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Differential diagnoses of thyroid neoplasms: Molecular and histological features and the impact on follow-up

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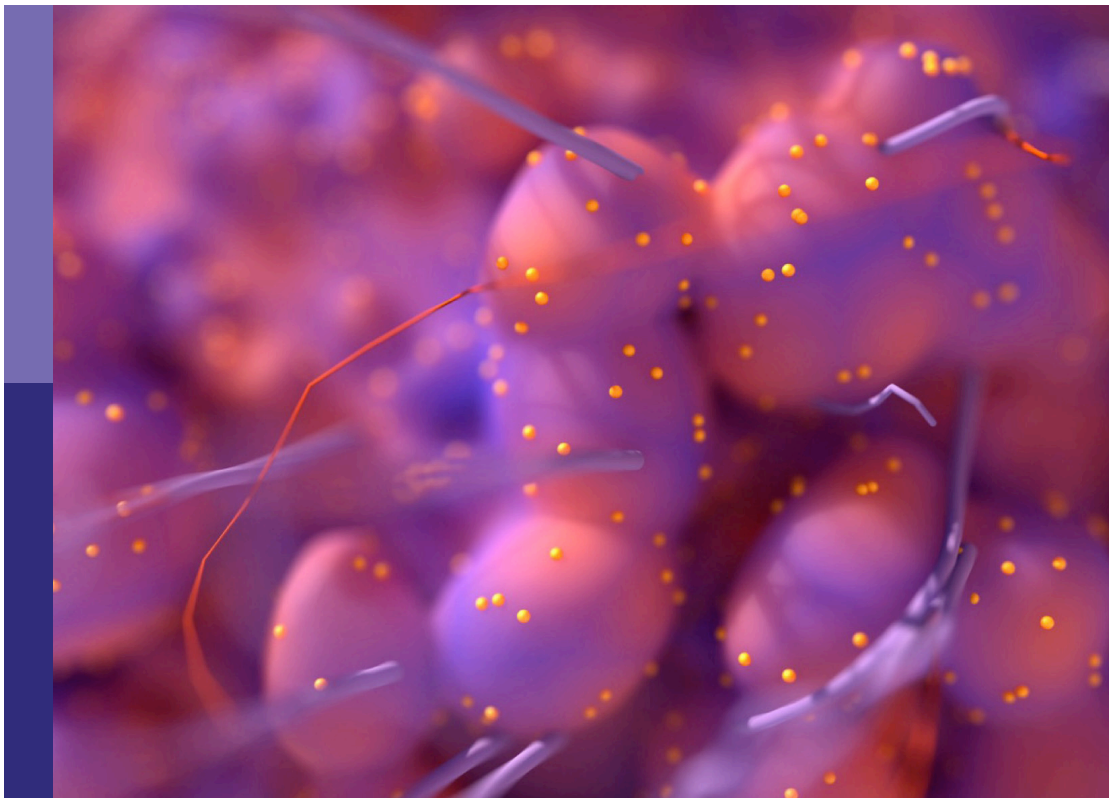

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Differential diagnoses of thyroid neoplasms: Molecular and histological features and the impact on follow-up

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Editorial: Differential diagnoses of thyroid neoplasms: Molecular and histological features and the impact on follow-up

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Editorial on the Research Topic
Differential diagnoses of thyroid neoplasms: Molecular and histological features and the impact on follow-up

Thyroid carcinoma (TC) is among the most frequent cancers in women (1–3). Its annual incidence increased over the last decades, mainly due to the improved ability to diagnose malignant transformation in small non-palpable thyroid nodules, to decline in more recent years in both sexes at a combined rate of 2.5% per year (1). Most of the epithelial TC are denoted as differentiated TC (DTC), including the papillary TC (PTC) and the follicular TC (FTC) histotypes, which, following dedifferentiation, are thought to give rise to the more aggressive poorly differentiated thyroid carcinoma (PDTC), and the incurable anaplastic TC (ATC) (4). Although derived from the same cell type, the different TC show specific histological features, biological activities and degree of differentiation due to different genetic alterations. Recently, multiple aspects of the clinical management of patients affected by thyroid nodules or thyroid malignancies, including diagnosis, treatment modalities and follow-up, are rapidly changing with the aim to resolve the still present clinical uncertainties (5–8).

Over the last years, a great advance in the comprehension of the molecular pathogenesis underlying TC progression, particularly in PTC (representing the most common thyroid malignancy), has led to a new classification of thyroid lesions into molecular subtypes with potential positive impact on the diagnostic accuracy of thyroid lesions, prediction of disease outcome, and patient's tailored therapy (9–11). In addition, ultrasound (US) assessment of thyroid parenchyma has witnessed over the last decade a dramatic improvement with the introduction of new US software, such as contrast-enhanced US and US-elastography (USE), now recognized by the World Federation for Ultrasound in Medicine and Biology (WFUMB) and the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) as an essential part of thyroid nodule US examination (12–16). Moreover, the introduction of minimally invasive or remote-access surgical approaches for tumor ablation, design of small molecules inhibitors for the treatment of more aggressive TC, and a patient's tailored follow up led to better disease outcomes and improved patient's quality of life (17–21).

In the present issue, new insights into molecular pathogenesis, diagnosis, therapy and follow-up of TC patients are presented. Zhao et al. reported on the clinical significance of the co-stimulatory molecule B7-H3 expression in PTC. They showed that the level of B7-H3 could represent an independent biomarker for predicting lymph node metastases and disease recurrences in PTC patients, thus providing a new potential molecular parameter to refine risk-adapted therapeutic strategies, and also a putative novel drug target for patients affected by aggressive disease (22).

The autoimmune Hashimoto's thyroiditis has been frequently shown to associate with DTC, but the liaison amongst these two clinical entities has yet to be elucidated. In this issue, Cappellacci et al. reported their single center experience on the incidence of Hashimoto thyroiditis in DTC patients, and assessed how this autoimmune disorder influences the risk of cancer recurrence (23).

The observation that both benign and malignant thyroid diseases (TD) frequently associate with extra-thyroidal malignancies has raised a considerable clinical interest (22–25). In particular, this relationship suggests: *i*) the need of an increased surveillance of TD patients at higher risk of developing extra-thyroidal malignancies; *ii*) the presence of common underlying molecular mechanism(s) responsible for these diseases, the comprehension of which could lead to a better and possibly personalized management of patients (22–25). In this context, Bellini et al. made a systematic review of the current evidence on the bidirectional relationship between thyroid and renal cancers. The authors showed that obesity and family history were the utmost common risk factors, and that genetic susceptibility was also present.

As above mentioned, USE is becoming an essential tool in the evaluation of thyroid nodules (12–16). Cantisani et al. performed a systematic review and a meta-analysis including 72 studies for a total of 13,505 patients and 14,015 thyroid nodules. In this study, the authors compared the diagnostic performances of qualitative, semi-quantitative and quantitative USE, and found that qualitative and semi-quantitative USE had very similar diagnostic accuracy, and that both of them were superior to the quantitative USE, with pooled sensitivity, specificity and AUC of 84%, 81%, and 0.89 respectively for qualitative USE, and 83%, 80%, and 0.93 for semi-quantitative USE. These data corroborate the valuable diagnostic role of USE in thyroid nodule evaluation, in accordance with the above reported guidelines from WFUMB and EFSUMB.

Although surgery is considered the gold standard in the treatment of papillary thyroid microcarcinoma (PTmC), active surveillance has become, over the last few years, an alternative option for PTmC patients. This approach was initially implemented in Japan to be then applied in other countries, but at present it has not yet been validated by the major Western Scientific Societies. In the work by Orlando et al. here reported, the results of nine studies published from 2017 to 2020 on this subject were analyzed. The authors concluded that, although data from western countries are still limited, active surveillance of PTmC appears to be a feasible strategy deserving further studies to confirm its usefulness in the clinical management of these patients. On the other hand, surgery is fundamental for the treatment of DTC and their more advanced forms (5). For the latter, however, no clear and specific guidelines have been drawn up to date. In the present issue, Bulfamante et al. retrospectively analyzed 111 patients with advanced DTCs, investigating the rate of radical excision, peri-procedural and post-procedural complications, quality of life, persistence, recurrence rates, and survival rates. The results were compared with those reported in the literature.

Among TC, ATC is a rare but highly aggressive and incurable form. Although some information on its molecular pathogenesis is available, little is known about risk factors. In their manuscript, Graceffa et al. reviewed the literature concerning risk factors for ATC, and examined analogous data from their own database. They found that ATC, in addition to being peculiar of elderly people, has a higher prevalence in individuals with a low level of education and a long history of multinodular goiter.

As previously stated, the increased comprehension of the genomic landscape of TC, and the possibility of identifying genetic alterations underlying more advanced diseases paved the way for a personalized therapy tailored on single patient characteristics. The last two manuscripts included in this special issue deal with the targeted therapies in patients with advanced DTC, PDTC and ATC (33, 34). In the first manuscript, Elia et al. describe a number of drugs approved by the Food and Drug Administration (FDA) for the therapy of the more aggressive and radioiodine (RAI)-resistant DTC and medullary thyroid cancer. In the second manuscript Macerola et al., besides providing a comprehensive review of the currently available targeted treatments for TC, describe the related predictive markers and testing methodologies.

In conclusions, the articles contained in the present special issue offer valuable information related to the clinical management of TC patients, which we hope will meet the interest of Frontiers in Oncology readers.

Author contributions

All authors contributed to the initial draft of the manuscript, its revisions and all approved the final submitted version.

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This editorial is dedicated to our colleague Prof. Antonio Catania, a gentleman and passionate surgeon who passed away prematurely in October of this year.

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.

Publisher's note

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SYSTEMATIC REVIEW
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US-Elastography With Different Techniques for Thyroid Nodule Characterization: Systematic Review and Meta-analysis

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Background: Thyroid nodules are frequent in adult population and thyroid cancer incidence has increased dramatically over the past three decades. The aim of this systematic review and meta-analysis was to evaluate the US-Elastosonography (USE) diagnostic performance in assessing the thyroid nodules malignancy risk.

Methods: PubMed and Embase databases were searched from January 2011 to July 2021. We extracted data from selected studies and calculated the overall diagnostic accuracy of qualitative USE, semi-quantitative USE and quantitative USE. Summary receiver operating characteristic (ROC) curve was elaborated to show the results. All statistical tests were performed using Metadisc and Medcal software package.

Results: Finally 72 studies with 13,505 patients and 14,015 thyroid nodules (33% malignant) undergoing elastography were included. The pooled sensitivity, specificity and AUC were 84%, 81%, and 0.89 respectively for qualitative USE; 83%, 80%, and 0.93 for semi-quantitative USE and 78%, 81% and 0.87, for quantitative USE. The qualitative and semiquantitative USE present very similar diagnostic accuracy values and both better than the quantitative USE.

Conclusions: USE is a useful imaging tool for thyroid nodule characterization. In accordance with recent guidelines and meta-analyses, the USE could be used daily in thyroid nodule malignancy risk stratification.

Systematic Review Registration: PROSPERO: CRD42021279257.

Keywords: thyroid, USE, shear wave elastography, strain elastography, meta-analysis, real-time elastography

INTRODUCTION

Thyroid nodules are frequent in adult population up to 60%, with a prevalence of cancer as 5% (1, 2). Since the incidence of thyroid cancer has mostly increased in the last decade (3, 4) the initial assessment of these patients is a hot topic and ultrasound (US) represents the first line imaging modality in this context. In fact, the US features such as micro- or macrocalcifications, marked hypo echogenicity, taller than wide shape, and thick irregular or lobulated margins are recognized as associated with malignancy (5), but they are not highly predictive: US sensitivity and specificity have high variability ranging between 52 and 97% and 26.6 and 83%, respectively. In addition, low reproducibility and operator-depending performance might reduce US diagnostic value. Thus, the only US images are suboptimal to actually diagnose a thyroid cancer.

To reduce or delete these limitations, several Thyroid Imaging Reporting and Data Systems (TIRADS) (6–9) have recently been proposed as a tool for uniform reporting and consistent evaluation.

This risk stratification should guide the indication for fine needle aspiration biopsy (FNAB) that is required when a suspicious nodule is identified, with normal thyroid stimulating hormone. FNAB presents a specificity of 60 to 98% and sensitivities from 54 to 90% (10, 11), so it is not such an accurate exam. In fact, non-diagnostic and indeterminate responses are common (12–15). Consequentially, on one hand, a significant number of patients have to repeat the procedure with incremented costs and on the other hand, some patients could receive unnecessary thyroid surgery, more for diagnostic than for therapeutic purposes. Considering these points and the known risks of thyroid surgery, improving the techniques for thyroid nodules diagnosis is mandatory. Among the different techniques, in the present paper we will address the role of Ultrasound elastography (USE) for thyroid characterization. Based on the fact that a suspicious nodule is at palpatory firm or hard in consistency, stiffness was adopted as indicator of malignancy for elastography (16, 17).

In this way, USE was utilized, and by the beginning encouraged literature data were obtained and as a consequence it was suggested very soon as an additional tool for thyroid nodule differentiation, in combination with conventional US and FNAB (18, 19).

Consequently, USE methods have been incorporated into international guidelines published by the WFUMB (World Federation for Ultrasound in Medicine and Biology) (20) and the EFSUMB (European Federation of Societies for Ultrasound in Medicine and Biology) (21); in the above-mentioned Guidelines, technical details, advantages and limitations for strain elastography (SE) and quantitative 2 D ultrasound shear wave elastography (SWE) have extensively been reported.

However, technology improvements, and open issues already reported by guidelines were reported to be addressed. To the best of our knowledge, presently, few studies have investigated the diagnostic performance of various thyroid Ultrasound elastography (UE) methods as applied in the clinical context and shown variable results.

Hence, this present, updated systematic review (registered in the international prospective register of systematic reviews PROSPERO: CRD42021279257) and meta-analysis assesses and summarizes current evidence on the diagnostic performance of various thyroid USE software in differentiating benign and malignant thyroid nodules.

MATERIALS AND METHODS

Literature Search

The following electronic databases were searched: PubMed and EMBASE.

The search strategy was based on the PICOS framework to identify search key words relating to the population, intervention, and outcomes in the different databases. The search concepts were: 1. Thyroid nodule AND 2. ultrasound 3. elastography OR elastosonography 4. SWE OR Shear wave elastography, 5. Strain 6. ARFI OR acoustic radiation force, and their related terms as MeSH terms, keywords and/or EmTree terms.

The search was conducted between January 2011 and July 2021 and only in English language.

Inclusion and Exclusion Criteria

From all retrieved references, duplicates were eliminated and the remaining records were screened.

All references identified were independently assessed by two authors, first by means of title and abstract, then by the review of the complete paper.

All the studies analyzed had to meet the following criteria: 1. The study involved only human subjects; 2. The study investigated the diagnostic performance of USE techniques as Strain and Shear wave for differentiation of benign and malignant thyroid nodules in a clinical setting; 3. Use of an appropriate reference standard (FNAC or histopathology); and 4. Diagnostic performance outcomes of interest were reported in terms of sensitivity, specificity, negative predictive values (NPV), positive predictive values (PPV), diagnostic accuracy, and/or area under receiver operator characteristic curve ROC curve (AUROC).

Exclusion criteria were: 1. Case reports, editorial letters, or commentaries; 2. Studies that included less of 50 thyroid nodules 3. Non-English; and 4. Insufficient diagnostic accuracy outcomes and studies without values of sensitivity, specificity, NPV and PPV; 5. paper related to specific categories such as Indeterminate nodules at Cytology.

Data Extraction

Two independent readers extracted the data in a pre-specified form. For each article, the following data were extracted: bibliographic data, type of study, type of setting, number of patients, demographic/clinical data (age, type of lesions, percentage of men and women), and number of nodules and prevalence of malignant nodules. Furthermore, for each USE techniques true (TP) and false

positive (FP), true (TN) and false negative (FN) were retrieved or calculated from sensitivity/specificity.

Quality Assessment

The quality of the studies included in the meta-analysis was assessed with a checklist based on the Quality Assessment for Studies of Diagnostic Accuracy (QUADAS 2) tool (22). Two investigators performed a quality assessment of the included studies independently, and disagreements were resolved by discussion.

Data Analysis Approach

The statistical pooling of test accuracy studies presents an added level of complexity as accuracy is usually quantified by two related statistics (sensitivity and specificity) rather than one, and meta-analysis must allow for the trade-off between the two. Positive and negative likelihood ratios (LRs) (that allow for this trade-off) were pooled with weighted averages applied, in which the weight of each study was its sample size. For each pooled estimate, a 95% confidence interval (CI) was calculated using random effects model. Positive and negative LRs (representing likelihood of malignancy in case of positive or negative results of index USE technique) could be interpreted as in **Table 1** (23).

A symmetric summary receiver operating characteristic (ROC) curve, as described by Moses et al. (24) was constructed to summarize the results; the area under this curve (AUC) was calculated.

Study heterogeneity was assessed by the I^2 index, which describes the percentage of total variation across studies that is due to heterogeneity rather than chance. A value of greater than 50% may be considered indicative of significant heterogeneity.

AUCs were compared with a z-test of the ratio between difference of AUC and square root of the variance of the difference (25).

Furthermore, a sub-analysis regarding prospective and retrospective papers was carried out.

All statistical tests were performed using Metadisc (26) and Medcal (27) software package.

RESULTS

We retrieved 437 records (113 in PubMed and 324 in Embase) that were 353 after removing the duplicates; of them 72 full-text were carefully examined and all of them, from whom TP, FP FN and TN were retrievable for single USE techniques, were included in meta-analysis. Quality of studies was generally high. Mean age of the 13,505 patients was 46 years; mean percentage of men was 24%.

The total thyroid nodules included in our study was 14,015.

A high malignancy rate (33%) was observed compared to the general population and with a pooled malignancy of 32% for qualitative USE, 29% for semi-quantitative USE and 33% for quantitative USE. The pooled sensitivity, specificity and AUC were 84% (95% confidence interval (CI), 0.83–0.85), 81% (95% CI, 0.80–0.82) and 0.89 (95% CI, 0.87–0.91) respectively for qualitative USE; 83% (95% CI, 0.81–0.84), 80% (95% CI, 0.79–0.82) and 0.93 (95% CI, 0.91–0.95) respectively for semi-quantitative USE and 78% (95% CI, 0.76–0.79), 81% (95% CI, 0.80–0.82) and 0.87 (95% CI, 0.86–0.88), respectively for quantitative USE. The positive likelihood ratios (PLR) and negative likelihood ratios (NLR) were 4.7 (95% CI, 3.5–6.3) and 0.24 (95% CI, 0.17–0.34) for qualitative USE; 6.5 (95% CI, 4.2–10.1) and 0.19 (95% CI, 0.13–0.27) for semi-quantitative USE; 4.4 (95% CI, 3.6–5.5) and 0.28 (95% CI, 0.24–0.33) for quantitative USE.

The results are synthesized in **Tables 2**–**4** and **Figures 1**–**3**.

Furthermore, a sub-analysis regarding prospective and retrospective works was carried out (**Tables 5**, **6**) showing followings results:

- from the prospective studies analysis resulted a pooled sensitivity, specificity and AUC of 74.4%, 82.3%, and 0.87 respectively for qualitative USE; 83.9%, 87.8%, and 0.94 respectively for semi-quantitative USE and finally, 78.0%, 80.9%, and 0.88 respectively for quantitative USE;
- from the retrospective studies analysis resulted a pooled sensitivity, specificity and AUC of 89.0%, 79.7%, and 0.92 respectively for qualitative USE and 78.7%, 81.8%, and 0.87 respectively for quantitative USE.

The retrospective papers analysis on semi-quantitative USE was not carried out due to the few papers.

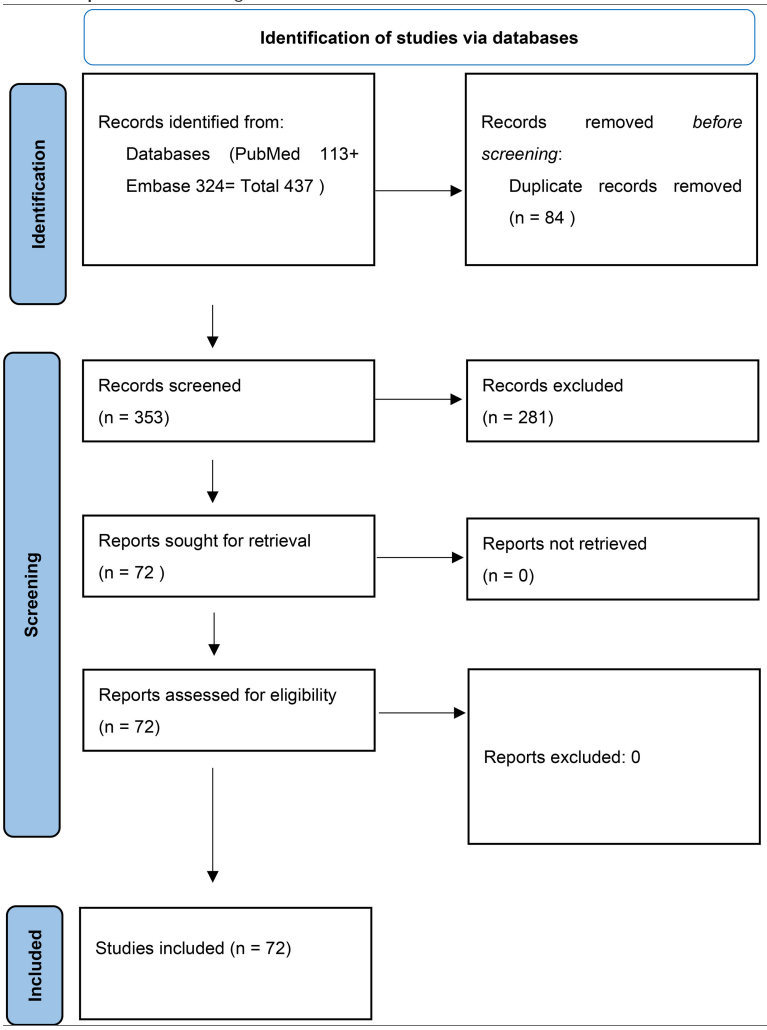
The area under the SROC curve was higher than 90% only for semi-quantitative USE ($p = 0.19$ for semi-quantitative USE vs qualitative USE; $p = 0.41$ for quantitative USE vs qualitative USE; $p = 0.01$ quantitative USE vs semi-quantitative USE).

The USE techniques with higher PLR (according to **Table 1** classification could be judged as useful) and lower NLR

TABLE 1 | Interpretation of the LR.

LR	Effect on Post-test Probability of Disease	Comment
Values between 0 and 1 decrease the probability of disease		
0.1	Large decrease	Conclusive
0.2	Moderate decrease	Useful
0.5	Slight decrease	Moderately useful
1	None	Not useful
Values >1 increase the probability of disease		
1	None	Not useful
2	Slight increase	Moderately useful
5	Moderate increase	Useful
10	Large increase	Conclusive

TABLE 2 | PRISMA flow diagram of articles included.



(according to **Table 1** classification could be judged as useful) was the semi-quantitative USE. Regarding the single dimensions of accuracy, the pooled specificity is equal among USE techniques while sensitivity is lower in quantitative USE than in strain elastography.

The qualitative and semiquantitative USE present very similar diagnostic accuracy values but both better than the quantitative USE. In particular, semi-quantitative USE AUC was statistically higher than quantitative USE one (p-value <0.05).

DISCUSSION

In addition to the clinical-laboratory evaluations, the clinical–therapeutic management of thyroid nodule is based on the ultrasound examination, which is the preferred thyroid imaging modality due to its non-invasiveness, wide availability and low cost. Several ultrasound features are used to classify thyroid nodules, each of them carrying a more or less high risk of malignancy (84). Trying to standardize the ultrasound estimate of thyroid nodules malignancy risk, it was introduce a risk-score called TIRADS (8, 19, 85, 86). The TIRADS lexicon is based on echo structure (solid, mixed or cystic), echogenicity (hyper, iso, hypoechoic or markedly hypoechoic), margins (regular, microlobulated; irregular/spiculate), internal components (micro or macro calcifications; cystic areas), and the shape [oval; taller than wide (87)] on ultrasound evaluation.

The main advantage of the routine TIRADS use is to identify with a great accuracy suspected thyroid nodules worthy of cytological investigation (88) and to exclude those not deserving at that time, thus reducing the total number and costs of FNA procedures (88, 89). However, TIRADS have limitations: there are many and different TIRADS with similar but non-overlapping classifications, accuracy is far less than 100% they are rarely used in real-life practice [in about 27.2% of the Italian reports (90)]. Therefore, fine-needle aspiration cytology (FNAC) still represents the gold-standard technique for classification of thyroid nodules, due to its high specificity (60–98%) to identify malignant thyroid nodules, but with variable sensitivity (54–90%) (10–14, 91).

In the last decades, many studies and meta-analyzes have demonstrated the effectiveness of new ultrasound techniques such as CEUS (contrast ultrasound) and, above all, USE (US-

TABLE 3 | Included studies details.

First author	Year	Elastography	Estimate of sensitivity	Estimates of specificity	Prospective	Retrospective
Chong Y. (28)	2013	qualitative USE	66%	75%	x	
		semi-quantitative USE	66%	54%		
Cantisani V. (29)	2013	semi-quantitative USE	93%	92%	x	
Refaat R. (30)	2014	qualitative USE	79%	81%	x	
		semi-quantitative USE	86%	90%		
Tatar I.G. (31)	2014	semi-quantitative USE	100%	76%	x	
Grazhdani H. (32)	2014	quantitative USE	91%	75%	x	
Mona A. (33)	2014	semi-quantitative USE	88%	86%	x	
Jun-Mei X. (34)	2014	qualitative USE	74%	91%		x
		quantitative USE	68%	77%		
Cakir B. (35)	2014	semi-quantitative USE	99%	96%	x	
Bederina E.L. (36)	2014	qualitative USE	95%	98%	x	
Erman Çakal. (37)	2014	qualitative USE	76%	96%	x	
		semi-quantitative USE	83	95		
Cantisani V. (38)	2015	semi-quantitative USE	92%	93%	x	
Huang X. (39)	2015	qualitative USE	74%	90%		x
		quantitative USE	82%	77%		
		quantitative USE	74%	90%		
Azizi G. (40)	2015	quantitative USE	79%	72%	x	
Cetin N. (41)	2015	semi-quantitative USE	56%	86%	x	
		qualitative USE	75%	81%		
Dobruch-Sobczak K. (42)	2016	quantitative USE	60%	70%	x	
Duan S.B. (43)	2016	quantitative USE	84%	78%	x	
Friedrich-Rust M. (44)	2016	qualitative USE	56%	81%	x	
		semi-quantitative USE	58%	78%		
Ping X. (45)	2016	quantitative USE	81%,	74%	x	
Seong M. (46)	2016	qualitative USE	29%	77%		x
		semi-quantitative USE	50%	57%		
Chen B.D. (47)	2016	quantitative USE	85%	87%		x
Ahmed E.E. (48)	2017	qualitative USE	83%	91%,	x	
Mohammed M.D. (49)	2017	qualitative USE	94%	77%	x	
Liu B.J. (50)	2017	quantitative USE	77%	80%	x	
Liu Z. (51)	2017	quantitative USE	81%	83%		x
Wang D. (52)	2017	quantitative USE	70%	81%		x
Kyriakidou G. (53)	2018	qualitative USE	73%	73%	x	
		quantitative USE	91%	79%		
		quantitative USE	73%	67%		
Wahab S. (54)	2018	qualitative USE	97%	83%	x	
Cantisani V. (55)	2019	semi-quantitative USE	83%	93%	x	
		quantitative USE	67%	83%		
Huang Y. (56)	2019	qualitative USE	80%	57%	x	
Yang Q. (57)	2019	quantitative USE	73%	85%	x	
		semi-quantitative USE	82%	88%		
Aghaghazvini L. (58)	2020	quantitative USE	90%	79%	x	
Li H. (59)	2020	qualitative USE	92%	61%		x
		semi-quantitative USE	81%	50%		
Huang S.T. (60)	2020	quantitative USE	69%	91%		x
Goel S. (61)	2020	quantitative USE	75%,	96%	x	
Shufang P. (62)	2020	qualitative USE	74%	81%		x
		qualitative USE	90%	92%		
		qualitative USE	94%	94%		
		qualitative USE	79%	96%		
Yavuz A. (63)	2020	quantitative USE	81%	94%	x	
Jinru Y. (64)	2017	qualitative USE	90%	86%		x
		semi-quantitative USE	90%	93%		
Yeon E.K. (65)	2020	quantitative USE	93%	30%		x
Tuan P.A. (66)	2020	quantitative USE	74%	90%	x	
Hu L. (67)	2021	quantitative USE	77%	65%	x	
Cantisani V. (68)	2012	semi-quantitative USE	97%	92%	x	
Cantisani V. (69)	2013	semi-quantitative USE	93%	89%	x	
Sohail S. (70)	2020	quantitative USE	81%	92%	x	
Idrees A. (71)	2020	semi-quantitative USE	90%	90%		x
Liao L.J. (72)	2019	qualitative USE	81%	70%	x	

(Continued)

TABLE 3 | Continued

First author	Year	Elastography	Estimate of sensitivity	Estimates of specificity	Prospective	Retrospective
		quantitative USE	81%	65%		
Fukuhara T. (73)	2018	qualitative USE	64%	66%	x	
		quantitative USE	80%	86%		
Wojtaszek-Nowicka M. (74)	2017	semi-quantitative USE	86%	88%	x	
Kim H. (75)	2013	quantitative USE	67%	72%		x
Wang H. (76)	2013	qualitative USE	85%	78%	x	
		semi-quantitative USE	81%	91%		
Wang H.L. (77)	2012	qualitative USE	78%	80%	x	
		semi-quantitative USE	88%	92%		
Veyrieres J.B. (78)	2012	quantitative USE	80%	90%	x	
Ning C.P. (79)	2012	qualitative USE	81%	72%	x	
		semi-quantitative USE	81%	84%		
Cakir B. (80)	2011	qualitative USE	58%	71%	x	
Hakan B. (81)	2021	quantitative USE	96%	95%	x	
Chen M. (82)	2016	quantitative USE	85%	84%		x
Liu B.X. (83)	2014	qualitative USE	79%	84%		x
		quantitative USE	68%	87%		

USE, Ultrasound Elastography.

Elastography) to improve the B-mode assessment of the thyroid nodule (38, 55, 92–100).

Recently USE was introduced in the last guidelines as an additional tool for stratifying the thyroid nodules malignancy risk, in combination with conventional US and FNA. In particular, the EFSUMB (European Federation of Societies for Ultrasound in Medicine and Biology) guidelines assert that Strain Ratio Elastography (SRE) should be part of the thyroid work-up due to its high diagnostic accuracy (92, 93).

The WFUMB guidelines (World Federation for Ultrasound in Medicine and Biology) state that both qualitative and semi-quantitative USE can be used for the evaluation of thyroid nodules and in particular qualitative USE which improves the B-mode ultrasound specificity but semi-quantitative USE is more easily learned (20). Furthermore, they state that SWE also improves the conventional US specificity, particularly in subcentimeter thyroid nodules (20).

Already several papers and meta-analyses assert that US-elastography is superior or similar to conventional ultrasound, in particular the following studies:

– in 2015, Nell et al. published a meta-analysis with 20 articles and 3,908 thyroid nodules assessed by qualitative USE using Asteria elastography (ES) classification. They showed a sensitivity and specificity of 85 and 80% respectively, using an elasticity score threshold between 2 and 3, and a sensitivity and specificity of 99 and 14% respectively, using an elasticity score threshold between 1 and 2. In conclusion they affirm that qualitative elastography can detect benign nodules with a high accuracy (101);
– in 2014, Ghajarzadeh et al. published a metanalysis with 12 articles and 1,180 thyroid nodules assessed by qualitative US-elastography. They showed a sensitivity and specificity of 86 and 66.7% respectively, using an elasticity score threshold between 2 and 3, and a sensitivity and specificity of 98.3 and 19.6% respectively, using a elasticity score threshold between 1 and 2. In conclusion they affirm that USE could be used as thyroid nodule screening tool (102).

Almost in parallel, articles began to be published comparing qualitative and semi-quantitative USE and in particular:

– in 2016, the metanalysis of Tian showed the better SRE accuracy than qualitative USE, with a sensitivity and specificity of 86.5% vs. 81.8% and 86.6% vs. 81.7% respectively (103);

TABLE 4 | Diagnostic performance of each USE methods.

Method	Studies,n	Benign,n	Malignant,n	Sens, % (CI, %) (I-square, %)	Spec, % (CI, %) (I-square, %)	PLR (CI)	NLR (CI)	AUC (CI, %)
Qualitative USE	26	4,635	2,640	84 (83–85) (91.1)	81 (80–82) (95.5)	4.7 (3.5–6.3)	0.24 (0.17–0.34)	89.0 (86.8–91.2)
Semi-quantitative USE	22	3,801	1,889	83 (81–84) (83.3)	80 (79–82) (96.7)	6.5 (4.2–10.1)	0.19 (0.13–0.27)	92.9 (91.0–94.7)
Quantitative USE	32	4,236	2,507	78 (76–79) (58.6)	81 (80–82) (89.5)	4.4 (3.6–5.5)	0.28 (0.24–0.33)	87.0 (85.7–88.2)

USE, Ultrasound Elastography.

– in 2014, Sun et al. published a metanalysis with 31 papers and 6,544 thyroid nodules assessed by real-time ultrasound elastography. They showed the better SRE accuracy than qualitative USE with a sensitivity and specificity of 85% vs 79% and 80% vs 77% respectively. In conclusion they affirm that the SRE and qualitative USE accuracy are similar although SRE diagnostic value is slightly higher than elasticity score (104);
– in 2013, Razavi et al. published a metanalysis with 24 papers and 3,531 thyroid nodules (2,604 benign and 927 malignant) assessed by qualitative and semiquantitative USE. They showed the better accuracy of SRE assessment than

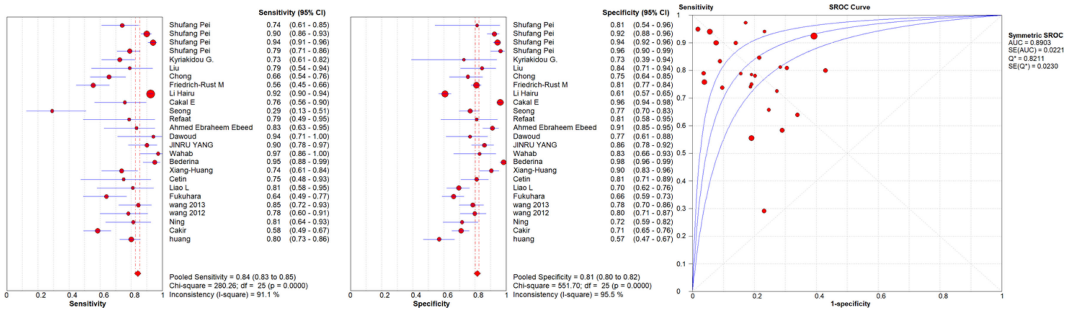


FIGURE 1 | Sensitivity and specificity forest plots and SROC curve for the qualitative USE. The circle size represents the nodule population of the selected articles; the line represents the confidence interval of the selected articles.

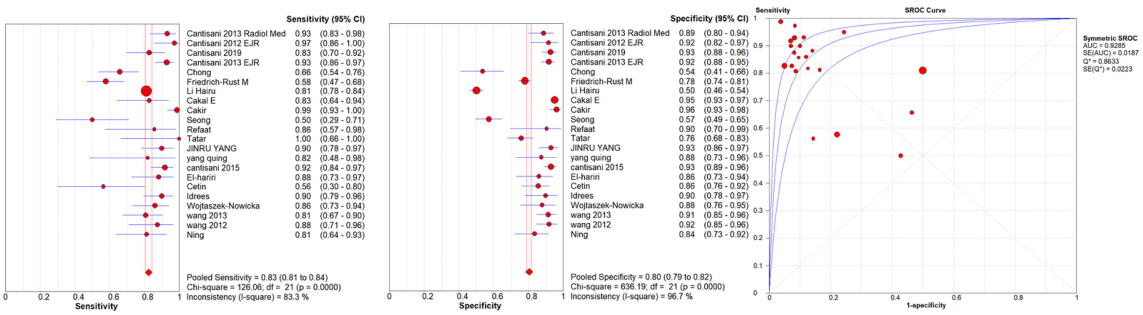


FIGURE 2 | Sensitivity and specificity forest plots and SROC curve for the semiquantitative USE. The circle size represents the nodule population of the selected articles; the line represents the confidence interval of the selected articles.

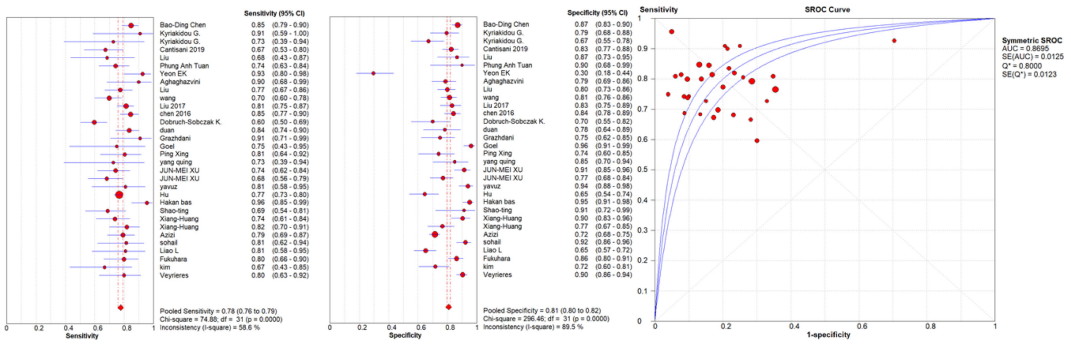


FIGURE 3 | Sensitivity and specificity forest plots and SROC curve for the quantitative USE. The circle size represents the nodule population of the selected articles; the line represents the confidence interval of the selected articles.

TABLE 5 | Pooled diagnostic performance of qualitative USE using only prospective papers. USE, Ultrasound Elastography.

Method	Sens, % (CI, %) (I-square, %)	Spec, % (CI, %) (I-square, %)	PLR (CI) (I-square, %)	NLR (CI) (I-square, %)	AUC (CI, %)
Qualitative USE	74.4 (71.5–77.2) (81.7)	82.3 (80.9–83.7) (94.7)	4.10 (2.98–5.66) (90.7)	0.30 (0.22–0.40) (79.8)	87.1 (84.1–90.1)
Semi-quantitative USE	83.9 (81.2–86.2) (83.9)	87.8 (86.5–88.9) (90.6)	7.36 (4.78–11.34) (92.6)	0.16 (0.10–0.27) (88.4)	94.3 (92.8–0.95.8)
Quantitative USE	78.0 (76.1–79.8) (55.1)	80.9 (79.5–82.2) (88.6)	4.60 (3.71–5.70) (84.0)	0.26 (0.22–0.32) (63.7)	87.8 (86.3–89.2)

elasticity score evaluation with a sensitivity of 89% vs. 82%, respectively, but with same specificity (82%) (105).

After the introduction of new elastosonographic techniques based on shear wave speeds, new studies and various meta-analyses were published to evaluate the SWE diagnostic performance compared to gold-standards, in particular:

- in 2015, Zhan et al. published a meta-analysis with 16 papers and 2,436 thyroid nodules (1,691 benign and 745 malignant) assessed by ARFI (acoustic radiation force impulse) imaging. They showed a sensitivity and specificity of 80% and 85% respectively, affirming SWE could help identify which patients should be treated surgically (106);
- in 2018, Chang et al. published a meta-analysis with 20 papers and 3397 thyroid nodules, assessed by quantitative SWE. They showed a sensitivity and specificity of 68 and 85% concluding that SWE is very accurate in distinguishing malignant and benign nodules (107);
- in 2020, Filho et al. published a meta-analysis with 17 papers and 3,806 thyroid nodules (2,428 benign and 1,378 malignant) assessed by 2D–SWE elastosonography by various manufacturers. They showed a sensitivity and specificity of 77 and 76% respectively for T–SWE (Toshiba shear-wave elastography); a sensitivity and specificity of 72 and 81% respectively for VTIQ (Virtual Touch tissue imaging and Quantification); and a sensitivity and specificity of 63 and 81% respectively for S-SWE (SuperSonic shear-wave elastography). In conclusion they affirm that 2D–SWE could rule out the malignant thyroid nature (108).

Furthermore, some meta-analyses which compare these two different elastosonographic techniques were published:

- in 2017, Hu et al. published a meta-analysis with 22 original articles and 2,661 thyroid nodules (2,063 benign and 598 malignant) assessed by SE (Strain Elastography) and SWE. They showed a sensitivity and specificity of 84 and 90% respectively for SE and a sensitivity and specificity of 79 and 87% respectively for SWE. In conclusion, they state that SE has a better sensitivity than SWE (0.84 vs. 0.79) with p-value <0.05 and above all a statistically better specificity than SWE (0.90 vs. 0.87 with p <0.05) (109);
- in 2016, Tian et al. published a meta-analysis with 54 papers with 10,001 thyroid nodules (7,380 benign and 2,621 malignant) assessed by SE (Strain Elastography) and SWE. They showed a sensitivity and specificity of 82.9 and 82.8% respectively for SE, and sensitivity and specificity of 78.4 and 82.4% for SWE. In conclusion they affirm that the SE sensitivity is better than SWE one (0.829 vs. 0.784) but with similar specificity (103).

The USE role is not limited to the thyroid cancer diagnosis but it is also useful in the detection of cervical lymph node metastases and to guide interventistic procedures (110). In fact, the EFSUMB guidelines state that USE can identify the most suspicious lymph nodes and the most suspicious internal areas worthy of cyto-histological investigation (92).

Our meta-analysis is the first meta-analysis since 2016 that individually takes into consideration the diagnostic performance of different USE types in the characterization of the thyroid

TABLE 6 | Pooled diagnostic performance of qualitative USE using only retrospective papers.

Method	Sens, % (CI, %) (I-square, %)	Spec, % (CI, %) (I-square, %)	PLR (CI) (I-square, %)	NLR (CI) (I-square, %)	AUC (CI, %)
Qualitative USE	89.0 (87.5–90.4) (92.1)	79.7 (77.7–81.6) (96.8)	6.10 (2.81–13.24) (96.2)	0.20 (0.10–0.39) (96.4)	92.3 (89.3–95.3)
Quantitative USE	78.7 (76.0–81.2) (63.1)	81.8 (79.8–83.8) (89.3)	4.26 (2.71–6.68) (93.8)	0.28 (0.23–0.34) (58.1)	86.5 (84.7–88.2)

Retrospective papers analysis about semiquantitative USE was not performed because too few papers. USE, Ultrasound Elastography.

nodule however using studies with at least 50 thyroid nodules because the smaller ones may have low precision (wide confidence interval of the estimates), may be of low quality and may increase heterogeneity.

In particular we examined qualitative USE, semiquantitative USE and quantitative USE and demonstrate that all of them are useful in the thyroid nodule characterization with high accuracy values and especially the same specificity. However, the semiquantitative and qualitative elastosonography showed the best diagnostic performance compared to SWE with the following sensitivity and specificity values 84 and 81% for qualitative USE, 83 and 80% for semiquantitative USE and 78 and 81% for quantitative USE.

Our results about strain-based USE techniques are similar with no significant statistical difference (p-value >0.05).

By contrast, the AUCs evaluation slightly favors the SRE over others (semiquantitative USE AUC: 0.93; qualitative USE: 0.89; quantitative USE: 0.87) with statistically significant values between semiquantitative USE and quantitative USE (p-value <0.05).

Our metanalysis results are quite in line with this recent meta-analyses and guidelines that indicate SRE the most accurate USE method in the malignancy risk stratification of the thyroid nodules (10).

Although in 2017 the Hu et al. meta-analysis showed the better qualitative USE sensitivity and specificity than SWE ones (0.84 vs. 0.79 with p >0.05 and 0.90 vs. 0.87 with p <0.05, respectively), the semiquantitative USE was poorly represented in their paper and not distinguished from qualitative USE in the statistical analysis (109).

In 2016 the meta-analysis of Tian concluded asserting that the SE (Strain Elastography) diagnostic performance (both qualitative and semiquantitative USE) was better than SWE with a p <0.05 and among the SE techniques the SRE (strain ratio elastography) accuracy was better than SE with elasticity score with sensitivity and specificity values of 86.5% vs 81.8% and 86.6% vs 81.7% (103).

These differences could be explained because the main qualitative USE limitation is the operator-dependence related to the subjective diagnostic evaluation based on different eye-type scales without agreement about the score to be used (55). In literature, several qualitative USE color pattern involving five, four, or two color score are used, but showing different diagnostic performances without having a better one (55).

SRE improves the subjective assessment of the nodule stiffness, in some cases it is not feasible due to the presence of micro-macrocalcifications, pathological changes in the surrounding parenchyma such as in autoimmune thyroiditis or when the nodule is so large as to replace the entire gland without healthy parenchyma to compare. Furthermore, there is no agreement on the SRE cut-off to choose, and therefore without having a real standardization of this method.

SWE is the quantitative USE technique based on the Shear-wave speeds measurement and so less affected by a subjective interpretation. But to date, the current and recent papers showed a worse SWE diagnostic accuracy than SE one. SWE can evaluate thyroid nodule also in presence of autoimmune thyroiditis (111) and so when SE is unfeasible for the pathological changes of peri nodular surrounding thyroid parenchyma.

I^2 quantify the effect of heterogeneity, describing the percentage of total variation across studies that is due to heterogeneity rather than chance. In our results it is very high for all parameters (>80%) except for the sensitivity of the SWE which is instead about 55.1%. This could lead to think that it is a technique less influenced by interobservational variability but nevertheless its I^2 is too high (>50%) and not low enough to affirm a good homogeneity between the different studies. One explanation could be that the qualitative and semi-quantitative USE techniques have been used for a long time and therefore the resulting studies are older and more heterogeneous. Other reasons should explain it such as a possible more homogeneous population or settings. The interobservational variability of the different USE techniques is beyond our purposes but to date it has been evaluated by few studies. Therefore, further studies are needed, especially prospective and with a large population.

In our study there are some limitations: at first, calcified and/or cystic nodules are not included by some studies for possible artifacts generation; secondly, the heterogeneity of the articles included may represent a source of bias as no consensus about the optimal elastosonographic methodology as the preferential use of carotid or freehand pulsation in the strain elastography; the non-univocal qualitative USE score to use (score 1–2; score 1–4 or score 1–5) and different Strain Ratio cut-off values; thirdly, the possible selection bias. In fact our thyroid nodules population presents a high pooled malignancy rate (33%) deriving from the studies published by various research institutes considered as a reference center for thyroid pathology and so with many patients with already suspected thyroid nodule. All this might have contributed to have misleading results.

In addition, we have to mention that we did not evaluate the inter-observational variability between the different USE techniques and secondly specific papers on indeterminate nodules at FNAC have not been.

Noteworthy, although FNAC is the gold standard for the thyroid nodule classification, it can show cellular atypia of undetermined significance (TIR3 category) in the 5–20% of cases (112). Therefore a fairly large number of patients undergo thyroidectomy for diagnostic rather than therapeutic purposes, with increased costs and possible complications. Therefore, in recent years, efforts have been made to better evaluate the cytologically indeterminate nodule and reduce the number of these thyroidectomies as only up to 30% of these patients harbor indeed thyroid cancer.

In this regard, MPUS tries to better characterize indeterminate thyroid nodules and with encouraging results (96, 106, 107, 113).

CONCLUSIONS

In conclusion, this comprehensive meta-analysis shows that all USE methods (quantitative, semi-quantitative and quantitative

USE) have a good sensitivity and specificity in differentiating malignancy from benignancy, with a slight better performance by means of qualitative USE.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

Conceptualization, VC, VDA, and CC Methodology, VC, DF, VS, ADS, CD, and GG Investigation, ADS, VS, DF, PP, EP, GP, and OG Resources, SS, PT, CD, and MT Data curation, DF, VC, CD, GG, and PT Writing—original draft preparation, DF, CD, VC, AL, RC, OG, and PT Writing—review and editing, VC, AMI, CD, GG, and EG Supervision, VC, CC, VDA, PT, and CD All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Papillary Thyroid Microcarcinoma: Active Surveillance Against Surgery. Considerations of an Italian Working Group From a Systematic Review

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Introduction: Active surveillance is considered a viable option for papillary thyroid microcarcinoma. Since the last decade of the 20th century, this method has spread from Japan to other countries, but has not yet been fully accepted and validated by the major Western Scientific Societies. In 2016, a systematic review on the results of active surveillance was published, based on two articles that showed encouraging results. Other reviews published subsequently, were mainly based on articles from the Far East. The aim of this review is to assess the most recent results published from 2017 to 2020 on this subject.

Materials and Methods: A systematic literature search was performed on MEDLINE *via* PUBMED, Web of Science, and Scopus according to PRISMA criteria. The MESH terms "papillary thyroid microcarcinoma" and "active surveillance" were adopted. Tumor progression, secondary localizations, and quality of life were the main benchmarks.

Results: Nine studies met the inclusion criteria. The increase in volume ranged from 2.7% and 23.2%; the occurrence of lymph node metastases from 1.3% to 29%; QoL was improved in both articles that addressed this topic. The level of evidence is considered low due to the retrospective and uncontrolled nature of most of the studies included in the review.

Conclusion: The evidence from the literature currently available on AS falls into two strands: a robust data set from the Japanese experience, and an initial experience from Western countries, whose data are still limited but which show a lack of substantial alerts against this practice. Further data is useful to validate the spread of Active Surveillance.

Keywords: papillary thyroid microcarcinoma, active surveillance, thyroidectomy, thyroid cancer, quality of life, lymph node metastasis

INTRODUCTION

Papillary Thyroid Microcarcinoma (PTmC) is a thyroid cancer measuring 1 centimeter in diameter at most. In most cases, it is diagnosed as an unforeseeable finding after pathology examination of a specimen removed for benign disease in most cases. It is diagnosed less frequently as a suspected infracentimetric thyroid nodule discovered during a routine neck ultrasonography or CT scan, and its presence is rarely revealed after palpable lymph node neck metastases, or in exceptional cases, distant metastases, have been discovered (1). Actually, this tumor is considered the main cause of the increase in the incidence of Papillary Thyroid Carcinoma (PTC) since the widespread use of high resolution ultrasounds has increased the diagnosis of PTmCs (2). Surgery is usually considered the gold standard in treatment of PTmC, although active surveillance (AS) began to take on (3) and has been described in several articles published since this procedure was introduced by Miyauchi at Kuma Hospital in Kobe (Japan) in 1993 and obtained approval by other surgeons (4) until it was adopted at the Cancer Institute Hospital in Tokyo (Japan) in 1995 (5). As a background, epidemiologic data strongly supported this option: in autopsy studies published before 1993, the prevalence of occult PTmCs ranged from 5,7% to 35,6% (1); Takebe (6) showed that the prevalence of PTC in a population undergoing a screening for breast cancer and that was submitted at the same time to a screening for thyroid cancer was 1000-fold the prevalence of clinically evident PTC in the same country and period. These findings led the teams of Kobe and Tokyo to introduce the practice of AS with the aim of identifying cancers growing during observation, considering that the consequent treatment delay did not worsen prognosis and systematic surgery for PTmC has more complications than advantages (5).

In 2016, Alhashemi published the first systematic review on active surveillance for management of low-risk Papillary Thyroid Carcinoma T1 N0 M0. Although the purpose of this article was to evaluate results of AS in all low-risk T1 papillary thyroid carcinomas (less than 2 cm in diameter), all in all it included two articles that evaluated literature concerning AS PTmC (7). Some more recent reviews (8, 9) have emphasised the benefits of this practice, but most of these papers come from Far East countries, often from the same working groups, or groups close in lifestyle and culture.

The aim of this systematic review is to assess the findings of the literature from the period 2016-2020 from an observation point outside the culture and environment in which this practice originated and developed. This is especially so now as a number of Western and European groups have published the first data on their experience with this topic.

MATERIALS AND METHODS

This systematic review was performed according to PRISMA criteria (10). According to these guidelines, we selected the studies included in this review as follows.

A systematic literature search was performed on MEDLINE *via* PUBMED, Web of Science and Scopus databases by four independent investigators (AC, IV, GR, and SR) who searched for articles published in English since 2017. We excluded previous papers because we considered Alashemi's review to be completely exhaustive and in no way improvable, in the reporting period considered (7). The MeSH terms adopted were: papillary thyroid microcarcinoma; active surveillance with Boolean operators AND or OR. We also included all relevant articles cited as references for selected articles and not found with our search. Non-English language articles, reviews, case reports, case series, editorials, and repeated or redundant manuscripts were excluded in advance. In the case of disagreement between investigators on the value of selected papers, a supplementary confrontation was crucial in decision making. Titles of papers were evaluated for the 91 manuscripts retrieved. The abstracts of papers that appeared to be in agreement with the aim of review were read and, if even after this step, the article appeared to be in line with our aims, it was downloaded and read in full.

The search process is reported in **Figure 1**.

The main benchmark of the included studies was progression, both in terms of volume increase beyond the parameters of significance (> 3 mm) and in terms of the appearance of secondary localisations. In two studies, of which one was a survey, we also assessed the quality of life (QoL) of patients undergoing active surveillance.

The data obtained did not undergo meta-analysis because they are still considered limited from the point of view of overall numbers, at least for those from Western countries, and in any case, they are not comparable, at the moment, with those already well established from the Kuma Hospital in Kobe and the Tokyo Cancer Institute (4, 5).

RESULTS

Overall, nine articles met the inclusion criteria for this review.

A retrospective, uncontrolled study by Kwon et al. (11), involving 192 patients with a median follow-up of 31.2 months, aimed at assessing the clinical outcomes of AS, showed an increase in volume in 14% of cases (27 patients). In 12.5% of cases (24 patients), surgical indication was given, 7 of which were due to lymph node metastases.

An uncontrolled retrospective study by Miyauchi et al. (12) of 1211 patients, with a median follow-up of 6.2 years and focusing on the probability of progression, showed an increase in lesion volume for 72 patients, lymph node metastases in 18 patients, and both occurrences in 4 patients.

Kim et al. (13) reported, in an uncontrolled retrospective study of 126 patients, the progression of lesion size in 25, of whom 4 went on to surgery. A further 10 subjects (a total of 14, or 11.1%) were not referred for surgery for another reason. It should be noted that, for the first time, a correlation was found between increased TSH values and progression.

An uncontrolled multicentre study by Oh in 370 patients observed with a median follow-up of 32.5 months and focused on the natural history of the disease, showing 23.2% of volumetric progression, 8.2% of lymph node metastases, and 15.7% of subjects requiring surgery overall due to anxiety caused by the disease condition (14).

Results from the groups in which AS was originally developed have, of course, continued to show encouraging results. The Kuma Hospital group (15) published results from 2288 patients

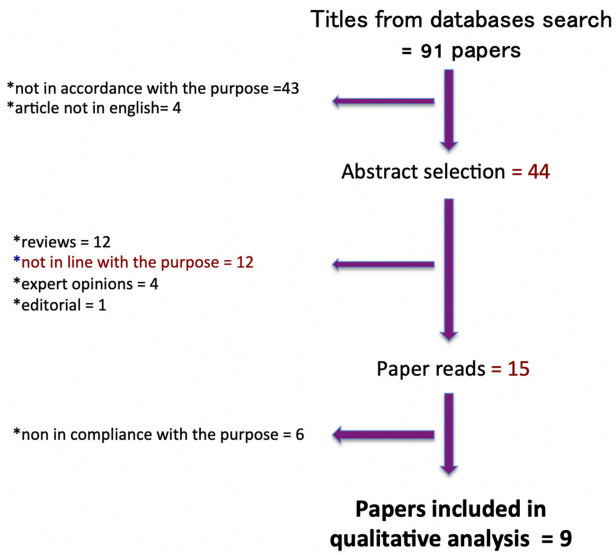

FIGURE 1 | Prisma diagram detailing the literature search process and article selection.

undergoing AS from 2005 to 2017, which showed that disease progression affected 57 patients, while 43 still preferred conversion to surgery during follow-up and 62 patients were operated on during observation on the observer's indication, either because of the onset of parathyroid disease, or for other reasons. The indication for AS increased significantly after 2011, and a parallel reduction in indications for conversion was observed during the same period.

In recent years, a number of referral working groups have also started practising AS outside the context of South-East Asia and Japan, publishing their first results, especially in the last two years. Rosario (2019) published the first South American study carried out on 77 patients, with a 30-month follow-up. Of these, only 1 patient showed tumour progression, with the appearance of lymph node metastases (16).

A recent study on 93 patients with a follow-up of 19 months (range: 6-54), showed a progression in only 3 of them; however, a further 19 patients withdrew their consent to active surveillance, requiring thyroidectomy. Of these, 9 were operated at the same centre and, among them, 1/9 patients showed minimal extrathyroidal extension and 1/9 a tall cell variant. Ten patients were lost at the follow-up. An increase in volume from 50% to 251% was also observed in 15/93 patients, of whom 2 were among those undergoing conversion from observation to surgery. The remaining 13, despite the substantial increase in volume, are still under observation (17).

Quality of life has been assessed in some studies concerning AS in PTmC. Both agree in the improvement of this parameter in subjects undergoing AS compared to the control group (patients undergoing thyroidectomy). However, it should be highlighted that both studies lack randomisation and have a short follow-up (18, 19).

On the other hand, a survey carried out by Yoshida, from the University of Tokyo, showed that patients' point of view on AS is strongly influenced by the physician and, conversely, understanding the patient's expectations is crucial for a shared decision making (20).

Table 1 is the panel of results of the present research. **Table 2** summarizes the average range of the main outcomes evaluated in the articles included in this review. Concerning the patients scheduled for surgery, we consider it useful to specify that not in all articles it is possible to distinguish the need established by the multidisciplinary team from the patient's choice. QoL was improved in both articles that addressed this topic.

As it is easy to observe, the overall level of evidence of the systematic review cannot be fully satisfactory, as the quality of the evidence of the individual papers is low, since most of them are uncontrolled, non-randomised, and retrospective studies.

DISCUSSION

A recent survey published by Sugitani (21) highlights that, at present, more than 50% of low risk PTmCs in Japan are kept under observation (21).

Active surveillance is currently considered, especially in eastern countries, to be a viable option that, in most cases, has a favorable impact on quality of life and costs in the medium to long term (22–24).

On the contrary, small thyroid carcinomas can benefit not only from thyroidectomy, but even from more or less extensive lymph node dissections, depending on their aggressiveness. In order to quantify the number of patients with PTmC requiring enlarged excision, we took into account the large case series published by Lombardi et al. (25), which showed that out of

TABLE 1 | Panel of results of systematic review.

Author/Year	Country	Trial	Sackett	Follow-up	Benchmark	Results of active surveillance
Miyauchi et al. 2017 (12)	Japan	Retrospective non-controlled **1211 patients**	IV	6,2 years	disease progression	1) >volume: 72 pts§ **(5,95%)** 2) LN met.*: 18 pts§ **(1,49%)** 1+2: 4 pts§ **(0,33%)**
Kwon et al. 2017 (11)	South Korea	Retrospective non-controlled **192 patients**	IV	31,2 months	Size increase Progression	>volume: 27pts§ **(14,06%)** surgery: 24pts§ **(12,5%)** LN met*: 7/24 **(3,65%)**
Kim et al. 2018 (13)	South Korea	Retrospective non controlled **126 patients**	IV	5 years	correlation TSH/disease progression	progression: 25 pts§ **(19,84%)** surgery: 14 pts§ **(11,1%)** *correlation TSH levels/ disease progression*
Oh et al. 2018 (14)	South Korea	Retrospective multicentric non-controlled **370 patients**	IV	32,5 months	disease progression	> volume: **23,2%** Surgery: **15,7%** LN met*: **8,2%**
Jeon et al. 2019 (18)	South Korea	Non-randomized 148 @lobectom **43 observed**	III	18-24 months	QoL	*better QoL*
Rosario et al. 2019 (16)	Brazil	Prospective non-randomized **77 observed** 18 surgery	III	30 months	disease progression	1 LN metastasis **(1,3%)**
Molinaro et al. 2020 (17)	Italy	Prospectively collected data non-controlled **93 patients**	IV	19 months	-disease progression -consent discontinuation	progression: 3 pts§ **(3,22%)** discontinuation: 19pts§ **(20,43%)** -9 operated at the same institution **(9,67%)**, of which: *1 minimal extrathyroidal extension *1 tall cell
Yoshida et al. 2020 (20)	Japan	Cross-sectional survey: - **20 pts**§ AS^^ -30 pts§	IV	4,1 years	PTmC related symptoms QoL	reduced state of anxiety improved QoL
Sasaki 2021 (15)	Japan	Surgery Retrospective non-controlled **2288 patients**	IV	2005-2017	disease progression	-progression: 57 pts§ **(2,49%)** -pts§ choice for surgery: 43 **(1,88%)** -need of surgery: 62pts§ **(2,71%)**

The data reported in this table came from articles included in the systematic review.
Sackett: Level of evidence based on Sackett's scale: I, meta-analysis or large randomized trials (clear cut-off results and low risk for error); II, small randomized trials and moderate/high risk for error; III, non-randomized but prospective with contemporaneous control trials; IV, non-randomized trials with historical controls or retrospective analysis; V, case series without control; expert opinion.
met, metastasis; Pts§, patients; @lobectom, thyroid lobectomy; QoL, Quality of Life.*
AS^^, Active Surveillance.
Bold values = number of patients enrolled for active surveillance.

more than 900 PTmC operated on, 9.6% were locally advanced (pT3), 5.6% were N1a and 1.1% N1b (25–27).

Again, a comparison of the data from the Sicilian Thyroid Cancer Registry (SRRTC) with those from a US epidemiological registry (SEER) shows that lymph node metastases are present in PTmC in 27.4% of cases in the former and in 28.9% in the latter; that extrathyroidal extension is present in 7.5% in the former and in 6.2% in the latter; and that, finally, multifocality is observed in 26% in the former and in 33.5% in the latter (28).

It should also be noted that micro pT3 makes up 25% of all pT3, and up 15% of all PTmC. In the light of current knowledge, more aggressive treatment is considered mandatory for these tumors (29, 30).

If these epidemiological data suggest that PTmCs should be assimilated to potentially aggressive forms of thyroid cancer, there are other data that could overturn the judgement: The frequency of incidental microcarcinomas in autopsy case histories is well known (1). Moreover, the results of the screening offered for low-cost thyroid nodular disease to the South Korean population in the early 2000s are well known, which showed a real "surge" in the number of PTC cases detected, with no change in mortality in the subsequent periods (31).

At present, the problem lies in the impossibility of identifying risk criteria for PTmC. In other words, we do not know of any biohumoral, radiological, or genetic indicators that would allow us to identify the limited subset of PTmCs that are destined for neoplastic progression (32).

Promising studies are underway to assess the real clinical impact of molecular tests for the management of thyroid nodules. These studies focus on the analysis of multiple genes and, if the results were

TABLE 2 | Average range of the main outcomes of active surveillance.

OUTCOMES	AVERAGE RANGE
Volume increase/progression	2,49 – 23,2
Lymph node Metastasis (n° of patients)	1,3 – 15,7
Scheduled for surgery	11,1 – 20,43

to confirm the hypotheses, a complete and extremely high-performance genetic identification could be able to define with much greater accuracy than at present the true nature of samples taken from FNA in patients with thyroid nodules. This could improve the detection of thyroid carcinomas with a more favourable prognosis and therefore candidates for less aggressive treatment or, in selected cases, even for AS. These studies, which from another perspective could redefine the TNM of any thyroid tumour, have not yet had sufficient development in the specific field of PTmC, and therefore can only be considered as a fascinating heuristic hypothesis (33).

These considerations ended up converging with the decades-long experience of Miyauchi et al., published in its evolution since the early nineties and founded on certain theoretical cornerstones, based on the non-variation of prognosis in the case of delayed surgery and the observation that systematic surgery can produce more complications than advantages in terms of prognosis. In this new vision, observation assumes the burden of detecting carcinomas which, as they progress, show potentially more aggressive behavior (4–6).

The prerequisite for the implementation of such a protocol, which substantially modifies the current recommendations in the Western world (ATA 2015), is the systematic bioethical sampling of all 5 mm nodules, in order to purify PTmC from benign nodules. Among the malignant ones, a distinction will be made between "high risk" (lymph node or distant metastases, cytological orientation for high malignancy, suspected invasion of the recurrent), PTmC "not suitable" for AS (protruding from the glandular profile, adherent to the airway or digestive tract with respect to which they determine a right or obtuse dihedral angle) and "suitable," ideally centroparenchymal, far from the inferior laryngeal nerve, with "reassuring" cytology (5).

The interest aroused by these initial data on this practice has led to a careful evaluation of these results.

The review published by Alashemi in 2016 (7) was carried out after a rigorous selection of 2375 papers, which resulted in only 2 papers being considered suitable. All the remaining literature was based on further reviews, often unsystematic, on data reported by other authors, reiterated or redundant, on unclear reasons and procedures for observation, or other causes of inadequacy. This study selected a number of cases of 1235 (published by Ito) and 322 (published by Sugitani) that allowed us to show a conversion rate to surgery of 15.5% and 8.7%, respectively, on a 5-6.5 year follow up, with a percentage of nodules progressing of about 5% (growth > 3 mm) plus about 1-1.5% presenting lymph node metastases. Despite a mortality rate of 0 in the follow-up period, the author pointed out some weaknesses in the documentation from these papers: there was a lack of clarity about the patients' reasons for choosing surgery, no data on quality of life, no indicators of disease progression, and finally, no data on TSH suppressive treatment.

The costs were another benchmark that we considered noteworthy, although not consistent with the main aims of this review. A study by Lin was set up on a real group of 349 patients with PTmC recruited from 1985 to 2017 from the institutional registry compared to a hypothetical group of subjects undergoing AS. The study showed that the costs of AS are equivalent to those of surgery after a little more than 16 years, and they are higher thereafter. Moreover, the incidence of permanent complications was also measured in 3.7% of patients undergoing thyroidectomy. This may lead to the conclusion that it is not economically viable to perform AS in younger patients, as they would be destined to a prolonged observation protocol (34).

A recent systematic review developed a cost-effectiveness analysis based on five retrospective trials. This study does not come to any definitive conclusions, but emphasizes the difficulties of carrying out evaluations of this kind, given that the costs evaluated (examinations, ultrasound investigations, surgery, etc.) have an extremely wide variability between the different national reference realities. However, even taking into account these limitations of the study, the authors are inclined to conclude, also in this case, that a younger age could make the cost-effectiveness assessment more favorable for surgery (35).

It should also be noted that AS requires more extensive diagnostic protocols than European standards. In particular, the restrictive indications for FNAB in many European countries conflict with the need to refer a large number of patients with suspicious subcentimetric nodules when adopting a strategy aimed at AS (36).

These considerations are further confirmed by the fact that thyroidectomy can now be performed effectively using minimally invasive techniques (37).

Along with these data, which are undoubtedly discouraging, we should highlight data that strongly supports AS in terms of disease progression and aggressiveness. In a large cohort of patients, drawn from the SEER US database from 1975 to 2015, staged T1-4 N0 M0 and dichotomously stratified between non-surgical (1453) and surgical (54718), Ho et al. (38) showed that there was an overlapping disease-specific survival (DSS), in the two arms of the study, for 'age < 55 years, for any size of T, while a high statistical significance ($p<0.001$) was detected only in patients > 75 years with T > 6 cm. Therefore, this study, although retrospective and with populations not specifically divided according to the strict criteria of active surveillance, seems to show that PTC exposes patients to a realistic scale of risk, which increases with tumour size and age (38).

On the other hand, some recent studies, carried out even outside the Far East context, have crossed the 1 cm size threshold to refer patients to AS, demonstrating the feasibility and reliability of this practice even in 1.5 cm diameter tumours and even for any "T" size (39, 40). Nevertheless, it has been reported that tumour size > 1 cm may be associated with a higher rate of lymphovascular invasion (41), so exceeding the original "classical" threshold for AS seems to need further validation studies. Ultimately, it should also be considered that AS should be compared, rather than total thyroidectomy, with haemithyroidectomy, which is itself a low-impact operation for the patient, both in terms of severe complications and resulting quality of life (24). Finally, we consider it useful to stress the importance of the tumor size: in fact, some studies have previously reported that PTMC <5 mm is usually not aggressive, whereas tumors >6 mm present a higher risk of lymph node metastasis (42).

CONCLUSION

We conclude that, to date, the scientific literature on AS in PTmC seems to be essentially divided into two different groups: a substantial series of results, mostly from the countries where AS was developed (Kuma Hospital in Kobe and Cancer Institute in Tokyo) and neighbouring sites, and initial, albeit encouraging, European and South American experiences.

The number of patients enrolled in Western trials is still too limited to draw conclusions of possible generalization, but available data allow us to draw some conclusions, which will have to be the subject of more robust and exhaustive evaluations:

-confirmation of PTmC indolence, at least in the vast majority of cases, is an optimal assumption for AS;

-none of the studies published so far, either in the Far East or in Western countries, has shown significant alerts such as to justify a hostile attitude towards AS, which, in the light of these data, could appear preconceived and anti-scientific;

-the studies that are to be published in the near future, and which will be welcome, in addition to progression-related parameters, should investigate the acceptance of AS by patients, taking into account the cultural differences that distinguish Far Eastern countries from Western ones, which are likely to have significant effects on the quality of life.

In any case, it should be taken into account that, in a large proportion of patients who still undergo surgery for PTmC, conservative surgery (haemithyroidectomy) performed with minimally invasive techniques remains a strong argument in favour of surgery.

Further evaluation in a larger scale of patient needs to be performed in order to validate AS in global settings with more robust data.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

AUTHOR CONTRIBUTIONS

Each author made substantial contributions to the work, has approved the submitted version and agrees to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated, resolved, and documented in the literature. Conceptualization: GO and GM. Methodology: GSc and SR. Validation: GSa and GG. Formal analysis: GSa. Investigation: AC. Resources: IV. Data curation: FC and AC. Writing: GO. Original draft preparation: AC and IV. Writing – review & editing: GM and GSc. Visualization: SR. Supervision: GM. All authors contributed to the article and approved the submitted version.

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Clinical Significance of the Expression of Co-Stimulatory Molecule B7-H3 in Papillary Thyroid Carcinoma

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Background: B7-H3, also known as CD276, an important immune checkpoint member of the B7-CD28 family, is confirmed as a promising target after PD-L1 in clinical trials. Although the overexpression of B7-H3 has been associated with invasive metastatic potential and poor prognosis in multiple types of cancer, nothing is known regarding the expression profiles of B7-H3 in papillary thyroid carcinoma (PTC). In this study, we carried out a large-scale analysis of B7-H3 expression in PTC patients and evaluated the potential clinical significance of B7-H3.

Methods: In total, data from 1,210 samples, including 867 cases from TCGA and four GEO datasets, were collected for B7-H3–related transcriptome analyses, and 343 postoperative, whole-tumor sections were collected from patients with PTC at our institute for B7-H3–specific immunohistochemistry (IHC) staining. The statistical analysis was primarily accomplished using the R project for statistical computing.

Results: B7-H3 positivity was found in 84.8% of PTC patients (291/343), and the mRNA and protein expression levels of B7-H3 in PTC were markedly higher than those of para-tumor tissues ($p < 0.001$), demonstrating that B7-H3 can serve as a potential diagnostic biomarker for PTC. The significant upregulation of B7-H3 in PTC is caused by distinct patterns of CNVs and CpG DNA methylation. Functional enrichment analysis confirmed that high B7-H3 expression was significantly associated with specific immune features and angiogenesis. High B7-H3 protein expression was associated with tumor size ($p = 0.022$), extrathyroidal extension (ETE) ($p = 0.003$), and lymph node metastasis (LNM) ($p < 0.001$). More importantly, multivariate analysis confirmed that B7-H3 was an independent predictor of relapse-free survival (RFS) ($p < 0.05$). In the subgroup analysis, positive B7-H3 staining was associated with worse RFS in patients with primary tumor size ≥2 cm ($p < 0.05$), age ≥55 years ($p < 0.05$), LNM ($p = 0.07$), multifocality ($p < 0.05$), and ETE ($p < 0.05$). In addition, Circos plots indicated that B7-H3 was significantly associated with other immune checkpoints in the B7-CD28 family.

Conclusion: This is the first comprehensive study to elucidate the expression profile of B7-H3 in PTC. Our observations revealed that B7-H3 is a novel independent biomarker for

predicting LNM and disease recurrence for PTC patients, and it thus may serve as an indicator that could be used to improve risk-adapted therapeutic strategies and a novel target for immunotherapy strategies for patients who undergo an aggressive disease course.

Keywords: papillary thyroid cancer, B7-H3, immune checkpoint, immunotherapy, metastasis, recurrence

INTRODUCTION

In recent years, encouraging developments have been reported for patients undergoing antibody treatments against immune checkpoints in the B7-CD28 family, such as CTLA4 and PD-1/PD-L1, which have brought about revolutionary changes in cancer treatment (Gong et al., 2018; Andrews et al., 2019). These achievements piqued our interest regarding whether immune checkpoints could be exploited as therapeutic targets for papillary thyroid carcinoma (PTC) treatment. Therefore, we conducted an exploration of public databases containing data regarding B7-CD28 family members in PTC patients.

B7-H3, known alternatively as CD276, belongs to the B7-CD28 immunoregulatory protein superfamily (Flem-Karlsen et al., 2018). Although the identities of its binding partners remain unclear, a wide range of B7-H3 expression has been reported in multiple tumor types, including non–small cell lung cancer (Carvajal-Hausdorf et al., 2019a), prostate cancer (Yuan et al., 2011), and renal cell cancer (Crispen et al., 2008). Although B7-H3 was initially characterized as a T cell co-stimulating protein, most recent studies have reported that B7-H3 is a T cell inhibitor that promotes tumor aggressiveness and proliferation (Picarda et al., 2016; Flem-Karlsen et al., 2018). Critically, B7-H3 seems to play vital roles in tumor growth and metastasis, and B7-H3 expression is associated with poor prognosis (Tekle et al., 2012). However, reports regarding B7-H3 expression in PTC are lacking; therefore, in this study, we performed a comprehensive analysis of B7-H3 expression in PTC.

MATERIALS AND METHODS

Publicly Available Data

We collected data for 867 PTC cases from five public datasets culled from The Cancer Genome Atlas (TCGA) (https://genomecancer.ucsc.edu) and Gene Expression Omnibus (GEO) (http://www.ncbi.nlm.nih.gov/geo), including 555 RNA-seq datasets (Illumina HiSeq 2000) from TCGA, 40 microarray cases from GSE29265, 95 microarray cases from GSE33630, 116 microarray cases from GSE35570, and 61 microarray cases from GSE60542. The microarray datasets from the GEO were log2-transformed and quantile-normalized prior to the analysis.

Patients and Samples

To verify the discoveries made by studying the public databases listed above, 343 patients with PTC who underwent surgery from 2003 to 2018 at the Department of Head and Neck Surgery, National Cancer Center, were included in this study. This study was approved by the Ethics Committee of the National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences. Informed consent requirements were waived due to the retrospective nature of this study, and all data were analyzed anonymously.

All 343 eligible patients were pathologically confirmed as PTC patients. All histological slides were independently re-reviewed according to the current WHO criteria by pathologists who were blind to disease outcomes. The data for 343 patients were extracted from the Department of Medical Informatics database to ensure the availability of an adequate medical history. At the time of diagnosis, all patients enrolled in this study had no history of previous thyroid surgery. The subjects with other types of malignancies or other histological types of thyroid cancer, for example, anaplastic thyroid cancer were systematically excluded.

Patient Characteristics and Clinical Outcomes

The clinicopathological variables of all patients, including sex, age at time of diagnosis, maximum tumor size, multifocal lesions, extrathyroidal extension (ETE), and lymph node metastasis (LNM), were evaluated comprehensively. TNM staging was classified based on the American Joint Committee on Cancer (AJCC, eighth edition) criteria for differentiated thyroid cancer. RFS was specified as the period from the date of first surgery to the date of recurrence, metastasis, or the time of last censoring. Recurrence was considered the appearance of disease proven by biopsy/confirmed by secondary surgery.

The clinicopathological features of 343 PTC patients are summarized in **Table 1**; their median age at the time of surgery was 47 years and ranged from 12 to 67. The majority of subjects were female (76%). Lobectomy and total thyroidectomy (TT) were performed for 161 (46.9%) and 182 (53.1%) patients, respectively, as the initial surgical treatment for the primary tumor. Central lymph node dissection (CLND) was performed for every patient, and lateral lymph node dissection (LLND) was performed for 132 (38.5%) patients. According to the final pathological evaluations, 175 (51.0%) patients with LNM were identified among all patients in the study. No patients had a history of undergoing head and neck radiation treatment or surgery at the initial diagnosis. The mean follow-up duration was 121 months (ranging from 13 to 161 months). Upon conclusion of the follow-up evaluation, 26 subjects suffered recurrence of the disease, but none died from PTC as the specific cause.

TABLE 1 | Relationship of B7-H3 expression by clinicopathological factors in PTC.

Characteristics	Total (N = 343)	B7-H3 expression in PTCs		
		Negative (N = 132, 38.5%)	Positive (N = 211, 61.5%)	*p*-value
Sex, n (%)				0.111
Male	78 (22.7)	24 (18.2)	54 (25.6)	
Female	265 (77.3)	108 (81.8)	157 (74.4)	
Age, y, n (%)				0.361
<55	286 (83.4)	107 (81.1)	179 (84.8)	
≥55	57 (16.6)	25 (18.9)	32 (15.2)	
Multifocality, n (%)				0.88
Yes	116 (33.8)	44 (33.3)	72 (34.1)	
No	227 (66.2)	88 (66.7)	139 (65.9)	
Extrathyroidal extension, n (%)				0.003
Yes	215 (62.7)	70 (53.0)	145 (68.7)	
No	128 (37.3)	62 (47.0)	66 (31.3)	
Lymph node metastasis, n (%)				<0.001
Yes	175 (51.0)	39 (29.5)	136 (64.5)	
No	168 (49.0)	93 (70.5)	75 (35.5)	
Recurrence, n (%)				0.001
Yes	27 (7.9)	2 (1.5)	25 (11.8)	
No	316 (92.1)	130 (98.5)	186 (88.2)	
Hashimoto thyroiditis, n (%)				0.018
Yes	57 (16.6)	14 (10.6)	43 (20.4)	
No	286 (83.4)	118 (89.4)	168 (79.6)	
Length, cm, n (%)				
<1	143 (41.7)	68 (51.5)	75 (35.6)	0.022
1–2	159 (46.4)	53 (40.2)	106 (50.2)	
2–4	34 (9.9)	10 (7.6)	24 (11.4)	
>4	7 (2.0)	1 (0.7)	6 (2.8)	
T stage, n (%)				0.028
I	29 (8.5)	15 (11.4)	14 (6.6)	
II	49 (14.3)	23 (17.4)	26 (12.3)	
III	69 (20.1)	44 (33.3)	25 (11.9)	
IV	196 (57.1)	50 (37.9)	146 (69.2)	

Initial Treatment

All patients underwent preoperational ultrasonography. The patients with suspected lateral LNM underwent additional CT scans. Lobectomy and TT were selected for each patient based on the condition of the primary tumor. All the subjects received CLND. Modified lateral lymph node dissection included levels II-IV as the minimal range. Intraoperative frozen section (FS) histology was used to aid surgeons in determining the scope of surgical plans. All the patients underwent postoperative thyroid-stimulating hormone (TSH) suppressive treatment.

Immunohistochemistry

Immunohistochemistry (IHC) staining for B7-H3 was interpreted by two pathologists in a blinded manner. Representative 4-μm-thick, formalin-fixed, paraffin-embedded (FFPE), resected specimens were placed onto the glass slides after deparaffinization, rehydration, antigen retrieval, endogenous peroxidase inactivation, and blocking of nonspecific binding. B7-H3 was stained overnight at 4°C using the D9M2l rabbit monoclonal antibody (1:200, clone D9M2l, catalog 14058s, Cell Signaling Technology, Danvers, MA, United States). Finally, the reaction products were imaged using a DAKO EnVision Detection System (Dako), and hematoxylin was used for counterstaining.

Quantification of B7-H3 Expression

The B7-H3 staining intensity was scored as follows: negative (–), faint/weak (+), medium (++), or strong (+++). The staining extent was scored according to the percentage of positively stained areas in tumor cells (TCs). The staining intensity and percentage positivity scores for TCs were then multiplied to compute the immunoreactivity score. The final B7-H3 staining pattern was scored as 0, 1, 2, and 3 in the following manner: 0, no membranous staining or <1% TCs with faint/weak membranous staining; 1, ≥1% TCs with faint/weak membranous staining or <1% TCs with medium membranous staining; 2, ≥1% TCs with medium membranous staining or <1% TCs with strong membranous staining; and 3, ≥1% tumor cells with strong membranous staining. The tissue samples with a final staining score of 0 or 1 were assigned to the B7-H3–negative group, while the specimens with a final staining score of 2 or 3 were assigned to the B7-H3–positive group.

Statistical Analysis

All statistical analyses and figure generation were carried out in SPSS version 25.0, GraphPad Prism version 8.0, and R version 3.5.1. Chi-square tests or Fisher's exact tests were used to assess the relationships between B7-H3 status and clinicopathological characteristics. The Mann–Whitney *U*-test was used to calculate

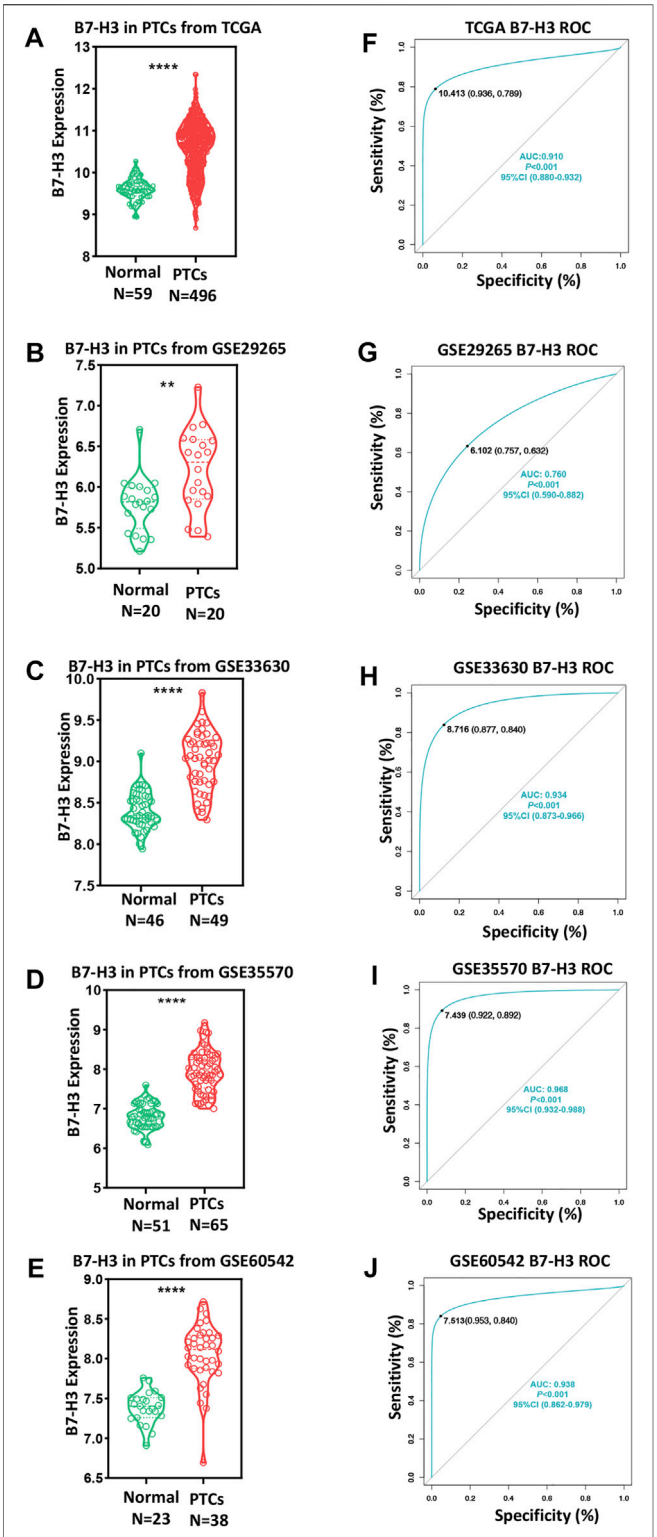

FIGURE 1 | B7-H3 expression of PTC and para-tumor tissue in TCGA and GEO datasets **(A–E)**. ROC curve of B7-H3 expression in TCGA and GEO datasets **(F–J)**. ** and **** represent $p < 0.01$ and $p < 0.0001$, respectively.

the distribution of B7-H3 in different groups. The difference between the survival rates of the B7-H3–positive and B7-H3–negative groups was assessed using the Kaplan–Meier method with a log-rank test. Additional figures were created in the R statistical software environment using several graphics packages, including *ggplot2*, *pROC*, and *survival*. The threshold for statistical significance was $p < 0.05$ for all statistical methods.

RESULTS

B7-H3 Is Overexpressed in PTC Tumors

To investigate the role of B7-CD28 family members in PTC, we assessed TCGA datasets to analyze the mRNA levels of transcripts encoding B7-CD28 family member proteins. The results showed that the B7-H3 mRNA level was higher than that of any other member of the B7-CD28 family in PTC (**Supplementary Figure S1**), indicating that B7-H3 may play a preponderant role in the development of PTC. We next determined the B7-H3 expression profiles of PTC tumors and para-tumor tissue samples from 555 patients. The results from both TCGA and GEO datasets showed that expression of B7-H3 was significantly upregulated in PTC tumors in comparison with para-tumor tissue (**Figures 1A–E**). ROC analysis was then used to investigate the potential diagnostic value of B7-H3 expression in PTC. The AUCs of B7-H3 in TCGA and four GEO datasets were 0.91, 0.76, 0.93, 0.97, and 0.94, which demonstrated aberrant B7-H3 expression, as we expected (**Figures 1F–J**). This finding prompted us to investigate the biological mechanism underlying abnormal B7-H3 expression in PTC tumors. Therefore, we performed further analysis based on TCGA datasets, which showed that the abnormal expression of B7-H3 in PTCs was closely related to distinct patterns of CNVs and CpG DNA methylation (**Supplementary Figure S2**).

B7-H3 Expression and Clinicopathological Features

In order to further explore our findings from TCGA, we examined the expression of B7-H3 by IHC in 343 PTC tumors and 159 para-tumor tissue samples. The results showed that B7-H3 staining in PTC TCs was mainly located in the cell membrane, and the corresponding para-tumor tissues showed extremely weak staining. In contrast, B7-H3 expression was relatively high in PTC TCs; among the PTC TC samples, 24 samples were scored as 0 (15.1%), 38 samples were scored as 1 (23.9%), 68 samples were scored as 2 (42.8%), and 29 samples were scored as 3 (18.2%) (**Figures 2A–E**).

Overall, the B7-H3 expression levels of nontumor tissue samples were significantly lower than those of their paired thyroid tumor tissue samples ($p < 0.001$) (**Figure 2F**). Next, we performed ROC analysis based on the IHC score of each sample. The results of the ROC analysis suggested that the B7-H3 protein level has diagnostic value for PTC patients (**Figure 2G**).

To further analyze the correlations between B7-H3 protein expression and clinical parameters, we classified IHC staining

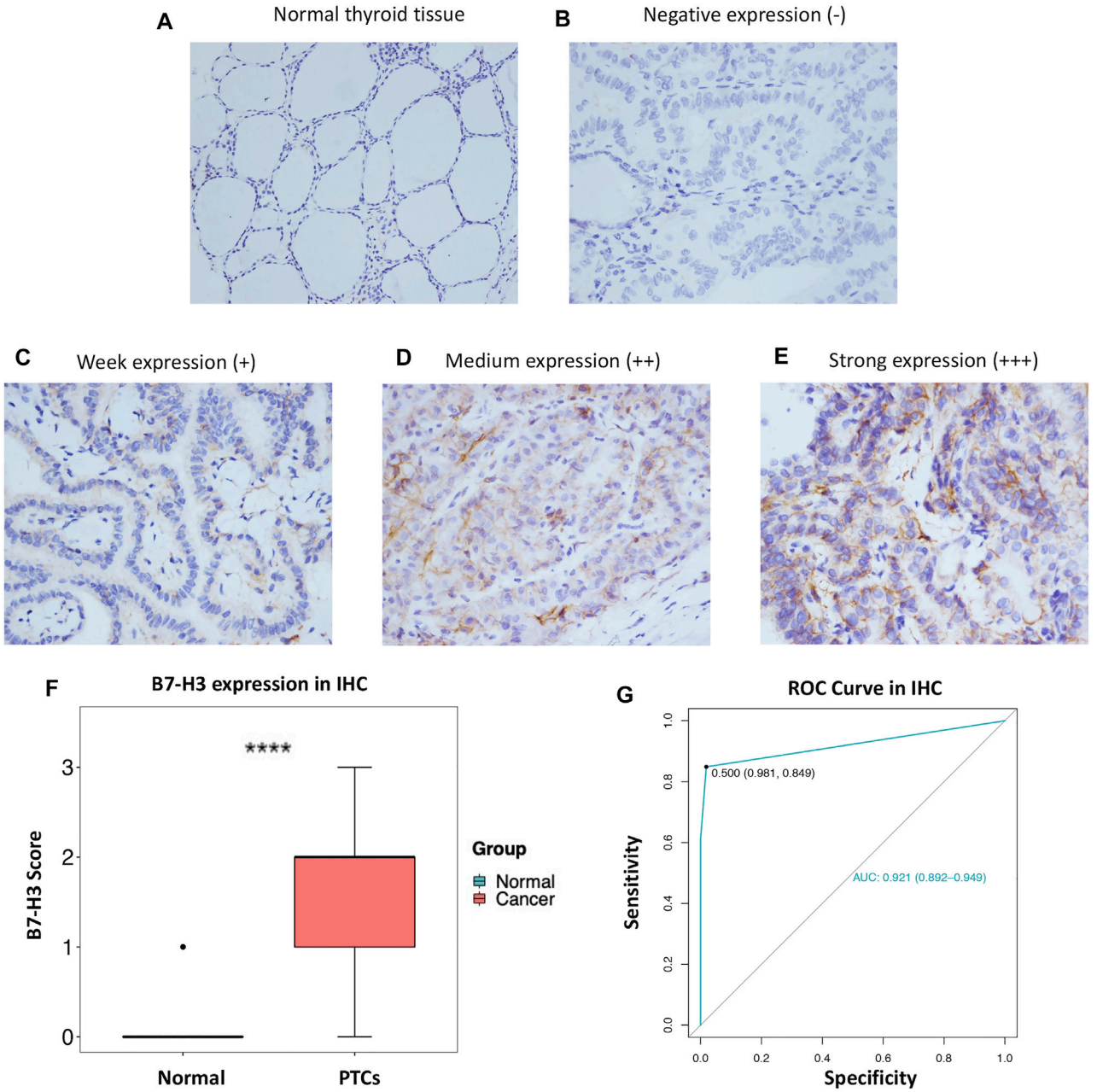

FIGURE 2 | Protein expression profile of B7-H3 in PTC (200 ×) and normal thyroid tissue (100 ×) **(A–E)**. B7-H3 expression score in PTC and normal tissue **(F)**. ROC curve of B7-H3 protein expression in PTC **(G)**. **** represents ***p*** <0.0001.

scores of 2 and 3 as positive B7-H3 staining, whereas scores of 0 and 1 were classified as negative B7-H3 staining. Using this method, B7-H3 expression was validated at the protein level for the total cohort of 343 specimens, among which 52 (15.2%) samples were scored as 0, 80 (23.3%) samples were scored as 1, 137 (39.9%) samples were scored as 2, and 74 (21.6%) samples were scored as 3. Therefore, 132 (38.5%) samples were assigned to the B7-H3–negative group and 211 (61.5%) samples were assigned to the B7-H3–positive group. The relationships between B7-H3 expression and clinical features are shown in **Table 1**. B7-H3 protein expression in PTC was found to be closely correlated with tumor length ($p = 0.022$), ETE ($p = 0.003$), LNM ($p < 0.001$), and recurrence ($p = 0.001$), but it was uncorrelated with sex and age ($p > 0.05$).

B7-H3-Related Biological Processes and Pathway in PTCs

Given that B7-H3 expression was found to be significantly correlated with tumor aggressiveness, we explored the

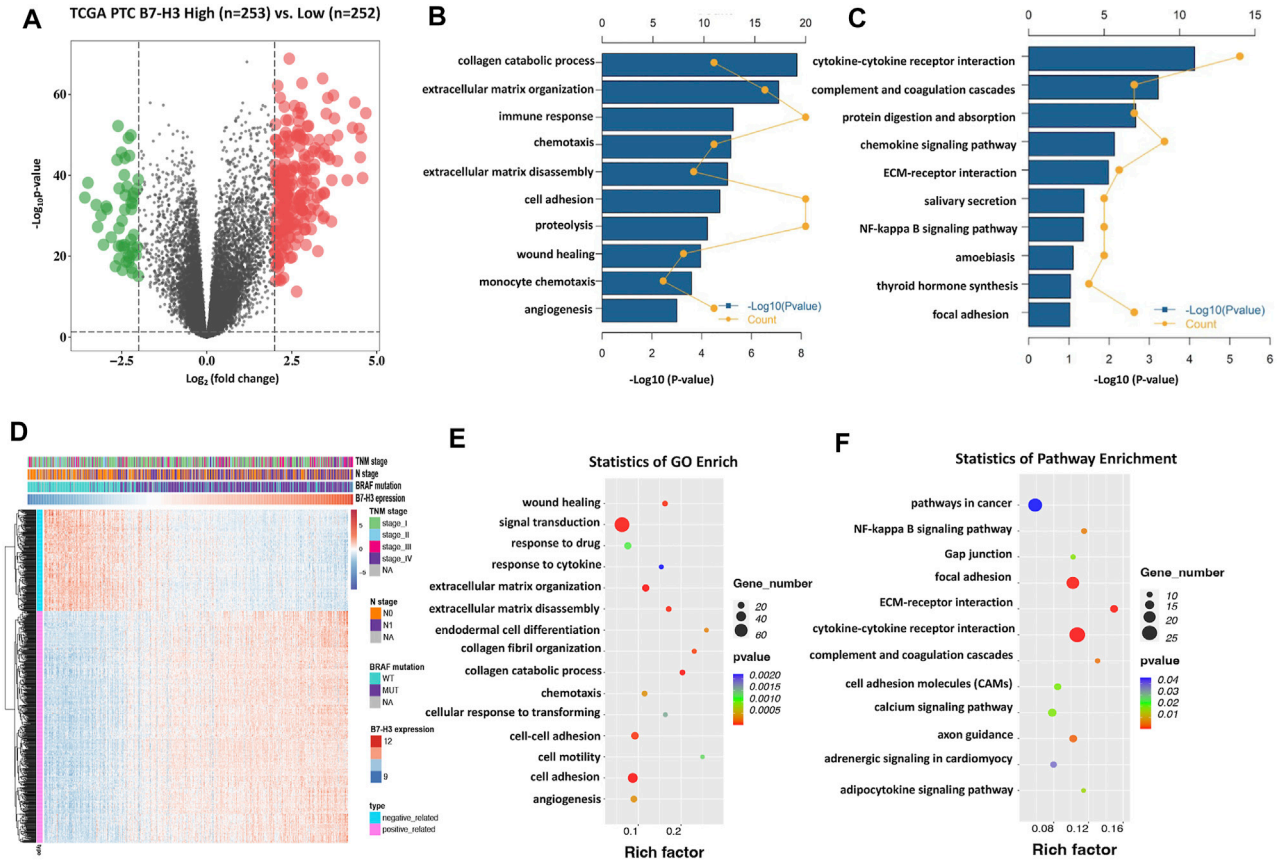

FIGURE 3 | B7-H3–related biological process and pathways in PTC. Differentially expressed genes in Volcano plots from TCGA dataset **(A)**. Gene enrichment with GO terms of selected genes **(B)**. Gene enrichment with KEGG terms of selected genes **(C)**. Particulars of risk score and most-related genes in heat map **(D)**. The bubble plots showing that B7-H3 is closely correlated with cell invasion, immune response and angiogenesis **(E,F)**.

underlying mechanism linking this clinical feature with B7-H3 expression in PTCs. Here, two different methods were used to reveal the B7-H3–specific biological landscape in PTCs. First, based on the median expression level of B7-H3 from TCGA datasets, the test subjects were divided into two groups: a B7-H3 high-expression group (N = 253) and a B7-H3 low-expression group (N = 252). Next, we identified 266 differentially expressed genes (DEGs) between the B7-H3 high- and low-expression groups using the following criteria: fold-change > 4 and $p < 0.0001$ (**Figure 3A**). Finally, 216 upregulated and 50 downregulated 50 genes were obtained for the B7-H3 high group, after which Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were conducted based on these DEGs. The results of the GO and KEGG analyses showed that the selected DEGs were likely to be involved in the immune response, extracellular matrix disassembly, and angiogenesis (**Figures B,C**). Next, we selected the set of genes with the most significant correlation with B7-H3 expression in PTC TCs ($|R| > 0.6$, $p < 0.0001$) (**Figures 3D–F**), which included 508 positively related genes and 223 negatively related genes. GO and KEGG analyses of the selected gene sets showed that they were mainly enriched in cell adhesion–related pathways and participated in the process of extracellular matrix decomposition, cell motility, and angiogenesis, suggesting that B7-H3 expression may be associated with PTC metastasis.

Prognostic Features of B7-H3 Expression in PTCs

Next, we assessed the association between B7-H3 expression and PTC prognosis. First, we performed a survival analysis using TCGA dataset. The results showed that the B7-H3–positive status was strongly associated with increased risks of relapse (**Figure 4A**, $p < 0.001$) and death (**Figure 4B**, $p < 0.001$) in comparison with B7-H3–negative status. Verification of these results at the protein level revealed that B7-H3–positive subjects showed dramatically worse RFS in comparison with that of B7-H3–negative subjects (**Figure 4C**, $p < 0.001$). Moreover, the analysis of patient prognosis suggested that the B7-H3 IHC staining score was positively correlated with poor prognosis (**Figure 4D,** $p < 0.001$). In addition, the B7-H3–related survival analysis was conducted in patient subgroups that were

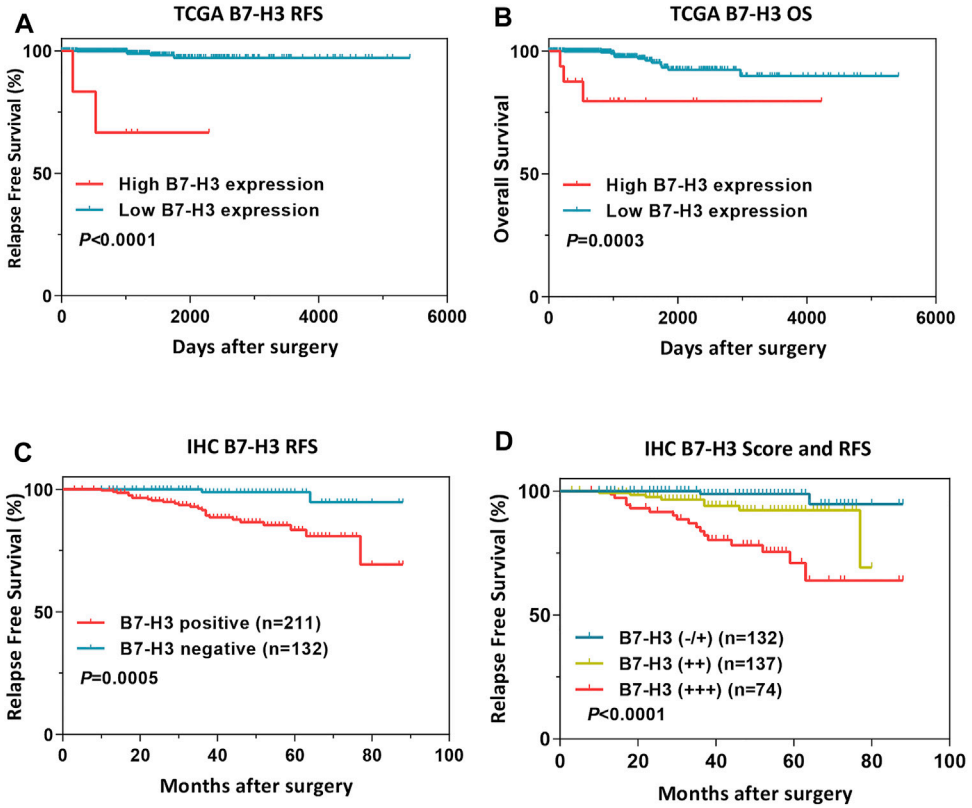

FIGURE 4 | Survival analysis of B7-H3 in PTC of TCGA dataset **(A,B)**. B7-H3 survival analysis in PTC of IHC **(C,D)**. Survival analysis based on B7-H3 expression in clinical subgroups **(E–P)**. $p < 0.05$ is considered to be statistically significant., ,

stratified using different clinicopathological variables. Using the Kaplan–Meier method and log-rank test, it was determined that B7-H3 positive staining was correlated with worse RFS in the following subgroups: tumor size ≥2 cm ($p < 0.05$), male ($p < 0.05$), age ≥55 years ($p < 0.05$), multifocality ($p < 0.05$), and ETE ($p < 0.05$) (**Figures 4E–P**).

Finally, we explored the utility of B7-H3 expression as an independent prognostic factor for PTC patients by conducting univariate and multivariate Cox regression analyses. The univariate analysis revealed that tumor length ($p < 0.001$), multifocality ($p = 0.024$), LNM ($p < 0.001$), and B7-H3 positivity in PTCs ($p = 0.004$) were significantly related to RFS, and the multivariate analysis demonstrated that these four characteristics were also independent predictors of RFS (**Table 2**, $p < 0.05$). The other selected variables were not significantly associated with PTC recurrence.

The Relationship Between B7-H3 and B7-CD28 Family Checkpoint Members

The results described above suggest that B7-H3 is a promising target for PTC treatment. The relationship between B7-H3 and B7-CD28 family checkpoints was assessed using TCGA datasets. The results showed that B7-H3 expression was significantly correlated with the expression levels of most B7-CD28 family members, and the correlations with the expression levels of PD-L1, PD-L2, CTLA4, and VTCN1 were particularly strong. These findings suggest that these markers may exert synergistic effects in the context of PTC, indicating that multicheckpoint blockade strategies may be promising treatment methods for PTC patients (**Figure 5**).

DISCUSSION

Given the high risk of metastasis and relapse associated with PTC and the limited available treatment options and lack of biomarkers for identifying potential high-risk patients, novel treatment strategies are urgently needed for the patients afflicted with this disease. The recent widespread success of immunotherapy methods targeting the B7-CD28 family members in a variety of malignancies, specifically the immune checkpoint B7-H3, has engendered hope that these strategies could also be applied to other types of cancer, including PTC. Although recent studies suggest that B7-H3 shows significant potential as a treatment target for patients with several different types of solid malignancies and aberrant B7-H3 expression has been documented in various human cancers (Benzon et al., 2017; Nehama et al., 2019; Carvajal-Hausdorf et al., 2019b; Cai et al., 2020),' a comprehensive analysis of B7-H3 expression in PTC had

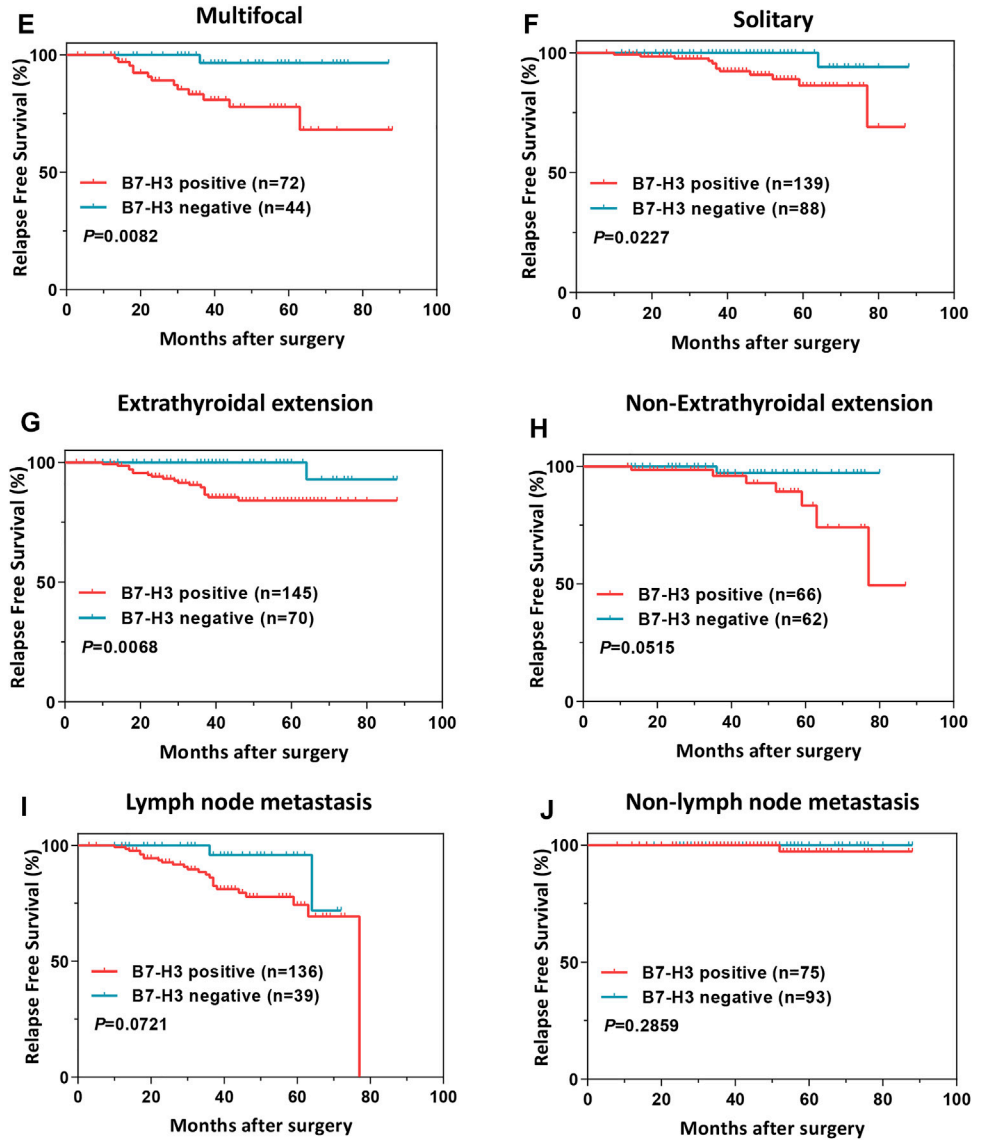

FIGURE 4 | Continued.

not been performed prior to this study. Herein, to explore B7-H3 expression profiles in PTC patients, data from a total of 1,210 samples, including 867 cases from TCGA and four GEO datasets, were collected for B7-H3–related transcriptome analyses, and 343 postoperative, whole-tumor sections were obtained from PTC patients at our institute for B7-H3–specific IHC staining.

Our analysis of TCGA and GEO datasets revealed that B7-H3 expression in PTC was conspicuously higher than that of other B7-CD28 family members, suggesting that targeting B7-H3 could be a more effective treatment strategy in comparison with targeting other B7-CD28 family members. Previous studies reported positive expression rates of B7-H3 in other human tumor tissues ranging from 37 to 76% due to the use of different scoring systems and antibodies for B7-H3 detection (Sun et al., 2006; Cheng et al., 2018; Inamura et al., 2018; Nehama et al., 2019; Kim et al., 2020). In our study, we found that B7-H3 expression was upregulated in the PTC tissues at the protein and mRNA levels, and IHC staining revealed B7-H3 protein expression in **most** of the PTC simples. In addition, our results revealed that abnormal B7-H3 expression in PTCs was closely correlated with distinct patterns of CNVs and CpG DNA methylation. These findings provide a potential mechanistic link between CNV/DNA methylation and increased expression of B7-H3, and they provide hope that the treatment strategies targeting DNA methylation regulators in combination with the administration of anti–B7-H3 monoclonal antibodies may exhibit superior curative effects for PTC patients in comparison with the currently available methods.

Given that B7-H3 protein was highly expressed on TCs and tumor-infiltrating blood vessels, while its expression level in

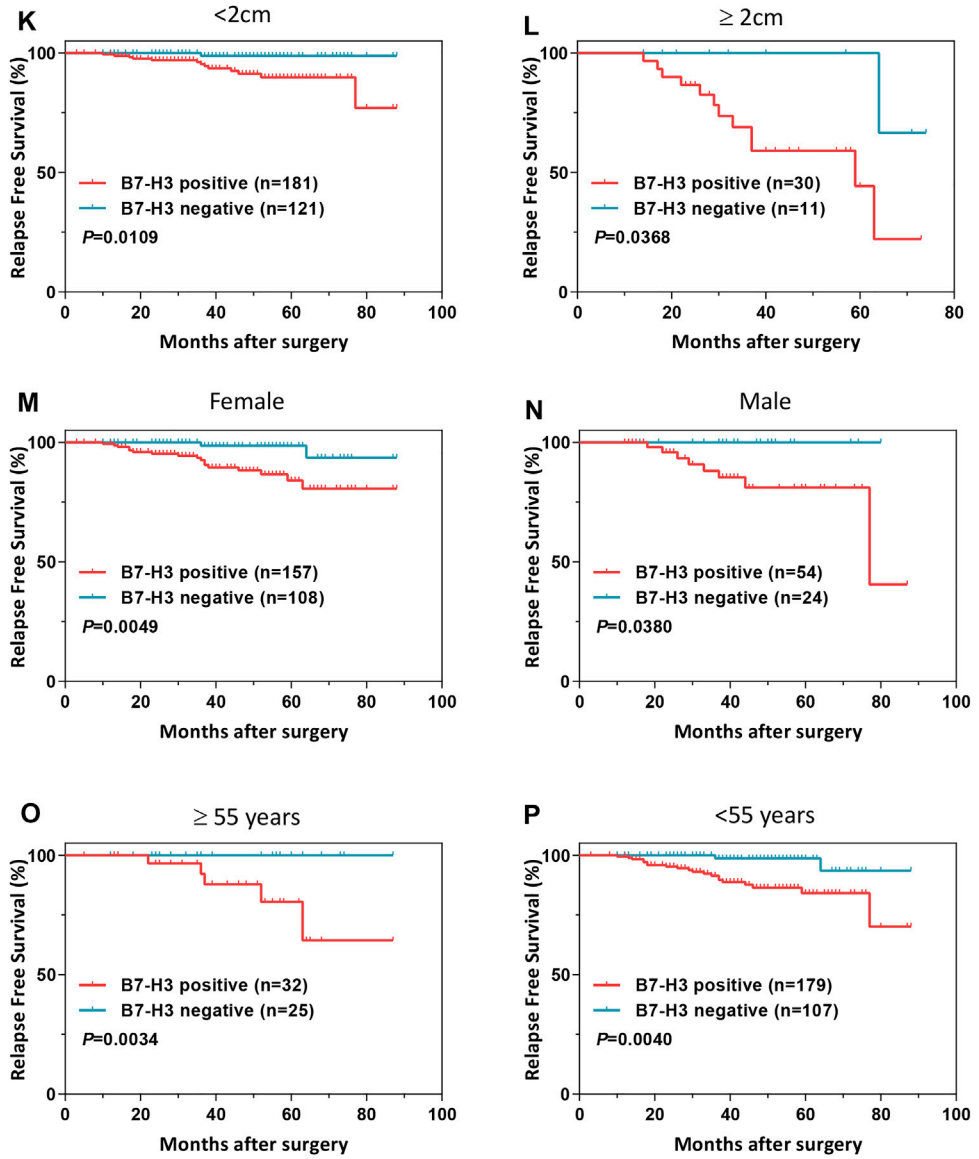

FIGURE 4 | Continued.

TABLE 2 | Univariable and multivariable Cox regression analyses for RFS in PTC patients.

Variable		Univariate analysis	*p*-value	Multivariate analysis	*p*-value
		HR (95%CI)		HR (95%CI)	
Sex	Male/female	0.698 (0.305–1.596)	0.394	1.374 (0.545–3.465)	0.501
Age	≥55/<55	1.111 (0.420–2.934)	0.832	1.057 (0.332–3.368)	0.925
Length	≥2/<2	9.034 (4.176–19.540)	**<0.001**	5.617 (2.335–13.513)	**<0.001**
Multifocality	Yes/no	2.393 (1.125–5.094)	**0.024**	3.512 (1.517–8.132)	**0.003**
Extrathyroidal extension	Yes/no	1.364 (0.597–3.118)	0.461	1.145 (0.384–3.412)	0.807
Hashimoto thyroiditis	Yes/no	0.454 (0.107–1.927)	0.285	0.383 (0.085–1.737)	0.214
Lymph node metastasis	Yes/no	40.095 (5.307–302.900)	**<0.001**	25.842 (3.015–221.522)	**0.003**
B7-H3	Positive/negative	8.369 (1.981–35.351)	**0.004**	4.566 (1.056–19.744)	**0.042**

p-value < 0.05 in bold are statistically significant. Abbreviations: HR, hazard ratio; CI, confidence interval; RFS, recurrence-free survival; PTC, papillary thyroid carcinoma.

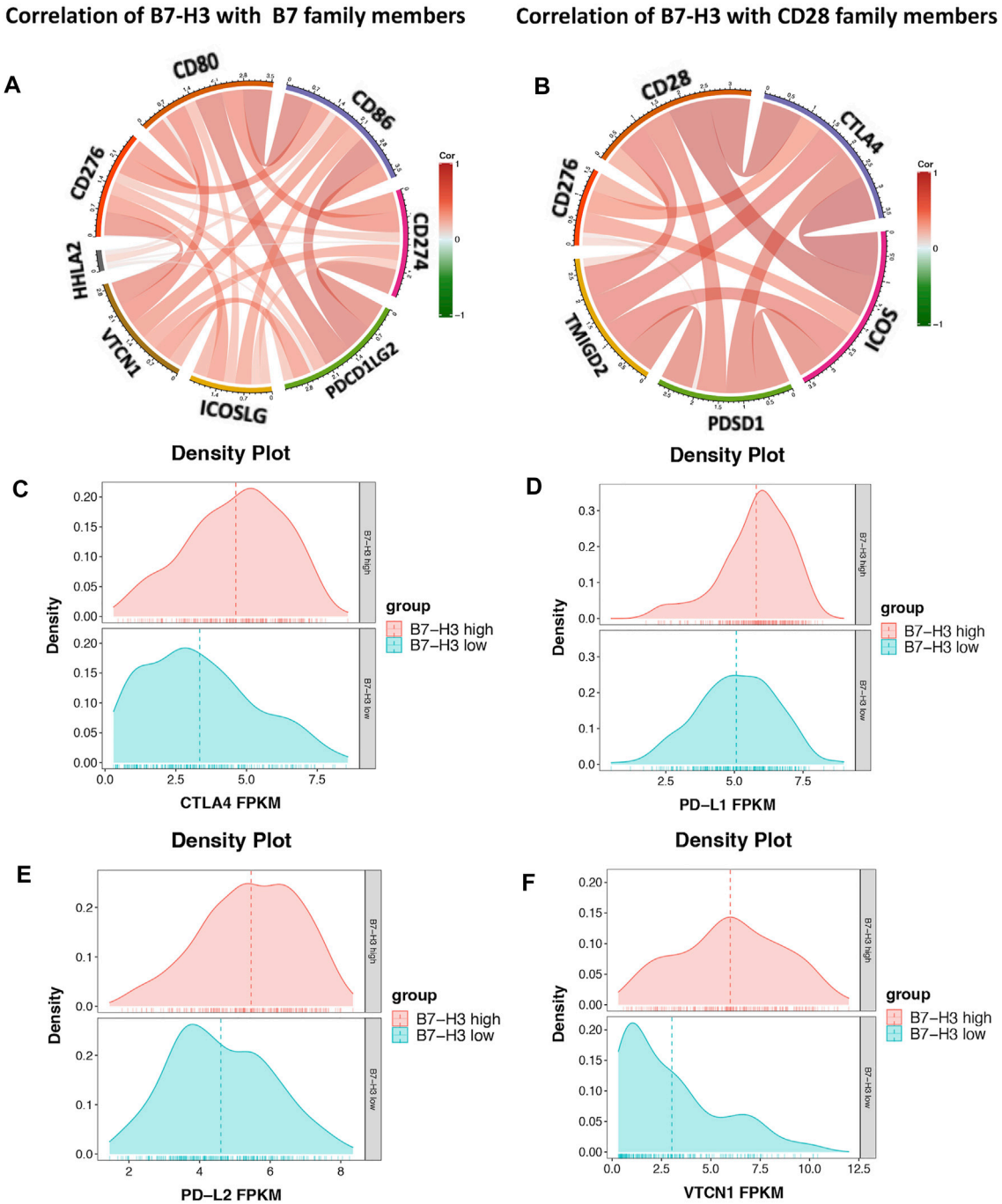

FIGURE 5 | Correlation of B7-H3 and B7 family members **(A)**. Correlation of B7-H3 and CD28 family members **(B)**. Distribution of PD-L1, PD-L2, CTLA4, and VTCN1 in high- and low-expression groups of B7-H3 **(C–F)**.

normal human tissue was low, therapeutic strategies targeting B7-H3 may be able to achieve high therapeutic efficacy against cancer cells together with low toxicity in normal tissues (Seaman et al., 2007; Cheng et al., 2018). Our GO and KEGG analyses showed that the B7-H3–related gene sets were enriched in genes related to angiogenesis and cell motility. Therefore, we speculated that high B7-H3 expression may be related to PTC lymph node metastasis and poor patient prognosis (Nilsson and Heymach, 2006; Zeng et al., 2018). Our IHC results confirmed that B7-H3 expression was associated with tumor size, ETE, LNM, and recurrence, indicating that B7-H3 plays specific roles in tumor invasion and controlling the tumor microenvironment. Many reports have shown that B7-H3 expression is related to poor prognosis in various tumors (Picarda et al., 2016; Flem-Karlsen et al., 2018). In this study, we found that high B7-H3 expression was

correlated with shorter RFS in PTC patients. The results of our survival analyses using TCGA datasets and IHC staining show that positive B7-H3 expression status is closely associated with poor prognosis for PTC patients. To verify this result, we explored the relationship between B7-H3 expression status and patient prognosis for subgroups stratified by age, ETE, LNM, and other features. Interestingly, although the B7-H3 expression status was correlated with patient prognosis for most of the subgroups, B7-H3 expression was not significantly associated with differences in RFS for the LNM and nonmetastasis subgroups (p = 0.0721). It is possible that no statistically significant relationship between RFS and B7-H3 expression was identified in this comparison because the B7-H3 expression levels of both groups were relatively high, and few subjects had low B7-H3 expression levels. More importantly, multivariate analysis was used to confirm that B7-H3 can serve as an independent prognostic factor for shorter RFS. These findings give us confidence that regardless of clinical features, B7-H3 can be used as a stable and effective prognosticator for identifying high-risk PTC patients.

Many studies have shown that B7-H3 predominantly acts as a molecular co-inhibitor at tumor sites to generate an immunosuppressive microenvironment (Suh et al., 2003; Zhang et al., 2018). The results of our study demonstrate that the expression of B7-H3 is closely related to that of most other B7-CD28 family members in PTC TCs. Promising results have been achieved with checkpoint inhibitors such as nivolumab and ipilimumab, resulting in U.S. FDA approval and widespread use in clinical settings (French et al., 2017; Larkin et al., 2019). Although the antibodies against B7-H3 are not currently used in clinical settings, the significant prognostic value of B7-H3 suggests that anti–B7-H3 monoclonal antibodies could provide significant benefits for PTC patients, especially those with advanced and iodine-resistant tumors.

In summary, we performed large-scale analyses exploring the clinical and immune features of B7-H3 in PTC patients. Our results demonstrate that the B7-H3 status of PTC TCs may serve as a predictive biomarker and facilitate the improvement of risk-adapted therapeutic strategies. However, the present analysis is limited by the potential effects of noise on our results and conclusions, so additional experiments should be performed to validate our findings.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/**Supplementary Material**. The other data used and/or analyzed during the current study are available from the corresponding author via reasonable request.

ETHICS STATEMENT

This study involving human participants were reviewed and approved by the institutional ethics committee of Cancer Hospital, Chinese Academy of Medical Sciences (NCC2021C-397). Informed consent requirements were waived due to the retrospective nature of this study.

AUTHOR CONTRIBUTIONS

BZ and ZH designed the study, performed the statistical analysis, interpreted the data, and drafted the manuscript. XZ (3rd author) and HL performed the IHC and assisted in drawing. HC, YH, XZ (6th author), and ZZ assisted in data interpretation. ZL, HL, LN, and CA supervised the study. 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/fcell.2022.819236/full#supplementary-material

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REVIEW
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Predictive Biomarkers in Thyroid Cancer

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In molecular pathology, predictive biomarkers identify which patients are likely to respond to targeted drugs. These therapeutic agents block specific molecules directly involved in cancer growth, dedifferentiation and progression. Until few years ago, the only targeted drugs available for advanced thyroid cancer included multi-tyrosine kinase inhibitors, mainly targeting the MAPK pathway and the angiogenic signaling. The administration of these drugs does not necessarily require a molecular characterization of tumors to assess the presence of predictive alterations. However, the availability of new selective targeted drugs for thyroid cancer patients is changing the diagnostic strategies for the molecular characterization of these tumors. The search for targetable alterations can be performed directly on tumor tissue by using a variety of methodologies, depending also on the number and type of alterations to test (i.e. single nucleotide variation or gene rearrangement). Herein, a comprehensive review of the currently available targeted treatments for thyroid cancer, related predictive markers and testing methodologies is provided.

Keywords: thyroid cancer, molecular marker, predictive marker, targeted therapy, molecular pathology

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INTRODUCTION

Thyroid cancer includes several histo-pathological entities, which are characterized by different histological, molecular, and clinical features. Carcinomas that originate from parafollicular cells, i.e. medullary thyroid carcinoma (MTC), should be distinguished from those derived from follicular cells. Medullary and non-medullary thyroid cancers are characterized by profound differences in terms of morphology, molecular landscape, clinical staging, and treatment (**Figure 1**).

A major distinction of thyroid carcinoma derived from follicular cells can be made depending on the degree of differentiation; these tumors can be classified as well-differentiated (WDTC), poorly differentiated (PDTC) and anaplastic thyroid carcinoma (ATC) (1). WDTCs include papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC) and Hürthle cell carcinoma (HCC). Each histotype presents peculiar morphological and molecular characteristics. Moreover, treatment strategies and prognosis could differ considerably among tumor types. PTC is the most common endocrine malignancy, and its standard treatment (thyroidectomy followed or not by radioiodine ablation) represents a definitive cure for most patients; 10-year overall survival is higher than 90% (2, 3). On the other hand, ATC is rare and frequently shows locally advanced disease and metastatic spread; survival of patients with ATC is dramatically short, with an overall survival after diagnosis of six months (4).

Until few years ago, targeted treatment options for advanced and metastatic disease included only nonselective tyrosine kinase inhibitors (TKI) (5). These agents block multiple tyrosine kinases, mostly participating to the mitogen-activated protein kinase (MAPK) pathway, frequently upregulated in thyroid cancer. Moreover, nonselective TKI have anti-angiogenic effects, targeting molecules as the vascular endothelial growth factor (VEGF) receptors. The administration of these drugs is independent of the presence of specific somatic molecular alterations.

The landscape of targeted treatments for thyroid cancer has been recently expanded. Agents that selectively block altered *RET* and *NTRK* receptors have been approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) (6). The search for predictive biomarkers has then become mandatory in thyroid cancer patients eligible for treatment.

The aim of this review is to provide a comprehensive view of predictive biomarkers that currently guide thyroid cancer patient management, taking into account the histological, molecular and clinical differences among thyroid tumor types.

THYROID CANCER: HISTOTYPES TO BE SCREENED FOR PREDICTIVE BIOMARKERS

In this section, a summary of thyroid cancer histological patterns, molecular aspects, and therapeutic protocols is provided to give an idea of which tumors deserve molecular screening for predictive purposes.

Well-Differentiated Thyroid Carcinoma (WDTC)

Among follicular-derived tumors, WDTC are common, with PTC being the most frequent malignancy affecting the thyroid gland (1). A pre-operative diagnosis of WDTC can be made by considering ultrasound and cytological findings. Then, the standard treatment protocol for this tumor includes a surgical removal of the lobe or the entire gland, with or without central and lateral neck dissection. After a post-operative assessment of disease status, radioactive iodine (RAI) therapy for minimizing the risk of disease recurrence can be either considered or not (2). The great majority of patients show an excellent response to standard treatments. Nevertheless, some WDTC patients do not respond or develop resistance to RAI therapy (3, 4). These patients frequently show aggressive tumors in terms of histo-morphology, loco-regional invasiveness, and clinical stage. For example, there are histological variants known to confer a more unfavorable outcome, such as the tall cell and the hobnail variants of PTC, and widely invasive FTC/HCC (1, 7). Although WDTC are generally characterized by a slow progression, RAI resistant advanced tumors can suddenly become locally aggressive and prone to metastatic spread and dedifferentiation (8).

The molecular landscape of WDTC deserves separate definitions for PTC, FTC, and HCC. In PTC, the most

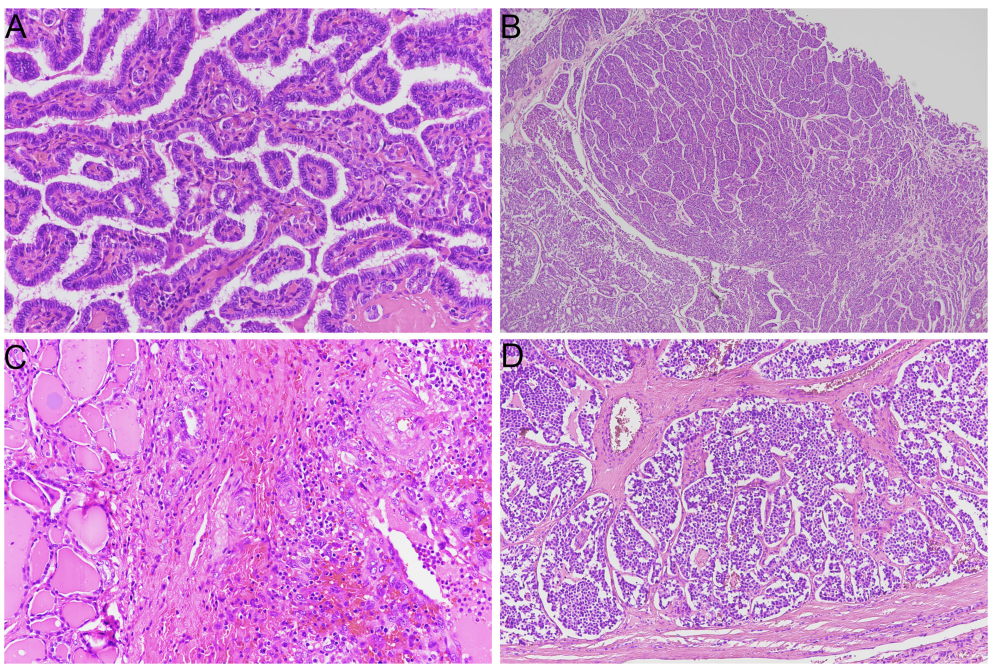

FIGURE 1 | Representative histological slides of thyroid cancer subtypes (hematoxylin/eosin stain). **(A)** papillary thyroid carcinoma (original magnification 20X); **(B)** poorly differentiated thyroid carcinoma with insular growth pattern (original magnification 4X); **(C)** anaplastic thyroid carcinoma (original magnification 20X); **(D)** medullary thyroid carcinoma (original magnification 10X).

frequently encountered molecular alteration is by far the *BRAF* p.V600E mutation (*BRAF*^{V600E}), detected in 45-50% of cases (9). However, *BRAF*^{V600E} prevalence largely varies across PTC variants. In tall cell and hobnail variants, *BRAF*^{V600E} can be present in more than 70-80% of cases; in follicular variant PTC, in less than 10% (10, 11). Based on genomic and transcriptomic data, The Cancer Genome Atlas (TCGA) study on PTC highlighted that they can be distinguished into *BRAF*^{V600E}-like and *RAS*-like tumors. *BRAF*^{V600E}-like tumors harbor also *RET* fusions, while *RAS*-like tumors show *RAS* mutations, the *BRAF*^{K601E}, and *PPARG* and *THADA* fusions (12).

By extending this definition to other WDTCs, FTCs can be considered as *RAS*-like tumors. In fact, FTCs show a high prevalence of *RAS* mutations (13). HCCs have a different set of genetic alterations, including also mitochondrial DNA mutations and aneuploidies (14, 15). In WDTCs, secondary mutations (i.e. *TP53* and *TERT* promoter mutations) are generally detected with a low frequency, which become higher in aggressive variants and in advanced stage, RAI resistant and metastatic tumors (9).

Biomarkers able to predict RAI responsiveness of WDTC have been proposed. In particular, *TERT* promoter mutations, especially when detected in co-occurrence with *BRAF* or *RAS* mutations, have been associated to RAI resistance (16–18). However, no molecular testing to guide RAI therapy administration is currently recommended by clinical guidelines.

On the contrary, in the advanced setting, patients are more likely to become potentially eligible for treatment with targeted drugs, therefore they should be screened for predictive molecular alterations. Although there is controversy on the optimal timing for performing molecular testing, a molecular screening of all WDTCs, independently of the therapeutic implications, is not currently recommended (4).

In practical terms, based on the molecular hallmarks, FTC and HCC are subtypes with a very limited set of targetable alterations (19). In fact, in these tumors, the *BRAF*^{V600E} is virtually absent, and actionable rearrangements are extremely rare, independently of the tumor stage and aggressiveness. On the other hand, PTCs harbor more frequently targetable alterations, but these are restricted to *BRAF* and *RET* fusions and mostly limited to classic and tall cell subtypes (20).

Poorly Differentiated Thyroid Carcinoma (PDTC) and Anaplastic Thyroid Carcinoma (ATC)

PDTC and ATC are aggressive tumors characterized by partial or total loss of differentiation, respectively. PDTCs show a poorer prognosis compared to WDTC, but a better 5-year overall survival (60-85%) compared to ATC (4, 21). For this reason, PDTCs are often considered morphologically and clinically halfway tumors between WDTC and ATC. In the past, a multistep evolution model for explaining thyroid cancer progression was proposed. According to this model, ATC and PDTC could derive directly from their well-differentiated counterparts, through the progressive acquisition of genetic alterations (5, 22, 23). More recently, the evidence derived from extensive molecular characterization of coexisting DTC and ATC, sustains the hypothesis of an early, independent evolution of tumor clones (24). It can be hypothesized that some ATCs emerge after a molecular and morphological evolution of DTCs, while others have a DTC-uncorrelated origin.

PDTC and ATC commonly harbor *BRAF* and *RAS* mutations, and often these mutations coexist with secondary genetic alterations, such as *PIK3CA*, *TP53* and *TERT* promoter mutations (9). However, the typical molecular frame of PDTC and ATC is different. For instance, PDTCs are more frequently gene fusion-driven tumors, while in ATCs gene fusions are rare. In addition, ATCs show a significantly higher prevalence of mutations in *TP53* gene (22, 23, 25, 26).

There are no dedicated treatment protocols for PDTC; thyroidectomy followed by RAI therapy offers a good local disease control, but the main cause of death is the metastatic spread (21, 27). Since PDTC show a relatively high frequency of gene rearrangements, molecular testing for potentially targetable alterations could offer important therapeutic options.

For patients with ATC, treatment options can have both therapeutic and palliative purposes. Tumor resection is considered depending on the extent of local invasion. In patients with systemic disease, thyroidectomy can help avoid future complication (i.e. airway obstruction). In unresectable tumors, neoadjuvant radiotherapy and/or chemotherapy can be considered (28). The use of chemotherapy regimens as systemic therapy for disease control highly depends on the established goals of care. Since targeted treatments are available for ATC patients, the recent American Thyroid Association guidelines recommend that molecular screening is performed at the time of diagnosis, so that clinicians can rapidly plan the most appropriate therapeutic strategy (28).

Medullary Thyroid Carcinoma (MTC)

MTC is a rare neuroendocrine tumor originating from parafollicular cells. MTCs can be either hereditary or sporadic (20-25% and 75-80% of all cases, respectively) (29); histologically, there is no morphological difference between the two forms, but familial MTCs show more frequently c-cell hyperplasia and tumor multifocality (30). Hereditary MTC can be diagnosed either alone (familial MTC) or as part of the spectrum of Multiple Endocrine Neoplasia (MEN) type 2 syndromes, which are characterized by the presence of germline mutations in *RET* gene. Therefore, all inherited MTCs are virtually *RET*-driven (31). In sporadic MTCs, *RET* mutations are detected at somatic level in about 50-60% of cases, while *RAS* mutations have a prevalence of about 20-30% (31, 32).

MTC treatment and prognosis highly depend on disease stage at presentation. When there is no evidence of systemic involvement, thyroidectomy represents an effective strategy to minimize the risk of local recurrence (31). The extent of surgery (central and lateral compartment dissection) is established upon ultrasound findings and serum calcitonin levels. In locally advanced and metastatic setting, thyroidectomy is considered after careful cost-benefit evaluation; systemic therapies can help

the control of disease. In children at risk of developing MTC (i.e. *RET* carriers), a prophylactic thyroidectomy can be also considered (31).

The algorithm for molecular diagnostics can be different for hereditary and sporadic MTC. It has to be specified that patients with suspected hereditary or familial MTC should be subjected to germline *RET* testing and genetic counselling. Also patients with presumed sporadic MTC should be referred to germline *RET* testing since, although rarely, they could be actually affected by hereditary forms (31). In case of advanced unresectable or metastatic MTC with germline *RET* testing not available or negative, a molecular screening should be performed on tumor tissue to search for somatic *RET* mutations (33).

TARGETED THERAPY FOR THYROID CANCER

In oncology, the terms "targeted therapy" indicate the use of anti-cancer drugs that target specific proteins involved in cancer growth and survival. The goal of these treatments is to inhibit tumor cell proliferation, with limited effect on normal cells.

Inhibitors that act on multiple kinases (multi-TKIs), i.e., nonselective targeted drugs, have been FDA-approved for treatment of both medullary and non-medullary thyroid cancer patients in advanced/metastatic setting. Efficacy of these inhibitors has been demonstrated in several clinical trial in terms of improved progression-free survival compared to placebo (5, 34).

Although predictive biomarkers of response to these drugs have been proposed and are still under investigation, no molecular characterization of tumors is needed before treatment. In fact, no clear association has been demonstrated between the presence of *BRAF*/*RAS* mutations and drug efficacy (4).

FDA-approved nonselective inhibitors for treatment of thyroid cancer are shown in **Table 1** (https://www.fda.gov, last accessed on 07/03/2022). Most of these agents not only act as inhibitors on tumor growth and proliferation, but they have also antiangiogenic effects.

On the other hand, in the context of appropriate clinical indications, there are targeted drugs that act selectively on specific altered molecules, and thus are administered only if the tumor harbors specific molecular alterations. In other words, the presence of certain molecular characteristics makes the patient eligible for a specific treatment. In this case, molecular testing of tumor become mandatory.

Predictive Biomarkers in Thyroid Cancer: Must-Test Genes

In this paragraph, targeted drugs currently approved for patients harboring specific genetic alterations are treated. According to OncoKB database (https://www.oncokb.org, last accessed on 17/03/2022), the number of actionable alterations in thyroid cancer is very limited. In detail, genes associated with predictive alterations with level of evidence 1, i.e., FDA approved drug in the specific indication, are listed in **Table 2** (https://www.fda.gov). There are no actionable genes with level of evidence 2 (biomarkers that are not FDA-recognized but indicated as predictive of response to an FDA-approved therapy by clinical guidelines).

BRAF inhibitors such as dabrafenib and vemurafenib have been largely investigated in the treatment of advanced thyroid cancer. The efficacy of these agents in DTC patients seems promising, especially in the light of the overall high frequency of *BRAF* mutations in follicular-derived thyroid cancer. Nonetheless, the only histotype with a specific anti-$BRAF^{V600E}$ approved inhibitor is ATC in locally advanced or metastatic setting. In $BRAF^{V600E}$-driven ATC patients, dabrafenib in combination with trametinib, a MEK inhibitor, has shown 69% overall response rate (35).

Selective *RET* inhibitors have been approved for advanced *RET*-altered thyroid cancer and non-small cell lung cancer. These inhibitors seem highly effective in the treatment of medullary and non-medullary thyroid cancers, and they also show acceptable safety profiles (36–40).

The approval of drugs for tumor-agnostic treatment, virtually accessible to all patients with advanced stage tumors, highlighted that molecular characterization of tumor is becoming increasingly important for patients' management. The agnostic approval of *NTRK*-rearranged selective inhibitors has derived from two clinical trials demonstrating their safety and efficacy in terms of response rate in several solid tumors (41–43).

TABLE 1 | List of nonselective TKIs that are currently approved by FDA for the treatment of advanced thyroid cancer patients.

Drug name	Agent type	Main targets	Biological effects	Indication
Vandetanib	Multi-TKI	*EGFR*, *VEGFR*	Inhibition of tumor growth and angiogenesis	Unresectable locally advanced/metastatic MTC
Cabozantinib	Multi-TKI	*MET*, *VEGFR*, *AXL*, *RET*, *ROS1*, *TYRO3*, *MER*, *KIT*, *TRKB*, *FLT-3*, *TIE-2*	Inhibition of metastasis, angiogenesis, and maintenance of tumor microenvironment	Progressive, metastatic MTC; locally advanced/metastatic RAI-refractory DTC progressing following VEGFR-targeted therapy
Sorafenib	Multi-TKI	*BRAF**, *KIT*, *FLT3*, *RET*, *VEGFR*, *PDGFRB*	Inhibition of tumor cell signaling, angiogenesis and apoptosis	Locally recurrent/metastatic, progressive RAI-refractory DTC
Lenvatinib	Multi-TKI	*VEGFR*, *FGFR*, *PDGFRA*, *KIT*, *RET*	Inhibition of angiogenesis, tumor growth and progression	Locally recurrent/metastatic, progressive, RAI-refractory DTC

TKI, tyrosine kinase inhibitor; MTC, medullary thyroid cancer; RAI, radioactive iodine; DTC, differentiated thyroid cancer.
**Including mutant BRAF*

TABLE 2 | List of selective targeted drugs FDA-approved for thyroid cancer treatment.

Targeted agent	Target	Predictive marker	Thyroid cancer histotype*
Dabrafenib and Trametinib in combination	*BRAF*, *MEK*	*BRAF* p.V600E	locally advanced/metastatic ATC
Selpercatinib, Pralsetinib	*RET*	*RET* mutation *RET* fusion	Advanced/metastatic MTC Advanced/metastatic, RAI-refractory thyroid cancer
Entrectinib**, Larotrectinib	*TRKA*, TRKB, *TRKC*	*NTRK* fusion	Unresectable/metastatic tumor progressing following prior treatment (tissue-agnostic)
Pembrolizumab	*PD-1*	MSI-H, TMB-H	Unresectable/metastatic tumor progressing following prior treatment (tissue-agnostic)

**As indicated by the FDA; includes tumor-agnostic drugs.*

***Entrectinib has also activity on ROS1 and ALK receptors.*

The predictive alteration and the specific indication for drug administration are also indicated. ATC, anaplastic thyroid cancer; MTC, medullary thyroid cancer; RAI, radioactive iodine; DTC, differentiated thyroid cancer; MSI-H, microsatellite instability – high; TMB-H, tumor mutational burden – high.

When considering predictive marker testing, it is necessary to specify that molecular screening should be guided also by (i) the prevalence of a predictive alteration in each tumor type and (ii) the evidence of drug efficacy in a specific clinical setting. For example, treatment of thyroid cancer with NTRK selective inhibitors seems to induce potent and durable responses (44, 45). *NTRK1* and *NTRK3* fusions are detected in 2-4% of thyroid tumors in adults, while *NTRK2* fusion have never been described (9).

As regards immunotherapy targeted agents, data on treatment efficacy in thyroid cancer patients is limited. Response rates in patients with advanced DTC and ATC for the anti-PD-1 monoclonal antibody drug (pembrolizumab) monotherapy are generally low (46–48). Currently, several phase II clinical trials evaluating immunotherapy agents in thyroid cancer, including pembrolizumab monotherapy, are ongoing (49–51).

As regards immunotherapy predictive biomarkers, it is known that thyroid tumors generally do not show a high tumor mutational burden (TMB-H) and high microsatellite instability (MSI-H) (23, 52–54). For ATC, it has been hypothesized that response to pembrolizumab is independent of MSI status and TMB (55). Alternative predictive biomarkers to immunotherapy response have been largely investigated. Specifically, the expression of PD-L1 assessed by immunohistochemistry represents a useful biomarker for other cancer subtypes, but, also in this regard, evidence on its predictive role in DTC and ATC are limited (56).

Another crucial aspect to consider for immunotherapy is that, currently, the EMA does not recommend using pembrolizumab in a tumor-agnostic setting (https://www.ema.europa.eu, last accessed on 07/03/2022). For all these reasons, testing of TMB and MSI status is not always performed in thyroid cancer.

Selective Inhibitors in Thyroid Cancer: New Clinical and Molecular Challenges

Although targeted drugs demonstrated highly effective in the treatment of advanced thyroid cancer, there are several issues that deserve further discussion.

First, targeted agents such as TKIs have generally positive effects on patients' survival, but they do not allow a full recovery. Duration of response is always limited, and drug resistance cannot be avoided; the development of acquired resistance to TKI treatment invariably causes disease progression. The available data are related to resistance mechanisms emerging after TKI therapy in other tumor types, mainly in non-small cell lung cancer. It is known that molecular mechanisms causing resistance to selective TKIs can either activate alternative signaling pathways – off-target alterations – or directly interfere with drug binding – on-target alterations. In *RET*-rearranged non-small cell lung cancer, both types of resistance mechanisms have been described in patients treated with *RET*-inhibitors (i.e., *RET* mutations, *KRAS* and *MET* amplification) (56, 57). In *RET*-mutant MTC patients, acquired *RET* mutations have been associated with resistance to selpercatinib (57–59). Similarly, resistance mutations in *NTRK* genes emerging following targeted therapy have been described in several cancer types (60, 61). In thyroid cancer patients treated with selective BRAF inhibitors, the emergence of acquired mutations in RAS genes has been described as a resistance mechanism (62).

The development of resistance mechanisms represents an important challenge not only for clinicians, but also in molecular pathology. The laboratory should provide clinicians with rapid and usable results: to limit tissue re-biopsy, the use of liquid biopsy should be implemented; multi-target, highly sensitive methodologies should be used; laboratories should be able to test also uncommon alterations, such as copy number variations and tumor microenvironment alterations. In fact, the identification of resistance mechanisms can guide strategies to counteract cancer progression (63). In lung cancer, the emergence of resistance mutations in plasma can predict disease progression even before it becomes clinically evident (64).

The second most important downside of TKIs is the not negligible portion of patients experiencing adverse events. Although TKIs show lower toxicity profiles than conventional systemic therapies, adverse effects affecting multiple organs have been reported (5, 65, 66). The main adverse events include hypertension, gastrointestinal toxicity, fatigue; the most common endocrine-related toxicity affects thyroid function and thyroid hormone metabolism (67). The clinical spectrum of toxicities is variable, from minimal side effects manifestations to severe toxicities that lead to treatment discontinuation. Highly selective TKIs seems to show lower toxicity profiles compared to nonselective TKIs, likely due to a reduced off-target activity (38, 68).

Targetable Alterations Outside of Approved Indication

Molecular testing can reveal the presence of predictive alterations for targeted drugs that are approved in other settings. If a targeted drug is approved for tumor type "A", treatment is virtually inaccessible to patients with tumor type "B", even though the specific predictive alteration is detected. In case of advanced, progressing thyroid tumors with no satisfactory alternative therapies, patients with predictive alterations can be enrolled either in clinical trials or in compassionate programs – if available, and if the inclusion criteria are met. Evidence of response rates of thyroid cancer patients following targeted drugs administration outside of approved indications mostly derives from case reports. In this context, treatment with *ALK* inhibitors in *ALK*-rearranged thyroid cancer patients showed promising results in terms of response rate and disease control (19, 69, 70).

A prospective, non-randomized clinical trial (NCT02925234) is enrolling patients with potentially targetable alterations who have exhausted alternative treatment options. This trial can be defined as a pan-cancer multi-drug program, in which patients carrying a druggable alteration have access to the specific targeted agent (71). The aim of the protocol is to evaluate response to treatment and survival rates of several anti-cancer drugs in different settings. The results of this clinical trial are likely to influence future tumor-agnostic drugs approval.

TESTING METHODOLOGIES AND PLATFORMS

Predictive biomarkers can be tested by a variety of methodologies and platforms. Commercial products both designed for thyroid cancer and pan-cancer panels are available. To give a short but practical overview on this topic, testing platforms can be divided according to the number and type of targets that should be tested. First, the number of actionable alterations in thyroid cancer is very limited, as already mentioned. Therefore, samples do not need particularly extensive molecular characterization. Moreover, the molecular landscape of thyroid tumors consists in mutually exclusive driver alterations that recur in few genes (12). In this regard, a cost-effective strategy can be represented by a first level analysis including *BRAF* and *RAS* genes, followed by further analyses in case of negative results. The cost-effectiveness of this kind of strategy compared to a deeper, multi-target molecular characterization performed up front highly varies according to different institutions. In fact, it is influenced by the number of samples, available facilities, type of gene panels, reimbursement policies and many other factors. Moreover, it has to be highlighted that an extensive molecular characterization could provide information on uncommon but targetable alterations (72). In advanced MTCs with negative or unavailable germline *RET* testing, the search for *RET* mutations should be conducted on tumor tissue; *RAS* mutations can be also detected in these tumors (33). Since it is advisable to analyze the entire coding region of *RET* gene, a next-generation sequencing (NGS) analysis can be more suitable compared to single target techniques.

Considering the type of predictive alterations, the recently approved targeted drugs against rearranged *RET* and *NTRK* genes complicated the picture of must-test alterations. Gene rearrangement analysis can be performed at three different levels: a) on DNA, by using NGS platforms, or *in-situ* methodologies; b) on RNA, with reverse-transcription PCR, by using specific primer pairs, NGS, or digital counting systems (nCounter nanoString); c) on protein, with immunohistochemistry (IHC) analysis, by using specific antibodies detecting an aberrant protein expression. Moreover, techniques can be either one-gene-one-test (such as IHC and fluorescent *in situ* hybridization, FISH) or multi-gene (NGS, nCounter system). Each technique has advantages and limitations. A screening based on IHC analysis would be extremely useful to select samples deserving molecular confirmation. However, in thyroid cancer, IHC could be considered not sufficiently accurate for *RET* and *NTRK* rearrangements (33, 73, 74). FISH represents a valuable *in situ* technique. The use of break-apart probes allows to identify a fusion event with no prior knowledge of the involved partner. It requires one tissue section per gene; therefore, it could be a sub-optimal choice in case of multiple targets to investigate. Moreover, FISH could miss small/intrachromosomal rearrangements, which are relatively frequent in *RET* gene (75). Several commercial RT-PCR tests have been validated for diagnostic use. Usually, specific primer-probe pairs are designed to anneal directly on the breakpoint region. The possibility to include probe pairs detecting multiple rearrangements in a single assay is technically limited, therefore several assays are necessary to cover the main fusion events. Therefore, FISH and RT-PCR could miss important information, but represent valuable instruments when NGS analysis is not available or not successful.

In molecular pathology, NGS analysis is becoming increasingly widespread. NGS is a high throughput technique that can be performed both on DNA and RNA. Several gene panels, also validated for diagnostic use, and different testing platforms are available. Accordingly, the number of targets is variable, but virtually NGS allows to analyze simultaneously different types of clinically relevant alterations, including single nucleotide variations, deletions, insertions and also gene fusions. Therefore, NGS represents a valuable resource for the analysis of all the required predictive biomarkers in thyroid cancer. Nonetheless, the analysis of rearrangements by NGS presents some disadvantages. For instance, DNA-based panels could miss rearranged cases compared to RNA-based ones (76). This is mainly due to the involvement of large intronic regions that make rearrangement detection technically challenging. Moreover, DNA analysis does not give information on the functionality nor on the expression of the fusion. NGS analysis performed on RNA can overcome these issues, but in turn presents an important technical limitation: it requires good quality RNA. RNA purified from formalin-fixed paraffin-embedded tissue is generally highly fragmented. Poor RNA

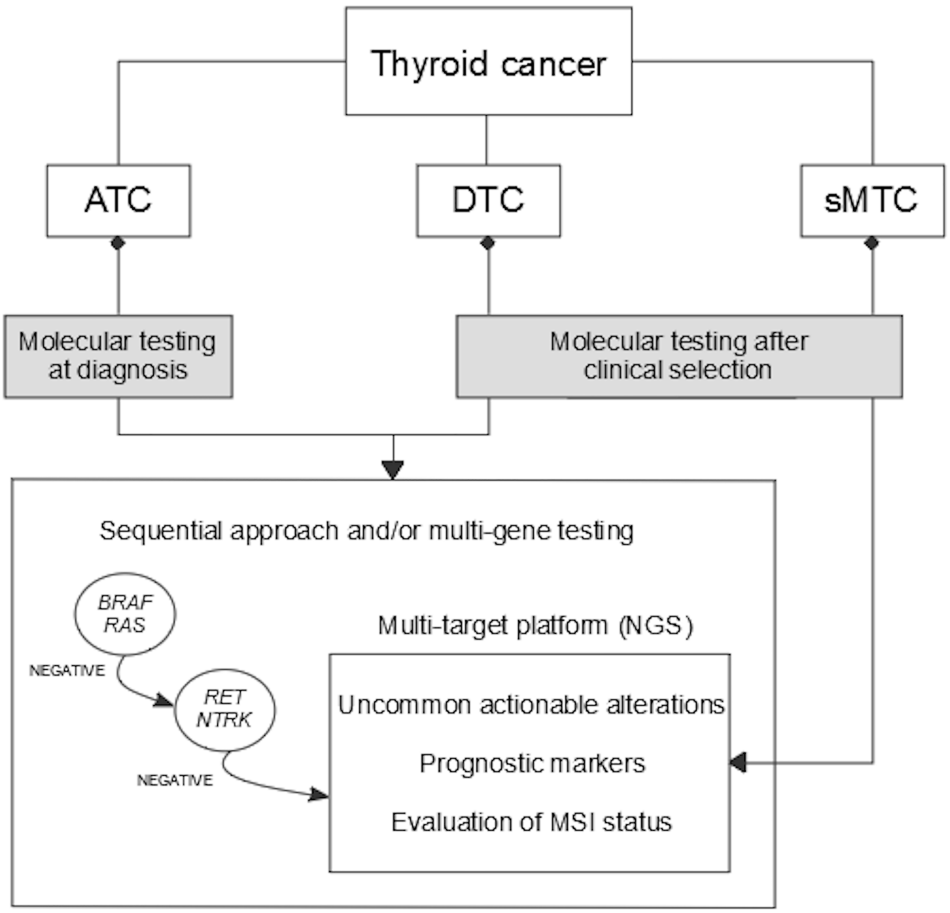

FIGURE 2 | Algorithm for molecular diagnostics of predictive markers in thyroid cancer. ATC, anaplastic thyroid cancer; DTC, differentiated thyroid cancer; sMTC, sporadic medullary thyroid cancer; MSI, microsatellite instability; NGS, next-generation sequencing.

quality can cause sequencing failure and also false negative results. In this regard, optimization of pre-analytical processing of tissue specimens is highly required (77, 78).

CONCLUSIONS

During the last 10 years, the therapeutic strategies for patients with advanced thyroid cancer have been expanded thanks to the availability of new, effective targeted drugs. In this setting, the molecular screening of tumors has acquired a crucial role in the management of patients, as also highlighted by the introduction of agnostic drugs, namely agents that are effective on tumors carrying a specific genetic alteration, regardless of cancer histotype.

Herein, a global view of predictive biomarkers analysis in thyroid cancer is given. An overall summary of which, where and when predictive biomarkers should be tested is illustrated in **Figure 2**. A molecular screening including the must-test alterations should be always performed in patients who are clinically eligible for targeted drug treatment. In addition, an extensive molecular analysis of tumors can be performed to eventually reveal the presence of uncommon actionable alterations, which might offer relevant therapeutic options.

However, as highlighted in the present review, for a not negligible portion of patients who lack the main targetable molecular alterations, treatment alternatives have been not significantly improved. The investigation of novel targeted agents and a better identification of escape pathways involved in drug resistance can help overcome the current limitations of targeted treatments.

AUTHOR CONTRIBUTIONS

Conceptualization, EM and FB; literature search, EM, AMP, PV, and AB; data analysis, EM, AMP, PV, AP, CU, LT, and AB; data curation, all authors; writing, original draft preparation, EM; manuscript review and editing, EM, AMP, PV, and AB; supervision, FB, FS, and RE. All authors have read and approved the content of the manuscript.

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Advanced Differentiated Thyroid Cancer: A Complex Condition Needing a Tailored Approach

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Differentiated thyroid cancers (DTCs) are slow-growing malignant tumours, including papillary and follicular carcinomas. Overall, prognosis is good, although it tends to worsen when local invasion occurs with bulky cervical nodes, or in the case of distant metastases. Surgery represents the main treatment for DTCs. However, radical excision is challenging and significant morbidity and functional loss can follow the treatment of the more advanced forms. Literature on advanced thyroid tumours, both differentiated and undifferentiated, does not provide clear and specific guidelines. This emerges the need for a tailored and multidisciplinary approach. In the present study, we report our single-centre experience of 111 advanced (local, regional, and distant) DTCs, investigating the rate of radical excision, peri-procedural and post-procedural complications, quality of life, persistence, recurrence rates, and survival rates. Results are critically appraised and compared to the existing published evidence review.

Keywords: thyroid, thyroid cancer, papillary carcinoma, follicular carcinoma, differentiated carcinoma, thyroidectomy, lymph node dissection

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INTRODUCTION

Thyroid cancer accounts for less than 1% of all malignancies, but for about 5% of all thyroid nodules (1). Among thyroid cancers, differentiated thyroid carcinoma (DTC) is the most common and includes papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), and Hürthle cell thyroid carcinoma (HTC) (2). DTC has an annual incidence of 1/10,000 with a female-to-male ratio of 3:1 (3, 4). DTC incidence is increasing, most probably due to the continuous improvement offered by early diagnostic tools (4–6). DTCs have a favourable prognosis, with a 5-year survival rate of approximately 93% and 88% in women, and men, respectively (4), although unfortunately, 5% of DTCs are fatal (3). Surgery represents the initial treatment of choice (7, 8), followed in most cases by radioactive iodine (RAI) I^{131} to eradicate microscopical disease or distant metastases. In addition, multikinase inhibitors (MKIs), including sorafenib and lenvatinib, are rising as alternative medical treatments in the most advanced forms, especially in RAI-refractory differentiated thyroid cancer (RR-DTC). Advanced disease accounts for 13%–15% of DTCs and is characterized by a worse

prognosis and more challenging management (5). Advanced DTC (ADTC) is defined by the existence of one or more of the following conditions: local invasion, bulky cervical nodes, and/or distant metastases. A recent review by Russel et al. describes all the issues associated with advanced thyroid cancer, from the lack of adequate guidelines and the need for a multidisciplinary and tailored approach. This is necessary to overcome the challenges of the distinct DTC subtypes, such as those related to surgical radicality, meaning not only total thyroidectomy and lymphadenectomy, but even tracheal ring resection, partial or total laryngectomy, or thoracic access. Based on the aforementioned study (4), the aim of the present manuscript is to critically review our single-centre experience of advanced thyroid cancer (local, regional, and distant), but focusing specifically on ADTC, thus not dealing with medullary, anaplastic carcinoma, or other undifferentiated cancers, because of their worst prognosis and the limited therapeutic options and surgical strategies currently available. Herein, we provided our DTC 30-year experience on therapeutic approach, peri-procedural and postsurgical management, quality of life disease recurrence, and survival rates.

MATERIALS AND METHODS

A retrospective analysis of medical and electronic charts of a single-centre series comprised 656 thyroid tumours, 627 of which are DTCs (333 PTC, 92 FTC, 202 papillary microcarcinoma), 21 medullary thyroid carcinomas, 1 squamous cell carcinoma, and 7 anaplastic tumours. An exception from Institutional Review Board evaluation was granted due to the study's retrospective nature. Our experience herein reported involved 111 consecutive patients diagnosed with ADTC who underwent a surgical assessment between May 1992 and September 2021 at the Outpatient Endocrine Surgery Service, Thyroid and Parathyroid Surgery Unit, in San Paolo Hospital, Milan, Italy. Before surgery, our protocol included blood tests, with particular attention to the TSH, calcium, PTH, 25 – OH vitamin D, and calcitonin blood levels. Neck ultrasound was always the first imaging examination, to evaluate for thyroid nodules and cervical lymph nodes. All patients with ultrasonographical suspicion of nodules and/or lymph node involvement underwent ultrasound-guided fine-needle aspiration (FNA) to confirm DTC cytologically. In case of suspicion of infiltration of surrounding tissues, further targeted radiological examinations, such as neck and chest CT, were performed.

All patients underwent a preoperative evaluation with video-laryngoscopy by an expert otolaryngologist. All ADTC cases were discussed pre- and postoperatively by a multidisciplinary team (MDT) consisting of an endocrinologist, an endocrine surgeon, an otolaryngologist, a pathologist, a radiologist, and a nuclear medicine physician (**Figure 1**).

Risk factors in the patient's medical history, such as family and personal history of thyroid disease, cancer, goitre endemics, and radiation exposures, were also analysed. Symptom onset was identified when the presence of local complaints such as dysphagia, dysphonia, dyspnoea, sensation of cervical encumbrance and cervical dysmorphism, lymphadenopathies, or distant metastases appeared. Patients were divided into the following classes according to the surgery performed: total thyroidectomy with adjacent structure resection and centra cervical lymphadenectomy (TT + CCL), total thyroidectomy and central and unilateral cervical lymphadenectomy (TT + CUCL), ;total thyroidectomy and central and bilateral cervical lymphadenectomy (TT + CBCL), unilateral cervical lymphadenectomy (UCL), and bilateral cervical lymphadenectomy (BCL) after previously performed thyroidectomy. "Cervical lymphadenectomy" was intended as modified radical neck dissection, including II, III, IV, and V levels. Only five patients, underwent additional resection of distant metastases. We systematically started the use of intraoperative inferior laryngeal nerve neuromonitoring in 2018. We also analysed the rate of complications and divided them into transient hypocalcaemia (TH), persistent hypocalcaemia (PH), recurrent laryngeal nerve transient unilateral palsy (RLNTUP), recurrent laryngeal nerve transient bilateral palsy (RLNTBP), recurrent laryngeal nerve persistent unilateral palsy (RLNPUP), recurrent laryngeal nerve persistent bilateral palsy (RLNPBP), superior laryngeal nerve deficit (SLD), and other complications. Moreover, we analysed the total excision rate and indications to perform postoperative RAI therapy or other adjuvant treatments. Of the 111 patients recruited, 53 were on regular follow-up (FU) at our Centre. Complete data were available on disease-free survival, total survival, and recurrence. Of the remaining 58 patients, we could not obtain up-to-date information, and they were therefore considered lost to follow-up. After treatment, all patients received levothyroxine

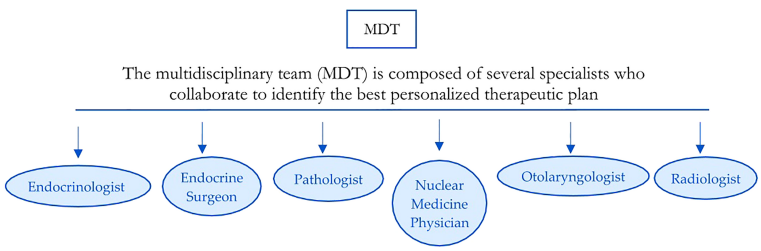

FIGURE 1 | Multidisciplinary team.

TSH suppressive therapy and were entrusted to the attending endocrinologists for follow-up treatment. All ADTC patients were considered high-risk and underwent periodic blood checks consisting of TSH, thyroglobulin, and antithyroglobulin antibodies, as well as a cervical ultrasonography (US) every 6–12 months, for an average period of 15 years (range 2–30 years).

RESULTS

Population

A total of 111 patients were enrolled in our study, 74 women and 37 men. The median age is 45.8 ± 19.5 SD years of age. Women were in the age ranges of 19 to 93, and men ranging between 11 and 81 years, with women's mean age of 46.4 ± 20.4 and men's 44.4 ± 17.9. We enrolled and treated exclusively in the abovementioned centre 96 patients, while 15 had previous thyroid surgery in other Italian centres.

Clinical History

Family history of cancer thyroid disease was present in 26/111 patients (23.42%), in particular 7 men (18.92%) and 19 women (25.68%). We identified 53 patients (47.75%) coming from areas known for endemic goitres; 17 are men (45.95%), and 36 are women (48.65%). Of our sample, 35 women (47.30%) were menopausal at the time of diagnosis. The clinical characteristics of the patients enrolled in the study are briefly summarized in **Table 1**.

Clinical Presentation

At the time of diagnosis, 76/111 (68.5%) patients had lymph node metastases, and the presence of a cervical mass was the problem that initiated the diagnostic process, which then led to the discovery of DCTs.

In the other cases, at the time of diagnosis, 22/111 patients (19.8%) presented with a locally advanced tumour characterized by the involvement of adjacent anatomical structures. All of them presented with local symptomatology and thus were referred for clinical evaluation. In this group of 22 patients, 15 were women and 7 were men, respectively 18.92% and 21.62% of their groups. The most frequent symptoms were dysphonia (8/22 cases), dysphagia (3/22 cases), and cervical encumbrance sensation (19/22 cases).

For six out of 111 (5.4%) patients, the tumour was occasionally detected incidentally during clinical examination conducted for other reasons and they were asymptomatic at the time of diagnosis.

In our study, seven out of 111 (6.3%) patients were diagnosed with DCTs after clinical examinations for distant metastasis presence, initially unknown, but later identified as a repetition from DCT. The distant metastases included the following groups: bone metastasis of the mandible, liver metastasis, lung and liver metastasis, two isolated lung metastases, one multiple lung metastasis, and multiple bone metastasis of three ribs.

After preoperative blood tests, five out of 111 cases of reduced TSH levels were detected (normal range 0.15–3.5 U/ml); two out of 111 patients had increased TSH levels, while the remaining patients were within the normal range. Only four out of 111 patients were hypocalcemic, but three previously underwent TT at different Italian centres. Only a single patient was hypercalcemic.

TABLE 1 | Clinical features of patients (n 111).

Clinical features	Value
Sex *n* (%)	74 (66.7%)
Female (F)	37 (33.3%)
Male (M)	
Age at surgery	45.8 ± 19.5
Mean ± DS	46 (11–93)
Median (range)	
Pre-operative TSH	1,97 ± 0,17
Mean ± DS	2,1 (0.0015–6.99)
Median (range)	
Preoperative fT_3	2.91 ± 0.07
Mean ± DS	3.1 (2.1–30)
Median (range)	
Preoperative fT_4	1.02 ± 0.18
Mean ± DS	1.2 (0.34–45.81)
Median (range)	
Presentation with	76 (68.47%)
Lymph node metastases	22 (19.82%)
Locally advanced	7 (6.3%)
Distant metastases	
Surgical treatment *n* (%)	21 (18.9%)
TT + CCL	70 (63.1%)
TT + CUCL	5 (4.5%)
TT + CBCL	10 (9.0%)
UCL	5 (4.5%)
BCL	

Treatment and Complications

Surgical procedures consisted of 21 (18.9%) TT and CCL, 70 (63.1%) TT and CUCL, 5 (4.5%) TT and CBCL, 10 (9.0%) UCL, and 5 (4.5%) BCL after previous thyroidectomy in other Italian centres. None of the patients enrolled in the study underwent hemithyroidectomy. In the case of local advanced tumours or distant metastases, patients received tailored treatments based on their individual clinical condition. For details, see **Table 2**.

The final histological examination on the samples obtained during surgery reported 9/111 (8.1%) cases of follicular carcinoma and 102/111 (91.9%) cases of papillary carcinoma.

After the surgery, 87/111 patients (78,3%) developed a temporary or persistent complication as summarized in **Table 3**.

Postsurgical Treatments and Follow-up

A total of 110 patients received adjuvant radioiodine therapy after surgery; only one patient died 3 weeks after surgery, as reported in **Table 3**. They underwent from one to five cycles of treatment, with a mean of 2.4 ± 1.3, and of 98 ± 19 mCu total radiation dose. Of the 111 patients in our cohort, 53 remained in regular FU at our centre.

The three out of 53 patients that underwent TT and CCL presented peculiar conditions, such as liver, lung, or rib metastasis. After thyroid surgery, they all received surgical treatment for metastatic lesions (left robotic hepatectomy, atypical lung resection, and rib removal).

Of a total of 53 patients, 46 underwent TT and CUCL, 5 of them with LN resection, and 4 underwent TT and CBCL, 2 of

TABLE 2 | Detailed treatment received by patients in case of local advanced tumour or distance metastases. In brackets are the reported number of cases.

Clinical condition	Treatment
Complete unilateral vocal cord palsy (6)	Unilateral laryngeal nerve resection
Unilateral vocal cord hypomobility (3)	Laryngeal nerve preservation
Intraoperative evidence of nerve hypofunction with neuromonitoring in extensive tumour infiltration (1)	Unilateral laryngeal nerve resection
Laryngeal and tracheal massive infiltration (1)	The patient refused to undergo total laryngectomy and tracheal resection preoperatively. Surgical excision was therefore subtotal followed by radioiodine treatment.
Laryngeal minimal infiltration (1)	Thyroid nodules identified during routine carotid echo doppler: PTC infiltrating proximal tissues. During TT+CCL, evidence of millimetric laryngeal infiltration was left in place. Radioiodine treatment. In 5 months, the patient developed a massive neck mass, with extensive laryngeal invasion. The patient underwent total laryngectomy + BCL.
Lung metastasis (8)	One patient underwent atypical thoracoscopic lung resection, with metastasis excision. All the other patients received only adjuvant radioiodine treatment.
Mandibular metastasis (1)	Nontoxic thyroid goitre, not in regular follow-up. Clinical evaluation was requested for a mandibular lesion, which showed to be an FTC metastasis. The patient underwent TT+CBCL and mandibular resection. After radioiodine evidence of lung, clavicle, spine, femoral, and skull base metastasis.
Rib metastasis (1)	Three bone metastases in three different ribs at diagnosis. The patient underwent TT+CCL and rib resection.
Brain metastasis (1)	PTC, treated with TT+UCLC. After 11 months, the patient underwent contralateral CLC for tumour relapse. After 13 months, a brain metastasis was detected, followed by trans-cranial excision.
Liver metastasis (1)	Evidence of liver lesion during examinations for other reasons, FTC metastasis. The patient underwent TT+CCL, and after full recovery (2 months), she underwent robotic left hepatectomy.

which with LN resection. Moreover, one of these four patients presented mandible metastasis as symptom onset. She underwent mandible resection, with total metastasis gross excision.

Only 8/53 (15.1%) (3 men and 5 women, mean age 69 years old) are deceased, while 45/53 (84.9%) are currently alive (16 men and 29 women, mean age 42 years old). Patients currently still alive have a mean FU of 165 ± 101.2 months.

A total of 39/45 (86.7%) never developed recurrences; they all underwent TT and CUCL. Serum Tg and Ab-Tg never showed an increase during the follow-up period. Neck ultrasound always resulted negative. Five out of 45 (11.1%) have developed relapse, and one (2.2%) had persistence. The persistence was subjected to the neck radiotherapy cycle (5550 Mbq). The five relapses included heterogeneous cases (**Table 4**).

Three of eight patients deceased underwent TT and CCL. Three out of eight underwent TT and CUCL, and two out of eight underwent TT and CBCL. They had a mean survival of 47 months, ranging from 14 days to 151 months. All these patients presented distinctive characteristics as shown in **Table 5**.

In our series, only one patient refused surgery and was therefore not included in the above discussion. He underwent treatment with lenvatinib and is now in good clinical condition with tumour regression 20 months after diagnosis. The 45 patients still alive and in regular follow-up are in good clinical condition. During a telephone interview specifically for the present study, they reported living a normal life with good quality of life, taking into account age and other pathologies. They are all in thyroxine suppression therapy and present a mean TSH level of 0.54 μIU/ml (range 0.0015–3.61 μU/ml), mean Tg 0.07 ng/ml (range 0.04– 0.59 ng/ml), and Ab anti Tg 11.26 IU/ml (0–20 IU/ml), and all have neck ultrasound negative for thyroid bed and lymph node recurrence.

DISCUSSION

There is no standard definition for ADTC in the literature. In the ATA guidelines published in 2016 (9), there is a session entitled

TABLE 3 | Post-surgical complications developed in our series.

TH	53/111	45 patients have total spontaneous recovery
PH	8/53	Patients have not recovered, needing persistent pharmacological support
RLNTUP	3/111	Full spontaneous recovery in about 6 months
RLNPUP	8/111	7 of them were "obliged" nerve resections, as shown in **Table 2**
RLNTBP	1/111	The patient underwent tracheostomy
SLD	1/111	Persistent nerve deficit
Hypoglossal nerve deficit	1/111	Total spontaneous recovery in 4 months
Unilateral spinal accessory deficit	2/111	Persistent nerve deficit
Brachial plexus deficit	1/111	Total spontaneous recovery in 3 months
Marginal mandibular branches of the facial nerve deficit	2/111	Total spontaneous recovery in about 6 months
Oedemas of the surgical wound	4/111	
Postoperative bleeding	1/111	Immediate revision surgery
Pulmonary embolism	1/111	Massive PE 3 weeks after TT. *Exitus*

TH, temporary hypocalcemia, PH, persistent hypocalcemia; RLNTUP, recurrent laryngeal nerve transient unilateral palsy; RLNTBP, recurrent laryngeal nerve transient bilateral palsy; RLNPUP, recurrent laryngeal nerve persistent unilateral palsy; RLNPBP, recurrent laryngeal nerve persistent bilateral palsy; SLD, superior laryngeal nerve deficit.

TABLE 4 | Clinical history and treatment of the five relapsed patients.

Patient 1	During radioiodine therapy, development of cervical lymph node metastasis (left V level). UCL + removal of a portion of infiltrated sternocleidomastoid.
Patient 2	Recurrence at the thyroid bed 1 year after surgery. Revision surgery, followed by external radiotherapy and radioiodine
Patient 3	V level lymph node metastasis from papillary carcinoma 12 years after surgery, treated with UCL.
Patient 4	Contralateral lymph node localization 11 months after TT+CUCL. He underwent a second UCL.
Patient 5	Contralateral lymph node localization 13 months after TT+CUCL. He underwent a second UCL and a new course of radioiodine therapy (130 mCu)

"DTC: Long-Term Management and Advanced Cancer Management Guidelines," but the definition of ADTC is missing. In our series, we defined ADTC according to Russel et al. (5): invasion of thyroid adjacent structures (local invasion), laterocervical lymph nodes, metastasis, distant metastases, and more than one of the previously mentioned characteristics simultaneously.

The first consideration in the analysis of the present study is treatment. The different therapeutic choices have been tailored to the characteristics of each patient, and this approach has led to encouraging results: 84.9% of the 45 patients with available follow-up are currently alive, with a median FU > 10 years, i.e., 165 months (range 12–345 months). All of them were in the group with regional lymph node metastases, more in detail, eight with locally advanced tumours. Among these 45, four (11.1%) have developed relapse, in all cases localized in the neck, that was subsequently successfully treated by re-surgery and adjuvant therapy. None of the 45 patients developed distant metastases. Two of these relapses had at the time of their first intervention one RLN infiltration and the other tracheal cartilage infiltration. Only one (2.2%) has persistence in the thyroid bed, without disease progression. For relapse and persistence, the histological result was PTC. Of the eight patients who died, only four died of neoplastic causes, two of them after more than 10 years, 151 months, and 111 months, both with histological results of PTC. The two patients with the worst evolution were, one, already diagnosed with multiple lung and bone metastases for follicular carcinoma, and the other, showed trachea and larynx involvement for papillary carcinoma.

According to literature (10, 11), local invasiveness, albeit with a limited number of local ADTC in our case series, had a greater impact on survival than cervical lymph node involvement. However, DTCs are generally slow-evolving tumours, even if advanced. This aspect should be the first to be considered in the therapeutic choice of ADTC. With regard to complications associated with surgery, we observed a higher percentage of definitive hypoparathyroidism and persistent RLNUP in thyroidectomies performed for diseases other than ADTC carcinoma (12, 13). This may be related to the fact that central neck dissection, always performed in all patients included in this study, is a well-known risk factor for persistent hypoparathyroidism. Regarding the definitive RLNUP, the locally advanced situation of the tumour, with the involvement of the RLN in 10 cases, preoperative palsy of the vocal cord and intraoperative nerve infiltration in 6, and intraoperative infiltration in 4 cases, leading to sacrifice in one, has increased the number of definitive vocal cord paralysis. In the case of locally advanced tumours (LADTC), the most commonly involved structure in our experience was the recurrent laryngeal inferior nerve (RLN), more specifically in 10 cases.

In agreement with the literature, treatment of the infiltrated RLN was based on the knowledge of preoperative vocal function (14, 15), magnitude of neural invasion, and occurrence of loco-regional or distant metastases not surgically removable. There is a general agreement that attempts should be made to preserve RLN when preoperative vocal cord function is normal, and intraoperatively it appears only to be superficially invaded. The location of the RLN invasion should be taken into account in the decision to preserve it or not: resection should be performed when neural invasion occurs near the point where the RLN enters the larynx. This is because an incomplete resection of the tumour at this point can lead to the progression of the tumour along the nerve and its spread into the larynx, with subsequent indications for laryngectomy (5). In addition to the location, the extent of neural invasion should influence intraoperative

TABLE 5 | Clinical history and treatment of the eight deceased patients.

Patient 1	Thyroid cartilage infiltration, revealed during TT. Adjuvant radioiodine therapy alone to preserve the organ. After 8 months, massive cervical metastasis associated with local laryngeal progression, which led to total laryngectomy. The patient died 15 months later secondary to other medical conditions.
Patient 2	Left vocal cord palsy at diagnosis. TT+CCL+ resection of the left lower laryngeal recurrent nerve. The patient died 22 months following another clinical condition.
Patient 3	Macroscopic laryngo-tracheal tumour penetration at the time of diagnosis, but the patient refused total laryngectomy and tracheal resection. Surgical excision was subtotal (TT+CCL) followed by radioiodine treatment. The patient died 5 months after secondary to another clinical condition.
Patient 4	TT+CCL. The patient died at 14 days for pulmonary embolism.
Patient 5	TT+CUCL. Local disease recurrence, in the thyroid bed and in an area of oesophageal infiltration and a delayed onset lymph node metastasis. The patient died 151 months after due to tumour progression.
Patient 6	TT+CUCL. Multiple lung and bone metastases at the time of diagnosis. The patient died 111 months after due to tumour progression.
Patient 7	TT+CBCL. Five months later, the patient showed a local macroscopic recurrence with the involvement of trachea and larynx, treated with a total laryngectomy. The patient also underwent therapies with tyrosine kinase inhibitors but died 12 months later.
Patient 8	TT+CBCL. Multiple lung metastases at the time of diagnosis. Progressive development of bone and brain metastasis. The patient died 55 months after due to tumour progression.

decision-making. When only the epineurium is infiltrated, a shave excision may be performed (14–16). Even if the nerve shaving may cause partial layer resection, most nerves demonstrate a long-term recovery of neural function (14). On the contrary, if the perineurium, endoneurium, and neural fibres are invaded, the resection should be considered. In this case, the simple shaving of the nerve without the complete removal of the tumour may be considered, as we did in the three cases abovementioned, that is, if adjuvant RAI therapy may treat the residual neoplastic tissue (14, 16–18). When intraoperative neuromonitoring (IONM) is routinely used during surgery, it is important to evaluate the possibility of proximally stimulating the nerve (18): the ability to stimulate the proximal side of an infiltrated RLN suggests some maintenance of neural function, even if paralysis of the vocal cord is documented preoperatively. As reported by Kamani et al., 60% of invaded nerves can be stimulated with IONM (19) and the resection of invaded RLN with preoperative paralysis of the vocal cord, according to some authors (20), may worsen glottic function. For this reason, the ability to stimulate the proximal segment of the invaded nerve may be a parameter for intraoperative decision to preserve or not the nerve (14). A very important aspect to consider about the treatment of an infiltrated RLN is that resecting or preserving it would not seem to change the prognosis (14, 17, 18, 21–23). For this reason, if possible, it would be best to preserve the sRLN, because the integrity of the nerve corresponds to the good functioning of the vocal cord, which has a great impact on the quality of life. A negative impact on the quality of life may occur when there is an infiltration of viscera adjacent to the thyroid, such as airways (larynx and/or trachea), oesophagus, and vascular structures. In our experience, among ADCT not lost at follow-up, five had invasions of the airway, two had laryngeal invasion, and three had tracheal invasion. One patient with laryngeal infiltration refused laryngectomy and died after 25. Another patient had infiltration of the larynx and the first two tracheal rings. She underwent laryngectomy 5 months after TT, and she died 12 months later. The others are alive respectively after 37, 77, and 101 months, without resecting trachea. They were treated with tracheal shaving during TT and CCL as well as postoperative RAI, referring to good quality of life. In the presence of visceral structure invasion, the objective of surgery should be to remove the majority of the tumour mass. ATA guidelines recommend surgery for tumours invading the upper aerodigestive tract along with ^{131}I (RAI) and/or external beam radiation therapy (ERBT) (9, 24, 25). In some patients, surgery may represent a viable cure effort, while in others it represents a palliative approach to reduce symptoms such as asphyxia and haemoptysis (26, 27). In the case of tracheal infiltration, surgical techniques depend on the type of tumoral invasion. The extent of tracheal invasion has been classified by Shin, as well as others (21, 27, 28), in four stages: perichondrial invasion with adherence to the trachea (stage I), cartilaginous invasion but without mucosal involvement (stage II), mucosal infiltration (stage III), and extension into the lumen (stage IV). In the suspicion of tracheal involvement, besides CT scan, preoperative bronchoscopy is mandatory, to assess mucosal intraluminal involvement. Shave resection of the airway may be appropriated in cases of limited cartilage invasion, involving tiny segments. In the presence of more extensive or intraluminal tumour infiltration, tracheal resection is suggested (21, 29). In the case of larynx infiltration, shave excision is possible when the tumour does not extend into the larynx. Otherwise, more aggressive treatment, such as laryngectomy, may be required (27). In our series, patients with laterocervical lymph node metastases, not lost at follow-up, were 44. All had a functional cervical dissection, including II, III, IV, and V levels. Five of them had recurrences, and 32 were alive following a mean follow-up of 210 months. Treatment of distant metastases should consider that both morbidity and mortality are increased in patients with distant metastases and that individual prognosis is influenced by a number of factors, including histology of the primary tumour, distribution and number of metastatic sites (e.g., brain, bone, lung), age at diagnosis, and RAI avidity (30–38). Treatment of a specific metastasis should be grounded on patient status and on the presence of other sites of disease (39–42). According to ATA (9), the sequence of therapeutic choices for the treatment of metastatic disease should be in order:

i) surgical excision of loco-regional metastasis in potentially curable patients;
ii) ^{131}I therapy for RAI-responsive disease;
iii) ERBT or other directed treatment modalities for metastases;
iv) TSH-suppressive thyroid hormone therapy for patients, with stable or slowly progressive asymptomatic disease;
v) systematic therapy with kinase inhibitors, especially for patients with significantly progressive macroscopic refractory disease.

In the management of the patient with lung metastases, therapeutic decisions should consider the following: size of metastatic lesions (i.e., macro-nodular detected by chest radiography, micro-nodular detected by CT, lesions below CT resolution); avidity for RAI; response to previous RAI therapy; stability or not of metastatic lesions. In view of the high rate of the observed complete remission, lung micro-metastases should be treated with RAI therapy and eventually be repeated every 6–12 months, if the disease continues to concentrate RAI and respond clinically (41, 42). Also, radioiodine-avid macro-nodular metastases should be treated with RAI when objective benefit is demonstrated (i.e., decrease in the size of the lesions, decreasing Tg). In the latter, however, complete remission is uncommon and survival remains poor. It is also worth considering the risks associated with repeated doses of RAI such as bone marrow suppression or pulmonary pneumonitis and fibrosis. Solitary lung metastases could be resected *via* surgery, even though the potential benefits compared with the risk of surgery remain unclear. With regard to bone metastasis, RAI therapy may be considered for those that are iodine-avid. In these cases, the therapy has been associated with improved survival, although RAI is rarely curative. Regardless, some patients with RAI-avid bone metastases may still benefit from this therapy (35–37). These patients may also be considered for directed therapy in cases

where metastases are visible on anatomical imaging. Directed therapies include surgery, EBRT, and other focal treatment modalities discussed below. Systemic therapy with bone-targeted agents may also be considered in these patients. The therapeutic approaches to brain and liver metastases are similar to those previously mentioned for lungs and bone metastases (9). As mentioned, several focal treatments other than surgery, for lung, bone, liver, and brain DTC metastases, are available. In certain patients, these treatment modalities, alternative to surgery, may be effective for local tumour control as first-line treatment with similar efficacy to that of surgical resection. In general, such treatments may be indicated in case of high anaesthesiologic risk, lung metastasis in patients with insufficient respiratory reserve, multiple previous surgical resections, local recurrence after surgery, and refusal of additional surgery. In our experience, we treated seven metastatic patients: two had pulmonary metastases, three had bone metastases, one had liver metastases, and one had brain metastases. Of the two patients with lung metastases, one was a young patient for whom the diagnosis of DTC was made through the removal of a solitary pulmonary nodule, with pathological examination showing PTC metastasis as a result. Then, only a single sub-centimetre nodule was found during neck ultrasound and she underwent total thyroidectomy with central neck dissection. After these two interventions, she underwent RAI with no evidence of further lesions. She remains disease free at her 89-month follow-up. The other one showed multiple pulmonary areas of uptake at post-thyroidectomy ^{131}I total body scan, after total thyroidectomy for follicular carcinoma. During a chest CT, she showed multiple micro-metastases. She underwent three cycles of RAI therapy. She is alive and persistent but reduced for many metastases at the 125-month follow-up. Of the three patients with bone metastases, two were treated: patients with ADTC are treated by RAI. The dose of RAI, the number of treatments, and the time between treatments should be personalized depending on unique patient characteristics such as disease response to therapy, patient's age, and the onset of side effects (i.e., radiation lung damage, bone-marrow suppression, and salivary gland damage). Therapy should be repeated at least 6–12 months after the previous treatment (5). Finally, systemic therapy may be considered in patients with locally advanced and/or metastatic RAI refractory disease not suitable for local therapies with the purpose to reduce tumour growth and/or metastatic spread (43). The first-line systemic therapy is represented by the multikinase inhibitors lenvatinib and sorafenib, approved by FDA and EMA (44–49). In our series, we have two patients enrolled by endocrinologists for systemic therapy with lenvatinib, as described before: one for local advanced and metastatic disease who refused intervention and the other for multiple recurrent metastatic DTCs (4). Nowadays, traditional chemotherapy is indicated only in selected cases: when multikinase inhibitors are ineffective or cannot be used. If ^{131}I-refractory, metastatic patients should be maintained only with TSH-suppressive thyroid hormone therapy if they are asymptomatic or stable or have a minimally progressive disease without clinically significant complications. These patients need serial radiographic imaging every 3–12 months (9).

CONCLUSIONS

In the case of locally and/or metastatic ADTC, there are many therapeutic options available. The best treatment modality must take into consideration various aspects related to the patient and the pathology at hand. Moreover, the benefits of surgical excision of a slow-evolving tumour, such as DTC, in terms of local control, risk of persistence/recurrence, and survival, must always be carefully weighed against the morbidity of surgery, which may require aggressive resection of extrathyroidal tissues. For these reasons, a single standardized approach for local and/or metastatic ADTC could not be established, but rather a tailored treatment must be defined for each patient. The best treatment certainly requires a multidisciplinary group, consisting of an endocrinologist, an expert endocrine surgeon, an otolaryngologist, a radiologist, a nuclear medicine doctor, and an oncologist, all operating in a high-volume centre. Multicentre studies from high-volume centres would be desirable, to optimize the choice of various therapeutic options discussed before, and to offer the best tailored treatment to the patients affected with locally and/or metastatic ADTC.

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 CONTRIBUTION

Conceptualization: LP; methodology: LP, AB and MB; validation PL, LC and AS; investigation: AB, EB and LP; writing—original draft preparation: AB, LP, MB and EL; writing—review and editing: MB, EL, EF, CP, CR and GF. All authors have read and agreed to the published version of the manuscript. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Risk Factors for Anaplastic Thyroid Carcinoma: A Case Series From a Tertiary Referral Center for Thyroid Surgery and Literature Analysis

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Anaplastic thyroid carcinoma (ATC) is a very rare and extremely aggressive disease with a very poor prognosis. Several risk factors have been hypothesized, but there is no clear-cut literature data on it. We reviewed the literature concerning risk factors for ATC and analyzed the institutional database from 2005 to 2022. In total, 15 papers were suitable for review, while the retrospective data collection search, conducted on our institutional database, provided 13 results. In our experience, in agreement with literature data, ATC seems to be a neoplasm peculiar to old age (in our database, mean age is 72 years), with a higher prevalence in subjects with a low level of education and a long history of multinodular goiter (MNG). The role of cigarette smoking and blood group, hypothesized on some literature data, was more uncertain, although the small sample size evaluated probably had a great influence on these results. The higher incidence of the disease in individuals with a history of MNG could suggest more aggressive choices in the treatment of a benign disease, in contrast to current practice. However, this may be a highly questionable point considering that ATC accounts for no more than 2% of all thyroid neoplasms in surgical departments, even those dedicated to endocrine neck surgery. Further studies are therefore necessary for a step forward in this direction.

Keywords: anaplastic thyroid carcinoma, risk factors, multinodular goiter, thyroidectomy, prognosis

INTRODUCTION

Anaplastic thyroid carcinoma (ATC) is a very rare and extremely aggressive disease with a very poor prognosis.

It accounts for 2%–3% of all thyroid neoplasms. It is composed of undifferentiated thyroid follicular cells, which require immunohistochemical and ultrastructural support to determine their epithelial origin. Beyond significant local invasion, ATC often presents with local and distant lymph node metastatic spread (1).

In Europe, the estimated incidence, during the years 2000–2007, was 0.1/100,000 subjects per year (2).

It is more frequent in the elderly, with only 25% of patients under 60 years of age and more than 90% are over 50 years old, mostly in their 70s and 80s.

ATC has a higher prevalence in women, with an estimated female-to-male (F:M) ratio of 1.5–3:1. (3, 4)

This cancer may arise *de novo* or in patients with a long history of multinodular goiter (MNG) or may represent the evolution of an unrecognized differentiated carcinoma (20%–25% of cases) (5).

At the time of diagnosis, about 80% of patients have locally advanced disease and almost 50% already have distant metastases (lung, bone, brain). The median survival from diagnosis is 5 months, and less than 20% of patients survive 1 year (6).

The literature shows that more than one-third of patients with ATC have a history of goiter before diagnosis (median duration, 8.5 years) (3, 7) and that this cancer is twice as common in areas with endemic goiter. Over the last few years, there has been a reduction in the incidence of ATC: in an effort to provide a possible explanation for this finding, it seems logical to relate this decline to iodine prophylaxis, which would act in the same direction by reducing the incidence of benign goitrogenic disease and, concurrently, of ATC (8–12).

Insofar that a specific risk context for this neoplasm is not yet well-defined, in reviewing our case series, we asked ourselves:

- Given that only 20%–25% represent the evolution of a differentiated thyroid carcinoma (DTC), are the risk factors the same as for a DTC, and more importantly, what other risk factors determine the evolution of a DTC into ATC?
- Is it possible to identify ATC-specific risk factors in order to achieve early diagnosis in patients suffering from MNG?
- Furthermore, in the era of increasing conservatism in thyroid surgery, including neoplastic disease, what is the actual risk of anaplastic carcinoma?

Clarifying risk factors could make disease prevention possible through early treatment of precursor conditions.

We reviewed the literature on specific risk factors for ATC and analyzed risk factors of ATC cases diagnosed by cytology and/or histology occurring in the surgical department of our university hospital from 2005 to 2022. These data were obtained from the pathology database of our institution and compared with literature. The intermediate endpoint of the study was to establish, on the basis of literature data and our institution's experience, potential risk factors for ATC diagnosed by histology. The final endpoint was to assess whether there are risk criteria that would change the current indications for thyroid surgery in defined risk settings.

MATERIALS AND METHODS

We searched for specific studies on risk factors related/correlated to ATC, including all types of English language articles in PubMed, Web of Science, and Scopus articles published from 2000 to 2022.

The following MeSH terms were adopted: "anaplastic thyroid cancer risk factors" or "anaplastic thyroid carcinoma risk factors." The search resulted in 139 and 108 articles, respectively. Once duplicate papers were excluded, the abstracts were read, and the unsuitable papers were excluded. We included only papers whose topic was specifically addressed on risk factors for anaplastic carcinoma, excluding papers on risk factors for benign and malignant nodular disease in general.

After this literature review, the number of articles considered eligible was only 15 (**Figure 1**).

After extrapolating from the literature review the risk factors recurrent in the enrolled studies, we have assessed the variables most frequently found in the literature: demographic data (sex, age, level of education) and smoking status. In addition, variables such as history of thyroid disease and possible suppressive therapy with L-T4, personal history, such as history of head and neck irradiation, therapy with radioiodine-131, diabetes mellitus (DM), neoplasms in other sites, body mass index (BMI), blood group, and hormonal history in women were evaluated.

In this study, we aimed to perform a retrospective assessment of potential risk factors for ATC in a group of patients with definite histologic diagnosis.

This study examines 13 cases of patients with ATC that occurred in the surgical department of Policlinico Universitario "P. Giaccone" of Palermo, a third-level center for thyroid surgery, from 2005 to 2022. By recruiting patients from surgical databases, we obtained a group of patients, albeit limited, but with a definite diagnosis.

Patients with a plausible diagnosis were excluded, even if strongly suspected from a clinical point of view, in the absence of a histological diagnosis. Therefore, patients were selected on the basis of a double search, one based on the databases of the General and Emergency Surgery and Oncological Surgery units of the Policlinico Universitario "P. Giaccone" of Palermo and the other on the database of the Surgical Pathology Service of the same institution. Therefore, we excluded patients who, although registered as ATC or other similar terms, were not part of the databases of the abovementioned services.

We also excluded cases of advanced papillary and follicular carcinomas, poorly differentiated tumors, and insular carcinomas.

In the present study, we aimed to analyze the data available at our institution on potential risk factors for ATC and to compare them with literature data. The surgery performed on each individual patient, the adjuvant therapy, and the outcome are summarized in **Table 1**. Given the small sample of patients enrolled, the statistical evaluation was only descriptive.

RESULTS

Demographic Data

Analysis shows that the majority of our ATC patients are women, with an F:M ratio of 2.25:1 (9 women and 4 men).

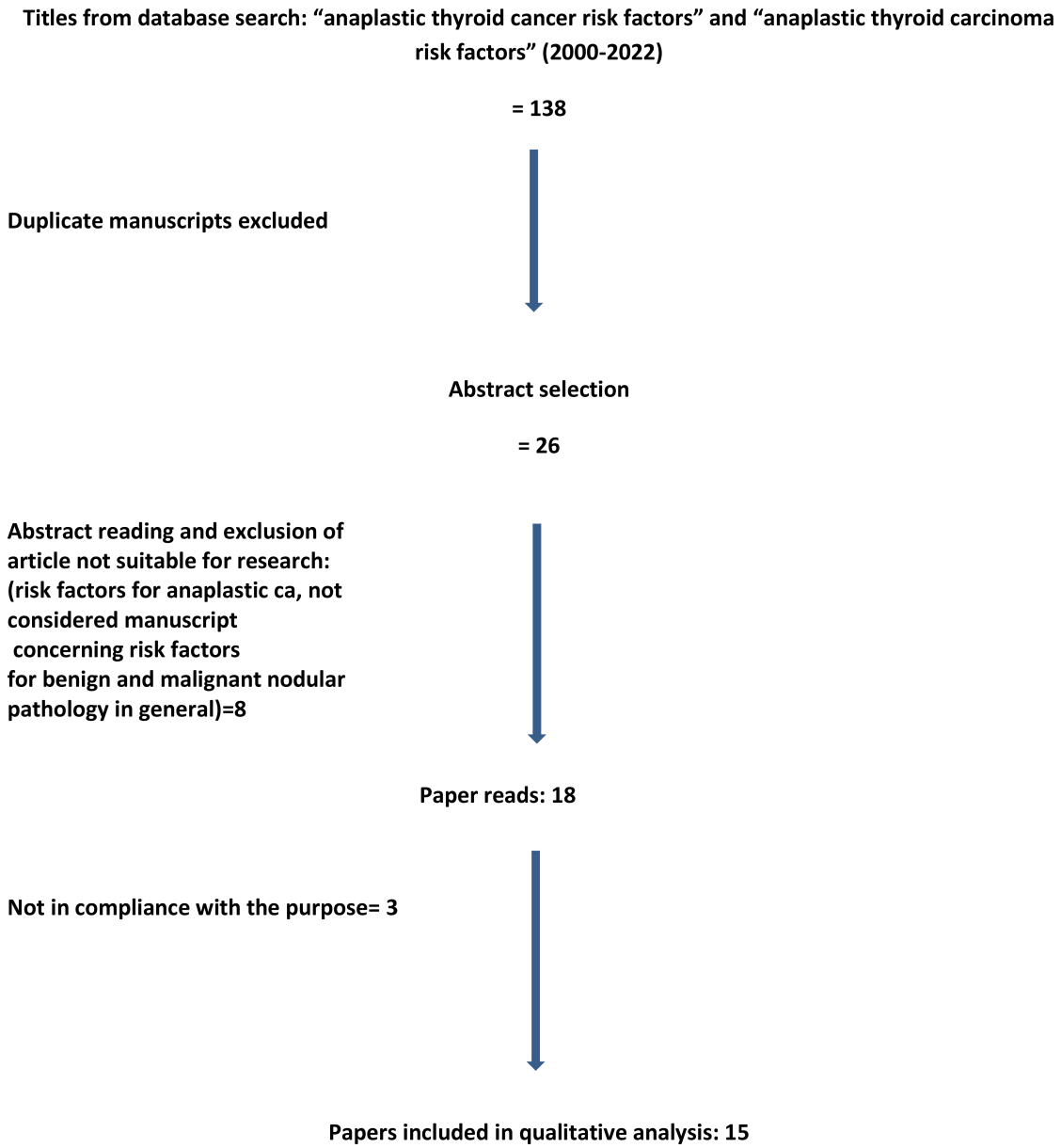

FIGURE 1 | Prisma diagram detailing the literature search process and article selection.

Almost all were over 60 years old, with a mean age of 72 years (range: 36–83) and a median age of 76 years. Only one patient was 36 years old at the time of diagnosis. She was a young pregnant woman at the 11th week of gestation when, due to the recent appearance of a painful swelling in the neck associated with dysphagia, she underwent an ultrasound examination and a nodule was found in the left lobe of the thyroid gland, Bethesda 6 on Fine Needle Aspiration Biopsy (FNAB). She underwent total thyroidectomy and lymphadenectomy of the left central hemi-compartment at the end of pregnancy, including a histological diagnosis of anaplastic carcinoma of the left lobe of the thyroid (transverse diameter maximum 3.1 cm) with anaplastic microfocus in the right lobe (1.9 × 1.2 mm) and 1/9 lymph nodes as site of metastasis.

Regarding the level of education, most had a low level of education: 54% had only a primary school certificate or no educational degree, 23% had a lower secondary school diploma, and only 23% had a high school diploma; no one had a college or university degree (**Figure 2**).

Concerning the smoking habit, out of 13 patients, 5 were ex-smokers, 2 were smokers, and 6 had never smoked.

- History of thyroid disease/MNG/levothyroxine suppressive therapy

TABLE 1 | Pt, patient; TT, total thyroidectomy; CND, central neck dissection; LND, lateral neck dissection; TS, tracheal stent; CT, chemotherapy; RT, Radiotherapy.

Pt	Age	Sex	Surgery	Chemotherapy/Radiotherapy (CT/RT)	Survival (months)
1.	71	F	TT + CND	None	20
2.	83	M	TT	CT	24
3.	82	F	None	None	1,5
4.	77	F	TT + CND + right LND + tracheostomy	CT	4
5.	77	M	None	CT	3
6.	36	F	TT + CND	Unknown	Lost at follow-up
7.	61	F	Biopsy	RT	3
8.	81	F	Debulking	CT + RT	6
9.	75	M	TT + LND	CT	40
10.	67	F	Biopsy	CT	2
11.	76	M	Biopsy + TS	None	1
12.	75	F	Biopsy + TS	None	1
13.	79	F	Biopsy + TS	None	2

Almost all of the patients had a history of known thyroid nodular disease.

Of 13 patients, 10 (76.9%) had a history of long-standing MNG (>8 years).

- Personal medical history: history of head and neck irradiation, therapy with Radium Iodine-131, DM, neoplasms in other locations

Only one female patient had a history of DM (7.7%). One male patient had a history of head and neck irradiation; one patient had a history of thyrotoxicosis treated with Radium Iodine-131.

Three patients had a personal history of malignancy in other sites (one with previous prostate cancer, one with a history of uterine malignancy, and another one had a colic stromal tumor treated 4 years previously).

- BMI, blood group, and hormonal history in women

The mean BMI of patients with ATC was 28.2 with a range from 16.8 to 39.9 and a median of 29 (**Figure 3**).

We observed a history of recent major weight loss in two female patients with BMI of 16.8 and 18.8.

Concerning the blood group, 4 patients had blood group A+, 5 patients had blood group 0 (1 0 RH negative and 4 0 RH positive), 2 had blood group B+, and 2 AB (**Figure 4**).

Regarding the hormonal history in women, the mean age of menarche = 12.8 years (range 10–16 years).

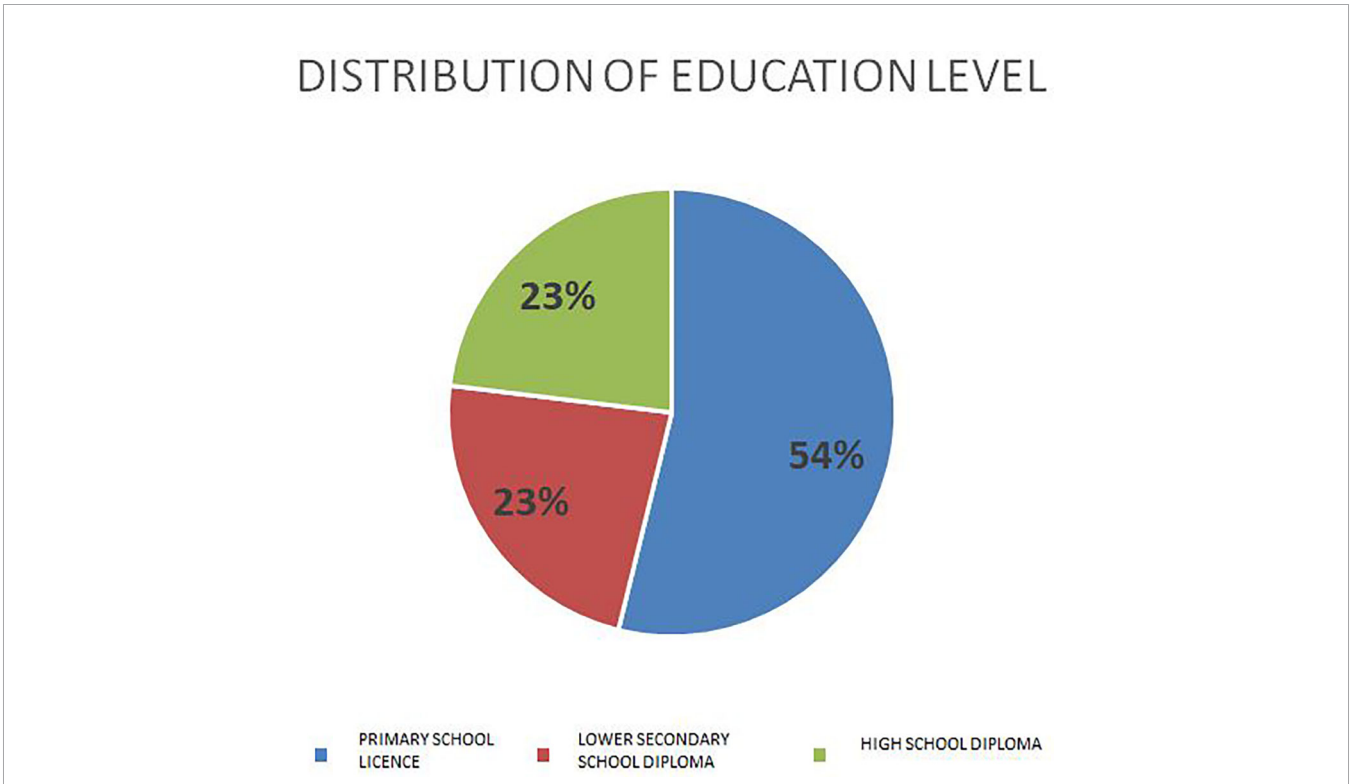

FIGURE 2 | Level of education.

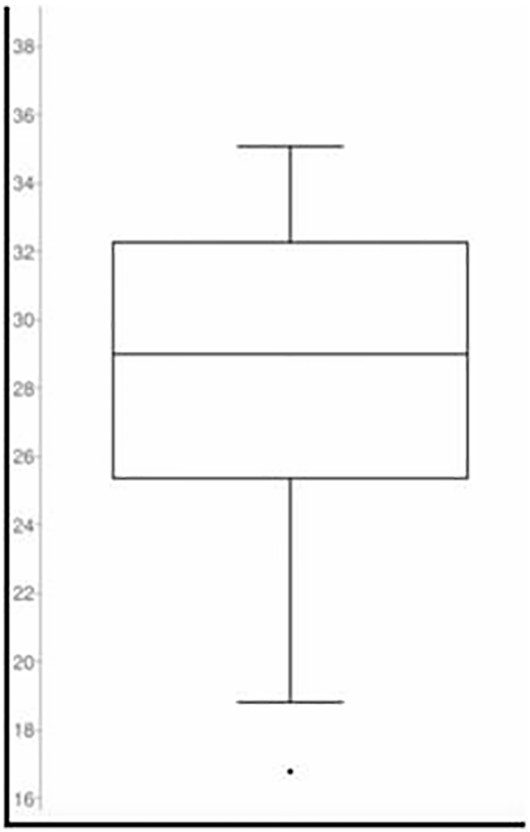

FIGURE 3 | BMI grouping in Box Plot.

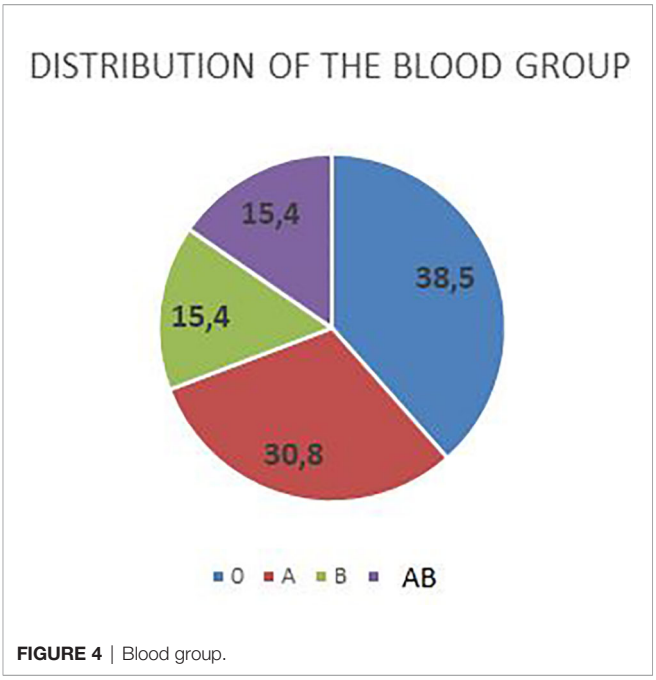

FIGURE 4 | Blood group.

The mean age of menopause = 53.4 years (range 50–57 years).

The demographic data of the institutional case series are summarized in **Table 2**.

DISCUSSION

In the literature review, the first case–control study on ATC risk factors is a Serbian study that started in 2004 and continued 3 and 4 and 10 years later.

The first part of the study (started/published in 2004) involved a smaller number of cases and a control group represented by patients recruited at the hospital for non-autoimmune rheumatic diseases. According to this study, independent risk factors for ATC were a clinical history of goiter, living in a goiter-endemic area, a personal history of other non-thyroid neoplasms, DM, and a low level of education (13).

The second part of the study (started/published in 2007) involved a larger number of cases and a control group of goiter patients in order to identify any independent risk factors. The only significant difference between cases and controls was a longer history of MNG in ATC cases (>10 years). According to the results obtained in this Serbian study, with patients with goiter as control, anaplastic cancer is associated with a lower level of education, a personal history of other neoplastic diseases, blood group B, late menarche, and early first full-term pregnancy. These two reproductive factors (late menarche and early first full-term pregnancy) were found to be statistically significant as risk factors associated with ATC but cannot be considered as independent risk factors, as they are generally risk factors for thyroid cancer, the incidence of which is several times higher in women than that in men (14–16).

In the case of the control group represented by patients with papillary carcinoma, no statistically significant independent risk factors for ATC were found except for the age factor (17).

In 2014, a new study was conducted in which the results of the previous studies were briefly summarized, and new results were presented, including a control group based on the general population with the same residence of the cases. This is the only long-standing case–control study aimed to identify potential risk factors for the development of ATC found on PubMed. The results show that the majority of patients with ATC are women with an F:M ratio of 1.5:1, with a mean age of 67 years (37–88 years). Most of them are in the seventh decade of life (51.2%). In addition, 69.8% lived in urban areas. There were no significant differences in habits such as cigarette, alcohol, and coffee consumption. Living in a goiter-endemic area was not statistically significant. The study identified three independent risk factors for ATC compared with previous studies in which five risk factors were identified, thus excluding goiter because the control group in the 2007 study consisted of patients with goiter and excluding MNG-endemic area because the control group in this study was composed of patients living in the same areas. The three independent risk factors for ATC identified by this study are diabetes, a personal history of other non-thyroidal malignancies, and type B blood group, as well as a low level of education. The search for independent risk factors for ATC is

TABLE 2 | Demographic data.

Variable	Men, N (%)	Women, N (%)	Total (%)
Sex	4 (30.8%)	9 (69.2%)	13 (100%)
Age (median)		72.3 (76)	
BMI (median)		28.2 (29)	
Diabetes	Yes, N (%)	No, N (%)	Total (%)
	1 (7.7%)	12 (92.3%)	13 (100%)
Goiter	Yes, N (%)	No, N (%)	Total (%)
	10 (76.9%)	3 (23.1%)	13 (100%)
Neck irradiation	Yes, N (%)	No, N (%)	Total (%)
	1 (7.7%)	12 (92.3%)	13 (100%)
History of cancer	Yes, N (%)	No, N (%)	Total (%)
(other than thyroid)	3 (23.1%)	10 (76.9%)	13 (100%)
Menarche		Mean age (Median)	
		12.8 (13)	
Menopause		Mean age (Median)	
		53.4 (54)	
L-T4 treatment	Yes, N (%)	No, N (%)	Total (%)
	3 (23.1%)	10 (76.9%)	13 (100%)

intended to reduce bias; however, one of the major problems with these studies is that they consist of a relatively small size of the group with ATC (18).

However, these risk factors are too general, given the epidemiology of DM in the western world and the increased incidence of malignancy with aging. There are no other data in the literature correlating anaplastic thyroid cancer with blood group, although there are data on other cancers at other sites.

Some older studies have correlated ATC with I131 and L-thyroxine therapy.

The study by Zivaljevic et al. (18) does not demonstrate this association (only one patient was previously operated for well-differentiated thyroid cancer with subsequent therapy with 131I Radioiodine before anaplastic transformation occurred; five patients in the case group were on L-thyroxine replacement or suppressive therapy).

A background of MNG and a history of radioactive iodine therapy is not uncommon (19), although these risk factors cannot be identified as independent.

ATC most frequently affects the elderly with a long history of MNG, reported in up to 50% of cases, and in 20% of cases, patients have a history of DTC, usually papillary type (20, 21).

Chandrakanth and Shaha (22), in a 2006 review, demonstrated that previous or concomitant thyroid disorder (benign or DTC) is a risk factor for the development of ATC (8, 10, 23–29) and that ATC is twice as common in goiter-endemic areas and that the decline in recent years may be due to iodine prophylaxis (8–12) and improvements in socioeconomic status, which have been shown to be associated with a reduction in the incidence of ATC (11). This study shows that the incidence of ATC is higher in goiter-endemic areas and in patients with previous papillary or follicular cancer who were not adequately treated (8, 26, 30–33). This has led to the suggestion that aggressive oncological treatment may be responsible for reducing the incidence of ATC by eliminating its transformation potential (8).

The coexistence of DTC and ATC is very well documented (31, 34, 35). Indeed, some studies have reported DTC to ATC transition zones in the same sample and even findings of tiny DTC foci within ATC and *vice versa* (36, 37).

Although any DTC type can be found in association with ATC, papillary cancer is the most common (8, 10, 26, 28, 32, 38). Within papillary cancer, the biologically more aggressive forms (insular and high cell type) are most commonly found associated with ATC, reinforcing the theory of neoplastic transformation.

The pathogenesis of de-differentiation from papillary carcinoma to ATC has been attributed, in some cases, to the acquisition of additional genetic changes including mutation in the p53 oncosuppressor gene (21). Increased expression of the urokinase-type plasminogen activator (uPA) system has been reported to be associated with tumor invasion, neo-angiogenesis, and metastatic spread in thyroid cancers, and Aurora kinases are associated with severe mitotic abnormalities. Based on this evidence, uPA and Aurora kinases are considered potential therapeutic targets for the treatment of ATC (38, 39).

In some cases, it has been suggested that radiotherapy of differentiated tumor (DTC) may play a role in the transformation to undifferentiated tumor (20, 40).

Maatouk et al. (19) described a case report of a 90-year-old patient with a long history of MNG (>45 years) and several bouts of thyrotoxicosis treated with I131 radiotherapy and subsequent diagnosis of advanced ATC that caused his immediate death (2 days after diagnosis), suggesting that a long history of MNG and radioiodine therapy are not uncommonly associated with ATC.

In the few cases where ATC occurred after radioactive iodine treatment for papillary carcinoma, p53 abnormalities were documented in both primary tumor and ATC by immunohistochemical evaluation (21).

After these studies, the issue of radioactive iodine as a cause of further induced p53 mutation was raised (questioned).

Leitzmann et al. (41) in a 2010 US prospective study analyzed the relationship between BMI/adiposity and physical activity and thyroid cancer with evidence of a positive relationship between increased BMI and thyroid cancer, particularly papillary,

follicular and anaplastic, non-medullary thyroid cancer. This association is greater in men than that in women. No significant relationship between physical activity and DTC was demonstrated, although there was some evidence of a positive relationship with the anaplastic cancer (41).

Engeland et al. (42) in a Norwegian study showed a stronger association between BMI and follicular carcinoma and anaplastic carcinoma than papillary thyroid cancer.

This hypothesis is supported by a recent study suggesting that an obesity-inducing high-fat diet may play an important role in thyroid carcinogenesis. This study, using a ThrbPV/PV/Pten+/- mouse model, points out that such a diet can cause increased proliferation of thyroid cancer cells, induced anaplastic changes, and increased serum leptin levels (43) through activation of the Janus kinase 2/signal transducer and activator of transcription (3 JAK2-STAT3) signaling pathway. Activation of Janus kinase 2/ signal transducer and activator of transcription (3 JAK2-STAT3) by other cytokines such as Interleukin 6 (IL-6) has also been reported in gastric carcinoma and colon cancer.

These observations suggest that activated Janus kinase 2/ signal transducer and activator of transcription (3 JAK2-STAT3) signaling could be a possible mechanism to mediate transformation or de-differentiation of thyroid cancer cells.

However, the biological mechanisms by which BMI in humans may relate to thyroid cancer remain speculative to date. Of these, the most convincing links adiposity excess and the consequent increase in insulin production, which is responsible for tumor growth by increasing free insulin-like growth factor (IGF)-1, which in turn stimulates cell proliferation and suppresses apoptosis and has been positively linked to thyroid cancer (44).

Although hyperinsulinemia *per se* has not been directly linked to thyroid carcinogenesis, hyperglycemia has been linked to thyroid cancer by some studies (45).

Furthermore, adiposity may increase the risk of thyroid cancer through its effects on thyroid-stimulating hormone (TSH) (46), which is an independent predictor of thyroid malignancy (increased visceral fat correlates with increased serum fT3, a possible expression of adaptive thermogenic phenomena, and TSH with likely altered negative feedback from circulating thyroid hormones).

BMI also correlates inversely with circulating adiponectin levels, and thyroid tumors express receptors for adiponectin. However, in the absence of a major direct effect of adiponectin on thyroid cancer cell lines *in vitro*, the negative association could be attributed to indirect effects of adiponectin possibly through metabolic regulation and insulin resistance (47).

Stansifer et al. (48), in a 2014 retrospective study considering 467 patients with thyroid tumors (404 papillary, 47 follicular, 13 medullary, and 3 anaplastic tumors) and 255 controls, show no positive correlation between obesity and thyroid cancer risk.

Other studies found a correlation between a high BMI and a more advanced stage, at the time of diagnosis, of thyroid cancer and also with a more aggressive histopathological subtype (49, 50), but not all studies agree that this actually increases the risk of developing thyroid cancer (51, 52). In any case, this study considers thyroid tumors in general, including 3 anaplastic ones, so statistically not significant for ATC (only 3 cases).

Schmid et al. (53), in a 2015 review, show a statistically significant 25% higher risk of thyroid cancer in overweight individuals and a 55% higher risk of thyroid cancer in obese individuals compared to their normal-weight peers. When assessed by histological type, obesity was significantly positively correlated with papillary, follicular, and anaplastic thyroid cancers, while it revealed an inverse association with medullary thyroid cancer. Both general adiposity and abdominal adiposity are positively associated with thyroid cancer. However, the relationships with BMI vary significantly depending on the histological type of the tumor (53).

Ma et al. (54), in a 2015 meta-analysis, correlate obesity with a significantly increased risk of thyroid cancer in both men and women, young and old, especially Caucasians and Asians. In a histological subgroup analysis, an increased risk of papillary, follicular, and anaplastic thyroid cancer was observed. However, obesity was associated with a reduced risk of medullary thyroid cancer. As this meta-analysis was considering only obesity, the possibility that the observed associations may be confounded by other lifestyle factors, such as reduced physical activity or dietary factors, cannot be excluded (54).

Leitzmann et al., (41) in a 2010 prospective study, show a weak and inconsistent positive association between BMI and thyroid cancer risk, as weak and inconsistent is the association with certain hormonal and reproductive factors among women, such as advanced age at menarche, advanced age at first delivery, a history of miscarriage of the first pregnancy, and use of oral contraceptives. In contrast, dietary intake of fish and cruciferous vegetables and cigarette smoking was inversely related to thyroid cancer (41).

Despite the fact that numerous studies in the past have made strong indications for multinodular goiter surgery, the current trend is still linked to obvious compressive symptoms, hyperfunction, or a real suspicion of cancer (41, 55).

The studies included in this review are summarized in **Table 3**.

Considering the scarcity of bibliographic data, the studies include a heterogeneous literature (retrospective, epidemiological, experimental, case–control, and review studies).

Finally, further studies are needed to clarify the potential biological mechanisms underlying the possible positive relationship between adiposity and thyroid cancer risk; currently, no specific correlation has emerged with ATC.

The persisting uncertainties about the very nature of this disease are among the causes of a difficult therapeutic approach, which to this day remains largely unsuccessful, given the extreme severity of the prognosis in spite of aggressive and combined treatments, as suggested in literature (56). Better therapeutic prospects may come, in the near future, from the identification of target therapies, which are still under study (57) since surgical resection does not improve survival in cases of advanced anaplastic cancer (58). Multinodular thyroid disease is

TABLE 3 | Summary of studies included.

	Authors	Study	Year	No. of patients
1.	Maatouk, J.,et al. (19)	Case report	2009	1
2.	Chandrakanth, A., et al. (22)	Review	2006	1,556
3.	Zivaljevic, V., et al. (13)	Case Control Study	2004	110
4.	Zivaljevic, V., et al. (13)	Case Control Study	2008	126
5.	Kim, W. G., et al.	Epidemiologic Study	2013	Animal and Laboratory studies
6.	Leitzmann, M. F., et al. (41)	Experimental study	2010	3,490,300
7.	Zivaljevic,V., et al. (13)	Case Control Study	2014	126
8.	Mitsiades, N., et al. (47)	Epidemiologic/Case Control Study	2011	175
9.	Stansifer, K. J., et al. (48)	Retrospective Study	2015	467
10.	Paunovic, I. R., et al. (3)	Retrodpective Study	2015	150
11.	Zivaljevic, V., et al. (13)	Case Control Study	2010	126
12.	Kitahara, C.M., et al. (52)	Risk Assessment Study	2012	197,710
13.	Engeland, A., et al. (42)	Case Control Study	2006	3,046
14.	Apostolou, K., et al. (59)	Observational Study	2021	3,233
15.	Ma, J., et al. (54)	Metanalysis	2015	12,620,676

Considering the scarcity of bibliographic data, the studies include a heterogeneous literature (retrospective, epidemiological, experimental, case control and review studies).

associated with a significant rate of incidental thyroid cancer, sometimes underestimated. For this reason it is necessary to know other risk factors to assess the malignant potential of a MNG estimated as benign (59).

CONCLUSION

The low incidence and poor prognosis within a few months of diagnosis negatively influence the finding of specific risk factors for ATC: rarity and exit within a few months of diagnosis compromise the collection of specific data.

The identification of independent or additional risk factors in the anaplastic transformation of a DTC and the understanding of the pathways of anaplastic transformation could help in the development of strategies for the early diagnosis and treatment of ATC.

A more aggressive surgical attitude in case of DTC could prevent the anaplastic transformation of the 20%–25% of ATC cases arising on diagnosable DTC. But is an aggressive approach in 98% of patients with DTC conceivable to prevent less than 2% of anaplastic thyroid cancers?

There are few epidemiological studies on this type of cancer.

A long observation period is needed to collect data on a few cases, given their rarity, in order to implement prevention and early detection strategies.

Therefore, it is particularly important to obtain epidemiological information on ATC on a large scale, from large databases, through multicenter studies involving high-volume centers worldwide.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

Ethical review and approval were 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

Each author made substantial contributions to the work, approved the submitted version, and agreed to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated, resolved, and documented in the literature. Conceptualization: GG and GSc. Methodology: GSa. Validation: GO. Formal analysis: PR, AC, and SM. Investigation: MP, SC, and FS. Resources: RT and GM. Data curation: GO and GM. Writing: GSc and GSa. Original draft preparation: GS. Statistical analysis: SM. Writing—Review and editing: GSc. Visualization: GO and GG. Supervision: GSc. All authors contributed to the article and approved the submitted version.

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Association between hashimoto thyroiditis and differentiated thyroid cancer: A single-center experience

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Hashimoto's thyroiditis is the most common cause of hypothyroidism in the iodine-sufficient areas of the world. Differentiated thyroid cancer is the most common thyroid cancer subtype, accounting for more than 95% of cases, and it is considered a tumor with a good prognosis, although a certain number of patients experience a poor clinical outcome. Hashimoto's thyroiditis has been found to coexist with differentiated thyroid cancer in surgical specimens, but the relationship between these two entities has not yet been clarified. Our study aims to analyze the relationship between these two diseases, highlighting the incidence of histological diagnosis of Hashimoto thyroiditis in differentiated thyroid cancer patients, and assess how this autoimmune disorder influences the risk of structural disease recurrence and recurrence rate.

KEYWORDS

hashimoto thyroiditis, differentiated thyroid cancer, thyroid surgery, papillary thyroid cancer, thyroid

Introduction

Hashimoto's thyroiditis (HT) is the most common autoimmune inflammatory pathology of the thyroid and is the leading cause of hypothyroidism in iodine-sufficient areas of the world. It is characterized by a lymphocytic infiltrate, in particular T-cells, able to determine the follicular destruction of the gland, its fibrous involution, and consequent hypothyroidism (1–5).

Thyroid cancer is the fifth most common cancer in the USA, and over 44 000 new cases occurred in men and women in 2021. The incidence of thyroid cancer is still rising worldwide, mostly due to the increased use of diagnostic imaging and surveillance, and

the diffusion of more accurate cytological classifications (6–12). Differentiated thyroid cancer (DTC) is the most common subtype, accounting for more than 95% of cases, and it is generally considered a tumor with a good prognosis, although a certain number of patients experience a poor clinical outcome (6, 8, 13–15).

Hashimoto's thyroiditis has been found to coexist with DTC in surgical specimens, but the relationship between these two entities has not yet been clarified in the literature (3, 16–25). Many authors suggest that patients with a diagnosis of HT, especially those with the nodular variant, should undergo a strict follow-up, as DTC seems to be more frequently encountered in those cases. Many studies report the frequent coexistence of these two pathologies in pathological examinations, but the clinical mechanisms linking these two pathologies and whether HT could be a protective or promoting factor for DTC progression remain unclear, as some authors report a higher frequency of multifocality in DTC with concomitant HT, while others suggest that the coexistence of HT in DTC patients is associated with favorable clinical outcomes (3, 17, 20–22).

Our study aims to analyze the relationship between HT and DTC, assessing the incidence of HT in DTC patients, and to evaluate whether HT influences the risk of structural disease recurrence, as described in 2015 ATA guidelines (26), and the disease-free survival rate in patients with DTC.

Methods

Study design

This was a retrospective cohort study that included patients who underwent thyroid surgery between January 2016 and December 2019 in our Unit of General and Endocrine Surgery. Patients were identified from a prospectively maintained institutional database. Data from 1981 consecutive patients were screened. Patients with a histological diagnosis of medullary or anaplastic thyroid cancer, those with a preoperative diagnosis of Graves' disease or toxic goiter, or those with incomplete data were excluded from this study. A total of 839 patients met the inclusion criteria and were enrolled in this study.

First, we assessed the incidence of HT in patients with a pathological diagnosis of benign or malignant thyroid disease.

Histological diagnosis of HT was made based on the presence in surgical specimens of the following characteristics: diffuse lymphoplasmacytic infiltration, germinal centers, and enlarged epithelial cells with large nuclei and eosinophilic cytoplasm (Askanazy or Hürthlecells). Non-specific lymphocytic thyroiditis occurring immediately adjacent to a tumor could not be differentiated from perineoplastic inflammation and was not considered HT.

Then, we focused our attention on patients with DTC. Particularly, we divided the patients into two groups according to the presence (HT-DTC group) or absence (non-HT-DTC group) of HT, with the aim of assessing the influence of HT on the risk of structural disease recurrence, as described in 2015 ATA guidelines (26), vascular invasion, multifocality (defined as the presence of two or more tumor sites), tumor size, gross and microscopic extrathyroidal invasion, microcarcinomas (defined as tumors < 1cm in larger diameter), nodal metastasis (calculated as more than 5 lymph node per patient), and type of DTC (Papillary Thyroid Cancer (PCT), aggressive variant of PTC [i.e. tall cell, hobnail variant, columnar cell) and Follicular Thyroid Cancer (FTC)].

Endpoints

The primary endpoint was to evaluate the incidence of HT in patients with benign or malignant thyroid disease to evaluate whether HT is an independent risk factor for DTC; the secondary endpoint was to assess whether HT is associated with aggressive forms of DTC through the analysis of pathological diagnosis, the ATA class risk of disease recurrence and the incidence of disease recurrence.

Postoperative management and follow-up

All patients were referred to endocrinologists for postoperative management, from which we obtained information regarding post-operative radioactive iodine (RAI) therapy administration, number of RAI therapy cycles, evidence of recurrence and type of recurrence (local and/or nodal).

Disease-free status was defined as a No Evidence of Disease (NED) and included the following features: no clinical evidence of tumor, no imaging evidence of disease by RAI imaging and/or neck ultrasound, and low serum thyroglobulin (Tg) levels during TSH suppression (Tg < 0.2 ng/mL) or after stimulation (Tg < 1 ng/mL) in the absence of interfering antibodies.

Statistical analysis

Univariate analysis was conducted using the chi-square test, or Fisher exact test when appropriate, for categorical variables, and Student's t-test for continuous variables. Factors with a p-value ≤0.10 in univariate analysis were considered potentially significant and were included in the multivariate analysis. Logistic regression analysis was used to identify independent risk factors for developing DTC; the results are presented as odds ratios (OR) with 95% confidence intervals (CIs). The results were considered statistically significant for p-value <0.05.

Disease-free survival was defined as the time from initial surgery to the detection of recurrence; the log-rank test was used to estimate the differences in Kaplan-Meier curves for independent risk factors. Calculations were performed with MedCalc R vers. 19.1.3. Continuous variables are reported as the mean ± standard deviation of the mean.

Results

Among the 839 patients included in our study, 229 (27.3%) were males, with an M/F rate of 1/3. The mean age was 52.1 years (SD 14.9, range 13-87 years). A total of 399 (47.6%) patients were included in the DTC group and 440 (52.4%) were included in the non-DTC group. The characteristics of the sample, including mean age, male sex, presence of HT, and preoperative administration of levothyroxine therapy for hypothyroidism are summarized in Table 1. In the univariate analysis, HT was significantly associated with DTC: its incidence was 50.1% in patients with malignant disease, and 38.6% in patients with benign disease ($p < 0.001$). Moreover, DTC patients were significantly younger than non-DTC patients: in fact, the mean age in the DTC group was 49.5 ± 15 years vs 54.4 ± 14.6 years in the non-DTC group ($p < 0.001$). No difference in terms of sex or preoperative administration of levothyroxine therapy for hypothyroidism was found.

In the multivariate analysis, both age (OR = 0.9805, 95% CI 0.9712 to 0.9899, $p < 0.001$) and HT (OR = 1.7784, 95% CI 1.3332 to 2.3722, $p < 0.001$) were found to be independent risk factors for developing DTC. Data from the multivariate analysis are reported in Table 2.

As already described in the methods, we performed a subsequent analysis on patients with DTC. Full data are reported in Table 3. The mean age in the HT-DTC group was 48.9 ± 14.8 years, while in the non-HT-DTC group was 50.1 ± 15 years (p=0.4131). There were 38 (19%) male patients in the HT-DTC group and 75 (37.7%) in non-HT-DTC group ($p < 0.0001$). Significant differences were found in terms of multifocality (40.5% in HT-DTC group, vs 29.6% in non-HT-DTC group; p=0.02), tumor size (13.7 ± 11.9 mm vs 17.6 ± 16.5 mm respectively, p=0.007) and microcarcinomas (44% vs 34.2% respectively, p=0.05). No differences in extrathyroidal extension, vascular invasion, metastatic lymph nodes, or type of DTC were found between the HT-DTC and non-HT-DTC groups.

After stratifying the HT-DTC and non-HT-DTC groups according to the ATA risk of structural disease recurrence, 125 and 120 patients were listed as low risk, 57 and 60 patients as intermediate risk, and 18 and 19 patients as high risk, respectively. No statistically significant difference was found (p=0.9033). The results are shown in Table 4.

Follow-up results of DTC patients are reported in Table 5. Sixty patients (15%) were lost to follow-up. The mean follow-up was 50.5 ± 13.8 months (median 50, range 14-76). RAI therapy was administrated to 225 (66.4%) patients. Overall, 12 patients experienced recurrent disease (3.0%). Three patients had local recurrences and 6 patients had nodal recurrences. The mean time between surgery and diagnosis of recurrence was 16.4 ± 13.4 months; the overall survival was 99.5%; only 2 patients died due to DTC. A statistically significant difference was observed in terms of the administration of RAI therapy between the HT-DTC group, which was given to 109 (63.7%) patients vs 116 (69%) patients in the non-HT-DTC group (p=0.04). We found no difference between HT-DTC and non-HT-DTC patients regarding recurrence rates (2.5% vs 3.5%, respectively; p=0.5746), site of recurrence and survival rate. Kaplan-Meier

TABLE 1 Univariate analysis between DTC and non-DTC patients.

	Non-DTC (Benign, 440)	DTC (Malign, 399)	*p*
Mean Age	54.4 ± 14.6	49.5 ± 15	**< 0.001**
Sex male	116 (24.4%)	113 (28.3%)	0.5769
Concomitant HT	170 (38.6%)	200 (50.1%)	**< 0.001**
Levothyroxine Therapy	45 (10.2%)	40 (10%)	0.9859

DTC, Differentiated Thyroid Cancer HT, Hashimoto Thyroiditis. Bold values simply indicate a statistical significative p value.

TABLE 2 Multivariable analysis between DTC and non-DTC group.

Variable	Coefficient	OR	95% CI	*p*
Age	-0,019685	0,9805	0,9712 to 0,9899	**<0,0001**
Male Sex	0,27206	1,3127	0,9539 to 1,8063	0,0948
Levothyroxine Therapy	-0,23410	0,7913	0,5169 to 1,2113	0,2812
HT	0,57570	1,7784	1,3332 to 2,3722	**0,0001**

Significance level p < 0,0001 AUC 0.608.
DTC, Differentiated Thyroid Cancer. HT, Hashimoto Thyroiditis. OR, Odds Ratio. CI, Confidence Intervals). Bold values simply indicate a statistical significative p value.

TABLE 3 Differentiated thyroid cancer patients.

	DTC (399)	HT-DTC (200)	Non-HT-DTC (199)	*p*
Age (years)	49.5 ± 14.9	48.9 ± 14.8	50.1 ± 15	0.4131
Male Sex	113 (28.3%)	38 (19%)	75 (37.7%)	< **0.0001**
Gross extrathyroidal extension	28 (7%)	12 (6%)	16 (8%)	0.4410
Microscopic extrathyroidal extension	20 (5%)	8 (4%)	12 (6%)	0.3703
Multifocality	140 (35.1%)	81 (40,5%)	59 (29.6%)	**0.0233**
Vascular invasion	19 (4.8%)	7 (3.5%)	12 (6%)	0.2509
Tumor size (mm)	15.6 ± 14.5	13.7 ± 11.9	17.6 ± 16.5	**0.0074**
Microcarcinoma (tumor size < 10 mm)	156 (39.1%)	88 (44%)	68 (34.2%)	**0.05**
Patients with 5 or more metastatic lymph node	17 (4.3%)	10 (5%)	7 (3.5%)	0.6214
PTC	220 (55.1%)	117 (58.5%)	103 (51.8%)	0.0691
Aggressive variants of PTC	90 (22.6%)	48 (24%)	42 (21.1%)	
FTC	89 (22.3%)	35 (17.5%)	54 (27.1%)	

DTC, Differentiated Thyroid Cancer. HT, Hashimoto Thyroiditis. PTC, Papillary Thyroid Cancer. FTC, Follicular Thyroid Cancer. Bold values simply indicate a statistical significative p value.

curves for independent factors are reported in Figure 1. The log-rank test in Kaplan-Meier curves did not show any significant difference between the HT-DTC and non-HT-DTC groups (p=0.5548).

Discussion

Since its first observation in 1955 by Dailey et al., the link between HT and DTC remains a controversial subject. Despite many studies in the literature having exploited this topic, there is not unanimous scientific opinion, and the debate is still open. Several studies reported an association between these two diseases (3, 16, 17, 22), while others did not find any significant relationship (27, 28). In 2013, Chui et al. demonstrated that autoimmune thyreopathy and chronically present phlogistic infiltrates could lead to the alteration of the follicular epithelium of the thyroid, which in fact could be responsible for the dysplastic transformation of the follicular epithelium and to the formation of so-called "zones of follicular dysplasia". This is a preneoplastic lesion that can evolve into papillary thyroid cancer (3, 29). Particular attention is given in the literature to the research of novel prognostic markers in thyroid cancer and the possible role of HT as a risk factor in the development of DTC; therefore, its role as a favorable prognosis factor continues to keep the discussion alive among the experts (3, 15–17, 20, 22, 30–36).

The aim of our study was to examine the relationship between HT and DTC, evaluate whether HT could be an independent risk factor for DTC, and assess the characteristics of DTC with concomitant HT in terms of risk of structural disease recurrence and disease-free survival when compared to DTC without concomitant HT.

In our study, the frequency of concomitant HT with DTC on surgical specimens was significantly higher than in non-DTC patients: approximately 50.1% of DTC patients were diagnosed with a concomitant HT, while only 38.6% of non-DTC patients were found to have a concomitant HT diagnosis on pathological examination ($p < 0.001$). These results are concordant with the most recent literature, as other studies before ours have pointed out an association between HT and DTC (3, 16, 17, 20, 22).

Notably, our study found no difference in terms of preoperative administration of substitutive levothyroxine therapy between the DTC and non-DTC groups; furthermore, DTC patients were significantly younger than non-DTC patients. This difference could be explained by a slightly earlier diagnosis of DTC with HT, as those patients are likely more prone to close follow-up, as already pointed out by Battistella et al. (22). It should also be emphasized that our sample is represented entirely by surgical patients; thus, a selection bias

TABLE 4 Risk of Structural Disease Recurrence between differentiated thyroid cancer with concomitant Hashimoto thyroiditis (HT-DTC group) and differentiated thyroid cancer without concomitant Hashimoto thyroiditis (non-HT-DTC group).

	Low Risk	Intermediate Risk	High Risk	p
HT-DTC	125	57	18	0.9033
Non-HT-DTC	120	60	19	

DTC, Differentiated Thyroid Cancer; HT, Hashimoto Thyroiditis.

TABLE 5 Differentiated thyroid cancer follow-up.

Variable	DTC (399)	HT-DTC (200)	Non-HT-DTC (199)	*p*
FU losses (%)	60 (15%)	29 (14.5%)	31 (15.6%)	0.8720
Mean duration of FU (months)	50.5 ± 13.8	49.5 ± 13.8	51.5 ± 13.8	0.1913
RAI therapy	225 (66.4%)	109 (63.7%)	116 (69%)	**0.04**
RAI therapy cycles (median)	1	1	1	1
Disease recurrence	12 (3.5%)	5 (2.9%)	7 (4.2%)	0.5746
Mean time from operation to recurrence diagnosis (month)	16.4 ± 13.4	16.8 ± 12.6	16.1 ± 15	0.9379
Local Recurrence	3 (0.9%)	2 (1.2%)	1 (0.6%)	1
Nodal Recurrence	6 (1.8%)	2 (1.2%)	4 (2.4%)	0.4489
Death due to DTC	2 (0.5%)	1 (0.5%)	1 (0.5%)	1

DTC, Differentiated Thyroid Cancer. HT, Hashimoto Thyroiditis. RAI, Radio-Active Iodine. FU, Follow-up. Bold values simply indicate a statistical significative p value.

could be present regarding age: it is possible that the age difference is due to earlier intervention in DTCs than in patients with benign disease.

In the multivariable analysis we found that the presence of HT was an independent risk factor for developing DTC (OR 1.778, 95% CI 1.333 to 2.372, $p < 0.001$). These results are similar to those published by Apostolou et al. in their 2021 manuscript, which shows an OR for thyroid cancer development of 2.31 when concomitant HT was diagnosed (95% CI 1.85-2.89, $p < 0.001$) (37).

Many authors have reported a considerably better prognosis of DTC when HT is also present; however, these data are not uniform in the various studies, as some report a greater presence of multifocal carcinomas and a similar recurrence rate to non-HT tumors (20, 21), while others show a greater presence of microcarcinomas, with a smaller average tumor size and a lower incidence of lymph node metastases and extrathyroidal extension, leading to significantly better disease-free survival for DTCs with concomitant HT than for those without HT (16, 22, 23, 38).

The 2015 ATA guidelines proposed a classification of risk of structural disease recurrence for DTCs, based on histological features of aggressiveness, such as extrathyroidal extension, vascular invasion, nodal metastasis, multifocality and aggressive variants. According to that, DTCs could be divided into three categories: low risk, intermediate risk and high risk, which identify a progressive risk of disease recurrence (26). As reported in table 4, we stratified DTC patients based on this classification. However, no statistically significant difference was found between HT and non-HT carcinomas.

We subsequently proceeded to analyze tumor characteristics with and without the coexistence of HT in surgical specimens,

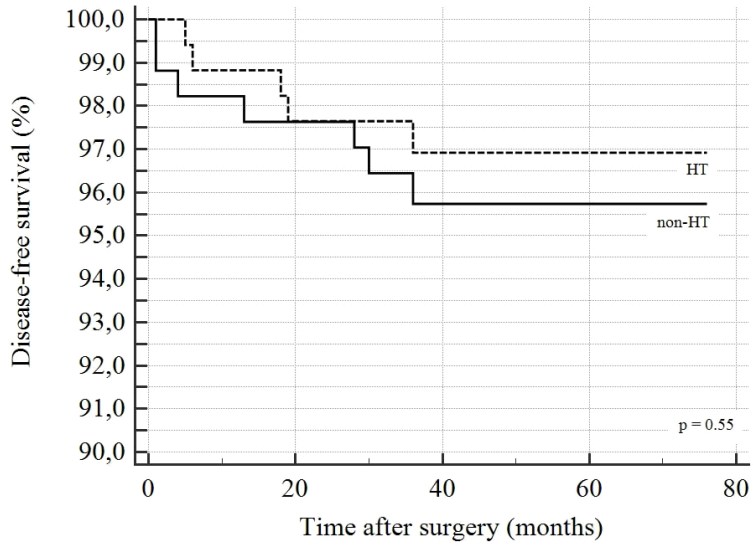

FIGURE 1
Kaplan-Meier curves estimating disease-free survival in HT-DTC and non-HT-DTC.

HT-DTC, and non-HT-DTC groups respectively. The results are shown in Table 3. A clear female predominance was present in the HT-DTC group, as widely foreseeable given the presence of HT. A statistically significant difference was found in tumor multifocality, which was present in 40.5% of HT-DTC, while only 29.6% of non-HT-DTC tumors were classified as multifocal (p=0.02). Our results are concordant with the studies in the literature, which report a slightly higher presence of multifocal thyroid cancers when HT is present (20, 21). Furthermore, HT-DTC tumor size was significantly smaller than non-HT-DTC, 13.7 ± 11.9 mm vs 17.6 ± 16.5 mm (p=0.007). According to that, 44% of HT-DTC was a microcarcinoma, compared to only 34.2% of the non-HT-DTC group (p=0.05). These results are similar to those reported by Battistella et al., Uhliarova et al., and Liang et al. (16, 20, 22) and support the thesis that DTCs with concomitant HT exhibit less aggressive histological features, although our study did not report statistically significant differences in terms of lymph node metastases and extrathyroidal extension in HT carcinomas, while some authors report a lower incidence of these aggressive features in HT-carcinomas, as pointed out before in this paragraph.

Globally, our overall survival was 99.5%, and our recurrence rate was 3%, with a mean time between surgery and diagnosis of recurrence of 50.5 ± 13.8 months. No statistically significant difference between HT-DTC and non-HT-DTC groups was found regarding recurrence rate and overall survival. Our study found a significant difference in terms of post-operative administration of RAI therapy, which was given in 63.7% in the HT-DTC group and in 69% in the non-HT-DTC group (p=0.04). These results are in line with those reported in the literature in the study of Lau et al., where the authors found that HT patients tend to have fewer cycles of RAI therapy and correspondingly have excellent response to treatment. Although our study found no influence of HT on recurrence and survival rates, this is probably due to the relatively small sample size; in the literature, a recent meta-analysis performed by Xu et al. showed a better prognosis of HT carcinomas than non-HT carcinomas, with a reduction both in mortality rate and recurrence rate (21).

Our study has some limitations. First, it is a retrospective study, even if we want to emphasize that data were extracted from a prospectively maintained database. The second limitation, as previously reported, is that our sample is based only on patients already submitted to thyroid surgery; therefore, it may not be fully representative of the general population. Finally, the real incidence of disease recurrence could be underestimated in our study, considering that the mean follow-up is 50.5 months, that these kinds of tumors are generally indolent, and that recurrences can appear up to 10 years after surgery.

Conclusion

In conclusion, the relationship between HT and DTC is far from explained. HT is frequently coexistent with DTCs in surgical specimens, and in our study, HT was found to be an independent risk factor for developing DTC. Furthermore, DTCs developed with concomitant HT appear to be smaller tumors than DTCs developed without HT, and even if our study finds no difference in risk of structural disease recurrence and recurrence rates between HT-DTC and non-HT-DTC, data in the literature suggest that DTCs with concomitant HT are characterized by less aggressive histological features and better prognosis than those without HT. Given our data, and considering those already present in the literature, we think that a strict follow-up of HT patients is advisable.

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 Independent Ethics Committee, A.O.U. Cagliari. The patients/participants provided their written informed consent to participate in this study.

Author contributions

FC: study conception and design, acquisition of data, analysis and interpretation of data, drafting of the article, final approval of the version to be submitted. GC: acquisition of data, drafting of the article, final approval of the version to be submitted. ML: analysis and interpretation of data, final approval of the version to be submitted. EL: analysis and interpretation of data, revision of the article for important content, final approval of the version to be submitted. MB: acquisition of data, analysis and interpretation of data, final approval of the version to be submitted. FB: analysis and interpretation of data, revision of the article for important content, final approval of the version to be submitted. FM: study conception and design, acquisition of data, drafting of the article, final approval of the version to be submitted. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

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Thyroid and renal cancers: A bidirectional association

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There is a deep interrelation between the thyroid gland and the kidney parenchyma, with dysfunction of the first leading to significant changes in renal metabolism and *vice versa*. Given the recognition of cancer as a systemic disease, the raise of thyroid tumors and the common association of several malignancies, such as breast cancer, prostate cancer, colorectal cancer, and other, with an increased risk of kidney disease, public health alert for these conditions is warranted. A systematic review of the current evidence on the bidirectional relationship between thyroid and renal cancers was conducted including 18 studies, highlighting patient's characteristics, histology, time for secondary malignancy to develop from the first diagnosis, treatment, and follow-up. A total of 776 patients were identified; median age was 64 years (range: 7–76 years). Obesity and family history were identified as the most common risk factors, and genetic susceptibility was suggested with a potential strong association with Cowden syndrome. Controversy on chemo and radiotherapy effects was found, as not all patients were previously exposed to these treatments. Men were more likely to develop kidney cancer after a primary thyroid malignancy, with 423/776 (54%) experiencing renal disease secondarily. Median time after the first malignancy was 5.2 years (range: 0–20 years). With the advancement of current oncological therapy, the prognosis for thyroid cancer patients has improved, although there has been a corresponding rise in the incidence of multiple secondary malignancy within the same population, particularly concerning the kidney. Surgery can achieve disease-free survival, if surveillance follow-up allows for an early localized form, where radical treatment is recommended.

KEYWORDS

thyroid cancer, renal cancer, multiple cancer, cancer surveillance and screening, cancer risk

Abbreviations: ccRCC, clear cell renal cell carcinoma; CS, Cowden syndrome; PTC, papillary carcinoma of the thyroid; RCC, renal cell carcinoma; TC, thyroid cancer.

Introduction

Thyroid cancer (TC) is one of the most rapidly increasing malignancies in Western countries, with an annual incidence rate of 5.4% in men and 6.5% in women (1). Much of this rise is largely due to early detection using more sensitive diagnostic procedures, including Artificial Intelligence, performed for other medical reasons and able to identify incidental small thyroid nodules, otherwise missed (2–4). Certain risk factors for TC are female sex, family history of TC, radiation exposure, lymphocytic thyroiditis, and reduced iodine intake (5, 6). On the basis of the histological and the clinical behavior, TCs are divided into well differentiated and poorly differentiated; well-differentiated TCs include the papillary and follicular histotypes (7). Surgery, either lobectomy or total thyroidectomy, represents the standard therapeutic approach for well-differentiated TC; radioactive iodine therapy is recommended for high-risk patients (5). Ablation and active surveillance are of increasing importance in patients who refuse surgery or are unfit for.

Improvements in the detection of TC and therapeutic strategies have likewise resulted in a more favorable course for this disease. Because the mortality rates for TC remained stable at around 0.5 deaths per 100,000, the number of patients surviving is on the rise (8, 9).

On the other hand, renal cancer, or renal cell carcinoma (RCC), is the 9th common cancer in men and the 14th one in women. RCC frequently presents incidentally; in fact, it is asymptomatic in most cases. Therefore, the diagnosis of patients with localized renal cancer, which is potentially treatable only with surgery or ablation, is almost always accidental (10). Identified risk factors include male sex, smoking tobacco, obesity, and hypertension (10, 11). RCC comprises an heterogeneous group of histological subtypes: Clear cell renal cell carcinoma (ccRCC), papillary, and chromophobe are the most common solid RCC (11). Nephron-sparing surgery or partial nephrectomy has evolved as the standard of care in patients with localized RCC; ablation and active surveillance are traditionally alternatives for patients who are unfit for surgery (11).

Thyroid interrelation with the kidney is well known (12); on the one hand, this gland is necessary for renal cells growth and for the maintenance of hydro-electrolyte homeostasis; on the other hand, the kidney eliminates thyroid hormones and regulates their serum level. There is therefore a deep interrelation among the two organs, with thyroid dysfunction causing significant changes to renal metabolism and *vice versa* (13).

Cancer is a systemic disease, and many common cancers, such as breast cancer, prostate cancer, colorectal cancer, and other, are associated with an increased risk of kidney cancer development, especially within the first 5 years after their diagnosis (14). Because the risk of second cancers after the diagnosis of primary TC is elevated (15), too, the aim of this manuscript is to review the current state of knowledge on the interrelationship between thyroid and renal cancers.

Methods

This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement (PRISMA) (16). The search was run in February 2022 across PubMed, Web of Science, and Scopus databases and was restricted to articles written in English only. References were cross-checked for additional relevant studies. The retrieved lists were exported to a reference manager (EndNote™) to eliminate duplicates, as shown in Figure 1.

Keywords "thyroid cancer" and "renal cancer" were used to include studies evaluating TC characteristics in patients previously affected by kidney cancer or kidney cancer characteristics in patients previously affected by TC. Reports of metastases were excluded from the analysis.

The research was performed by two independent investigators; subsequently, the results were compared and combined; in case of disagreement on the value of the selected

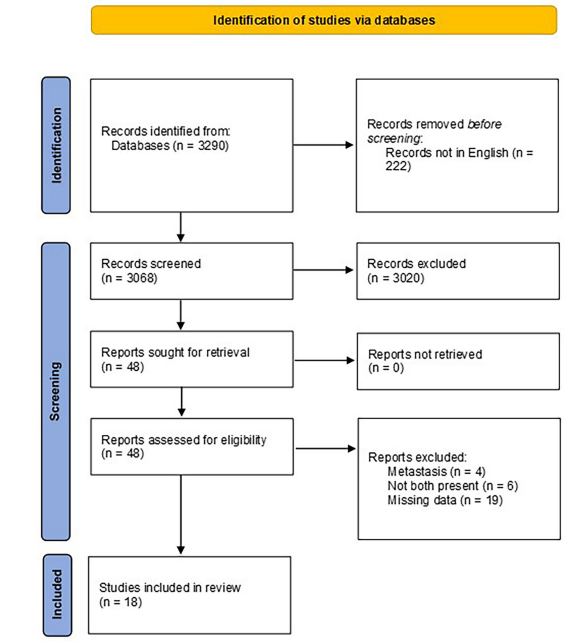

FIGURE 1
PRISMA diagram.

papers, an additional comparison was crucial in the decision-making process. Only published literature was included, and no date limits have been set. Only English language articles were included; reviews, editorials, and repeated or redundant manuscripts were excluded. Only registry analyses and retrospective studies, mostly case reports, were found and included in the present review.

Data extraction was performed thereafter, including the details of title, authors, date of publication, country, research design, patients' characteristics, and outcomes.

A risk of bias assessment was performed using the Newcastle–Ottawa Scale quality assessment star system (Table 1), in which a paper is judged on the selection of the study groups, the comparability of the groups, and the ascertainment of either the exposure or the outcome of interest for case control or cohort studies, respectively (24).

Results

A total of 3,290 manuscripts were retrieved from the search; following exclusion based on title and abstract screening (n = 3,020) and after full text read (n = 48), the remaining studies included in the review were 18 (Table 2). The majority (11/18) were case reports. A total of 776/64,187 patients were identified.

Patients' characteristics

Median age was 64 years (range: 7–76 years). The association of thyroid and renal malignancies was more often observed in the male population. After evaluating the time between the two malignancies for all the studies included in the review, in no case, a significant difference was detected: The median interval

TABLE 1 Newcastle–Ottawa Scale (NOS) quality assessment star system.

NOS[17]

COHORT STUDY

Article	Selection				Comparability	Outcomes			Total
	Selection of nonexposed cohort	Representativeness of exposed cohort	Ascertainment of exposure	Outcome not present at the start of the study		Assessment of outcomes	Length of follow-up	Adequacy of follow-up	
Canchola et al. (17)	☆	☆	☆	☆	☆☆	☆	☆	☆	9/9

CASE CONTROL

Article	Selection				Comparability	Outcomes			Total
	Case Definition	Representativeness of the Cases	Selection of Controls	Definition of Controls		Ascertainment of Exposure	Non-Response Rate	Same methods of Ascertainment for cases and control	
Abdel-Rahman, et al. (14)	☆	☆	☆	☆	☆	☆	☆	☆	8/9
Antonelli, et al. (18)	☆	☆	☆	☆	☆☆	☆	☆	☆	9/9
Carhill et al. (19)	☆	☆	☆	☆	☆☆	☆	☆	☆	9/9
Murray, et al. (20)	☆	☆	☆	☆	☆☆	☆	☆	☆	9/9
Murray S, et al. (21)	☆	☆	☆	☆	☆	☆	☆	☆	8/9
Ngeow, et al. (22)	☆	☆	☆	☆	☆☆	☆	☆	☆	9/9
Van Fossen, et al. (23)	☆	–	☆	☆	☆	☆	☆	☆	7/9

The stars mean the grading according to the Newcastle-Ottawa scale.

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TABLE 2 Results.

Article	Year	Type of study	Case	Sex	Age	Histology	Genetic syndrome	Risk factors	Thyroid cancer	Interval to second cancer	Conclusion
Abdel-Rahman (14)	2017, Egypt	Case control	341/9861	N/A	N/A	N/A	N/A	Treatment factors (radiation) Common etiology factors (smoking) Rare hereditary cancer syndromes	Primary	5 years	Beyond 5 years, patients with primary thyroid cancer have an enhanced risk to develop a second primary kidney cancer. This link may be an expression of a particular genetic makeup determining patients' susceptibility to both cancers.
Albores-Saavedra, et al. (25)	2014, Mexico	Case report	2/2	F	72 54	Papillary urothelial carcinoma and PTC	N/A	No specific risk factors were identified.	Second Second	14 years 1,5 years	These malignant neoplasms do not apparently share similar risk factors.
Antonelli, et al. (18)	2012, Italy	Case control	15/285	N/A	N/A	N/A	N/A	No specific risk factors were identified.	Second	N/A	The risk of development of a second neoplasia in patients with RCC increases with aging.
Canchola et al. (17)	2005, USA	Cohort study	16/10932	F	55	PTC and RCC not otherwise specified	N/A	Obesity increases the risk of both thyroid and kidney cancer	Primary	3 years	Increased surveillance is warranted for kidney cancer among women with thyroid cancer.
Carhill et al. (19)	2014, USA	Case control	117/23514	N/A	N/A	Papillary thyroid carcinoma (85%) and ccRCC (79%)	N/A	Genetic susceptibility, implication of clinical therapy	N/A	6 years	The association between thyroid and kidney cancer needs further investigation.
Oh, et al. (26)	2015, Korea	Case report	1/1	M	50	ccRCC and PTC	N/A	Family history of thyroid cancer	Synchronous	0	No specific risk factor or genetic syndrome were identified.
Atta, et al. (27)	2016, Egypt	Case report	1/1	F	76	ccRCC and PTC	No mutations were detected	Family history of colon, lung, kidney and thyroid cancer.	Primary	14 years	No genetic mutation was detected, despite the family history.
Kim, et al. (15)	2020, Canada	Case report	1/2	M	22	Chromophobe RCC and PTC	Cowden syndrome (CS)	Family history of kidney and thyroid cancer.	Primary	12 years	Thyroid neoplasia and RCC are minor diagnostic criteria for CS.
Klain, et al. (28)	2021, Italy	Case report	1/2	M	64	ccRCC and PTC (tall-cell variant)	N/A	No specific risk factors were identified	Second	20 years	No specific risk factor or genetic syndrome were identified.
Ma, et al. (29)	2014, China	Case report	1/1	F	35	ccRCC+ SFT and PTC + follicular thyroid carcinoma	N/A	Negative family history of neoplasia	Synchronous	0	No specific risk factor or genetic syndrome were identified.
Malchoff et al. (30)	1999, USA	Family report	31/31	N/A	N/A	Papillary renal carcinoma and PTC	Mutation of a gene that maps to 1q21	No specific risk factors, except for	N/A	N/A	Familial association of PTC with papillary renal neoplasia defines a distinct familial tumor syndrome.

(Continued)

TABLE 2 Continued

Article	Year	Type of study	Case	Sex	Age	Histology	Genetic syndrome	Risk factors	Thyroid cancer	Interval to second cancer	Conclusion
								genetics, were identified			
Murray, et al. (20)	2016, USA	Case control	12/3066	6 F 6 M	53 64	PTC and RCC not otherwise specified	N/A	No specific risk factors were identified	Second	N/A 7 years	The rate of thyroid cancer in both women and men surgically treated for RCC was significantly higher. Observed association is unlikely due to treatments effects because primary treatment in renal cancer is surgical.
Murray S, et al. (21)	2013, USA	Case control	3/433	N/A	N/A	N/A	N/A	Older and radiation exposure	Synchronous	0	Papillary thyroid cancer is the most frequent histologic type associated to RCC.
Ngeow, et al. (22)	2014, USA	Case control	2/114	M F	7 36	N/A	PHTS	PTEN mutation	Primary	14 8	A bidirectional association between thyroid and renal cancers suggests shared genetic and environmental risk factors.
Peng, et al. (31)	2019, China	Case report	1/1	M	58	ccRCC and micro-papillary thyroid carcinoma	N/A	No specific risk factors were identified	Primary	1 years	Integrin αvβ6 is positively expressed in multiple primary cancer, also in patients with RCC and thyroid cancer.
Samarasinghe, et al. (32)	2020, USA	Case report	1/1	F	56	ccRCC and PTC + medullary thyroid cancer	RET mutational analysis was negative	Family history of breast cancer and RCC	Primary	2 years	RET mutational analysis was negative.
Song, et al. (33)	2017, Canada	Case report	1/1	M	72	ccRCC and PTC	N/A	N/A	Synchronous	0	Tumour-to-tumour metastasis of a thyroid cancer into a primary renal neoplasm is extremely rare and maybe resulting from rich vascularity and perfusion to enable successful delivery and deposition of metastatic tumour cells.
Van Fossen, et al. (23)	2013, USA	Case control	230/15940	90 M 60 F	N/A	N/A	N/A	N/A	60 primary 80 second	N/A	This study demonstrated a bidirectional association between thyroid and renal cancers. This association is more likely explained by shared genetic and environmental factors.

ccRCC, clear cell renal cell carcinoma; PTC, papillary thyroid carcinoma; N/A, not available; F, female; M, male.

between first and second cancer was 5.2 years (range: 0–20 years).

Most patients presented a TC as first primary malignancy (423 out of 776; 54%), and 110 patients (14%) developed a TC as second primary malignancy; a renal tumor was synchronous in only six patients out of 776 (0.78%).

Histopathological characteristics

With regards to histology, the papillary phenotype of TC was found in all patients. Sporadic cases of follicular carcinoma (29) and medullary (32) thyroid carcinoma have also been identified; however, both of them were also associated with a papillary thyroid carcinoma. On the contrary, with regards to renal carcinoma, a greater variety was observed concerning histology. In the series evaluated, the most represented type was ccRCC; however, sporadic cases of other renal malignancy cases were also reported, namely, urothelial (25), chromophobic (34), and papillary renal carcinoma (30).

Sex

With regard to TC, female sex was universally identified as a risk factor (17, 20); on the contrary, male sex is associated to the

development of RCC (17). From the report by Van Fossen et al. (23), female TC patients had a twofold increase in the prevalence of a subsequent renal cell cancer (23), and female renal cell cancer patients had a 1.5-fold increase in the prevalence of TC; male patients with TC had 4.5-fold prevalence increase of subsequent RCC, and male patients with RCC had an increased threefold prevalence of subsequent TC. Male sex emerged as a risk factor of association between thyroid and kidney cancers (18).

Common identified risk factors

Identified common risk factors between thyroid and kidney cancers are few (19, 25), with obesity remaining a unique denominator to develop malignancy in general and in particular for these two; see Figure 2. Family history (29) of both cancers is also well recognized, and as previously mentioned, male sex as well as aging increased the risk to develop both cancers and, in particular, RCC (18).

Although radiation exposure is a known risk factor for the development of neoplasms and, in particular, for TC, no correlation between radiotherapy and the development of both TC and renal cancer was identified in any of the studies included in the review. Murray et al. (21) observed, in fact, that only 35% of patients with TC and an additional primary cancer, whether it is RCC or not, reported radiation exposure in their medical history. Given the recognized increased risk of TC following other primary malignancies, particularly RCC, it seems unlikely that the increased incidence could be due to the carcinogenic effect of radiations (14).

Although many chemotherapeutic agents are known to be carcinogenic, the patients have not undergone chemotherapy, and it is therefore not possible to evaluate the carcinogenic effects of these drugs.

Genetic syndrome

TC is associated with a heterogeneous pattern of genetic mutations involving the mitogen-activated protein kinase (MAPK) pathway (6). The main genetic mutations are represented by the oncogenes RAS and BRAF; in particular, $BRAF^{V660E}$ is present in about half of the PTCs (35).

Excluding the familial forms of medullary thyroid carcinoma, such as familial medullary thyroid carcinomas and multiple endocrine neoplasia (MEN), the familial forms of thyroid carcinoma are numerous and can be divided into syndromes with a prevalence of non-thyroid neoplasms and syndromes with a prevalence of TC. The first group includes also familial adenomatous polyposis, Cowden syndrome (CS), Werner syndrome, Carney complex, and Pendred syndrome; the second group includes pure familial papillary thyroid carcinoma with or without oxyphilia and familial papillary thyroid carcinoma with papillary RCC or with multinodular goiter (36).

There are also numerous genetic alterations involved in the development of RCC, in particular, the most important mutations involving the tumor-suppressor Von Hippel-Lindau (VHL), observed in about 80% of ccRCC (9). Hereditary forms of RCC include von Hippel-Lindau syndrome, hereditary papillary RCC, Birt-Hogg-Dubé syndrome, hereditary leiomyomatosis, and tuberous sclerosis (11).

The link between thyroid and kidney cancer may be an expression of genetic makeup that increases patients' susceptibility to both malignancies (26, 27). Although this

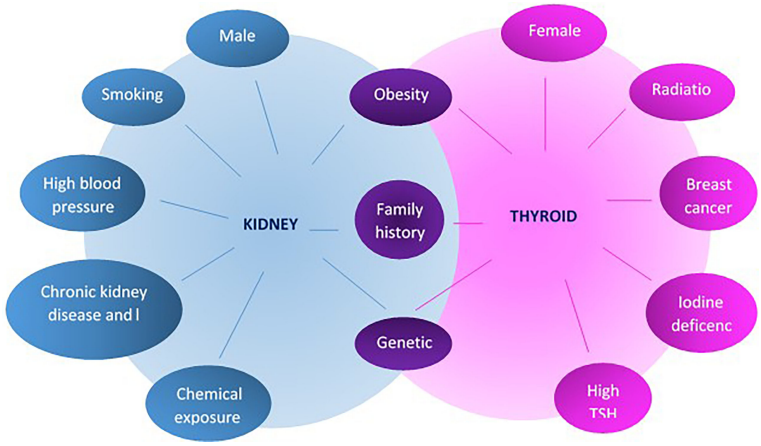

FIGURE 2
Risk factors for the development of thyroid and kidney cancer.

condition seems the most likely to occur, few studies have identified genetic syndromes or specific mutations, which are associated with the increased susceptibility to these two tumors, highlighting a familiar link (23, 32).

In the present review, CS (15, 22) was confirmed a risk factor leading to thyroid and renal cancers. CS is part of the Phosphatase and tensin homolog (PTEN)-hamartoma tumor syndrome, a disorder caused by a germline mutation of PTEN, a tumor suppressor gene. This syndrome is associated with the development of a variety of tumors, both benign and malignant (11): Thyroid carcinoma is one of major diagnostic criteria for CS, whereas RCC is part of the minor criteria.

PTEN mutation, even in the absence of the CS, was identified as risk factor (22), with Integrin αvβ6 positively expressed in multiple primary cancer, among which TC and RCC (31). The genetic mutation 1q21 (30) was identified in forms where PTC was associated to papillary renal neoplasia tumors, highlighting in this way a peculiar familial tumor syndrome (Table 2).

Time of second cancer occurrence

According to the analysis of the data presented in Table 2, the median interval between first and second cancer was 5.2 years (range: 0–20 years), with no substantial difference in the time interval considering one or the other cancer as the first presented.

Treatment and follow-up

For both thyroid and kidney cancers, treatment of choice was represented by surgical excision (28), with no differences if they were primary, synchronous, or second malignancies. In most cases, the selected patients were affected by localized neoplasms; thus, no need for systemic therapy was required. Furthermore, as they were detected at an early stage, surgery had a curative effect. In case of synchronous malignancy, radical nephrectomy first and then total thyroidectomy with lymphoadenectomy were carried out (33).

In general, management of PTC remained equivalent, regardless of whether or not the patient had a synchronous or antecedent non-thyroidal neoplasia (21).

In most of the reported cases, the second cancer was identified during follow-up, except for the few cases of synchronous tumors, for which the pre-operative investigations made it possible to identify the second neoplasm at an earlier stage (33). In consideration of the increased risk of developing a second tumor after the primary cancer, all the authors recommended to keep this risk in mind during the follow-up of thyroid and kidney malignancies.

Discussion

The present review evaluated the association between thyroid and renal cancers, regardless of which cancer occurred first, highlighting that each primary thyroid or renal malignancy increases the relative risk of subsequent malignancy in the remnant organ of the survived patients. This applies to both sexes, particularly relevant in men (23), even if other reports document an increase only in treated female TCs (37).

Although there is a risk of a second primary tumor following primary invasive neoplasms and, specifically, there is a reciprocal association between thyroid and renal cancers, the estimated risk for the development of both cancers is low, with an incidence of about 1% according to Van Fossen et al. (23) For this reason, it is not considered necessary to include diagnostic screening tests in the follow-up of these neoplasms, compared with what is already foreseen for general population. If a more targeted preventive screening is deemed appropriate (7, 38, 39) in the presence of additional risk factors, ultrasound scans of the neck and kidneys may be indicated.

A bidirectional association between thyroid and renal cancers can be explained by shared genetic and/or common environmental risk factors including recognized etiological factors (i.e., smoking and obesity), or rare genetic syndromes predisposing to both events and regardless of the use of any forms of radiation treatment (14). Furthermore, individuals who develop both thyroid and renal carcinomas may represent a unique subset of cancer patients (19).

TC is associated with a number of genetic mutations leading to a different aggressive behavior. BRAF and RAS rearrangements remain the principal oncogenes, although other mutations, namely, TERT promoter and in TP53, as well as PIK3CA–PTEN–AKT–mTOR pathway and SWI–SNG complex (40), synergistically concur to worse outcomes and can be used in tumor prognostication (41). In the case of medullary carcinoma, RET mutation is commonly identified, supporting a distinct clonal origin in the case of a coexisting papillary tumor, as different cellular types might be affected simultaneously (42, 43).

The majority of renal carcinomas are sporadic, and numerous are the genetic alterations involved; in particular, the most important mutations involve the tumor-suppressor VHL, observed in about 80% of ccRCC (9). A genetic predisposition accounts for around 4% of the incidence of this malignancy, namely, in people affected by von Hippel-Lindau disease, hereditary papillary renal cancer, hereditary leiomyomatosis and renal cancer, and Birt-Hogg-Dubé syndrome. Other studies have also proposed possible genetic correlations between thyroid and renal cancers; Malchoff et al. (30) identified a distinct familial tumor syndrome linked to a germline mutation in chromosome

1q21 and characterized by a familial association of papillary TC, nodular thyroid disease, and papillary renal neoplasia. TC of follicular origin and renal cancer have also been found with greater frequency in CS, a hereditary cancer syndrome associated with a germline mutation in PTEN (44) and characterized by the presence of multiple hamartoma and dermatologic manifestations such as acral keratosis and facial trichilemmomas. For CS, thyroid carcinoma is one of the major diagnostic criteria, whereas RCC is part of the minor criteria.

Interestingly, as our review reported, the phenomenon of increased genetic instability and reduction of tumor immunity in multiple cancer patients was confirmed by the case of the woman with medullary, papillary, and RCC (32), a very rare combination, where even if the patient had no previous endocrine history, her mother was affected by breast cancer, another disease deeply connected to TC (4, 45–47) and the brother presented with RCC, too. A triple malignant tumor was also reported in a male of the same age with thyroid, kidney, and colon being affected (31), demonstrating common expression paths with integrin avß6 in multiple primary cancer (29).

Treatment of choice is surgery, regardless of whether or not the patient had a synchronous or antecedent neoplasia (21). The prognosis is in relation to the biological characteristics of each cancer, with the extent of the surgical procedure aiming to radically excise the mass and potentially reduce the incidence of subsequent cancers (48), without additional chemo and/or radiotherapy. If the patient is unfit for surgery, ablation *via* interventional radiology might represent a valid alternative (14).

Although radiation exposure is a known risk factor for the development of neoplasms and in particular for TC, and many chemotherapeutic agents are known to be carcinogenic, no correlation between radiotherapy or chemotherapy and the development of both TC and renal cancer was identified in any of the studies included in the review. This is mainly because the treatment of localized kidney cancer is primarily surgical, such as the treatment of well-differentiated TC; in fact, the association between kidney and TC is probably not related to radio and chemotherapy treatments (22) but rather to a shared genetic makeup or other environmental factors.

Considering the low risk of developing kidney cancer after TC and *vice versa*, it does not seem necessary to change the follow-up of patients with one of the two cancers to monitor the onset of the other one; however, it is important to always keep in mind that there is an increased risk of developing a second malignancy. It is always necessary to remember that, in subjects with a genetic syndrome that increases the risk of developing tumors, the follow-up should be structured taking into account the underlying genetic pathology.

Limitations

In consideration of the practical difficulties associated with evaluating the research question in prospective settings, only registry analyses and retrospective studies, mostly case reports, were included in the present review, limiting the evidence achieved. High-quality population databases are recommended with prospective analysis to elucidate on the bidirectional association between thyroid and kidney cancers.

Conclusions

As for TC, the advancement of diagnostic methods has led to an early treatment and an improvement in prognosis; in the same way, for kidney cancer, the increase in the diagnosis of neoplasms in the early stages has led to an increased survival; therefore, there has been a corresponding rise in the incidence of multiple primary cancers. A bidirectional association between thyroid and renal cancers has been identified and can be explained by shared genetic and common environmental risk factors. Even if there is an association, the coexistence of primary thyroid and RCC is rare. The standard treatment for both thyroid and kidney cancers remains surgery, which does not need to be associated with adjuvant therapies in the early stages, and the follow-up does not require special attention from clinicians or screening tests, except in cases of known genetic syndromes.

Data availability statement

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

Author contributions

Conceptualization: MB, EL, SS and DP. Methodology: MB, SS, FF, AL, DT, MA and MV. Investigation and Data curation: EL, DP, VC, VD, EB and SU. Writing—original draft preparation: MB and EL. Writing—review and editing: MB, EL, SS, DP, FF, AL, DT, MA, MV, VC, EB and SU. Supervision: SS, DP, FF, SU and AL. 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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Molecular features of aggressive thyroid cancer

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Poorly differentiated thyroid cancer (PDTC) and anaplastic thyroid cancer (ATC) have a worse prognosis with respect to well differentiated TC, and the loss of the capability of up-taking ^{131}I is one of the main features characterizing aggressive TC. The knowledge of the genomic landscape of TC can help clinicians to discover the responsible alterations underlying more advance diseases and to address more tailored therapy. In fact, to date, the antiangiogenic multi-targeted kinase inhibitor (aaMKIs) sorafenib, lenvatinib, and cabozantinib, have been approved for the therapy of aggressive radioiodine (RAI)-resistant papillary TC (PTC) or follicular TC (FTC). Several other compounds, including immunotherapies, have been introduced and, in part, approved for the treatment of TC harboring specific mutations. For example, selpercatinib and pralsetinib inhibit mutant RET in medullary thyroid cancer but they can also block the RET fusion proteins-mediated signaling found in PTC. Entrectinib and larotrectinib, can be used in patients with progressive RAI-resistant TC harboring TRK fusion proteins. In addition FDA authorized the association of dabrafenib ($BRAF^{V600E}$ inhibitor) and trametinib (MEK inhibitor) for the treatment of $BRAF^{V600E}$-mutated ATC. These drugs not only can limit the cancer spread, but in some circumstance they are able to induce the re-differentiation of aggressive tumors, which can be again submitted to new attempts of RAI therapy. In this review we explore the current knowledge on the genetic landscape of TC and its implication on the development of new precise therapeutic strategies.

KEYWORDS

aggressive thyroid cancer, genetic mutations, molecular features, RAI refractioness, targeted therapy

Introduction

Thyroid cancer (TC) is a highly diffuse endocrine tumor affecting especially the female gender with a low death rate but increasing worldwide (1–4). TCs classification is based on the cells of origin with an incidence that changes according to the different histotypes. The differentiated TC (DTC) is the most common tumor, which arises from thyroid follicular cells, and represents with papillary TC (PTC), and follicular TC (FTC) about 85–95% of all TCs. Hürthle cells TC and poorly differentiated TC (PDTC) account for 2–5% of all TCs, and the anaplastic TC (ATC) comprises about 1.7% of all cases of TC. Medullary TC (MTC) arises from para-follicular C cells of neuroendocrine origin, accounting for 3–5% of all TCs (5).

Patients with PDTC and ATC have a worse prognosis with respect to well differentiated TC (WDTC), and a lower overall survival (OS) rate with a mean survival of about 3.2 years and 6 months, respectively (6). High rate of disease relapse is registered in PDTC patients, who report frequent local invasion of the disease at the level of trachea and/or esophagus, and also distant progression to the liver, lungs, bone and brain (7–9).

Some PDTC tumors are characterized by refractoriness to T4-mediated TSH suppression or to the therapy with radioiodine (RAI) (7).

ATC is a very aggressive cancer usually originating from DTCs or PDTCs, and is characterized by a quickly growth that can vary from days to several weeks; it is often associated to dysphagia, acute hoarseness, dyspnea, and/or neck pain (7, 10).

Thyroid ultrasound (US) helps in stratifying the risk of malignancy of thyroid nodules, that according to their morphological features (shape, size, echogenicity, margins, the presence of microcalcifications) can be further examined by the fine needle aspiration cytology (FNAC) (11).

The criteria defining the risk of malignancy for biopsied nodules and their subsequent clinical management follow the Bethesda classification system (12).

However, it is often challenging make the right therapeutic decision with indeterminate thyroid nodules, and molecular testing of genetic mutations related to TC can improve the risk stratification supporting the decision-making process in order to avoid unnecessary invasive procedure, such as surgery, and predicting possible adverse clinical outcomes in the post-operative phase (11, 12).

Thyroid cancer molecular alterations

Some of the genetic TC alterations are called "driver" mutations that promote the normal cell transition into cancerous one, whereas the "passenger" mutations are the consequences of carcinogenesis and of loss of differentiation (13, 14). About 90% of alterations are mutually exclusive activating oncogene *BRAF* (~60%), *RAS* (~13%), and rearrangements [ALK, RET, and NTRK genes (~5%)]; whereas the other 10% are loss-of-function of tumor suppressor genes (including PPARγ, PTEN, and TP53) (15–17). The Cancer Genome Atlas (TCGA) documented aberrations of genes in 97% of PTCs, including driver genes *CHEK2, EIF1AX*, and *PPM1D*, members of the phosphoinositide 3-kinase (PI3K) pathway and other gene fusions (17), however 3% of PTCs (called "dark matter") still are genetically undefined (18). The molecular mechanisms that guide the progression to a more aggressive pattern are not largely elucidated (19). The genetic alterations per tumor found in ATC are higher in comparison to PTC and FTC (16); and according to TCGA, PDTC has also a higher mutational burden compared to PTC, but lower than ATC. Genomic instability in PDTC and ATC involve both somatic driver mutations and gene fusions (17, 20). Parallel sequencing studies have been carried out on both PDTCs and ATCs, in order to study their molecular features and discovering the differences between these two types of tumors. Elevated frequencies of TERT promoter, TP53, PTEN and PIK3CA mutations have been observed in ATCs with respect to PDTCs; ATCs also have NF1, NF2, ATM, CDKN2A, CDKN2B and RB1 mutations. Instead, PDTCs showed a higher frequencies of gene fusions (RET, ALK, NTRK1, NTRK3) (21). Recently, next-generation sequencing (NGS) studies, have revealed molecular clues underlying the progression of DTC to PDTC and ATC (15, 16).

Genetic pathways, and epigenetic mechanisms implicated in TC pathogenesis and progression

Most of the TC primary driver oncogenes activate the mitogen activated protein kinase (MAPK) and the PI3K pathways (22–24); the alterations involving these pathways are the most found in ATC and PDTC (20). $BRAF^{V600E}$ and RAS-like mutations, including three highly homologous isoforms (NRAS, KRAS and HRAS) are the most common found in TC.

According to the TCGA, $BRAF^{V600E}$ is the most frequent driver mutations associated with PTC (1, 17, 25); found in 25% of ATC, and associated with tumor aggressiveness, and a bad prognosis (26, 27). Moreover, it is related to an absent or reduced expression of various genes, such as those encoding thyroid-peroxidase, the sodium-iodide symporter, Tg, TSH receptor, and pendrin genes (SLC26A4) (1, 28, 29). Therefore, it is suggested as a predictive marker of PTC persistence or recurrence, decreased efficacy of RAI therapy (30), and reduction of the OS (13, 31–33).

Mutated BRAF PTC has been related to different clinical-pathological conditions with a negative prognostic impact, and a more aggressive behaviour (extra-thyroidal extension, lymph node metastases, advanced disease stage) (1, 34, 35). Other

studies showed no kind of correlation between $BRAF^{V600E}$ and any of the PTC aggressiveness features (13, 36). However, the detection of $BRAF^{V600E}$ in FNAC improves the diagnostic accuracy of PTC reducing also false-negative results (13).

RAS genes mutations are mainly found in FTC and in follicular variant PTC (FVPTC) (30-45%), in PDTC (20-40%), in a less percentage of ATC (10-20%), and also in benign follicular thyroid adenoma (20-25%), while rarely in classical PTC (1, 37). These mutations are more commonly related to indolent behaviour, follicular growth, encapsulation, and a lower incidence of nodal metastasis (11, 38). However, RAS mutations are believed to worsen TC prognosis and life expectancy inducing the passage from a WDTC to a de-differentiated type, the development of distant metastases, and recurrence (13). Furthermore, the de-differentiation effect has been supported by the chromosome instability because of mutant RAS (1, 39, 40).

Association with clinical-pathological manifestation is controversial (41, 42). Disease-specific death risk is 2.9 times higher in subjects harbouring RAS mutation with respect to those without RAS mutation (43). The detection of RAS mutation in FNAC has an important clinical meaning for indeterminate nodules, with a predictive value for cancer ranging between 74% and 88% (13).

The gradual passage or de-differentiation of WDTC to ATC it has been hypothesized to be induced by the accumulation of genetic alterations, particularly of BRAF or RAS mutations (6, 44, 45).

Point mutations have been also identified in TERT promoter, resulting in a telomerase activation that is up-regulated in 80-90% of TC; whereas it is not present in normal thyroid cells (1, 46, 47).

Duan et al. studies found that: 1) ATC with PTC components is typically characterised by a BRAF mutation, and at least one late mutation event (TP53, TERT, or PIK3CA); 2) RET fusion is more frequently associated to PDTC with PTC components. In subjects with PDTC/ATC a worse OS is related to TERT and concurrent PIK3CA mutations (6). The prognostic effect related to TERT promoter mutations was not present when BRAF mutation occurred separately, showing that the co-existence of both mutations is determinant for tumor aggressiveness (40).

Other alterations mostly found in PDTC (10-14%) than in ATC (3-5%) are the genes fusions (48). RET represents the most frequent genetic fusion, especially RET/PTC1 and RET/PTC3; NTRK, ALK and BRAF fusions are unusual (20, 48). Post-radiation exposure TC, and children, reporting or not a previously irradiation history, display a high frequency of RET/PTC1 and RET/PTC3 rearrangements. RET/PTC3 is related with the tendency for aggressive behaviour and advanced stage, higher rates of extra-thyroidal extension and lymph node metastases (13). It seems that RET/PTC is a leading mutation in thyroid carcinogenesis (49–51), it is especially related to the classic PTC subtype (51). However, according to TCGA, RET/PTC is considered a primary genetic event in only 6.8% of the PTC cohort (1, 17). The diagnostic and predictive value of RET/PTC is controversial; in fact, in cases of indeterminate cytology it is still not routinely examined by molecular testing (13, 52, 53).

As regard the NTRK1/3 rearrangements, their encoded protein is constitutively active, causing the activation of the pro-oncogenic pathways PI3K/AKT, phospholipase C (PLC-γ), and MAPK (1, 7, 54).

Also ALK, a transmembrane tyrosine kinase, when activated can trigger downstream signalling pathways, including MAPK, JAK/STAT, and PI3K/AKT. ALK gene alterations lead to disease progression and aggressiveness, and they are more detected in PDTCs, ATCs than in PTCs (1, 55).

The mechanism of age-associated genetic alterations is not still fully understood, however chromosomal rearrangements are strongly related to the exposition to ionizing radiation, while $BRAF^{V600E}$ point mutations may be associated to excess dietary iodine intake or exposure to chemical disruptors in volcanic regions (56, 57). DNA fragility and impaired repair mechanism are both implicated in radiation-induced genetic damaged or stocastic oncogenic fusion (58–60). Young children might develop more frequently uncoupled double-stranded breaks and translocation with partner genes because they seem to be more vulnerable to the ionizing radiation activity and to the loss of the DNA repair capacity (60).

Only 2.3–2.5% of FTCs display microsatellite instability (MSI), which derived from persistent oxidative stress and subsequent impairment of DNA mismatch repair gene(s) encoding MutL-homolog DNA mismatch repair enzymes PMS1, PMS2, and MLH1, MLH3 (61–63). Since tumors harboring MSI are susceptible to anti-programmed cell death ligand 1 (PD-L1) immunotherapy, additional efforts are needed to clarify the role of mismatch repair gene deficiency in TC (64).

Epigenetic alterations influence gene expression: hyper-methylation of gene promoter sequences lead to heritable inhibition of transcription, while unmethylation results in increased gene transcription (65). Thyroid-specific tumor suppressor genes can promote cell de-differentiation if wrongly methylated during the first steps of tumorigenesis. If cell lines, with TSHR gene silenced by hyper-methylation, are treated with a demethylating agent, they can in part restore TSHR expression and subsequent TSH-induced iodine uptake and effectiveness of RAI (66–68). Other tumor suppressor genes silenced by aberrant methylation are genes encoding cyclin-dependent kinase inhibitors p15INKa and p16INK4b (69), RASSF1A (70), ECAD, RARβ-2, NIS-I, DAPK, ATM, SLC26A, SLC5A8, and TIMP3 (71, 72). It has been suggested that the latter four are associated with aggressive features (18).

Molecular driven therapies

Several molecular driven therapies have been evaluated in aggressive TC (Table 1) (73). Systemic treatments for unresponsive metastatic non-anaplastic follicular cell derived TC include the antiangiogenic multitargeted kinase inhibitor (aaMKIs) sorafenib and lenvatinib (7, 23, 74–77). The Food And Drug Administration (FDA) authorized these aaMKIs, because they can improve progression-free survival as emerged from phase III randomized double blinded crossover clinical trials. Although non tested in a "head-to-head" trial, lenvatinib showed a longer progression-free median survival (18.3 months vs 3.6 months of the placebo group, $p < 0.001$) compared to sorafenib and to placebo group (10.8 months vs 5.8 months respectively, $p < 0.0001$), becoming the first-choice agent among oral aaMKIs (78, 79). Most patients demonstrated disease stabilization or minor/partial responses, which lasted mean period of 12-24 months (78). Lately, also Cabozantinib, an aaMKI previously approved by FDA for the treatment of MTC, has been authorized in case of failure of first-line therapy with lenvatinib and sorafenib, since it improves progression free survival as a second-line agent (80). These compounds do not require mutation profiling of the tumors and they can be also administered when specific targetable mutation (eg, *NTRK*, *ALK*, *RET*, or *BRAF*) have not been identified. As they target primarily the angiogenic vascular endothelial growth factor receptor (VEGFR) signaling, the side effects may include fatigue, hypertension, diarrhea, hand-foot skin reaction and other rashes, thyroid dysfunctions, hepatotoxicity, renal toxicity and fistula formation in the gastrointestinal tract and/or in other locations.

On the other hand, if specific driver mutations are identified (eg, *NTRK*, *ALK*, *RET*, *BRAF*), new mutation-specific kinase inhibitor should be considered which have been FDA-approved, specifically for TCs or for any tumor type harboring the same molecular target (7, 23, 81, 82). For this reason, these compounds require the tumor mutation profiling to prove their pertinence to a specific patient. For example, selpercatinib and pralsetinib inhibit mutant RET in MTC but they can also block the RET fusion proteins-mediated signaling found in PTC and other types of tumor (such as lung cancer) as documented by enduring high partial response and several complete responses rates in Phase III trials (83, 84). These RET inhibitors appear also to be better tolerated than the aaMKIs. However, emerging over time new RET mutations can cause therapeutic resistance by blocking drugs access to the active site or through other mechanisms (85, 86). The clinical trials performed for TRK inhibitors, entrectinib and larotrectinib, have documented activity also for TC (87, 88) and they can be used in some patients with progressive RAI-resistant TC harboring TRK fusion proteins. In addition, the FDA, according to a small cohort study in which ~50% of patients had partial responses to therapy, authorized the association of dabrafenib ($BRAF^{V600E}$ inhibitor) and trametinib (MEK inhibitor) for the treatment of $BRAF^{V600E}$-mutated ATC (89, 90). A subgroup of patients of that cohort displayed a prolonged responses of several years (89). Based on these data, it is recommended to obtain rapid $BRAF^{V600E}$ testing in all patients with ATC (91). Regarding $BRAF^{V600}$-mutant PTC, off-label administration of a BRAF inhibitor could be considered especially for whom aaMKI therapy is contraindicate. Furthermore, $BRAF^{V600E}$ inhibitors have showed promising results for advanced DTC in phase II studies (92, 93). Ultimately, the activity of FDA-approved immune checkpoint inhibitors (such as anti-PD1 and anti-PDL1) is also routinely tested in TC samples and predictors of response are the detection of MSI and high mutational burden (24).

RAI-R development and redifferentiation strategies

The loss of the capability of up-taking ^{131}I is one of the main features characterizing aggressive TC. The cancer therapy with RAI is based in the exploiting of Na/I symporter (NIS). NIS is primarily regulated by TSH through the cAMP pathway, and it is necessary to transport the iodide against a concentration gradient in thyroid follicular cells to synthetize thyroid hormone (94). This mechanism is lost in case of NIS downregulation or loss of function.

RAI refractoriness can be defined by different scenarios, such as the absence of RAI uptake at the initial whole body scan (WBS) or in metastatic lesions, or the loss of the capacity to uptake RAI after a previous WBS showing avidly uptake RAI metastases; a progression of the disease in a subject who has previously received RAI, or a cumulative activity of 600 mCi of ^{131}I; the presence of locally advanced disease that cannot be treated by surgery or evaluated by RAI uptake (95, 96). Genetic and epigenetic alterations in the RTK/BRAF/MAPK/ERK and PI3K-AKT-mTOR pathways underly the diminished NIS signalling/activity that lead to RAI refractoriness and to a more aggressive behaviour (97): their identification can be useful to investigate new compounds able to act against these aberrant molecular mechanisms overcoming the standard cancer therapy resistance.

In vivo studies in mice focused on the disruption of $BRAF^{V600E}$-driven MAPK signaling and found an increase of the iodine uptake (29). According to these findings a clinical trial has been conducted on RAI-resistant metastatic TC subjects who had undergone a whole body I^{124} PET/CT, who were then treated with selumetinib (a MEK inhibitor) for 4 weeks, and subsequently underwent a second scan (98). A partial response has been obtained in approximately 62.5% of the treated subjects, whereas the others had stable disease over a year. It has been observed a difference in the response of the patients

TABLE 1 FDA-approved therapies for thyroid cancer.

Drugs (commercial name)	Targets	Type of cancers	Ongoing/completed Trials in the last 5 years
Lenvatinib (Lenvima®)	VEGFR-1-2-3, FGFR1-2-3-4; PDGFRα, KIT, and RET	Poorly Differentiated/ATC	NCT04731740 (study suspended) in combination with Pembrolizumab (Pembrolizumab +Lenvatinib or Pembrolizumab +Chemotherapy)
		Locally Advanced Invasive TC	NCT04321954 (recruiting)
		RAI-R TC	NCT04858867 (recruiting)
		Stage IVB Locally Advanced and Unresectable or Stage IVC Metastatic ATC	NCT04171622 (recruiting) in combination with Pembrolizumab
		Recurrent, metastatic RAI-R DTC.	NCT03573960 (Active, not recruiting)
		In bone-predominant metastatic RAI-R DTC	NCT03732495 (recruiting) in combination with Denosumab
		Radioactive Iodine-Sensitive DTC	NCT03506048 (terminated) (Study has been abandoned for lack of accrual)
Sorafenib (Nexavar®)	BRAF, ^{V600E}BRAF, c-KIT, FLT-3, CRAF, VEGFR-2; VEGFR-3, PDGFR-β	ATC	NCT03565536 (recruitment status unknown)
		TC	NCT03630120 terminated (Lack of efficacy) in association with Lenvatinib; Cabozantinib or Vandetanib for MTC
Cabozantinib (Cabometyx®)	MET, VEGFR, GAS6, RET, ROS1, TYRO3, MER, KIT receptor, TRKB, FLT3, TIE-2	Advanced DTC	NCT03914300 (Active, not recruiting) in combination with Nivolumab and Ipilimumab
		RAI-R DTC	NCT03690388 (Active, not recruiting)
		Advanced and progressive tumors from endocrine system (ATC, etc)	NCT04400474 (recruiting) in association with atezolizumab
		Advanced Cancer and HIV	NCT04514484 (recruiting) in association with nivolumab
Selpercatinib (Retsevmo®)	RET, VEGFR1-3, FGFR-1-2-3	Progressive, Advanced, Kinase Inhibitor Naïve, RET-Mutant MTC	NCT04211337 (recruiting)
		Advanced Solid Tumors including RET Fusion-positive Solid Tumors, MTC and other Tumors with RET Activation	NCT04280081 (Active, not recruiting)
		RET-Altered TC	NCT04759911 (recruiting)
		Pediatric Patients With Advanced RET-Altered Solid (MTC, PTC, etc) or Primary Central Nervous System Tumors	NCT03899792 (recruiting)
Pralsetinib (Gavreto®)	RET	RET-Mutated MTC	NCT04760288 (Not yet recruiting)
		Unresectable or Metastatic NSCLC or MTC	NCT04204928 (Approved for marketing)

(Continued)

TABLE 1 Continued

Drugs (commercial name)	Targets	Type of cancers	Ongoing/completed Trials in the last 5 years
Entrectinib (Rozlytrek®)	TRKA, TRKB, TRKC, ROS1, and ALK	Solid Tumors Harboring NTRK 1/2/3 (Trk A/B/C), ROS1, or ALK Gene Rearrangements (Fusions) (PTC, etc)	NCT02568267 (recruiting)
Larotrectinib (Vitrakvi®)	TRKA, TRKB, TRKC	Solid Tumors Harboring NTRK Fusion	NCT02576431 (recruiting)
		Advanced Refractory Solid Tumors, Lymphomas, or Multiple Myeloma	NCT02465060 (recruiting) MATCH Screening Trial
Dabrafenib (Tafinlar®) Trametinib (Mekinist®)	RAF kinase MEK	Locally Advanced or Metastatic, RAI-R $BRAF^{V600E}$ Mutation-positive DTC	NCT04940052 (recruiting)
		ATC	NCT04238624 (recruiting)
		RAI-R TC	NCT05182931 (recruiting)
		RAI-R TC	NCT04554680 (recruitment status unknown)
		Metastatic TC	NCT04619316 (recruiting)
		BRAF-positive ATC	NCT04739566 (recruiting)
		BRAF Mutated ATC	NCT03975231 (recruiting) in association with IMRT
		BRAF Mutated ATC	NCT04675710 (recruiting) in association with Pembrolizumab
		RAI-R TC	NCT04544111 (recruiting) in association with PDR001
Trametinib (Mekinist®)	MEK	Advanced Solid Tumor Patients with a BRAF V600 Mutation	NCT05275374 (not yet recruiting) in combination with XP-102
Ipilimumab (Yervoy®)	anti-CTLA-4	Relapsed or Refractory Ovarian Cancer, Triple Negative Breast Cancer (TNBC), ATC, Osteosarcoma, or Other Bone and Soft Tissue Sarcomas	NCT03449108 (recruiting) in association with Nivolumab and other drugs
Nivolumab (Opdivo®)	anti-PD-1	Metastatic RAI-R BRAF V600 Mutant TC	NCT04061980 (recruiting) Encorafenib and Binimetinib with or without Nivolumab
		Advanced Solid Tumors (PTC, etc)	NCT04731467 (recruiting) in combination with CM-24
Pembrolizumab (Keytruda®)	anti-PD-1	Metastatic or Locally Advanced Anaplastic/Undifferentiated TC	NCT05119296 (recruiting)
		Poorly Chemo-responsive Thyroid and Salivary Gland Tumors	NCT03360890 (recruiting) in combination with Docetaxel
		DTC	NCT02973997 (Active, not recruiting) in combination with Lenvatinib
		ATC	NCT05059470 (recruiting)
		Malignant Neoplasms of Thyroid and Other Endocrine Glands, and other malignant cancer	NCT03435952 (recruiting) in association with Clostridium Novyi-NT and Doxycycline
		Advanced/Metastatic Solid Tumors (TC, etc)	NCT04234113 (recruiting) in combination with SO-C101
Atezolizumab (Tecentriq®)	anti-PD-L1	Advanced Solid Tumors (TC, etc)	NCT05253053 (recruiting) To Evaluate Efficacy and Safety of

(Continued)

TABLE 1 Continued

Drugs (commercial name)	Targets	Type of cancers	Ongoing/completed Trials in the last 5 years
			TT-00420 as Monotherapy and Combination
Selumetinib (Koselugo®)	MEK 1/2	Malignant Neoplasms of Thyroid and Other Endocrine Glands, and other Malignant Neoplasms	NCT03162627 (active, not recruiting) The most recently 2017 in combination with Olaparib

°All the cited trials have been obtained from the site: https://clinicaltrials.gov.
ALK, Anaplastic lymphoma kinase; ATC, Anaplastic thyroid cancer; CTLA-4, Cytotoxic T-Lymphocyte Antigen 4; DTC, Differentiated thyroid cancer; FGFR, Fibroblast growth factor receptors; FLT3, Fms-like tyrosine kinase-3; IMRT, Intensity-Modulated Radiation Therapy; MEK, Mitogen-activated protein kinase kinase; MTC, Medullary thyroid cancer; NTRK, Neurotrophic tyrosine receptor kinase; NSCLC, Non-small-cell lung cancer; PD-1, Programmed cell death protein 1; PD-L1, Programmed Death Ligand-1; PTC, Papillary thyroid cancer; PDGFR, Platelet derived growth factor receptor; RAI-R TC, Radioiodine-refractory thyroid cancer; RTK, Receptor tyrosine kinase; TC, Thyroid cancer; TRK, Tropomyosin receptor kinase VEGFR, Vascular endothelial growth factor receptors.

according to their mutational status, in fact those harboring RAS mutations responded more frequently with respect to those with the $BRAF^{V600E}$ mutations.

Other studies have been carried out by using different drugs including $BRAF^{V600E}$, TRK, and RET inhibitors in selected patients according to their genomic tests (58, 99–101).

A study enrolled non-genomically identified patients for first RAI therapy after surgery, who were randomly assigned in a "receiving selumetinib group" and in a "no selumetinib group" and benefits in response rates between the groups were not reported (102). The redifferentiation approach could be in the future a useful strategy to delay long-term treatment with kinase inhibitors using RAI therapy.

These results suggest the use of genomic tests for treatment decisions (24).

Conclusion

De-differentiated TC and ATC have a worse prognosis with respect to WDTC and the loss of the capability of up-taking ^{131}I is one of the main features characterizing de-differentiated and aggressive TC. The knowledge of the genomic landscape of TC can help clinicians to discover the responsible alterations underlying more advance diseases and to address more tailored therapy (103–109). In fact, to date, the aaMKIs sorafenib, lenvatinib, and cabozantinib, have been approved for the therapy of aggressive RAI-resistant PTC or FTC. Several other compounds, including immunotherapies, have been introduced and, in part, approved for the treatment of TC harboring specific mutations. For example, selpercatinib and pralsetinib inhibit mutant RET in MTC but they can also block the RET fusion proteins-mediated signaling found in PTC. Entrectinib and larotrectinib, can be used in some patients with progressive RAI-resistant TC harboring TRK fusion proteins. In addition FDA authorized the association of dabrafenib and trametinib for the treatment of $BRAF^{V600E}$-mutated ATC (89, 90).

Tyrosine kinase inhibitors drugs can act against different altered pathways implicated in the pathogenetic process of aggressive TC. However, patients can't have a good therapeutic response to the therapies with activation of other pathways able to evade the drugs antitumoral effect. Moreover, patients can experience important side effects that can lead to the interruption of the therapy.

New therapies strategies are under investigations, with drugs against immune checkpoint inhibitors.

A good therapy strategy is knowing the molecular pattern of each patient that could aid in the choice of right therapies avoiding the administration of ineffective drugs. A personalized therapy is the challenge of the precision medicine. This challenge can be largely support by *in vitro* drug tests performed on primary tumor cells obtained from patients, that reflect the *in vivo* behavior with a predictive positive value of 60%, and negative predictive value of 90% (110–112). Furthermore, *in vitro* studies can be performed in cells obtained by using the non-invasive technique of FNAC, without the use of surgery (113–115).

Therefore, additional studies about molecular implications involved in the development of aggressive cancer, as well as about each individual patients response to chemotherapeutics will pave the way in the battle against thyroid aggressive cancer.

Author contributions

GE, AP, SB, GM, CS, AA, PF, SMF conceived the paper. GE, AP, SMF specifically wrote the paper and controlled references. All authors reviewed and approved the final version of the manuscript. GE and AP equally contributed as first authors.

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.

Publisher's note

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