Université de Montréal

The relevance of preoperative ultrasound cervical mapping in thyroid cancer

by

Davit Kocharyan

Research Director: Edgard Nassif

Department of Surgical Oncology (CHUM)

Faculty of Medicine

This thesis is being presented in order to obtain a

Master's Degree in Biomedical Sciences

Option: Clinical Research

December 2016

RÉSUMÉ

Pendant les trente dernières années, le taux d'incidence du cancer de la thyroïde chez l'homme et la femme a considérablement augmenté partout dans le monde. Cependant, on estime que d'ici à 2019 le cancer de la thyroïde deviendra le troisième cancer le plus répandu chez les femmes dans tous les groupes d'âge en raison de la tendance d'augmentation plus dramatique chez elles. En général, il n'y a aucune raison claire qui explique l'augmentation mondiale de l'incidence du cancer de la thyroïde et il est émis l'hypothèse que cette recrudescence de l'incidence a une étiologie multifactorielle. Bien qu'il soit clair que le progrès technique des modalités de l'imagerie diagnostique telle que l'échographie peut amener à une augmentation du taux de détection du cancer de la thyroïde secondaire au sur-diagnostic des maladies sous-cliniques, il existe des preuves fortes indiquant une vraie augmentation du cancer de la thyroïde. La cartographie cervicale échographique préopératoire est un outil important dans l'algorithme diagnostic du cancer de la thyroïde. Elle aide à identifier l'étendue des métastases ganglionnaires cervicales afin de guider la dissection chirurgicale anticipée. La dissection chirurgicale du cou orientée selon les compartiments anatomiques et guidée par la cartographie cervicale échographique peut amener à une réduction des risques des complications postopératoires et des récidives tumorales locorégionales.

Nous avons effectué une analyse qualitative et quantitative de la cartographie cervicale échographique afin d'évaluer la fiabilité diagnostique de ce test. Nos résultats ont démontré une valeur prédictive positive assez élevée de cette modalité diagnostique ainsi q'une association quantitative forte entre les données de la cartographie échographique et les résultats de l'histopathologie. Nous suggérons que l'utilisation de la cartographie cervicale échographique

cible les patients présentant un risque plus important d'une maladie persistante / récidivante.

Mots-clés : cancer de la thyroïde, cartographie cervicale échographique, valeur prédictive positive, analyse qualitative et quantitative.

ABSTRACT

Over the last 30 years, the incidence rate of thyroid cancer has drastically increased in both genders all over the world. However, due to a more dramatic pattern in females, it is estimated that by 2019 it will become the third most prevalent cancer in women of all ages.

Overall, there are no clear reasons behind the worldwide increase in thyroid cancer incidence and it is hypothesized that this upsurge has a multifactorial etiology. Despite the fact that recent advances in imaging modalities such as ultrasound can lead to thyroid cancer overdiagnosis by improving the detection rate for subclinical disease, there is strong evidence indicating a true increase in the occurrence of thyroid cancer as well.

Preoperative ultrasound cervical mapping, an important tool in the diagnostic algorithm of thyroid cancer, helps to identify metastatic spread in cervical lymph nodes and guides the surgeon for subsequent surgical dissection. Compartment oriented neck dissection directed by ultrasound mapping decreases locoregional tumor recurrence and lowers the risk of postsurgical complications.

We conducted a qualitative and quantitative analysis of ultrasound mapping to evaluate this test's diagnostic reliability. Our results demonstrated that the positive predictive value of this diagnostic modality was sufficiently high and that there was a strong quantitative association between ultrasound mapping and histopathology results. We therefore recommend that ultrasound mapping be used to target patients with a higher risk of persistent or recurrent thyroid cancer.

Keywords: Thyroid cancer, ultrasound cervical mapping, positive predictive value, qualitative and quantitative analysis.

Contents

List of Tables

List of Figures

List of Acronyms

AAPC: Average annual percentage change

AJCC: American Joint Committee on Cancer

APC: Annual percentage change

ASIR: Age-standardized incidence rate

ATC: Anaplastic thyroid carcinoma

BPA: Bisphenol A

CC: Central compartment

CEA: Carcinoembryonic antigen

CGRP: Calcitonin gene-related peptides

CHUM: Centre hospitalier de l'Université de Montréal (University of Montreal hospital center)

CI: Confidence interval

CT: Computerized tomography

DIT: Diiodotyrosine

DSVPTC: Diffuse sclerosing variant of papillary thyroid carcinoma

DTC: Differentiated thyroid carcinoma

FNA: Fine needle aspiration

FNA-Tg: Fine needle aspiration – thyroglobulin

FTC: Follicular thyroid carcinoma

FV-PTC: Follicular variant of PTC

LC: Lateral compartment

LN: Lymph node

M: Metastasis

MEN: Multiple endocrine neoplasias

MIT: Monoiodotyrosine

MRI: Magnetic resonance imaging

MTC: Medullary thyroid cancer

N: Node

NIS: Sodium/iodine symporter

P/R: Persistent/recurrent

PCB: Polychlorinated biphenyls

pCND: Prophylactic central neck dissection

PDTC: Poorly differentiated thyroid carcinoma

PHAH: Polyhalogenated aromatic hydrocarbon

PLN: Positive lymph node

PMC: Papillary microcarcinoma

PPV: Positive predictive value

PTC: Papillary thyroid cancer

RLN: Recurrent laryngeal nerve

SD: Standard deviation

SEER: Surveillance, Epidemiology, and End Results

SES: Socioeconomic status

SLN: Suspicious lymph node

T: Tumor

T3: Triiodothyronine

T4: Thyroxine

TBG: Thyroxine-binding globulin

TFC: Thyroid follicular cell

Tg: Thyroglobulin

TPO: Thyroid peroxidase

TRH: Thyrotropin-releasing hormone

TSH: Thyroid-stimulating hormone

TT: Total thyroidectomy

TTR: Transthyretin

U.S.: United States

U/S: Ultrasound

WHO: World Health Organization

Introduction

Historical background (1)

The thyroid gland was first described by anatomist Andrea Vesalius in 1543 in his inauguration of modern anatomy "De Humani Corporis Fabrica" published in Switzerland.

Later, Julius Casserius (1545–1616) drew the first distinct image of the thyroid gland in a classical horseshoe shape. In 1656, Thomas Wharton named it "glandula thyreoidea" not because of its shape (thyreos in Greek means "shield") but because it resembles nearby anatomical structures such as the thyroid cartilage of the larynx [1, 2]. The first surgical removal of the thyroid gland was described by German surgeon Lorenz Heister in 1742 [2].

Embryology (2)

The thyroid gland is the first endocrine structure to appear during the embryological development of the human body starting around the 4^{th} week of gestation [1, 3]. It is a composite of two different cell types which have two distinct embryological origins. The major part of the thyroid is composed of epithelial cells which have an endodermal origin and arise from the primitive pharynx. Later in development, these cells become thyroid follicular cells (TFCs) which are responsible for producing thyroid hormones such as triiodothyronine (T3) and thyroxine (T4). The thyroid gland's second embryological component is the neural ectoderm from the ultimobranchial body (fifth pharyngeal pouch) which gives rise to parafollicular cells or calcitonin-producing C cells [4-6]. Precursors of the C cells migrate from the neural crest to

the fourth pharyngeal pouch which is symmetrically located on both sides of the neck [5]. C cells subsequently reach their final position within the upper third of the thyroid lobes where they comprise only 1-2% of the thyroid cell mass [4].

Several developmental steps take place before mature thyroid gland formation [5]. Around the 20^{th} embryonic day, the thyroid anlage develops as part of the endodermal epithelium in the midline of the primitive pharynx [5].

The foramen caecum, which is the endodermal bud between the first and second branchial arches, gives rise to the principle cells responsible for the thyroid gland's structural and functional integrity [3]. The foramen caecum lies at the intersection of the tongue's midline and the sulcus terminalis which defines the border between the oral (anterior two-thirds) and pharyngeal (posterior third) parts of the tongue [2, 3]. In adults, the circumvallate papillae delineate this border which also corresponds to the tongue's ectodermal-endodermal boundary [3]. The TFC proliferation results in the formation of the initial thyroid primordium. The formation then starts to thicken and invaginates inferiorly, and this transformation forms a tubular structure called the thyroid diverticulum [3, 7]. As it continues to migrate caudally, it transforms into a bilobed and solid structure which eventually reaches its final position on the inferior aspect of the anterior neck. The migration process is completed by the 45^{th} day and the thyroid gland lies inferiorly to the larynx, around the anterior and lateral sides of the trachea [3]. The migratory route from the foramen caecum to the final cervical position is called the thyroglossal tract [8]. During late embryological development, the thyroglossal duct regresses and becomes fibrotic. The foramen caecum then involutes into a vestigial pit that can be visualized in 60% of adults at the base of the tongue [3]. During the process of migration,

different deviations from the normal development route may occur. Occasionally, the thyroglossal duct fails to atrophy, leaving accessory or ectopic thyroid tissue along the migratory path. These islands of ectopic tissue transform into neoplasia in rare cases. More commonly, they may become metabolically active but the amounts of produced hormones are minimal without the main gland [3]. Rarely, the thyroid gland fails to descend into the neck, resulting in a lingual thyroid [7]. In this condition, a mass is noted in the posterior oral tongue, and the patient may complain of dysphagia, dysphonia, and dyspnea [3, 8]. Although a maldescended thyroid may not function appropriately, it is essential to keep in mind that a lingual thyroid may be the only functional thyroid tissue present [3, 8]. If it is removed due to its mass effect, subsequent replacement therapy should be administered to prevent further aggravation of the patient's hypothyroid state. Cysts and sinuses are other morphological patterns of failed involution of the thyroglossal duct. These lesions are typically located in the midline, around the level of the hyoid bone. Infections and inflammatory processes are common pathologic manifestations of those congenital malformations, and antibiotics along with planned surgical intervention are the standard of care in advanced cases.

Simultaneously to the thyroid's migration process, the C cells in the 4^{th} pharyngeal pouch are positioned in the ultimobranchial body. First, the ultimobranchial bodies migrate from their lateral origin to a medial position on either side of the neck. By week 10, the main histophysiologic units of the mature and differentiated thyroid gland such as TFCs and C cells have merged at the front of the cricoid cartilage on the trachea. The thyroid gland then starts to enlarge and the TFCs, which notably outnumber the interspersed C cells, begin to organize into follicles. The final dispersion of C cells within the thyroid is not uniform, as they are mostly

localized in the middle and upper thirds of the gland's lateral lobes [5]. The C cells comprise only 1-2% of the thyroid cell mass [4]. The final stage in normal organogenesis of the gland is a functional differentiation of the TFCs, which later lead to hormone production [5].

Functional differentiation of TFCs and C cells occurs along with the thyroid migration process and is regulated by relevant controller genes. The natural drive of thyroid morphogenesis is closely related to transcription factors such as TTF-1, TTF-2, Foxe1, Hhex, and PAX-8, which are essential for the thyroid's normal development and migration [5, 8]. The simultaneous presence of these genes is essential for recruiting TFCs and organizing the thyroid bud. They continue to promote further development of the thyroid until it has completed migration and starts to enlarge [5]. At this point, other genes take over the functional development, and pathways for hormone synthesis start to activate. There are three functional stages in thyroid development: precolloid, colloid, and follicular. They occur approximately at 7 weeks to 12 weeks, 13 weeks to 14 weeks, and after 14 weeks. Hormone production in the thyroid starts at around 12 weeks [8].

Histology (3)

Histologically, the main architectural units of the thyroid gland are numerous hollow sacs called thyroid follicles. The connective tissue septa separates the thyroid gland into groups of 20-40 follicles [9]. The follicles contain colloid at their center and a simple cuboidal epithelium composes their main lining. Staining with hematoxylin and eosin produces a uniformly pink

color in the colloid lumen. The follicular cells' principal role is to produce thyroid colloid, which contains high quantities of thyroglobulin and iodide.

The colloid is then extruded into the follicular lumen for future use. Subsequently, thyroid-stimulating hormone (TSH), which is the main regulator of the gland's functional activity, stimulates the conversion of the colloid precursor into mature thyroid hormone. Although follicular cells are single layered and usually cuboidal in shape, their architecture may be significantly affected by the general metabolic state of the gland. In the event of high metabolic demand for thyroid hormones, the amount of colloid goes down as a consequence of its increased conversion into mature thyroid hormones. The follicular cells may also change their shape and become columnar. In cases of relative hypoactivity of the gland, the follicular lumen becomes larger as more colloid is stored in preparation for future use. Decreased metabolic activity causes the follicular cells to flatten and become smaller in size. Although most authors believe the monoclonal origin of thyroid cells, there is some evidence showing the polyclonal origin of thyroid cells with different malignant potential for each group of cells [10]. The next level of the thyroid's functional and structural organization is the parafollicular cells (C cells) which produce calcitonin. They are situated in the stroma, between thyroid follicles. To better view these cells, immunostains such as immunoperoxidase are the preferred method [3].

Anatomy (4)

The mature thyroid gland is an H- or U-shaped bilobed, butterfly-shaped endocrine organ which is located on the anterolateral aspect of the cricothyroid membrane, cricoid and trachea [3, 5, 9]. Depending on gender and age, the normal thyroid in an adult weighs around 12-25 g, which corresponds to a volume of 12-20 ml [4, 5]. Usually, it is slightly larger in men. The isthmus of the gland connects the right and left lobes of the thyroid. On ultrasonography the average size of each lobe is 4 cm long vertically and 1-1.5 cm wide with a thickness of 1 cm. [3]. In some cases there is an extension of thyroid tissue which arises from the isthmus. This tissue, known as the pyramidal lobe, goes superiorly along the midline and is a remnant of the embryologic thyroglossal duct [3].

There are three main anatomical borders separating the thyroid from neighboring structures. The trachea and esophagus are the demarking posteromedial boundaries; the carotid sheath and sternocleidomastoid muscles are the limiting lateral and anterolateral boundaries respectively [5]. The thyroid extends from the level of the fifth cervical vertebra to the first thoracic vertebra.

An extension of the pretracheal fascia (Berry's ligament) composes the fibrous capsule of the thyroid which serves as a supporting structure. The septae of the capsule divide the gland into macroscopic lobules. Arterial and venous branches run along the septae within the connective tissue. The visceral part of pretracheal fascia is attached anteriorly to the crycoid and thyroid cartilage [5, 8]. The muscular part of the pretracheal fascia encloses three pairs of infrahyoid muscles, namely the sternohyoid, sternothyroid, and thyrohyoid [8]. Because of its firm attachments, the thyroid may move superiorly as a consequence of tracheal elevation during

swallowing [3]. This has some diagnostic value as it helps to differentiate thyroid masses (such as thyroid nodules) and congenital malformations (such as thyroglossal duct cysts). In contrast to lymphadenopathy or branchial cleft cysts which remain stationary during swallowing, thyroid nodules and thyroglossal duct cysts are freely movable [3].

Blood Supply (4.1)

The arterial blood supply to the thyroid is the richest per gram of tissue [3, 4]. The external carotid artery and the thyrocervical trunk give rise to the superior and inferior thyroid arteries respectively. The superior thyroid artery supplies the upper pole and passes in a caudal direction from the external carotid artery [4, 8]. The inferior thyroid artery supplies the posterior part of the gland and runs in a cranial direction [4]. There may be a third, relatively uncommon, arterial supply from the brachiocephalic trunk called the arteria thyroidea ima (3%-10%) [2, 5, 8]. While arterial blood is circulated by two pairs of arteries, venous outflow is through three main venous pathways named the superior, middle, and inferior thyroid veins. The superior thyroid vein accompanies the superior thyroid artery and, along with the middle thyroid vein, drains into the internal jugular vein [2, 3]. There is some risk of rupture of the middle thyroid vein during thyroidectomy as it has a posterior course and can be damaged in forward traction of the gland [2]. Usually, there are several inferior thyroid veins that drain either into the internal jugular or the brachiocephalic veins.

Lymph Drainage (4.2)

The thyroid has a very rich lymphatic system. It consists of an intra and extraglandular network with developed lymph vessel anastamoses that drain each part of the gland in multiple different directions [4, 8, 11]. This can explain the frequent intraglandular spreading and multifocal distribution of thyroid neoplasias.

The lateral and medial aspects of the gland have some topographic differences in their lymphatic drainage. The lymphatic vessels of the gland's lateral part pass along the arteries. They may ascend across the superior thyroid artery or descend across the inferior thyroid artery before reaching their final pool in the jugular chain of cervical nodes. Between the superior and inferior thyroid arteries, lymphatic vessels may drain directly into the jugular nodes. The lymphatic vessels of the gland's medial part also drain in two directions: superiorly to the digastric nodes and inferiorly to the paratracheal and brachiocephalic nodes [2, 6]. In the midline, just above the isthmus of the thyroid, lies the Delphian node (named after the Oracle of Delphi). An abnormal looking Delphian node may have some diagnostic value as nodal metastases are common in thyroid cancer [8].

There are two main drainage pathways for the periglandular lymph network. The first pathway originates from lymph nodes and lymph vessels in the central zone of the neck. It is positioned between the carotid sheaths and extends in a downwards direction including pre and paratracheal regions. From this point, the lymph drainage may branch with the mediastinal lymphatic system.

The second lymph drainage pathway (secondary drainage) arises from the lateral region of the neck. It involves the lymph vessels and lymph nodes along the jugular vein as well as lymphatic

structures extending up towards the submandibular region. Lymph nodes in the supraclavicular triangle are also part of the secondary drainage network [4].

The thyroid's dense lymphatic network is closely linked to the concept of a stepwise progression of nodal metastasis. This has enormous clinical relevance, as a nodal metastasis from one nodal station to another is directly correlated with the extent of the neck dissection and radiation therapy in patients with thyroid cancer [11]. Sentinel lymph node studies about the pattern of thyroid lymphatic drainage revealed some consistency in the dispersion of metastasis in different malignancies. The first metastases extend into the nodes of the central compartment of the neck (pre and paratracheal stations) followed by the nodes of the superior mediastinum and the lateral cervical nodes. The neck's lymphatic mapping is often described in terms of "levels" ranging from level I to level VI. Level VI nodes are located in the central compartment which is bounded by the hyoid bone superiorly, the suprasternal notch inferiorly, and the carotid arteries laterally. There are three nodal groups in the level VI compartment that are responsible for thyroid drainage: paralaryngeal, paratracheal, and prelaryngeal (Delphian). Levels III, IV, and V are part of the lateral cervical compartments which are commonly affected by bilateral metastasis. Level III consists of lymph nodes located around the middle third of the internal jugular vein. It extends from the lower side of the body of the hyoid bone (superiorly) to the lower side of the cricoid cartilage arch (inferiorly). From anterior to posterior this zone is limited by the lateral border of the sternohyoid muscle and the posterior border of the sternocleidomastoid muscle[12, 13]. Level IV consists of LNs located around the lower third of the internal jugular vein. It extends from the lower side of the cricoid cartilage arch (superiorly) to the level of clavicle (inferiorly). The anterior boundary is the lateral border of the sternohyoid muscle, and the

posterior boundary is the posterior border of the sternocleidomastoid muscle[12-14]. Level V is comprised of the LNs in the posterior triangle. The boundaries of the triangle are the anterior border of the trapezius muscle posteriorly, the posterior border of the sternocleidomastoid muscle anteriorly, and the clavicle inferiorly[12-14]. Lymph nodes lying superiorly to the innominate vein in the superior mediastinum compose level VII [11].

Nerves (4.3)

The recurrent laryngeal nerve (RLN) provides motor and sensory innervations of the larynx. The RLN is responsible for phonation as well as swallowing, and any significant damage to this nerve can cause dysphonia and varying degrees of dysphagia [4, 5]. Considering the close location of the thyroid gland to the RLN path, there is a risk of RLN infiltration or compression by thyroid cancers, followed by impairment of function [4]. The RLN is a branch of the vagus which has different courses on both sides of the neck. On the right, the nerve loops posteriorly to the subclavian artery, then extends up until it reaches the tracheoesophageal groove. In contrast, the nerve on the left side loops posteriorly to the arch of the aorta, followed by an ascending course in the tracheoesophageal groove [2, 7]. Along its path the RLN also innervates the trachea and esophagus by multiple small branches [2]. The RLN carries motor, sensory, and parasympathetic fibers. The external branch of the nerve is responsible for the motor function of 4 intrinsic muscles (lateral cricoarytenoid, posterior cricorytenoid, transverse and oblique interarytenoid and thyroarytenoid) of the larynx. The internal branch of the RLN supplies sensation to the vocal cords and subglottic region. The RLN also sends branches to the inferior

constrictor and cricopharyngeus muscles before entering the larynx [15]Another important branch of the vagus is the superior laryngeal nerve which runs in a caudal direction along the superior thyroid artery. After passing behind the internal carotid artery, it divides into external and internal branches. The motor/external branch supplies the cricothyroid muscle, and damage to this nerve also results in voice changes [4].

Physiology (5)

The principal purpose of the thyroid gland is to provide adequate amounts of L-thyroxine (T4) and, to a much lesser extent, L-triiodthyronine (T3) which are essential for normal body metabolism, growth, and development. Both T4 and T3 are iodinated hormones which require the thyroid to be able to extract circulating iodide from the bloodstream for further organification and incorporation into the thyroid hormone molecules [9]. The thyroid's level of activity and the amount of produced hormones are tightly regulated on several levels, both within and outside the gland. Those regulating mechanisms may have first line clinical relevance in thyroid cancer as the cancerous cells originate from normal thyroid cells. Depending on the level of dedifferentiation, the cancerous cells may use cellular mechanisms that are identical to those of normal cells in order to remain viable. In clinical settings these similarities help to identify, characterize, and finally treat different malignancies of the thyroid [9].

In order for the thyroid gland to provide the necessary amount of physiological hormones, an adequate supply of dietary iodine is essential. Both high and low iodine-containing diets may cause a vast range of serious problems. One of the most detrimental complications of over or

undersupply of dietary iodine is thyroid cancer. There is some evidence showing a higher incidence rate of papillary thyroid cancer in areas where there is an abundance of dietary iodine [16]. Meanwhile, low iodine intake may increase the proportional incidence of follicular thyroid cancer [8]. Administration of excess pharmacologic doses of iodine may inhibit iodide organification and thyroglobulin (Tg) proteolysis followed by an acute decrease in thyroid hormone production and release. This phenomenon, known as Wolff–Chaikoff effect, is explained by oversaturation of the sodium-iodine symporter (NIS). However, this effect does not last long as the gland adapts quickly to the new conditions and downregulates the uptake of iodine despite its continuously high plasma levels [9, 11].

One of the most critical steps in thyroid hormone formation is the transportation of the iodine into TFCs against an electrochemical gradient [11]. This is a saturable, energy dependent process which requires oxidative phosphorilation [11]. The main system that provides the necessary influx of iodine into the TFCs is NIS, which is a transmembrane protein located in the basolateral membrane of the thyrocyte [9, 11]. NIS cotransports sodium and iodide in a 2:1 ratio using the transmembrane sodium gradient as a driving force in order to stimulate iodide uptake into follicles [3]. The expression level of NIS on TFCs is responsive to different regulatory factors including TSH, and this mechanism is widely used in clinical settings. After intense TSH stimulation, malignant thyrocytes of papillary, follicular, and Hurthle cell cancers increase the uptake of I-131 as NIS is also present in their membrane [11]. However, in some types of thyroid cancer, NIS either does not have enough sensitivity or is completely absent which makes treatment with radioactive iodine practically useless [11]. Apart from therapeutic purposes, the NIS transporter concept can serve as a valuable diagnostic tool in patients with

differentiated thyroid cancers (papillary and follicular cancers) who underwent a thyroidectomy. In this case, the whole body scan with radiotracer can discover residual functioning thyroid tissue or actively functioning metastasis which can be retreated with larger doses of radioactive iodine [8]. Another way to use NIS for diagnostic purposes is by staining removed thyroid specimens with labeled antibodies against the symporter. This can provide valuable information in terms of the cancer's aggressiveness and response to radioactive iodide [8].

It is worth mentioning that similar iodide transport mechanisms can be found in different tissues, including the stomach, salivary glands, and occasionally breasts. This can explain some side effects of radioactive iodine such as radiation-induced sialadenitis or xerostomia in patients treated for thyroid cancer [8, 9].

The next step in the synthesis of thyroid hormones is the organification of the iodide, which is carried out by the thyroid peroxidase (TPO) enzyme along with hydrogen peroxide. After the oxidation process, newly formed organic iodine iodinates the tyrosyl residues in thyroglobulin (Tg) [2, 9]. The whole process takes place in the opposite surface of TFCs where the apical membrane faces the colloid. The final stage of thyroid hormone formation is the coupling of the iodotyrosine molecules within thyroglobulin. Depending on the number of coupled iodine atoms, different types of thyroid hormone can be formed. While T4 is formed by coupling two molecules of diiodotyrosine (DIT), T3 is formed by coupling one molecule of DIT and one molecule of monoiodotyrosine (MIT). Antithyroid medications target the thyroperoxidase enzyme, which in turn blocks the formation of mature, end stage hormones.

The primary storage of thyroid hormones is the thyroid follicle colloid where the completely formed hormone is linked to thyroglobulin (Tg) [2, 9, 11]. Since it is one of the largest, active biological molecules in the human body and plays a central role in thyroid hormone synthesis and storage, thyroglobulin tends to have high diagnostic value in patients with different thyroid disorders including metastatic differentiated thyroid cancers [8]. High Tg levels indicate an increased probability of metastatic disease, especially in bones, lungs, and lymph nodes. Undetectable Tg levels combined with a negative whole body iodine scan are very reliable followup tests showing the absence of residual cancerous tissue [8].

The primary stimulator for thyroid hormone secretion is TSH which promotes proteolysis of thyroglobulin, followed by the release of free hormones into circulation [2, 9]. The equilibrium between the unbound (metabolically active) and bound (metabolically inactive) fractions of the released hormone is regulated by carrier proteins such as thyroxine-binding globulin (TBG), transthyretin (TTR), albumin, and lipoproteins [11].

The thyroid's growth and functional activity, starting with the formation of thyroid hormones and ending with their secretion, is tightly regulated by the hypothalamo-pituitary-thyroid axis. The primary signal comes from the hypothalamus: thyrotropin-releasing hormone (TRH) stimulates TSH synthesis and release from the anterior pituitary which in turn stimulates the growth and hormone secretion of the thyroid itself. This mechanism is widely used in treating thyroid cancer as chronic suppression of TSH mitogenic activity by exogenous thyroxine is crucial in preventing cancerous cell activity [9].

The second hormone produced by the thyroid gland (C cells) is calcitonin which is a 32-amino acid polypeptide belonging to a family of related molecules called the calcitonin gene-related peptides (CGRP) [5, 9]. Calcitonin is one of the compounds regulating calcium levels in the blood. In conditions causing elevated levels of blood calcium, C cells secrete calcitonin which downregulates osteoclast-mediated bone resorption and by doing so it decreases high serum calcium levels [5]. Calcitonin may also serve as a tumor marker in medullary thyroid carcinoma which is part of Sipple syndrome (MEN type 2A) and is related to RET proto-oncogene.

Epidemiology (6)

With 140,000 newly diagnosed cases worldwide, thyroid cancer is the most common endocrine tumor and accounts for 2.5% of all human cancers [17-19]. Its incidence was relatively stable until the early 1990s. During the last three decades the incidence of thyroid cancer has increased drastically all over the world [20-24]. This trend is observed on all continents except Africa which probably is related to insufficient detection [24]. Between 1973 and 2009 the incidence of thyroid cancer more than doubled worldwide including such developed countries as the U.S., Canada, France and Australia [17, 22]. During the last 20 years the overall incidence rate has gone up by more than 6% annually [22]. While 20 years ago thyroid cancer was ranked only 14th, currently it is the 5th most common cancer in women. In Italy, it is the second most prevalent cancer among women in the age group below 45 [16, 24].

Rates of thyroid cancer are rising in both genders, but due to the more dramatic pattern in females it is estimated that by 2019 thyroid cancer will surpass the rates of common cancers and

will become the third most prevalent cancer in women of all age groups and the second most common cancer in women younger than 45 in the U.S. [21, 25]. In 2014, there were 62,980 new cases of thyroid cancer in the U.S. Every year thyroid cancer accounts for around 3.8% of all newly diagnosed cancer cases in the U.S. Overall, its incidence increased from 4.9 to 14.7 per 100,000 population in 2011. According to the Surveillance, Epidemiology, and End Results (SEER) program conducted in the U.S., the average annual percentage change (AAPC) for thyroid cancer is +5.2 in men and +5.7 in women for the 2002-2011 time interval [16]. The SEER database, which is a population-based cancer registry involving around 10% of the U.S. population, provides epidemiologic data on different characteristics of tumors as well as treatment approaches and survival rates [21]. It summarizes relevant epidemiologic information collected from 1973 to 2012. The higher incidence rate has been mostly attributable to papillary cancer, with only a slight increase in other histotypes [22]. Due to thyroid cancer's indolent nature, the mortality rate is not as high as in other more common cancers. In 2014, estimated mortality from thyroid cancer was around 1,890 individuals. In comparison, the primary "killers" such as breast and lung cancers cause hundreds of thousands of deaths each year [20]. However, based on the SEER program's results, the thyroid cancer mortality rate rose slightly for the 2001-2010 period with an AAPC of 0.9 [16]. In fact, the observed upshift in thyroid cancer mortality is not only statistically significant, it also surpasses the increase in mortality rate of any other cancer, except liver [26]. This increase in the mortality rate has occurred in spite of the introduction of better diagnostic and therapeutic measures [24].

Epidemiology: Canada (6.1)

Canada reflects the same trend: the overall incidence of thyroid cancer increased by 156% between 1991 and 2006 [27]. A study based on data from the Canadian Cancer Registry showed that age-adjusted incidence rates doubled between 1970-72 and 1994-96 [28]. Another study revealed that the rise in thyroid cancer incidence in Manitoba reached 373% from 1970-2010 [23]. The same trend was observed in an Ontario-based retrospective study indicating that the incidence rate was up 146% from 1990 to 2001 [29]. In 2007, more than 4,000 Canadians were diagnosed with thyroid cancer, or nearly 12 cases per 100,000 population, which accounts for around 2.5% of all malignant tumors [30]. In 2012, the numbers were even worse as an estimated 5,650 Canadians were diagnosed with thyroid cancer for the first time [23]. Characteristic features of thyroid cancer are an incidence rate that is three times higher in women than in men, along with the relatively young age of affected patients [30]. Around 75% of all cases are diagnosed before age 60, and nearly 40% of all thyroid cancers are found before the age of 45. With such a high incidence rate among the young population, thyroid cancer is currently ranked as the second most common cancer in Canadians aged 15 to 44 and is the most common cancer diagnosis in those aged 15 to 29 [30]. While papillary cancer is the most widespread histotype, composing 86% of all diagnosed cases, follicular, medullary, and anaplastic thyroid cancers represent 6%, 2%, and 1% respectively. Thyroid cancer has the most rapidly rising incidence rate in Canada. The age-standardized incidence rate (ASIR) jumped 5.7% per year in males (from 2.0 to 5.2 per 100,000 population) and 7.3% per year in females (from 6.8 to 17.9 per 100,000 population) for the 1992-2007 period. The most vulnerable category in terms of the highest spike in ASIR was women aged 30 to 59, with an annual ASIR of 8.2% [30]. The increasing ASIR trend was observed in all Canadian provinces and territories over the last 16

years. This progression varies widely across the various provinces. In 2007, the highest ASIR was in Ontario (15.2 per 100,000) and the lowest were in Saskatchewan (5.2) and British Columbia (5.8) [30]. For the same period, the annual percentage change (APC) for Quebec was 7.3% and the ASIR was around 11 per 100,000 population which is not much different from the Canadian average of 11.6 per 100,000.

The situation is completely different when it comes to the mortality rate from thyroid cancer: it has remained exceptionally low and stable [23, 30]. For the 1992-2007 period, there were an average of 142 deaths per year in Canada with even a slight decrease (less than 1% per year) in the age-standardized mortality rate from 0.5 per 100,000 in 1992 to 0.4 per 100,000 in 2007 [30]. Thyroid cancer's low mortality rate is reflected in the five-year relative survival rate which is the highest of all cancers in Canada, reaching 97% for the 2001-2003 timeframe [30] and 98% in 2011 [23]. Increased incidence of thyroid cancer with low mortality causes increased proportional prevalence, with two- and five-year prevalence exceeding 9% per year from 1999/2000 to 2008 [31]. Only liver cancer has higher prevalence (8.5%) for a 10-year duration [31].

According to the Canadian Cancer Society, thyroid cancer is the 5th most common cancer in women and accounted for 5% of all cancers in 2015 [32]. In 2015, out of 6,300 newly diagnosed thyroid cancer cases, 4,800 were in women (ASIR 23.1 per 100,000) and 1,450 in men (ASIR 6.7 per 100,000). By comparison, in 1986 the ASIR for women was 5.2 per 100,000 population which proves once again the tremendous spike in thyroid cancer incidence [32].

The Canadian Cancer Society has also indicated that Quebec is second after Ontario with around 360 men and 1,200 women being newly diagnosed with thyroid cancer in 2015. These values correlate with an ASIR of 7 and 25 per 100,000 population in men and women respectively [32].

Increasing incidence hypothesis (7)

Overdiagnosis of subclinical disease reservoir (7.1)

There are multiple controversial explanations for the worldwide increase in thyroid cancer. Although various hypotheses have been put forward to pinpoint the principal mechanism behind such a dramatic upsurge in the thyroid cancer incidence rate, many authors agree that the main question that needs to be answered is whether the alarming increase reflects a true change in incidence or is simply due to the "overdiagnosis" of subclinical disease [18, 20, 22, 24, 33]. This distinction may have a very important clinical application in terms of prevention and management. For instance, a real upsurge in the incidence rate should raise concerns about environmental factors that may be responsible for this phenomenon. Meanwhile, the management approaches will have to be reviewed in case the incidence rate has been falsely pushed up by "overdiagnosing" a previously subclinical pool of the disease. This would help avoid unnecessary therapeutic interventions in an otherwise harmless, clinically insignificant disease [33].

Based on autopsies of people who died from other causes, the estimated prevalence range of papillary thyroid cancer varies from 3% to about 35% depending on the country and the pathology methods adopted to examine the specimens [16, 20, 22]. Moreover, incidental thyroid

cancers can be found in up to 50% of thyroid specimens removed for benign causes. These findings, along with the observation that the higher incidence is mostly ascribed to greater detection of cancers which are too small to be detected by conventional physical exams, indicate the existence of a large reservoir of subclinical papillary thyroid cancer. Overdiagnosis of this otherwise clinically insignificant reservoir of thyroid cancer can explain the "apparent" upsurge in the incidence rate [16].

Many recent studies show strong evidence in favor of the hypothesis that the rising incidence is due to increased diagnostic scrutiny and widespread access to highly sensitive radiological testing [34-36]. In the last 30 years there has been a dramatic increase in the use of all kinds of diagnostic imaging modalities including cross-sectional imaging, with a twofold increase in CT scans and a threefold increase in MRI scans [33, 37]. In their study, Udelsman and Zhang concluded that "the epidemic of thyroid cancer" in the U.S. is largely attributable to increased detection. They found a statistically significant direct linear correlation between the frequency of cervical ultrasonography and the thyroid cancer incidence rate [34]. In another study, Zevallos et al. presumed that the doubling of thyroid cancer incidence within the U.S. Veterans Affairs healthcare system between 2000 and 2012 could be related to a nearly fivefold and sevenfold increases in the use of thyroid ultrasound and fine needle aspiration respectively [36]. It should be noted that the prevalent use of ultrasound has boosted the detection of small thyroid nodules which otherwise would remain undiscovered without any further diagnostic or therapeutic interventions [38].

Data analysis from the National Cancer Institute's SEER program showed that there is a large gender discrepancy in thyroid cancer detection. The incidence rate in women is 4 times greater

than in men even though the autopsy incidence of thyroid cancer is higher in men. This correlation is probably related to the overdiagnosing phenomenon in women [39]. Some authors suggest that even the age of the diagnosing physician may impact the thyroid cancer incidence rate, with more experienced doctors reporting fewer cases because they tend to use ultrasound and FNA less [40]. More aggressive approaches in terms of diagnosis and treatment, such as more frequent biopsies and an increase in the number and extent of surgeries especially total thyroidectomy [41], provide larger specimens and more opportunities for pathologists to find cancer [20]. The pathology guidelines for managing thyroid specimens have also changed, and thyroidectomy specimens are now examined in their entirety compared with the past practice of examining only representative sections of the specimen. More detailed examination of each thyroid specimen increases the chances of identifying small lesions thereby raising the incidence rate [20].

Other important factors that may have contributed to the increased incidence of thyroid cancer detection are access to healthcare services as well as socioeconomic status (SES) [20, 42]. Morris et al. showed a strong correlation between the markers of access to the healthcare system and the incidence of papillary thyroid cancer [22]. Countries with higher SES tend to have higher rates of screening procedures with a subsequent increase in thyroid cancer detection. The positive association between thyroid cancer and various socioeconomic indicators, such as high levels of income, education, and white-collar employment, would seem to indicate an increased screening effect [22, 24].

Arguments in favor of a true increase in thyroid cancer incidence (7.2)

Although most studies agree that the more widespread application of highly sensitive diagnostic modalities has greatly contributed to the detection of a previously "hidden" pool of subclinical disease, it is impossible to ignore the evidence indicating that such an upsurge in thyroid cancer incidence cannot be explained solely by the overdiagnosis phenomenon and that there must be other factors causing a true rise in the number of thyroid cancer cases [16, 20, 24].

The main argument against the overdiagnosis hypothesis is the stable or even decreasing incidence of other types of cancers diagnosed with highly specialized imaging techniques and diagnostic biomarkers [16]. According to data from the SEER registry, the rates of some common cancers, such as prostate, colorectal, and breast cancer, have dropped while the incidence rate of thyroid cancer increased by as much as 60% for the period of observation from 2002 to 2011.

Assuming that improved detection is the principal mechanism of increased incidence, then the diagnosis and treatment of early-stage, small tumors should be accompanied by a significant decrease in larger, more advanced tumors [24]. Although the upsurge in the detection rate for small thyroid tumors (≤1 cm) certainly surpasses the rate for larger, more advanced tumors (≥4 cm), many studies have shown a significant increases in thyroid cancer in all size categories [16, 17, 20, 33]. In their recent study, Malone et al. demonstrated that 38% of large thyroid cancers (≥4 cm) are found incidentally on imaging studies done for other reasons [43]. Since it seems very unlikely that 38% of all large tumors were being missed in the past, a real increase in the incidence rate could be the reason.

Another interesting observation is related to the histotypes of thyroid cancer. While improved detection would affect all histotypes, the rise in incidence is, in fact, almost exclusively due to papillary thyroid cancer (PTC) [17, 24].

Moreover, in their study, Cramer et al. state that "improved detection techniques may explain the 8.0%/year difference between the increase in microPTC compared with the average increase in neoplasms larger than 1 cm; however, changes in the methods of detection cannot explain the overall increase in all sizes of PTC" [17].

If all these hypotheses and studies are correct, there must be some underlying driving forces which could explain this profound increase in thyroid cancer.

Risk factors causing a true increase in thyroid cancer incidence (7.3)

Exposure to ionizing radiation is an extensively studied and well-documented risk factor for thyroid cancer due to the thyroid's ability to concentrate iodine and the relatively higher irradiation because of its localization [16, 20, 24, 33]. The mechanisms behind this phenomenon are DNA strand breakages, the development of somatic mutations, chromosomal rearrangements, and intra-chromosomal inversions causing RET proto-oncogene to fuse with other genes such as H4 or ELE 1 [20, 33, 44]. Once exposed to ionizing radiation, young subjects (<20 y/o) have a higher probability of developing thyroid cancer due to greater radiosensitivity.

During the last 20 years, the lifetime dose of ionizing radiation for many U.S. residents has doubled as a result of medical and dental diagnostic procedures [20, 24, 33]. Although CT scans

account for only 15% of all radiological diagnostic interventions, they are the source of more than half of the radiation doses that U.S. residents are exposed to [24]. Some studies conducted in pediatric populations showed a weak but nonnegligible correlation between the risk of thyroid malignancies and CT scan exposure. Moreover, there is an inverse correlation between the risk of thyroid cancer and the age at exposure. Children aged <5 years have the highest risk and there is a significant decline in risk after the age of 20 [33, 45]. Nevertheless, the appreciable increase in thyroid cancer incidence cannot be ascribed solely to CT scans because most CT scans are performed in patients aged >50. Another major source of irradiation is nuclear medicine where radioactive iodine has been widely used recently for both diagnostic and therapeutic purposes in different thyroid diseases [24]. Diets containing supplemental iodine are another potential risk factor for thyroid cancer as they interfere with iodine organification and thyroid hormone synthesis [24]. Another possible mechanism is the increased incidence and severity of chronic thyroid conditions such as chronic lymphocytic thyroiditis and autoimmune thyroiditis [16].

Enviromental pollutants, such as asbestos, benzene, formaldehyde, pesticides, bisphenol A (BPA), polychlorinated biphenyls (PCBs), and polyhalogenated aromatic hydrocarbons (PHAHs), may also contribute as both genotoxic and nongenotoxic carcinogens [24]. In addition, chronic metabolic conditions, such as diabetes and obesity-related metabolic disorders, are among the proposed risk factors related to the higher incidence of thyroid cancer [20, 33]. Those conditions lead to low grade inflammation triggered by alterations in adipokines. This occurs along with increased circulating leptin and an adiponectin deficiency which is independently and inversely associated with thyroid cancer risk [46]. All these metabolic changes are linked to higher activity in the mitogen-activated protein kinase pathway which has

a crucial role in carcinogenesis by activating mitogenesis [33, 46, 47]. Other potential risk factors, including estrogen exposure and dietary nitrates, still need to be investigated since there is a lack of conclusive data in the literature [20, 33].

Overall, the reasons behind the worldwide increase in thyroid cancer are still unclear. Most likely, the observed exponential upsurge has a multifactorial etiology. Certainly, the increased detection rate can partly explain the phenomenon but, at the same time, there is strong evidence indicating a true increase in thyroid cancer incidence. In summary, further studies are warranted in order to investigate the real reasons and determine the potential carcinogens and their mechanisms of action [24].

Histologic classification (8)

Based on their cellular origin, characteristics, and prognosis, thyroid cancers are classified into several histological types and subtypes. Thyroid cancers are mostly epithelial tumors originating from thyroid follicular cells (TFCs) and parafollicular cells (C cells) or from nonepithelial stromal elements [48]. On the basis of architectural, cytologic, and histogenetic features, the World Health Organization (WHO) classifies primary thyroid tumors into epithelial or nonepithelial and benign or malignant categories. According to the same classification, lymphomas and miscellaneous tumors are defined as separate groups [48-51].

Papillary thyroid carcinoma (PTC) (8.1)

Papillary thyroid carcinoma (PTC) is the most common differentiated carcinoma, accounting for 80-90% of all primary thyroid malignancies [9, 48, 50]. Generally, papillary carcinomas are indolent, slowly growing tumors. Women aged 25-45 have a three times higher risk of developing PTC [8]. Because of the carcinoma's indolent nature, most cases have an excellent prognosis but in up to 10% of cases recurrent disease may occur in the form of cervical lymph node metastasis and/or distal metastasis [48, 50]. The main mode of PTC spreading is via the lymphatic system. Dispersion of cancerous cells within the thyroid may lead to "multifocal" disease and cervical node metastasis. It has been estimated that in over 50% of cases, PTCs have nodal metastasis at initial diagnosis [48, 50]. Since venous invasion rarely occurs, metastasis outside the neck is unusual [52]. In 5-7% of cases, PTC may cause distant metastasis in lungs and bone [52]. PTC's tendency to spread locally in the neck may cause tracheal compression and involve the recurrent laryngeal nerve [5]. Although most PTC show a papillary growth pattern, the main distinguishing feature is malignant cytologic (nuclear) changes which are important diagnostic hallmarks [11, 50, 52]. The typical nuclear appearance of PTC includes enlargement, hypochromasia, intranuclear cytoplasmic inclusions, nuclear grooves, and distinct nucleoli [11]. The PTC nuclei have also been described as clear, ground glass or "Orphan-Annie eyed" [50, 52]. Psammoma bodies, which are another microscopic characteristic of PTC, are laminated calcifications that may occur in about 40% of cases [9, 11, 52]. The psammoma bodies are formed when calcium is deposited on the dying cells after infarction of the papillae. Another theory relies on the intracellular accumulation of calcium by tumor cells which later leads to

their death and the calcium's release. Further deposition of free calcium leads to lamellation [52].

Microscopically, papillary carcinoma has multiple histotypes, the most important of which are papillary microcarcinoma (PMC), follicular variant, tall cell variant, and diffuse sclerosing type [49, 52].

According to the World Health Organization (WHO), the term papillary microcarcinoma refers to thyroid cancer measuring 1 cm or less in diameter [53]. According to the American Thyroid Association, PMC detection accounts for almost 50% of the thyroid cancers diagnosed in the past three decades. The incidental detection of PMC in the thyroid gland is a frequent finding in autopsies and may indicate the indolent nature of this tumor without having any clinical relevance [53]. However, PMCs may lead to lymph-node metastasis in the central and lateral compartments of the neck [54]. Despite the incidence of locally metastatic disease, the prognosis for PMC is excellent and 10 year mortality rates in patients with this tumor are less than 1% according to the American Thyroid Association [54].

The follicular variant of PTC (FV-PTC) is one of the most common subtypes among all PTC histotypes, accounting for up to 41% of all PTC cases [55]. Although FV-PTC contains a mixed histology of both PTC and FTC, microscopically its architecture is predominantly follicular [55]. Despite some unique clinical behavior, FV-PTC seems to be quite a distinctive entity, sharing clinical features with both PTC and FTC. Having more favorable clinicopathologic features and a better tumor risk group profile does not impact the long-term outcome of FV-PTC, which is similar to that of conventional PTC [48, 56].

The diffuse sclerosing variant of PTC (DSVPTC) is an unusual variant, with prevalence ranging from 0.7-6.6% of all PTCs. A macroscopic feature of DSVPTC is the extensive involvement of the thyroid gland without forming a dominant mass [57]. Its hallmark on microscopic examination is the extensive fibrosis, squamous metaplasia and numerous psammoma bodies. Since DSVPTC has a higher incidence of lymph node metastasis at presentation than conventional PTC, along with a distant metastasis rate of around 5%, aggressive treatment protocols are required in managing DSVPTC [57]. Nevertheless, cancer recurrence and cancer-related mortality have been reported at 14% and 3% respectively [57].

The tall cell variant of PTC, which has 10% prevalence and a 10-year mortality rate of up to 25%, is the most common aggressive variant of PTC [50, 58]. Microscopically, tall cells are twice as tall as their width and should represent at least half of the papillary carcinoma cells [50]. This variant has a high prevalence among patients with radioactive iodine refractory disease and is associated with a high rate of B-RAF mutations, making the latter a potential target in terms of treatment strategies [58].

Follicular thyroid carcinoma (FTC) (8.2)

Follicular thyroid carcinoma (FTC) is the second most common malignant neoplasm arising from epithelial cells. It accounts for 5-10% of thyroid cancers in non-iodine deficient areas and ranges up to 30-40% in iodine deficient areas [48]. Because it is difficult to distinguish follicular carcinoma from follicular adenoma based on the results of fine needle aspiration (FNA), the

term “follicular neoplasm” is used for these tumors. Other methods, such as immunohistochemistry, morphometry, cytogenetic, and oncogen markers, have not provided reliable information regarding the distinction between follicular adenoma and follicular cancer on FNA [48]. Excisional biopsy (hemithyroidectomy) may be warranted in order to establish the precise histologic diagnosis on the basis of capsular and vascular invasion which helps discriminate between benign and malignant lesions [6]. On microscopic examination, follicular carcinoma is usually composed of microfollicles (minimal amount of colloid within the small follicles) or solid nests of tumor [8]. Depending on the degree of invasiveness, there are two major groups of follicular carcinomas: minimally invasive and widely invasive [48]. Follicular cells may invade either the tumor capsule and/or the blood vessels, with vascular invasion having a worse prognosis. Other recognized risk factors, such as age >45, gender, extrathyroid invasion, greater tumor size, and the presence of distant metastasis at presentation, are also associated with poorer prognosis [32]. Nevertheless, metastases to cervical lymph nodes are rare and are often accompanied by direct extrathyroidal extension of the cancer [9].

Hurthle cell carcinoma (8.3)

One of the variants of follicular carcinoma is Hurthle cell carcinoma which is composed predominantly (>75%) of oncocytic cells and is thought to have a worse prognosis than the usual follicular carcinoma [48, 50]. The benign variant of Hurthle cell lesions represented by Hurthle cell adenoma is generally indistinguishable from the malignant variant based only on the results

of cytological analysis. Thus, once a Hurthle cell lesion is detected by FNA, an excision biopsy may be indicated for further histological analysis [5]. Metastasis to cervical lymph nodes in Hurthle cell carcinoma is more common compared to the usual follicular carcinoma [9].

Poorly differentiated thyroid carcinoma (PDTC) (8.4)

Poorly differentiated thyroid carcinoma (PDTC) characterizes a heterogeneous group of malignant neoplasms. It was recognized as a distinct pathologic entity by the WHO in 2004 [59]. The macroscopic hallmarks of PDTC are large (>5 cm in diameter), solid, unencapsulated, nodular or multinodular, gray-white tumors which are likely to penetrate perithyroidal tissues. On microscopic examination, these tumors represent a trabecular, solid or insular growth pattern [48]. Due to its heterogeneity, PDTC has different biologic behavior and various histologic patterns of growth. Although PDTC accounts for only 1-15% of all thyroid cancers, it is the main cause of death from "nonanaplastic follicular cell-derived thyroid cancer" [59, 60].

Medullary thyroid cancer (MTC) (8.5)

Medullary thyroid cancer (MTC) is a neuroendocrine tumor of the thyroid gland's parafollicular or C cells and it accounts for up to 10% of all malignant thyroid tumors [48]. Although approximately 80% of all MTCs are sporadic cases, there are also some hereditary cases resulting from RET proto-oncogene mutation which is the underlying defect in Multiple Endocrine Neoplasia type 2 (MEN2) [61]. Despite MTC's morphological diversity, the

particular type of histology does not seem to influence the disease's course [62]. The primary manifestation of sporadic MTC in most cases (75-95%) is a solitary nodule, usually located in the upper portion of the thyroid lobe [61, 63, 64]. In the majority of cases, the MTC has already metastasized at presentation, with 50% of patients having clinically detectable cervical lymph nodes, up to 15% having upper aerodigestive tract compression signs, and 5% having distal metastatic disease [63]. A characteristic feature of MTC is the production of calcitonin which can serve as a tumor marker because its concentration usually correlates with tumor mass and is almost always abnormally elevated in patients with a palpable tumor [63]. The MTC cells are also capable of secreting carcinoembryonic antigen (CEA) which may indicate advanced disease if levels are abnormally high [65].

Anaplastic thyroid carcinoma (ATC) (8.6)

Anaplastic thyroid carcinoma (ATC) is an extremely aggressive malignant thyroid tumor which accounts for less than 2% of all thyroid malignancies [66, 67]. Compared to differentiated thyroid cancers, ATC's disease-specific mortality rate surpasses 90%, with an average survival duration of 6 months after diagnosis [66]. Having a mean age of 65 at initial presentation, most patients with ATC are older than those with differentiated thyroid carcinoma [67].

The major etiologic factors of ATC are pre-existing thyroid diseases or other types of thyroid cancer such as PTC, FTC, or poorly differentiated carcinoma. This observation supports the theory that ATC develops from more differentiated tumors following the dedifferentiating events [68]. This hypothesis might be supported by the fact that both well differentiated thyroid

cancers and ATC have the same underlying molecular development mechanism. In both cases, mutations in BRAF and RAS play a central role in the early events of the progression pathway. Although ATC has multiple histopathological features, there is no evidence showing the impact of a particular histotype on the prognosis [66]. Around 90% of patients have regional or distant metastasis at the time of initial presentation, with lungs being the most common site of distal metastasis [69, 70].

Thyroid cancer staging (9)

According to the American Cancer Society, the TNM system adopted by the American Joint Committee on Cancer (AJCC) is the most frequently used system to describe thyroid cancer stages. Based on three key parameters, the TNM system indicates the size of the primary tumor (T), describes the extent of metastatic disease to the regional lymph nodes (N) and specifies whether there are distant metastases to other organs (M).

Primary tumors (T) for nonanaplastic thyroid cancer might be subdivided into several subcategories, the first being Tx where the tumor cannot be assessed due to missing information and the most advanced being T4b where the tumor has invaded prevertebral fascia or encased the carotid artery or mediastinal vessels [71, 72]. All anaplastic thyroid carcinomas are considered T4 tumors at initial diagnosis, with T4a having only intrathyroidal expansion (surgically resectable) and T4b growing outside the thyroid gland (surgically unresectable) [71, 72].

The N parameter describes the metastasis into the regional lymph nodes, consisting of the central compartment, lateral cervical, and upper mediastinal lymph nodes. This parameter is also divided into several subcategories, with the most advanced being N1b which is associated with unilateral, bilateral, contralateral cervical, or superior mediastinal lymph node metastasis [71].

There are only three subcategories for M when evaluating distant metastasis. M1 indicates that the cancer has spread to distant lymph nodes and internal organs [71].

After the TNM values have been determined (table I, page 54), the combined results are used to assign the stage (ranging from I to IV). Differentiated thyroid cancers (PTC and FTC) are further grouped into stages in a way that the patient's age is taken into consideration as a dichotomous variable. The cutoff value is age 45 and all patients below that age are either Stage I (no distant metastases) or Stage II (distant metastases present), which means they should have an excellent prognosis [11, 71, 72].

In contrast to PTC and FTC, age is not a factor in MTC staging, and all ATCs are considered Stage IV tumors, reflecting the poor prognosis for this type of cancer [71, 72].

Molecular Pathogenesis (10)

Several types of genetic alterations and epigenetic changes have been shown to play a fundamental role in the tumorigenesis of different variants of thyroid cancer [48, 73, 74]. An understanding of the pathways, such as gene mutation, aberrant gene methylation, and gene translocation, can open up new perspectives in terms of molecular diagnostics, treatment, and prognosis prediction [74].

One of the most common pathogenetic mutations of differentiated thyroid carcinoma (DTC) is the point missense mutation in exon 15 of BRAF which occurs in around 45% of sporadic PTCs [50, 73, 74]. This mutation is generated when glutamic acid is replaced by the amino acid valine at residue position 600. It leads to the expression of the BRAF-V600 mutant protein, causing constitutive activation of serine/threonine kinase [73, 74]. Over 90% of BRAF alterations are due to a V600 mutation. The most common subtype of PTC carrying a BRAF mutation is the tall cell variant (77%), with FVPTC having the lowest percentage (12%) of this particular mutation [50, 73].

Several studies have observed a correlation between BRAF-V600 mutation and the clinicopathological features of PTC [75]. BRAF mutation is more common in older patients and is associated with extrathyroidal invasion, advanced stages, and poorer clinical outcomes including higher recurrence rates and refractoriness to radioiodine treatment since BRAF mutation is responsible for suppressing the sodium/iodide symporter (NIS) [50, 73].

Rat sarcoma oncogene (RAS) mutations are second in prevalence after BRAF mutations. There are three RAS isoforms: H-RAS, K-RAS, and N-RAS [50, 73, 74]. The most frequent RAS

mutations linked with thyroid cancer are the point mutations in codon 61 of H-RAS and N-RAS. These mutations cause RAS to lose its intrinsic GTPase activity, which means that RAS is in a permanent GDP-bound active state [73, 74]. Although RAS has a classic dual stimulator impact on the MAPK and PI3K–AKT pathways, in tumorigenesis RAS activates the PI3K–AKT pathway instead [74]. Despite the fact that RAS mutation is relatively rare (0-20%) in conventional PTC, approximately half of FTCs and FVPTCs may carry RAS mutations. In addition, 20% of follicular adenomas may harbor a RAS mutation, suggesting that RAS mutation is an early event in tumorigenesis [50]. PTCs carrying RAS mutations are predisposed to a more benign disease course with a lower rate of lymph node metastasis and greater likelihood of encapsulation [73].

Chromosomal rearrangement of RET proto-oncogene is an example of the gene translocations which were first observed in PTC, thus, it is called RET/PTC. The rearranged RET/PTC is located on chromosome 10q11-12 and encodes a transmembrane receptor RTK [73, 74]. Although there are more than 10 types of RET–PTC translocation, RET/PTC1 and RET/PTC3 are responsible for most of the rearrangements found in PTC [73, 74]. These rearrangements cause constitutive activation of several downstream pathways including MAPK and PI3K-AKT [50]. It is worth noting that RET/PTC chromosomal rearrangements are observed in 15-45% of conventional PTCs and 80% of radiation induced PTCs [50, 76].

The balanced translocation between chromosomes 2 and 3 results in the PAX8/PPAR fusion oncogene which is detected in 36.5% of FTCs (from 11% to 63%), 9.5% of follicular adenomas, and 13.2% of FVPTCs. This is the most common genetic abnormality involved in FTC [77].

Epigenetic modulations, such as DNA methylation, histone deacetylation, and chromatin remodeling, have been implicated in DTC tumorigenesis as well [75].

The mechanism of MTC molecular pathogenesis is also associated with RET proto-oncogene which is detected in almost all patients with hereditary MTC and in 30-50% of sporadic cases. MTC aggressiveness can be predicted by the presence of certain RET mutations along with designated levels of calcitonin and carcinoembryonic antigen (CEA). Low calcitonin levels in combination with high CEA levels or a rapid CEA/calcitonin doubling time indicates a poorer prognosis and the disease's progression [78].

In a clinical setting, stratifying patients with germline RET mutations into specific risk groups may help target the patients with higher risk of malignant transformation. In those patients, a prophylactic thyroidectomy represents the best chance of curing the familial MTC [78].

The most common mutations reported in ATC are p53, RAS, and BRAF [79]. Overall, the p53 tumor suppressor gene is the most frequently affected gene in human cancer. It is assumed that p53 genetic alterations are late events in the thyroid tumorigenesis cycle and might be linked to a poor prognosis [48].

Diagnostic modalities in managing DTC (11)

According to the American Thyroid Association Management Guidelines [80], the main goals of initial therapy in patients with DTC are to improve overall and disease-specific survival, diminish the risk of persistent/recurrent disease and associated morbidity, and provide the

necessary conditions for accurate disease staging and risk stratification. At the same time, precautions should be taken in order to minimize treatment-related morbidity and unnecessary therapeutic interventions. To reach those goals, the American Thyroid Association strongly recommends performing preoperative staging with diagnostic imaging that may or may not include laboratory tests [80]. The frequency of micrometastasis is high but it does not have as much clinical significance as macrometastasis. Preoperative ultrasonography of the central and lateral compartments may identify abnormal lymph nodes in up to 35% of patients [81]. These ultrasound (U/S) findings may alter the planned surgical approach in up to 20% of cases by facilitating complete resection of the disease and potentially minimizing locoregional recurrence [82, 83]. However, preoperative U/S identifies only 50% of the lymph nodes affected in the central compartment because the overlying thyroid gland does not allow adequate visualization [84]. Although there is no single sonographic feature with adequate sensitivity and specificity, evaluating a combination of such characteristics as size, loss of fatty hilum, shape, echogenicity, cystic changes, calcifications, margins, compressibility and peripheral vascularity may help when making decisions about further managing suspicious lymph nodes [80, 85]. Among all those features, microcalcifications have the highest specificity. Any lymph node with microcalcification should therefore be considered abnormal [84]. In their study, Chung et al. showed an exponential increase of metastasis rate associated with the increased number of suspicious sonographic features. Most notably, in the presence of 4 suspicious features on U/S, the rate of metastasis was 100% [86].

The location of suspicious lymph nodes on U/S may also be of some benefit in decision making as malignant lymph nodes are more likely in levels III, IV, and VI [87].

One obvious advantage of U/S compared with other imaging modalities is the possibility of proceeding with U/S guided fine needle aspiration (FNA) for sonographically suspicious lymph nodes [88].

FNA for the cytologic evaluation of thyroid cancer was first used by Martin and Ellis at New York Memorial Hospital for Cancer and Allied Diseases in 1930 [89]. Undefined thyroid nodules are a common clinical problem as 4-7% of the U.S. adult population may have palpable thyroid nodules [89]. The evidence shows that the probability of developing thyroid cancer from a clinically solitary thyroid nodule or a multinodular goiter is about 5% in non-endemic areas [89]. The principal indications for FNA are therefore thyroid nodules or cervical lymph nodes that are sonographically suspicious for thyroid cancer. More widespread use of this procedure has reduced the number of diagnostic thyroid surgeries for suspicious thyroid nodules by 60-85% [89]. The recommendations known as the Bethesda System for Reporting Thyroid Cytopathology were adopted as a way to report thyroid nodule FNA cytology. The system consists of the following diagnostic categories: nondiagnostic or unsatisfactory, benign, atypia of undetermined significance or follicular lesion of undetermined significance, follicular neoplasm or suspicious for a follicular neoplasm, suspicious for malignancy, malignant. The cancer risk estimate within each of the 6 categories is determined based on literature review and expert opinion [80].

Although it may be difficult to interpret results in patients with an intact thyroid gland, adding FNA-Tg (thyroglobulin) washout to the diagnostic workup of suspicious cervical lymph nodes may be of some benefit in selected patients [80]. A cutoff value of 1 ng/ml for FNA-Tg provides 100% sensitivity, 96.2% specificity, and 97.2% positive predictive value [90]. FNA-Tg washout

may be especially useful in cases where FNA is not diagnostic due to inadequate cytologic evaluation, cystic degeneration of the lymph node, or equivocal results from cytologic and sonographic evaluations. In those cases, adding FNA-Tg may help overcome the limitations of FNA because combining 1 or 2 suspicious U/S features with FNA-Tg may significantly increase sensitivity [86].

Other imaging modalities such as CT and MRI with contrast can be used in addition to U/S in advanced disease cases where there are invasive tumors or clinically apparent involvement of multiple LNs. Ahn et al. showed that although U/S has very high accuracy when used for per patient analysis, CT's even higher accuracy in terms of per level analysis may enable it to play an adjunctive role in decision making about surgical extent in selected patients with thyroid cancer [91]. Another study showed that preoperative mapping with combined U/S and CT was significantly superior to U/S alone in primary and revision patients both in the central and lateral compartments [92]. MRI has relatively low sensitivity (30-40%) for detecting cervical lymph node metastasis [80].

Preoperative ultrasound cervical mapping (12)

There is an accepted opinion that although clinically apparent and/or palpable LNs should be removed, very small cancerous LNs do not need to be resected as the subsequent radioiodine therapy can resolve the issue in those groups of patients. Other patient groups may harbor nonpalpable metastasis located in both the central and lateral neck compartments. In those

patient groups, U/S is an indispensable tool for detecting and managing occult metastasis [83, 93].

In DTC, the incidence of cervical LN metastasis at the time of diagnosis is about 20-50%, and the incidence of micrometastases may approach 90% depending on the detection method used [91, 93]. Although there is some controversy regarding the prognostic impact of cervical LN involvement, the evidence shows that patients with cervical LN metastases at initial presentation tend to have more locoregional recurrence than those without LN involvement [93-95].The locoregional recurrence rate of DTC ranges between 5% and 30% within 10 years of initial diagnosis [82, 96]. However, given the indolent nature of DTC, 5 and 10 years survival rates are 95% and 90% respectively [97]. Despite a high overall survival rate, local P/R disease caused by LN metastasis continues to present management challenges especially in the central compartment. In contrast to the lateral compartment, clinical and radiological detection of central neck nodal metastasis is not sufficiently reliable at pre-operative assessment. Prophylactic central neck dissection (pCND) is performed in order to reduce the risk of P/R disease in the central compartment [98]. Nevertheless, the role of pCND remains controversial in managing DTC due to a lack of prospective randomized controlled studies [80]. In their retrospective study, Barczynski et al. showed statistically significant improvement in the survival rate of a group of patients who underwent total thyroidectomy (TT) with pCND versus TT alone (98% and 92.5% respectively over the 10-year period) [99]. The same study also showed a significant reduction in the 10-year local recurrence rate of TT with pCND versus TT alone [99]. However, different meta-analysis did not definitively show any significant risk reduction in the local recurrence rates of TT with pCND in comparison to TT alone [100].

In MTC, LN involvement at the time of initial diagnosis ranges from 35% to 50% [101]. Due to the more aggressive course of the disease, the locoregional recurrence rate of MTC is about 45% by the 10 years followup point[102]. However, the overall prognosis for patients with MTC is good because the 10 years survival rate ranges from 75% to 85%[101].

As indicated by the data, in many cases patients may have undetected macroscopic LN metastases at the time of initial surgery. Detecting and resecting these LNs may prevent subsequent reoperation due to recurrent disease and may reduce the risk of postoperative morbidity [82]. Therefore, it is crucial to detect metastatic LNs before the surgery because accurate preoperative mapping of the cervical nodal levels is essential for adequate surgical planning, which in turn may reduce the risk of postsurgical complications from repeated surgery [91]. U/S is currently the preferred imaging modality for preoperative detection and assessment of cervical nodal metastases [91]. It has several advantages as it is radiation-free, easy to perform, and is widely available [88]. At the same time, this modality has some drawbacks: it is subjective, operator dependent, and subject to anatomical limitation of the examination field [91].

Nevertheless, some authors point out that positive preoperative U/S findings for LN metastases may indicate a more aggressive course for the disease which will necessitate additional surgical intervention in the future [103] .

Considering the relevance of this imaging modality, we decided to evaluate the qualitative and quantitative reliability of preoperative U/S lymph node detection based on cervical nodal levels, which is otherwise known as “ultrasound cervical mapping.”

ARTICLE

The relevance of preoperative ultrasound cervical mapping in patients with thyroid cancer

Kocharyan D, Schwenter F, Bélair M, Nassif E.

Can J Surg. 2016 Apr;59(2):113-7.

PMID: 27007092

Correspondence to: E. Nassif, Department of Surgical Oncology, 1560 Sherbrooke St. East, Montreal, QC, H2L 4M1; edgard.nassif@umontreal.ca

Abstract

Background

Cervical lymph node involvement in thyroid cancer is associated with locoregional recurrence and decreased disease-free survival. Preoperative lymph node mapping helps in planning surgery for neck dissection and improves patient outcomes. The aim of this study was to perform a qualitative and quantitative analysis of ultrasound mapping for thyroid cancer and evaluate the significance of this exam in terms of identifying the group of patients who would benefit most from subsequent surgical dissection.

Methods

Retrospective review of 263 patients who underwent thyroid surgery between 2009 and 2013 was performed. The positive predictive values of ultrasound mapping of both the lateral and central compartments together and the lateral or central compartment individually were calculated. A quantitative analysis was performed by comparing the number of positive lymph nodes at ultrasound imaging with histopathologic evaluation.

Results

136 cases in 120 patients met the inclusion criteria for ultrasound mapping analysis. The positive predictive values (PPV) were: lateral and central compartments PPV=83.82 (95% CI 0.76-0.89), lateral compartment PPV=85.39% (95% CI 0.76-0.91), central compartment PPV=80.48% (95% CI 0.7-0.87). When comparing the positive lymph nodes at ultrasound

imaging with histopathologic evaluation using the chi-square test, the result was X^2=10.33 (p=0.006).

Conclusion

This single-institution study indicated that preoperative ultrasound mapping is an accurate imaging procedure for predicting lymphatic spread in differentiated and medullary thyroid cancer. Ultrasound mapping can be used as an efficient tool for surgical planning and prognosis determination, as well as for identifying the group of patients who would benefit most from subsequent surgical intervention.

Introduction

Differentiated thyroid carcinomas are the most common types of thyroid malignancies. Papillary carcinoma (PTC) accounts for approximately 80% of all thyroid neoplasms [104, 105]. PTC has shown a permanent increase in its incidence [106], with the fastest increase in women [104], making it now the sixth most common cancer among women. There are several major risk factors for developing PTC, such as exposure to ionizing radiation and a family history of thyroid cancer. PTC is also associated with oncogenes mutations such as BRAF, RET/PTC, RAS and TRK [104].

Despite a high survival rate with 5- and 10-year overall survival of 90% and 95%, respectively [97], about 20%-50% of patients require additional treatments for lymph node (LN) metastasis [93, 97, 107]. In 80%-90% of patients, micrometastasis can be found in meticulous bilateral neck dissection [93, 104, 108]. Although cervical LN metastasis has almost no impact on short-term survival, it greatly affects long-term survival as a major risk factor for locoregional tumor recurrence [97]. LN metastasis is related to several predisposing risk factors such as male gender, age over 45, tumor size more than 1 cm, and lymphovascular and extrathyroidal invasions [109]. Moreover, PTC located in the upper neck was shown to present a higher rate of lateral neck metastasis than lower neck PTC [109].

Despite an increased rate of recurrence in patients with PTC and LN metastasis, there is no evidence that radical neck or routine central compartment dissection can improve disease-free survival, particularly in papillary thyroid microcarcinomas measuring less than 10 mm [110]. Since neck dissection is associated with such complications as hypocalcaemia, recurrent laryngeal nerve palsy, hematoma, chyle leakage and spinal accessory nerve dysfunction, the indications for surgical intervention should be thoroughly investigated [111, 112].

LN involvement can be detected by physical examination, with a sensitivity as low as 15%-30% [109, 113], while neck imaging is highly efficient, in particular high-resolution ultrasound (U/S) which is able to detect nodules as small as 5 mm**.** CT scans can also be used for staging LN in thyroid carcinoma. Although most studies agreed that US is the first choice [83, 114-116] some investigators found comparable sensitivity with CT and even slightly higher than US for the central compartment [91, 117]. In those studies, it is interesting to observe that the sensitivity of the US is lower (under 80 %) than what was previously reported. CT necessitates the injection of a high quantity of iodine contrast which could interfere in the follow up investigation and treatment of the patient. Indeed after receiving iodine contrast, the patient must wait between 3-4 months before being able to receive radioactive-iodine treatment or a thyroid iodine uptake scan. It is important to be aware of the patient therapy and coordinate the CT with the medical team. This is clearly a disadvantage for this modality. Using U/S also gives the opportunity to practice FNA (fine needle aspiration) on LN that are undetermined and influence the extent of the dissection.

An U/S-based preoperative evaluation of the primary tumor's extent as well as LN involvement has become an essential procedure, which can modify the overall surgical approach in up to 40% of cases [85, 110]. This approach is recommended in the guidelines of the American Thyroid Association [118].

The aim of this study is to evaluate the diagnostic reliability of preoperative U/S mapping of thyroid cancers in our institution by calculating the positive predictive value (PPV) of the test and trying to determine whether there is a quantitative association between U/S mapping and pathological analysis. The interpretation of such an association may help to target the group of patients who would benefit most from the subsequent surgical procedure and at the same time

help avoid unproductive surgical dissection in patients with minimal risk of disease recurrence. To our knowledge, no study has been done to evaluate the existence of such a quantitative association between cervical LN mapping and pathological results.

Materials and methods

The medical records of 263 patients who underwent surgery for thyroid cancer at the Centre hospitalier de l'Université de Montréal (CHUM) between 2009 and 2013 were retrospectively reviewed. The only exclusion criterion was the absence of U/S mapping. Electronic institutional database and Microsoft Access software were used for data collection. Surgeries were performed by nine surgeons (one endocrine surgeon and eight otorhinolaryngologists). Ten radiologists were involved in the study but more than 80% of cases were examined by one radiologist with advanced skills in U/S mapping of thyroid cancer. An U/S system equipped with a high-resolution (13 MHz) linear probe was used. Parameters including operative procedures, pathological analyses and mapping results according to neck compartments were collected.

For the purpose of quantitative analysis, patients were divided into two groups based on the number of suspicious LNs at U/S mapping (group A: 1 or 2 LNs; group B: 3 or more LNs). Concerning the histopathological results, three groups were created according to the number of positive LNs (group 1: 0 LNs; group 2: 1 or 2 LNs; group 3: 3 or more LNs). The LN location in the neck was divided into central (CC) and lateral compartments (LC). CC is defined as the space between the medial margins of bilateral carotid arteries, corresponding to level VI, while

LC extends from the carotid arteries to the medial border of the trapezius muscle and involves levels II, III, IV and V, according to the definitions of the American Head and Neck Society and the American Academy of Otolaryngology – Head and Neck Surgery [109, 119, 120].

The imaging suspicion of LN involvement was based on various criteria such as cystic changes, hyperechogenicity, loss of hilar echogenicity (fat center), internal microcalcifications, poorly defined irregular borders, shape/dimension ratio, compressibility, vascularity, size >5 mm in its shortest diameter and round shape (Figure 1, page 58) [85, 104, 107, 121, 122]. In our study, a node was considered suspicious if at least one of these criteria was met. As for loss of hilar echogenicity on nodes smaller than 5 mm, an additional criterion was needed to reach the level of suspicious LN. The surgical exploration was guided by U/S mapping. Suspicious LNs were removed based on the compartment-oriented approach without further dissection. On histopathology, LNs with a diameter less than 5 mm were examined by one-slice specimen. Two-slice specimen examination was used for the LNs having a diameter greater than 5 mm. Statistical analyses were performed using SPSS software, version 20. Continuous variables were analyzed with the mean ± standard deviation, and X^2 was used for categorical data.

Results

Among 263 patients who underwent thyroid surgery, 261 had undergone U/S mapping. Suspicious LNs had been found in 154 cases. Despite positive U/S mapping, 18 cases were excluded from the study due to the absence of LNs in the surgical specimen in 3 patients, the absence of pathological results in 1 patient, the absence of neck compartment dissection in 11 patients, and a change in surgical planning in favor of palliative tracheotomy in 1 patient and hemithyroidectomy in 2 patients. Therefore, results were obtained after analyzing 136 cases of positive U/S mapping in 120 patients (Table II, page 55).

The patients' mean age was 49.9 (16-90, SD 16.2). Among those patients, 89 were female (74.1%) and 31 male (25.9%). Nine patients had been operated twice, two patients three times, and one patient four times. Out of 120 patients, histopathological analysis indicated 110 PTCs, 8 medullary cancers, 1 follicular cancer and 1 Hurthle cell tumor. In 87 cases, compartment-oriented surgical excision of suspected LNs along with thyroidectomy had been performed (primary surgeries) and 49 procedures had been carried out for recurrent disease (secondary surgeries). During the data collection period (02/2014-09/2014), radiological findings of locoregional recurrent disease were noted in 26 patients (21.6%). On U/S mapping, there were 40 cases in group A (29.4 %) and 96 cases in group B (70.6%). On histopathology, there were 22 cases in group 1 (16.2%), 41 cases in group 2 (31.1%) and 73 cases in group 3 (53.7%) (Table III, page 56). Among these groups, the chi-square test resulted in $X^2 = 10.33$ ($p = 0.006$).

The PPVs of ultrasound mapping were calculated for LC alone, CC alone and both LC/CC. For LC, 76 cases out of 89 were confirmed positive on pathological examination, resulting in LC-PPV=85.39% (95% CI 0.76-0.91). For CC, there were 66 positive cases out of 82, resulting in CC-PPV=80.48% (95% CI 0.7-0.87). Finally, for both LC/CC, 114 out of 136 cases were confirmed positive, with a LC/CC-PPV=83.82 (95% CI 0.76-0.89). Positive results on histopathology were derived following the examination of 1766 and 659 LNs removed from the LC and CC (Table IV, page 57).

Discussion

This study examined the qualitative and quantitative reliability of U/S mapping of thyroid cancer in a single institution. Our results confirmed that U/S mapping is a reliable tool for detecting affected LNs. With an LC/CC-PPV value of 83.82% together with advantages such as simplicity, ease in terms of performance, wide availability, comparatively low price, non-invasiveness and lack of radiation [113], U/S mapping is an excellent tool for surgery guidance. The relatively low rate of detection of metastatic LNs in the CC might be explained by anatomical limitations in that area of the neck, such as the clavicle, sternum and tracheal air shadow [106, 118]

Micrometastases (<2 mm) in patients undergoing prophylactic LN dissection were found in up to 80% of individuals [113, 123]. Nevertheless, such micrometastases are not clincially relevant, as most patients with PTC do not develop palpable lymphadenopathies. On the contrary, patients presenting with macrometastases (> 2 mm) have 5% to 40% persistent/recurrent (P/R) disease after surgery [124, 125]. P/R disease may be detected by TSH-stimulated serum thyroglobulin levels followed by confirmatory U/S guided FNA cytology [124, 126]. P/R disease has a tremendous impact on quality of life and despite the additional therapeutic interventions, 10% of patients die from it [113]. Reoperation of P/R disease involves many difficulties because the extensive scarring from previous surgery can obscure normal anatomy, which in turn contributes to longer operative times and increased morbidity [127]. U/S mapping guides the surgeon for precise neck dissection, resulting in decreased locoregional tumor recurrence and lower risk of postsurgical complications due to reoperation. To analyze this test more thoroughly, we decided to go one step further in our study by evaluating not only PPV but also the quantitative reliability

of the test, which, to our knowledge, had not been done before. Such an approach seemed highly valuable since patients with multiple positive LNs have been shown to be at greater risk of recurrence [128]. Based on the quantitative results of U/S mapping, patients can be stratified according to the level of risk of recurrent disease, which in turn will help in better selecting surgical candidates. The use of U/S mapping to plan compartment-oriented LN dissection could therefore benefit patients undergoing thyroid cancer surgery, as recognized by the American Thyroid Association [128]. In this study, results showed a statistically significant association between U/S mapping analysis and LN pathological examination, indicating that U/S mapping is an effective tool in the armamentarium of quantitative prediction for lymphatic spread in thyroid cancer. Another notable observation was derived by analyzing the false positive cases on U/S mapping. In 10 out of 22 cases (45.4%), patients were suffering from Hashimoto's thyroiditis which was mimicking affected LNs in thyroid cancer. The retrosternal localization and small size (<5 mm) of the affected LNs made it extremely difficult to distinguish them from metastatic LNs. Consequently, we believe that Hashimoto's thyroiditis might be considered a limiting factor for U/S mapping in patients with suspicion of thyroid cancer. Another major subject of discussion is the test's specificity and sensitivity. In fact, it is impossible to calculate either sensitivity or specificity since negative findings on U/S cannot be considered true negatives because of the lack of supporting "gold standard" results. In the literature, these two values have a very wide range (from 29% to 100%) depending on the study design and chosen "gold standard" methods, as shown by Wu et al. in their meta-analysis [129]. We therefore believe that the best way to solve this contradiction is to conduct a prospective study with long-term followup of negative cases on U/S mapping.

Conclusion**:** U/S mapping is a reliable tool for guiding surgical dissection of the neck, both for a primary tumor and for P/R disease. It has a sufficiently high PPV and strong quantitative association with histopathological analysis which makes it possible to focus on patients with higher risk of recurrent disease. Meanwhile, there might be some limitations to the test in cases such as thyroiditis, and further research needs to be done to obtain more reliable values for both sensitivity and specificity.

Table I: AJCC 7th Edition/TNM Classification System for Differentiated Thyroid Carcinoma

T0	No evidence of primary tumor
T1a	Tumor ≤1 cm, without extrathyroidal extension
T1b	Tumor >1 cm but ≤2 cm in greatest dimension, without extrathyroidal extension
T2	Tumor >2 cm but ≤4 cm in greatest dimension, without extrathyroidal extension
T3	Tumor >4 cm in greatest dimension limited to the thyroid or Any size tumor with minimal extrathyroidal extension (e.g., extension into sternothyroid muscle or perithyroidal soft tissues)
T4a	Tumor of any size extending beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, esophagus, or recurrent laryngeal nerve
T4b	Tumor of any size invading prevertebral fascia or encasing carotid artery or mediastinal vessels
N0	No metastatic nodes
N1a	Metastases to level VI (pretracheal, paratracheal, and prelaryngeal/Delphian lymph nodes)
N1b	Metastases to unilateral, bilateral, or contralateral cervical (levels I, II III, IV, or V) or retropharyngeal or superior mediastinal lymph nodes (level VII)
M0	No distant metastases
M1	Distant metastases

Table II: Surgical and histopathological characteristics of patients who underwent ultrasound mapping followed by neck dissection

No. of patients/cases	120/136
Gender (F/M)	89(74.1%)/31(25.9%)
Mean age ± SD/Min-Max	49.9±16.2/16-90
No. of primary/secondary surgeries	87/49
No. of postsurgical complications	3
Locoregional recurrence on U/S followup	26 (21.6%)
Histopathology (patients/result)	110/papillary 8/medullary 1/follicular 1/Hurthle cell

Table III: Cross-tabulation of the results among the groups of U/S mapping and histopathology

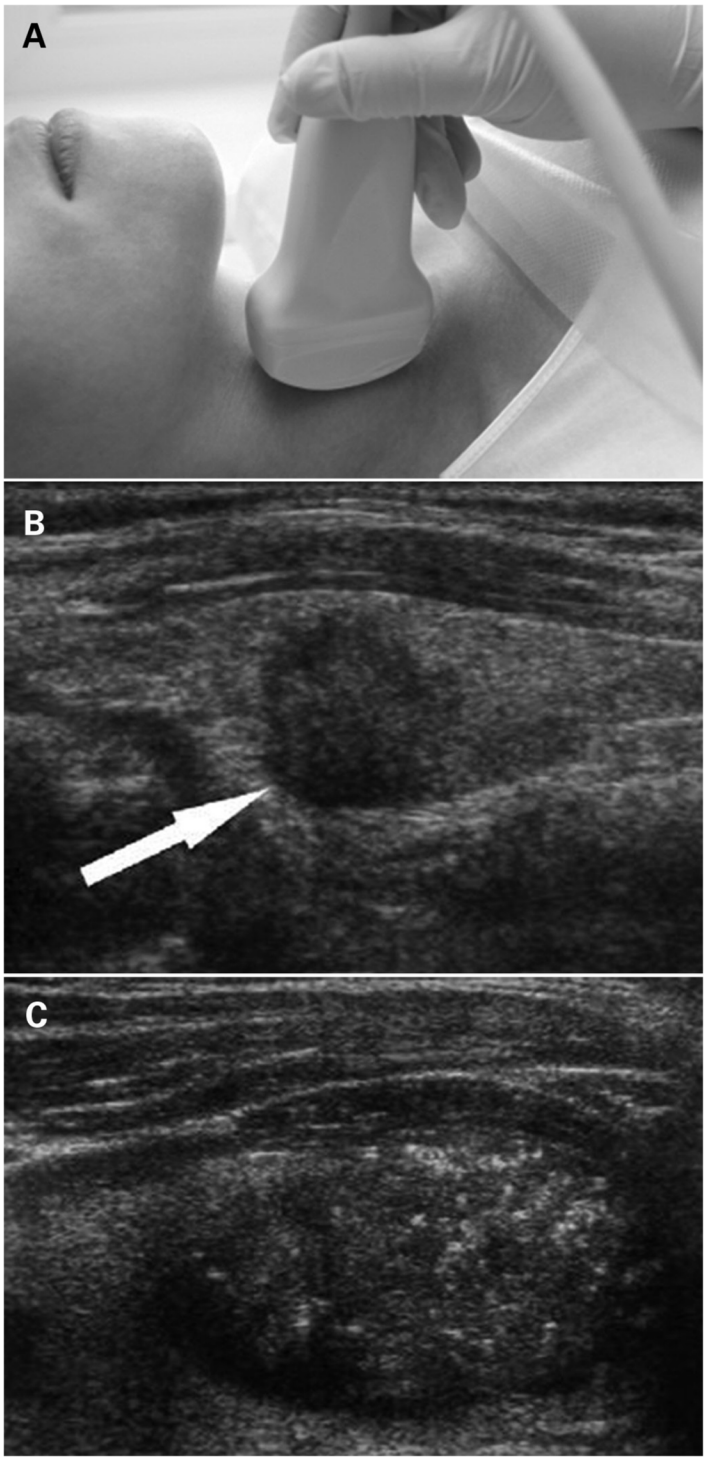
Mapping	Histopathology			
	1 (0 PLN)	2 (1 or 2 PLN)	3 (3< PLN)	Total
A (1 or 2 SLN)	10	17	13	40
B (3≥ SLN)	12	24	60	96
Total	22	41	73	

SLN: suspicious lymph nodes. PLN: positive lymph nodes

Table IV: Descriptive statistics of the removed LNs according to compartments

	Lateral compartment		Central compartment	
No. of dissected compartments	91		93	
	Positive LN	Total LN	Positive LN	Total LN
Mean No. of LN ± SD	4.21 ± 0.66	19.41± 1.6	3.24± 0.42	7.09 ±0.61
Median No. of LN (Min/Max)	2(0/49)	17(1/72)	2(0/24)	5(1/34)
Sum of LN	383	1766	301	659

Figure 1: (A) Ultrasonography of the neck. (B) Cancerous nodule detected on ultrasound (arrow). (C) Microcalcifications in the cancerous nodule



Discussion

The main goal of this study was to evaluate the diagnostic reliability of preoperative ultrasound (U/S) cervical mapping and highlight the relevance of this tool as a primary imaging modality for detecting and localizing metastatic nodal propagation in patients with thyroid cancer. Considering the results we obtained, it is fair to assert that U/S mapping is a powerful weapon in experienced hands because it can alter surgical planning and ameliorate overall patient outcomes by helping to minimize the risk of P/R disease.

Although many studies have tried to describe comprehensive qualitative evaluation by calculating not only the PPV of U/S cervical mapping but also its specificity and sensitivity, we appear to be the first group to adopt the quantitative evaluation method for this test by estimating the correlation between imaging results and histopathology. The idea behind this approach is strongly associated with prognostic factors for P/R PTC. According to some authors, several characteristic features of nodal metastases such as the number of metastatic lymph nodes (LNs), their size, and the presence of extranodal extension are the principal factors impacting the incidence of P/R disease [130, 131]. In particular, Ito et al. showed that patients with 5 or more clinically apparent metastases have a higher risk of locoregional recurrence [131]. Furthermore, the histopathology results of the neck dissection specimens confirmed this hypothesis as the patients with multiple positive nodes proved to be at greater risk of P/R disease [128]. This observation allows us to use the number of suspicious LNs on U/S mapping as useful criteria

for targeting the patient group with higher risk of P/R disease, which in turn may alter the treatment plan according to the risk group.

Our study showed that U/S mapping is an excellent tool for predicting the number of possibly affected LNs, which helps us stratify patients according to their risk of P/R disease and then adjust the extent of surgery based on that risk level.

Regarding the qualitative analysis, our results corroborate previously published outcomes. U/S mapping was shown to be a very reliable test with a PPV of over 80% and 85% in the central and lateral compartments respectively. The difference in the detection rate between the two compartments is due to anatomical limitations in the central compartment.

Although we obtained good results in terms of the ability of U/S mapping to detect suspicious LNs, the current study's design did not allow us to calculate either test sensitivity or specificity, which is one of the major limitations of our study. In the literature, these two features of the test have a very wide range [129] as there is no universal agreement on what should be considered the "gold standard" which could confirm or refute the negative results of preoperative lymph node staging by U/S mapping. Another weakness of our study is its limited generalizability, which is otherwise known as external validity. Our data originated from a single institution, which raises some questions about the extent to which the results can be applied in other clinical environments. This concern is supported by the fact that U/S mapping is a very operator dependent procedure, and this can impact the test's predictive reliability depending on the operator's experience, which can vary at different hospitals.

During the data analysis we also had some unexpected findings. In almost half of the false positive U/S mapping cases, the patients were suffering from Hashimoto's thyroiditis. After

investigating possible explanations, we concluded that the small size (<5 mm) and localization of the affected LNs did not allow for adequate visualization by U/S, in which case the inflamed LN could mimic a cancerous one.

Conclusion

In patients with thyroid cancer, preoperative LN staging has enormous relevance as it impacts surgical planning and overall outcomes. Preoperative U/S cervical mapping helps in risk stratification for P/R disease. It is useful in identifying the patients who would benefit most from subsequent compartment-oriented neck dissection. Nevertheless, further study may be warranted to calculate both the specificity and sensitivity of the test.

We recommend that U/S mapping be used as a routine imaging modality in order to prevent P/R disease and reduce morbidity caused by repeated surgeries.

References

1. Stathatos, N., *Thyroid Physiology.* Medical Clinics of North America, 2012. **96**(2): p. 165-173.
2. Daniel Oertli, M.D. and M.D. Robert Udelsman, M.B.A., *Surgery of the Thyroid and Parathyroid Glands*. Second ed. 2012: Springer.
3. Heymann, W.R., *Thyroid Disorders with Cutaneous Manifestations*. 2008: Springer.
4. Nyström, E., et al., *Thyroid Disease in Adults*. 2011: Springer.
5. Johnathan G.H. Hubbard, William B. Inabnet, and C.-Y.L. (Eds), *Endocrine Surgery (Principles and Practice)*. 2009: Springer.
6. McDougall, I.R., *Thyroid Cancer in Clinical Practice*. 2007, Springer.
7. Policeni, B.A., W.R.K. Smoker, and D.L. Reede, *Anatomy and Embryology of the Thyroid and Parathyroid Glands.* Seminars in Ultrasound, CT and MRI, 2012. **33**(2): p. 104-114.
8. McDougall, I.R., *Management of Thyroid Cancer and Related Nodular Disease*. 2006: Springer.
9. Leonard Wartofsky, M., MPH, MACP and M. Douglas Van Nostrand, FACP, FACNP, *Thyroid Cancer: A Comprehensive Guide to Clinical Management*. Second ed. 2006: Springer.
10. Jovanovic, L., et al., *Thyroid Gland Clonality Revisited: The Embryonal Patch Size of the Normal Human Thyroid Gland Is Very Large, Suggesting X-Chromosome Inactivation Tumor Clonality Studies of Thyroid Tumors Have to Be Interpreted with Caution.* The Journal of Clinical Endocrinology & Metabolism, 2003. **88**(7): p. 3284-3291.
11. ROBERT J. AMDUR, M.E.L.M., MD, *ESSENTIALS OF THYROID CANCER MANAGEMENT.* Springer, 2005.
12. Daniel G. Deschler, M.M.G.M., MD; Richard V. Smith, MD, *Quick Reference Guide to TNM Staging of Head and Neck Cancer and Neck Dissection Classification.* American Academy of Otolaryngology—Head and Neck Surgery; American Head and Neck Society, 2014.
13. Manual, A.C.S., *Cancer Staging Manual*. 2002, Springer.
14. Silverman, P.M., *Lymph node imaging: multidetector CT (MDCT): Monday 3 October 2005, 14:00–16:00.* Cancer Imaging, 2005. **5**(Spec No A): p. S57-67.
15. Ardito, G., et al., *Revisited anatomy of the recurrent laryngeal nerves.* Am J Surg, 2004. **187**(2): p. 249-53.
16. Vigneri, R., P. Malandrino, and P. Vigneri, *The changing epidemiology of thyroid cancer: why is incidence increasing?* Curr Opin Oncol, 2015. **27**(1): p. 1-7.

17. Cramer, J.D., et al., *Analysis of the rising incidence of thyroid cancer using the Surveillance, Epidemiology and End Results national cancer data registry.* Surgery, 2010. **148**(6): p. 1147-52; discussion 1152-3.
18. Lalmi, F., J.L. Sadoul, and V. Rohmer, *Les cancers de la thyroïde : de l'épidémiologie à la biologie moléculaire.* Annales d'Endocrinologie, 2015. **76**(6): p. S19-S28.
19. Leenhardt, L. and P. Grosclaude, *[Epidemiology of thyroid carcinoma over the world].* Ann Endocrinol (Paris), 2011. **72**(2): p. 136-48.
20. Davies, L., et al., *American Association of Clinical Endocrinologists and American College of Endocrinology Disease State Clinical Review: The Increasing Incidence of Thyroid Cancer.* Endocr Pract, 2015. **21**(6): p. 686-96.
21. Kuo, J.H., J.A. Chabot, and J.A. Lee, *Breast cancer in thyroid cancer survivors: An analysis of the Surveillance, Epidemiology, and End Results-9 database.* Surgery, 2016. **159**(1): p. 23-30.
22. Morris, L.G., et al., *The increasing incidence of thyroid cancer: the influence of access to care.* Thyroid, 2013. **23**(7): p. 885-91.
23. Pathak, K.A., et al., *The changing face of thyroid cancer in a population-based cohort.* Cancer Med, 2013. **2**(4): p. 537-44.
24. Pellegriti, G., et al., *Worldwide increasing incidence of thyroid cancer: update on epidemiology and risk factors.* J Cancer Epidemiol, 2013. **2013**: p. 965212.
25. Aschebrook-Kilfoy, B., et al., *The acceleration in papillary thyroid cancer incidence rates is similar among racial and ethnic groups in the United States.* Ann Surg Oncol, 2013. **20**(8): p. 2746-53.
26. Rahib, L., et al., *Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States.* Cancer Res, 2014. **74**(11): p. 2913-21.
27. Guay, B., et al., *Incidence of differentiated thyroid cancer by socioeconomic status and urban residence: Canada 1991-2006.* Thyroid, 2014. **24**(3): p. 552-5.
28. S Liu1, R.S., A-M Ugnat2 and Y Mao2, *Increasing thyroid cancer incidence in Canada, 1970–1996: time trends and age-period-cohort effects.* British Journal of Cancer, 2001.
29. Kent, W.D., et al., *Increased incidence of differentiated thyroid carcinoma and detection of subclinical disease.* CMAJ, 2007. **177**(11): p. 1357-61.
30. A. Shaw, M.R.S., MSc; L. Mery, MSc, *Thyroid cancer in Canada.* Chronic Diseases and Injuries in Canada, 2014. **34**.
31. Wilkins, L.F.E.a.K., *Canadian trends in cancer prevalence.* Statistics Canada, 2012.
32. Podda, M., et al., *Follicular thyroid carcinoma: differences in clinical relevance between minimally invasive and widely invasive tumors.* World Journal of Surgical Oncology, 2015. **13**: p. 193.
33. Ukrainski, M.B., E.A. Pribitkin, and J.L. Miller, *Increasing Incidence of Thyroid Nodules and Thyroid Cancer: Does Increased Detection of a Subclinical Reservoir Justify the Associated Anxiety and Treatment?* Clin Ther, 2015.
34. Udelsman, R. and Y. Zhang, *The Epidemic of Thyroid Cancer in the United States: The Role of Endocrinologists and Ultrasounds.* Thyroid, 2013. **24**(3): p. 472-479.
35. Bruel, A.V.d., et al., *Regional Variation in Thyroid Cancer Incidence in Belgium Is Associated With Variation in Thyroid Imaging and Thyroid Disease Management.* The Journal of Clinical Endocrinology & Metabolism, 2013. **98**(10): p. 4063-4071.

36. Zevallos, J.P., et al., *Increased thyroid cancer incidence corresponds to increased use of thyroid ultrasound and fine-needle aspiration: A study of the Veterans Affairs health care system.* Cancer, 2015. **121**(5): p. 741-746.
37. Smith-Bindman, R., D.L. Miglioretti, and E.B. Larson, *Rising use of diagnostic medical imaging in a large integrated health system.* Health Aff (Millwood), 2008. **27**(6): p. 1491-502.
38. Mazzaferri , E.L., *Management of a Solitary Thyroid Nodule.* New England Journal of Medicine, 1993. **328**(8): p. 553-559.
39. Davies, L. and H. Welch, *CUrrent thyroid cancer trends in the united states.* JAMA Otolaryngology–Head & Neck Surgery, 2014. **140**(4): p. 317-322.
40. Nagar, S., et al., *Age of diagnosing physician impacts the incidence of thyroid cancer in a population.* Cancer Causes & Control, 2014. **25**(12): p. 1627-1634.
41. Sun, G.H., S. DeMonner, and M.M. Davis, *Epidemiological and economic trends in inpatient and outpatient thyroidectomy in the United States, 1996-2006.* Thyroid, 2013. **23**(6): p. 727-33.
42. Li, N., et al., *Impact of enhanced detection on the increase in thyroid cancer incidence in the United States: review of incidence trends by socioeconomic status within the surveillance, epidemiology, and end results registry, 1980-2008.* Thyroid, 2013. **23**(1): p. 103-10.
43. Malone, M.K., et al., *Thyroid cancers detected by imaging are not necessarily small or early stage.* Thyroid, 2014. **24**(2): p. 314-8.
44. Marina N. Nikiforova, 2 James R. Stringer,3 Ruthann Blough,5 Mario Medvedovic,4 James A. Fagin,2 Yuri E. Nikiforov1*, *Proximity of Chromosomal Loci That Participate in Radiation-Induced Rearrangements in Human Cells.* Science, 2000. **290**(5489): p. 138-141.
45. Tipnis, S.V., et al., *Thyroid Doses and Risks to Adult Patients Undergoing Neck CT Examinations.* American Journal of Roentgenology, 2015. **204**(5): p. 1064-1068.
46. Mitsiades, N., et al., *Circulating Adiponectin Is Inversely Associated with Risk of Thyroid Cancer: In Vivo and in Vitro Studies.* The Journal of Clinical Endocrinology & Metabolism, 2011. **96**(12): p. E2023-E2028.
47. Pappa, T. and M. Alevizaki, *Obesity and thyroid cancer: a clinical update.* Thyroid, 2014. **24**(2): p. 190-9.
48. Scopa, C.D., *Histopathology of Thyroid Tumors. An Overview.* HORMONES, 2004.
49. CHRISTOPH HEDINGER, M., " E. DILLWYN WILLIAMS, MD,t AND LESLIE H. SOBIN, MD, *The WHO Histological Classification of Thyroid Tumors.* Cancer Causes & Control, 1989.
50. Hiroshi Katoh*, K.Y., Takumo Enomoto and Masahiko Watanabe, *Classification and General consideration of thyroid cancer.* Annals of Clinical Pathology, 2015.
51. Nikiforov, Y.E., *Thyroid Tumors-Classification, Staging, and General Considerations.*
52. LiVolsi, V.A., *Papillary thyroid carcinoma: an update.* Mod Pathol, 2011. **24 Suppl 2**: p. S1-9.
53. Pisanu, A., et al., *Risk Factors for Nodal Metastasis and Recurrence Among Patients with Papillary Thyroid Microcarcinoma: Differences in Clinical Relevance Between Nonincidental and Incidental Tumors.* World Journal of Surgery, 2009. **33**(3): p. 460-468.
54. Noguchi, S., et al., *Papillary microcarcinoma.* World J Surg, 2008. **32**(5): p. 747-53.

55. Yu, X.M., et al., *Follicular variant of papillary thyroid carcinoma is a unique clinical entity: a population-based study of 10,740 cases.* Thyroid, 2013. **23**(10): p. 1263-8.
56. Lang, B.H., et al., *Classical and follicular variant of papillary thyroid carcinoma: a comparative study on clinicopathologic features and long-term outcome.* World J Surg, 2006. **30**(5): p. 752-8.
57. Pillai, S., et al., *Diffuse sclerosing variant of papillary thyroid carcinoma—an update of its clinicopathological features and molecular biology.* Critical Reviews in Oncology / Hematology. **94**(1): p. 64-73.
58. Ghossein, R. and V.A. Livolsi, *Papillary thyroid carcinoma tall cell variant.* Thyroid, 2008. **18**(11): p. 1179-81.
59. Cherkaoui, G.S., et al., *Poorly differentiated thyroid carcinoma: a retrospective clinicopathological study.* Pan Afr Med J, 2015. **21**: p. 137.
60. Ibrahimpasic, T., et al., *Outcomes in patients with poorly differentiated thyroid carcinoma.* J Clin Endocrinol Metab, 2014. **99**(4): p. 1245-52.
61. Wells, S.A., Jr., et al., *Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma.* Thyroid, 2015. **25**(6): p. 567-610.
62. Desai, S.S., S. Sarkar, and A.M. Borges, *A study of histopathological features of medullary carcinoma of the thyroid: cases from a single institute in India.* Indian J Cancer, 2005. **42**(1): p. 25-9.
63. Kebebew, E., et al., *Medullary thyroid carcinoma: clinical characteristics, treatment, prognostic factors, and a comparison of staging systems.* Cancer, 2000. **88**(5): p. 1139-48.
64. Dottorini, M.E., et al., *Multivariate analysis of patients with medullary thyroid carcinoma. Prognostic significance and impact on treatment of clinical and pathologic variables.* Cancer, 1996. **77**(8): p. 1556-65.
65. Machens, A., et al., *Abnormal carcinoembryonic antigen levels and medullary thyroid cancer progression: a multivariate analysis.* Arch Surg, 2007. **142**(3): p. 289-93; discussion 294.
66. Hirokawa, M., et al., *Histopathological analysis of anaplastic thyroid carcinoma cases with long-term survival: A report from the Anaplastic Thyroid Carcinoma Research Consortium of Japan.* Endocr J, 2016.
67. Nagaiah, G., et al., *Anaplastic thyroid cancer: a review of epidemiology, pathogenesis, and treatment.* J Oncol, 2011. **2011**: p. 542358.
68. Ricarte-Filho, J.C., et al., *Mutational profile of advanced primary and metastatic radioactive iodine-refractory thyroid cancers reveals distinct pathogenetic roles for BRAF, PIK3CA, and AKT1.* Cancer Res, 2009. **69**(11): p. 4885-93.
69. McIver, B., et al., *Anaplastic thyroid carcinoma: a 50-year experience at a single institution.* Surgery, 2001. **130**(6): p. 1028-34.
70. Carcangiu, M.L., et al., *Anaplastic thyroid carcinoma. A study of 70 cases.* Am J Clin Pathol, 1985. **83**(2): p. 135-58.
71. *AJCC Cancer Staging Manual*. 2002, Springer.
72. *Thyroid Cancer Detailed Guide*, ed. A.C. Society. 2014.
73. Kim, J.G., *Molecular pathogenesis and targeted therapies in well-differentiated thyroid carcinoma.* Endocrinol Metab (Seoul), 2014. **29**(3): p. 211-6.
74. Xing, M., *Molecular pathogenesis and mechanisms of thyroid cancer.* Nature Reviews Cancer, 2013. **13**(3): p. 184-199.

75. Shah, D.A.L.a.M.H., *Thyroid cancer: pathogenesis and targeted therapy.* Therapeutic Advances in Endocrinology and Metabolism, 2011.
76. Bounacer, A., et al., *High prevalence of activating ret proto-oncogene rearrangements, in thyroid tumors from patients who had received external radiation.* Oncogene, 1997. **15**(11): p. 1263-73.
77. Kroll, T.G., et al., *PAX8-PPARgamma1 fusion oncogene in human thyroid carcinoma [corrected].* Science, 2000. **289**(5483): p. 1357-60.
78. Almeida, M.Q. and A.O. Hoff, *Recent advances in the molecular pathogenesis and targeted therapies of medullary thyroid carcinoma.* Curr Opin Oncol, 2012. **24**(3): p. 229-34.
79. Smallridge, R.C. and J.A. Copland, *Anaplastic thyroid carcinoma: pathogenesis and emerging therapies.* Clin Oncol (R Coll Radiol), 2010. **22**(6): p. 486-97.
80. Haugen, B.R., et al., *2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer.* Thyroid, 2016. **26**(1): p. 1-133.
81. Shimamoto, K., et al., *Preoperative staging of thyroid papillary carcinoma with ultrasonography.* Eur J Radiol, 1998. **29**(1): p. 4-10.
82. Kouvaraki, M.A., et al., *Role of preoperative ultrasonography in the surgical management of patients with thyroid cancer.* Surgery, 2003. **134**(6): p. 946-54; discussion 954-5.
83. John M. Stulak, M.C.S.G., MD; David R. Farley, MD; Geoffrey B. Thompson, MD; and M.I.D.H. Jon A. van Heerden, MD; Carl C. Reading, MD; J. William Charboneau, *Value of Preoperative Ultrasonography in the Surgical Management of Initial and Reoperative Papillary Thyroid Cancer.* ARCH SURG, 2006.
84. Leboulleux, S., et al., *Ultrasound Criteria of Malignancy for Cervical Lymph Nodes in Patients Followed Up for Differentiated Thyroid Cancer.* The Journal of Clinical Endocrinology & Metabolism, 2007. **92**(9): p. 3590-3594.
85. Lew, J.I., S.E. Rodgers, and C.C. Solorzano, *Developments in the use of ultrasound for thyroid cancer.* Curr Opin Oncol, 2010. **22**(1): p. 11-6.
86. Chung, J., et al., *Optimal indication of thyroglobulin measurement in fine-needle aspiration for detecting lateral metastatic lymph nodes in patients with papillary thyroid carcinoma.* Head Neck, 2014. **36**(6): p. 795-801.
87. Kuna, S.K., et al., *Ultrasonographic differentiation of benign from malignant neck lymphadenopathy in thyroid cancer.* J Ultrasound Med, 2006. **25**(12): p. 1531-7; quiz 1538-40.
88. Wong, K.T. and A.T. Ahuja, *Ultrasound of thyroid cancer.* Cancer Imaging, 2005. **5**: p. 157-66.
89. Nguyen, G.K., et al., *Fine-needle aspiration of the thyroid: an overview.* Cytojournal, 2005. **2**(1): p. 12.
90. Snozek, C.L., et al., *Serum thyroglobulin, high-resolution ultrasound, and lymph node thyroglobulin in diagnosis of differentiated thyroid carcinoma nodal metastases.* J Clin Endocrinol Metab, 2007. **92**(11): p. 4278-81.
91. Ahn, J., et al., *Diagnostic Accuracy of CT and Ultrasonography for Evaluating Metastatic Cervical Lymph Nodes in Patients with Thyroid Cancer.* World Journal of Surgery, 2008. **32**(7): p. 1552-1558.

92. Lesnik, D., et al., *Papillary thyroid carcinoma nodal surgery directed by a preoperative radiographic map utilizing CT scan and ultrasound in all primary and reoperative patients.* Head Neck, 2014. **36**(2): p. 191-202.
93. Hwang, H.S. and L.A. Orloff, *Efficacy of preoperative neck ultrasound in the detection of cervical lymph node metastasis from thyroid cancer.* Laryngoscope, 2011. **121**(3): p. 487-91.
94. AlNoury, M.K., et al., *Preoperative imaging modalities to predict the risk of regional nodal recurrence in well-differentiated thyroid cancers.* Int Arch Otorhinolaryngol, 2015. **19**(2): p. 116-20.
95. Kim, D.W., et al., *Sonographic features of cervical lymph nodes after thyroidectomy for papillary thyroid carcinoma.* J Ultrasound Med, 2013. **32**(7): p. 1173-80.
96. Na'ara, S., et al., *Contemporary Management of Recurrent Nodal Disease in Differentiated Thyroid Carcinoma.* Rambam Maimonides Med J, 2016. **7**(1).
97. Conzo, G., et al., *Predictive value of nodal metastases on local recurrence in the management of differentiated thyroid cancer. Retrospective clinical study.* BMC Surg, 2013. **13 Suppl 2**: p. S3.
98. Glover, A.R., et al., *The pros and cons of prophylactic central neck dissection in papillary thyroid carcinoma.* Gland Surg, 2013. **2**(4): p. 196-205.
99. Barczynski, M., et al., *Prophylactic central neck dissection for papillary thyroid cancer.* Br J Surg, 2013. **100**(3): p. 410-8.
100. Hennessy, M. and D. Goldenberg, *The Role of Prophylactic Central Neck Dissection in the Treatment of Differentiated Thyroid Cancer.* Rambam Maimonides Medical Journal, 2016. **7**(1): p. e0007.
101. Sippel, R.S., M. Kunnimalaiyaan, and H. Chen, *Current management of medullary thyroid cancer.* Oncologist, 2008. **13**(5): p. 539-47.
102. Tuttle, R.M. and I. Ganly, *Risk stratification in medullary thyroid cancer: Moving beyond static anatomic staging.* Oral Oncology, 2013. **49**(7): p. 695-701.
103. Wang, L.Y., et al., *Preoperative neck ultrasound in clinical node-negative differentiated thyroid cancer.* J Clin Endocrinol Metab, 2014. **99**(10): p. 3686-93.
104. Cisco, R.M., W.T. Shen, and J.E. Gosnell, *Extent of surgery for papillary thyroid cancer: preoperative imaging and role of prophylactic and therapeutic neck dissection.* Curr Treat Options Oncol, 2012. **13**(1): p. 1-10.
105. Li, Q.S., et al., *Papillary thyroid carcinoma on sonography.* Clin Imaging, 2010. **34**(2): p. 121-6.
106. Iyer, N.G. and A.R. Shaha, *Management of thyroid nodules and surgery for differentiated thyroid cancer.* Clin Oncol (R Coll Radiol), 2010. **22**(6): p. 405-12.
107. Yoon Se Lee, Y.-S.L., Jin-Choon Lee, Soo-Geun Wang, Seok-Man Son, Sang-Soo Kim, In-Ju Kim, Byung-Joo Lee, *Ultrasonographic findings relating to lymph node metastasis in single micropapillary thyroid cancer.*
108. Giacomini, C.P., R.B. Jeffrey, and L.K. Shin, *Ultrasonographic evaluation of malignant and normal cervical lymph nodes.* Semin Ultrasound CT MR, 2013. **34**(3): p. 236-47.
109. Choi, Y.J., et al., *Clinical and imaging assessment of cervical lymph node metastasis in papillary thyroid carcinomas.* World J Surg, 2010. **34**(7): p. 1494-9.

110. Kim, K.E., et al., *Preoperative prediction of central lymph node metastasis in thyroid papillary microcarcinoma using clinicopathologic and sonographic features.* World J Surg, 2013. **37**(2): p. 385-91.
111. Conzo, G., et al., *Clinicopathological pattern of lymph node recurrence of papillary thyroid cancer. Implications for surgery.* Int J Surg, 2014. **12 Suppl 1**: p. S194-7.
112. Lee, D.W., et al., *Roles of ultrasonography and computed tomography in the surgical management of cervical lymph node metastases in papillary thyroid carcinoma.* Eur J Surg Oncol, 2013. **39**(2): p. 191-6.
113. Sakorafas, G.H., D. Sampanis, and M. Safioleas, *Cervical lymph node dissection in papillary thyroid cancer: current trends, persisting controversies, and unclarified uncertainties.* Surg Oncol, 2010. **19**(2): p. e57-70.
114. Pedro Weslley Souza Rosário, P., Sérgio de Faria, MD,, et al., *Ultrasonographic Differentiation Between Metastatic and Benign Lymph Nodes in Patients With Papillary Thyroid Carcinoma.* J Ultrasound Med, 2005.
115. Langer, J.E. and S.J. Mandel, *Sonographic imaging of cervical lymph nodes in patients with thyroid cancer.* Neuroimaging Clin N Am, 2008. **18**(3): p. 479-89, vii-viii.
116. King, A.D., *Imaging for staging and management of thyroid cancer.* Cancer Imaging, 2008. **8**(1): p. 57-69.
117. Na, D.K., et al., *Evaluation of cervical lymph node metastasis in thyroid cancer patients using real-time CT-navigated ultrasonography: preliminary study.* Ultrasonography, 2015. **34**(1): p. 39-44.
118. Choi, J.S., et al., *Staging of papillary thyroid carcinoma with ultrasonography: performance in a large series.* Ann Surg Oncol, 2011. **18**(13): p. 3572-8.
119. Machens, A., S. Hauptmann, and H. Dralle, *Lymph node dissection in the lateral neck for completion in central node-positive papillary thyroid cancer.* Surgery, 2009. **145**(2): p. 176-81.
120. Sugitani, I., et al., *Prospective outcomes of selective lymph node dissection for papillary thyroid carcinoma based on preoperative ultrasonography.* World J Surg, 2008. **32**(11): p. 2494-502.
121. Lew, J.I. and C.C. Solorzano, *Use of ultrasound in the management of thyroid cancer.* Oncologist, 2010. **15**(3): p. 253-8.
122. Shuzhen, C., *Comparison analysis between conventional ultrasonography and ultrasound elastography of thyroid nodules.* Eur J Radiol, 2012. **81**(8): p. 1806-11.
123. Lewis K. Shin, M., Eric W. Olcott, MD, R. Brooke Jeffrey, MD, and Terry S. Desser, MD, *Sonographic Evaluation of Cervical Lymph Nodes in Papillary Thyroid Cancer.* Ultrasound Quarterly, 2013. **29**.
124. Binyousef, H.M., et al., *Preoperative neck ultrasonographic mapping for persistent/recurrent papillary thyroid cancer.* World J Surg, 2004. **28**(11): p. 1110-4.
125. Lim, Y.S., et al., *Lateral cervical lymph node metastases from papillary thyroid carcinoma: predictive factors of nodal metastasis.* Surgery, 2011. **150**(1): p. 116-21.
126. Udelsman, R., *Treatment of persistent or recurrent papillary carcinoma of the thyroid--the good, the bad, and the unknown.* J Clin Endocrinol Metab, 2010. **95**(5): p. 2061-3.
127. McCoy, K.L., et al., *Same-day ultrasound guidance in reoperation for locally recurrent papillary thyroid cancer.* Surgery, 2007. **142**(6): p. 965-72.
128. Nixon, I.J., et al., *The impact of nodal status on outcome in older patients with papillary thyroid cancer.* Surgery, 2014. **156**(1): p. 137-46.

129. Wu, L.M., et al., *The accuracy of ultrasonography in the preoperative diagnosis of cervical lymph node metastasis in patients with papillary thyroid carcinoma: A meta-analysis.* Eur J Radiol, 2012. **81**(8): p. 1798-805.
130. Leboulleux, S., et al., *Prognostic factors for persistent or recurrent disease of papillary thyroid carcinoma with neck lymph node metastases and/or tumor extension beyond the thyroid capsule at initial diagnosis.* J Clin Endocrinol Metab, 2005. **90**(10): p. 5723-9.
131. Yasuhiro ITO, M.F., Chisato TOMODA, Hiroyuki INOUE, Minoru KIHARA,, T.U. Takuya HIGASHIYAMA, Yuuki TAKAMURA, Akihiro MIYA, Kaoru KOBAYASHI,, and F.M.a.A. MIYAUCH, *Prognosis of Patients with Papillary Thyroid Carcinoma Having Clinically Apparent Metastasis to the Lateral Compartment.* Endocrine Journal, 2009.