (C)TRA since an R0 resection with postoperative permanent tracheotomy (and usable voice) is always better than both persistent airway disease or total laryngectomy with/without tracheoesophageal voice prosthesis insertion (26). Of note, one should consider that, in case of anterior cricoid arch resection, the ensuing bilateral loss of vocal cords tension for lack of crico-thyroid muscles, if associated with bilateral RLN palsy, may make posterior cordotomy with/without arytenoidectomy useless in the attempt of getting a patent airway without tracheostomy. Last but not least, placing a tracheostomy below the anastomotic line after (C)TRA with bilateral RLN palsy may significantly increase the risk of postoperative complications such as anastomotic dehiscence, stenosis or tracheo-innominate fistula.

Radical comprehensive approaches like (C)TRA, able to maintain a good quality of life, are strongly recommended in patients with DTC even in the presence of a limited burden of asymptomatic distant metastases (8, 26). However, general health status (age, comorbidities, compliance) and willingness to undergo surgery play a prominent role in selecting patients amenable for such a major surgical procedure.

Role of Endoscopy and Imaging in Evaluating Shin Stage

The first endoscopic examination to be performed in every TC patient should include a flexible videolaryngoscopy, even in the absence of an appreciable hoarseness: in fact, finding a unilateral RLN palsy should prompt to the request of more targeted investigations (such as tracheoscopy and cross-sectional imaging studies) to determine and precisely evaluate potential airway involvement, both quantifying its radial (depth) and cranio-caudal extents (22). This also applies to other common signs and symptoms of advanced TC such as hemoptysis, dyspnea, dysphagia, thyroid fixation or clinically enlarged lymph nodes: even though infrequent, these findings should prompt adequate

imaging to exclude aerodigestive tract invasion and quantify it for appropriate surgical treatment planning.

Transcutaneous US can detect the depth of airway invasion, reliably distinguishing superficial (Shin I-II) vs. deeper (Shin III-IV) infiltration with a diagnostic accuracy potentially reaching 93% (79–81). However, US is generally considered highly operator-dependent and less reliable for tumors larger than 4 cm or with major intralesional calcifications, as well as with significant retrosternal extension.

CT is considered superior to US and definitely more reproducible for precise three-dimensional assessment of airway invasion (22), with a mean sensitivity, specificity, and accuracy in detecting tracheal invasion of 59.1%, 91.4%, and 83.2%, respectively (82). It is important to emphasize that the CT should be performed with contrast, to give the most precise information. In particular, the most quoted CT diagnostic criteria are tumor in contact for 180° or more of tracheal circumference, deformity of the airway lumen (i.e. indentation due to pressure effect) at the level of such a contact, focal irregularity, thickening or bulging of the mucosal lining and, finally, presence of tumor within the tracheal lumen (82).

MR seems to have lower diagnostic accuracy than US and CT, with a tendency to overestimate the actual depth of airway invasion (83). Others report superior outcomes with MR compared to other imaging techniques (84). However, a tumor-airway contact exceeding 135° of the tracheal circumference seems to efficiently predict some degree of cartilaginous involvement (85).

Laryngo-tracheoscopy allows appreciation of airway invasion when the airway submucosa is reached (Shin III-IV), thus appearing as a subtle localized or diffuse mucosal redness, with elevation, edema, presence of telangiectasias and vascular engorgement, with focal erosions or endoluminal vegetations in the most obvious scenario (41). This is in line with the experience of the first author, who missed Shin II tracheal invasion in 11% of his series by endoscopy and imaging (26). The sensitivity of this tool for tracheal invasion evaluation is, in fact, reported to be around 85%, with a mean underestimation of the actual cranio-caudal tumor extent of an average of 0.8 (maximum 2) tracheal rings compared to postoperative histopathologic specimens (86).

Endobronchial US (EBUS) is the latest imaging technique for assessment of the presence and degree of airway invasion by TC. Recent reports highlight an accuracy significantly higher than those reported by CT and/or MR, with a sensitivity and specificity of 92% and 83%, respectively (84). However, EBUS is still relatively infrequently used in most medical facilities due to some inherent drawbacks such as increased invasiveness, high cost, and limited utility in evaluating tumors infiltrating at the level of the thyroid upper lobe (82).

It would therefore appear that the most adequate diagnostic algorithm for advanced TC with suspicious airway invasion should be based on careful endoscopy of the larynx and trachea, with US and subsequent CT or MR depending to the local facilities and expertise.

Oncologic Prognosticators

Predictors of survival in advanced TC involving the airway may be patient-related (age, gender), tumor-related, and treatment-related. Among tumor-related factors, micro- vs. macroscopic extrathyroidal extension, limited to one vs. multiple organs has been recently demonstrated to play an important role (7). Strap muscles (T3b) and RLN invasion (T4a) have no prognostic influence on survival, but they do affect recurrence in contrast to laryngeal, tracheal, and esophageal involvement which heavily impact both local recurrence and survival rates (9, 87). Tracheal and esophageal invasion (T4a) present no prognostic differences when all tumor tissue can be removed within negative margins (R0 resection). By contrast, invasion of the larynx (T4a) reflects a more aggressive behavior of disease (47), even though no clear distinction is usually made in the literature with respect to the specific anatomic site(s) of TC infiltration. Intuitively, anterior cricoid involvement has a very limited impact on radicality of tumor resection and possibility of organ preservation compared to lateral and/or posterior cricoid infiltration (26). The same holds true when considering invasion of the inferior border of the thyroid laminae compared to transgression of their lateral edges in close proximity with the piriform sinus, or when dealing with a superficial (external perichondrium) vs. a full-thickness thyroid cartilage invasion.

General consensus has been reached on preserving the RLN whenever it is preoperatively functioning, even though encased by tumor, as long as it is not directly infiltrated by TC and all gross disease is removed, adding postoperative adjuvant therapy in the form of RAI or EBRT as indicated (22). Sacrifice of the RLN is generally only justified when preoperatively already non-functioning or when its preservation would inevitably leave behind gross residual disease (R2 resection) (88). In this case, RLN reinnervation by direct suture of healthy stumps at frozen sections, interposition of a nerve graft or suturing the ansa hypoglossi to the distal RLN stump may be considered to maintain vocal muscle tone and improve functional outcomes for voice rehabilitation (89–91).

Tumor histopathological type is a strong predictor of survival in TC invading the aerodigestive tract: the 5-year survival rate in DTC is around 75%, while it declines below 60% in medullary TC, and to 20% in undifferentiated tumors (26, 47).

Among procedure-related prognosticators, the most powerful seems to be R status: a number of series have confirmed better 5-year survival in R0 compared to R2 resections (90-78% vs. 50-35%) (35, 92, 93). Even R1 resections, while apparently presenting similar 5-year survivals (71, 93), in the long run are invariably associated with a higher rate of recurrence (46, 70, 71, 94). Moreover, when comparing immediate R0 resection with R1 resection followed by delayed radicalization, even considering non-organ sparing surgery, 10- and 20-year disease-free survival decrease from 67% and 50% to 7% and 0%, respectively (46). However, the absence of high-quality prospective data does not allow to solve the discrepancy between the above mentioned data and those reported by others, with no statistically significant survival differences between R0 and R1 resections (54, 61).

Role of RAI and EBRT

It should be emphasized at the outset that postoperative RAI and/or EBRT do not replace adequate surgery with R0 resection due to the high failure rates of these adjuvant therapies in

controlling residual R2 disease, especially at the level of the airway. Adjuvant therapy by RAI after (C)TRA for T4a DTC is widely used whenever sufficient iodine uptake is demonstrated. However, response to RAI often cannot be established before surgery and is not uniform especially for microscopic residual disease (R1 resection) on the airway surface (95).

Special consideration should be paid to patients who have already received RAI in the past and experience further disease progression, since tolerance to and further potential benefit from RAI is questionable in such a clinical scenario. Moreover, EBRT as initial mode of therapy in TC invading the airway deeper than its cartilaginous framework should not be offered given the limitation it places on wound healing in case of a subsequent (C)TRA (26) and the low probability of appropriate response of bulky TC invading the airway (5). Still controversial is the potential role of adjuvant EBRT after segmental R0 airway resection for DTC, especially when the laryngo-tracheal axis was the only site of macroscopic extrathyroidal extension. Since no survival analysis was available regarding the impact of both RAI and EBRT as adjuvant therapies after (C)TRA, no conclusion on their prognostic role can be withdrawn from the present systematic review.

Study Limitations

The most notable limits of our paper are the retrospective nature and relatively low number of patients included in each case series, *per se* potentially flooded by selection biases and a wide array of geographic, therapeutic, and epidemiologic differences. Lack of details concerning the histotypes and use of adjuvant treatment protocols reduces the possibility to infer their impact on prognosis of patients treated by (C)TRA. Moreover, the evolution in the diagnostic and therapeutic strategies occurred during the long time span of our systematic review must be taken into proper consideration.

CONCLUSIONS

The current literature is still devoid of prospective clinical trials addressing optimal management of T4a TC invading the crico-tracheal axis. However, based on the retrospective case series analyzed, even though characterized by the common biases related to the relatively small number of patients recruited in a long period of time, (C)TRA appears to be a reproducible major surgical procedure, which is able to ensure both good oncological outcomes as well as a reasonable chance of laryngeal function preservation for TC invading the trachea deeper than the level of its external perichondrium and less than 5.5 cm in length. However, the non-negligible mortality and complication rates should prompt management of these advanced tumors by skilled surgical teams in tertiary referral centers with the adequate multidisciplinary expertise, after proper diagnostic work-up and patient selection.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

CP, AD'C, DH, LK, GR, AR, JS, AS, RS, VV, MZ, and AF contributed to conception and design of the study. CP, DL, and MT organized the database and performed the statistical analysis. CP, DL, and MT wrote the first draft of the manuscript. CP, AD'C, DH, LK, GR, AR, JS, AS, RS, VV, MZ, and AF wrote the final draft of the manuscript. All authors contributed to the article andapproved the submitted version.

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High Incidence of Distant Metastasis Is Associated With Histopathological Subtype of Pediatric Papillary Thyroid Cancer - a Retrospective Analysis Based on SEER

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Objective: Children with papillary thyroid cancer (PTC) have a higher invasive rate and distant metastasis rate, but the mortality rate is lower with unknown reasons. The majority of PTC cases comprise classical papillary thyroid carcinoma (CPTC) and follicular variant papillary thyroid carcinoma (FVPTC). This study aimed to determine the relationship between histopathological subtype and rate of distant metastasis and investigate factors influencing distant metastasis in pediatric PTC.

Methods: A total of 102,981 PTC patients were recruited from SEER registry, 2004-2015. Proportion of distant metastasis between children (≤18 years) and adults with different histopathological subtypes was compared by propensity score matching. The cut-off age for distant metastasis in children was calculated by receiver operating characteristic (ROC) curve, and the risk factors for distant metastasis in pediatric patients were analyzed by logistic regression models.

Results: Among the 1,484 children and 101,497 adults included in the study, the incidence of CPTC patients with distant metastasis in children was higher than that in adults ($p<0.001$). The ROC curve was calculated, which yielded a cut-off age for distant metastasis in CPTC children as 16 years old. In CPTC, the proportion of young children (2-16 years) with distant metastasis was higher than that of adolescents (17-18 years) and adults (>18 years) (both $p<0.001$). While there was no such trend in FVPTC. In young children (2-16 years), the incidence of CPTC with distant metastasis was higher than FVPTC ($p=0.006$). There was no difference between the proportion of CPTC and FVPTC with distant metastasis in adolescents (17-18 years) and adults. Logistic regression models revealed that extrathyroidal extension, lymph node metastasis and CPTC

histopathological subtype were risk factors for distant metastasis in young children aged 2 -16 years.

Conclusions: In CPTC, the incidence of distant metastasis in young children (2-16 years) was significantly higher than that in adolescents (17-18 years) and adults (>18 years). In patients with distant metastasis aged 2-16 years, the proportion of CPTC was higer than that of FVPTC. Extrathyroidal extension, lymph node metastasis, and CPTC histopathological subtype were risk factors for distant metastasis in young children aged 2-16 years.

Keywords: papillary thyroid carcinoma (PCT), histopathological subtype, distant metastasis, pediatrics - children, SEER, FVPTC, CPTC

INTRODUCTION

Thyroid cancer is the most common endocrine cancer in the pediatric population (1). In a cross-sectional study based on the Surveillance, Epidemiology, and End Results (SEER) database including individuals younger than 20 years who had a diagnosis of thyroid cancer, the incidence of pediatric thyroid cancer increased by 1.1% per year from 1973 to 2006, with a significant increase of 9.5% per year from 2006 to 2013 (2). Compared to adult thyroid carcinoma, the prognosis of pediatric papillary thyroid cancer is generally fair (3). The reported mortality rate of pediatric papillary thyroid cancer (PTC) is very low with a higher rate of distant metastasis (DM) in most series despite more advanced disease at presentation and a higher risk of recurrence (4–6). A study involving patients aged 12 to 83 years showed that approximately half of patients with well-differentiated thyroid cancer with DM die of disease within 5 years of initial diagnosis despite thyroid surgery and radioactive iodine (RAI) (7). Indeed, the presence of DM is reported to be an independent predictor for poor overall survival (8). Therefore in a disease with a low mortality rate such as PTC, it is critical to identify tumors at initial presentation that are at risk of developing DM to assist in clinical decision making. Despite incidence of DM of PTC in children (≤18 years) was higher than in adults (>18 years), the American Thyroid Association Guidelines for the Treatment of Thyroid Nodules and Differentiated Thyroid Carcinoma do not differentiate children from adults in diagnosis and treatment, thereby calling for further studies to guide treatment strategies. Pediatric PTC patients aged less than 18 years might benefit from tailored disease management by cut-off age and expect better prognosis.

Some published studies have suggested that the frequency of DM was different among the various histopathological subtypes (8), the majority of PTC cases comprise classical papillary thyroid carcinoma (CPTC) and follicular variant papillary thyroid carcinoma (FVPTC) histopathological subtypes (9), which account for 67-74% and 26-30% of cases, respectively (9, 10). According to previous reports, extrathyroidal extension (ETE) and cervical lymph node metastases (LNM) are more common in CPTC than in FVPTC (11). The current consensus among thyroid academia is that there are only a few differences between CPTC and FVPTC. Moreover, the overall management of the two malignancies is similar, and patients with CPTC and FVPTC have similar long-term outcome (11). However, despite these similarities, minimal ETE and aggressive histopathological features, such as thyroid capsule infiltration, are significantly more common in CPTC than in FVPTC (11). Besides, patients with mETE showed significantly higher rates of lymph node metastases in the neck (12) and all levels of ETE, including microscopic ETE, were associated with increased risk for lymph node metastasis and DM (13). Thus, we propose the following hypothesis: The high DM rate in pediatric PTC aged less than 18 years old is associated with histopathological subtype.

Although there were some reports focusing on those young children had a greater degree of ETE and lymph node involvement than adolescents and were more prone to DM (4). Due to the lack of pediatric patients who are necessary to determine the explanation for the differences in clinicopathological outcomes observed in pediatric PTC patients, the focus on the reason why young children and adolescent patients show this difference has been rarely explored. More unfortunately, little attention was paid to differences in pathological subtypes among young children and adolescents. To assess our hypothesis and calculate the cut-off age for DM in children, as well as investigate factors influencing distant metastasis in pediatric PTC, we conducted a retrospective analysis at the first time using a population-based data set with a large sample.

MATERIALS AND METHODS

Data Source

Data for this study were extracted from the National Cancer Institute's SEER data (Surveillance, Epidemiology, and End Results), which is one of the most representative large oncology registry databases in North America, covering 34.6% of the U.S. population and collecting case information from 18 population-based cancer registries (14).

Abbreviations: AUC, area under curve; B, regression coefficient; CI, confidence interval; CPTC, classical papillary thyroid carcinoma; CSS, cancer-specific survival; DM, distant metastasis; ETE, extrathyroidal extension; FVPTC, follicular variant papillary thyroid carcinoma; LNM, lymph node metastasis; OR, odds ratio; OS, overall survival; PSM, propensity score matching; PTC, papillary thyroid carcinoma; RAI, radioactive iodine; ROC, receiver operating characteristic; SD, standard deviation.

Study Participants

The study cohort included patients diagnosed with PTC between 2004 and 2015. All included patients were identified using histopathological codes of the International Classification of Disease for Oncology, Third Revision (ICD-O-3). Histopathological codes were listed below CPTC included 8050/3 (papillary carcinoma not otherwise specified, NOS), 8260/3 (papillary adenocarcinoma, NOS), and 8343/3 (papillary carcinoma, encapsulated); FVPTC included 8340/3 (papillary carcinoma, follicular variant); 8342/3 (papillary carcinoma, oxyphilic cell), 8344/3 (papillary carcinoma, columnar cell), and 8450/3 (nonencapsulated sclerosing carcinoma) were classified as other (15). Exclusion criteria for this study included: (1) age or race unknown; (2) incomplete/missing information on tumor size, tumor invasion, multifocal, LNM, or DM. This study was based on the American Joint Committee on Cancer (AJCC) (Eighth Edition). Data in the SEER database at the time of extraction was based on AJCC (Sixth Edition) and AJCC (Seventh Edition). We performed a second extraction of the data, converting variables defined by the AJCC (Sixth Edition and Seventh Edition) into AJCC (Eighth Edition). Our analysis included demographic variables: sex; age at diagnosis; ethnicity; histopathological subtype; tumor size; ETE; LNM; DM; foci; cancer-specific survival (CSS); and overall survival (OS); survival months.

Age was categorized as ≤18 years and >18 years. The patients aged ≤ 18 years were regarded as children and patients aged >18 years were regarded as adults. Ethnicity was categorized according to the record in the SEER database as Black, White and other. Histopathological subtype was based on ICD-O-3. Tumor size less than 15cm are preserved. ETE was based on "CS extension (2004-2015)" codes. DM and LNM were based on AJCC (Eighth Edition). Foci was based on "CS site-specific factor 1" codes.

Statistical Analysis

The database materials were obtained by SEER*Stat 8.3.9 software, processed by WPS 2.7.1 software and statistically analyzed by SPSS 26.0 (IBM) software. Patient information, including demographic data and cancer-related information, was compared with histopathological subtypes. In our data, only two histopathological subtypes, CPTC and FVPTC, were present in patients ≤ 18 years old with DM. Therefore, this study focused on CPTC and FVPTC. Categorical variables were reported as frequency and proportion. We used the chi-square test or Fisher's exact test to compare these variables. To minimize selection bias, propensity score matching (PSM) was performed on ETE and LNM when comparing the proportion of DM in children and adults with different histopathological subtypes. The 1:1 matching scheme was used for matching, and a caliper of 0.05 SD for the probit value. The age cut-points at which CPTC and FVPTC developed DM in children were calculated by receiver operating characteristic (ROC) curve. Logistic regression analysis was used to calculate the risk factors for DM in children and regression coefficient (B), odds ratio (OR), the 95% confidence interval (CI) was used for reporting. Bilateral p value <0.05 was considered statistically significant difference. Variables with p value <0.05 in univariate analysis were included in multivariate analysis.

RESULTS

Demographic and Clinicopathological Characteristics

There were 102,981 patients diagnosed with PTC between 2004 and 2015, who met the inclusion criteria. **Table 1** summarizes the demographic and clinicopathological features of these patients. A total of 101,497 adult patients aged >18 years (98.56%) and 1,484 pediatric patients aged ≤18 years (1.44%) were included. 79,431 (77.13%) were female and 23,550 (22.87%) were male. Of these, 67,614 (65.66%) were CPTC patients, 33,862 (32.88%) were FVPTC patients, and 1,505 (1.46%) were patients with other histopathological subtypes. DM occurred in 999 patients (0.97%), ETE occurred in 18,204 patients (17.68%), LNM occurred in 24,636 patients (23.92%), and 43,804 patients (42.54%) had multifocal tumors. CSS was 98.84% and OS was 92.79%.

Table 2 describes the demographics and clinicopathological features of PTC patients with DM. DM occurred in a total of 999 patients, of whom 41 (4.10%) were children (≤18 years) and 958 (95.90%) were adults (>18 years). Of the pediatric patients with DM, 36 (87.80%) were CPTC patients, 5 (12.20%) were FVPTC patients, and no DM was found in pediatric patients with other histopathological subtype. 28 (68.29%) patients had ETE, 40 (97.56%) patients had LNM, and 25 (60.98%) patients had multifocal tumor, while no patient died. Of the adult patients with DM, 617 (64.41%) were CPTC, 302 (31.52%) were FVPTC, 39 (4.07%) were other histopathological subtypes, 612 (63.88%) had ETE, 629 (65.66%) had LNM, 502 (52.40%) had multifocal tumors, and 451 (47.08%) died, of which 302 (31.52%) died of PTC.

Propensity Score Matching Compares the Proportion of DM Occurring Among Different Histopathological Subtypes in Children and Adults

1,484 children (≤18 years) and 101,497 adults (>18 years) were included in the study. 41 (2.76%) children had DM, 958 (0.94%) adults had DM (p<0.001) (**Table 1**). In patients who had DM, histopathological subtypes were compared between children and adults: CPTC was observed in 36 (87.80%) children and 617 (64.41%) adults, FVPTC was observed in 5 (12.20%) children and 302 (31.52%) adults, other histopathological subtype was only observed in 39 (4.07%) adults (**Table 2**). In CPTC patients, DM was observed in 36 (3.32%) children and 617 (0.93%) adults (p<0.001). After the propensity score was matched with ETE and LNM, the proportion of DM was 75% in children, 49.05% in adults(p<0.001). In FVPTC patients, DM was observed in 5 (1.29%) children and 302 (0.90%) adults (p=0.408). After the propensity score was matched with ETE and LNM, the proportion of DM was 55.56% in children, 49.92% in adults (p=1.000) (**Table 3**).

TABLE 1 | Baseline clinicopathological characteristics of patients with PTC.

	All patients [no. (%)]	Children [no. (%)]	Adults [no. (%)]	P-value
No. of patients	102,981 (100.00)	1,484 (1.44)	101,497 (98.56)	
Gender				<0.001
Female	79,431 (77.13)	1,224 (82.48)	78,207 (77.05)	
Male	23550 (22.87)	260 (17.52)	23,290 (22.95)	
Ethnicity				=0.003
White	85,052 (82.59)	1,263 (85.10)	83,789 (82.56)	
Black	6,522 (6.33)	63 (4.25)	6,459 (6.36)	
Other	11,407 (11.08)	158 (10.65)	11,249 (11.08)	
Histological subtype				<0.001
CPTC	67,614 (65.66)	1,083 (72.98)	66,531 (65.55)	
FVPTC	33,862 (32.88)	388 (26.14)	33,474 (32.98)	
Other	1,505 (1.46)	13 (0.88)	1492 (1.47)	
DM				<0.001
Presence	999 (0.97)	41 (2.76)	958 (0.94)	
Absence	101,982 (99.03)	1,443 (97.24)	100,539 (99.06)	
ETE				<0.001
Presence	18,204 (17.68)	380 (25.61)	17,824 (17.56)	
Absence	84,777 (82.32)	1,104 (74.39)	83,673 (82.44)	
LNM				<0.001
Presence	24,636 (23.92)	774 (52.16)	23,862 (23.51)	
Absence	78,345 (76.08)	710 (47.84)	77,635 (76.49)	
Foci				0.15
Solitary	59,177 (57.46)	880 (59.30)	58,297 (57.44)	
Multifocal	43,804 (42.54)	604 (40.70)	43,200 (42.56)	
CSS				=0.001
Alive/Dead (other causes)	101,785 (98.84)	1,481 (99.80)	100,304 (98.82)	
Thyroid cancer	1,196 (1.16)	3 (0.20)	1,193 (1.18)	
OS				<0.001
Alive	95,551 (92.79)	1,472 (99.19)	94,079 (92.69)	
Dead	7,430 (7.21)	12 (0.81)	7,418 (7.31)	
Survival months (mean ± SD)	79.33 ± 41.53	83.52 ± 41.21	79.27 ± 41.53	<0.001

PTC, papillary thyroid carcinoma; CPTC, classical papillary thyroid carcinoma; FVPTC, follicular variant papillary thyroid carcinoma; DM, distant metastasis; ETE, extrathyroidal extension; LNM, lymph node metastasis; CSS, cancer-specific survival; OS, overall survival; SD, standard deviation.

Cut-Off Age for DM in Children With CPTC and FVPTC Calculated by Receiver Operating Characteristic (ROC) Curve and Difference Comparison in the Proportion of Histopathological Subtypes Above and Below the Cut-Off Age

The ROC curve was calculated, which yielded a cut-off age of CPTC children with DM as 16 years old, with the area under the curve of 0.707, sensitivity of 0.750 and specificity of 0.606 (**Figure 1A**). Therefore, we divided patients into young children (2-16 years), adolescents (17-18 years), and adults (>18 years). In CPTC, DM was observed in 31 (5.16%) young children, 5 (1.04%) adolescents and 617 (0.93%) adults (p<0.001) (**Table 4-1**). In young children who had DM, 31 (93.94%) patients were CPTC, 2 (6.06%) patients were FVPTC (p=0.006). There was no difference between the proportion of CPTC and FVPTC in adolescents and adults who had DM (**Table 4-2**).

Risk Factors of Distant Metastasis in PTC Patients Calculated by Logistic Regression Models

Logistic regression univariate analysis showed that ETE (B 1.824, OR 6.194, 95% CI 2.900–13.228, p<0.001), LNM (B 3.330, OR 27.925, 95% CI 3.797–205.354, p=0.001), multifocal tumor (B 1.081, OR 2.947, 95% CI 1.410–6.161, p=0.004), and the CPTC histopathological subtype (B 1.775, OR: 5.901, 95% CI: 1.400-24.867, p=0.016) were risk factors for DM in young children (2-16 years) with PTC. The above meaningful variables for univariate analysis were included in logistic regression multivariate analysis, which showed that ETE (B 1.015, OR 2.759, 95% CI 1.240–6.140, p=0.013), LNM (B 2.568, OR 13.039, 95% CI 1.682–101.086, p=0.014), and CPTC histopathological subtype (B 1.460, OR 4.308, 95% CI 1.006–18.447, p=0.049) were risk factors for DM in young children (2-16 years) with PTC (**Table 5-1**). The CPTC histopathological subtype was not a risk factor for DM in adult patients (>18 years) (**Table 5-2**).

DISCUSSION

Thyroid cancer is the most rapidly increasing cancer in the United States (16). The incidence rates of pediatric thyroid cancer (patients younger than 20 years) increased more rapidly from 2006 to 2013 than from 1973 to 2006 (2). The incidence of DM is significantly more frequent in children (<19 years) than in adults, but the prognosis is generally good (3) with unclear reasons. By analyzing the demographic information and clinicopathological characteristics of a large number of PTC patients, this study confirmed that the incidence of DM in pediatric PTCs

TABLE 2 | Baseline clinicopathological characteristics of PTC patients with distant metastasis.

	Children [no. (%)]	Adults [no. (%)]	*P*-value
No. of patients	41 (4.10)	958 (95.90)	
Gender			=0.009
Female	30 (73.17)	501 (52.30)	
Male	11 (26.83)	457 (47.70)	
Ethnicity			0.675
White	34 (82.92)	738 (77.04)	
Black	2 (4.88)	67 (6.99)	
Other	5 (12.20)	153 (15.97)	
Histological subtype			=0.008
CPTC	36 (87.80)	617 (64.41)	
FVPTC	5 (12.20)	302 (31.52)	
Other	0 (0.00)	39 (4.07)	
ETE			0.564
Presence	28 (68.29)	612 (63.88)	
Absence	13 (31.71)	346 (36.12)	
LNM			<0.001
Presence	40 (97.56)	629 (65.66)	
Absence	1 (2.44)	329 (34.34)	
Foci			0.281
Solitary	16 (39.02)	456 (47.60)	
Multifocal	25 (60.98)	502 (52.40)	
CSS			<0.001
Alive/Dead (other causes)	41 (100.00)	656 (68.48)	
Thyroid cancer	0 (0.00)	302 (31.52)	
OS			<0.001
Alive	41 (100.00)	507 (52.92)	
Dead	0 (0.00)	451 (47.08)	
Survival months (mean ± SD)	85.00 ± 40.35	55.55 ± 42.00	<0.001

PTC, papillary thyroid carcinoma; CPTC, classical papillary thyroid carcinoma; FVPTC, follicular variant papillary thyroid carcinoma; ETE, extrathyroidal extension; LNM, lymph node metastasis; CSS, cancer-specific survival; OS, overall survival; SD, standard deviation.

TABLE 3 | Comparison of children and adults with distant metastasis in CPTC and FVPTC.

	CPTC				FVPTC			
	No. of patients	With DM [no. (%)]	Without DM [no. (%)]	*P*-value	No. of patients	With DM [no. (%)]	Without DM [no. (%)]	*P*-value
Children	1,083	36 (3.32)	1,047 (96.68)	<0.001	388	5 (1.29)	383 (98.71)	0.408
Adults	66,531	617 (0.93)	65,914 (99.07)		33,474	302 (0.90)	33,172 (99.10)	
after propensity score matching ETE and LNM								
Children	48	36 (75.00)	12 (25.00)	<0.001	9	5 (55.56)	4 (44.44)	1.000
Adults	1,258	617 (49.05)	641 (50.95)		605	302 (49.92)	303 (50.08)	

CPTC, classical papillary thyroid carcinoma; FVPTC, follicular variant papillary thyroid carcinoma; DM, distant metastasis; ETE, extrathyroidal extension; LNM, lymph node metastasis.

(≤18 years) was higher than that in adults, and the incidence of pediatric CPTCs (≤18 years) was also higher than that in adults. We compared the proportion of children (≤18 years) and adults with DM in CPTC and FVPTC respectively by propensity score matching. The results showed that in CPTCs, children had a higher rate of DM than adults. However, in FVPTCs, there was no difference in the incidence of DM between children and adults. We also used ROC curve to calculate the cut-off age for DM in CPTC children, and compared the difference in the proportion of histopathological subtypes above and below the cut-off age. According to our calculations, CPTCs had a higher rate of DM than FVPTCs in both children (≤18 years) and young children (2-16 years). There was no difference in DM rate between CPTC and FVPTC in adolescents (17-18 years) and adults. These findings further validated the correlation between CPTC and DM in young children. Finally, logistic regression models revealed that ETE, LNM, and CPTC histopathological subtype were risk factors for DM in young children aged 2-16 years. However, CPTC histopathological subtype was not a risk factor for DM in adolescents aged 17-18 years and in adults aged older than 18 years.

PTC is more common in female than in male in a research including patients aged 0-24 years and the incidence of thyroid cancer increased with age (17). For pediatric population, difference in gender starts just above age 10, with increasing distinction above age 15 (18). There had been previous studies dividing thyroid cancer patients into pediatrics (<13 years) and adolescents to compare the metastasis and disease progression (19). Within the pediatric group under 18 years of age, special attention should be paid to male patients under 15 years old, as they are associated with a more advanced disease at diagnosis (5).

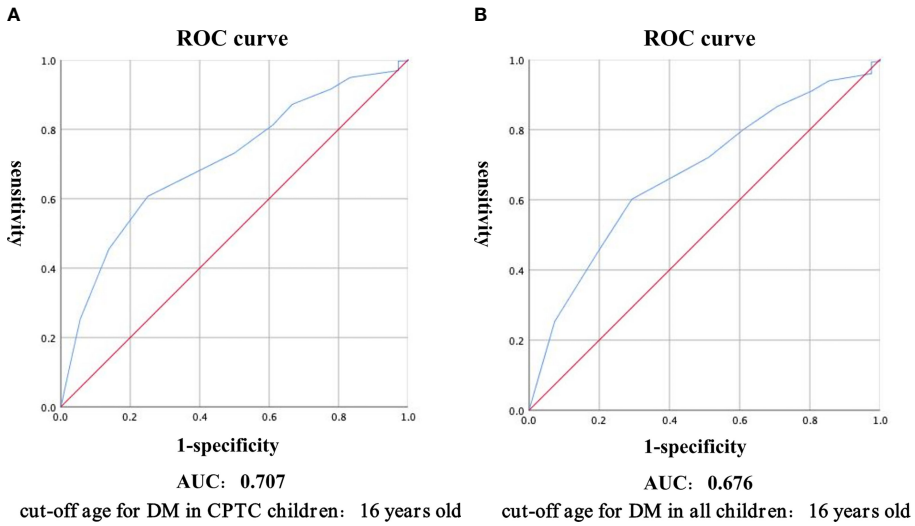

FIGURE 1 | Receiver Operating Characteristic curve was used to calculate the cut-off age for distant metastasis in CPTC children **(A)**, and all children **(B)**. ROC, receiver operating characteristic; CPTC, classical papillary thyroid carcinoma; AUC, area under curve.

TABLE 4-1 | Comparison of different age groups with distant metastasis of CPTC.

	No. of patients	With DM [no. (%)]	Without DM [no (%)]	*P*-value	*P*-value		
					(1)	(2)	(3)
2-16years (1)	601	31 (5.16)	570 (94.84)	<0.001	–	<0.001	<0.001
17-18years (2)	482	5 (1.04)	477 (98.96)			–	0.990
Adults (3)	66,531	617 (0.93)	65,914 (99.07)				–

CPTC, classical papillary thyroid carcinoma; DM, distant metastasis.

TABLE 4-2 | Comparison of histological subtype in different age groups with distant metastasis of PTC.

	Histological subtype	With DM[no. (%)]	Without DM [no. (%)]	*P*-value
2-16 years	CPTC	31 (93.94)	570 (72.43)	0.006
	FVPTC	2 (6.06)	217 (27.57)	
	No. of patients	33	787	
17-18 years	CPTC	5 (62.50)	477 (74.18)	0.731
	FVPTC	3 (37.5)	166 (25.82)	
	No. of patients	8	643	
Adults	CPTC	617 (67.14)	32,857 (33.16)	0.694
	FVPTC	302 (32.86)	66,229 (66.84)	
	No. of patients	919	99,086	

PTC, papillary thyroid carcinoma; CPTC, classical papillary thyroid carcinoma; FVPTC, follicular variant papillary thyroid carcinoma; DM, distant metastasis.

Tumors were generally infiltrative in patients younger than 15 years (20). Therefore, we believe that it is necessary to take the age into consideration to divide young children separately from adolescents during the development and disease progression of PTC. We calculated the cut-off age for DM in children as 16 years old by ROC curve, with the area under the curve of 0.676, sensitivity of 0.602 and specificity of 0.707 (**Figure 1B**), and we found that adolescent patients aged 17-18 years performed similar to adult patients regardless of the proportion of DM or the distribution of histopathological subtypes, while young children aged 2-16 years exhibited significantly different features from adolescent patients aged 17-18 years and adult patients. Therefore, further studies are needed to provide better individualized treatment for pediatric PTC patients.

A previous study had shown that CPTC and FVPTC were different in driving somatic genetic alterations and cell signal transduction, which leaded to the poor differentiation and strong invasiveness of CPTC (21). CPTC was a strong predictor of high

TABLE 5-1 | Risk factors of distant metastasis in pediatric patients aged 2-16 years calculated by logistic regression models.

Variables	Univariate analysis				Multivariate analysis			
	B	OR	95% CI	*P*-value	B	OR	95% CI	*P*-value
Gender								
Female			ref					
Male	0.484	1.623	0.739-3.564	0.227				
ETE								
Absence			ref				ref	
Presence	1.824	6.194	2.900-13.228	**<0.001**	1.015	2.759	1.240-6.140	**0.013**
LNM								
Absence			ref				ref	
Presence	3.330	27.925	3.797-205.354	**0.001**	2.568	13.039	1.682-101.086	**0.014**
Foci								
Solitary			ref					
Multifocal	1.081	2.947	1.410-6.161	**0.004**	0.489	1.630	0.752-3.532	0.215
Size								
≤1cm			ref					
>1cm	1.984	7.270	0.986-53.636	0.052				
Ethnicity								
Black			ref					
White	-0.285	0.752	0.172-3.285	0.705				
Other	0.185	1.204	0.224-6.483	0.892				
Histological subtype								
FVPTC			ref				ref	
CPTC	1.775	5.901	1.400-24.867	**0.016**	1.460	4.308	1.006-18.447	**0.049**
Other		0.000		0.999				

B, regression coefficient; OR, odds ratio; CI, confidence interval; ETE, extrathyroidal extension; LNM, lymph node metastasis; FVPTC, follicular variant papillary thyroid carcinoma; CPTC, classical papillary thyroid carcinoma; ref, reference. Bold values indicate statistical significance.

TABLE 5-2 | Risk factors of distant metastasis in adult patients calculated by logistic regression models.

Variables	Univariate analysis				Multivariate analysis			
	B	OR	95% CI	*P*-value	B	OR	95% CI	*P*-value
Gender								
Female			ref				ref	
Male	1.133	3.104	2.732-3.527	**<0.001**	0.823	2.277	1.999-2.595	**<0.001**
ETE								
Absence			ref				ref	
Presence	2.147	8.563	7.498-9.780	**<0.001**	1.501	4.486	3.866-5.206	**<0.001**
LNM								
Absence			ref				ref	
Presence	1.850	6.362	5.563-7.275	**<0.001**	1.119	3.062	2.629-3.566	**<0.001**
Foci								
Solitary			ref					
Multifocal	0.400	1.491	1.313-1.694	**<0.001**	-0.091	0.913	0.800-1.041	0.174
Size								
≤1cm			ref				ref	
>1cm	1.619	5.048	4.147-6.146	**<0.001**	0.849	2.337	1.904-2.868	**<0.001**
Ethnicity								
Black			ref					
White	-0.165	0.848	0.659-1.090	0.198				
Other	0.274	1.315	0.986-1.756	0.063				
Histological subtype								
FVPTC			ref				ref	
CPTC	0.028	1.028	0.895-1.181	0.694	-0.477	0.621	0.537-0.718	**<0.001**
Other	1.081	2.948	2.104-4.132	**<0.001**	-0.103	0.902	0.637-1.278	0.562

B, regression coefficient; OR, odds ratio; CI, confidence interval; ETE, extrathyroidal extension; LNM, lymph node metastasis; FVPTC, follicular variant papillary thyroid carcinoma; CPTC, classical papillary thyroid carcinoma; ref, reference. Bold values indicate statistical significance.

recurrence risk and high cancer-specific mortality, with a worse prognosis than FVPTC (9). In a study including 163 patients aged less than 18 years diagnosed as PTC, BRAF mutations and RET and NTRK fusions were detected mainly in CPTCs (20). The presence of a BRAFV600E mutation was reported to be correlated significantly with the need for a second treatment during the follow-up in patients under 18 years of age, and BRAF mutations might be associated with more aggressive clinical features and a higher risk of recurrence or persistence of disease in the pediatric population (5). Furthermore, the fusion-driven tumors, in general, displayed a lower thyroid differentiation score than mutation-driven samples (≤18 years), suggesting that gene fusion-positive pediatric PTCs are less differentiated (22). Besides, fusion gene-positive pediatric PTC cases (6-20 years) had more aggressive disease with more frequent extrathyroidal extension and lymph node and distant metastases than patients without fusion genes (23). This finding prompted us to speculate that the poor outcome in classical PTCs compared to FVPTCs was largely attributable to higher proportion of BRAF V600E mutations and RET and NTRK fusions in the former group. Clinically, the prevalence of high-risk parameters was significantly different among the two subtypes. Risk factors including ETE, LNM, stages III/IV, disease recurrence, radioiodine treatment, as well as mortality were lower in FVPTC (24). After studying a subgroup of FVPTC with an intact tumor envelope and very good prognosis, it is referred to as "non-invasive follicular thyroid tumor with papillary features" (NIFTP) and is classified as a non-cancerous tumor (25). In conclusion, the biological behaviors and disease prognosis of CPTC and FVPTC differ significantly, and they should be distinguished in diagnosis and treatment.

It has been reported that CPTCs were less represented in patients aged less than 15 years than in patients aged 15-18 years, while FVPTCs occurred more frequently in the former group (20). However, our results showed that the proportion of CPTCs was higher than that of FVPTCs, both in children aged ≤18 years and in young children aged 2-16 years regardless of with or without DM. The reason for this difference may be that our study is based on a large sample size, but the previous study included only 163 samples. In the other hand, our logistic regression analysis showed that tumor diameter > 1 cm was not a risk factor for DM in patients aged 2-16 years (p>0.05). This indicates that although children, when compared to adults, had larger primary tumors (26) and the tumor diameter is related to the poor prognosis of FVPTC (9), but tumor diameter is not a risk factor for DM in patients aged 2-16 years, which may be one of the reasons why the proportion of FVPTCs in patients aged 2-16 years with DM is lower than that of CPTCs.

The molecular biological characteristics of the pathogenesis of thyroid cancer in children and adults may explain the differences in clinical manifestations and prognosis (27). Despite this, the clinical assessment and treatments used in pediatric thyroid cancer are the same as those implemented for adults (18). Histopathological subtypes have recently been shown to play an important role in determining the persistence and/or recurrence of disease (28). Our data show that the incidence of DM in young children (2-16 years) with CPTC is significantly higher than that in adolescents (17-18 years) and adults, and that the same histopathological subtype presents different clinical and histopathological features in different age groups. ETE, LNM, and CPTC subtype are risk factors for DM in pediatric patients aged 2-16 years. We suggest that the histopathological subtypes of CPTC and FVPTC should be classified and managed separately in patients aged 2-16 years to cope with the persistent or recurrent risk of disease, but further studies are needed to expand our findings, which may guide therapeutic strategies.

This study has certain limitations. First, it was a retrospective analysis based on the SEER database. There was an inherent selection bias. To control for selection bias, we adopted a rigorous scientific study design, clarified the inclusion criteria and exclusion criteria of subjects, and unified the disease diagnosis. Second, not all data are available from the SEER database, such as patient recurrence information, not allowing analysis of the subsequent DM occurrence in different histopathological subtypes. Third, the use of stratified analysis in this study would cause statistical deviation due to the small number (41 cases) of pediatric patients with DM. Finally, data in this study were extracted from the SEER database from 2004 to 2015, but the 2017 WHO classification introduced the NIFPT terminology for encapsulated FVPTCs. As a result, a minority of the "FVPTCs" reported in the SEER database may in fact be NIFPTs. It might affect the differences in outcome between bona fide FVPTCs vs CPTCs within an acceptable range.

However, our study is the first with a large sample to investigate the role of common histopathological subtypes in determining DM of pediatric PTC and the reasons of the high DM incidence in children. We propose that pediatric PTC patients should be divided into patients aged 2-16 years and patients aged 17-18 years. CPTC patients aged 2-16 years might be treated more aggressively. Further studies in the future may be helpful to guide the treatment strategies for PTC in pediatric patients.

In conclusion, this study showed that in CPTC, the incidence of distant metastasis in young children (2-16 years) was significantly higher than that in adolescents (17-18 years) and adults (>18 years). There was no such difference among patients with FVPTC. In patients with distant metastasis aged 2-16 years, CPTC patients had a higher rate of DM than FVPTC patients. Extrathyroidal extension, lymph node metastasis, and CPTC histopathological subtype were risk factors for distant metastasis in young children aged 2-16 years.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

XZ, ZW, ZG, JX, MC, WS, LH, WD, JH, DZ, CL, TZ, LS, PZ, and HZ contributed to this study. ZW contributed to the conception and design of this study. XZ collected data. XZ and ZG performed the statistical analysis. XZ and ZW drafted and

wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Head-to-Head Comparison of ^{68}Ga-PSMA-11 and ^{131}I in the Follow-Up of Well-Differentiated Metastatic Thyroid Cancer: A New Potential Theragnostic Agent

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Introduction: Thyroid cancer is the main endocrine neoplasia worldwide, for which ^{131}I therapy is the cornerstone treatment. One of the main problems of follow up in patients with this type of cancer, is the need for thyroglobulin stimulation, not to mention the poor availability of ^{123}I or ^{124}I, to perform studies with a higher degree of sensitivity. Prostatic Specific Membrane Antigen (PSMA) PET/CT has demonstrated to be quite useful in a diversified number of neoplasms, on behalf of its capacity of evaluating the extent of type II carboxypeptidase expression in vascular endothelium. The end point of this article is to assess whether this novel image method possesses applicability in thyroid neoplasms follow up, for diagnostic and potentially therapeutic purposes.

Methods: We retrospectively evaluated well differentiated metastatic thyroid cancer patients, who underwent a post therapeutic ^{131}I dose whole body scan (WBS) and complementary SPECT/CT, as well as ^{68}Ga-PSMA–11 PET/CT.

Results: Ten patients with differentiated thyroid cancer were included, of whom 80% were women and 20% men, mean age was 58 years old (± 11.6). Sixty-four metastatic lesions were analyzed, 67.19% had papillary histology and 32.81% were follicular type, the most affected site of metastases was bone in 57.81%, followed by lung 17.19%, lymph nodes 7.81%, postoperative thyroid bed 4.69%, brain 4.69% and others 7.81%. ^{68}Ga PSMA-11 PET/CT detected 64/64 lesions, all of them also identified by computed tomography (CT), whereas ^{131}I SPECT/CT detected 55/64 lesions. Discrepant lesions were localized in lung 44.4%, brain 22.2%, postoperative thyroid bed 11.1%, lymph nodes 11.1% and bone 11.1%. The degree of correspondence among observers was outstanding for both radiotracers, but close upon perfect for PSMA-11 ($\kappa = 0.98$; 95% CI, 0.80 – 0.91), as opposed to ^{131} I ($\kappa = 0.86$; 95% CI, 0.71 – 0.76).

Conclusions: ^{68}Ga-PSMA PET/CT showed an utterly superior capability for metastatic lesion detection when compared to ^{131}I SPECT/CT. These findings suggest that PSMA PET/CT could possibly and precociously identify radioiodine refractoriness. PSMA uptake values not only expedite diagnosis, but also award it the ability to be used for therapeutic intents.

Keywords: thyroid cancer, PSMA, iodine, theragnostic, PET

INTRODUCTION

Thyroid cancer is the most common endocrine malignancy, with approximately 62,000 new cases per year in the USA (1). Differentiated thyroid cancer (DTC) is a slow growing tumor with a very low disease-specific mortality rate for local-regional disease (5 – year overall survival of 99.9% and 98.3% for localized and regional metastatic disease respectively), however distant metastatic disease is associated with significantly worse prognosis (5 – year overall survival of 54.9%) (1). It is estimated that approximately 2,200 cancer deaths will occur among patients with thyroid carcinoma in the United States (2). Oncological outcomes in thyroid cancer depend on histologic subtypes, age, gender, tumor characteristics, molecular features, and lymph node or distant metastases. Most thyroid cancer patients have a favorable response to surgery and risk-adapted postoperative therapy with thyroid hormone suppression and radioactive iodine (RAI) therapy, in selected situations (3).

Standard of care imaging modalities include diagnostic ^{131}I – WBS after surgery to assess the completeness of thyroidectomy and whether residual disease is present. Post-treatment ^{131}I whole body imaging must be done, considering that up to 25% of scans exhibit lesions that may be clinically important, which were not detected by other diagnostic imaging methods (4). However, some of the inherent limitations to this method, include patient preparation (mainly levothyroxine withdrawal, which decreases quality of life due to the exacerbation of hypothyroidism symptoms, among others) and low diagnostic accuracy (planar scan has a 41% sensitivity and 68% specificity, while sensitivity of SPECT reaches up to 45% with an 89% specificity) (5).

DTC can become resistant to RAI therapy, and in those cases, treatment options are limited (6). ^{18}F-FDG PET/CT scan is indicated for patients with a negative ^{131}I WBS, who have suspicion of structural disease based on other imaging methods and/or elevated thyroglobulin (Tg) suggestive of distant metastases (7). Thus, imaging and therapy in thyroid cancer would largely be benefited from improvement.

Novel molecular radiotracers utilizing PSMA ligand uptake measured with PET/CT have emerged as a new generation imaging modality. PSMA is a type II transmembrane glycoprotein highly expressed in prostate cancer (PCa) and is the upcoming imaging modality for staging, re-staging and response assessment in PCa (8). It has showed better diagnostic accuracy than conventional imaging in high-risk PCa patients, and therapeutic benefits (with safety and efficacy) when combined with ^{177}Lu (9, 10). Despite its name, it is not specific to prostate; PSMA has been found to be expressed in the neovasculature of multiple solid tumors, and increased uptake of ^{68}Ga-PSMA PET/CT has been demonstrated in several non-prostatic malignancies, including thyroid cancer (11, 12). Specifically, histological studies have verified the expression of PSMA in the microvasculature of thyroid cancers (13, 14) where, PSMA expression was related to malignant disease, poor prognostic factors and poorer progression free survival (PFS) (15). This evidence suggests the potential use of PSMA PET/CT as a theragnostic and prognostic imaging biomarker. Given this clinical and technical background, we report our experience in a head-to-head comparison of these two imaging procedures for the detection of disease in patients affected by thyroid cancer.

MATERIALS AND METHODS

Study Population

This retrospective study was approved by our hospital's Local Research Ethics Committee; due to its retrospective condition, informed consent from patients was not necessary.

This study incorporates patients that were referred to the nuclear medicine department in the period comprehended between 2019 – 2020, with the following inclusion criteria: (1) patients with histologically proven well differentiated thyroid cancer, (2) who had received radioiodine therapy, and (3) no other anti-tumor therapy prior to PET/CT. Exclusion criteria were patients (1) who had other primary malignancies at the time of examination, (2) with prior history of neck radiotherapy. FDG PET/CT was not performed on any patient, since we were not looking for tumoral dedifferentiation.

Every patient that received an ^{131}I ablative or therapeutic dose, was prepared according to ATA guidelines, verifying TSH levels (above 30 μUI/L) prior to the radiopharmaceutical administration. None of them received rhTSH.

Image Acquisition

WBS and SPECT/CT from vertex to pelvis (Siemens SPECT/CT Symbia T6, Siemens Healthineers, Knoxville, TN, US) were performed 7 days after therapeutic oral administration of ^{131} I (3,700 to 7,400 MBq), according to the clinical standard protocol. Regarding the timing protocol for this study, we followed ATA guidelines recommendations of performing WBS in a period ranging from 2 to 12 days post therapy, considering the dose of ^{131}I given, and this radiotracer's half-life. The SPECT reconstruction datasets were: 128 × 128 matrix (ordered subset expectation maximization [OSEM]) algorithms with 8 subsets and 4 iterations, followed by an 8 – mm Gaussian filter.

Whole – body (from top of head to mid – thigh) PET/CT was performed after 6 weeks from SPECT/CT acquisition, approximately 60 minutes after the intravenous injection of ^{68}Ga PSMA–11 (148 – 185 MBq) according to the clinical standard protocol for tumor imaging. A Biograph mCT 20 Excel PET/CT scanner was used (Siemens Healthineers, Knoxville, TN, US). The PET reconstruction datasets were 400 × 400 matrix (pixel size: 1.5625 × 1.5625 × 2.78 mm^3) with Time of Flight (TOF) OSEM algorithms with 21 subsets and 3 iterations, followed by a 6 – mm Gaussian filter. CT was acquired using 140 mA, 130 kV, 5 mm width and a 1 mm pitch.

Image Analysis

Two experienced nuclear physicians evaluated the resulting images in consensus. Both studies of each patient were compared, identifying the areas of greatest radiotracer uptake, and associating them with corresponding CT findings. Lesions were classified by regions in lymph nodes, postoperative thyroid bed, bone, brain, lung, and others (muscle and kidneys). Quantified lung lesions were larger than 1 cm, a maximum of 4 lesions.

Regarding SPECT/CT, counts of each lesion were quantified, as well as those from background (pectoralis major muscle) to obtain tumor–to–background ratios (TBR = lesion counts/ background counts). As for PET/CT, the SUVmax was measured with isocontour volume of interest (VOI), along with the determination of the background with a spherical VOI of 1 cm^3 in the pectoralis major muscle, which was used as a reference to calculate the TBR (TBR = SUVmax lesion/SUVmax tissue reference).

Reference Standard

Lesions that went through biopsy, or that presented tomographic alterations associated with uptake of at least one of the radiopharmaceuticals, were taken as true positives. Positive lesions in PET and SPECT were considered those with TBR > 1.

Statistical Analysis

Collected data was analyzed using the statistical software STATA 14.0. The univariate analysis carried out included the clinical characterization of the patients. Descriptive statistic was performed, providing frequencies for categorical variables; for continuous variables, the standard deviation (SD), mean and median were provided.

The sample size was determined by convenience, due to the retrospective characteristic.

RESULTS

We evaluated 10 patients with differentiated thyroid cancer: 80% (n = 8) were women and 20% (n = 2) men, mean age was 58 years old (± 11.6). The characteristics of the population, including histology, are summarized in **Table 1**.

Sixty-four metastatic lesions were analyzed, 67.19% (n = 43) had papillary histology and 32.81% (n = 21) were follicular type, the most affected site of metastases was bone in 57.81% (n = 37), followed by lung 17.19% (n = 11), lymph nodes 7.81% (n = 5), postoperative thyroid bed 4.69% (n = 3), brain 4.69% (n = 3) and others 7.81% (n = 5).

64/64 of lesions (100%) fulfilled ^{68}Ga PSMA – 11 PET/CT positivity criteria. The main image criterion of positivity was the presence of focal uptake areas in one or more locations and/or higher than in the surrounding tissue background regardless of the presence or absence of lesion in the corresponding CT images. whereas ^{131}I SPECT/CT detected 55/64 lesions (85.9%). Discrepant lesions (n = 9) were localized in lung 44.4% (n = 4) (**Figure 1**), brain 22.2% (n = 2), postoperative thyroid bed 11.1% (n = 1), lymph nodes 11.1% (n = 1) and bone 11.1% (n = 1) (**Figure 2**). In PET/CT median values resulted as follows: SUVmax 7.25, SD 11.8, (range from 1.8 to 70.5), TBR 5.8, SD 6 (1.8 – 35). While in SPECT/CT median value of counts were 40, SD 4,364.6 (0 – 35,000), TBR 6.1 and SD 114.8 (0 – 714). Higher uptake of PSMA was seen in brain metastases (**Figures 3**–**5**), crosswise it showed a lower uptake in malignant lymph nodes (**Figures 6**, **7**). In contradistinction, greater uptake was seen with ^{131}I imaging in residual thyroid tissue (**Figure 8**), whilst diminished uptake was observed in lung lesions with this radiotracer (**Figure 9**). Although median TBR was greater in ^{131}I SPECT/CT, SD was also higher; this was since in patient number 1, residual thyroid tissue was identified, which had substantial radioiodine avidity, causing an enormous amount of scattering data (**Figure 10**). In the rest of the patients, TBR from PET/CT was greater, meaning they had a much better target–to–background ratio (**Figure 11**). The degree of correspondence among observers was outstanding for both radiotracers, but close upon perfect for PSMA – 11 (κ = 0.98; 95% CI, 0.80 – 0.91), as opposed to ^{131} I (κ = 0.86; 95% CI, 0.71 – 0.76).

DISCUSSION

This study describes to the best of our knowledge, the first experience regarding ^{68}Ga-PSMA PET/CT vs ^{131}I SPECT/CT imaging in patients with well – differentiated metastatic thyroid carcinoma. PSMA PET/CT detected various types of lesions including central nervous system, lymph nodes, pulmonary nodules, and bone metastases. These findings are similar to those of Verma et al. and Lütje et al., who also detected these and various other lesions (16, 17). PSMA expression is frequently observed in the tumor-associated neovasculature of multiple neoplasms, including thyroid cancer. A systematic review by Bertagna et al. on thyroid incidentalomas with ^{68}Ga – PSMA, showed that up to 23% of the detected lesions, corresponded to malignant – type lesions (18). There are other reviews that demonstrate a high expression of PSMA receptors in the microvasculature of thyroid neoplastic cells, which has risen the feasibility to evaluate this transmembrane protein; nevertheless, some studies carried out in microarrays have shown a variable uptake in well differentiated histologies. Apparently, the expression of PSMA is related to size and vascular invasion in follicular histologies (19, 20). Moore et al.,

TABLE 1 | Patient characteristics.

Patient	Age (years)	Gender	Histopatology	Thyroglobulin (ng/mL)	Anti- Thyro-globulin anti-bodies (U/ML)	Radioiodine cumulative dose (MBq)	Treatment received	Metastatic lesion localization	RAI lesion uptake (TBR)	PSMA lesion uptake (TBR)
1	64	Male	Papillary thyroid carcinoma, classic variant	264.5	4000	7400	Total Thyroidectomy (TT) + Radioactive iodine (RAI) therapy	Lymph nodes, thyroid bed, lung and bone.	0 – 714.2	3 – 14.9
2	42	Male	Papillary thyroid carcinoma, classic variant	114	12	16650	TT + RAI	Lymph nodes.	0	1.8
3	52	Female	Papillary thyroid carcinoma, classic variant	1000	17.7	7400	TT + RAI	Brain, bone, muscle.	0 – 544	1.4 – 35.2
4	73	Female	Papillary thyroid carcinoma, classic variant	546	2.3	14800	TT + RAI	Lung and retrotracheal implant.	0 – 7.5	3.3 – 6.5
5	61	Female	Follicular thyroid carcinoma, insular differentiation (20%)	3922	1.55	25900	TT + RAI	Bone and paravertebral implant.	1.9 – 10.3	6 – 11.3
6	53	Female	Follicular thyroid carcinoma, angioinvasive with oxyphilic switch	1444	1.2	33300	TT + RAI	Bone.	3 – 10.5	2.5 – 4.9
7	65	Female	Papillary thyroid carcinoma, classic variant	253	2	14800	TT + RAI	Thyroid bed and lymph nodes.	3 – 12.6	3.3 – 5.6
8	34	Female	Papillary thyroid carcinoma, with solid areas (10%) and hobnail micropapillary (5%)	219	1	29600	TT + RAI	Thyroid bed and lung.	1	3 – 5
9	55	Female	Papillary thyroid carcinoma, classic variant	1384	10.5	22200	TT + RAI	Lymph nodes, bone, kidneys.	0 – 13	2.2 – 17
10	63	Female	Follicular variant of Papillary thyroid carcinoma, tall cell variant (10%), insular variant (20%)	2046	174.6	46250	TT + RAI	Lymph nodes, lung and brain.	1.6 – 14.8	8 – 74

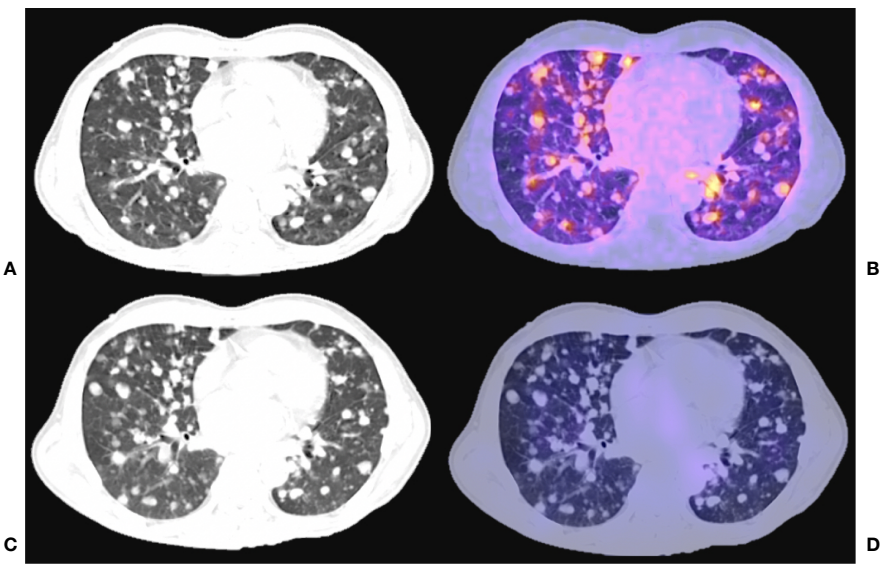

FIGURE 1 | 55 years old female, papillary thyroid carcinoma classic variant, treated with thyroidectomy + RAI (22200 MBq cumulative dose). Thyroglobulin 1384 ng/ml. Fused hybrid and simple CT thoracic images. **(A)** CT acquired for PET fusion **(B)** ^{68} Ga - PSMA PET/CT. **(C)** CT acquired for SPECT fusion **(D)** ^{131}I -SPECT/CT.

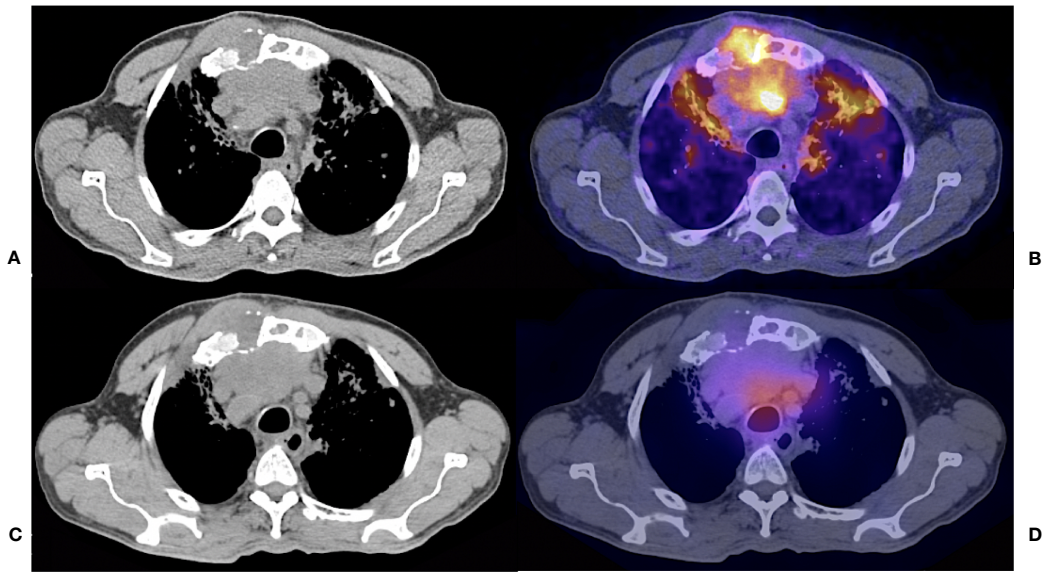

FIGURE 2 | 61 years old female with follicular thyroid carcinoma, insular differentiation (20%), treated with TT + RAI (cumulative dose 25900 MBq); Thyroglobulin 3922 ng/dl. Fused hybrid and simple CT thoracic images shows a lytic lesion in sternum with soft tissue component and prevascular conglomerate. **(A)** CT acquired for PET fusion **(B)** ^{68}Ga – PSMA PET/CT with focal uptake of the radiotracer. **(C)** CT acquired for SPECT fusion **(D)** ^{131}I – SPECT/CT shows diffuse uptake of the radiotracer.

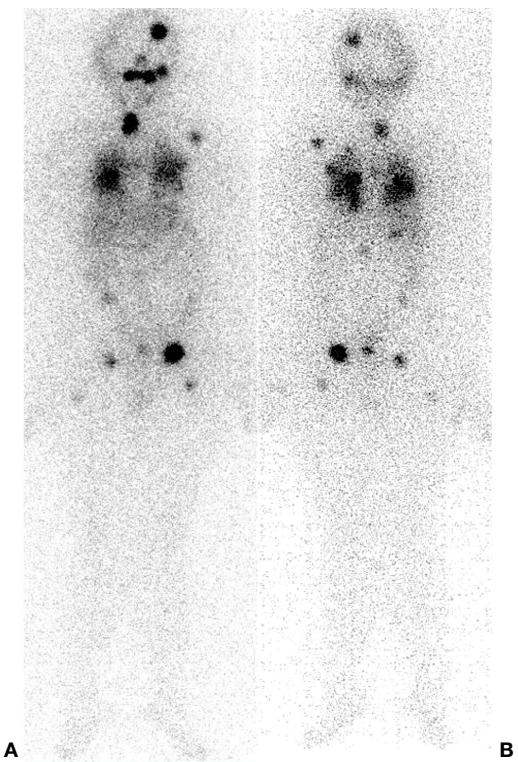

FIGURE 3 | ^{131}I – Post Therapy Whole Body Scan. 42 years old male, papillary thyroid carcinoma classic variant. Thyroglobulin 114. RAI cumulative dose (16650 MBq). **(A)** Anterior projection. **(B)** Posterior projection.

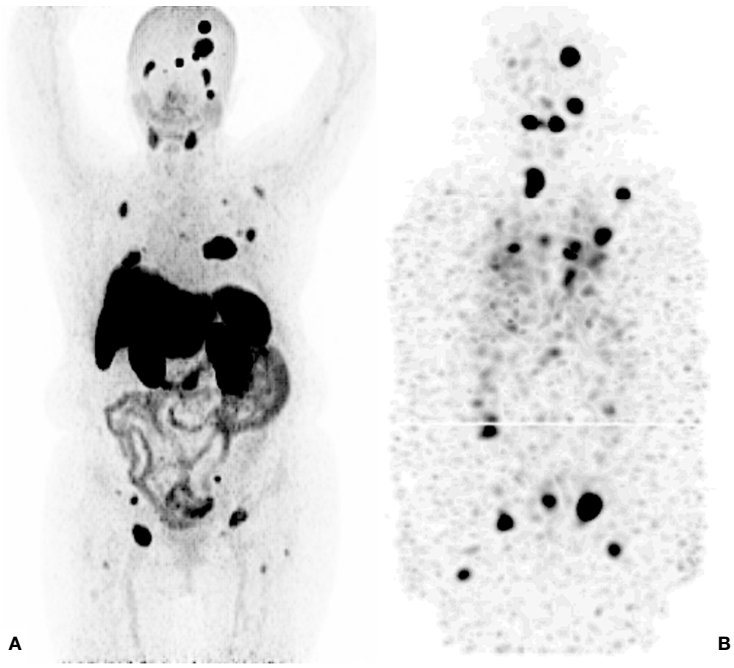

FIGURE 4 | ^{131}I – Post Therapy Whole Body Scan. 42 years old male, papillary thyroid carcinoma classic variant. Thyroglobulin 114. RAI cumulative dose (16650 MBq) Maximum Intensity Projections. **(A)** ^{68} Ga - PSMA PET/CT. **(B)** ^{131}I - SPECT/CT.

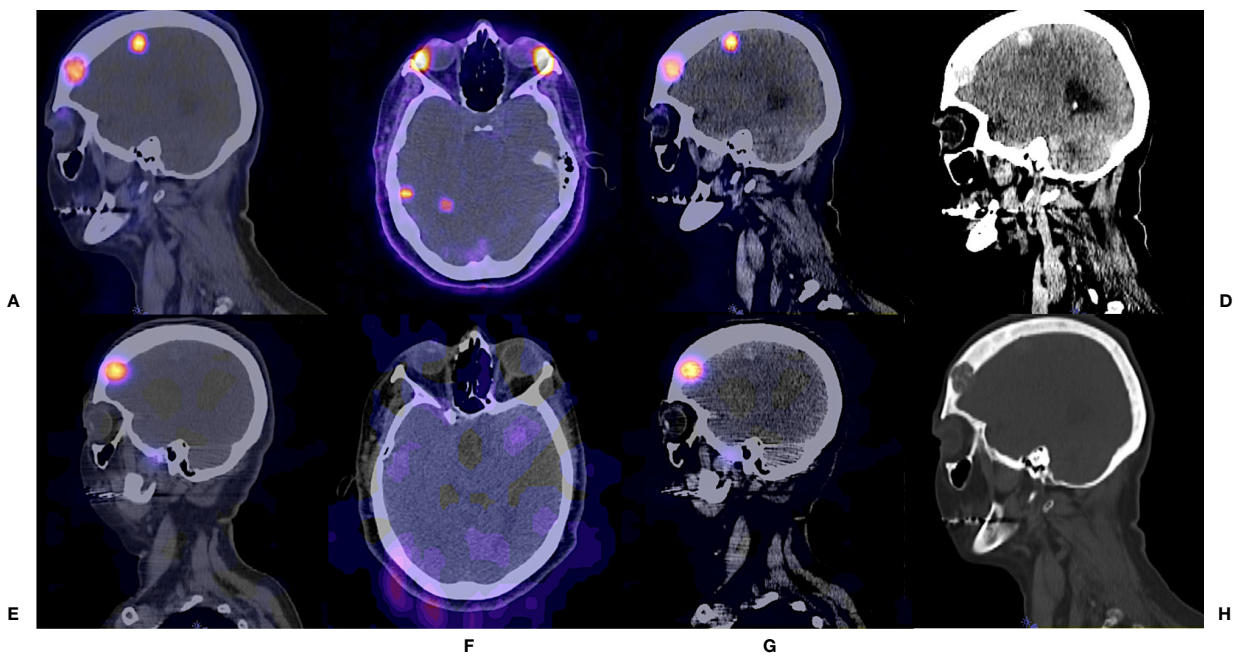

FIGURE 5 | 52 years female, papillary thyroid carcinoma classic variant. Thyroglobulin 1000; radioiodine cumulative dose 7400 MBq. Fused hybrid and simple CT head and neck images. **(A–C)** show ^{68} Ga – PSMA PET/CT sagittal **(A, C)** and axial **(B)** views in soft tissue **(A, B)** and brain windows **(C)**; while **(E–G)** demonstrate ^{131}I – SPECT/CT images in homologous reconstructions. Notice how PSMA PET/CT illustrates more intraparenchymal metastatic lesions, which also show higher tracer uptake than ^{131}—SPECT/CT. It also can be visualize a bone lytic lesión in frontal bone. Images on the right represent simple CT sagittal recontructions in brain **(D)** and bone **(H)** windows, where morphologic aspects of the lesions are better characterized.

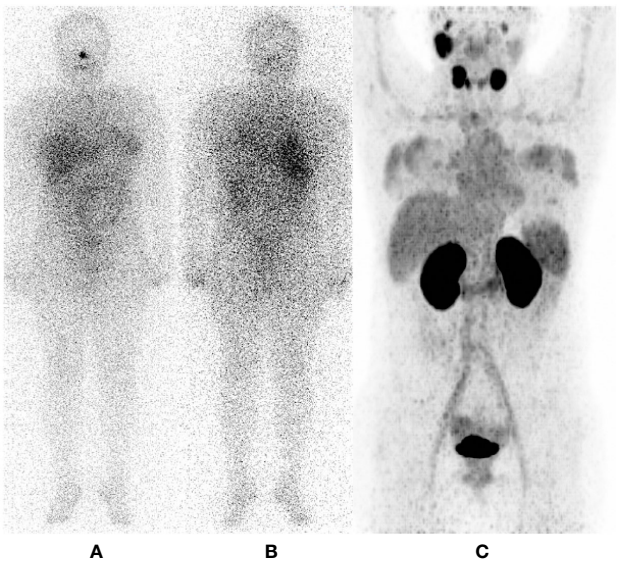

FIGURE 6 | 73 years old female treated with total thyroidectorny + RAI (cumulative dose 14800 MBq); Thyroglobulin 546 ng/ml. **(A, B)** ^{121}I – post therapy whole body scan shows no uptake other than usual biodistribution. Physiological uptake variant was observed in mamary glands with both tracers. **(C)** ^{68}Ga – PSMA PET/CT MIP, on the other hand, conspicuously shows a central cervical compartment adenopathy.

demonstrated the immunohistochemical PSMA expression in benign and malignant thyroid tissue, as well as in metastatic tissue and infiltrated lymphatic nodes, the first of them, resulting in a higher degree of the antigen expression. It has also been observed that PSMA expression depends on histology; moderate to high grade expression of PSMA has been documented in classic papillary, follicular and papillary with follicular variant histologies, as well as in radioiodine – refractory subtypes

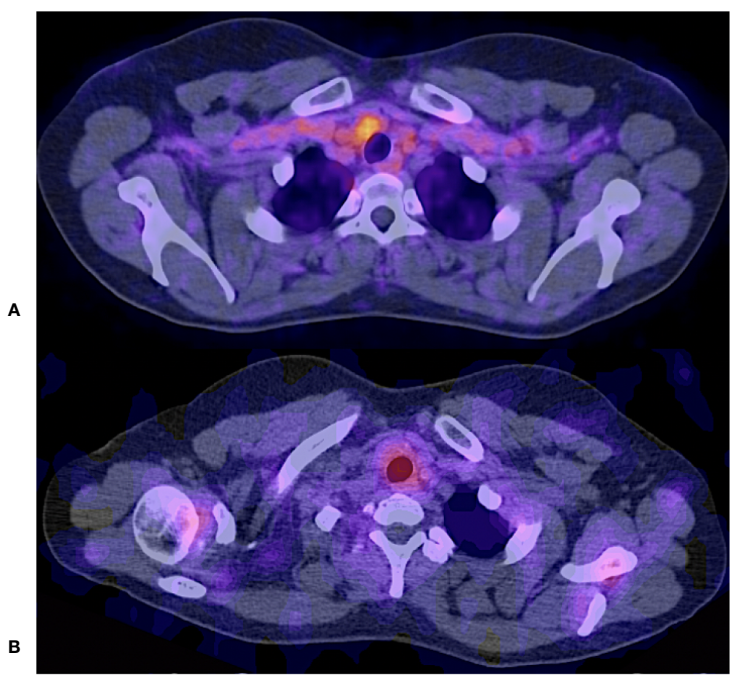

FIGURE 7 | Fused hybrid images **(A)** ^{68}Ga PSMA PET/CT shows focal radiotracer uptake in the cervical level VI located adenopathy. **(B)** ^{131}I – SPECT/CT shows diffuse radiotracer uptake in the same adenopathy.

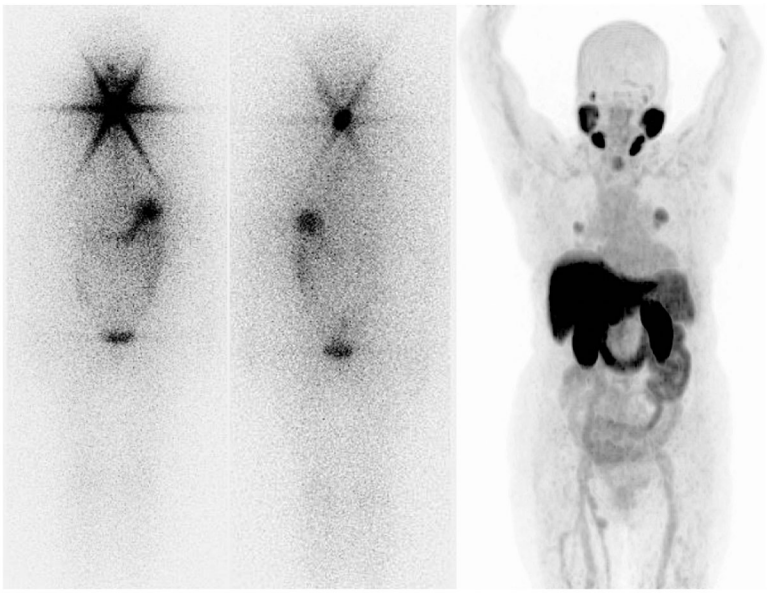

FIGURE 8 | 65 years old female; Thyroglobulin 253 ng/dl; treated with TT+RAI (14800 MBq). First two images show an ^{131}I – post therapy whole body scan in **(A)** anterior and **(B)** posterior projections, with a star – shapped intense uptake. The image on the right **(C)**, shows ^{68}Ga – PSMA PET/CT maximum intensity projection [MIP], which manifests a glaring superiority regarding spacial resolution, and target–to–background ratio.

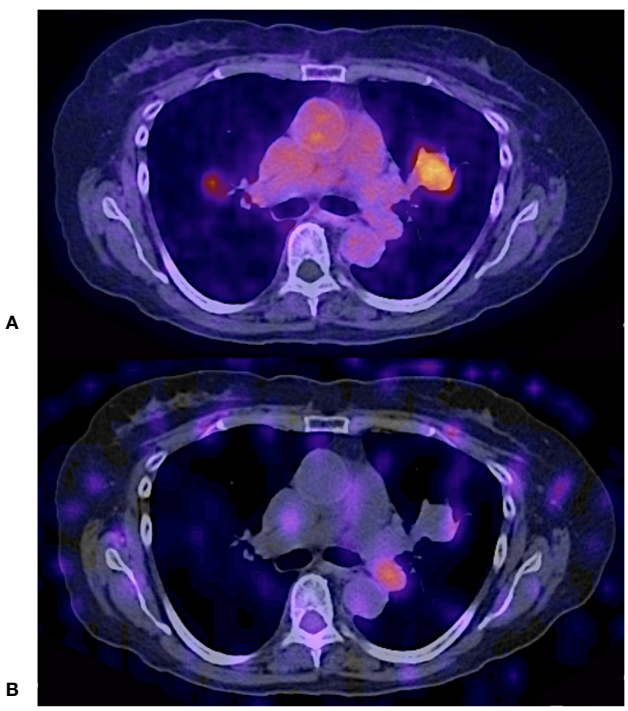

FIGURE 9 | 65 years old female; Thyroglobulin 253 ng/dl; treated with TT+RAI (cumulative dose 14800 MBq) **(A)** ^{68}Ga - PSMA PET/CT **(B)** ^{131}I – SPECT/CT.

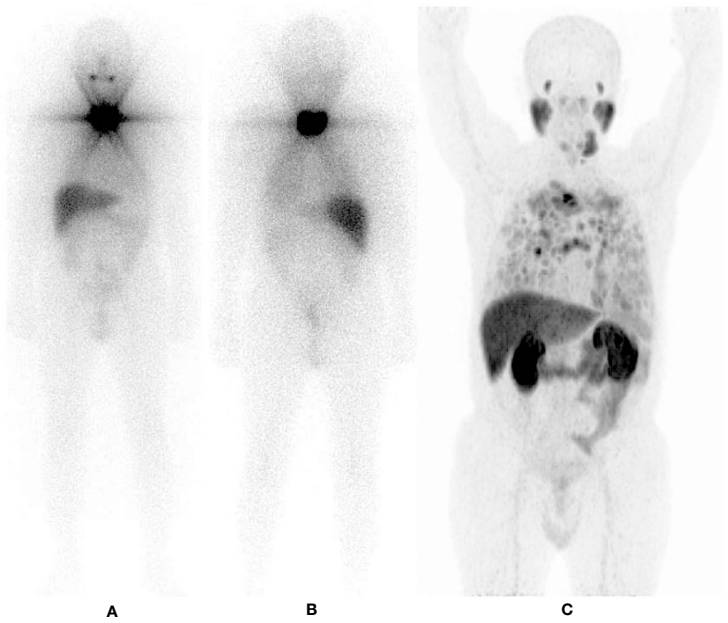

FIGURE 10 | 64 years old male, papillary thyroid carcinoma classic variant; treated with TT+RAI (cumulative dose 7400 MBq); Thyroglobulin 264.5 ng/dl. **(A, B)** ^{131}I – post therapy whole body scan shows a focal uptake in thyroid bed and difusse concentration in lungs. **(C)** ^{68}Ga – PSMA PET/CT maximum intensity projection with multiple lung and lymph node lesions PSMA avid.

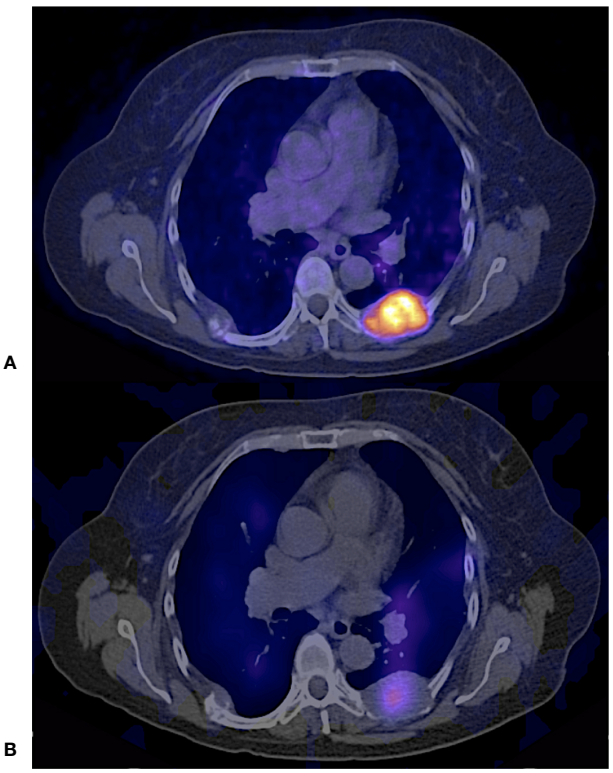

FIGURE 11 | 61 years old female with follicular thyroid carcinoma, insular differentiation (20%), treated with TT + RAI (cumulative dose 25900 MBq); Thyroglobulin 3922 ng/dl. Paravertebral implant. **(A)** ^{68}Ga - PSMA PET/CT avid lesion. **(B)** ^{131}I – SPECT/CT showed diffuse uptake of the radiotracer.

(RAIR); and weak to null expression in anaplastic thyroid carcinoma (21). PSMA is also implicated in the generation of glutamate *via* its enzymatic action on N–acetyl–aspartyl–glutamate (NAAG). This transmembrane protein is required for liberation of glutamate from tumor – derived NAAG, although this relationship has not yet been established in the context of PCa. It has been shown that PSMA generates a localized reservoir of glutamate from NAAG and activates tumor growth in some neoplastic cells, like in high – grade ovarian serous adenocarcinoma (22). It is described that PSMA uptake may have some false positives related to inflammatory processes and non-prostatic malignancies such as clear cell renal carcinoma, hepatocarcinoma, among others (23). In the case of our study, all of the lesions were correlated with tomographic findings.

Our results demonstrate that PSMA detected more metastatic lesions than ^{131}I, which could be related to the expression of type II carboxypeptidase in the vascular endothelium (19). All the lesions manifested PSMA uptake regardless of their iodine avidity; despite it, there was a single patient who received an ^{131}I ablation dose, in whom postoperative thyroid bed showed more radioiodine avidity. The pathology report revealed negative post-surgical margins, therefore, the intense uptake of iodine, corresponds to the presence of non-tumoral thyroid tissue, which is why it did not exhibit PSMA avidity. This displays congruity with the information published in *The Atlas of Human Proteins*, where it is observed both at RNA and proteomic levels, that benign thyroid tissue has no expression of FOLH1 (24, 25).

We recognize that patients involved in this study do not have homogenous characteristics, which certainly represents a weakness in our study. As it is a pilot retrospective study, we only evaluated molecular phenotype characteristics of PSMA uptake. Also, one of the main problems regarding SPECT/CT in patients with well differentiated thyroid cancer and their follow-up, is spatial resolution, and that there are currently some other iodine radioisotopes that allow PET/CT realization, such as ^{124}I, which would also permit a better head–to–head comparison; however, there is very few availability worldwide and it is also quite expensive. Although both radiotracers studies had 6 weeks interval, progression is not likely, since patients had just received an ^{131}I therapeutic dose. Furthermore, there is evidence that sustains mean time-to-progression is 2 years (26).

Although the few numbers of patients evaluated was a limitation, this was compensated by the significant number of lesions that were observed in the study. These findings indicate the potential clinical usefulness of ^{68}Ga – PSMA PET/CT; not only because it is able to depict tumor lesions in various locations, but also because it may detect lesions which are not visualized by ^{131}I SPECT/CT. Furthermore, ^{68}Ga – PSMA PET/CT could be used for theragnostic application in selected patients for possible therapy with ^{177}Lu – PSMA–617. Vries et al., described the experience of employing ^{68}Ga – PSMA PET/CT imaging and subsequent therapy of metastatic RAIR DTC using ^{177}Lu – PSMA–617, where one of the two patients who underwent radioligand therapy, showed slight, temporary response (27). Literature is scarce regarding ^{177}Lu – PSMA therapy in patients with RAIR DTC, Assadi and Ahmadzadehfar presented another case report in which both ^{177}Lu – PSMA and ^{177}Lu – DOTATATE was given to a patient (28). Since PSMA uptake mechanism does not depend on iodine – sodium (I – Na) symporters, TSH stimulation is not necessary for theragnostic purposes. This evidence suggests that PSMA could be an alternative for diagnosis and treatment in patients diagnosed with thyroid cancer; nonetheless, future prospective studies should be performed.

CONCLUSIONS

In patients with well differentiated thyroid cancer, ^{68}Ga – PSMA PET/CT detects a greater number of lesions than ^{131} I, acknowledging CT as reference. In none of the cases, were there lesions observed by radioiodine imaging, that were not detectable by CT. Since PSMA uptake mechanism is I – Na symporter independent, its application in patients with this type of cancer, may have several benefits and/or advantages over the actual SOC imaging modality, being the first of them not requiring levothyroxine suppression, as well as identifying precociously, lesions with a more aggressive potential that could be going through radioiodine refractoriness. Furthermore, taking into consideration the degree of PSMA uptake by metastatic lesions, theragnostic purposes ought to be considered in future prospective trials.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comité de Etica del Instituto Nacional de Cancerología. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

QP-C, JV-A, SG-R, and FG-P conceptualized the study. FG-P, QP-C, and LT-A analyzed and interpreted the data and wrote the manuscript. QP-C, JV-A, SG-R, IS-G, EG-A, LT-A, and FG-P participated in scientific discussions and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Glucose-to-Lymphocyte Ratio (GLR) as a Predictor of Preoperative Central Lymph Node Metastasis in Papillary Thyroid Cancer Patients With Type 2 Diabetes Mellitus and Construction of the Nomogram

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Background: Detection of metastasis of central lymph nodes in papillary thyroid cancer is difficult before surgery. The role of routine or preventive central lymph node dissection in the management of papillary thyroid cancer remains inconclusive. Moreover, glucose metabolism and systemic inflammation are related to the aggressiveness of several malignant tumors and the prognoses of these patients. This study aimed to construct a nomogram based on the readily available preoperative clinical features for predicting the occurrence of preoperative central lymph node metastasis in patients with papillary thyroid cancer and type 2 diabetes mellitus. The findings may underlie clinical implications for determining the appropriate treatment strategies for these patients.

Methods: A total of 419 patients were enrolled. We used the receiver operating characteristic curves to determine the best cut-off value and converted the continuous into categorical variables. Next, a single-factor logistic analysis for the independent variables was performed, following which a multivariate regression analysis was conducted for the selected significant risk factors. Finally, the nomogram was constructed and verified using external data; the existing data were compared with the original model.

Results: According to the receiver operating characteristic curves, the best cut-off values for glucose-to-lymphocyte ratio and tumor size were 4.23 cm and 0.95 cm, respectively. Findings from the multivariate logistic regression analysis suggested that age, bilateral tumors, maximum tumor size, and the ratio of glucose-to-lymphocytes were independent risk factors for preoperative central lymph node metastasis. The C-indexes in the training and the external validation data sets were 0.733 and 0.664, respectively. Both calibration curves and the Hosmer-Lemeshow tests indicated that the model was well-calibrated.

Through decision curve analysis, the predictive model was estimated to have strong clinical applicability and greater benefits. To compare the performance of the new with that of the original model, we performed a net reclassification index and the integrated discrimination improvement analyses, both of which indicated that the new model had a better predictive ability.

Conclusion: In patients with type 2 diabetes mellitus and papillary thyroid cancer, a high preoperative glucose-to-lymphocyte ratio was an independent predictor of the preoperative central lymph node metastasis. The nomogram so constructed could better predict the preoperative central lymph node metastasis in these patients.

Keywords: GLR, PTC, nomogram, NRI, IDI, DCA

INTRODUCTION

Diabetes is a common chronic metabolic disease, with annually increasing morbidity and mortality rates (1). Diabetes and cancer are closely related in that diabetes can cause an increase in the death rate among cancer patients (2–4). Previously, two large-scale studies have shown that a history of diabetes may be related to the occurrence of thyroid cancer (5, 6). Patients with diabetes mellitus (DM) are at a higher risk of developing thyroid cancer (7–9); relative to those without DM, the incidence of thyroid cancer in patients with DM increases by 20% (8). People with DM and elevated fasting blood sugar levels are more likely to develop thyroid cancer, thereby indicating a positive correlation. Consequently, elevated blood sugar may affect the survival time and clinical outcomes in cancer patients (10–12). Previous studies also show that the development of cancer increases the risk of diabetes (13). The concentrations of inflammatory cytokines, such as TNF-a, CRP, and IL-1 in diabetic patients are substantially higher relative to non-diabetic patients (14–17). In addition, it is generally believed that chronic inflammation is indispensable in the occurrence and development of diabetes, along with the pathogenesis of its complications (18). Different cell types secrete several inflammatory cytokines that are released into the blood circulation and produce differential effects on varying tissue types (19–21).

In several human cancer types, recently, systemic inflammation and the tumor microenvironment play indispensable roles in activating tumor cell proliferation, invasion, and metastases (17, 21–24). Moreover, the peripheral blood parameters of these markers, including white blood cells, lymphocytes, neutrophils, platelet count, and monocytes, are cheap and can be easily obtained to further evaluate the prognoses of various human cancers. Several studies on hematological indicators have been published, some of which show that glucose-to-lymphocyte (GLR) is a reliable prognostic marker for pancreatic (25) and gallbladder cancers (26). However, there are no published reports on the association of the risk of preoperative central lymph node metastasis (CLNM) and GLR in papillary thyroid cancer (PTC) patients with type 2 DM (T2DM). Surgical resection is the most effective treatment for PTC but the role of prophylactic central lymph node dissection (CLND) in PTC remains controversial (27, 28), and the patients with CLND experience higher rates of postoperative hypocalcemia (27, 29). Therefore, identification of an effective way to exclude low-risk patients from CLND is necessary. The purpose of this study was to construct the nomogram for improving the accuracy of predicting the possibility of lymph node metastasis in the central region before surgery using easily accessible and inexpensive hematological indicators.

PATIENTS AND METHODS

Patient Recruitment

A retrospective analysis was performed for a total of 419 patients with PTC and T2DM who underwent thyroid lobectomy and lymph node dissection in two clinical centers; among them, 319 were enrolled from the First Affiliated Hospital of Wenzhou Medical University between 2017 and 2020, and 100 from the Zhejiang Provincial People's Hospital between 2018 and 2020. The information on the clinical pathology of patients was collected. None of the patients showed central or lateral lymph node enlargement, assessed by preoperative imaging examinations or biopsy scans.

The exclusion criteria were as follows:

1) Incomplete clinicopathological characteristics.
2) Patients whose pathology suggested the presence of malignant PTC without lymph node dissection.
3) Patients with poor blood sugar control after admission; the standard range for poor blood glucose control was hBA1c> 7% and those who could not be operated upon.
4) Patients with hyperthyroidism, history of thyroid radiation, or thyroid surgery.
5) The preoperative imaging examinations, using the results of the preoperative ultrasound, CT, MRI, or biopsy scans, suggested the presence of swollen lymph nodes in the central and lateral areas.
6) Patients with other malignancies.
7) Patients with a history of infections or other inflammation (excluding Hashimoto's thyroiditis).

Data Acquisition

All the selected patients underwent a comprehensive preoperative evaluation, including thyroid ultrasound, blood indicator assessment, and thyroid function tests. The basic information, including age, gender, height and weight, and diabetes-related medical history (including medications for the treatment of diabetes) were obtained.

Thyroid ultrasound and cervical CT examinations were used to assess the maximum tumor size, lateral position, multifocal, and chronic lymphocytic thyroid inflammation. The results of intraoperative rapid freezing and postoperative paraffin pathology were also analyzed. Hematology indicators were typically collected within a week before the operation. The formula for GLR calculation was as follows: GLR=glucose count/lymphocyte count.

Statistical Analysis

We used the ROC curves to determine the best cut-off values for the variables and stratified them. Using the chi-square test, we evaluated if the two sets of data were comparable. Single- and multi-factor logistic analyses were used to identify the factors that were significantly related to preoperative CLNM and the predictive model was constructed. We calculated the area under the ROC curve (AUROC) for the training and the validation sets. The calibration curve was used to evaluate the agreement between actual observations and predicted values, and further, visualize these results. The Hosmer-Lemeshow test was used to assess the significance of the calibration curves (p>0.1 indicated good consistency). The DCA curves were used to measure the net benefits for different threshold probabilities, thereby aiding the determination of the clinical benefits of using the nomogram in clinical settings. The NRI and IDI values were used to quantitatively analyze and evaluate the improvements in the diagnostic performance of the new relative to the original model. NRI>0 suggested improvement; NRI<0 indicated negative improvement, while NRI=0 showed no improvement. IDI>0 implied improvement; IDI<0 was a negative improvement, and IDI=0 indicated no improvement. All the statistical analyses were performed using R (version 4.0.2) and SPSS 25.0 software. $P < 0.05$ was considered statistically significant.

RESULTS

Clinical Characteristics and Basic Information

In this study, we included 419 confirmed cases of PTC and T2DM from the above-mentioned two hospitals. All the data were divided into two based on their source as the training (n=319, 76%) and external test (n=100, 24%) data sets. For objectivity, ROC analysis was used to select the best cut-off value for each continuous variable. The results of the chi-square analysis, as shown in **Table 1**, indicated that the two data sets were not significantly different (P>0.5), and thus, were consistent and comparable.

TABLE 1 | Baseline characteristics of patients in the training and external validation data sets.

Variables	Training Data Set N=319	Validation Data Set N=100	P-value
Age			
>55Y	181	51	0.314
≤55Y	138	49	
Gender			
Female	211	68	0.731
Male	108	32	
Medicine for diabetes			
Metformin(-)	173	62	0.172
Metformin(+)	146	38	
BMI(kg/m2)			
>32.49	3	3	0.13
≤32.49	316	97	
Maximum diameter of mass			
>9.5mm	107	34	0.933
≤9.5mm	212	66	
Multifocality			
Solitary	251	73	0.236
Multiple	68	27	
CLNM			
CLNM(-)	185	55	0.597
CLNM(+)	134	45	
Laterality			
Unilateral	289	89	0.639
Bilateral	30	11	
Hashimoto's thyroiditis			
Absent	283	89	0.937
Present	36	11	
TC(mmol/L)			
>7.235	14	5	0.798
≤7.235	305	95	
TG(mmol/L)			
>3.975	36	16	0.212
≤3.975	283	84	
glucose(mmol/L)			
>7.25	190	60	0.938
≤7.25	129	40	
Albumin(g/L)			
>42.15	212	68	0.775
≤42.15	107	32	
AGR			
>1.25	260	82	0.911
≤1.25	59	18	
neutrophil(x10^9)			
>4.395	101	32	0.949
≤4.395	218	68	
mononuclear(x10^9)			
>0.355	207	68	0.568
≤0.355	112	32	
lymphocyte(x10^9)			
>1.435	275	80	0.132
≤1.435	44	20	
lymphocytepercent(%)			
>37.35	70	25	0.524
≤37.35	249	75	
TSH(mIU/L)			
>0.855	255	82	0.65
≤0.855	64	18	
GLR			
>4.23	161	47	0.545
≤4.23	158	53	

Association Between Clinical Features and GLR in the Two Cohorts

The best cut-off value for GLR was 4.23. Therefore, we divided the patients into two according to their GLR values as the low GLR level group (≤4.23), comprising 211 patients (50.4%) and the high GLR level group (>4.23), comprising 208 cases (49.6%). As listed in **Table 2**, the GLR levels were significantly related to age (P=0.004), metformin consumption (P=0.026), blood sugar (P<0.001), lymphocyte count (P<0.001), and lymphocyte percentage (P<0.001). The higher GLR levels were associated with older age, no metformin consumption, high blood sugar, higher lymphocyte counts, and lower lymphocyte percentage.

Univariate and Multivariate Analyses for Preoperative CLNM Variables

In univariate analysis, age (P=0.054), maximum tumor diameter (P<0.001), tumor bilaterality (P<0.001), and GLR (P=0.063) were found to be significantly associated with preoperative CLNM (**Table 3**). Further, a multivariate logistic regression analysis was performed to identify the important variables that were significantly related to preoperative CLNM. The results showed that age (≤55 years, P=0.019), maximum tumor diameter (>0.95cm, P<0.001), bilateral tumor (P<0.001), and high GLR (P=0.041) were independent risk factors for preoperative CLNM (**Table 3**).

Construction of the Nomogram

The independent factors selected based on the results of the multivariate analysis were used to construct the nomogram for predicting the individual risk of preoperative CLNM (**Figure 1**). The scores for each independent predictor were plotted and serially summed up to obtain the total score for confirming the possibility of preoperative CLNM.

Evaluation of the Nomogram

To evaluate the ability of the nomogram for predicting the preoperative CLNM in PTC and comorbid T2DM patients, we used an R package to perform ROC analysis and obtained an AUROC of 0.733 in the internal training set (**Figure 2A**) and an AUROC of 0.664 in the external validation set (**Figure 2B**). The calibration curves indicated a high degree of consistency between the predicted value and the real situation, as shown in **Figures 3A, B** (by Hosmer-Lemeshow test, internal training set, p =0.5416304>0.1 and external validation set p=0.5803292>0.1). **Figures 4A, B** show the DCA diagrams for the internal and the external data sets, respectively. The dotted line in the figure represents the net benefit, while the gray line shows the net clinical benefit that these patients received (with the assumption that all patients had undergone treatment for CLND). The black color represents the net clinical benefit, assuming that all patients did not undergo the treatment for CLND. Through DCA, the net clinical benefit for patients who were predicted to and underwent selective CLND through this nomogram was found to be markedly higher than that for all patients who had undergone or not received treatment for CLND. The training data were concentrated between approximately 20% and 90% of the distribution, while the external validation data were between 30% and 90%.

TABLE 2 | Correlations between GLR and the clinical characteristics of patients in all datasets.

Variables	GLR ≤ 4.23N=211	GLR>4.23N=208	P-value
Age			
>55Y	102	130	0.004
≤55Y	109	78	
Gender			
Female	141	138	0.917
Male	70	70	
Medicine for diabetes			
Metformin(-)	107	128	0.026
Metformin(+)	104	80	
BMI(kg/m2)			
>32.49	4	2	0.421
≤32.49	207	206	
Maximum diameter of mass			
>9.5mm	73	68	0.68
≤9.5mm	138	140	
Multifocality			
Solitary	162	162	0.787
Multiple	49	46	
CLNM			
CLNM(-)	126	114	0.31
CLNM(+)	85	94	
Laterality			
Unilateral	189	189	0.656
Bilateral	22	19	
Hashimoto's thyroiditis			
Absent	185	187	0.47
Present	26	21	
TC(mmol/L)			
>7.235	9	10	0.79
≤7.235	202	198	
TG(mmol/L)			
>3.975	23	29	0.345
≤3.975	188	179	
glucose(mmol/L)			
>7.25	66	184	<0.001
≤7.25	145	24	
Albumin(g/L)			
>42.15	145	135	0.407
≤42.15	66	73	
AGR			
>1.25	168	174	0.287
≤1.25	43	34	
neutrophil(x10^9)			
>4.395	61	72	0.21
≤4.395	150	136	
mononuclear(x10^9)			
>0.355	151	124	0.1
≤0.355	60	84	
lymphocyte(x10^9)			
>1.435	202	153	<0.001
≤1.435	9	55	
lymphocytepercent(%)			
>37.35	73	22	<0.001
≤37.35	138	186	
TSH(mIU/L)			
>0.855	172	165	0.572
≤0.855	39	43	

TABLE 3 | Univariate and multivariate analyses for logistic regression in the training data set.

Variables	Univariate Analysis of preoperative CLNM			Multivariate Analysis of preoperative CLNM		
	OR	95% CI	P-value	OR	95% CI	P-value
Age	0.581	0.334-1.009	0.054	0.0542	0.324-0.904	0.019
>55Y						
≤55Y						
Gender	0.917	0.506-1.662	0.774			
Female						
Male						
Medicine for diabetes	0.691	0.407-1.174	0.172			
Metformin(-)						
Metformin(+)						
BMI(kg/m2)	2.334	0.411-13.258	0.339			
>32.49						
≤32.49						
Maximum diameter of mass	4.195	2.403-7.323	<0.001	4.243	2.499-7.205	<0.001
>9.5mm						
≤9.5mm						
Multifocality	0.745	0.389-1.423	0.372			
Solitary						
Multiple						
Laterality	9.022	2.803-29.035	<0.001	8.115	2.603-25.301	<0.001
Unilateral						
Bilateral						
Hashimoto's thyroiditis	1.391	0.615-3.148	0.428			
Absent						
Present						
TC(mmol/L)	1.846	0.501-6.801	0.357			
>7.235						
≤7.235						
TG(mmol/L)	1.361	0.577-3.208	0.481			
>3.975						
≤3.975						
Albumin(g/L)	1.278	0.701-2.329	0.423			
>42.15						
≤42.15						
AGR	1.682	0.794-3.563	0.175			
>1.25						
≤1.25						
neutrophil(x10^9)	1.581	0.874-2.859	1.13			
>4.395						
≤4.395						
mononuclear(x10^9)	1.291	0.692-2.409	0.422			
>0.355						
≤0.355						
TSH(mIU/L)	1.697	0.865-3.328	0.124			
>0.855						
≤0.855						
GLR	1.691	0.972-2.941	0.063	1.704	1.021-2.843	0.041
>4.23						
≤4.23						

Comparison With the Original Model

To further verify that the nomogram indeed had better predictive ability and accuracy, we compared the new with the original model. Through literature review, we obtained a nomogram published by Andrew M. Thompson et al., in 2014, for the prediction of preoperative CLNM in PTC patients (30). This nomogram includes age, gender, tumor size, and tumor multifocality as the parameters. The AUC obtained after substituting our data into the original model was 0.693 (**Figure 5A**), lesser than 0.733, the value obtained using the new model. We used NRI and the IDI quantitative analyses to evaluate the improved diagnostic performance of the new model relative to the original model. As shown in **Figure 5B**, NRI=0.04171037>0, (IDI=0.0902>0) indicated that the diagnostic performance of the new model improved significantly relative to the original model.

DISCUSSION

At present, surgeons routinely perform preventive dissection of lymph nodes in the central region of the neck in radical thyroid cancer surgery for PTC patients (28) but preventive CLND can

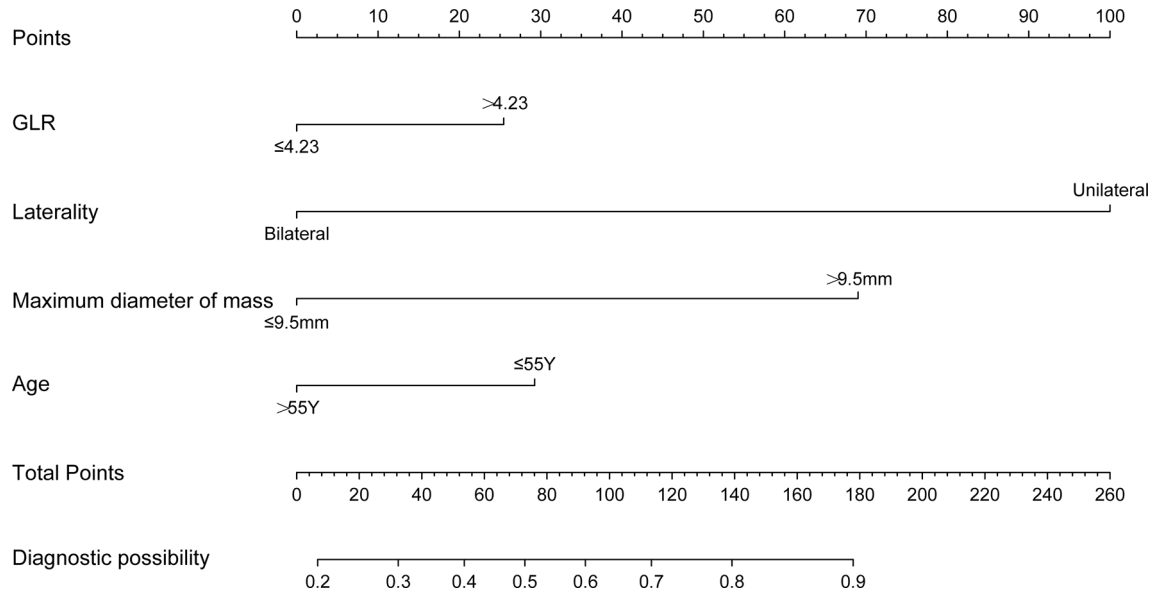

FIGURE 1 | Nomogram for predicting the preoperative CLNM in T2DM-PTC patients.

lead to an increased risk of postoperative hypocalcemia and other risks (27, 29). How to accurately identify patients who do not need preventive CLND before surgery needs to be addressed for effective clinical practice.

Many previous studies show that GLR can be used as a prognostic indicator for patients with pancreatic (25) and pT2 gallbladder cancers (26), and hospitalized patients with acute severe pancreatitis (31). However, there is no study on the role of GLR in PTC. Therefore, our important core hypothesis was as follows: preoperative GLR is significantly correlated with CLNM in PTC patients with T2DM.

In this study, we retrospectively analyzed the clinical data of 419 patients with PTC complicated by T2DM. Univariate and multivariate logistic regression analyses showed that age < 55 years, bilateral tumor, maximum tumor size > 9.5mm, and higher GLR ratio were all independent risk factors for CLNM. The inflammatory cytokines in diabetic patients are higher than those in non-diabetic patients (32). Systemic inflammation plays key roles in the occurrence, development, progression, metastasis, and recurrence of several solid tumors. Some documented literature suggests that lymphocytes can activate cell-mediated immune responses and tumor cell lysis (23, 24, 32). Thus, we

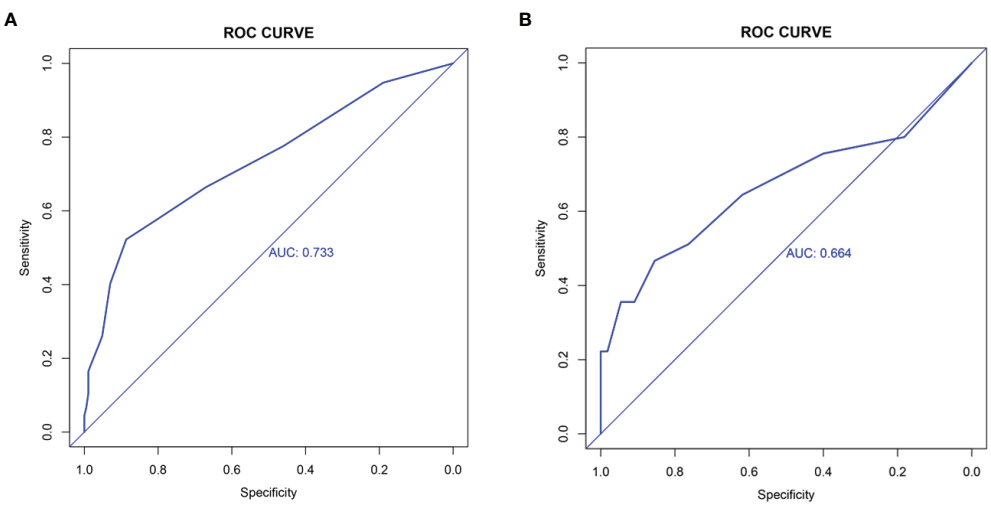

FIGURE 2 | Receiver operating characteristics (ROC) analysis in **(A)** training data set; **(B)** external validation data set. The AUC for the nomogram in the training data set is 0.733, while for the external validation data set is 0.664.

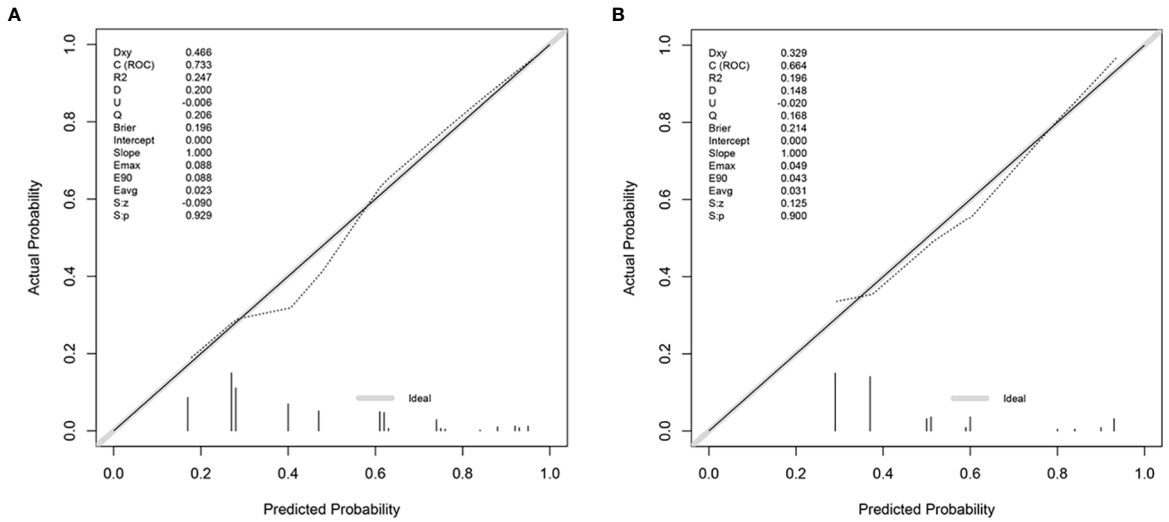

FIGURE 3 | Calibration curves for the nomogram for **(A)** training data set and **(B)** external validation data set. The calibration curves show a high degree of consistency between the predicted value and the actual situation, as the p-values are 0.5416304 (>0.1) and 0.5803292 (>0.1), respectively from the Hosmer-Lemeshow test.

speculated that GLR could predict the CLNM of PTC patients with T2DM by reflecting the levels of inflammation, wherein a high GLR value implied high blood glucose or low lymphocyte level. Since we excluded patients with hBA1c>7% or poor blood glucose control before surgery, high GLR may be more closely related to low lymphocyte levels. Lymphocytes are a major component of anti-tumor immunity, stimulating the release of cytokines such as interferons and TNF-α, thereby exerting protective effects (33). However, in our previous screening, no significant correlation was found between the number of lymphocytes or the blood glucose alone before surgery and CLNM in patients with PTC combined with T2DM. These results indicated that GLR was an independent significant new indicator for predicting preoperative CLNM in patients with PTC combined with T2DM. Relative to unilateral tumors, the incidence of CLNM in bilateral tumors increased by 8.115 times, consistent with previous studies (34). We speculated that this may be due to the more aggressive bilateral tumors. Moreover, results of both univariate and multivariate logistic regression analyses indicated that tumor > 9.5 mm was an independent risk factor for CLNM. The risk of CLNM increased

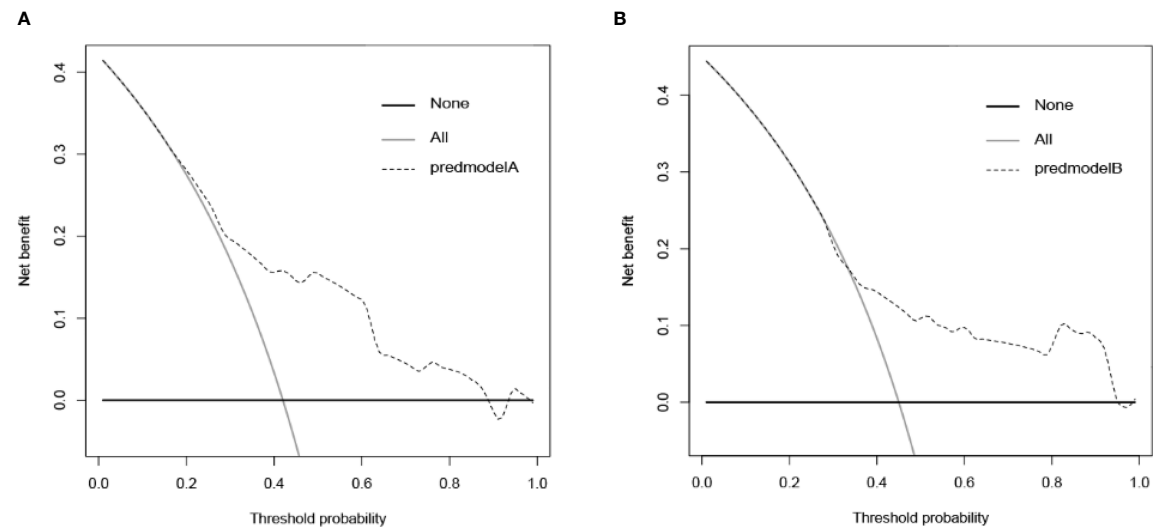

FIGURE 4 | Decision curve analysis (DCA) for the nomogram in **(A)** training data set and **(B)** external validation data set. The training data are concentrated between approximately 20% and 90% and the external validation data are approximately between 30% and 90% of the total distribution.

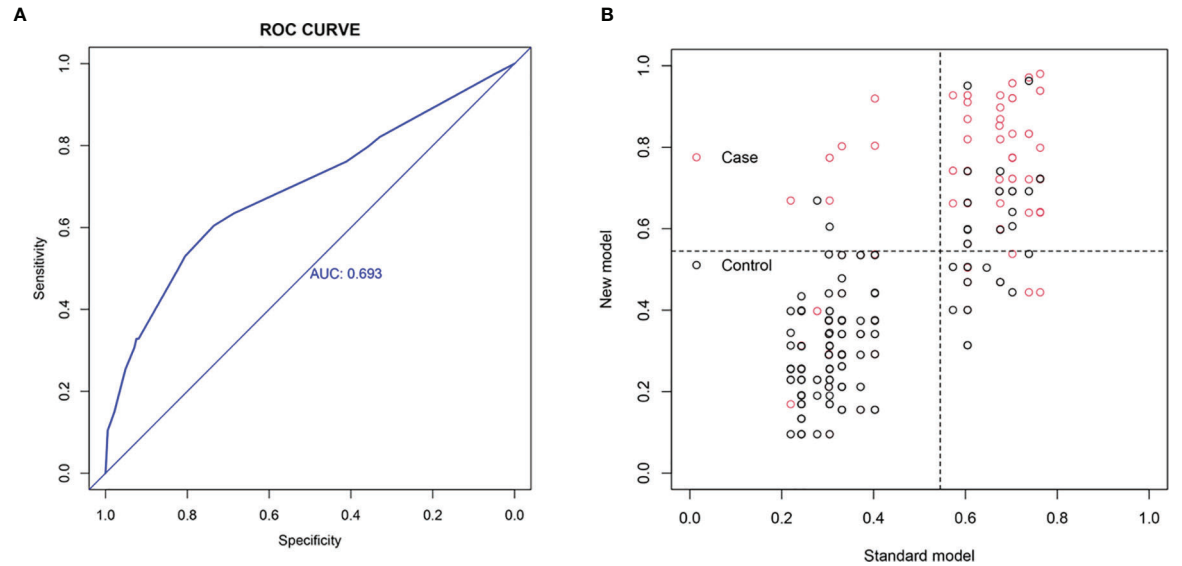

FIGURE 5 | Comparison with the original model. **(A)** ROC analysis. **(B)** Net reclassification index (NRI). NRI=0.04171037>0, (IDI=0.0902>0) indicates that the diagnostic performance of the new model is improved relative to the original model.

4.243-fold when the tumor diameter was greater than 9.5 mm. In addition, in both the training (≤ 9.5mm: > 9.5mm = 212:107) and the validation group (≤ 9.5mm: > 9.5mm = 66:34) the ratio, ≤ 9.5mm: > 9.5mm, was approximately 2:1. In the subgroup whereby the maximum diameter of mass was <9.5mm, the CLNM in the training group comprised 63 patients, and the CLNM in the external validation group of 23 patients, while in the subgroup whereby the maximum diameter of mass was >9.5mm, the CLNM in training group comprised 71 patients, and that in the external validation group of 22 patients. In a population more than twice the size of patient number whose maximum diameter of mass was > 9.5mm, there were similar proportions of CLNM cases. This further confirmed that in the patients whose maximum diameter of mass was <9.5mm, CLNM occurrence was difficult, consistent with previous literature (35–37). In terms of age, 55 years the recommended cut-off in the AJCC 8th edition thyroid cancer TNM stage (38), and less than 55 years was found to be an independent risk factor for CLNM, consistent with the literature (32, 35).

After confirming that high GLR was an independent risk factor for CLNM in PTC patients with T2DM, we further examined the clinical value of this new indicator for PTC patients with T2DM for improved prognoses, survival, treatment, and reduced surgical harm due to CLND. We further utilized GLR, unilateral and bilateral factors, tumor size, and age to construct a preoperative CLNM prediction model for the target population of PTC patients with T2DM. To verify that our model showed significantly improved CLNM for the target population of PTC patients with T2DM relative to the existing models, we compared our model with a known model. The preoperative CLNM prediction model described in Andrew M. Thompson et al., includes age, gender, tumor size, and tumor multifocality, and the target population was PTC patients (30). We input our data into the model of Andrew M. Thompson et al. and obtained an AUC of 0.693, smaller than our new model. This indicated that our model had a higher accuracy for distinguishing PTC patients with T2DM with or without CLNM. To further verify the good diagnostic efficiency of our model, we performed NRI and IDI quantitative analyses, and the results suggested that our new model including GLR and the excluding tumor multifocal and gender as factors, substantially improved the diagnostic efficacy relative to the model described by Andrew M. Thompson et al. Moreover, as compared to the existing models, the new model included fewer factors, calculating the risk scores more concise. Additionally, GLR is an easily obtained clinical indicator. Before each patient is admitted to the hospital for surgery, routine examinations for blood indicators, such as blood glucose and B-ultrasound are performed. Therefore, our model is universal and can be generalized. Without additional harm to patients or more tests, more meaningful clinical decisions and individualized treatment options can be taken in the future.

However, our study also has some limitations. The study has a retrospective design and some data bias may exist. The sample size was not large enough and more samples may be needed to further verify our model. We hope that in the future there will be more sensitive indicators to better predict CLNM and lymph node metastasis, to facilitate a solid theoretical basis for clinical decision making for the benefit of these patients.

In conclusion, GLR was found to be an independent risk factor for PTC and T2DM. Our proposed nomogram could be used to predict preoperative CLNM, which showed the benefits of good sensitivity and specificity.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the ethics committee of Zhejiang Provincial People's Hospital and the ethics committee of First Affiliated Hospital of Wenzhou Medical University. The patients/participants provided their written informed consent to participate in this study

AUTHOR CONTRIBUTIONS

All the authors have made significant contributions to the study design, data acquisition, analysis, and interpretation. They have substantially participated in drafting the manuscript and critically revising the important content and assume responsibility for all aspects of this work. All authors contributed to the article and approved the submitted version.

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CASE REPORT
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Internal Jugular Vein Thrombosis After Microwave Ablation of Cervical Lymph Node Metastasis in Papillary Thyroid Microcarcinoma: A Case Report

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In this study, two patients with papillary thyroid carcinoma and lymph node metastasis were treated by Dr. Shurong Wang's team and are reported. The two patients refused surgery and underwent microwave ablation (MWA) of the thyroid and lymph node lesions. Ultrasound review 2 days after MWA revealed internal jugular vein thrombosis. Patient #1 received low molecular weight heparin calcium injection, Xueshuantong injection, Xiangdan injection, and rivaroxaban. Patient #2 was treated with enoxaparin sodium injection, Xueshuantong injection, urokinase, and warfarin sodium tablet. The thrombus was successfully managed in each patient using anticoagulant treatment. Such complication of MWA has not been reported in many cases before. According to the relevant literature, thrombosis after thyroid cancer ablation might be related to subclinical hypothyroidism, increased heme oxidase 1 (HO-1) levels in the blood of patients with papillary thyroid cancer, and increased platelet content and mean platelet volume in patients with thyroid cancer. No specific cause of thrombosis was identified in the two cases reported here. No recurrence was observed after 1 (patient #1) and 4 (#2) years of follow-up. In conclusion, patients with papillary thyroid carcinoma and lymph node metastasis should undergo color Doppler ultrasound of the neck after MWA of thyroid lesions and neck metastasis.

Keywords: internal jugular vein, thrombosis, microwave ablation, lymph node, cervical, papillary thyroid carcinoma

INTRODUCTION

Thyroid cancer is the most common malignant tumor of the endocrine system (1). Papillary thyroid carcinoma is the most common type of thyroid cancer, with a high frequency of cervical lymph node metastasis but a slow course and low mortality (2). There were 60,220 cases of thyroid cancer in the United States in 2013 and 90,000 new cases of thyroid cancer in China in 2015 (1). Due to the good biological behavior and slow growth of papillary thyroid microcarcinoma, some patients carry lesions all their lives without disease progression. Therefore, some authors believe that some small

cancers can be observed without any treatment. For example, Ito et al. (3) reported the observation results of 340 patients with papillary thyroid microcarcinoma operated on or not; after an average follow-up time of 74 months, no significant differences were found in lymph node metastasis and prognosis between the two groups. Another study by Ito et al. (4) followed 1235 cases of thyroid microcarcinoma for 6 years; lesions increased in 58 cases (4.6%), and lymph node metastasis occurred in 19 cases (1.5%).

Minimally invasive thyroid treatment technologies are available for patients with microcarcinoma who refuse thyroidectomy for fear of postoperative pain or cosmetic reasons. Ultrasound-guided microwave ablation (MWA) is widely used in the alternative treatment of primary and metastatic liver cancer, lung, kidney, and adrenal malignancies (5). In addition, MWA in treating benign thyroid neoplasms has achieved considerable efficacy (6).

This paper reports two patients with papillary thyroid carcinoma and lymph node metastasis. Both patients developed internal jugular vein thrombosis after the MWA of thyroid carcinoma and lymph node metastases. Such complication of MWA has not been reported in many cases before.

CASE PRESENTATION

Case 1

A 28-year-old female patient was admitted to the Oncology Department of Yantai Affiliated Hospital affiliated to Binzhou Medical College because thyroid nodules were found during a physical examination on September 20, 2019. She had no family history of thyroid diseases. Blood cell analysis revealed red blood cells (RBC) at 4.79×10^{12}/L, hemoglobin (HGB) at 83 g/L ↓, hematocrit (Hct) at 29.1% ↓, mean corpuscular volume (MCV) at 60.8 fL ↓, mean corpuscular hemoglobin (MCH) at 17.3 pg ↓, average hemoglobin concentration (MCHC) at 285 g/L ↓, platelets (PLT) at 256×10^{9}/L, and mean platelet volume (MPV) at 7.0 fL. For thyroid function, free triiodothyronine (FT3) was 4.44 pmol/L, free thyroxine (FT4) was 13.50 pmol/L, thyroid-stimulating hormone (TSH) was 10.00 μIU/mL ↑, thyroglobulin antibody (TGAb) was 260.20 U/mL ↑, and thyroid peroxidase antibody (TPOAb) was >1300 U/mL ↑. Carbohydrate antigen 125 (CA125) was 35.6 U/mL ↑. For coagulation, plasma fibrinogen (Fib) was 1.59 g/L ↓, and D-dimer was 1.24 μg/mL ↑. Other tests were unremarkable. Laryngoscopy and chest radiography were negative. On ultrasound, a hypoechoic nodule of 1.10×0.75×0.63 cm could be seen in the right lobe of the thyroid gland, with irregular shape and unclear boundary (**Figure 1A**). Strong spot-like echoes could be seen inside. Hypoechoic nodules were seen in levels IV and VI of the right neck. The larger one was located at the right, with a size of about 1.32×0.63 cm, irregular in shape, an unclear boundary between the dermis and medulla, without hilum (**Figure 1B**). Biopsy of the thyroid nodule revealed papillary thyroid carcinoma (**Figure 1C**). Fine-needle aspiration (FNA) of the levels IV and VI lymph nodes revealed lymphocytes and epithelial cells with dysplasia, consistent with lymph node metastasis of papillary thyroid carcinoma (**Figure 1D**).

According to the ultrasound classification of thyroid nodules and cytology results, the patient was diagnosed with papillary thyroid carcinoma and cervical lymph node metastasis. Surgical treatment was recommended, but the patient was young and suffered from anemia, and she refused surgery. The physician strongly recommended ultrasound-guided MWA, which Dr. Shurong Wang performed on September 25, 2019. Expanded ablation was performed for papillary thyroid carcinoma of the right lobe of the thyroid (with a maximum ablation range of 2.26 × 1.23 cm) and four cervical lymph nodes. Postoperative contrast-enhanced ultrasound showed no enhancement of the thyroid nodules after ablation (**Figures 1E, F**). The thyroid ultrasound on the second day after ablation showed necrotic changes in the right lobe of the thyroid and right cervical lymph nodes. Heterogeneous hypoechogenicity was observed in the middle and lower segment of the right internal jugular vein, with a length of about 3.56 cm and a width of about 0.50 cm (**Figure 1G**). A small number of blood flow signals were observed around the hypoechogenic signals (**Figure 1H**). After thrombolytic therapy (low molecular weight heparin calcium injection 4100 IU, q 12 h, subcutaneous injection, 5 days; Xueshuantong injection 400 mg added into 0.9% sodium chloride injection 250 ml, qd, IVGTT, for 5 days; Xiangdan injection 20 ml added into 5% glucose injection 250 ml, qd, IVGTT, for 5 days; rivaroxaban 15 mg, bid, po, for 7 days), the venous thrombosis disappeared, and venous blood flow had returned to normal at the 1-month reexamination (**Figure 1I**). The patient was kept on suppressive treatment after ablation. No recurrent lesions were found in the cervical lymph nodes and thyroid during 1 year of postoperative follow-up.

Case 2

A 49-year-old female patient was admitted to the Department of Oncology of Yantai Affiliated Hospital of Binzhou Medical College because thyroid nodules were found during a physical examination on January 1, 2016. She had no family history of thyroid disease. The blood cell analysis showed a neutrophil ratio (Neut%) at 36.9% ↓, lymphocyte ratio (Lymph%) at 48.3% ↑, RBC at 4.20×10^{12}/L, HGB at 128 g/L, Hct at 37.2%, MCV at 88.6 fL, MCH at 30.4 pg, MCHC at 343 g/L, PLT at 196×10^{9}/L, and MPV at 10.1 fL. FT3 was 5.20 pmol/L, FT4 was 14.83 pmol/L, TSH was 2.28 μIU/mL, TGA was 38.90 U/mL, TPOAb was 53.80 U/mL, and CA125 was 8.70 U/mL. Preoperative laryngoscopy and chest radiography were negative. Ultrasound of the thyroid showed a hypoechoic nodule of 0.88×0.72×0.76 cm in the right lobe of the thyroid, with an irregular shape, unclear boundary, uneven internal echo, and spot-like strong echo (**Figure 2A**). Blood flow signals could be seen around and inside the nodule. Two lymph node lesions could be detected in level IV of the right neck; the larger was about 1.14×0.59 cm, with a regular shape, clear boundary, and uneven internal echo (**Figure 2B**). Contrast-enhanced ultrasound showed uneven and low enhancement of the nodules in the right lobe of the thyroid gland. Cytology results revealed papillary thyroid carcinoma with right cervical

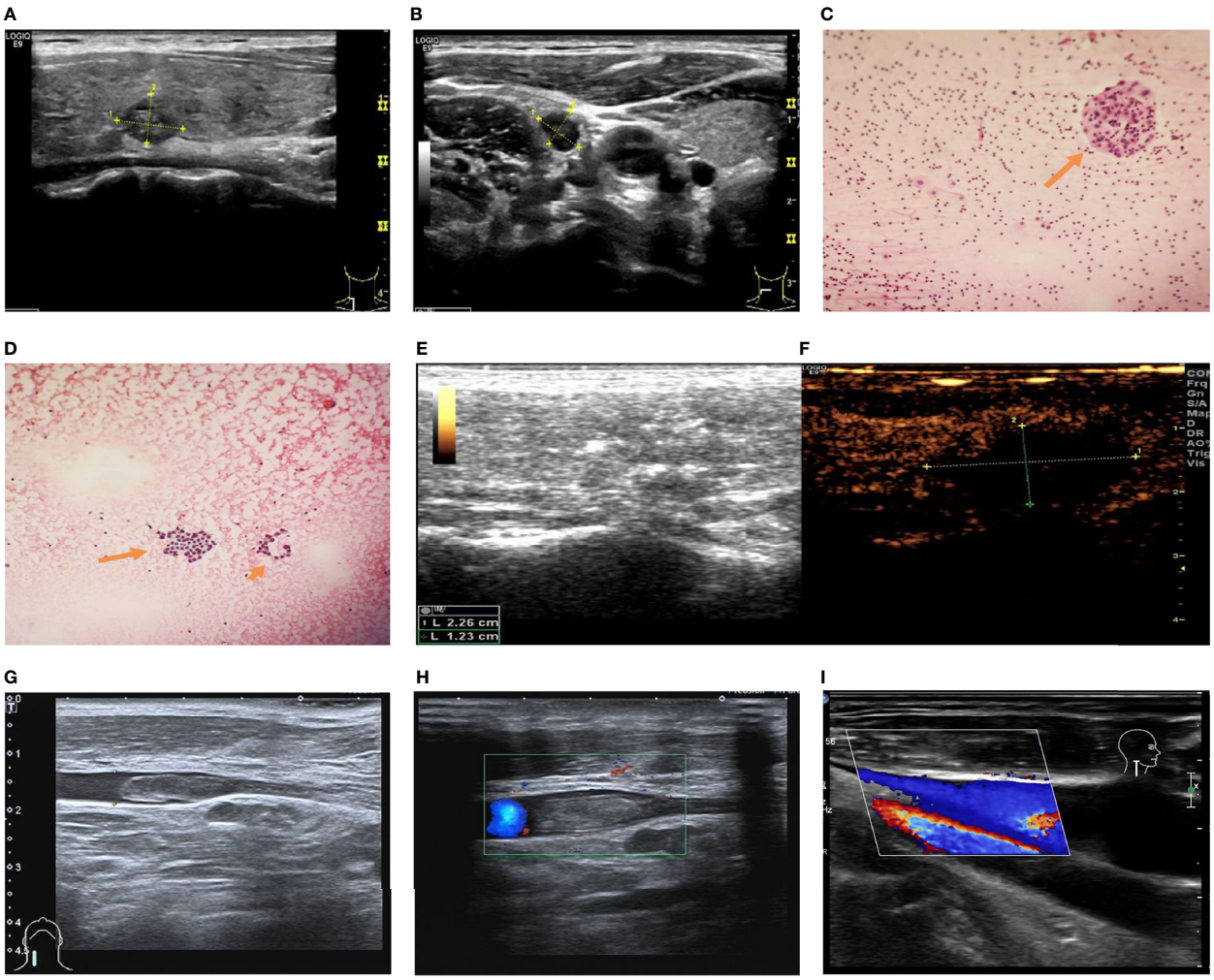

FIGURE 1 | Ultrasound and flow chart of Patient #1 after ablation. **(A)** Preoperative ultrasound of thyroid nodule. **(B)** Preoperative ultrasound of lymph node. **(C)** Thyroid FNA smear: epithelial cells with dysplasia (arrow). **(D)** Cervical lymph node FNA smear: lymphocytes and epithelial cells with dysplasia (arrow). **(E, F)** Ultrasound after extended ablation of papillary thyroid carcinoma of the right lobe of thyroid of the patient immediately after postoperative (local no contrast medium filling). **(G)** Two-dimensional ultrasonography of internal jugular vein thrombosis on the second day after ablation. **(H)** Thrombus flow diagram of the right internal jugular vein on the second day after ablation. **(I)** One month after thrombolysis, venous thrombosis disappeared, and venous blood flow returned to normal.

lymph node metastasis, galectin-3 (+), Ki67 (+), MC (+), CD56 (-), and CK19 (+).

Surgical treatment was recommended, but the patient refused the operation (for fear of pain and esthetic reasons) and insisted on MWA. The patient underwent ablation by Dr. Shurong Wang on January 5, 2016. Expanded ablation was performed for the papillary thyroid carcinoma of the right lobe of the thyroid and two cervical lymph nodes. Postoperative contrast-enhanced ultrasound showed no enhancement of thyroid nodules after ablation (**Figure 2C**). Heterogeneous hypoechogenicity was observed in the middle and lower segment of the right internal jugular vein, with a length of about 3.56 cm and a width of about 0.50 cm according to 2D ultrasound and color Doppler ultrasound (**Figures 2D, E**). Thrombolytic therapy was given (enoxaparin sodium injection 6000 IU, q 12 h, subcutaneous injection, 1 week; 0.5 g Xueshuantong added into 250 ml of normal saline, qd, and IVGTT, for 5 days; urokinase 200,000 IU added into 0.9% normal saline, bid and IVGTT, for 5 days; warfarin sodium tablet 2.5 mg, qd, po, started on the sixth day of medication, for one week). After 1 week, the thrombus disappeared, and venous flow was restored (**Figure 2F**). The patient was kept on suppressive treatment for 3 years after ablation. No recurrent lesions were found in the cervical lymph nodes and thyroid gland during the postoperative follow-up of >4 years

DISCUSSION

There are few reports of internal jugular vein thrombosis after MWA of thyroid lesions. The cervical lymph node metastasis was located behind the internal jugular vein in the two cases reported here. During the ablation of lymph nodes, normal saline

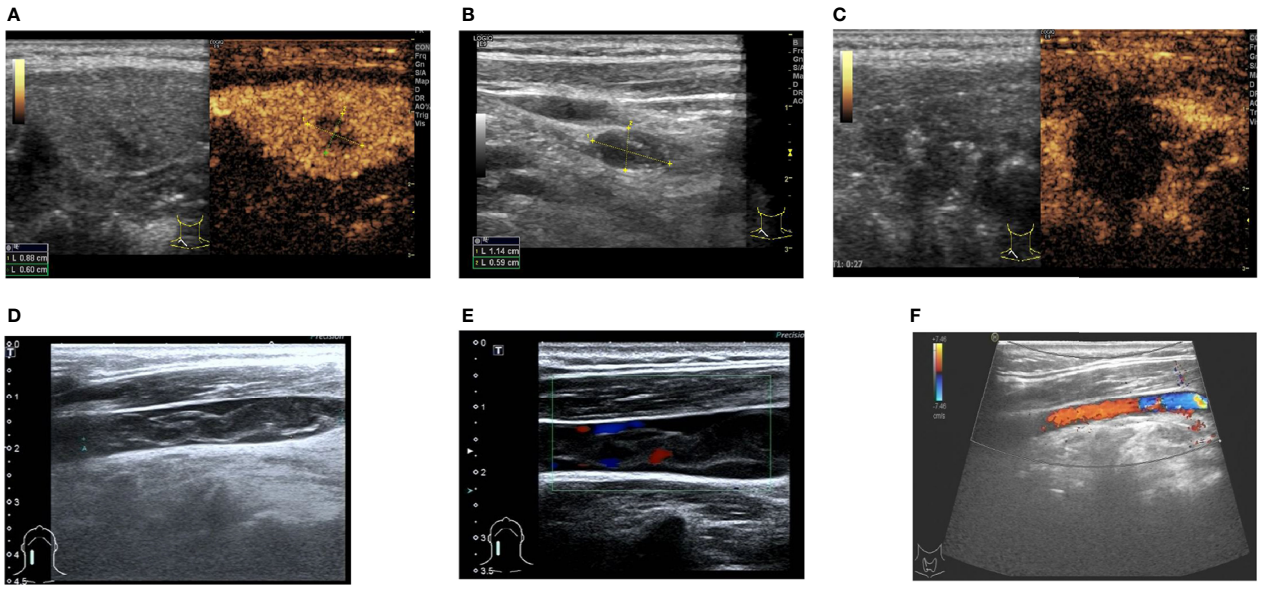

FIGURE 2 | Ultrasound and flow chart of Patient #2 after ablation. **(A)** Preoperative ultrasound of thyroid nodule. **(B)** Preoperative ultrasound of lymph node. **(C)** Ultrasound after extended ablation of papillary thyroid carcinoma of the right lobe of thyroid of the patient immediately after postoperative (local no contrast medium filling). **(D)** Two-dimensional ultrasonography of internal jugular vein thrombosis on the second day after ablation. **(E)** Thrombus flow diagram of the right internal jugular vein on the second day after ablation. **(F)** One week after thrombolysis, venous thrombosis disappeared, and venous blood flow returned to normal.

should be injected between the lymph node and the internal vein to protect the internal jugular vein from heat damage. The blood flow of the ipsilateral internal jugular vein was observed to be normal immediately after the operation. Routine local compression and cold compress were applied for 60 min after the operation. The internal jugular vein thrombosis in the two patients might be related to compression caused by edema and the cold compress. Still, many patients did not develop internal jugular vein thrombosis under the same conditions. In the past 5 years, the authors treated 54 patients with cervical lymph node metastasis of thyroid cancer with MWA. Thirty-eight of them (70.4%) were located in level IV of the neck. Each patient underwent postoperative cervical vascular ultrasound. The ablation needle models KY-2000 and KY-2450A-1 were used. The ablation power of MWA was 30 W in both patients. The ablation range was expanded by 0.5 cm around the thyroid cancer. The cervical lymph nodes underwent conformal ablation. The distance from the internal jugular vein was about 1 cm. In addition, sufficient spacer fluid was injected around the lymph nodes during the ablation to prevent heat damage. Therefore, the possibility of intraoperative jugular vein injury is very small.

According to the literature, internal jugular vein thrombosis after MWA might be related to the following factors. First, Erem et al. (7) reported that certain coagulation factors might be increased in the blood of patients with subclinical hypothyroidism (SH) (7). The main diagnostic indicator of SH is the elevation of TSH in blood, and studies showed that serum TSH levels are independently associated with thrombosis (8). TSH is related to the change of gene expression of endothelial cells, and the increase of TSH might influence the expression of the endothelial nitric oxide synthase (PGI2) gene and induce endothelial cell dysfunction and thrombosis (9). Patient #1 had SH, which might be related to venous thrombosis after MWA. Second, Chadarevian et al. (10) reported that patients with mild hypothyroidism had decreased DD levels, increased plasma Fib, and decreased fibrin degradation, which could easily lead to local thrombosis. In this study, Patient #1 showed a decrease in Fib and an increase in DD. Other coagulation items were all within the normal range. On the other hand, Patient #2 showed normal coagulation parameters. Therefore, it was inconsistent with the cases reported here. In addition, only patient #1 had SH. Third, elevated levels of heme oxidase 1 (HO-1) have been observed in the blood of patients with papillary thyroid carcinoma (11). During the process of heme catabolism, HO-1 promotes the production of carbon monoxide (12). Increased plasma carbon monoxide concentrations promote coagulation by adhesion to fibrinogen (13), which leads to thrombosis. Both patients in this study developed venous thrombosis after MWA of papillary thyroid carcinoma, which might cause an enhancement of the coagulation response induced by a series of physiological reactions of cancer cells or through some stimulation. Fourth, increased platelet content and mean platelet volume in patients with thyroid cancer could induce thrombosis (14). Both patients in this article were in the normal range. Fifth, thyroid cancer itself might be prone to thrombosis, and the side effects of levothyroxine inhibitory might increase VTE risk. Endothelial dysfunction, primary or secondary blood coagulation, and fibrinolysis pathway disorders can enhance the blood coagulation response (15, 16).

In this report, right internal jugular vein thrombosis occurred in two patients after MWA of papillary thyroid carcinoma neck metastases. It might be caused by impaired endothelial function after ablation. In this study, the common characteristics of the two cases were thyroid cancer and lymph node metastasis in level IV of the right neck, which was treated by MWA. The right cervical lymph nodes are located around the right internal jugular vein. Cryogenic isolating fluid was injected during the ablation process to compress the internal jugular vein. After the surgery, an ice bag was used to apply pressure on the neck, inducing thrombosis. Still, the exact causes remain to be determined.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by [Ethics Committee of Yantai affiliated hospital of Binzhou Medical University] [Approval number: F-KY-0059-20151001001-01]. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

S-RW and YL contributed to the conception and design of the study. X-JW, J-LW, L-HL, S-JY, and B-BY organized the database. S-RZ performed the statistical analysis. YL wrote the first draft of the manuscript. S-RW, Q-LX, and J-KL wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

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ORIGINAL RESEARCH
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Hashimoto's Thyroiditis Is Associated With Central Lymph Node Metastasis in Classical Papillary Thyroid Cancer: Analysis from a High-Volume Single-Center Experience

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Purpose: Central lymph node metastasis (CLNM) is regarded as a predictor for local recurrence in patients with papillary thyroid carcinoma (PTC) but the role of prophylactic central lymph node dissection (CLND) is controversial. Our study aims to identify the clinical factors associated with CLNM and develop a nomogram for making individualized clinical decisions.

Method: The perioperative data of 1,054 consecutive patients between Jan 2019 and April 2021, in our center, were reviewed and analyzed. A total of 747 patients with histopathologically confirmed classical PTC were included as the training cohort and 374 (50% training cases) patients were randomly selected to build a validating cohort *via* internal bootstrap analysis. Univariate and multivariate logistic regression were used to analyze the correlation between clinicopathological characteristics and CLNM.

Result: In the training cohort, 33.6% (251/747) of patients with classical PTC were confirmed with CLNM. And the CLNM was determined in 31.4% (168/535) of non-Hashimoto's thyroiditis (HT) patients versus 39.2% (83/212) in HT patients (p=0.043). Four factors including gender, age, size, and HT status were confirmed significantly associated with CLNM. The established nomogram showed good discrimination and consistency with a C-index of 0.703, supported by the internal validation cohort with a C-index of 0.701. The decision curve analysis showed the nomogram has promising clinical feasibility.

Conclusion: Our study suggested that classical PTC patients with features like male gender, age<55 years old, tumor size>1cm, and HT condition had a higher risk of CLNM. And the nomogram we developed can help surgeons make individualized clinical decisions in classical PTC patients during preoperative and intraoperative management.

Keywords: papillary thyroid carcinoma, Hashimoto's thyroiditis, central lymph node metastasis, nomogram, risk factor

INTRODUCTION

Over the past few years, the incidence rate of papillary thyroid carcinoma (PTC) has rapidly increased on a global scale, which has become the most prevalent endocrine-related malignancy (1–3). Despite a favorable long-term prognosis (4, 5), a minority of patients with central lymph node metastasis (CLNM) and even lateral lymph node metastasis (LLNM) showed higher recurrent risk and impaired disease-free survival (6–8). For instance, in one large-scale retrospective study from Italy, Sapuppo et al. determined that PTC patients with N1b had a higher risk of suffering distant metastases than N1a and N0 patients (7). Moreover, PTC patients with high-volume CLNM had a higher risk of local recurrence (7).

Currently, great achievements have been made in a range of works to determine the risk factors in the lymph node metastasis of PTC (9–11). Many clinical characteristics including but not limited to the male, younger age, larger tumor size, multifocal lesions, and extracapsular spread were regarded as the independent risk factors involving the lymph node metastasis of PTC.

Hashimoto's thyroiditis (HT), also known as chronic lymphocytic thyroiditis, is the most prevalent autoimmune disease nowadays (12). It causes chronic inflammation and damage in thyroid tissues *via* over-expression of thyroid globulin antibody (TgAb) and thyroid peroxidase antibody (TPOAb), leading to the condition of hypothyroidism in about 20-30% of patients, ultimately. Although a high concurrent rate of HT and PTC has been observed in the last century (13, 14), the relationship between these two diseases remains highly controversial. Some studies suggested that PTC patients who coexisted with HT had lower staging and better prognosis, compared with non-HT patients (15–19). The potential mechanisms are partly due to the modulation of the tumor microenvironment, the induction of abnormally immune responses and lymphocytic infiltrates in tumor tissue. However, some other studies reported that the coexistence of HT had no protective effect on patient outcomes (20, 21). Furthermore, some scholars even suggested that high thyroid antibody status, especially serum TgAb level, could be a risk factor in promoting the CLNM of PTC patients (22, 23). More clinical trials focusing on the effect of HT on the progression of PTC are merited.

The purpose of this retrospective study was to evaluate the role of clinicopathological factors including HT condition in predicting the CLNM for PTC patients. Also, we aimed to establish a prediction model based on the extracted clinicopathological factors to help surgeons preoperatively make individualized clinical decisions in terms of whether the prophylactic central lymph node dissection (CLND) is warranted.

MATERIAL AND METHOD

Data Source

Patients with the postoperative histopathological diagnosis of classical PTC at the Second Affiliated Hospital of Chongqing Medical University between January 2019 and April 2021 were reviewed and analyzed.

The inclusion and exclusion criteria among the training cohort were as follows: patients were enrolled during the study period using the following inclusion criteria: 1) aged between 18-75 years, and 2) underwent thyroidectomy and central lymph node exams. Patients were excluded during the study period using the following exclusion criteria: 1) no histologically proven PTC, 2) no lymph nodes found in the final pathological report, 3) other subtypes of thyroid cancer, 4) history or coexistence of other head and neck cancer, and 5) incomplete or missing medical records (**Figure 1**).

Surgical Extension

All of the surgical procedures (thyroidectomy and CLND) in our department were conducted by Prof. Yin, Prof. Ming, and Prof. Luo who are the senior specialists in general surgery, especially in terms of thyroid and breast surgery. The surgical extension of thyroid surgery followed the Chinese guideline (24). Specifically, central lymph node dissection is generally recommended, whereas lateral neck compartmental lymph nodes should be reasonably and selectively dissected. For tumors with a diameter> 4cm, or a naked thyroid infringement (clinical T4), or thyroid cancer patients with clinically significant local lymph node metastasis (clinical N1), first-proceeds should be total thyroidectomy. For the thyroid cancer patient with cN0 with a tumor diameter <4 cm, the treatment should be lobectomy, unless a clear indicator for resection of the contralateral gland, and the frozen biopsy was routinely performed in our department. Prophylactic or therapeutic unilateral central lymph node dissection was routinely performed in our department, whereas bilateral central lymph node dissection should be reasonably and selectively dissected (unless cN1 of contralateral central lymph node or multifocal lesions).

Variable Definition and Classification

The following information was collected to establish a retrospective database: gender, age, tumor size, extrathyroidal invasion, the status of CLNM and LLNM, HT status. HT was diagnosed based on one of the following criteria: i) the ultrasound examination revealed diffuse enlargement of thyroid with abundant blood flow combined with TPOAb > 34 IU/L or TgAb > 115 IU/L; ii) postoperative pathology confirmed diffusion lymphocytic thyroiditis), multifocality (more than two primary tumor focus) and $BRAF^{V600E}$ mutation testing results. All acquired surgical specimens were examined by the pathologists from the department of pathology of the Second Affiliated Hospital of Chongqing Medical University.

Statistical Analysis

The baseline characteristics by HT were compared using Chi-Square tests. Univariate and multivariate logistic regression analyses were used to identify the independent risk factors in patients. A two-tailed p-value of <0.05 was defined as the criterion for variable deletion when performing multivariate analyses. A nomogram for predicting the CLNM based on the results of the multivariate logistic regression

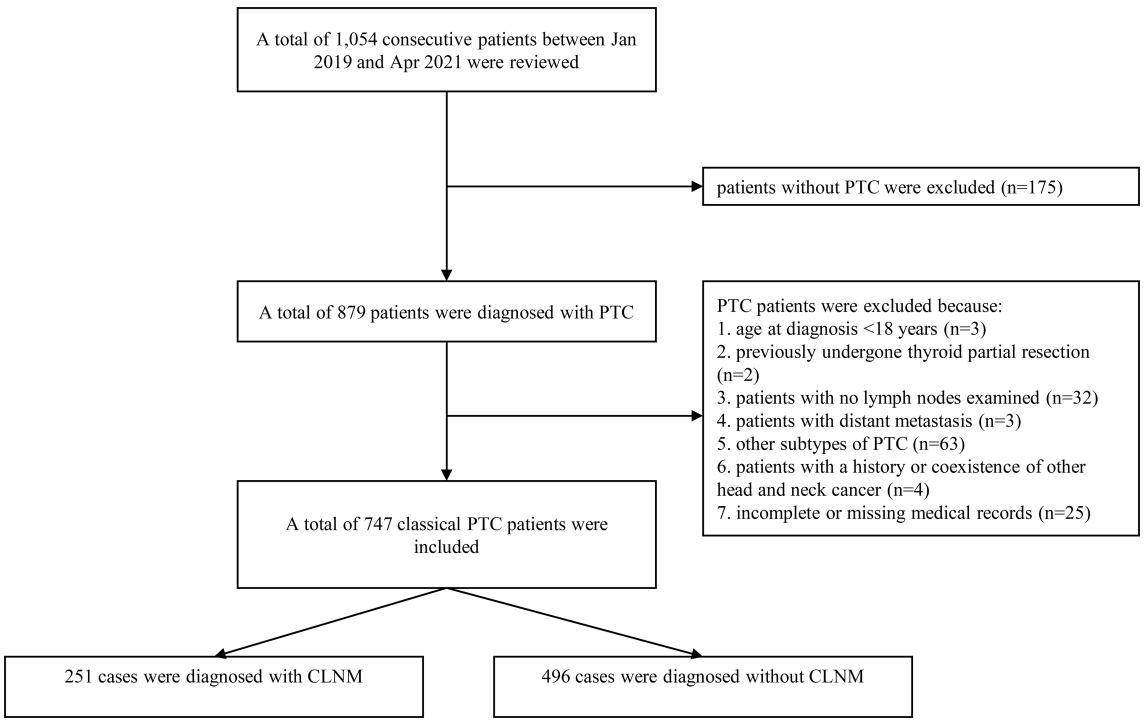

FIGURE 1 | The patients' selecting process in our department.

analysis was developed and evaluated by a decision curve analysis (DCA) curve. All analyses were performed using the "SPSS version 24.0" and "R version 4.0.2" software.

RESULTS

Baseline Clinicopathological Characteristics

A total of 747 (157 men; 590 women) classical PTC patients were enrolled in the training cohort and 374 patients were included as the validating cohort for developing and internally validating the clinical risk-stratifying model (**Table 1**). There were no significantly different clinicopathological characteristics observed in the two cohorts. However, a remarkable difference was determined in the clinical features of these patients when they were stratified by HT status (**Table 2**). Among these patients, female patients (590/747, 79%) accounted for most of the study group, compared with male patients (157/747, 21%). Additionally, the CLNM has ultimately been confirmed in 251 (33.6%) PTC patients, and 58 (7.8%) of patients were histopathologically confirmed with LLNM. Collectively, 212 (28.4%) PTC patients were diagnosed with coexisted with HT, and a significantly higher proportion (94.3%) of the female population was found in patients with concurrent HT (p<0.001). Besides, the CLNM was determined in 168 (31.4%) non-HT patients and 83 (39.2%) HT patients. However, there was no significant difference between these two datasets in terms of the prevalence of LLNM (p=0.889). Furthermore, there was no significant difference in age, tumor size, multifocality, extrathyroidal invasion, TNM stage, and $BRAF^{v600E}$ mutation.

Univariate and Multivariate Analysis of CLNM

We found that male gender (p<0.001), age<55 years old (p=0.014), tumor size>1cm (p<0.001), and presence of HT condition (p=0.044) were the potential risk factors associated with CLNM in PTC patients (**Table 3**). The multivariate analysis confirmed that male gender (OR=2.426, 95%CI: 1.628-3.614, p<0.001), tumor size (1cm<largest diameter ≤2cm, OR=3.315, 95%CI: 2.309-4.760; 2cm< largest diameter ≤4cm, OR=5.270, 95%CI: 2.908- 9.552; largest diameter >4cm, OR=5.072, 95%CI: 1.083-23.748; p<0.001), and HT condition (OR=1.678, 95%CI: 1.161-2.424, p=0.006) were defined as independent risk factors for CLNM. On the contrary, age ≥55 years old (OR=0.583, 95%CI: 0.382-0.890, p<0.001) was a protective factor for CLNM. Regarding to the $BRAF^{V600E}$ status, there was no significant relationship observed in $BRAF^{V600E}$ mutation and risk of CLNM in classical PTC patients (OR=1.162, 95%CI: 0.604-2.234, p=0.666).

Nomogram Construction and Validation for Predicting CLNM in PTC Patients

Based on the independent risk factors determined by multivariate analysis, a nomogram (**Figure 2**) was established for predicting the risk of CLNM in patients with PTC. Patients

TABLE 1 | The clinicopathological characteristics of classical PTC patients.

Variables	Subgroup	No. of patients		*P*
		Training cohort(n=747)	Validating cohort(n=374)	
Age (year)	<55	589	294	[a]0.938
	≥55	158	80	
Gender	male	157	76	[a]0.815
	female	590	298	
Race	Chinese	747	374	–
8th TNM Stage	I	702	356	[b]0.724
	II	40	16	
	III	5	2	
Multifocality	No	466	217	[a]0.173
	Yes	281	157	
Extrathyroidal invasion	No	685	344	[a]0.909
	Yes	62	30	
Primary surgical extension	TL+CLND	222	117	[a]0.182
	subTT+CLND	46	33	
	TT+CLND	479	224	
Tumor size (cm)	≤1	496	244	[b]0.924
	>1 and ≤2	189	95	
	>2 and ≤4	55	31	
	>4	7	4	
$BRAF^{V600E}$ mutation	No	45	26	[a]0.749
	Yes	587	287	
	N/A	115	61	
Clinical lymph node status	cN0	627	308	[a]0.551
	cN1	120	66	
HT	No	535	249	[a]0.085
	Yes	212	125	
TSH	/	*2.71 ± 5.26	2.42 ± 1.61	[c]0.292
fT3	/	4.85 ± 1.06	4.84 ± 0.86	[c]0.817
fT4	/	*16.78 ± 3.05	16.85 ± 2.95	[c]0.724
tT3	/	*2.01 ± 5.22	1.81 ± 1.05	[c]0.514
tT4	/	*99.94 ± 21.02	102.14 ± 22.36	[c]0.146
TgAb	/	*184.02 ± 496.64	152.79 ± 350.39	[c]0.282
TPOAb	/	*66.93 ± 119.85	73.41 ± 125.62	[c]0.403
TG	/	* 38.31 ± 88.79	44.21 ± 104.02	[c]0.344
Central LN examined	/	*4.20 ± 4.50	4.47 ± 4.93	[c]0.347
Central LN positive	/	*1.12 ± 2.19	1.32 ± 2.51	[c]0.175
Lateral LN examined	/	*1.21 ± 4.46	1.40 ± 4.85	[c]0.515
Lateral LN positive	/	*0.30 ± 1.43	0.33 ± 1.54	[c]0.723
Central LNM	No	496	239	[a]0.424
	Yes	251	135	
Lateral LNM	No	689	341	[a]0.563
	Yes	58	33	

**: Mean ± HT, Hashimoto's thyroiditis; PTC, papillary thyroid carcinoma; cN0, clinically lymph node-negative; cN1, clinically lymph node-positive; TL, thyroid lobectomy; CLND, central lymph node dissection; subTT, subtotal thyroidectomy; TT, total thyroidectomy; LNM, lymph node metastasis; LN, lymph node. [a]Pearson's Chi-squared test [b]Two-tail Fisher exact test [c]Student's two-tail t-test.*

with the risk factors including gender, age, tumor size, and HT were enrolled in our nomogram model (the score of each factor is shown in **Table 4**) to predict the presence of CLNM in PTC patients. A C-index of 0.703 was achieved in the present model. Moreover, the calibration curve for evaluating the accuracy of the nomogram showed a good internal consistency (**Figure 3A**). Besides, the AUC of the training cohort was 0.703, which was in accordance with the C-index (**Figure 3B**). In addition, an internal cohort with half the study population was developed via random bootstrap analysis, which was used to validate the feasibility of the nomogram and reached an AUC of 0.701 (**Figure 3C**). Furthermore, a decision curve analysis (DCA) was performed to compare the predictive ability between the combined clinical factors nomogram and the single-factor model. The standardized net benefits of the models were comparable and there was a significant overlap between these models. Collectively, the DCA curve showed that the prediction ability of the combined independent risk factors was superior to the single-factor in detecting CLNM for classical PTC patients which would be more effective than a treat-none or treat-all strategy when the threshold probability ranged from 0.2 to 0.8 (**Figure 4A**). To further evaluate the clinical significance of this prediction model, the clinical impact curve (CIC) was delineated (**Figure 4B**). As expected, the CIC results show that among the broad thresholds for CLNM

TABLE 2 | Clinicopathological features of 747 PTC patients with HT or without HT in the training cohort.

Variables	Subgroup	No. (%) of patients		*P*
		without HT (n=535)	with HT (n=212)	
Gender	male	145 (27.1)	12 (5.7)	**<0.001**
	female	390 (72.9)	200 (94.3)	
Age	<55	414 (77.4)	175 (82.5)	0.119
	≥55	121 (22.6)	37 (17.5)	
Tumor size (cm)	≤1	361 (67.5)	135 (63.7)	0.175
	>1 and ≤2	127 (23.7)	62 (29.2)	
	>2 and ≤4	40 (7.5)	15 (7.1)	
	>4	7 (1.3)	0 (0.0)	
Multifocality	positive	202 (37.8)	79 (37.3)	0.900
	negative	333 (62.2)	133 (62.7)	
Extrathyroidal invasion	positive	46 (8.6)	16 (7.5)	0.639
	negative	489 (91.4)	196 (92.5)	
BRAF mutation	No	30 (5.6)	15 (7.1)	0.719
	Yes	421 (78.7)	166 (78.3)	
	NA	84 (15.7)	31 (14.6)	
TSH	/	*2.54 ± 5.190	3.14 ± 5.436	¶0.158
fT3	/	*4.89 ± 1.075	4.75 ± 1.001	¶0.314
fT4	/	*16.74 ± 2.933	16.87 ± 3.321	¶0.348
tT3	/	*2.12 ± 6.128	1.69 ± 0.361	¶0.937
tT4	/	*100.10 ± 20.773	99.53 ± 21.728	¶0.162
TgAb	/	*64.21 ± 289.082	481.89 ± 727.597	**<0.0001**¶
TPOAb	/	*29.58 ± 57.723	160.40 ± 172.732	**<0.0001**¶
TG	/	*41.27 ± 89.908	31.05 ± 85.510	¶0.494
Central LN examined	/	*3.30 ± 3.739	6.46 ± 5.390	**<0.0001**¶
Central LN positive	/	*1.03 ± 2.120	1.33 ± 2.372	¶0.092
Lateral LN examined	/	*1.17 ± 4.555	1.31 ± 4.242	¶0.699
Lateral LN positive	/	*0.31 ± 1.555	0.25 ± 1.097	¶0.584
Central LNM	No	367 (68.6)	129 (60.8)	**0.043**
	Yes	168 (31.4)	83 (39.2)	
Lateral LNM	No	493 (92.1)	196 (92.5)	0.889
	Yes	42 (7.9)	16 (7.5)	
TNM stage	I	504 (94.2)	198 (93.4)	0.462
	II	19 (3.6)	11 (5.2)	
	III	12 (2.2)	3 (1.4)	

**: Mean ± SD; ¶: two-tailed t-test; HT, Hashimoto's thyroiditis; LNM, lymph node metastasis; NA, not mentioned. Bold values indicate statistical significance (p < 0.05).*

TABLE 3 | Univariate and multivariate analysis of 747 PTC patients for CLNM.

Variables	Subgroup	Univariable		Multivariable	
		Hazard ratio	*P*	Hazard ratio	*P*
Gender	female	Reference	**<0.001**	Reference	**<0.001**
	male	1.945 (1.357, 2.787)		2.426 (1.628, 3.614)	
Age	<55	Reference	**0.014**	Reference	**0.012**
	≥55	0.607 (0.409, 0.902)		0.583 (0.382, 0.890)	
Tumor size (cm)	≤1	Reference	**<0.001**	Reference	**<0.001**
	>1 and ≤2	3.202 (2.252, 4.552)		3.315 (2.309, 4.760)	
	>2 and ≤4	4.752 (2.667, 8.466)		5.270 (2.908, 9.552)	
	>4	4.224 (0.932, 19.142)		5.072 (1.083, 23.748)	
Multifocality	negative	Reference	0.372	/	
	positive	1.153 (0.844, 1.574)			
$BRAF^{V600E}$ mutation	No	Reference	0.666	/	
	Yes	1.162 (0.604, 2.234)			
	NA	0.969 (0.460, 2.042)			
HT	No	Reference	**0.044**	Reference	**0.006**
	Yes	1.406 (1.010, 1.957)		1.678 (1.161, 2.424)	

CLNM, central lymph node metastasis; HT, Hashimoto's thyroiditis; NA, not mentioned. Bold values indicate statistical significance (p < 0.05).

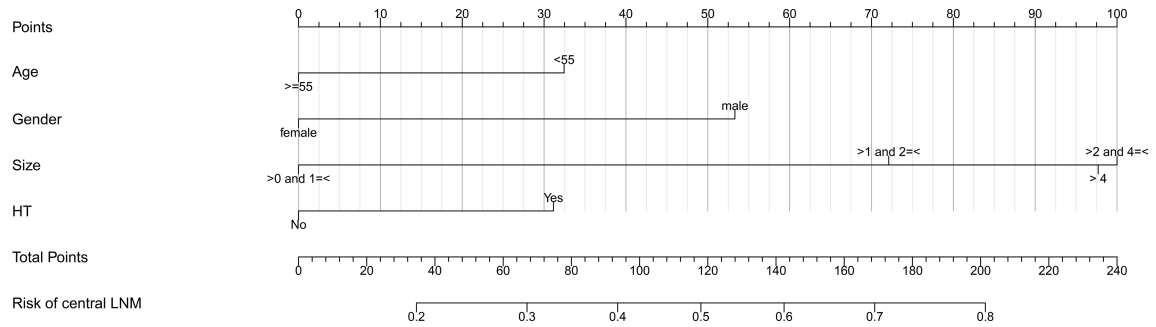

FIGURE 2 | Clinical factor-based nomogram used for predicting the CLNM in PTC patients. CLNM, central lymph node metastasis, HT, Hashimoto's thyroiditis.

(20–80%), the nomogram was classified as positive and the number of true positives was greater than those of the separated factor model.

DISCUSSION

To date, with an increasing prevalence and overdetection rate of PTC around the world, the management of this disease becomes more precise and particularly crucial. For preventing unnecessary diagnostic workup and possible surgical intervention, two medical societies from East Asia and North America (the Japan Association of Endocrine Surgery and the American Thyroid Association) updated the clinical practice guidelines regarding the indications and strategy for active surveillance (AS) of low-risk PTMC ($cT_1N_0M_0$) patients, especially for the elderly population (25, 26). It indicated that the treatment modality of differentiated thyroid carcinoma became more conservative in developed countries. Nevertheless, surgical intervention is still the pivotal initial treatment for PTC patients in China (24), especially in terms of patients with suspicious clinically CLNM.

In the present study, we aimed to investigate the risk factors in promoting the CLNM in PTC patients and further establish an individualized model for this subpopulation. The CLNM was histopathologically confirmed in 251 (33.6%) PTC patients which were consistent with other reports (15, 16, 27). Besides, 58 (7.8%) patients were diagnosed to suffer from LLNM which was consistent with Homma's study (8.4%) (6) but much lower than the observation result (25.6%) made by Yang et al. (27). This divergence might be due to the different study populations and the number of patients. We selected seven variables including gender, age, tumor size, multifocality, $BRAF^{V600E}$ mutation, and HT for univariate analysis. Neither multifocality nor $BRAF^{V600E}$ mutation was associated with CLNM. Due to these differences, approximately 15% of patients did not undergo the fine-needle aspiration (FNA) or $BRAF^{V600E}$ test before the surgery which was a common limitation in recent studies (27, 28). Besides, a relatively smaller sample size compared with other large cohort studies which also contributed to this difference. Hence, gender, age, tumor size, and HT were finally screened out for multivariable analysis, similar to the recently reported studies (29, 30). Interestingly, it is believed that tumor size was one of the pivotal risk factors in CLNM and the risk of CLNM increased as the diameter of the primary nodule increased. However, our data suggested that the highest risk ratio did not appear in tumors with a diameter larger than 4cm, instead of in tumors with a diameter larger than 2cm but smaller than 4cm (**Figure 1**). This phenomenon was potentially associated with the limited sample size (only seven cases) of PTC patients with a diameter larger than 4cm. Reviewing recent guidelines from Asian and western countries (24, 26), the role of prophylactic central lymph node dissection (CLND) in PTC continues to be debatable. For instance, as recommended by the clinical practice guideline derived from the American Thyroid Association, omitting CLND was safe and appropriate for patients with the small primary thyroid tumor, especially in terms of clinically node-negative (cN0) PTC. In addition, one multicenter study determined that there was no

TABLE 4 | The specific value of clinicopathological features in the nomogram.

Characteristics	Score
Age	
≥5 years old	0
<55 years old	32
Gender	
female	0
male	53
Size (cm)	
>0 and ≤1	0
>1 and ≤2	72
>2 and ≤4	100
>4	98
Hashimoto's thyroiditis	
Not present	0
present	31
Total point for predicting the CLNM	
0.2	34
0.3	67
0.4	93
0.5	118
0.6	141
0.7	169
0.8	201

CLNM, central lymph node metastasis.

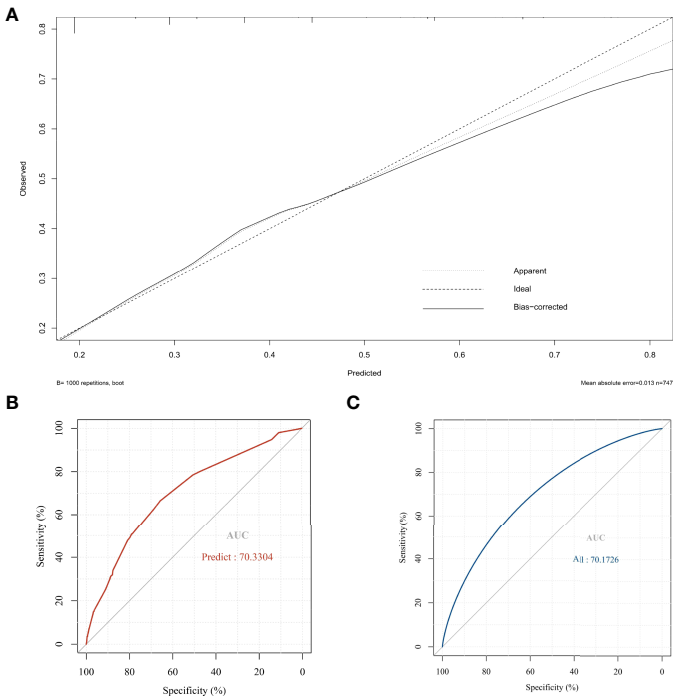

FIGURE 3 | **(A)** The calibration curve for evaluating the accuracy of the nomogram; **(B)** The receiver operating characteristics (ROC) curve and area under the ROC curve (AUC) in the training cohort; **(C)** The receiver operating characteristics (ROC) curve and area under the ROC curve (AUC) in the validating cohort.

significant difference in the risk of regional recurrence among total thyroidectomy (TT) alone, TT with ipsilateral CLND, and TT with bilateral CLND in dealing with cN0 differentiated thyroid carcinoma (DTC) but groups treated with TT alone presented the lowest incidence of postoperative complications (31). Notably, clinical evidence from one meta-analysis (32) report (3,331 cases involved) demonstrated that patients with prophylactic CLND presented a 35% reduction in risk of postoperative locoregional recurrence to those who undergo TT alone during the 5 year follow-up, whereas the overall complication rate including, but limited to, transient hypocalcemia, in the former group was much higher than the latter group. Similarly, a randomized controlled study by Viola et al. (33) also highlighted that there were no clinical advantages in performing prophylactic CLND in patients with PTC with cN0 at neck ultrasound. While the TNM staging system is a common method to predict the disease-specific prognosis of PTC, it could not provide preoperative guidance for surgeons to decide on the precision surgical extension (34). Consequently, a more comprehensive evaluation of risk factors for CLNM in the adult population might bring more clinically significant value for preoperatively clinical decisions in these patients with different risks.

Additionally, in our study, the diagnosis of HT was based on the pathology of the surgical specimen. We observed that there was a lower rate of coexisted HT in male PTC patients than in female PTC patients (7.6% vs. 33.9%, $p<0.001$), which was consistent with previous studies, and no significant difference was found between PTC patients with or without HT except for gender and CLNM. Based on multivariate analysis, the HT condition in our study was determined to be an independent risk factor in CLNM ($p=0.006$), which was partially different from the conclusion in the previous study. Therefore, the role of HT in the progression of PTC is worth discussing.

Currently, a range of works (15, 23, 35, 36), especially retrospective studies, have determined a high concurrent rate of HT and PTC from surgical specimens but the relationship between these two diseases, as well as HT and CLNM, has been controversial. Immunologically, emerging evidence has shown that an abnormal inflammatory response, especially the imbalanced subsets of T cells, NK cells, and cytokines were presented in HT condition (14, 37), which could potentially affect the tumor microenvironment and subsequent prognosis. For instance, *in vitro* experiment, Lubin et al. (38) verified that the presence of background HT contributed to a higher risk of CLNM *via* increased programmed death ligand-1(PD-L1). On the contrary, results from Hu et al. (19) suggested that enhanced MHC class I expression in HT could decrease the PD-L1 and further overcome the CLNM in PTC patients. Serologically, Wen et al. (23) conducted that different thyroid antibody status was significantly associated with the CLNM in PTC patients concurrent with HT. They concluded single TgAb was a risk factor in CLNM, whereas TPOAb played a protective role in preventing CLNM. By contrast, a few studies hold the opposite view on the role of serum TPOAb levels in CLNM (39). The inconsistent results in these studies inspired us to provide our own experience in the present work.

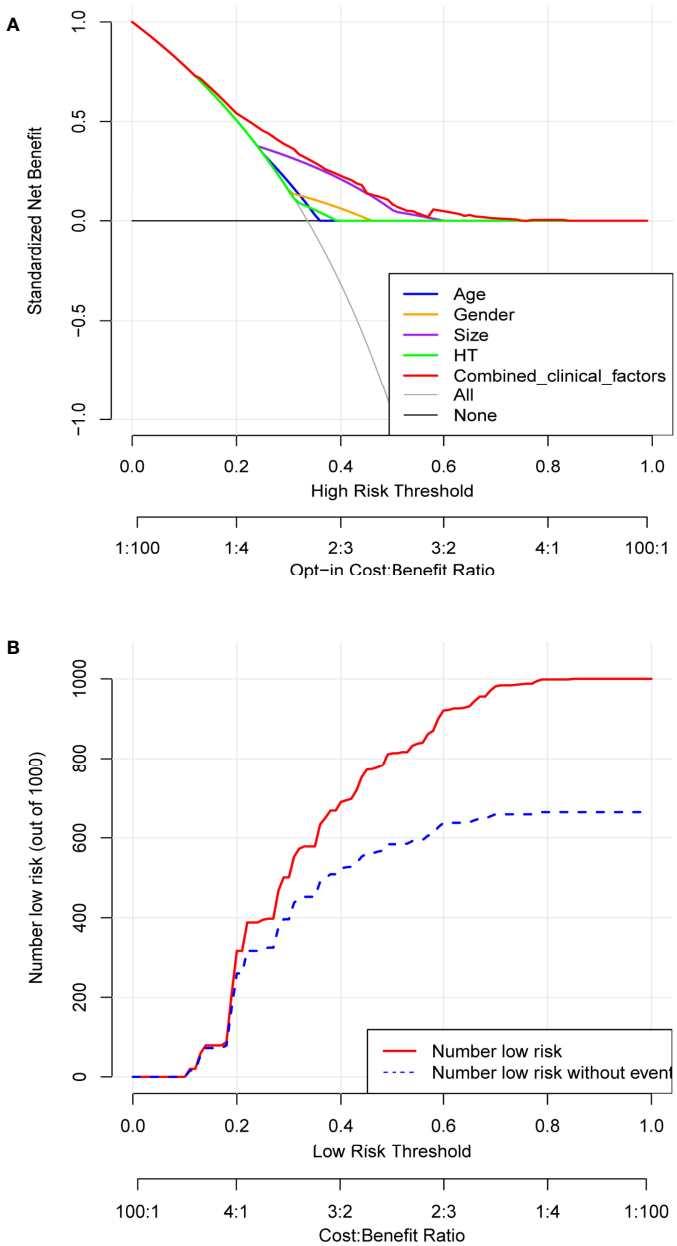

FIGURE 4 | Determination of decision points *via* Decision Curve Analysis (DCA) and Clinical Impact Curve (CIC). **(A)** DCA for the prediction model (EI-Score). The decision curve analysis graphically shows the clinical usefulness of the EI-Score based on a continuum of potential thresholds for central lymph node metastasis (CLNM) (x-axis) and the net benefit of using the EI-Score to stratify patients (y-axis). Net benefit curves are plotted across probability thresholds for 7 options: ''treat all' assume all patients have CLNM, 'treat none' assume no patients have CLNM, treat according to combined clinical factors (age, gender, tumor size, HT), Age, Gender, size, HT and EI-Score. Net benefit = (true positives/N)- (false positives/N) *(weighting factor). Weighting factor = Threshold probability/(1-threshold probability). **(B)** CIC for EI-Score. The red line shows the total number who would be deemed as low risk of CLNM for each risk threshold. The blue line shows how many of those would be true positive (without CLNM).

Reviewing similar works on predicting CLNM (15, 27, 28, 40), our study had a partial difference and takes it a step further. Our data indicated that HT was one of the independent risk factors in promoting CLNM which deserved further evaluation. Although the C-index in the previous study achieved 0.764 based on 914 PTC patients (41) and 0.854 based on a sample size of 1,252 PTC patients (27), the C-index of our nomogram was still more than 0.7, indicating that it also has sufficient discrimination ability. The DCA results show that the nomogram we developed has good clinical practical value. Combined with other established nomograms based on ultrasound signatures, our nomogram with clinicopathological characteristics with the strongest risk factors

including gender, age, size, and HT can increase the accuracy of predicting CLNM. These prognostic factors collected from preoperative testing could further help surgeons to decide the extent of the initial thyroidectomy and whether prophylactic central neck dissection is warranted.

Nonetheless, the results from the study need to be carefully interpreted and some limitations should be addressed in the following works. First and foremost, the weakness of this cohort is a lack of external validation which limits the clinical application. Thus, the external validation cohorts from multicenter countries are urgently demanded to further evaluate the feasibility of our nomograms. Second, this was a retrospective study from a single-center teaching hospital center which did introduce some selection biases. Moreover, there were only four clinical factors ultimately added to our nomogram, which indicated there might be potential variables waiting to be discovered that could make our nomogram complete and more reliable, including but not limited to body mass index (BMI), preoperative ultrasound signatures, and some laboratory testing results which were previously determined to be associated with CLNM in classical PTC patients (27, 28).

CONCLUSION

In summary, several clinical features including male gender, younger age, larger tumor size, and HT status were independent risk factors for CLNM in classical PTC patients. A predicting nomogram based on these clinical risk factors is established to help surgeons make individualized clinical decisions during intraoperative management.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Conception and design: BZ and YM. Administrative support: ZL. Provision of study materials or patients: YF, HC, and YM. Collection and assembly of data: BZ, YM, and KX. Data analysis and interpretation: BZ, YM, and YF. Manuscript writing: All authors. Final approval of manuscript: All authors.

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Ultrasonic Characteristics Improve Prediction of Central Lymph Node Metastasis in cN0 Unifocal Papillary Thyroid Cancer

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Background: Prediction of central lymph node metastasis (CLNM) is vital for clinical decision-making processes in clinically N0 (cN0) unifocal papillary thyroid carcinoma (PTC), but the sensitivity of preoperative detection of CLNM is limited. The aim of the present study was to determine whether there are ultrasonic (US) characteristics associated with CLNM.

Methods: In total, 1657 PTC patients (514 men and 1143 women) were enrolled in the present study between January 2018 and May 2021. The patients met the following inclusion criteria based on preoperative detection: suspected nodule confirmed as PTC by biopsy; the nodule was unifocal and less than 4 cm in diameter; no prior neck radiation exposure; no extrathyroidal extension; and no CLNM or distant metastases on imaging. All the enrolled patients underwent total thyroidectomy with prophylactic central lymph node dissection (CLND). A postoperative pathological diagnosis was made.

Results: CLNM was found in 58.4% of male patients and 36.9% of female patients. In univariate analysis, size, adjacent anterior capsule, distance to the lower pole and color Doppler flow imaging (CDFI) were considered risk factors for the male and female groups ($p < 0.05$). In multivariate analyses, size, adjacent anterior capsule, distance to the lower pole and CDFI were independent risk factors for male patients. For females, the independent risk factors included size, adjacent anterior capsule, distance to the lower pole and CDFI.

Conclusion: In the present cohort, US imaging characteristics, including size, adjacent anterior capsule, distance to the lower pole and CDFI, were identified to be potentially beneficial in preoperative clinical decision-making processes for cN0 unifocal PTC patients.

Keywords: ultrasonic characteristics, CLNM, cN0 PTC, unifocal, predictor

INTRODUCTION

Thyroid carcinoma is one of the most common neoplastic diseases (1), and its morbidity is increasing worldwide (2). The age-standardized incidence of thyroid carcinoma is over 5% in some Asian countries (3), and this carcinoma occurs approximately three times more often in women than in men (4). Papillary thyroid carcinoma (PTC) accounts for approximately 95% of thyroid carcinomas and generally has an excellent prognosis with 10-year survival rates approaching 90-95% (5). Nevertheless, because PTC represents approximately 95% of all cases, most cancer related mortality is due to PTC.

The primary treatment method of PTC is surgical ablation. The objectives of initial surgical therapy include removing the primary tumor and clinically significant cervical lymph nodes as well as minimizing treatment-related morbidity and the risk of recurrence or metastasis. Recent American Thyroid Association (ATA) guidelines and National Comprehensive Cancer Network (NCCN) guidelines note primary risk factors for preoperative determination of the thyroid resection extent (6, 7). Lobectomy is indicated if the following criteria are met: no prior radiation exposure, no cervical lymph node metastases, no extrathyroidal extension, no distant metastases and tumor size less than 4 cm in diameter. For these criteria-matched clinically N0 (cN0) unifocal PTCs, routine prophylactic central compartment lymph node dissection (CLND) is not recommended by both guidelines. However, central compartment lymph node metastasis (CLNM) is relevant to risk stratification and prognosis (8, 9). Currently, there are no non- or minimally-invasive methods that are completely reliable for detecting all of the potential metastases (10). Thus, an accurate preoperative evaluation of CLNM is vital for the management of PTC patients.

For cN0 unifocal PTCs, the accurate identification of CLNM is crucial. Nonetheless, CLNM is difficult to detect preoperatively, and the current assessment methods have limited power. Approximately 30–80% of PTCs are associated with CLNM (11–13), and some studies have suggested that CLNM is related to disease relapse and distant metastases (14–16). As CLNM is difficult to detect preoperatively and CLND is related to morbidity (17), the clinical decisions for treatment are controversial (18). Previous medical studies have suggested that CLND may reduce the recurrence of PTC, indicating a risk stratification for recurrence and distant metastases. In addition, the treatment procedure, such as radioactive iodine therapy, may be altered accordingly. For those criteria-matched cases, prophylactic CLND permits patients to obtain more active medical treatment and less hazardous reoperative surgical treatment (19, 20). However, prophylactic CLND increases the morbidity, such as hypoparathyroidism and recurrent laryngeal nerve injury (21, 22). The ATA and NCCN guidelines do not suggest prophylactic CLND, stating that prophylactic CLND may be considered in specific patients who have advanced primary tumors. There is also a viewpoint that more evidence is needed to support that prophylactic CLND is beneficial to reduce recurrence rates (23). Above all, a more accurate evaluation of CLNM is necessary for cN0 unifocal PTC patients to obtain better clinical decisions.

Ultrasonic (US) detection is the preferred diagnostic method for CNLM. Although it has many advantages, there are limitations. For example, the sensitivity of US detection in evaluating CLNM ranges from 20 to 60% (24–26). Although many studies have reported high-risk factors related to clinical and US characteristics predictive of CLNM of PTC patients, the conclusions are controversial. Some of the identified risk factors, such as tumor differentiation, extrathyroidal invasion and gene type, are only available postoperatively (27), indicating that they cannot provide reliable information for preoperative clinical decision-making processes. Consequently, the research on a noninvasive and valuable approach based on US detection for evaluating CLNM is essential but challenging.

The present study aimed to evaluate the US imaging characteristics of nodules associated with CLNM in cN0 unifocal PTC patients. The present conclusions may be useful in preoperative clinical decision-making processes.

MATERIALS AND METHODS

Patient Data and Ethical Approval

The studies involving human participants were reviewed and approved by the Ethics Committee of the Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences (Guangzhou, Guangdong Province, People's Republic of China), and they conformed to the provisions of the Declaration of Helsinki. Written informed consent from the patients was not required to participate in this study in accordance with national legislation and the institutional requirements. We evaluated the patients retrospectively with histologically confirmed PTC in our hospital between January 2018 and May 2021. The patients were enrolled according to the following criteria: (1) the suspected thyroid nodule was unifocal and less than 4 cm in diameter based on US examination; (2) the suspected nodule was confirmed to be malignant by ultrasound-guided puncture biopsy; (3) no extrathyroidal extension and no cervical lymph node metastases based on US examination; (4) patients were subjected to an initial thyroid surgery with CLND and were histologically confirmed as having PTC; and (5) no prior neck radiation exposure. Patients were excluded based on the following criteria: (1) having distant metastases or malignant tumors in other organs; (2) poorly differentiated based on postoperative pathology; (3) the US imaging information was incomplete, or the quality of the images was unclear; or (4) multifocal lesions based on postoperative pathology. **Figure 1** shows the patient recruitment process. Ultimately, 1657 patients (514 men and 1143 women) were included in the present study. The data were divided into CLNM-negative and CLNM-positive groups according to the pathology results.

US Equipment and Evaluation of US Characteristics

US examinations were performed using HI Vision 900, HI Vision Ascendus and HI Vision Preirus color US units (with US elasticity imaging capability) from Hitachi, and the probe frequency was 6.0–13.0 MHz. The US imaging features of every patient were retrospectively re-examined by two independent radiologists with more than 10 years of experience in thyroid US imaging; neither

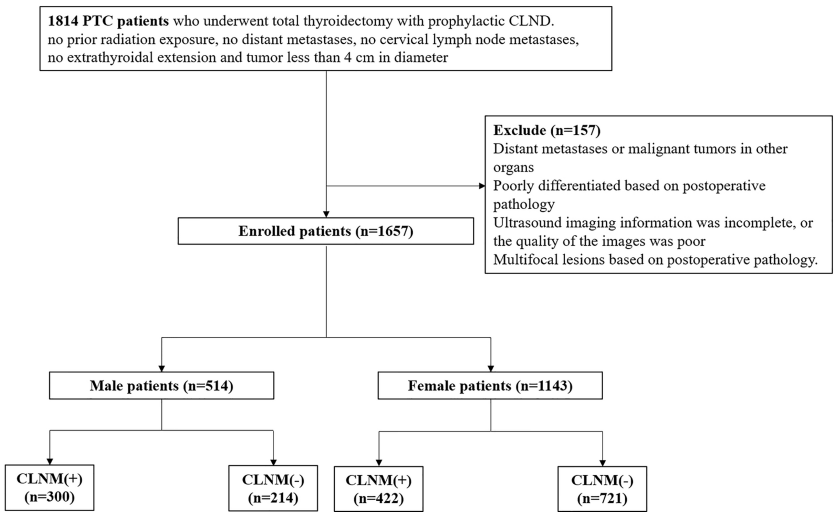

FIGURE 1 | Flow chart of the patients enrolled in our study.

observer knew the clinical nor the pathological outcomes. If the radiologists faced a dilemma, they would determine their final decisions by a consensus. The imaging characteristics of each nodule were as follows: tumor size; multifocality; aspect ratio (height divided by width on transverse views, A/T); tumor location; distance between the nodules and the adjacent capsule; microcalcification situation; border; US halo; tumor internal vascularity; and Hashimoto's thyroiditis. Many images of the longitudinal and transverse axes were fully evaluated. The tumor size refers to the maximum diameter (D) of the nodule, which was classified as follows: $D \leq 0.5$ cm, $0.5 < D \leq 1.0$ cm, $1.0 < D \leq 1.5$ cm and $D > 1.5$ cm. The A/T was classified as ≤ 1 or > 1. The location of the tumor was evaluated according to the following three aspects: location (left lobe, right lobe and isthmic), distance to the upper pole and distance to the lower pole. The distance between the tumor and adjacent capsule (anterior and posterior) was classified into three categories as follows: < 1 mm and not protruding outside the thyroid capsule; 1 ≤ and < 2 mm; and ≥ 2 mm. Tumor vascularity was classified from 0 to 3 and evaluated by color Doppler flow imaging (CDFI). Hashimoto's thyroiditis was diagnosed on the basis of US characteristics. Because the diagnostic performance of the present study depended on the accuracy of operator-reported imaging features, the interobserver reproducibility for US features was assessed. Regarding the preoperative identification of cervical lymph nodes (LNs), a LN was considered suspicious if it had one of the following characteristics: microcalcifications, hyperechoic change, loss of fatty hilum; round shape; and necrosis (28).

Statistical Analysis

Statistical analysis was performed with SPSS Statistics version 24.0 (IBM Corp.). Categorical variables are presented as numbers and percentages. A chi-square test or Fisher's exact test was used to assess differences between groups. A logistic regression model was used to evaluate the risk factors. The reported statistical significance levels were all two-sided with statistical significance set at 0.05.

RESULTS

Characteristics of Patients

Among the 1657 patients, there were 514 (31.0%) male patients and 1143 (69.0%) female patients. A significant difference was found in gender between CLNM-positive and CLNM-negative patients; 58.4% of males and 36.9% of females were CLNM-positive patients ($p < 0.05$). The gender disparities in incidence, prognosis and aggressiveness are well established for PTC, but the underlying causes remain poorly understood. Population-based studies have shown that reduced estrogen exposure favors PTC malignancy (29, 30). To adjust for the gender factor, we arranged two separate groups for these patients.

Risk Factors for Male and Female Patients With cN0 Unifocal PTC

The patient features and US imaging characteristics of thyroid nodules in the male and female cohorts are shown in **Tables 1** and **2**. Univariate and multivariate analyses were conducted to determine the differences in clinical and US imaging features between CLNM-positive and CLNM-negative groups. In univariate analysis, size ($p < 0.05$), adjacent anterior capsule ($p < 0.05$), distance to the lower pole ($p < 0.05$) and CDFI ($p < 0.05$) were considered risk factors for both male and female groups (**Tables 1** and **2**). In multivariate analyses, size, adjacent anterior capsule, distance to the lower pole and CDFI were considered independent risk factors (**Tables 3** and **4**). For male patients, size (0.5-1 cm, OR 2.62, 95% CI 1.18-5.81; 1-1.5 cm, OR 6.93, 95% CI 3.01-15.95; >1.5 cm, OR 12.12, 95% CI 5.21-28.18), adjacent anterior capsule (1-2 mm, OR 2.00, 95% CI 1.32-3.03;

TABLE 1 | Clinical and US imaging characteristics of male patients.

Characteristics	CLNM		P value
	+	-	
Total	300	214	
Size			< 0.05
0-0.5 cm	8	40	
0.5-1 cm	103	95	
1-1.5 cm	72	41	
> 1.5 cm	117	38	
Adjacent anterior capsule			< 0.05
< 1 mm	128	25	
1-2 mm	76	60	
≥ mm	96	129	
Distance to the lower pole			< 0.05
0-10 mm	120	43	
10-20 mm	102	67	
≥ 20 ram	78	104	
CDFI			< 0.05
02-Mar	129	179	
0-1	171	35	
Age (year)			0.27
≥ 55	145	114	
< 55	155	100	
A/T			0.83
> 1	171	120	
≥ 1	129	94	
Location			0.34
left lobe	127	95	
right lobe	155	100	
Isthmic	18	19	
Adjacent posterior capsule			0.17
< 1 mm	76	42	
1-2 mm	89	59	
≥ 2 mm	135	113	
Distance to the upper pole			0.9
0-10 mm	76	51	
10-20 mm	103	77	
≥ 20 mm	121	86	
Microcalcification			0.09
Present	225	146	
Absent	75	68	
Smooth border			0.32
Absent	283	206	
Present	17	8	
Ultrasonic halo			0.54
Absent	264	192	
Present	36	22	
Hypoechoic			0.34
Present	280	204	
Absent	20	10	
Hashimoto's thyroiditis			0.85
Present	17	13	
Absent	283	201	

<1 mm, OR 2.81, 95% CI 1.83-4.33), distance to the lower pole (10-20 mm, OR 1.52, 95% CI 1.02-2.27; 0-10 mm, OR 2.63, 95% CI 1.69-4.09) and CDFI (2-3, OR 0.69, 95% CI 0.48-1.00) were considered independent risk factors. For female patients, size (0.5-1 cm, OR 0.89, 95% CI 0.53-1.49; 1-1.5 cm, OR 1.43, 95% CI 0.81-2.52; >1.5 cm, OR 4.90, 95% CI 2.73-8.77), adjacent anterior capsule (1-2 mm, OR 3.21, 95% CI 2.21-4.46; <1 mm, OR 5.14, 95% CI 3.60-7.34), distance to the lower pole (10-20 mm, OR 2.06, 95% CI 1.44-2.94; 0-10 mm, OR 3.58, 95% CI 2.36-5.42) and CDFI (2-3, OR 1.82, 95% CI 1.33-2.48) were considered independent risk factors.

DISCUSSION

In the present study, we found and validated several US-based characteristics for predicting the probability of CLNM in cN0 unifocal PTC patients. The patients in the present study met the

TABLE 2 | Clinical and US imaging characteristics of female patients.

Characteristics	CLNM		P value
	+	-	
Total	422	721	
Size			< 0.05
0-0.5 cm	32	80	
0.5-1 cm	144	466	
1-1.5 cm	89	120	
> 1.5 cm	157	55	
Adjacent anterior capsule			< 0.05
< 1 mm	216	160	
1-2 mm	128	159	
≥ 2 nun	78	402	
Distance to the lower pole			< 0.05
0-10 mm	157	128	
10-20 mm	190	273	
≥ 20 mm	75	320	
CDFI			< 0.05
2-3	150	180	
0-1	272	541	
Age(year)			0.24
≥ 55	73	145	
< 55	349	576	
A/T			0.94
> 1	197	335	
≥ 1	225	386	
Location			0.19
left lobe	190	353	
right lobe	208	341	
Isthmic	24	27	
Adjacent posterior capsule			0.68
< 1 mm	104	162	
1-2 mm	88	150	
≥ 2 mm	230	409	
Distance to the upper pole			0.26
0-10 mm	25	61	
10-20 mm	176	283	
≥ 20 mm	221	377	
Microcalcification			0.17
Present	325	529	
Absent	97	192	
Smooth border			0.14
Absent	358	634	
Present	64	87	
Ultrasonic halo			0.18
Absent	406	681	
Present	16	40	
Hypoechoic			0.63
Present	405	696	
Absent	17	25	
Hashimoto's thyroiditis			0.62
Present	120	215	
Absent	302	506	

following criteria based on preoperative detection: the suspected nodule was confirmed to be PTC by biopsy; the nodule was unifocal and less than 4 cm in diameter; no prior neck radiation exposure; no extrathyroidal extension; no CLNM; and no distant metastases. The present findings indicated that these risk factors may improve the preoperative prediction of CLNM in a noninvasive manner. The sensitivity for detecting CLNM using preoperative neck US imaging is low (31, 32) due to air in the trachea, complex structures in the sternum and clavicle, which make it difficult for US imaging to detect CLNM.

For clinically N0 unifocal PTCs, the precise evaluation of CLNM is important. Barczynski et al. acknowledged that CLND promotes both a locoregional situation and 10-year disease-specific survival without increasing the risk of permanent morbidity (33). Hartl et al. reported that CLND does not enhance the incidence of morbidity, especially the permanent dissections (34), which may be due to the surgical skills of the surgeons reducing complications. In our study, all of the complications and side effects were documented and treated. The complications of surgery included hypocalcemia,

TABLE 3 | Multivariate logistic regression analysis of risk factors for male.

Characteristics	β	Odds ratio [95% CI]	*p*
Male size			
0.5-1 cm	2.62	1.18-5.81	<0.05
1-1.5 cm	6.93	3.01-15.95	<0.05
> 1.5 cm	12.12	5.21-28.18	<0.05
Adjacent anterior capsule			
1-2 mm	2	1.32-3.03	<0.05
< 1 mm	2.81	1.83-4.33	<0.05
Distance to the lower pole			
10-20 mm	1.52	1.02-2.27	<0.05
0-10 mm	2.63	1.69-4.09	<0.05
CDFI			
2-3	0.69	0.48-1.00	<0. 05

TABLE 4 | Multivariate logistic regression analysis of risk factors for female.

Characteristics	β	Odds ratio[95% CI]	*p*
Female			
size			
0.5-1 cm	0.89	0.53-1.49	<0.05
1-1.5 cm	1.43	0.81-2.52	<0.05
> 1.5 cm	4.9	2.73-8.77	<0.05
Adjacent anterior capsule			
1-2 mm	3.21	2.21-4.66	<0.05
< 1 mm	5.14	3.60-7.34	<0.05
Distance to the lower pole			
10-20 mm	2.06	1.44-2.94	<0.05
0-10 mm	3.58	2.36-5.42	<0.05
CDFI			
2-3	1.82	1.33-2.48	<0. 05

hoarseness, seroma, pain and choke. The complications were totally under control and there were no permanent injury caused. It is well-known that revision surgery in scarred areas promotes a high risk for recurrent laryngeal nerve (RLN) injury and parathyroid gland injury. Zhao et al. indicated that CLND significantly lowers LN recurrence (35). In addition, CLND helps surgeons assess the tumor-node-metastasis (TNM) stage of patients with PTC to determine the subsequent radioactive iodine (RAI) therapy (36). However, ATA and NCCN guidelines do not recommend prophylactic CLND. Nixon et al. found that the 5- and 10-year disease-free survival of patients with PTC who did not undergo prophylactic CLND is 100%; they considered an active observation of CLN safe and that it should be suggested for all patients with PTC considered before and during surgery without central neck metastasis (37). Furthermore, many researchers have suggested that prophylactic CLND may promote the complication rate of RLN and parathyroid gland permanent injury by approximately 2-fold (38, 39). Above all, CLND is important but should be implemented with care. Further, it is imperative to diagnose CLNM preoperatively for clinical decision-making processes.

In the present study, an US feature was generated using risk factors, including tumor size, for the prediction of CLNM. In the present study, tumors with a larger size on US examination were more likely to be related to CLNM, which was consistent with other reports (40). Tumor size is widely analyzed in many staging systems, including the American Joint Committee on Cancer (AJCC) staging system. The most used cutoff in risk stratification is 1 cm, which is widely accepted as a risk factor for CLNM and is associated with higher mortality (41). Many studies have utilized the largest diameter of the tumor as the tumor size, but there is no definitive conclusion at present (42). However, some studies have reported that tumor size is not an adequate independent predictor of CLNM (43). Several previous studies have set the size threshold between 5 and 10 mm; however, these studies have reported that when the tumor is less than this threshold, the rate of CLNM is still high, ranging from 26% to 55% (44, 45).

The location features of the nodule may also be important risk factors. A tumor adjacent to the anterior capsule and that has a short distance to the lower pole has a close association with CLNM (46). The thyroid gland is encapsulated by a thin fibroelastic (true) capsule, and this capsule is covered by a pretracheal fascia from the outside and is called a false capsule. The true capsule gives rise to septa deep into the parenchyma, dividing the thyroid gland into lobules. The septa makes room for blood vessels, nerves and lymphatics in the gland. The thyroid gland and its neighboring structures have many lymphatics, which drain the thyroid in almost every direction. Within the thyroid gland, lymphatic channels are present beneath the capsule and connect lobes through the isthmus. Most thyroid neoplasms drain directly to CLN basins, except for cancers in the superior third of the gland, which may drain to the lateral compartment (known as skip metastases) (47). This may be the reason for the association of a closer distance to the capsule and lower pole on US imaging with CLNM. In the present study, the CDFI was significantly different between the CLNM-positive and CLNM-negative groups; richer blood supply was correlated with a higher probability of CLNM (48).

The present study had several limitations, including those inherent to a retrospective study design. The present study was also a single-center historical cohort study, and our results may have been biased accordingly. Stringent external validation needs to be performed in larger, prospective multicenter clinical trials to obtain a more objective conclusion. In addition, a relatively small number of patients had large-volume CLNM, which did not allow us to demonstrate the key predictive factor of occult large-volume CLNM. The performance of our prediction depends on the accuracy of operator-reported imaging characteristics. The criteria used to evaluate the US features were subjective. However, the interobserver agreement for each feature in the present study was good. Although we did not evaluate the recurrence of PTC according to different clinical factors, our findings are still important for clinicians to make decisions on management strategies for cN0 unifocal PTC. The CLNM status is an indicator of aggressive behavior in PTC, but its evaluation has been limited in imaging studies. The present study suggested that the size, adjacent anterior capsule, distance to the lower pole and CDFI of cN0 unifocal PTC patients are good preoperative clinical factors that predict the occult CLNM status. Thus, it may be appropriate to perform more precise

inspection or surgical intervention rather than active surveillance for those patients.

In summary, the present study revealed several risk factors based on US imaging characteristics, suggesting that this easy-to-use method can be applied to facilitate preoperative individualized prediction of occult CLNM in cN0 unifocal PTC patients, which is in line with the current trend towards precision medicine.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Guangdong Provincial People's Hospital, Guangdong Academy of Medical Sciences (Guangzhou, Guangdong Province, People's Republic of China). Written informed consent was not required for this study, in accordance with the local legislation and institutional requirements.

AUTHOR CONTRIBUTIONS

YL, JH, and ZZ, have contributed equally to this work and share first authorship. They are responsible for research design, data collecting, analysis and writing. YH and JD are responsible for research design and data collecting. SW and ZW are responsible for research design, data analysis. All authors contributed to the article and approved the submitted version.

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Vascular Endothelial Growth Factor Receptor Inhibitors in Chinese Patients With Advanced Radioactive Iodine-Refractory Differentiated Thyroid Cancer: A Network Meta-Analysis and Cost-Effectiveness Analysis

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Introduction: Two targeted drugs (apatinib and lenvatinib) show clinical efficacy in first-line treatment of Chinese patients with radioactive advanced iodine-refractory differentiated thyroid cancer (RAIR-DTC) and are recommended by the Chinese Society of Clinical Oncology guidelines. Considering the high clinical cost of long-term vascular endothelial growth factor receptor inhibitor administration and to determine which of the two targeted drugs is preferable, we opted to conduct a cost-effectiveness analysis (CEA) and network meta-analysis (NMA).

Material and Methods: The results of NMA and CEA included in the two phase III randomized clinical trials REALITY (NCT03048877) and Study-308 (NCT02966093), in which Bayesian NMA and CEA were performed on 243 and 149 Chinese patients, respectively, were retrieved. Overall survival and progression-free survival (PFS) for apatinib versus lenvatinib were determined by NMA. CEA involved the development of a 20-year Markov model to obtain the total cost and quality-adjusted life-years (QALYs), and this was followed by sensitivity and subgroup analyses.

Results: Compared with lenvatinib, apatinib therapy provided a 0.837 improvement in QALY and $6,975 reduction in costs. The hazard ratio of apatinib versus lenvatinib and the cost of the targeted drugs had a significant impact on the model. According to the sensitivity analysis, apatinib was more cost-effective and had no correlation with willingness-to-pay in China. Subgroup analysis showed that apatinib maintained PFS more economically.

Conclusion: NMA and CEA demonstrated that apatinib was more cost-effective compared to lenvatinib in the first-line treatment of Chinese RAIR-DTC patients.

Keywords: advanced radioactive iodine-refractory differentiated thyroid cancer, apatinib, lenvatinib, cost-effectiveness analysis, network meta-analysis

INTRODUCTION

Thyroid cancer (TC) is the tenth most common cancer worldwide, with more than 580,000 new cases diagnosed and more than 43,000 deaths (1). More than 190,000 new cases have been reported in China (1). Differentiated TC (DTC) is the most prevalent, accounting for more than 90% of all TCs (2). The probability of recurrence or metastasis disease was close to 60% (3). Radioactive iodine therapy was the primary treatment for patients with advanced DTC, but 30% of patients become radioactive iodine-refractory (RAIR) cancers (4, 5), which have a 10-year survival rate of 10% (6).

Two phase III trials, DECISION (NCT00984282) and SELECT (NCT01321554), demonstrated that sorafenib and lenvatinib extend progression-free survival (PFS) by a significant 10.3 months and 18.3 months, respectively, compared with placebo in the whole population of RAIR-DTC patients (7, 8). Subsequently, sorafenib and lenvatinib have been approved by Chinese Society of Clinical Oncology (CSCO) and were added to their guidelines in 2017 and 2020, respectively, as standard treatments for Chinese patients with RAIR-DTC (9). Unfortunately, no significant increase in overall survival (OS) was observed in either study, which included populations from multiple countries (7, 8). Therefore, as China is a country of major RAIR-DTC prevalence, the limited treatment regimens cannot meet the demand. Treatment strategies for Chinese patients with RAIR-DTC need to be improved and widely applied in clinical practice.

Apatinib is a small-molecule angiogenic inhibitor of vascular endothelial growth factor receptor (VEGFR)-2 of high selectivity. Lenvatinib is a multitargeted tyrosine kinase inhibitor (TKI) specific for VEGFR-1, -2, and -3. Based on Chinese people with RAIR-DTC, two studies showed the significant clinical benefits of TKIs. The REALITY trial (NCT03048877) showed that apatinib significantly extended the median PFS (22.2 months; HR, 0.26; 95% CI, 0.14–0.47; $P < 0.001$) and OS (HR, 0.42; 95% CI, 0.18–0.97; $P = 0.04$) of Chinese patients in advanced stages of RAIR-DTC compared with placebo (10). Study-308 (NCT02966093) demonstrated that lenvatinib significantly improved the median PFS (23.9 months; HR, 0.16; 95% CI, 0.10–0.26; $P < 0.0001$) of Chinese patients with RAIR-DTC compared with placebo. However, there was no significant benefit in terms of OS (HR, 0.42; 95% CI, 0.18–0.97; $P = 0.04$) (11). Because of these findings, both apatinib and lenvatinib are recommended as advanced RAIR-DTC treatment in the 2021 CSCO guidelines (9). With the remarkable results of the two Chinese-patient-based studies, the concomitant cost-effectiveness of the two TKI types has become the focus of attention. To answer this question, we compared the cost-effectiveness of apatinib and lenvatinib for patients with advanced RAIR-DTC from the perspective of Chinese payers.

METHODS

Network meta-analysis and cost-effectiveness analysis (NMA and CEA) were guided by the PRISMA NMA checklist and the Economic Assessment Report Standard Statement (CHEERS) checklist, respectively (**Supplementary Material eTables 1**, **2**).

Network Meta-Analysis

Study Selection and Assessment of Bias Risks

We searched PubMed, Embase, Cochrane, and Web of Science for compliant English-language publications up to March 15, 2022, with the search terms "PD-1", "tyrosine kinase inhibitor", "vascular endothelial growth factor receptor inhibitors", "apatinib", "lenvatinib", "radioactive iodine-refractory differentiated thyroid cancer", and "clinical trial" (**Supplementary Material eTable 3**). Abstracts from meetings of the American Society of Clinical Oncology (ASCO) and the European Society of Medical Oncology (ESMO) were also reviewed. The eligible literature met the following criteria: (1) Phase III randomized controlled trials; (2) apatinib or lenvatinib were compared for Chinese patients with advanced RAIR-DTC; (3) the outcomes were OS and PFS; (4) details of treatment strategies and treatment-related adverse events (AEs) were included. Those not meeting the inclusion criteria were disregarded. Two reviewers (Y.W.Z. and K.L.) independently screened the selected studies to avoid missing articles and extract relevant data. The bias risk assessment for these clinical trials was performed using Cochrane RevMan (version 5.4, available: https://training.cochrane.org).

Statistical Analysis

We used R software (version 4.1.1, available: http://www.rproject.org) and software package "netmeta" for Bayesian network meta-analysis to obtain the HRs of OS and PFS for apatinib versus lenvatinib. However, due to the lack of data from the two studies that can provide information for assessing the heterogeneity between the tests, we chose the method of Su et al. and adopted the fixed-effect model for analysis (12).

Cost-Effectiveness Analysis

Patients and Treatments

The model patient cohorts from REALITY and Study-308 were used to form a patient simulation cohort. The REALITY trial enrolled 46 Chinese patients with advanced RAIR-DTC who received apatinib treatment from February 17, 2017, with the

data expiration date of March 25, 2020. Study-308 enrolled 103 Chinese patients with advanced RAIR-DTC who received lenvatinib treatment from January 11, 2017, with a data cut-off date of July 31, 2019. The baseline characteristics of patients and details of medications used are presented separately in **Supplementary Material eTables 4** and **5**. We assumed that the included Chinese patients were 60 years old, 65 kg in weight, 164 cm in height, and 1.72 m^2 in body surface area (13). Patients receiving apatinib and lenvatinib were assessed with computed tomography or magnetic resonance imaging every 2 and 8 weeks, respectively (10, 11). After progression, all patients received best supportive care (BSC). Finally, each patient who died was given terminal care.

Model Construction

A Markov model was developed using TreeAge (Version TreeAge Pro 2021, https://www.treeage.com) to evaluate the cost-effectiveness of apatinib versus lenvatinib for Chinese patients with RAIR-DTC. The Markov model included three health states: PFS, disease progression (PD), and death (**Supplementary Material eFigure 2**). The clinical trial treatment protocol and follow-up protocol were applied for 2 months, and when more than 99% of patients died, the time horizon was 20 years. We extracted survival curves from REALITY and Study-30 through GetData (Version 2.26; http://www.getdata-graph-digitizer.com/index.php) and used the survival curves to extract the transition probabilities. Then, the best fitting parameter model was selected from Weibull, Gompertz, exponential, log-logistic, and log-normal distribution using the Akaike information criterion and Bayesian information criterion. After selecting the Weibull fitting parameter model, two parameters were calculated with R software: Scale (λ) and Shape (γ). More details are shown in **Supplementary Material eFigure 3** and **eTable 6**. Our main results were total cost, quality-adjusted life-years (QALYs), which is a number derived from a weighted analysis of the quality and annual health discount rates associated with a patient's annual health status, and incremental cost-effectiveness ratio (ICER), and we used a willing-to-pay (WTP) threshold of $37,653/QALY (thrice China's per-capita gross domestic product 2021) to determine cost-effectiveness. When ICER < WTP, the increased cost is completely worth it, and the treatment option have cost-effectiveness; When ICER > WTP, the increased cost is not worth it, and the intervention measures are not cost-effective.

Utility and Cost Estimates

As health utility values were reported in the two studies, the published literature was adopted, assuming that the PFS status and PD status had utility scores of 0.80 and 0.50, respectively (14, 15). The disutility of AEs was also considered (16, 17) (**Table 1**).

We only considered direct medical costs, including drug treatment, administration, BSC, terminal care, laboratory, tumor imaging, and treatment-related AEs. Drug prices were sourced from Xiangya Hospital of Central South University in China. All remaining costs were derived from the published literature (12, 16, 18–21). All prices are expressed in US dollars, using the exchange rate *$1 = ¥6.3389* (March 14, 2022). Based on our consumer price index and a discount rate of 3% per year, healthcare-related costs were inflated to 2022 values in China (22) (**Table 1**).

Sensitivity Analyses

Sensitivity analyses were applied to resolve uncertainties in the model. One-way sensitivity analysis included relevant parameters and 20% variation ranges, and the probability sensitivity analysis involved 10,000 Monte Carlo simulations to obtain an acceptable curve (22).

We included subgroups of patients separated by age, sex, and pathological typing for analysis. We first performed network meta-analysis to obtain the HRs of existing subgroups of PFS (apatinib versus lenvatinib). Then, according to the method adopted by Dong et al., the ICER and cost-effectiveness probability of each subgroup were obtained (22).

RESULTS

Network Meta-Analysis

A total of 299 records were identified by searching major literature databases, and we eventually included two phase III randomized clinical trials (REALITY and Study-308) based on the criteria, with a total of 243 Chinese patients with advanced RAIR-DTC (**Supplementary Material eFigures 1**, **4**). In the REALITY trial, 92 patients received either apatinib or placebo. In the Study-308 trial, 151 patients were treated with lenvatinib or placebo (**Supplementary Material eTable 7**). NMA showed that the HRs of OS and PFS for apatinib compared with lenvatinib were 0.50 (95% CI, 0.16–1.57) and 1.63 (95% CI, 0.75–3.51), respectively (**Table 1**).

Cost-Effectiveness Analysis

Base-Case Analyses

For 149 Chinese patients with advanced RAIR-DTC, apatinib gained 5.905 QALYs at a total cost of $85,551. Apatinib regimes were accompanied by a relatively small improvement in QALY and lowered healthcare costs by $6,975 compared to lenvatinib. Hence, of the two treatment strategies, apatinib was the most efficacious and cost-effective (**Table 2**).

Sensitivity Analyses

One-way sensitivity analysis indicated that the HR for PFS (apatinib versus lenvatinib), the utility of PFS with apatinib, and the cost of TKIs were sensitivity factors for the model. The incidence of AEs had negligible effect (**Figure 1**). The cost-effectiveness acceptability curve demonstrated that the apatinib strategy was consistently cost-effective, regardless of WTP (**Figure 2**). Among all the included subgroups, apatinib performed better in prolonging survival, with an increase in the QALYs for apatinib versus lenvatinib ranging from 0.746 to 1.002. Apatinib showed dominant cost-effectiveness for a subgroup of patients that were ≤65 years of age, male, and had papillary TC (PTC) (**Supplementary Material eTable 8**).

TABLE 1 | Model parameters: baseline values, ranges, and distributions for the sensitivity analysis.

Parameters	Baseline value	Range		Reference	Distribution
		Minimum	Maximum		
Weibull survival model of apatinib					
OS	Scale= 0.0006137, Shape= 1.8950428	–	–	(10)	–
PFS	Scale= 0.003379, Shape= 1.821018	–	–	(10)	–
Weibull survival model of lenvatinib					
OS	Scale= 0.005721, Shape= 1.283299	–	–	(11)	–
PFS	Scale= 0.022440, Shape= 1.152276	–	–	(11)	–
HR for apatinib vs lenvatinib					
OS	0.50	0.16	1.57	Network meta-analysis	–
PFS	1.63	0.75	3.57	Network meta-analysis	–
Risk for main AEs in apatinib group					
Risk of hypertension	0.348	0.277	0.415	(10)	Beta
Risk of hand-foot syndrome	0.174	0.139	0.209	(10)	Beta
Risk of proteinuria	0.152	0.121	0.182	(10)	Beta
Risk of diarrhoea	0.152	0.121	0.182	(10)	Beta
Risk of hypocalcaemia	0.065	0.052	0.078	(10)	Beta
Risk for main AEs in lenvatinib group					
Risk of hypertension	0.621	0.497	0.745	(11)	Beta
Risk of proteinuria	0.233	0.186	0.280	(11)	Beta
Risk of hand-foot syndrome	0.097	0.078	0.116	(11)	Beta
Risk of diarrhoea	0.068	0.054	0.082	(11)	Beta
Risk of platelet count decreased	0.068	0.054	0.082	(11)	Beta
Utility					
PFS	0.80	0.64	0.96	(14, 15)	Beta
PD	0.50	0.40	0.60	(14, 15)	Beta
Disutility					
Platelet count decreased	0.020	0.016	0.024	(16)	Beta
Hand-foot syndrome	0.016	0.013	0.019	(17)	Beta
Diarrhoea	0.014	0.011	0.017	(17)	Beta
Hypertension	0	NA	NA	(17)	Beta
Hypocalcaemia	-[a]	–	–	–	–
Proteinuria	-[a]	–	–	–	–
Drug cost, $/per cycle					
Apatinib	1,850	1,480	2,220	Local Charge	Gamma
Lenvatinib	5,725	4,580	6,870	Local Charge	Gamma
Cost of AEs, $					
Apatinib	10	8	12	(12, 21)	Gamma
Lenvatinib	50	40	60	(12, 16, 21)	Gamma
Administration per cycle	36	29	43	(18)	Gamma
Best supportive care per cycle	440	352	528	(18)	Gamma
Terminal care per patient	2129	1,703	2,555	(18)	Gamma
Tumor imaging per cycle	145	116	174	(19)	Gamma
Laboratory per cycle	232	186	278	(20)	Gamma
Body surface area (meters^2)	1.72	1.38	2.06	(13)	Normal
Discount rate	0.03	0	0.05	(22)	Uniform

[a]*The disutilities with regard to hypocalcaemia and proteinuria were not reported.*
OS, overall survival; PFS, progression-free survival; AEs, adverse events.

TABLE 2 | Baseline results.

Outcomes	Apatinib	Lenvatinib
QALYs	5.905	5.068
Change in cost, $[a]	0.837	
Total cost $	85,551	92,526
Change in QALYs[a]	-6,975	
ICER $/QALY	Dominated[b] (-8,333)	
WTP $/QALY	37,653	

[a]*Change in cost and change in QALYs represent the results of apatinib minus lenvatinib.*
[b]*Apatinib showed higher effectiveness and lower cost, as compared with the lenvatinib.*
ICER, incremental cost-effectiveness ratio; LY, life-year; QALY, quality-adjusted life-year; WTP, willingness-to-pay.

DISCUSSION

TC is endocrine system malignancy (23). The morbidity, mortality, and burden of the disease continue to rise worldwide (24–26). China has one of the highest TC burdens in the world, with an average cost of $11,560 per patient in the first year after diagnosis (27, 28). Because of rising healthcare costs, treatment cost evaluations are necessary. TKI is currently on the radar of clinicians and patients with RAIR-DTC. Two previous studies, DECISION (NCT00984282) and SELECT (NCT01321554), demonstrated the significant clinical efficacy of TKIs (7, 8). Therefore, three economic assessments have been published based on two major studies. Huang et al. and Tremblay et al.

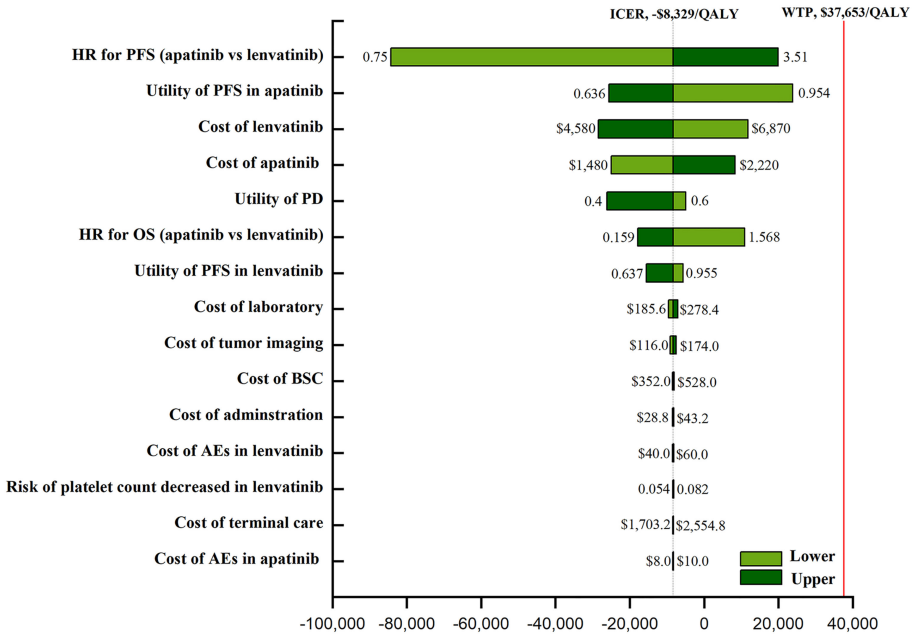

FIGURE 1 | The one-way sensitivity analyses of the apatinib vs Lenvatinib. PFS, progression-free survival; PD, disease progression; OS, overall survival; BSC, best supportive care; AEs, adverse events.

showed that the ICER generated by lenvatinib and sorafenib in patients with advanced RAIR-DTC were $103,925 per QALY and $95,695 per QALY, respectively, and that lenvatinib is cost-effective compared with sorafenib at a WTP of $150,000 per QALY and $100,000 per QALY, respectively, from the US perspective (29, 30). Leslie et al. showed lenvatinib to be more cost-effective than sorafenib (ICER = $25,275 per QALY) or placebo (ICER = $40,869 per QALY) and that sorafenib was also cost-effective compared to placebo (ICER = $64,067 per QALY) (15). The final analysis demonstrated lenvatinib to be the most cost-effective option for RAIR-DTC at a WTP of 100,000 per QALY, although both lenvatinib and sorafenib were more cost-effective than placebo (15). These studies evaluated the cost performance of the two TKIs based on the perspective of US patients and obtained consistent results that show lenvatinib may be more cost-effective. With the development of new drugs, Chinese physicians and patients are gradually paying attention to the cost and efficacy of TKIs. Therefore, using the Markov model and the clinical efficacy and safety data from two large, randomized phase III clinical trials, we estimated the cost-

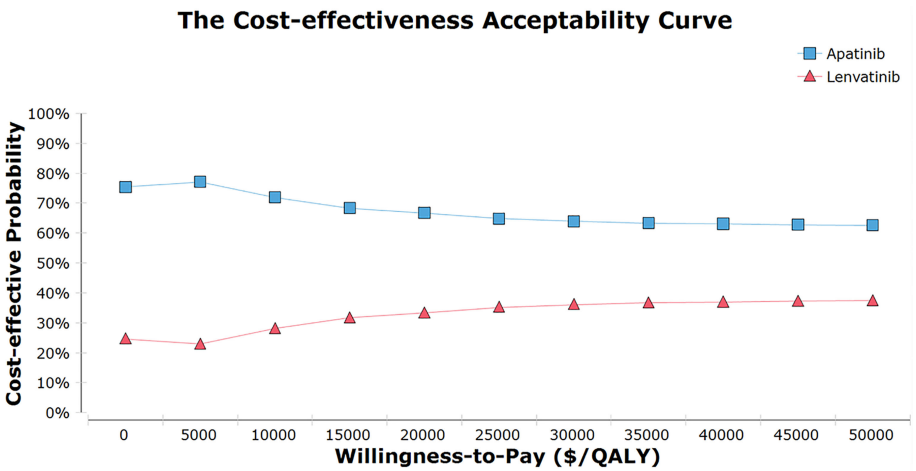

FIGURE 2 | The cost-effectiveness acceptability curves for the apatinib vs Lenvatinib. QALY, quality-adjusted life-year.

effectiveness over a 20-year time horizon for apatinib and lenvatinib as therapies for RAIR-DTC. This study of RAIR-DTC patients in China showed that apatinib therapy provided a 0.837 improvement in QALY and $6,975 reduction in costs compared with lenvatinib, resulting in ICER value is definitely lower than the WTP value. Deep meaning indicates that apatinib is a superior treatment strategy compared to lenvatinib, achieving higher efficacy as well as a lower healthcare cost. Therefore, apatinib was more cost-effective compared to lenvatinib in the first-line treatment of Chinese RAIR-DTC patients. Although, it is worth considering that the additional costs associated with lenvatinib are mainly due to follow-up, meaning that the same follow-up plan should be set up for the same cancer type.

We used sensitivity analysis to confirm model uncertainty. From the one-way sensitivity analysis, we deduced that the model's most influential parameter was the HR of PFS (apatinib versus lenvatinib), underscoring the need for robust head-to-head clinical data. It was also sensitive to the utility of PFS, and the analysis found that, for patients with a lower utility of PFS, apatinib had a more favorable economic outcomes compared with lenvatinib, but apatinib had a worse economic outcome for patients with a higher utility. Another important influencing variable was the price of the TKIs: apatinib's price increase of more than 56% and lenvatinib's price decrease of more than 46% mean lenvatinib is more cost-effective. Changing other parameters had virtually no influence on our results, and due to the high cost of TKIs, reducing the prices of apatinib and lenvatinib was considered the most practical measure in the context of cost-effectiveness and optimal logistics.

The results were consistent with baseline in the subgroup analysis, showing that the ICER (apatinib versus lenvatinib) was lower than the WTP. In the sensitivity analysis, apatinib was more cost-effective than lenvatinib 55%. It is worth noting that in the subgroups of Chinese patients of ≤65 years, male, and PTC, apatinib generation was associated with a higher efficacy at a lower cost. Changes in the QALYs of PFS in PTC were widely observed, leading to the possibility that TKI was more effective among many pathological types of PTC. This is consistent with the results of two previous retrospective studies, the multivariate analysis of which identified histological grade as a favorable prognostic factor. Lars et al. reviewed 173 patients with PTC, and their analysis showed that the 10-year survival rates of patients with low-and high-grade PTC were 95.3% and 75.1%, respectively (31). Allen et al. reviewed 37,858 cases of PTC and found the prognosis of moderately differentiated and poorly differentiated PTC was positively correlated with OS compared with highly differentiated PTC (HR, 1.21; 95% CI, 1.04-1.41; P = 0.02 and HR, 2.62; 95%CI, 2.23-3.08, P < 0.001) (32). Therefore, under the influence of cost-effectiveness, pathological typing to predict treatment prognosis needs to be performed.

In China, those approving innovative drugs to maintain our healthcare system should not only take into account the huge clinical and economic benefits but also realize the importance of prognostic marker biomarker analysis. Unfortunately, we did not analyze predictive markers. However, studies have found that thyroglobulin (Tg) and anti-thyroglobulin (TgAb) are important prognostic factors for guiding clinical treatment and are valuable parameters for long-term monitoring of DTC patients. Patients with Tg of <0.2 ng/mL or thyroid-stimulating hormone-stimulated Tg of <1 ng/mL responded well to treatment and had minimal levels of recurrence and an almost complete absence of disease-specific deaths (33–36). However, patients with higher than normal Tg levels (inhibition of Tg of ≥1 ng/mL or stimulation of Tg of ≥10 ng/mL) or elevated TgAb values after treatment had a low mortality rate, but a significant proportion of this group developed structural disease recurrence (33, 37). A prospective study involving serum from 249 patients showed that VEGF and VEGFR may have prognostic value for RAIR-DTC (38). Levels of VEGF showed a clear link to a lower risk of recurrence (overall response [OR], 0.08; 95% CI, 0.01-1.43; P = 0.018 and OR, 0.08; 95% CI, 0.01-1.37; P = 0.016) (38). In addition, molecular markers such as BRAF, EGFR, Ki67, and P53 were also potentially effective prognostic factors (39–42).

This study had several significant advantages. Firstly, we ascertained the cost-effectiveness of two TKI-based RAIA-DTC treatments over a 20-year time range using an economic model. As input for our model, clinical efficacy and safety data were extracted from high-quality phase III clinical trial datasets, and the costs were from the perspective of Chinese payers. We concluded that our model provides long-term cost and effectiveness predictions that are easily translatable to clinical practice. Secondly, we considered the disutility generated by AEs. We used the average health utility of PFS in patients with advanced RAIR-DTC and corrected it using the disutility generated by AEs. When evaluating the economic benefits of the two TKIs, only the negative utility generated by severe AEs had a strong correlation with the quality of life (QoL) (43). Finally, we compared the cost-effectiveness of the two TKIs and added three subgroups that might be useful in clinical practice.

The study had some limitations. Firstly, the survival data were obtained from the interim analysis results of the phase III clinical trials REALITY and Study-308. The survival data will mature with the extension of follow-up time, and the model will become more stable. Secondly, these two TKI schemes were not directly evaluated in any of the trials. Therefore, we compared the two TKIs schemes indirectly using the NMA findings from two phase III clinical trials with similar research content and characteristics of the included patients. However, this method comes with potential uncertainties. Thirdly, to simplify the calculation, we assumed that patients in both groups only received BSC after treatment with TKIs for recurrence, so the analysis may have underestimated the cost of PD. However, we discovered from the sensitivity analysis that the economic burden of PD had little effect on the model's outcomes. Fourthly, since neither the REALITY nor Study-308 reports provided QoL data, the health state utility in this model was obtained from previously published data and was based on patients in the US or UK. As the QoL of Chinese patients with RAIR-DTC has not yet been reported, this was an essential deviation. Including the QoL of Chinese patients in future studies means the economic results will be updated in time. Fifthly, because of the lack of subgroup survival curves in both studies, we were unable to run a complete model for each subgroup, and the original group equilibrium generated by NMA analysis may not apply to the subgroups. Therefore, the results of the subgroup analysis should be interpreted with caution. Finally, in this model, we

only considered the cost and corresponding disutility of treatment-related grade 3/4 and ≥5% AEs, which may have had some influence on the overall cost and utility. However, sensitivity analysis showed that the incidence and disutility of major AEs had little effect on the results.

CONCLUSIONS

In this network meta-analysis and cost-effectiveness analysis, apatinib was a more desirable treatment strategy than lenvatinib for Chinese patients with RAIR-DTC at any WTP threshold. Innovative therapies that provide significant results are pivotal, and lowering the prices of these drugs is critical to achieving their cost-effectiveness. Therefore, apatinib presents a new treatment option with an optimal cost-effective ratio for RAIR-DTC patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

YZ, KL, KW, and LP performed the experiments. YZ and KL analyzed the data. LP contributed materials and analysis tools. YZ, KL, KW, and LP wrote the manuscript. YZ and KL contributed equally. YZ, KL, KW, and LP approved the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.909333/full#supplementary-material

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