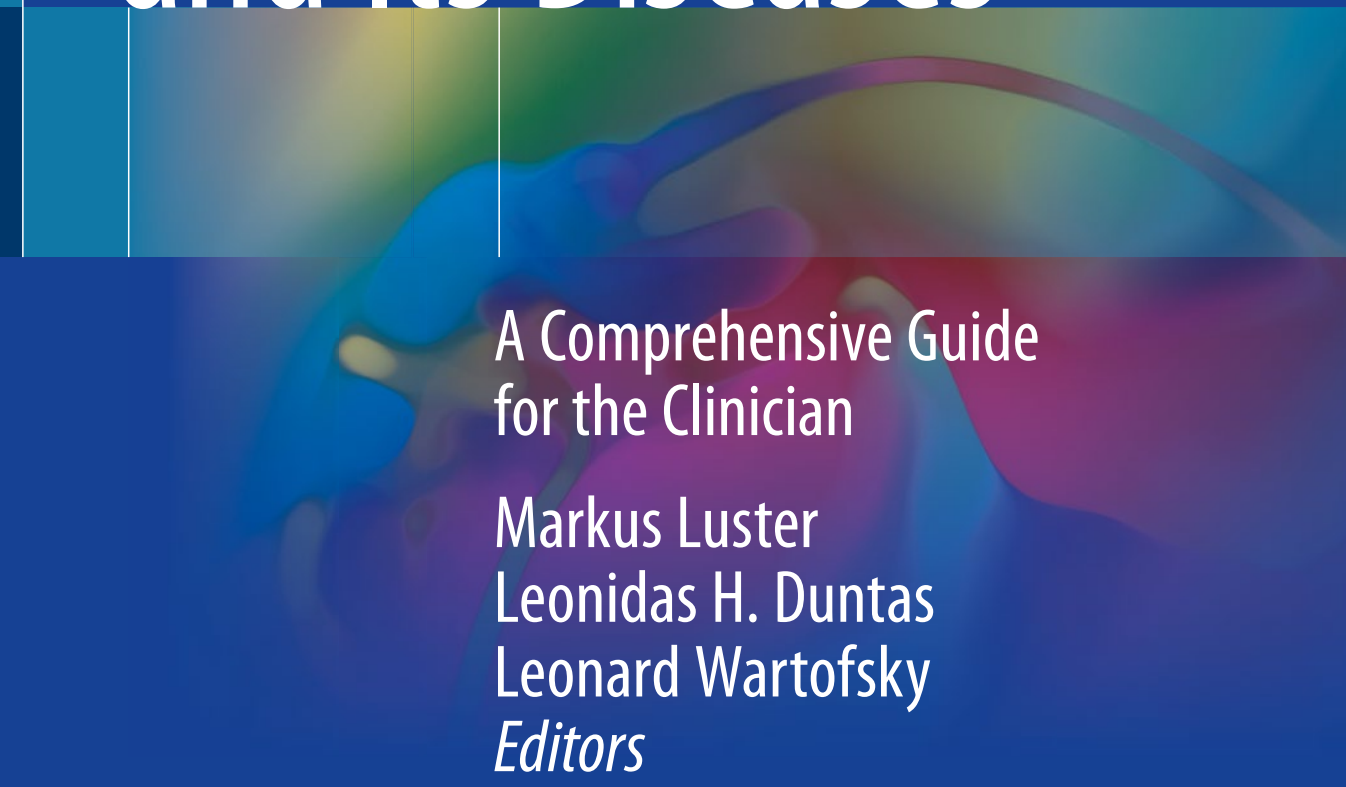
The Thyroid and Its Diseases



A Comprehensive Guide for the Clinician

Markus Luster
Leonidas H. Duntas
Leonard Wartofsky
Editors

Springer

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Part I

Introductory Section

Anatomy and Physiology of the Thyroid Gland

Nikolaos Stathatos

The thyroid gland is located in the lower part of the anterior neck, at the level of the second to third tracheal rings, inferior to the larynx. It is a shield-shaped organ, named after the thyroid cartilage of the trachea that was described as "shield-like" ("thyreos" is the Greek name for shield) [1]. It is made of two lobes, each laying on the corresponding side (right and left) of the tracheal wall. They are connected with a thin strip of thyroid tissue that extends across the anterior surface of the trachea called the isthmus (Fig. 1). Each lobe is about 3–4 cm long, 2 cm wide, and, in most cases, a few millimeters (mm) thick. The isthmus is usually only a few millimeters thick and up to 15 mm in height. Occasionally, an elongated, finger-like structure is also present that extends from the isthmus superiorly, called the pyramidal lobe. This represents the remnant of the thyroglossal duct, a structure that is formed at the time of the thyroid embryogenesis, the formation of the thyroid gland in the first trimester of pregnancy. The primitive thyroid gland (also called the thyroid enlarge) first appears during the fourth week of gestation in the floor of the primitive pharynx (at the level of the base of the tongue) [2]. There is however more recent evidence that suggests that molecular events critical for the development of the thyroid gland take place before the development of the thyroid enlarge: the expression of key transcription factors the presence of which is critical, such as the NK2 homeobox [Nkx2]-1 and the paired box [Pax]-8 [2]. Although the exact molecular pathways necessary for the proper development of the thyroid gland remain largely unknown, it is becoming more and more clear that defects during this very complex process can lead to problems from thyroid dysgenesis [3] to syndromes of reduced thyroid hormone sensitivity [4]. As embryonic development progresses, the thyroid gland descends inferiorly into the neck while retaining its connection to the tongue with a small duct. This is the thyroglossal duct mentioned above. The gland reaches its final location by week 7. In most cases, the thyroglossal duct has disappeared completely by week 10. There are however several variations to this sequence of events: As stated above, the pyramidal lobe forms at the location of the inferior most part of the thyroglossal duct, usually attached to the isthmus of the thyroid gland. This is considered a normal variation and requires no intervention. A much less frequent variation is that the thyroglossal duct remains present and patent. In such cases, the formation of cysts along the path of this duct is a common occurrence, most commonly at the level of the hyoid bone [5]. Most are asymptomatic and up to one third diagnosed later during the adult life of a patient. These represent the most

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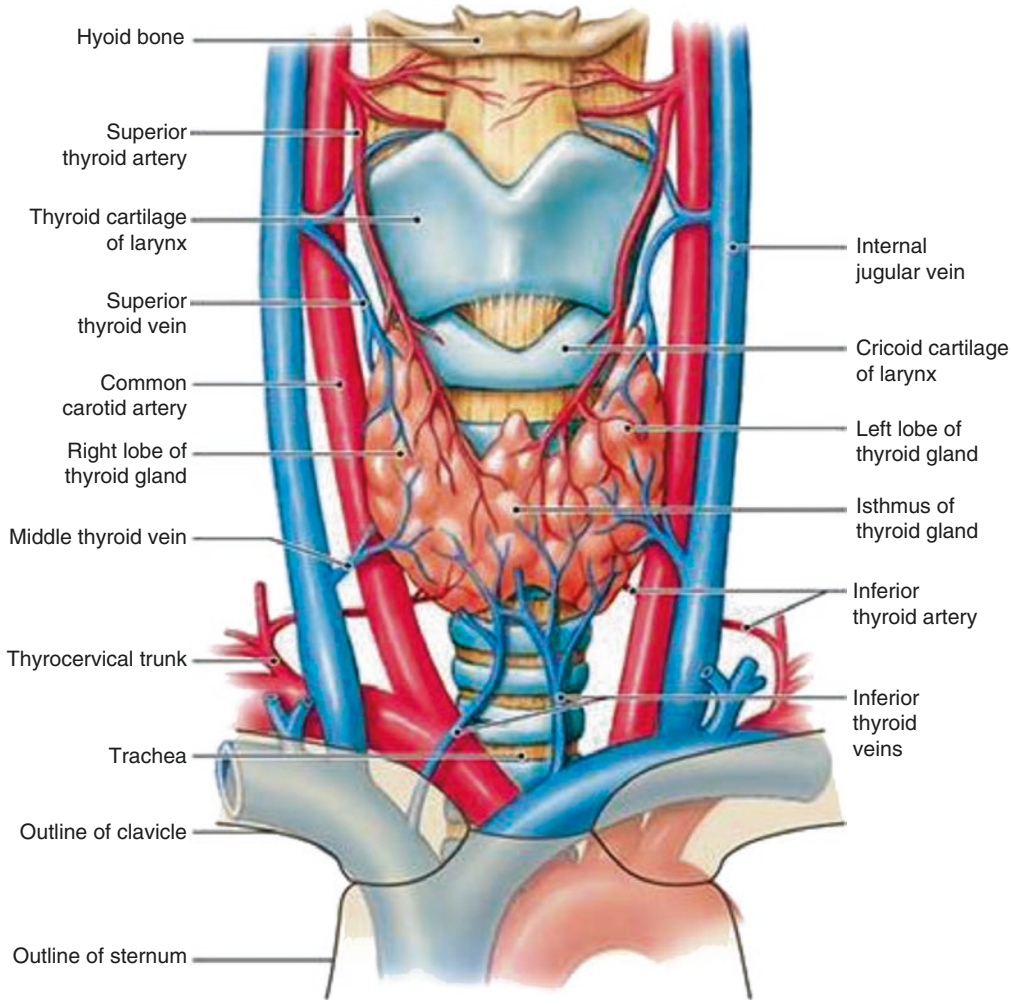

Fig. 1 Anatomy of the thyroid gland. Martini FH, Nath JL, Bartholomew EF. Fundamentals of Anatomy and Physiology, 10th ed. ©2015. Reprinted by permission of Pearson Education, Inc., New York, New York

common cystic lesions found in the neck, and because they are associated with thyroid malignancies [6], they should be carefully evaluated. More rare developmental events include a lingual thyroid, hemiagenesis or complete agenesis of the thyroid gland. A lingual thyroid is formed when the thyroid does not descent into the neck at all and remains at the base of the tongue [7]. It is often noted as a mass at the base of the tongue. In cases of thyroid agenesis, the thyroid gland does not form at all. In cases of hemiagenesis, only half or some part of the thyroid forms [3]. These conditions are very important to diagnose early because they are associated with thyroid dysfunction very early in life (often noted during neonatal screening for thyroid disorders). Recently, attempts to identify potential genetic etiologies have been undertaken but with limited success so far [8].

Traditionally, thyroid cells were thought to have a monoclonal origin, all originating from the same precursor cell. A very interesting concept has arisen recently, that of a polyclonal origin of thyroid hormone producing cells within the thyroid gland, possibly even with different malignant potential [9, 10].

Another embryonic structure incorporated into the thyroid gland during embryonic

development is the ultimobranchial body (itself derived from the brachial pouches). Cells of neural crest origin migrate into this structure prior to its incorporation into the thyroid gland. These are the cells that will ultimately produce calcitonin (also called parafollicular or C-cells). Because of the way the primitive thyroid and the ultimobranchial body interact with each other during this critical developmental process, the result is an uneven distribution of the calcitonin producing cells in the fully developed thyroid gland. They are restricted to the middle and upper third of the gland, while the poles and the isthmus are devoid of these cells. That way is very rare to find tumors originating from the C-cells in these parts of the thyroid gland.

The location of the thyroid gland has several significant clinical implications. Examples include that nodules located posteriorly and medially, near the wall of the trachea may more difficult to palpate and as a result can be more easily missed. Also, tumors that originate there are more difficult to detect and may invade surrounding structures like the trachea and/or esophagus prior to detection.

Several critical structures are located around the thyroid gland. The parathyroid glands are small glands (usually a few milligrams in weight) that are located posterior to each thyroid pole (upper and lower, right and left). Although they have no functional relationship with the thyroid gland, their close anatomical relationship has very important clinical implications. Their proper identification is critical at the time of thyroid surgery, because their accidental removal will result in permanent hypoparathyroidism, a condition with life-long morbidity and potentially even mortality.

Also located along the posterior aspect of the thyroid gland are the two recurrent laryngeal nerves, innervating the muscles of the vocal cords. Injury to these nerves at the time of thyroid surgery can result in a hoarse voice (if unilateral) to stridor and the need for a permanent tracheostomy (if bilateral). It is very important for a surgeon to be intimately familiar with the local anatomy as well as the many variations that have been described so that the possibility of a surgical complication is reduced to an absolute minimum. These nerves originate from the vagus nerve on each side at the level of the aortic arch. They run superiorly along the tracheoesophageal groove, posteriorly to the thyroid gland, although several variations have been described [11]. They are located close to the inferior thyroid artery but can be found posteriorly, anteriorly, or even in between the branches of the blood vessel. This close relationship of these nerves to the thyroid gland and its blood supply requires that the surgeon pays very close attention to identifying them through careful dissection in order to avoid damaging them. Recently, guidelines have been published to assist with the surgical management of thyroid nodules and cancer [12].

The blood supply of the thyroid gland comes from two arteries on each side: the superior thyroid arteries originate from the external branch of the carotid artery. Accompanied by the superior laryngeal nerve, they enter into the upper poles of the thyroid gland. Due to the close proximity of these blood vessels to the superior laryngeal nerves, it is usually recommended that the surgeon ligates the superior thyroid arteries as close to the thyroid gland as possible in order to avoid damaging the nerves. The inferior thyroid arteries are branches of the thyrocervical trunk and, as stated above, are in close proximity to the recurrent laryngeal nerves. Another artery called thyroidea ima artery occasionally provides blood supply to the thyroid gland. This can originate from either the thyrocervical trunk or the aortic arch. The superior, middle, and inferior thyroid veins provide the venous drainage of the thyroid gland. The superior and middle veins drain into the internal branch of the jugular vein, while the inferior thyroid veins anastomose with each other anteriorly to the trachea and drain into the brachiocephalic vein. The lymphatics of the thyroid gland drain into the lymph nodes of the central cervical (level VI) compartment. The paratracheal nodes are also involved in drainage of the thyroid gland. This is very important because it is these nodes that are the first to be involved in the metastatic process of many thyroid malignancies and need to be removed at the time of surgery.

As stated above, because of embryonic development of the thyroid gland, thyroid tissue can be found anywhere along the path of the thyroglossal duct, from the base of the tongue to the normal location of the adult thyroid gland. However, thyroid tissue has been described in many ectopic locations in the human body, including the carotid bifurcation [13], intracardiac [14, 15], the ascending aorta [16], the gallbladder [17, 18], the porta hepatis [19], intramesenteric [20], and the ovaries [21]. Thyroid cancer has also been described in many of these sites [22], although it is not clear if these represent a primary or metastatic focus.

The thyroid follicle is the characteristic histologic structure of the gland (Fig. 2). It consists of a single layer of thyroid cells ("thyrocytes"), surrounded by a basement membrane. The follicle is filled with a viscous honey-like fluid called colloid. It contains thyroglobulin, a very large glycoprotein (660 KDa) that represents the precursor and storage form of thyroid hormone. The size of the follicles can vary significantly, but their average size is about 200 μm. There are fibrous septa that separate follicles (usually into groups 20–40). The calcitonin producing C-cells are located between the thyroid follicles, usually in small groups in the middle and upper one third of the thyroid parenchyma.

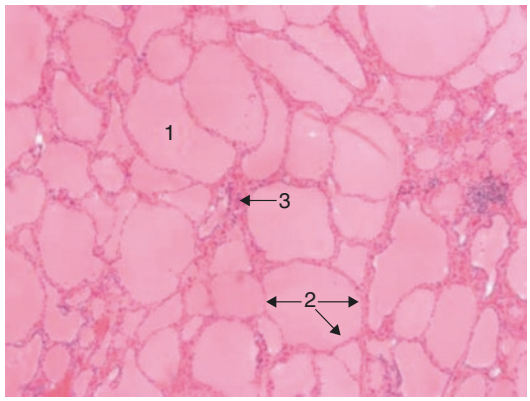

Fig. 2 Thyroid histology: (*1*) Thyroid follicle. (*2*) Follicular cells. (*3*) Parafollicular (C-) cells

Thyroid Physiology

The thyroid gland plays a critical role in the regulation of multiple bodily functions such as the metabolic rate, energy expenditure, and the function of organs like the heart and the brain. Levothyroxine is the main product of the thyroid gland, also known simply as thyroid hormone. It is the product of two tyrosine molecules, each carrying two atoms of iodine. In order to be able to synthesize adequate amounts of thyroid hormone, thyroid cells have developed complex mechanism to incorporate, concentrate, and store iodine from the circulation, even against a concentration gradient. A dedicated transporter, the sodium (Na)-iodine symporter (NIS), is located in the basal membrane of these cells (Fig. 3). Its function is to actively (at the expense of energy in the form of adenosine triphosphate—ATP) transport inorganic iodide present in the circulation inside the thyroid cells. This results in an iodine concentration inside these cells that is 20–40 times that of the serum iodine level. This symporter can also be found in the epithelial cells of salivary glands. This is significant because when patients with thyroid cancer are treated with radioactive iodine, this can concentrate inside these glands and in some cases cause radiation sialadenitis and even xerostomia. This symporter has recently become the focus of intense study in cases of poorly differentiated thyroid cancers. These cancers often do not express the sodium iodine symporter, or the symporter is not present in the basal membrane of these so that it can be functional. As a result, these cancer cells lose the ability to concentrate the radioactive iodine given to treat them, a step that is absolutely critical for the radioactive iodine to be effective. Several clinical trials are currently under way with pharmaceutical molecules targeting specific intracellular targets (e.g., MEK or BRAF), some with promising results, showing increased expression or functionality of the symporter and increased concentration of radioactive iodine in these cells [23].

Once the iodine enters the thyroid cells, it is organified; it is incorporated onto tyrosine residues present in the amino acid sequence of

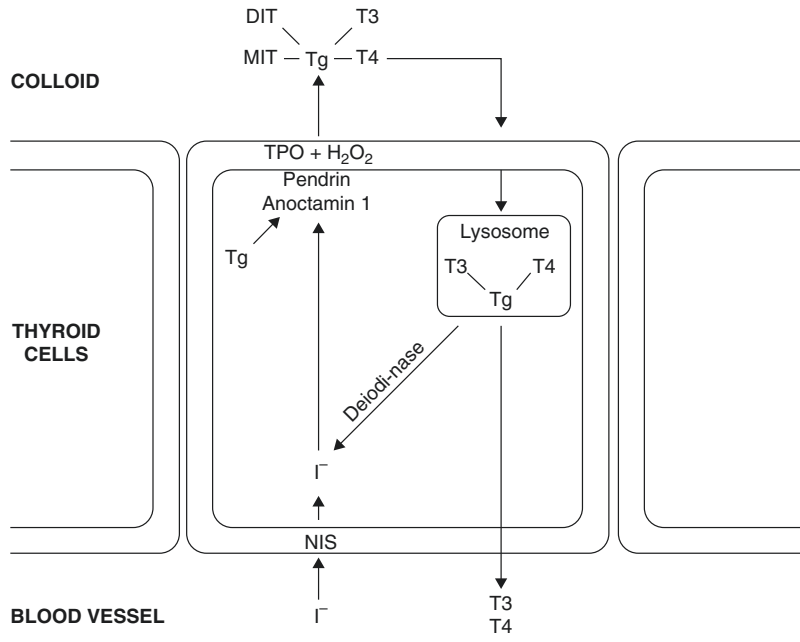

Fig. 3 Thyroid cell physiology, organification of inorganic iodide concentrated from the circulation: *NIS* sodium (Na)-iodide symporter, *TPO* thyroid peroxidase, *Tg* thyroglobulin, *DIT* diiodothyronine, *MIT* monoiodothyronine

thyroglobulin. This is accomplished at the apical membrane of the thyroid cell, with multiple key enzymes playing a critical role, such as the thyroid peroxidase (TPO) that requires the presence of hydrogen peroxide, pendrin, and possibly the calcium-activated anion membrane channel anoctamin 1 [24] that has been most recently associated with apical iodine efflux. Each thyroid hormone molecule can take up to four iodine atoms, forming the various forms of thyroid hormone. Once formed, thyroid hormone is stored in the colloid as part of the structure of thyroglobulin (Fig. 3).

When thyroid cells are stimulated by TSH, thyroglobulin enters the thyroid cell from the colloid (pinocytosis) where it is broken down in lysosomes, cleaved by endopeptidases, so that thyroid hormone that is incorporated into its amino acid structure can be released into the circulation, mostly in the form of levothyroxine. About 90% (75–100 μg/day) of the thyroid gland output is in the form of levothyroxine and about 10% (6 μg) in the form of triiodothyronine.

The thyroid peroxidase enzyme is the target of the immune system in cases of thyroid autoimmune disease, and detection of antibodies against this enzyme (anti-TPO antibodies) in the serum of patients is diagnostic of this process. It is however important to note here that it remains unclear if these antibodies are the cause or simply the result of the autoimmune process. A mutation of the other enzyme necessary for the organification of iodine, pendrin, results in a rare genetic disease called Pendred disease, manifested clinically by sensorineural deafness, a goiter, and hypothyroidism. Finally, thyroglobulin, which is also present in the circulation under normal circumstances, is often the target of the immune system. Up to 10% of the population with no evidence of thyroid disease have detectable antithyroglobulin antibodies in their blood. This is thought to be related to the extensive glycosylation of the thyroglobulin residues [25]. However, the clinical significance (if any) of these antibodies remains largely unknown, although they have been associated with increased risk of pregnancy loss [26]. The presence of these antibodies in the serum does make the measurement of serum thyroglobulin unreliable, which is often a significant limitation for some patients with thyroid cancer.

It is critical for the clinician that manages patients with thyroid diseases to have an in-depth understanding the role of iodine in thyroid

physiology. As discussed above, iodine plays a central role in the function of the thyroid gland. Iodine deficiency is a well-known cause of goiters and hypothyroidism, a finding that has led many countries in adopting iodine supplementation policies, such as fortifying salt. If, however, iodine-deficient patients are given iodine, their thyroid gland can produce large amounts of thyroid hormone, often in excess, causing hyperthyroidism. This is called the "Jod-Basedow" effect [27]. The rapid iodination of thyroglobulin that contains little iodine, mostly in the setting of a "toxic or hot" nodule or underlying Graves' disease is thought to be the potential cause although this remains to be proven.

On the other hand, the presence of excess amounts of iodine in the thyrocytes can inhibit the process of iodine uptake and organification (by inhibiting the NIS and TPO) and result in hypothyroidism. This is called the "Wolff-Chaikoff" phenomenon [28, 29]. This phenomenon is most often thought to take place in thyroid glands that are iodine sufficient. This inhibition is however temporary: the result of the Wolff-Chaikoff effect is the depletion of intracellular iodine that allows for the reset of the organification mechanism and synthesis of new thyroid hormone. This is called the "escape from the Wolff-Chaikoff effect."

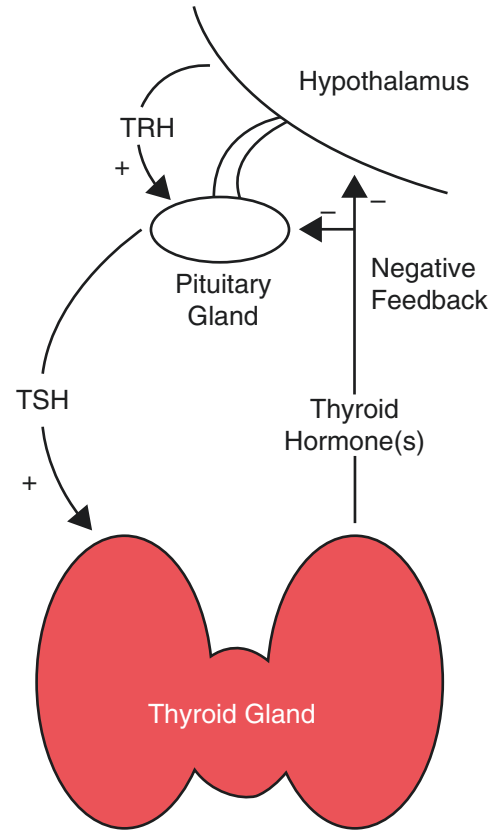

Fig. 4 Hypothalamic-pituitary-thyroid axis: negative feedback loop

Hypothalamic-Pituitary-Thyroid Axis

TSH (thyroid-stimulating hormone or thyrotropin) is the main regulator of thyroid function (Fig. 4). It is a peptide hormone produced in the anterior pituitary gland (adenohypophysis). TSH is in turn under the regulation of both TRH (thyrotropin-releasing hormone) that is produced in the hypothalamus and the circulating levels of thyroid hormone. It is actually sensitive to small changes on the levels of peripheral thyroid hormone. This is a classical feedback loop, frequently described in various endocrine axes. Thyroid hormone inhibits TRH production. This leads to decreased TSH synthesis and release from the pituitary gland and, in turn, decreased stimulation of the thyroid gland to uptake iodine and synthesize and release thyroid hormone. It has been shown that measuring serum TSH is the most sensitive method of assessing thyroid function and diagnosing most functional thyroid problems, like hypo- and hyperthyroidism [30], the rare exception being pituitary or hypothalamic diseases which represent more challenging forms of thyroid axis pathology [31, 32].

Peripheral Actions of Thyroid Hormone

Thyroxine (T4) and triiodothyronine (T3) are the two main forms of circulating thyroid hormone. They are mostly bound to various serum protein, the three major being thyroid-binding globulin (TBG), transthyretin, and albumin

[33]. Thyroid hormone has also been shown to bind to thyroid hormone autoantibodies [34] and lipoproteins [35]. These carrier proteins serve to both carry large amounts of thyroid hormones that are readily available when needed as well as to protect the different tissues from exposure to excess amounts of free thyroid hormones. Genetic defects of these carrier proteins can lead to altered binding of thyroid hormone and "abnormal" thyroid function tests [36]. It is very important for these conditions to be suspected and diagnosed early because patients are clinically euthyroid and require no treatment. One such example is a condition called familial dysalbuminemic hyperthyroxinemia. This is the result of a mutation of albumin that results in a dramatic 60-fold increase in the affinity of this protein for thyroid hormone. Carriers of such a mutation have an elevated level of serum thyroxine but a normal TSH and are clinically euthyroid [36].

Traditionally, thyroid hormone was thought to enter the target cells by simple diffusion through the cell membrane. However, several transporting proteins have been identified recently. These include the monocarboxylate transporter 8 (MCT8), MCT10, the organic anion-transporting polypeptide 1C1 (OATP1C1) [37], and the L-type amino acid transporters LAT1 and LAT2 [38]. The clinical significance of these transporters has been clearly shown because syndromes of reduced thyroid hormone sensitivity have been described in patients carrying mutations in one of these proteins [39].

As stated above, thyroxine is the main metabolic product of the thyroid gland, carrying four atoms of iodine. This is however thought of as a pre-hormone, which has to be metabolized to the active triiodothyronine, carrying three atoms of iodine in specific locations, which is the active hormone exerting all the peripheral effects of thyroid hormone. The conversion of T4 to T3 is a peripheral event that does not take place in the thyroid gland. This is achieved with the help of a selenium-containing enzyme called deiodinase. There is a total of three isoforms of the deiodinase enzyme: types 1, 2, and 3, each with apparently different functions.

Type 1 deiodinase (D1) has been described as a scavenger enzyme, given its higher affinity for reverse T3 (rT3). This is an inactive form of thyroid hormone, although there is some evidence to suggest it may actually be biologically active [40]. D1 has also more recently been shown to play a role in the synthesis of thyronamines, a class of endogenous compounds that seem to play a significant role on the actions of thyroid hormone [41], even with potential therapeutic possibilities, as in cases of acute stroke by inducing transient hypothermia [42].

The main deiodinase enzyme responsible for the conversion of T4 to the active T3 is the type 2 isoenzyme (D2) [43]. It also provides critical regulation of intracellular T3 actions by regulating the availability of its nuclear receptors.

Finally, D3 catalyzes the conversion of T4 and T3 to the inactive rT3 and to 3, 3′-diiodothyronine, which is also metabolically inactive. It seems to represent the physiologic inactivator of thyroid hormones [43]. Of interest, a very rare condition called consumptive hypothyroidism has been described where a hepatic hemangioma overexpresses D3. The result is a state of hypothyroidism because of excessive deactivation of T3 and T4 [44].

Thyroid hormone exerts its peripheral effects through both genomic and non-genomic pathways. Traditionally, they were thought to act by binding and activating specific nuclear receptors that in turn alter the expression of target genes (genomic actions of thyroid hormone). Two main groups of receptors have been identified so far: Thyroid receptor alpha (TRα) and beta (TRβ). Several isoforms of these receptors have also been described (TRα1, TRα2, TRβ1, TRβ2), each with tissue-specific expression and functions. The TRα1 receptor is widely expressed but has especially high expression in cardiac and skeletal muscles. The TRα2 is also widely expressed. The TRβ1 is mostly expressed in the brain, liver, and kidney, while the TRβ2 is limited to the hypothalamus and pituitary gland. Examples of isoform specific function include the effects on plasma cholesterol that are mediated by TRβ1 [45] and the cardiovascular effects mediated by isoform TRα1 [46]. Several attempts

to mimic these specific functions have been undertaken, mostly trying to lower serum cholesterol [47], unfortunately still with only limited success so far. The timing of these isoforms' expression during embryogenesis has also been shown to be critical with TRα being expressed widely and early during development, whereas the TRβ being restricted and expressed later [48].

Non-genomic actions of thyroid hormone have also been described [49]. These are mediated through a number of different membrane receptors that may or may not homologies to the their nuclear receptor counterparts. Examples include a truncated version of the TRα that seem to be essential for the non-genomic maintenance of the actin cytoskeleton by T4 [50] or a thyroid hormone receptor located on integrin αvβ3 that can activate the MAPK and PI3K pathways as well as regulate the intracellular trafficking of intact TRβ1 and MAPK from the cytoplasm to the nucleus [51, 52]. However, it has been recently shown that the postsynaptic effects induced after these membrane receptors have been activated by thyroid hormone may also include direct alteration of gene expression (including the genes for TRα, TRβ, and those encoding angiogenesis such as FGF2, MMP2, H1F1A, and COX2 [53, 54]), thus showing overlapping activity with the nuclear receptors.

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The Hypothalamic-Pituitary-Thyroid Axis: Physiological Regulation and Clinical Implications

Alina Gavrila and Anthony N. Hollenberg

Thyroid hormone (TH) has a key role in regulating the function of the majority of body tissues and organs, being essential for normal growth and differentiation as well as control of energy homeostasis and metabolism during adult life. TH acts primarily through nuclear receptors in different tissues to control a wide variety of genomic programs. TH levels need to be tightly regulated since even mild decreases or increases in circulating levels resulting in subclinical hypo- or hyperthyroidism, respectively, can negatively affect physiologic function. The hypothalamic-pituitary-thyroid (HPT) axis has the crucial role to maintain normal TH levels, and this has been achieved by the development of a neuroendocrine loop consisting of a negative feedback mechanism between circulating TH levels and the hypothalamus and pituitary gland [1–3]. Remarkably, there is significant interindividual variability with only narrow intraindividual variability in the HPT function under basal conditions in healthy people. Each individual has a physiological HPT axis set point where it functions optimally. This set point is determined mainly by genetic factors (40–60%) with some effect from environmental factors [4]. Numerous mutations or polymorphisms in the HPT axis that affect thyroid hormone production and function have been identified that could explain the different individual set point. To understand how the set point is defined and maintained, the following chapter will review the components of the HPT axis and their integration. Additionally, the regulation of the HPT axis in health and disease will be described.

Thyrotropin-Releasing Hormone (TRH)

TRH, produced in the hypothalamus, plays an essential role in the regulation of the HPT axis. Although not necessary for thyrotroph cell development in the pituitary gland, TRH is essential for both appropriate thyroid-stimulating hormone (TSH) and TH synthesis [5, 6]. Mice lacking the TRH gene develop central hypothyroidism [7]. TRH receptor mutations in humans have also been identified and cause central hypothyroidism [8].

TRH is a tripeptide synthetized from a larger inactive precursor preproTRH containing 242 amino acids in humans through several co- and posttranslational processes [3]. TRH is produced in several hypothalamic nuclei and other central nervous system (CNS) regions. Only the hypophysiotropic TRH-secreting neurons from the

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paraventricular nucleus (PVN) in the hypothalamus project to the median eminence (ME) and are part of the HPT axis playing a role in the regulation of TSH production [1, 3].

The PVN is located medially and symmetrically in the hypothalamus at the level of the upper third of the third ventricle and has a lateral magnocellular and medial parvocellular subdivision. The magnocellular area contains neurons that produce oxytocin and vasopressin and project in the posterior pituitary. The hypophysiotropic TRH neurons are located in the parvocellular area and project to the external zone of the ME, where the TRH is released from axon terminals into the portal capillaries. TRH is transported through the hypophysial portal system to the anterior pituitary, where it binds to TRH receptors (TRHR1) and regulates the production and secretion of TSH from thyrotrophs and prolactin from lactotrophs [1]. TRHR1 is the only TRH receptor present in the pituitary gland; a second TRHR2 with unknown function was found to be expressed mainly in the brain and is likely only functional in rodents [9].

TRH stimulates the synthesis, secretion, and biological activity of TSH. TRH stimulates the release of pre-synthesized TSH and also increases the synthesis of both the alpha TSH subunit common to all glycoprotein pituitary hormones [luteinizing hormone (LH) and follicle-stimulating hormone (FSH)] and the specific TSH-beta subunit which encodes for biologic specificity [1]. TRH also regulates TSH glycosylation/posttranslational processing of TSH oligosaccharide chains, which is important for folding, secretion, clearance, and biological activity of TSH. Animal and human studies have showed that TRH deficiency is associated with inappropriately low pituitary production of TSH, which also has decreased biological activity [10].

The location of the hypophysiotropic TRH neurons in the PVN is critical to their function in regulating the thyroid axis. The PVN receives and integrates multiple neuronal and humoral signals and adjusts the HPT axis to adapt to external and internal environmental changes, such as cold, starvation, and illness [1, 3]. Numerous neuronal axons from different hypothalamic and brain areas project and form synaptic associations with the TRH neurons in the PVN. In addition, the TRH neurons receive humoral signals from the circulation through the vascular supply of the ME or the PVN [1, 3].

In both rodents and humans, the PVN receives input from the arcuate nucleus (ARC) in response to peripheral feeding-related signals. Specifically, the ARC sends inhibitory signals from orexigenic neurons which co-secrete agouti-related peptide (AgRP) and neuropeptide Y (NPY) and stimulatory signals from anorexigenic neurons which secrete the alpha-melanocyte-stimulating hormone (alpha-MSH) from their axon terminals in the PVN. These signals are important in HPT axis response to starvation and illness and can result in the suppression of TRH production and secretion. The PVN also receives input from the catecholamine-producing brainstem neurons, which stimulates TRH gene transcription during cold exposure (2/3 adrenergic neurons and 1/3 noradrenergic neurons) [1, 3]. Input received from the dorsomedial nucleus (DMN) may be involved in the circadian regulation of the TRH neurons [1].

The role of the non-hypophysiotropic TRH neurons located in the hypothalamus and other CNS areas is not known; however, they may play a role in the autonomic regulation of thermogenesis by projecting to the brain stem and spinal cord and also in food intake/appetite/feeding behavior by projecting to the satiety center (ventromedial hypothalamus, VMH) and hunger center (lateral hypothalamus, LH) [1–3]. TRH administration to rodents is known to induce anorexia, to inhibit food and water intake without affecting the TSH [3]. TRH is also synthetized in peripheral organs, such as the intestinal tract, pancreas, and heart, which probably contribute to circulating TRH. Hypothalamic TRH is not thought to affect circulating TRH levels.

TRH and the Regulation of Prolactin Secretion

TRH binds to TRH receptors (TRHR1) and regulates the production and secretion of TSH from thyrotrophs and prolactin from lactotrophs in the

anterior pituitary [1]. Exogenous TRH administration increases prolactin production and secretion from the anterior pituitary in humans. Endogenous TRH is not important for the development or differentiation of fetal pituitary lactotrophs or in maintaining prolactin secretion under normal conditions [11]. In addition, only a small percentage of patients with hypothyroidism and increased endogenous TRH production in the hypothalamus have elevated prolactin levels [11]. It is recommended to measure thyroid function tests in patients who present with hyperprolactinemia, since thyroid hormone treatment in hypothyroid patients can result in normalization of prolactin levels.

Thyroid-Stimulating Hormone (TSH)

TSH is a glycosylated polypeptide produced in the anterior pituitary gland in cells termed thyrotrophs (Fig. 1). TSH is a heterodimeric glycoprotein consisting of an alpha subunit common for

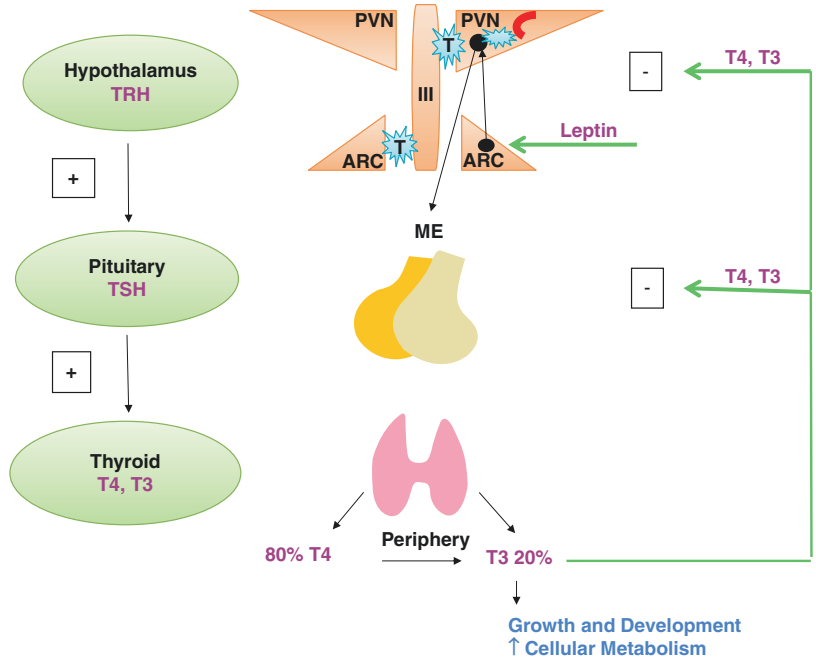

Fig. 1 The hypothalamic-pituitary-thyroid (HPT) axis. Hypophysiotropic thyrotropin-releasing hormone (TRH) neurons located in the paraventricular nucleus (PVN) of the hypothalamus close to the third ventricle (III) project to the median eminence (ME) and release TRH into the local portal system. Thus, TRH reaches the pituitary, where it binds to specific receptors and stimulates the thyroid-stimulating hormone (TSH) production and secretion into the circulation. Circulating TSH binds to specific receptors in the thyroid to stimulate the thyroid hormone (TH) production and secretion. Tetraiodothyronine (T4) represents 80%, while triiodothyronine (T3) represents 20% of the TH produced in the thyroid. T4 is a prohormone, which is converted in the periphery to T3, the active form of TH. TH regulates the function of the majority of body tissues and organs, playing an important role in normal growth and differentiation as well as control of energy homeostasis and metabolism during adult life. Circulating T4 is transported into specialized cells called tanycytes (T) located in the hypothalamus, which produce type 2 deiodinase (DIO2) and convert T4 to T3. Locally synthetized T3 binds to specific receptors in the hypophysiotropic TRH neurons and inhibit TRH synthesis. Large amounts of circulating T3 can directly cross into the brain and control TRH production. Similar to the hypothalamus, the majority of T3 that acts in the pituitary is produced locally by the DIO2, which is expressed in the pituitary in stellate cells located adjacent to thyrotrophs. The hypophysiotropic TRH neurons in the PVN receive projections from neurons in the arcuate nucleus (ARC) that express leptin receptors and respond to changes in circulating leptin levels during fasting/food intake. The ARC mediates the leptin-induced changes in the HPT axis

all of the glycoprotein hormones [TSH, LH, FSH, and human chorionic gonadotropin (hCG)] and a unique beta subunit that encodes for its biologic specificity. TRH plays an important role in TSH glycosylation, which does not interfere with the immunological TSH assay; however, it can affect TSH bioactivity in humans. Lack of TRH severely impairs the regulation of TSH secretion and its biological activity. In hypothalamic hypothyroidism, serum TSH levels are normal or only slightly increased, and the TSH has decreased bioactivity, which accounts for the low T4 levels [5, 9, 10].

While hypothalamic TRH and circulating TH are the most important regulators of TSH synthesis and secretion, other hormonal pathways also impact its production. Dopamine inhibits TSH production and secretion by activation of dopamine-2 receptor (D2R) in pituitary thyrotrophs, while at a lesser degree, it stimulates TRH secretion in the PVN. Somatostatin inhibits TSH release by activation of the SST2 and SST5 receptors expressed on thyrotrophs [12]. Glucocorticoids decrease TRH gene expression in the PVN and also directly suppress TSH production and secretion, thus resulting in low TSH levels. Physiological cortisol levels may play a role in the diurnal TSH rhythm, which is characterized by lower levels in the morning and higher levels at night, opposite to the cortisol rhythm [13]. Leptin acts directly and indirectly via alpha-MSH/CART expressing neurons from the ARC to stimulate TRH synthesis and secretion in the PVN [1]. Leptin and TSH secretion follows a similar pattern [12].

Negative Feedback Regulation of TRH and TSH Production by Thyroid Hormone (TH)

There are simultaneous changes at multiple levels in the HPT axis in response to circulating TH levels (Fig. 1). Indeed, the entire axis is designed to be activated when TH levels are low and suppressed when TH levels are high. For this reason the serum TSH has become the most important laboratory test in interpreting thyroid function in humans as it is easily measurable in the peripheral circulation.

In hypothyroidism, there are increased TRH production and secretion from hypophysiotropic TRH neurons in the PVN and also decreased expression of the TRH-degrading enzyme present in cells near the hypothalamus (tanycytes) and increased TRHR1 synthesis in the pituitary. Together, these changes allow TRH action to increase such that it leads to increased TSH production and release from the pituitary. Opposite changes are seen in hyperthyroidism [9]. While TRH is not required for the induction of TSH in hypothyroidism, it greatly accentuates its rise. Most of the changes caused in TRH production by TH are mediated at the mRNA level such that TRH mRNA is increased in hypothyroidism and decreased in hyperthyroidism. The actions of TH on TRH gene expression are rapid and can occur in hours [1].

The effects of TH in the hypothalamus are primarily mediated by T3. The majority of T3 is produced locally in the hypothalamus from circulating T4 that is transported into specialized cells called tanycytes located between the PVN and ME, which produce type 2 deiodinase (DIO2). When present in large amounts in the circulation, T3 itself can directly cross into the brain and regulate TRH gene expression. T3 binds to its receptors in the hypophysiotropic TRH neurons from the PVN, specifically the THR-beta2 isoform, and inhibits TRH mRNA expression [2]. While other thyroid hormone receptors exist, termed THR-alpha and THR-beta1, it is likely the TRH-beta2 which is of paramount importance in the hypothalamus and pituitary [14]. In the absence of T3, TRH gene expression is activated presumably by the TRH-beta2, which no longer has hormone bound to it. The mechanism by which the TRH-beta2 is able to repress in the presence of ligand and activate in its absence remains unknown.

As discussed, most T3 that acts on hypophysiotropic TRH neurons is produced locally in the hypothalamus by the conversion of T4 to T3 by DIO2 in tanycytes which line the third ventricle. Presumably, the T3 produced in this area is then able to access TRH neurons to allow for its regulation [6, 9]. The presence of this local generator of T3 allows for the DIO2 to be a target to control

TRH production and release. For example, in sepsis or other acute severe illnesses where the HPT axis is suppressed and low TH levels are seen in the presence of inappropriately normal or suppressed TSH levels, the expression of DIO2 in the hypothalamus is activated by inflammatory pathways leading to increased local T3 and suppressed TRH production [1].

In addition to its role in regulating TRH production, TH also directly controls TSH production in pituitary thyrotrophs. Similar to the hypothalamus, the majority of T3 that acts in the pituitary is produced locally by DIO2, which is expressed in stellate cells located adjacent to thyrotrophs in the pituitary. Like TRH, both the TSH-alpha and TSH-beta subunits of TSH are negatively regulated at the mRNA levels by T3 via the THR-beta2 receptor expressed in pituitary thyrotrophs. In the hypothyroid state, TSH subunit expression is activated by both increased TRH levels and the unliganded effects of THR-beta2. The converse occurs in hyperthyroidism. Interestingly, DIO2 is also regulated by TH, specifically by T4 and only minimally by T3. In the presence of high levels of T4, DIO2 is rapidly degraded at the protein level to prevent the conversion of T4 to T3 and inhibit further consequences of hyperthyroidism [1, 9, 14].

Clinical Implications

The regulation of TSH at multiple levels by local T3 has made it the most reliable biomarker for interpreting thyroid function. Initial models assumed a simple inverse linear correlation; however, more recent studies have showed an intraindividual log-linear relationship between TSH and circulating free T4 levels [15, 16]. There is an exaggerated TSH response to subtle TH changes; therefore, the TSH test is considered to be more sensitive than the free T4 test. The TSH assay is very reliable and cost-effective and is the main laboratory test used in the clinical setting to diagnose and monitor patients with hypo- and hyperthyroidism. Third-generation TSH assays are currently available, which have increased sensitivity and can separate low TSH levels in hyperthyroid patients from low normal TSH levels in euthyroid subjects. The advent of sensitive TSH assays has allowed for the identification of subclinical hypo- and hyperthyroidism. In these situations the TSH is abnormal with T4 and T3 levels still within normal range [15].

The most recent large cross-sectional studies examining the TSH-free T4 relationship over the entire thyroid function range, from hypo- to hyperthyroidism (instead of rather extrapolating results from euthyroid patients), showed a curvilinear shape with a steeper response of TSH to free T4 changes in the hypothyroid or hyperthyroid spectrum and a damped response in the middle in the euthyroid range; thus, the greater the deviation from optimum normal function toward hypo- or hyperthyroidism, the greater the TSH change in response to free T4 changes [15]. This challenges whether TSH is the best test to use to estimate thyroid function in euthyroid patients, when there are smaller TSH changes in response to free T4 changes.

Approximately 10–15% of hypothyroid patients requiring TH replacement report not feeling well, despite of normal thyroid function test results. This could be explained by the fact that the TSH on treatment returns to the normal population range; however, it does not achieve the genetically determined physiological set point [4]. In addition, in treated athyreotic patients, it is still unclear whether levothyroxine doses used to normalize TSH levels can still fully normalize T3 levels in all tissues [15, 17, 18]. There may be multiple reasons to explain this, but T3 action may be different in the hypothalamus and the pituitary than in other cell types, based on the thyroid receptor isoform present or whether different deiodinases are present.

Multifaceted Feedback Control

While the negative feedback regulation of the HPT axis by circulating TH levels was probably the first mechanism developed during evolution, other newer complex regulatory mechanisms of the HPT axis have emerged to allow for

adaptation to different environmental changes [14]. Several exogenous physiologic and pathologic factors can affect the HPT axis and result in deviations from the fixed set point-negative regulatory loop [19]. Indeed, there is a diurnal TSH rhythm with a nocturnal TSH surge, and prolonged fasting results in decreased in TSH and TH levels. When interpreting the TSH level in a clinical setting, we may need to take into consideration the time of the day and the fed status of the individual.

Circadian Rhythm of the HPT Axis

As outlined, TSH is secreted from thyrotrophs located in the pituitary gland into the circulation in a pulsatile manner. TSH has a circadian rhythm with the lowest levels in the afternoon between 1600 and 2000 h, followed by a rise during the evening and maximum levels between 0200 and 0400 h; there are no gender differences [12, 20]. No circadian rhythm has been observed for T4, probably because of its long half-life. T3 has a circadian rhythm that correlates with the TSH circadian rhythm, the peak T3 lagging 90 min behind the peak TSH level. This suggests that TSH plays a role in maintaining circulating T3 levels [21]. In primary hypothyroidism, there is an increased basal serum TSH concentration with preserved circadian rhythm and pulsatility; the total TSH secretion is increased 10-fold in subclinical hypothyroidism and 200-fold in severe hypothyroidism [12]. The physiological role of the TSH circadian rhythm is not known [14].

The suprachiasmatic nucleus (SCN) of the hypothalamus may play a role in the regulation of the diurnal TSH oscillations. SCN neurons project to the PVN, and ablation of this center affects the circadian TSH rhythm. Circulating TH does not affect the gene expression in the SCN; thus the diurnal TSH oscillations are independent of the negative feedback mechanism [14]. In addition, the dorsomedial nucleus (DMN) may be involved in the circadian regulation of TRH neurons which would contribute to the rhythmicity of TSH secretion [1].

The HPT Axis and Food Deprivation

It has been known for many years that fasting decreases circulating TH levels, which is thought to represent an adaptive mechanism to conserve energy**.** Food restriction results in decreased TRH mRNA expression in the PVN and, consequently, lower TSH and TH levels [3, 6]. More recently, leptin has been shown to play a critical role in this mechanism by affecting the function of one of the main hypothalamic centers controlling food intake and energy homeostasis, the ARC (Fig. 1). Fasting decreases leptin production, which results in increased appetite, energy conservation, and changes in the neuroendocrine axis. The ARC mediates the leptin-induced changes in the HPT axis during fasting [14].

The hypophysiotropic TRH neurons in the PVN receive projections from leptin-responsive neurons that express leptin receptors in the ARC. One group of ARC neurons produces the anorexic peptide alpha-MSH which binds to specific receptors on TRH neurons and prevents the fasting-induced suppression of the thyroid axis [3]. Food intake results in increased leptin levels and increased alpha-MSH expression. In contrast, a second group of ARC neurons co-express the orexigenic peptides, NPY and AGRP; leptin inhibits these neurons. Since fasting results in decreased leptin levels, these neurons are activated and elevate AgRP and NPY expression which, in turn, suppresses TRH mRNA expression, thus causing central hypothyroidism [3].

During fasting, there are also changes in the peripheral TH metabolism affecting the conversion of T4 into T3 and reverse T3 (rT3). Evidence exists for enhanced metabolism and excretion of T4 in the liver by type 3 deiodinase (DIO3), sulfotransferases, and glucuronidating enzymes and decreased T3 production by downregulation of the liver type 1 deiodinase (DIO1) [22–24].

The positive influence of leptin on the TRH axis could explain the thyroid function changes noted in patients with anorexia nervosa and obesity. Adolescents with anorexia nervosa have low fat stores resulting in low leptin levels, and they also have low TSH and free T3 levels. In agreement with this, obese patients have increased

body fat and leptin levels as well as slightly elevated TSH and free T3 levels. The leptin, TSH, and free T3 levels normalize with weight gain in patients with anorexia nervosa and weight loss in obese patients. These changes in the TRH axis may represent an adaptation mechanism that decreases energy expenditure to conserve energy in thin patients and increases energy expenditure thus reducing the conversion of energy into fat in obese patients. Pharmacological treatment to normalize the thyroid axis has not proved to be beneficial in patients with abnormal weight [25–27].

The HPT Axis and Cold Exposure

Exposure to cold stimulates the HPT axis through adrenergic inputs to the hypophysiotropic TRH neurons in the PVN. Catecholamine-expressing neurons from the medulla and pons project to the hypophysiotropic TRH neurons in the PVN (alpha2 adrenergic receptors) and also to the external layer of the ME (alpha1 adrenergic receptors). Cold exposure increases the prepro-TRH mRNA levels in the PVN within 30–60 min, increases TRH release from the ME, and stimulates TSH secretion from the pituitary gland, which results in increased circulating T4 and T3 levels [3]. However, despite these anatomic pathways being present, there is little clinical significance seen as circulating thyroid hormone levels do not vary with temperature.

The HPT Axis and Illness

Illness affects the negative feedback loop centrally resulting in downregulation of the HPT axis. This condition is characterized by initially low circulating T3 and in severe illness low T4, increased rT3, and inappropriately normal or low TSH levels and is called the "non-thyroidal illness syndrome" (NTIS) (also "euthyroid sick syndrome" or "low T3 syndrome").

TRH mRNA expression in the PVN of hypothalami collected from patients who died after prolonged illness was decreased and correlated positively with premortem serum TSH and T3 levels measured in samples collected less than 24 h before death [28]. The mechanism of these changes is not completely understood. As discussed, animal models for NTIS have showed a significant increase in DIO2 expression in tanycytes, which is thought to increase the local T3 production and through this decrease the TRH mRNA expression in both acute and chronic illness. Cytokines associated with illness, including interleukin 1 (IL1), IL6, and tumor necrosis factor-alpha (TNF-alpha), mediate the development of this syndrome [29, 30].

Animal models have showed that illness also results in decreased TSH-beta mRNA expression in the pituitary and low circulating TSH levels. The mechanism is unclear, since DIO2 expression in the pituitary is either increased or decreased in NTIS, depending on the species and type of illness studied [29].

The NTIS picture is complex, involving various illness-induced changes in the thyroid function and local TH metabolism of different organs in acute vs. chronic disease in addition to the central changes in the hypothalamus and pituitary. Cytokines released in NTIS may also inhibit TH synthesis and secretion from the thyroid gland as well as the TH transport inside peripheral cells. In addition, in NTIS, there is cytokine-mediated local regulation of TH metabolism in different organs independently of the circulating TH levels, mainly by affecting the expression of the different types of deiodinases present in different peripheral tissues. For example, in the liver, which is thought to be a major source of circulating T3, there is decreased DIO1 expression resulting in decreased local T3 production in acute inflammation, while the local T3 production does not seem to be affected in chronic inflammation. In skeletal muscle, there is increased DIO2 associated with decreased DIO3 expression resulting in increased local T3 production in acute inflammation, while there is increased expression of both DIO2 and DIO3, resulting in lower local T3 and higher T2 production in chronic inflammation [29, 30]. For example, changes in the local TH metabolism in the muscle during severe illness may play a role in

the myopathy associated with prolonged ventilator dependence [29].

The classical view is that in illness there is an adaptive decrease in body metabolism to preserve energy. The combination of low T3 and T4 levels is associated with poor prognosis in severely ill patients, and T3 and/or T4 administration in different conditions associated with NTIS to restore circulating T3 levels has not been beneficial and may result in a poorer outcome [23, 29, 31]. The only condition where T3 treatment may be beneficial is in NTIS associated with heart failure and cardiac surgery; however, further studies are required in this area before treatment can be recommended [32–34].

It is currently thought that the body changes occurring during the acute phase of illness are supportive but they become harmful during prolonged critical illness [29]. In critically ill patients supported with intensive medical care for several weeks, administration of exogenous TRH and growth hormone-releasing peptide (GHRP2) restored TSH and GH pulsatile secretion and resulted in increased anabolism [35]. This indicates that at least part of the wasting syndrome of protracted critical illness is caused by relatively insufficient secretion of GH and TSH and can be reversed by continuous infusion of the hypothalamic releasing factors [29, 35].

The HPT Axis and Aging

Population-based studies have showed that TSH levels tend to increase with age [36–38]. This seems to be beneficial since higher TSH levels in very elderly has been associated with decreased mortality [39, 40]. Therefore, the high prevalence of subclinical hypothyroidism in older people may be overestimated and result in unnecessary treatment that can be harmful. Using age-specific TSH reference intervals for TSH has been suggested [38]. In addition, serum T3 levels are reduced with preserved T4 levels, and there is a lesser TSH response to hypothyroxinemia in older individuals. It has been hypothesized that there is increased inhibition by T3 versus decreased TRH action on the TSH production [41, 42].

Drugs that Affect the HPT Axis

In addition to TH and cytokines, there are a number of other drugs used clinically that can affect the HPT axis at the level of hypothalamus or pituitary.

High doses of exogenous glucocorticoids act mainly on receptors located on the TRH neurons in the PVN and may decrease TRH gene expression but also on the pituitary directly to suppress TSH production and secretion. Together this can result in low TSH levels [13, 43]. A dexamethasone dose of only 0.5 mg can lower the serum TSH level, while 20–30 mg of prednisone or 100 mg of hydrocortisone or more per day is required to have this effect [13, 44, 45]. Physiologic levels of hydrocortisone may play a role in the diurnal TSH rhythm characterized by lower levels in the morning and higher levels at night [46]. Interestingly, long-term high doses of glucocorticoids or endogenous hypercortisolism in Cushing's disease do not result in central hypothyroidism, probably because the decrease in TH levels will overcome the glucocorticoid effect and increase TSH secretion [13]. In contrast, patients who present with Addison's disease may have a slightly high TSH level which reflects glucocorticoid deficiency [46].

Dopamine used in the ICU setting binds and activates D2Rs in the hypothalamus and pituitary. Dopamine is a natural catecholamine released from hypothalamic neurons located in the ARC in the local portal circulation that plays an important role in prolactin regulation and also affects TSH secretion in the pituitary. Although dopamine appears to stimulate the TRH release from the hypothalamus, it has a stronger effect on the pituitary where it decreases the TSH pulse amplitude, thus resulting in decreased TSH levels [13, 47]. The HPT axis suppression secondary to dopamine infusion will further worsen the suppression of this axis associated with nonthyroidal illness syndrome in critically ill patients [13].

Dopamine agonists, such as bromocriptine used to treat hyperprolactinemia, have similar

suppressive effects on the HPT axis, while dopamine receptor antagonists, such as metoclopramide or domperidone, have opposite effects during acute administration [13, 48, 49]. Interestingly, chronic treatment with these drugs will not affect the TSH secretion, probably because the decrease in TH levels will overcome their effect on the HPT axis [13].

Somatostatin is a peptide secreted in the periventricular nucleus and ARC in the hypothalamus that enters the local portal system and binds to five types of receptors (SST-1 to SST-5) in the pituitary, playing an important inhibitory role on GH secretion. Somatostatin also directly inhibits TSH secretion from the pituitary thyrotrophs [47]. Long-term administration of somatostatin analogues, such as octreotide in acromegaly, does not cause clinically significant central hypothyroidism [13]. However, somatostatin analogues are effective in treating patients with TSH-secreting pituitary adenoma in addition to surgery as well as patients with resistance to TH [50, 51].

Retinoids are vitamin A analogues that bind a nuclear hormone receptor, the retinoid X receptor (RXR). The RXR forms heterodimers with other nuclear transcription factors, including TH receptors resulting in activation of these receptors and inhibition of TSH secretion in the pituitary. Patients with subcutaneous T-cell lymphoma treated with bexarotene develop reversible central hypothyroidism [52, 53].

Several anticonvulsants (carbamazepine, oxcarbamazepine, and valproic acid) may result in central hypothyroidism in addition to increasing TH metabolism through the activation of the P450 system [54].

Tricyclic antidepressants, antipsychotic phenothiazines, as well as atypical antipsychotics may interfere with the HPT axis and decrease the TSH response to TRH [55]. The clinical significance is likely not important with this class of drugs.

Metformin can lower TSH level through an unknown mechanism in hypothyroid, however not in euthyroid patients who have an intact hypothalamic-pituitary feedback mechanism [56].

Summary and Conclusions

The HPT axis is obviously critical to maintaining the euthyroid state and can be interrogated rapidly with the TSH assay in order to determine the functional status of an individual patient. While this works in most clinical situations, it is imperative that the clinician be aware of other clinical states that can invalidate the normal regulation of the HPT axis. From a clinical perspective, the most important to consider are illness and co-administered medications, but as reviewed, many other physiologic states can and will influence the set point of the axis. It is likely that further work in each of these areas will help us better understand how to interpret the function of the HPT axis.

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"Thyroglobulin Storage, Processing and Degradation for Thyroid Hormone Liberation"

Klaudia Brix, Maria Qatato, Joanna Szumska, Vaishnavi Venugopalan, and Maren Rehders

Abbreviations

AFU	Angio-follicular unit
CH	Congenital hyperthyroidism
CNS	Central nervous system
Cts	Cathepsin gene
DIT	Diiodothyronines
DUOX	Dual oxidase
ER	Endoplasmic reticulum
ERAD	ER-associated degradation
fT_4	Free T_4
IYD	Iodotyrosine deiodinase
M6P	Mannose 6-phosphate
MIT	Monoiodothyronines
MPR	Mannose 6-phosphate receptor
NIS	Sodium-iodide symporter
PDI	Protein disulfide isomerase
rER	Rough endoplasmic reticulum
rT_3	Reverse T_3
T_2	3,3′-Diiodothyronine and 3,5-diiodothyronine
T_3	3,5,3′-Triiodothyronine
T_4	3,5,3′,5′-Tetraiodothyronine, thyroxine
TAM	Thyronamines
Tg	Thyroglobulin
TGN	*trans*-Golgi network
TH	Thyroid hormone
TPO	Thyroid peroxidase
TSH	Thyroid-stimulating hormone

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Introduction: The Thyroid Gland and Its Tasks

Thyroid-derived molecules are the classical TH 3,5,3′-triiodothyronine (T_3) and 3,5,3′,5′-tetraiodothyronine (thyroxine, T_4), their nonclassical metabolic derivatives 3,3′-diiodothyronine and 3,5-diiodothyronine, and possibly also the thyronamines (TAM), primarily 3-T_1AM and T_0AM. Collectively, these thyroid molecules from "T_4–T_0" have been referred to as the thyronome [1–3].

This review will focus on classical T_3 and T_4 [4–9]. They are delivered with the blood circulation to peripheral organs and to the central nervous system (CNS). Deiodinases of TH target cells are required to convert circulating T_4 upon uptake into the biologically active T_3. Alternatively, T_4 is converted into biologically inactive reverse T_3, which is part of the body's TH inactivation pathways. The TH deiodinating enzymes are selenoproteins, which are expressed in a tissue- and cell type-specific manner and which are also present in thyroid epithelial cells along with a number of other selenoenzymes that have critical functions, particularly during the

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iodination of Tg [10] (see section "Iodination of Tg in the Thyroid Follicle Lumen: A Unique Posttranslational Modification").

The thyroid gland's tasks are relevant to all body functions. This conclusion is derived from the notion that adequate TH levels are required for the development and proper functioning of nearly every organ in all phases of life, i.e., from early embryonic tissue morphogenesis to postnatal development, from childhood to adulthood, as well as aging.

To fulfill its tasks, the thyroid gland is built by functional units, the so-called thyroid follicles, which are composed of a monolayer of thyroid epithelial cells (thyrocytes) [11, 12], and few calcitonin-producing C cells [13, 14]. In addition, thyroid follicle cells are in intimate contact with endothelial cells of the blood vessels forming a basket-like, highly complex vasculature around each individual thyroid follicle [11]. Thyroid follicles with associated blood vessels are collectively referred to as angio-follicular units (AFU; [15, 16]). The microvasculature of thyroid follicles not only consists of blood vessels but also contains lymphatic vessels, typically surrounding clusters of few thyroid follicles [17].

Biosynthesis of Tg, Its Folding and Trafficking, and Acquisition of Different Posttranslational Modifications

Thyroglobulin (Tg) is synthesized at the rough endoplasmic reticulum (rER) of thyroid epithelial cells as a protein consisting of 2768 amino acids with a 19-amino-acid signal peptide [18–21]. Upon import into and folding within the ER lumen, Tg is transported via the Golgi apparatus and the *trans*-Golgi network (TGN) to the apical plasma membrane domain of thyroid epithelial cells for its subsequent secretion into the thyroid follicle lumen (Fig. 1). During its transport along the secretory pathway [22], Tg undergoes several posttranslational modifications, i.e., it becomes N- and O-glycosylated, mannose 6-phosphorylated, and sulfated, and eventually, upon its secretion into the extracellular lumen, Tg is iodinated in a reaction that is unique for the thyroid gland (see section "Iodination of Tg in the Thyroid Follicle Lumen: A Unique Posttranslational Modification"). Tg typically occurs as a soluble dimer, but additionally, it has the ability to multimerize (see section "Compaction and Storage of Tg as Covalently Cross-Linked Thyroid Globules"). The biosynthesis of Tg is regulated by thyroid-stimulating hormone (TSH) (see sections "Regulation of Tg Utilization for TH Liberation" and "Further Mechanisms of Thyroid Function Regulation").

Investigations on structure-function relationships and the molecular evolution of Tg have recently unraveled its multi-domain architecture to be unique among proteins of vertebrates [23]. The glycosylation of Tg is unusual in that it features an evolutionary early character, whereby complex, hybrid, high-mannose, and glucosamine types of carbohydrates are detected in N-glycosylated Tg, which is also O-glycosylated, and can bear a considerable proportion of up to 10% of its molecular mass as carbohydrate moieties [24–31].

The significance and consequence of Tg's manifold posttranslational modifications, including its species-specific glycosylation patterns, are not yet fully understood, but the posttranslational and pre-iodination modifications of Tg may help thyroid epithelial cells to monitor Tg's proper folding by chaperone-mediated ER quality control mechanisms and to regulate its intrathyroidal transport as well as its iodination upon secretion into the extracellular thyroid follicle lumen [22, 27, 30–34]. In specific forms of congenital hypothyroidism (CH), mutations in the *TG* gene can result in altered intracellular transport of Tg, eventually leading to defective TH synthesis [30, 33, 35–37]. In the context of intrathyroidal Tg transport, it is of particular interest to note that misfolding of Tg in the ER lumen can be the causative basis of such specific forms of CH in which quality control and the safe-guarding ER-associated degradation (ERAD) mechanisms are outcompeted [22, 33, 38–40]. The result is an overload of the thyrocyte's rER with an accumulation of excessive amounts of misfolded Tg, leading to a massive expansion of the ER and, eventually, to thyroid dysfunction.

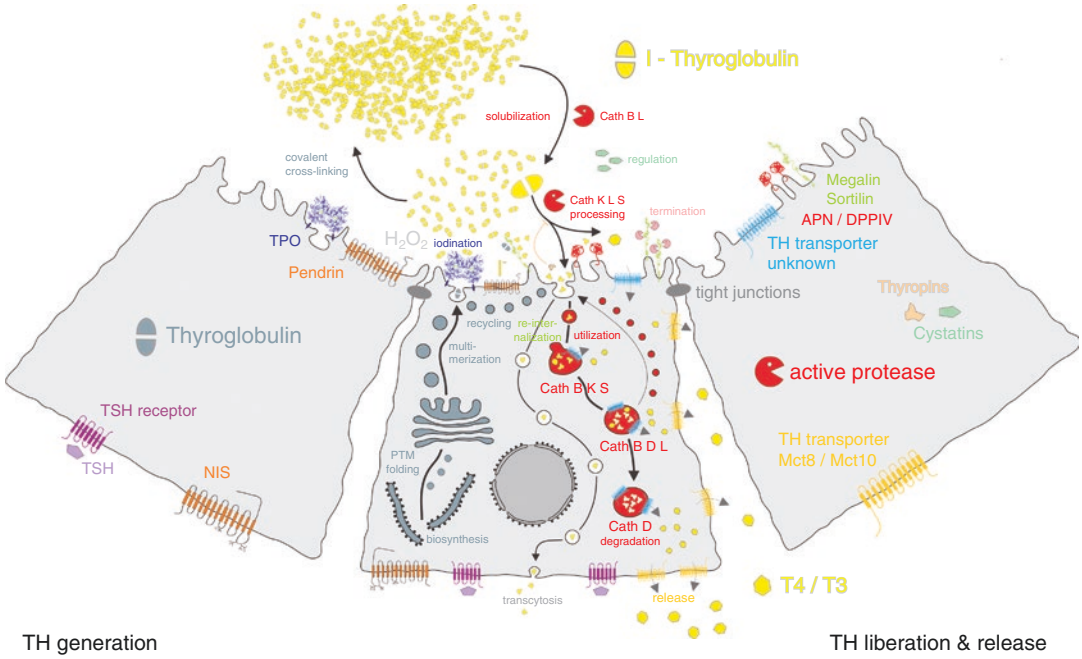

Fig. 1 Biosynthesis, iodination upon secretory release, luminal storage, processing, and degradation of thyroglobulin for TH generation, liberation, and release from thyroid follicles. Schematic diagram depicting molecules of thyroid epithelial cells that are important for (i) TH generation on the protein backbone of thyroglobulin (Tg), (ii) liberation of thyroxine (T_4) and some triiodothyronine (T_3) by extra- and intracellular means of proteolysis, and (iii) TH release from thyroid follicles into the surrounding blood circulation through Mct8- and Mct10-mediated transport across the basolateral plasma membrane domain of thyrocytes. Left: Tg biosynthesis at the rER is followed by its folding and acquisition of different posttranslational modifications (PTM), and multimerization before the TH precursor molecule becomes iodinated upon its secretory release. The basolateral sodium-iodide symporter (NIS) and the apical sodium-iodide anti-porter pendrin enable iodide transport across the epithelial sheet into the thyroid follicle lumen. Oxidative conditions are generated by DUOX and DUOX-associated proteins of the apical plasma membrane domain (not shown), thereby enabling the heme-containing thyroid peroxidase (TPO) to iodinate tyrosine residues and intramolecularly couple them to preform iodothyronines on Tg's backbone. A number of dehalogenases are important for recycling of surplus iodine (not shown). Tg is covalently cross-linked for efficient storage in the follicle lumen in a form that keeps the iodine-rich molecule osmotically inert. Extracellularly acting proteolytic enzymes first solubilize Tg from its high molecular mass storage forms, before T_4 can be liberated by a combinatory action of endo- and exopeptidases. Aspartic cathepsin D; cysteine cathepsins B, C, K, L, and S; as well as a plasminogen-like serine protease (not shown) and a metallopeptidase (not shown) have been shown to proteolytically process Tg before or after its endocytosis and delivery to endo-lysosomes, where it is completely degraded for its turnover. In particular, TH liberation from Tg and its degradation fragments can be performed by cathepsins K and S or by combined action of the cathepsins with the ectoenzymes aminopeptidase N (APN) and dipeptidylpeptidase IV (DPPIV) of the apical plasma membrane domain of thyrocytes. The proteolytic processing and TH liberation from Tg by extracellular means are regulated by endogenous cysteine peptidase inhibitors, the type 2 cystatins C, D, E, and F, which may further involve substrate-assisted means of regulation by thyropins (see Fig. 2). Tg re-internalization is believed to depend on binding proteins of low, medium, or high affinity, to which low-density lipoprotein (LDL)-related proteins such as megalin and sortilin belong. The thyroid hormones T_3 and T_4 are translocated across the membranes of thyroid epithelial cells by means of TH transporting molecules, such as the monocarboxylate transporters Mct8 and Mct10, that export T_4 and T_3 into the extracellular space from where they can enter the blood circulation to become bound to transporter proteins like the thyroxine binding globulin (TBG; not shown), which deliver the TH to their target organs in the body periphery and the central nervous system. Tg can also reach the blood circulation as an intact iodinated protein by transepithelial vesicular transport, i.e., transcytosis, which bypasses endo-lysosomal degradation. Thyroid functions are directed in many aspects by thyroid-stimulating hormone (TSH), released from pituitary cells upon a shortage in circulating TH levels, and negative feedback onto the hypothalamus to release the thyroid-releasing hormone thyroliberin (TRH; not shown). The schematic drawing omits the recently discovered G

(continued)

protein-coupled receptors of the apical plasma membrane domain at primary cilia of thyroid epithelial cells [200], namely, the trace amine-associated receptor 1 (Taar1), that is known to be triggered by a nonclassical, TH-related molecule, 3-iodothyronamine (3-T_1AM), which is generated from classical TH by decarboxylation and deiodination. It is not known to date whether 3-T_1AM is generated within thyroid follicles or by extra-thyroidal means. It was proposed very recently that Taar1 and TSH receptors co-regulate thyroid functions in mice [201]

Mannose 6-phosphorylation of proteins is believed to serve as a targeting signal for sorting into transport vesicles destined to late endosomes and lysosomes through interaction of the mannose 6-phosphate (M6P) moieties upon their addition in the *cis*-Golgi cisternae, with the cation-dependent 46 kDa M6P receptor (CD-MPR) in the *trans*-Golgi network (TGN) [41–46]. However, this potential targeting route is bypassed in fully functional thyrocytes because Tg trafficking typically skips recognition by the CD-MPR in the TGN and the protein is further transported along the secretory pathway, resulting in secretion of mannose 6-phosphorylated Tg into the extracellular space [47–50]. It may be speculated that the extensive and somewhat unusual N- and O-glycosylation of Tg explains why the M6P signal is masked and why the thyroid-specific protein Tg reaches the extracellular thyroid follicle lumen rather than being delivered directly to late endosomes and lysosomes for proteolytic degradation [38, 48, 51–53].

For thyroid function, the transport of Tg along the secretory pathway and its delivery at the apical plasma membrane are critically important because only then the protein can become iodinated [54]. Thus, only if Tg follows the secretory pathway all the way up to its secretion into the extracellular follicle lumen, it may serve as the TH precursor molecule that it is. Hence, it may be speculated that fully glycosylated Tg is a better substrate for thyroid-specific iodination reactions, while poorly glycosylated Tg might be suboptimal for iodination, thus yielding low-iodinated, poorly glycosylated, and potentially less stable Tg. However, it has been shown that altered posttranslational modifications—e.g., desialylation—and increasing concentrations of Tg paradoxically accelerate iodination [55].

Sulfation of Tg is not well studied, but it is known to happen in the Golgi apparatus of thyroid epithelial cells in a species-specific manner, yielding sulfated tyrosine residues and sulfated high-mannose carbohydrates on Tg [56]. The biological significance of sulfated Tg to thyroid physiology has been addressed only occasionally. However, it was proposed that sulfated tyrosine residues are likely affecting the efficiency of Tg iodination [57, 58]. Negatively charged chondroitin sulfate side chains, characterizing human Tg in particular, are less prevalent in papillary thyroid carcinoma [59] and, hence, must be considered a feature of the differentiated state of thyrocytes. All in all, these results show that further studies on the importance of Tg's posttranslational modifications, in context of subsequent iodination, are necessary.

Apart from the abovementioned general posttranslational protein modifications, a species-specific covalent cross-linkage of Tg occurs while traveling along the secretory route and upon its secretion into the extracellular thyroid follicle lumen [60–64] (see section "Compaction and Storage of Tg as Covalently Cross-Linked Thyroid Globules").

Finally, Tg undergoes a highly complex sequence of proteolytic processing, i.e., the irreversible posttranslational modifications that are initiated in the thyroid follicle lumen by extracellular proteolysis before complete Tg degradation continues in the compartments of the endocytic pathway [65–73]. Extracellular proteolytic processing of Tg results in the liberation of TH prior to Tg's re-internalization, thereby also yielding differently sized molecular mass fragments of the TH precursor molecule, which might involve in substrate-assisted pathways of thyroid autoregulation (see section "Tg Proteolysis by Extra- and Intracellular Means and Regulation of Tg Utilization for TH Liberation"). Thus, the bidirectional transport pathway that Tg follows in thyroid epithelial cells is typically completed with its endo-lysosomal degradation (Fig. 1). Alternatively, re-internalized Tg may bypass endo-lysosomes to reach the blood circulation as an intact molecule [32, 74]. Circulating Tg [20,

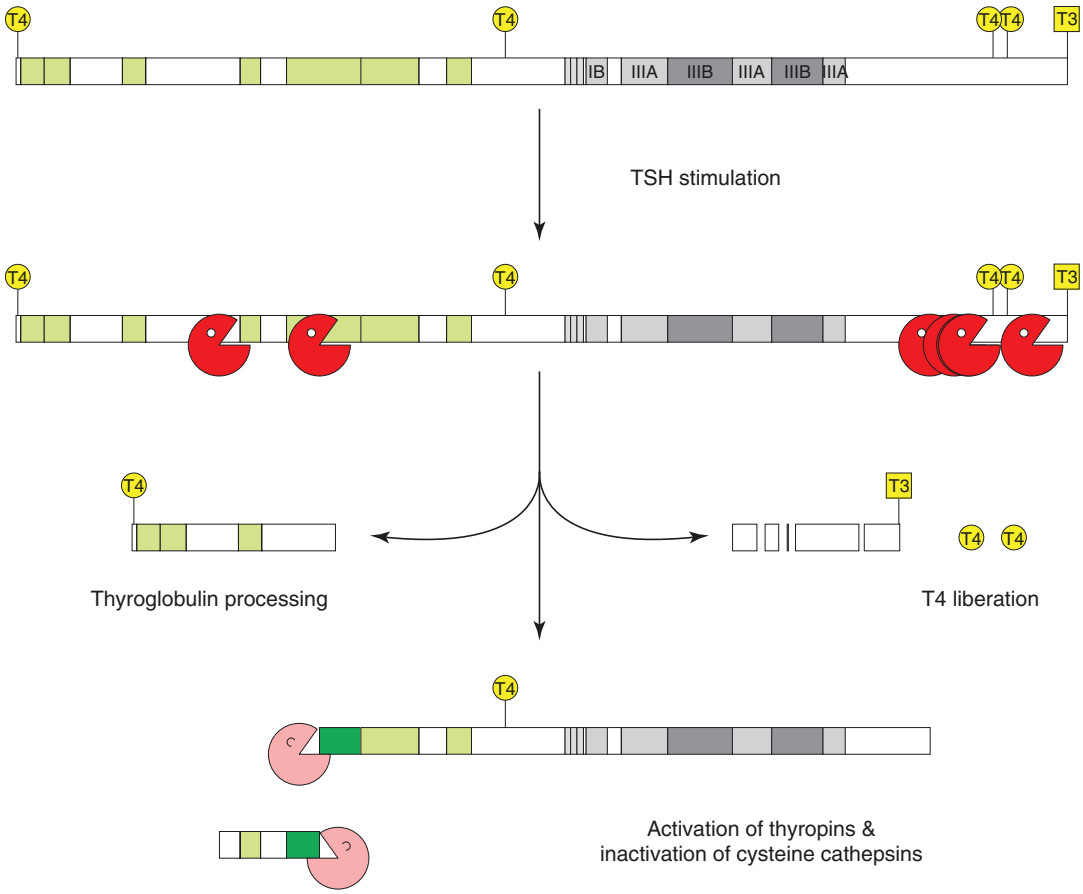

Fig. 2 Proposal of substrate-assisted regulation of Tg proteolysis. Schematic illustration of Tg proteolysis by cysteine cathepsins (*red*) which, upon cleavage of Tg internal sequences (*light green*), may unmask thyropins and render them inhibitory against Tg-processing enzymes in a substrate-assisted fashion (*bright green*). A cysteine cathepsin cleavage site in human Tg has been detected by an in vitro degradation assay which, if used, would yield activated thyropin sequences [72], hence, indicating the principle possibility of such a regulatory mechanism

75] reaches, e.g., the liver, where Kupffer cells have the ability to internalize circulating Tg and to liberate TH by extra-thyroidal means [76–78].

Iodination of Tg in the Thyroid Follicle Lumen: A Unique Posttranslational Modification

Tg iodination by thyroid peroxidase (TPO) for TH generation [10, 79–87] depends on both iodide trapping and an oxidative environment provided by the H_2O_2-generating system consisting of thyroid-unique dual oxidases (DUOXs) and their activating maturation factors, the DUOXAs [88]. However, the exact reaction mechanism of iodination at the side chains of tyrosine residues (involving radical iodine or anionic iodide) and the intramolecular coupling of the resulting iodotyrosyls to form Tg backbone-bound iodothyronines remains somewhat enigmatic [86, 89]. Moreover, it was emphasized that iodotyrosyl formation by iodination of Tg as such is a separate event, unassociated with the coupling reaction that yields iodothyronine formation [90]. Accordingly, iodotyrosine residues are distributed along the length of the Tg molecule, whereas coupling to form protein-bound mono-, di-, tri-, or tetraiodothyronines (preformed MIT, DIT, T_3, or T_4, respectively) happens in a directed fashion [91].

Iodine trapping begins with the entry of iodide through facilitated transport across the basolateral plasma membrane of polarized thyroid epithelial cells which is mediated by the sodium-iodide symporter (NIS) [85, 92]. At the opposite cell pole, the chloride-iodide anti-porter pendrin and the more recently identified calcium-activated anion channel protein anoctamin 1 are believed to enable iodide transport across the apical plasma membrane [85, 93, 94], thereby allowing iodide to reach the extracellular follicle lumen in which the apically located TPO then mediates iodine organification by iodotyrosyl formation on Tg. Iodination at tyrosine residues and coupling to iodothyronines, therefore the generation of T_3 and T_4, preformed at distinct positions in the Tg molecule, occur in the direct vicinity of the apical plasma membrane, because the reactive domain of TPO faces the thyroid follicle lumen and the strictly required oxidative conditions are generated in its pericellular, apically apposed region (Fig. 1). Excessive iodination at the hydroxyl side groups of tyrosine residues can be handled by a recently discovered flavoprotein, the iodotyrosine deiodinase IYD, which is also present at the apical plasma membrane domain of thyrocytes and serves in a salvage pathway that allows efficient iodide recycling [95].

It is important to note that iodination of Tg's tyrosine residues and their coupling to the preformed TH on the Tg backbone is a very complex reaction which is unique for the thyroid gland. It remains unanswered why iodine organification on Tg is such a thyroid-specific reaction—despite the notion of thyroid-specific iodide transporters—and why it does not occur elsewhere in the body, although iodination-competent peroxidases are present in several cell types, including other epithelial tissues. It may be speculated in this regard that the specific architecture of thyroid follicles promotes the iodination reaction on Tg, because the follicle lumen ensures a secluded extracellular environment that restricts all players in this complex and potentially cytotoxic chemical reaction to a small sub-follicular area. This notion and interpretation is also reflected by coining of the term "thyroxisome" which describes the iodination machinery including the H_2O_2-generating system [15, 96]. However, cell biologically speaking, the complex assortment of the molecules constituting the iodination machinery that acts in certain, spatially confined areas of the extracellular thyroid follicle lumen is not enclosed in a biological membrane.

Eventually, one preformed T_3 and four preformed T_4 molecules are positioned at the very N- and C-terminal ends and in the middle of the TH precursor molecule Tg [54, 97, 98]. Although the three-dimensional structure of iodinated Tg molecules is still not known, it is tempting to speculate that the preformed TH are strategically positioned and exposed at the periphery of the Tg molecule, such that their liberation by proteolytic processing is rendered an efficient and very fast process that does not necessarily require complete degradation of Tg [71, 72, 97, 98] (see section "Tg Proteolysis by Extra- and Intracellular Means"). Thus far it can be concluded that the protein sorting and trafficking mechanisms of thyroid epithelial cells are following noncanonical principles, and may even be considered unique, as in the instance of the iodination reaction that occurs just before Tg is compacted and stored in the extracellular follicle lumen and/or taken up by endocytosis for reentry into thyroid epithelial cells.

Compaction and Storage of Tg as Covalently Cross-Linked Thyroid Globules

Tg monomers, dimers, and calcium-compacted multimers are eventually secreted at the apical plasma membrane domain of thyroid epithelial cells and may be further compacted in the extracellular follicle lumen upon iodination. Thus, storage of Tg occurs at astonishingly high protein concentrations reaching up to 800 mg/mL [99, 100]. Luminal Tg is typically found in different hydrodynamical states of compaction [101, 102]. This is to say that the so-called thyroid colloid found in the follicle lumina of mammalian thyroid glands is typically made of multilayered, quasi-crystalline thyroid globules which are surrounded by a variably thick layer of soluble Tg (Fig. 3) [61, 62, 66]. Thyroid globules can occur

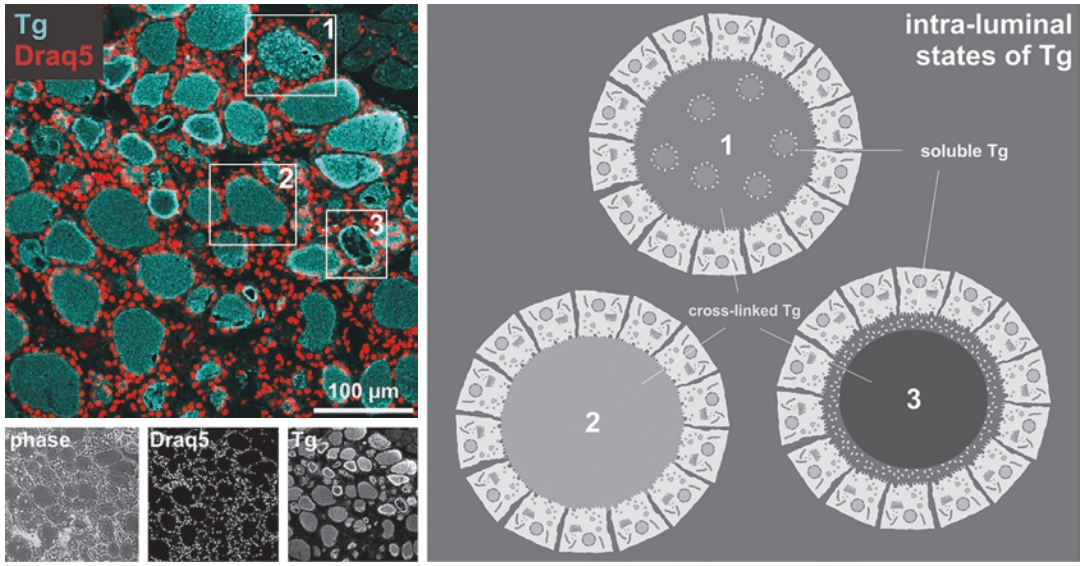

Fig. 3 Different levels of compaction and accessibility of Tg enable and represent the heterogeneity of functional states in which individual follicles exist across a thyroid lobe at any given time. Phase contrast and corresponding confocal fluorescence micrographs of mouse thyroid cryo-sections immunolabeled with Tg-specific antibodies, and counter-staining of nuclear DNA, as indicated (*left*). Schematic drawing illustrating how Tg is stored in the extracellular lumen of thyroid follicles in covalently cross-linked form, thereby acquiring heterogeneous states and different levels of compaction (*right*, cf. *left panel*). Luminal solubilization activity is seen by the formation of ringlike soluble Tg around compacted Tg forms (*box 1*). A uniform Tg labeling indicates a compact, non-solubilized colloid in its cross-linked form (*box 2*), whereas higher-intensity labeling implies solubilized Tg, which allows a higher degree of antibody accessibility to reactive Tg epitopes. In particular, an intense luminal periphery (*box 3*) indicates Tg solubilization by extracellularly secreted Tg-processing proteases at the apical-apposing pole

as one or several entities in the extracellular follicle lumen [61, 62, 66, 101], thereby reflecting the state of Tg utilization for TH liberation in each follicle of a thyroid lobe individually.

A range of enzymes are involved in Tg cross-linking, including disulfide bridges-forming protein disulfide isomerase (PDI), dityrosine-building peroxidase, and tissue transglutaminase which connects Tg molecules by isodipeptide linkages [60–62, 64]. The nature of the cross-linkage between Tg molecules depends to a certain extent on the species in which Tg multimerization occurs, where covalent cross-linkage differs in mouse, rat, porcine, and bovine thyroid glands [66]. Notably, disulfide bonds are most prevalent in human thyroid globules, which consist of several layers of covalently cross-linked Tg [61]. Moreover, especially in the human thyroid gland, self-assisted covalent cross-linkage of Tg is possible due to its ability to act as a disulfide-forming molecule through the thioredoxin "CXXC-boxes" located in the N-terminal half of the Tg molecule [63].

Concentric rings, representing onion-like layers of seemingly separate sheets made of Tg multimers, are easily detected in the thyroid follicle lumen when cryo-sections of thyroid tissue are inspected by phase contrast or immunofluorescence microscopy upon labeling with anti-Tg antibodies (Fig. 3; [66]). However, the concentric rings of Tg deposited in the thyroid follicle lumen are not so prominently observed when thyroid tissue is paraffin-embedded such as routinely performed for standard histological inspection. Thus, different preparation protocols might explain why Tg multimerization by covalent cross-linking had been overlooked for a long time before it was first described in the 1990s [62].

It has been suggested that covalently cross-linked Tg is a sign of aging and represents "insoluble" aggregates of compact Tg in the colloid, characterized by very low, if any, Tg turnover

[101–103]. Hence, thyroid follicles in which covalently cross-linked Tg is stored as "insoluble" aggregates would denote these follicles as older follicles of the heterogenous follicle population of a thyroid gland. However, originally, it was suggested that covalently cross-linked Tg serves to store Tg at high concentrations and in an osmotically inert, compacted form [61, 62]. Thyroid globules persist for long time intervals, before they eventually become solubilized, and Tg is subsequently utilized as TH precursor protein in times of high TH demand [65, 71, 72, 101, 104].

Hence, since its first description [62], the significance of Tg storage as thyroid globules consisting of covalently cross-linked Tg multimers has been discussed in the context of different concepts. The notion of a physiological significance of thyroid globule formation as an iodine storage pool is supported by the observation of the aforementioned concentric rings upon incubating isolated human thyroid globules with H_2O in vitro, which first results in swelling, followed by peeling off of the Tg multimers, layer by layer, from the isolated thyroid globules [61]. These findings indicate several rounds of Tg biosynthesis, deposition into the follicle lumen, and appositional compaction into thyroid globules, to alternate with rounds of Tg solubilization from the storage forms (discussed in [62, 66, 101, 104]). Such a concept would find further support if differently iodinated Tg would be found in the multilayered thyroid globules. This hypothesis is, however, difficult to test since covalently cross-linked Tg is not readily accessible in its intact form to immunolabeling where, in principle, antibodies against differently iodinated Tg could be used (see Fig. 3) [61, 66, 71, 101]. However, in times of high TH demand, thyroid globules gradually disappear from the thyroid follicle lumen [61, 101], supporting their role as iodine and TH storage pool rather than representing aged protein aggregates.

Tg Proteolysis by Extra- and Intracellular Means

Thyroid globules consisting of differently cross-linked Tg forms can easily reach up to ten times the size of a thyroid epithelial cell. Therefore, it was proposed that they are physiologically processed by means of extracellular protease-mediated Tg solubilization from the covalently cross-linked storage forms, a process preceding Tg's re-internalization for endo-lysosomal delivery [65, 66, 71, 73, 104].

Extracellular proteolysis within the thyroid follicle lumen was first suggested in 1941 [105] when the luminal content was aspirated and found to contain acidic proteinases. Several decades later, it was described that Tg can undergo limited proteolysis mediated by extracellularly acting cysteine peptidases, followed by the endocytic uptake of partially degraded Tg into thyroid epithelial cells [65, 71–73]. Thus, thyroid functions are enabled by the sequential proteolytic processing of Tg, resulting in the liberation of T_4 and, to a lesser extent, T_3. In this view, proteolytic processing of Tg for TH liberation starts already in the extracellular thyroid follicle lumen and is completed intracellularly within endosomes and lysosomes of thyroid epithelial cells (Fig. 1).

In situ proteolysis of both human and mouse Tg is mediated by the cysteine cathepsins B, K, L, and S [65, 66, 71–73, 104, 106]. In addition, a plasminogen-like serine protease was proposed to act specifically on Tg multimers of human thyroid follicles [103, 107]. Moreover, the dimeric cysteine cathepsin C and plasma glutamate carboxypeptidase, a metallopeptidase, presumably involve in Tg degradation for TH liberation in the thyroid gland of rats, as these proteases have been detected as secretory products of Fischer rat thyroid cells and have been shown to be able to process Tg in vitro [108]. Therefore, cysteine cathepsins constitute a major group of enzymes among the proteases known to process Tg. Notably, the thyroid functions of cysteine cathepsins are well in line with the notion that these proteases are widely expressed, playing essential roles in selective protein turnover for maintenance of tissue homeostasis and regulation of cell signaling [109–111].

Mice with deficiencies in distinct cysteine cathepsins are characterized by impaired proteolysis of Tg, resulting in its persistence in the thyroid gland of cathepsin B (*Ctsb*$^{-/-}$)- and L (*Ctsl*$^{-/-}$)-deficient animals [71]. The typical mul-

tilayered appearance of extracellularly stored Tg was retained in cathepsin K-deficient (*Ctsk*$^{-/-}$) mice only, suggesting cathepsins B and L to be the main proteases involved in solubilizing Tg from its covalently cross-linked storage forms [71]. Mice deficient in cathepsin L, or both cathepsins K and L, exhibit significantly reduced serum levels of free T_4 (fT_4), indicating that Tg utilization for T_4 liberation is mediated by a combinatory action of cathepsins K and L [71]. Besides, cathepsin K is special among the cysteine cathepsins as it is able to directly liberate T_4 from Tg, while the other Tg-processing enzymes require a combined action with additional exopeptidases to cleave off T_4 from the Tg peptides, derived by preceding proteolytic processing of Tg mono-, di-, or multimers [72, 73].

A rearrangement of the endocytic system with a redistribution of these Tg-processing enzymes toward extracellular locations, plus lysosomal swelling, was demonstrated in thyroid tissue of cysteine cathepsin-deficient mice [71]. Furthermore, cathepsin L was identified as a survival factor for thyroid epithelial cells because its absence resulted in increased numbers of dead cells accumulating in the extracellular follicle lumina of *Ctsl*$^{-/-}$ mice [71]. Cysteine cathepsin-deficient mice were further characterized by flat epithelia and increased follicle areas, correlating with reduced levels of serum fT_4. Thus, animals lacking cathepsin K and L functions feature altered Tg-processing abilities and exhibit a phenotype resembling thyroid goiter [71]. Accordingly, it is concluded that in the mouse thyroid, cathepsins B and L are the main enzymes responsible for Tg solubilization, while T_4 liberation from Tg is mediated by a combinatory action of cathepsins K and L [71]. Thus, in the thyroid gland of mice, cysteine cathepsins play pivotal roles in the utilization of the prohormone Tg for TH liberation via sequential extra- and intracellular Tg-processing and degradation [71, 104].

In an approach simulating the in situ situation of Tg proteolysis in the human thyroid gland, an assay was developed to test for proteolytic processing of human Tg, thereby accounting for redox potentials and pH values corresponding to the conditions expected in the extracellular space of the thyroid follicle lumen and in comparison to the conditions typically detected within endo-lysosomes of mammalian cells [72]. Cleavage patterns of human Tg by different combinations of cysteine cathepsins B, K, L, and/or S indicated distinct, compartment-specific processing of the prohormone [72]. Moreover, and similar to the findings in mice, the localization of cysteine cathepsins in the human thyroid gland was shown not to be restricted to endo-lysosomes, but they are also present in the thyroid follicle lumen [72, 104].

In addition, a plasminogen-like protein, present in the pericellular environment at the apical pole of human thyroid epithelial cells, has been suggested to contribute to Tg degradation by extracellular means, due to this serine protease's ability to specifically degrade "insoluble" Tg multimers [103, 107]. Accordingly, the plasminogen-like protein is likely to also be associated in the regulation of the composition of the luminal content of thyroid follicles in human. It was further proposed that the secreted plasminogen-like serine protease, by acting on "insoluble" Tg forms, prevents accumulation of the latter upon aging [103].

In addition to this likely scenario, the cysteine cathepsin-mediated Tg proteolysis pathways feature specific spatiotemporal action patterns, since cysteine cathepsins and other endopeptidases can act on both covalently cross-linked and soluble Tg alike [71, 104]. This depends on where they meet with their natural substrate, e.g., cysteine cathepsins B, K, L, and S cleave Tg in a limited fashion in the extracellular milieu of the thyroid follicle lumen for initial and fast T_4 liberation, before these enzymes, together with aspartic cathepsin D, completely degrade Tg within endo-lysosomes for liberation of T_3 and T_4 [25, 65–68, 71–73, 104, 112]. However, the prospect of TH liberation from Tg by cysteine cathepsins in the oxidizing milieu of the thyroid follicle lumen has been questioned [113], because reducing and acidic conditions are required for their optimal activity. In keeping with the notion of extracellular stabilization of cysteine cathepsins by an excess of substrate [114–116], experimental evidence was provided that cysteine cathepsins K and S, in particular, liberate T_4 from human Tg in non-favorable, neutral (pH 7.0), and oxidizing

conditions (−150 mV), such as expected for the extracellular thyroid follicle lumen [72].

Recently, a mouse model, i.e., cystinosin-deficient mice (*Ctns*$^{-/-}$), has underlined the necessity of proper redox conditions in thyrocytes as a precondition to both productive Tg folding in the rER and efficient Tg degradation [117]. Cystinosin is a cystine transporter of endo-lysosomal membranes whose deficiency results in lysosomal storage disease phenotypes, first manifesting in the kidney but also becoming prevalent, among other organs, in the thyroid and pancreas of the afflicted patients [118]. Cystinosin deficiency in mice results in hypothyroidism due to altered biosynthesis of Tg through ER stress and altered Tg-processing within endo-lysosomes [117]. Collectively, the results denote redox conditions as an important factor for proper Tg folding in the rER [22] and for the extent of Tg degradation by extra- and intracellular means [71, 72, 104, 117]. Accordingly, hematopoietic stem cell transplantation was eventually shown to rescue the hypothyroid phenotype of cystinosin deficiency, whereby the transplanted hematopoietic stem cells differentiated into macrophage-like cells that interacted with thyroid follicle cells via nanotubes, such that proper redox states were reconstituted in thyroid follicles, restoring the euthyroid state [119].

In conclusion, Tg-processing enzymes that are able to liberate TH from different Tg forms (intact or partially degraded monomers, dimers, or multimers, as well as thyroid globules) must be further studied in more detail, since a complete image appears to still be missing some important players. So far, obvious thyroid phenotypes (hypothyroidism) were described for *Ctsk*$^{-/-}$/*Ctsl*$^{-/-}$ mice [71] and for *Ctns*$^{-/-}$ animals [117]. The proteolytic enzymes, considered thus far as playing a role in Tg utilization, are able to process and degrade Tg either alone (cathepsins K and S) or in combination with other peptidases (cathepsins B, C, D, and L, plasma glutamate carboxypeptidase, and plasminogen-like protease). Therefore, it is likely that additional Tg-processing protease candidates will be identified in the future, when more organ-specific animal models are established, in particular thyroid-specific knockouts. The reason why single protease deficiency is often not enough to cause severe pathological phenotypes, with regard to the thyroid or any other organ, is viewed in the ability of proteases to functionally compensate each other through redundancy, i.e., by transcriptional and translational upregulation of related enzymes upon targeted gene deletion or pharmacological inhibition of a specific protease [71, 109, 111, 112].

A better understanding of the delicate balance between different proteases acting on distinct Tg forms in neutral, or acidic and oxidizing, or reducing environments of the follicle lumen and the endocytic compartments of thyrocytes, respectively, would certainly help to explain mechanistically the thyroid gland's autoregulative mechanisms.

Tg Re-Internalization

Tg reenters thyroid epithelial cells to follow different fates; first and of foremost importance for the TH-generating tasks of the thyroid gland, Tg is internalized upon TSH stimulation of thyroid epithelial cells [120–122], thereby reaching endosomes and lysosomes for its degradation, resulting in exhaustive TH liberation [25, 32, 66, 104, 113, 123]. Second, low-iodinated Tg is internalized in a TSH-independent manner for recycling and to eventually undergo another round of iodination. Different modes of Tg binding to cell surface constituents have been suggested in this regard [123–125], among these, sortilin-mediated recycling pathways of Tg have been shown to occur in male but, astonishingly, not in female mice [126]. Third, non-processed, variably iodinated Tg enters thyrocytes and bypasses endo-lysosomal delivery to become subsequently secreted at the basolateral plasma membrane domain [74, 127, 128], thereby eventually reaching the blood stream.

With regard to the canonical pathway of Tg utilization, Tg re-internalization from its storage compartment—the extracellular thyroid follicle lumen—for subsequent endo-lysosomal degradation is believed to occur either by receptor-

mediated endocytosis or, especially in rodents, by macropinocytosis. The latter uptake mechanism is similar to fluid-phase uptake (small volumes), which does not involve specific ligand receptors and is, therefore, nonselective. However, macropinocytosis is a process that also bears morphological characteristics of phagocytic uptake (large volumes), which typically involves receptors.

Beyond the discussion about the precise cell biological mechanisms and volumes of Tg entities taken up by thyrocytes, a number of potential Tg receptors have been proposed to act at the apical surface of thyroid epithelial cells in this pathway. Such receptors have been suggested to distinguish between different forms of Tg. Thus, Tg endocytosis and its potentially involved receptors constitute part of the "last come—first served" hypothesis [123, 127, 129], which predicts that newly synthesized Tg is taken up right after its iodination upon secretion into the follicle lumen and thus used immediately for TH liberation by lysosomal degradation. Such a mechanism would allow thyrocytes to distinguish high- from low-iodinated Tg, and the involvement of a receptor, specific for iodinated Tg, would indeed make sense to select between Tg molecules of different iodination states [25]. Conversely, it has also been argued that the apical pericellular space of the thyroid follicle lumen is so packed with high concentrations of soluble, mostly well-iodinated, Tg molecules and that thyrocytes would not necessarily need a Tg-specific receptor to further concentrate the TH precursor protein within clathrin-coated pits for its subsequent receptor-mediated uptake [49, 62, 123, 130, 131]. Nevertheless, the debate continues since decades, and a number of receptors have been proposed to be involved in the re-internalization of Tg.

The cation-dependent and cation-independent MPRs were suggested as Tg receptors, thereby considering the M6P modifications on Tg as interaction targets (see section "Biosynthesis of Tg, Its Folding and Trafficking, and Acquisition of Different Posttranslational Modifications") [50]. Such an uptake mechanism would imply that the MPRs are abundantly present at the apical plasma membrane domain of thyrocytes to overcome the fact that the M6P moieties are somewhat buried in the Tg structure, i.e., not well exposed for receptor binding (mannose 6-phosphorylated Tg skips recognition by the CD-MPR of the TGN, see section "Biosynthesis of Tg, Its Folding and Trafficking, and Acquisition of Different Posttranslational Modifications"). This proposition has consequently been ruled out, at least for porcine thyrocytes [49].

Alternatively, an asialoglycoprotein receptor (ASGPR) was suggested to specifically identify exposed galactose or N-acetylgalactosamine residues on desialylated and otherwise deglycosylated Tg [132–134]. It is important to note that the same ASGPR of thyrocytes has been proposed to contribute to pathways of thyroid autoregulation, whereby thyroid-specific gene expression is suppressed by luminal Tg (see section "Further Mechanisms of Thyroid Function Regulation") [135, 136].

Moreover, Tg re-internalization and its delivery to endo-lysosomes have been suggested to be mediated by low-affinity [49] or moderate- to high-affinity receptors of unknown identities [130].

Megalin/gp330 is an LDL receptor-like protein (LRP) [137] that is expressed at the apical plasma membrane of thyrocytes. It was proposed to serve as a receptor binding to the C-terminal portion of monomeric Tg, which is subsequently delivered to the blood circulation by following the transcytotic route of thyrocytes [123, 138–140]. Transcytosis across the thyroid epithelium explains mechanistically the appearance of intact forms of Tg that are frequently detectable in the blood circulation, albeit at low levels [127]. Whether or not such circulating Tg may serve as an extra-thyroidal source of TH is not yet fully understood, although it was shown that circulating Tg is taken up specifically by liver resident macrophages which are indeed capable of liberating TH from circulating Tg in the body periphery [76–78].

All in all, despite the different concepts that have been put forth offering attractive solutions as to how thyroid epithelial cells could efficiently handle the heterogeneous bulk of stored Tg from

the extracellular lumen, the question as to whether Tg re-internalization from the follicle lumen follows nonselective endocytic pathways, or is receptor-mediated, remains posed.

Recently, the genome-wide association study SHIP (Study of Health in Pomerania) has demonstrated that a few traits correlate significantly with thyroid volume and goiter risk. These include genetic loci upstream of or within *CAPZB*, i.e., a gene encoding an actin-binding protein important for the regulation of actin polymerization [141]. The same genetic locus was also found to associate with TSH levels in the SHIP, SHIP-TREND, CARLA, and HUNT studies of Germany and Norway, respectively [142]. Thus, genes coding for proteins that are well-known to be involved in enabling actin dynamics in cell migration and/or phagocytosis were found to correlate with hypothyroid phenotypes. However, further cell biological studies are required to verify and validate these interesting indications derived from the cohort studies. Hence, future studies might clarify whether the detected associations of *CAPZB* and hypothyroidism might nurture substantial support for a revival of the classical concept of macropinocytosis [131, 143, 144]. This cellular process is considered to serve as a fast means of Tg uptake in high quantities, thereby providing thyrocytes with an efficient (but nonselective) mechanism of TH liberation through endo-lysosomal Tg degradation.

Regulation of Tg Utilization for TH Liberation

From the above considerations, it becomes clear that the extracellular thyroid follicle lumen harbors a plethora of functions critical to TH generation. It is the side of Tg iodination for TH preformation on Tg's protein backbone. The follicle lumen serves as storage device in which compaction of Tg is realized through covalent cross-linkage for thyroid globule formation. In addition, the thyroid follicle lumen, together with the apical plasma membrane of thyrocytes, features molecular mechanisms by which the fate of stored Tg is determined toward its subsequent recycling, transcytosis, or degradation. Moreover, limited proteolysis of Tg for its solubilization from the covalently cross-linked thyroid globules and, finally, partial Tg utilization resulting in T_4 liberation is initiated in the thyroid follicle lumen by extracellularly acting proteases.

Another very important notion, however, is that TH liberation by proteolytic processing of Tg is tightly regulated, regardless of whether Tg is utilized extra- or intracellularly. The central regulation of thyroid functions by the so-called HPT axis (hypothalamus-pituitary-thyroid axis) [15, 145] involves activation of thyrocytes from their resting state by thyroid-stimulating hormone (TSH) binding to the basolateral TSH receptors [146]. These belong to the G protein-coupled receptor (GPCR) family [147]. The short-term effects of TSH activation of thyrocytes are mediated by $G_{\alpha q}$ signaling, whereby the phosphatidylinositol cascade is initiated, leading to enhanced cytosolic Ca^{2+} levels [148–150]. In particular, short-term TSH stimulation of thyrocytes causes rapid retrieval of Tg-processing proteases out of endo-lysosomes into transport vesicles destined to the apical cell surface for the subsequent secretion of the active enzymes into the thyroid follicle lumen [104, 106, 111, 151]. Hence, reallocation of vesicles containing, e.g., cysteine cathepsins B, K, and L from the perinuclear region to the apical plasma membrane of thyrocytes via retrograde trafficking initiates Tg solubilization and T_4 release extracellularly [65, 66, 71, 73, 151]. Tg is then re-internalized, further processed, and completely degraded within endo-lysosomes [71, 104]. As a consequence of long-term TSH stimulation, however, transcriptional regulation ensures the restoration of the luminal Tg storage pool via de novo biosynthesis of Tg, mediated by the $G_{\alpha s}$ signaling pathway [39, 121, 152]. In addition, chronic TSH stimulation contributes to transcriptional upregulation of the expression of Tg-processing proteases like the cathepsins B, D, and S [153–155].

Another pathway of regulating Tg utilization is provided by the protein itself. This is to say that specific domains of the Tg molecule, the so-called Tg type-1 domains, or thyropins [156–

158], are proposed to act as inhibitors of the Tg-processing aspartic and cysteine cathepsin proteases, namely, when these are cleaved off in a protease inhibitor-competent form from Tg's protein backbone (Fig. 2). Such a pathway of substrate-assisted autoregulation of TH liberation has been proposed by several groups, but, to the best of our knowledge, it has not been formally proven [66, 72, 104, 159].

Further proposed regulatory pathways of timely termination of Tg proteolysis in the extracellular follicle lumen involve the Tg-selective binding molecule megalin/gp330/LRP2 (see section "Tg Re-internalization"). It is tempting to speculate that megalin may also serve as a receptor that mediates reuptake of the Tg-processing proteases, because it belongs to the class of LDL receptor-related proteins. The LRPs provide "moonlighting" functions due to the multidomain structure of their extended extracellular domains, thereby enabling the binding of a broad range of molecules, including proteases and their inhibitors [160–162]. In the thyroid gland, megalin is strategically positioned at the apical plasma membrane domain of thyrocytes [163], such that its extracellular domain, with the many different ligand-binding properties, reaches out into the follicle lumen. Hence, megalin may not only serve as a Tg binding cell surface receptor, thereby determining the fate of internalized Tg destined to become either degraded or transcytosed (see section "Tg Re-internalization"), but it might also serve as a molecule which can interact with the Tg-processing proteases.

Future studies have to elucidate the molecular and cellular mechanisms that explain intrathyroidal balancing of proteolytic and anti-proteolytic activities acting on Tg as their natural substrate.

Further Mechanisms of Thyroid Function Regulation

TSH stimulation of thyrocytes can be considered uniform, since a uniform supply of this circulating pituitary glycoprotein hormone can be secured through the network of capillaries encompassing each follicle, as well as through a fairly homogeneous distribution of the TSH receptor at the basolateral plasma membrane domain of thyrocytes. Nevertheless, within the same thyroid lobe, individual follicles are typically found at different activation states at any given point in time. The prevalence of such functional heterogeneity across the thyroid, reflected in a heterogeneity in the follicular Tg content, introduces the concept of the "follicular cycle model," wherein the function of the thyroid gland is co-regulated by (1) TSH signaling from the basolateral pole of the thyrocytes and (2) Tg acting from the apical pole onto the thyrocytes of an individual thyroid follicle [135, 164–166].

Accordingly, Tg is believed to act as a negative regulator of thyroid-specific gene expression [167], while TSH is a well-known positive regulator of thyroid differentiation and function. This means that a thyroid follicle rich in luminal colloid content will attain a state of luminal Tg saturation that is "sensed" by self-regulatory means, where Tg acts as a negative self-regulator of its own biosynthesis. Additionally, the storage capacity of individual thyroid follicles is coupled to interfollicular differences in TSH-induced kinetics of Tg utilization. Thus, certain follicles would degrade Tg faster than others, thereby contributing more to the overall TH release from the thyroid gland [168].

Upon TSH stimulation, the initial response is an increased rate of Tg degradation that exceeds the rates of Tg biosynthesis, iodination, and storage. As TSH signaling persists but with luminal Tg content reduced in favor of TH liberation, the thyrocytes will eventually shift in their response in favor of restoring their Tg store, wherein the rate of Tg de novo biosynthesis overtakes the rate of Tg utilization [165, 168]. The morphological equivalent of these cyclic shifts of Tg deposition and Tg utilization is the concentric ringlike layered appearance of intraluminal Tg (see section "Compaction and Storage of Tg as Covalently Cross-Linked Thyroid Globules") [66]. In line with such a regulatory scenario, low follicular content of Tg is known to induce maximal pendrin expression, and is subsequently accompanied by enhanced iodide transport, thereby involving Tg iodination in the regulatory pathways directed by both TSH and Tg [169].

Another essential notion that must also be considered in this context is the "Wolff-Chaikoff effect" (reviewed in [165, 170]) that describes how excess iodide suppresses TH generation by a mechanism that diminishes iodine organification and, therefore, TH generation. This effect is eventually overcome through regulation at the level of iodide import into thyroid follicles, namely, by the downregulation of NIS at the basolateral plasma membrane. Thus, the concept emerges that Tg biosynthesis and iodine organification are mutually regulated by each other, resulting in temporally differing luminal contents of thyroid follicles.

It is concluded that Tg, in addition to TSH and iodine, must be viewed as an important player involving in thyroid autoregulation at the follicular, hence, the local sub-thyroidal level (Fig. 4).

Perspectives

As elegant as the described concepts of thyroid autoregulation are, important questions remain unanswered. Namely, how can the thyroid epithelial cell of one thyroid follicle differentiate between low-iodinated and high-iodinated forms of Tg, while the neighboring thyroid follicle might consume all of its luminally stored Tg at once, indicating that this is high-iodinated Tg giving rise to high amounts of TH eventually reaching the blood circulation? The answer to this question is likely to involve Tg receptors such as megalin. However, the expression of megalin appears astonishingly uniform across neighboring follicles of porcine thyroid tissue [171], and bearing in mind that the blood supply to follicles is uniform, the differences in functional activity between individual follicles of a given thyroid gland remain a mystery.

However, we propose to include the outflow of iodine from the thyroid gland via T_4 and T_3 release from thyroid follicles into the blood circulation by TH transporters as further means of thyroid autoregulatory mechanisms.

TH transporters facilitate selective TH transport across the plasma membrane of TH target cells [172–187]. The monocarboxylate transporter 8 (MCT8) has attracted the most attention in the past, since its functional absence, which leads to alterations in TH export from thyroid follicles and altered TH import into target cells, thus consequently, in the TH amount supplied to the central nervous system, results in severe X-linked psychomotor retardation in patients suffering from the Allan-Herndon-Dudley syndrome [188, 189].

In the human thyroid gland, the monocarboxylate transporter 8 (MCT8) and the type 2 L-amino acid transporter (LAT2) are the main TH transporting molecules [190], while in mice, Mct8, Mct10, Lat1, Lat2 in addition to the organic anion-transporting polypeptide Oatp1c1, and Oatp1a4 are expressed in thyrocytes [191]. The Mct8 protein, in particular, facilitates TH transport across the basolateral plasma membrane of thyrocytes and, thus, enables T_4 export from the thyroid gland [190, 192–195]. In addition, it has been proposed that not only TH export but also TH import into thyroid epithelial cells requires TH transporters [1, 196]. It is further predictable that specific molecules are required to facilitate TH transport across any biological membrane (see Fig. 1). Consequently, TH transporter molecules are not only required at the basolateral plasma membrane domain, but their presence is essential at the apical plasma membrane of thyroid epithelial cells as well as at endo-lysosomal membranes (see Figs. 1 and 4), i.e., at all sub-cellular locations where proteolytic liberation of TH from Tg can occur [197].

However, TH transporters at the apical plasma membrane and at endo-lysosomal membranes of thyroid epithelial cells and their functional relation to the basolateral TH transporting molecules (Mct8 and Mct10) have not yet been studied in sufficient detail. Here, we propose that the main T_4- and T_3-exporting molecules Mct8 and Mct10, respectively, are both present at the basolateral plasma membrane domain of thyroid epithelial cells, where they involve in intrathyroidal regulation of Tg-processing in mice. Indeed, an enhanced extent of Tg-processing by cathepsin proteases B, D, and L in thyroid tissue of Mct8- and/or Mct10-deficient mice was recently observed [198, 199]. Thus, TH transporters would

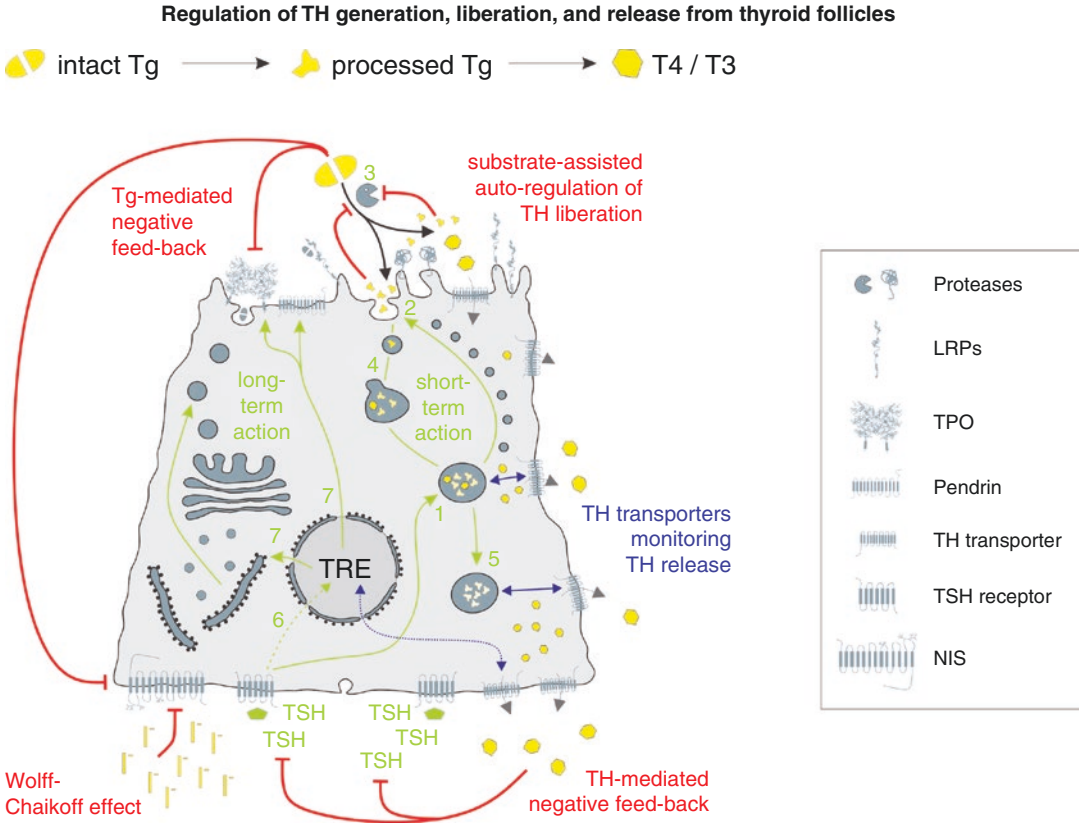

Fig. 4 Canonical and noncanonical feedback regulation of thyroid functions. Schematic drawing depicting different pathways of central and intrathyroidal regulation of thyroid functions. TSH, secreted by the pituitary gland in response to low levels of circulating TH, is delivered to thyrocytes via the blood circulation. It binds to TSH receptors located on the basolateral plasma membrane domain of thyrocytes. The TSH receptor activation results in the short-term TSH action mediated by the $G_{\alpha q}$ signaling pathway, leading to the reallocation of Tg-processing proteases and their subsequent secretion into the extracellular follicle lumen (1 and 2). Then, Tg solubilization begins in the follicle lumen and results in the initial liberation of T_4 (3). Partially degraded Tg molecules are reinternalized into the thyrocyte (4), in part in a receptor-mediated fashion. Intracellular Tg degradation is resumed in endo-lysosomal compartments, leading to further TH liberation (5). The TH is then transported into the cytosol and, next, into the extracellular space via specific TH transporters (e.g., Mct8) located on the basolateral plasma membrane of thyroid epithelial cells, where it can access the blood circulation. TH is then delivered to various target organs, including the central nervous system, where high TH levels negatively feedback on TRH and TSH release from hypothalamic neurons and pituitary cells, respectively. Persistent TSH stimulation activates eventually the $G_{\alpha s}$ signaling pathway, thereby promoting the "long-term" TSH effect, which primarily drives de novo Tg synthesis. This process is initiated by an upregulation in Tg biosynthesis and iodide transport genes (6). Newly synthesized Tg undergoes posttranslational modification in the ER, following which it is transported along the secretory route (7) to be finally secreted into the extracellular follicle lumen, where it undergoes iodination and is compacted into its cross-linked form for storage. Tg accumulation in the follicle lumen exerts negative regulatory feedback on its own biosynthesis genes, as well as on iodide transporters (i.e., NIS and pendrin). Additionally, Tg production is regulated at the level of iodine organification via the "Wolff-Chaikoff" effect

act as critical sensors of the amount of TH made available by the thyroid gland to the body periphery. Thus, it must be clarified how the levels and activities of Tg-processing proteases correlate to the expression levels and localization patterns of TH transporters in thyroid tissue and how TH transporter function correlates to TSH regulation of the thyroid gland (Fig. 4). Answers to these questions will allow a better understanding of how TH levels are sensed by thyroid epithelial

cells [200] and, subsequently, how autoregulation is achieved by integrating TSH-regulated TH liberation through Tg proteolysis and TH transport across the membranes of thyroid epithelial cells. In this regard, we proposed very recently that Taar1 and TSH receptors may co-regulate thyroid functions, namely TH liberation by Tg processing and TSH regulation of thyroid follicles, because Taar1-deficient mice feature mildly altered Tg processing, mislocalized TSH receptors and hyperthyropinemia [201]. This proposal awaits further experimental evidence to predict possible Taar1-TSH receptor co-regulation of thyroid states in species other than the mouse.

Several of the open questions discussed in this review on thyroid cell biology are currently being addressed in the framework of Thyroid Trans Act [196].

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Disorders of Thyroid Hormone Transporters and Receptors

W. Edward Visser

Introduction

Thyroid hormone (TH) is indispensable for normal development and metabolism of all tissues. Primary thyroid diseases characterized by abnormal serum TH concentrations result in a variety of clinical symptoms. Hypothyroid symptoms include cold intolerance, constipation, weight gain, and bradycardia, while thyrotoxic symptoms include heat intolerance, weight loss, anxiety, and increased heart rate. The importance of TH for development is illustrated by the consequences of untreated congenital hypothyroidism, resulting in severe growth failure and permanent intellectual disability [1].

Clinical effects of an altered thyroid state arise from changes in TH physiology at the cellular level. At TH target tissues, cellular TH homeostasis requires adequate function of (1) TH transporter proteins, (2) deiodinating enzymes, and (3) nuclear receptors (Fig. 1). Defects in any of these processes give rise to distinct syndromes, collectively called disorders of TH signaling. This chapter addresses clinical aspects of disorders due to defective transport and receptor function, while SBP2 defects are covered in the chapter by Rayman and Duntas.

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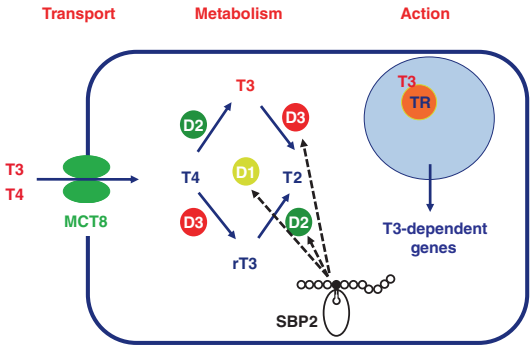

Fig. 1 Model of TH regulation at the cellular level. Transporters are required for uptake and release of T4 and T3. MCT8 is prototypic for cellular T3 and T4 transport. Three deiodinases catalyze activation or inactivation of TH. D2 and D3 are important for local TH regulation. D2 converts T4 to bioactive T3, whereas D3 degrades T3 to 3,3-T2. Insertion of the selenocysteine into the deiodinases requires SBP2. Ultimately, T3 binds to its nuclear receptors (TRs) and modulates gene expression of T3-target genes

Regulation of TH Bioactivity

The hypothalamus–pituitary–thyroid (HPT) axis principally regulates circulating serum TH concentrations. The hypothalamus produces thyrotropin-releasing hormone (TRH), which stimulates the pituitary to produce thyroid-stimulating hormone (TSH). TSH acts on the thyroid gland to synthesize and secrete predominantly the prohormone T4 and to a lesser extent the bioactive hormone T3. Circulating TH inhibits TRH

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and TSH synthesis and secretion, thereby completing the endocrine negative feedback loop.

The vast majority of TH in the bloodstream is bound to carrier proteins. As a consequence, approximately 0.02% of total T4 and 0.3% of total T3 concentrations are available as free hormone in the blood. The main binding proteins are thyroxine-binding globulin (TBG), albumin, or transthyretin (TTR), of which TBG is quantitatively the most important carrier. Mutations in these carrier proteins generally do not produce clinical symptoms but can affect thyroid function tests, which can be easily misinterpreted [2].

Transport of TH across the plasma membrane is a crucial first step to govern intracellular TH concentrations. Given the lipophilic nature of TH, it has been assumed for decades that translocation of TH across the lipid bilayer of cell membranes occurred by diffusion [3]. However, experimental evidence over the last 40 years and clinical studies in the last decade have clearly shown that TH traverses the cell membrane mainly through transporter proteins [4]. Many different transporters have been identified that are capable of TH transport, although only a few are highly specific for TH [4]. Monocarboxylate transporter 8 (MCT8, SLC16A2) has been shown to transport the iodothyronines T4, T3, rT3, and 3,3′-T2 [5]. In addition to aromatic amino acids, the highly homologous MCT10 (SLC16A10) transports T3 and to a lesser extent T4 [6]. MCT8 and MCT10 have a wide tissue expression. The organic anion-transporting polypeptide 1C1 (OATP1C1, SLCO1C1) transports T4 and is importantly expressed in the brain [7].

At the pre-receptor level, deiodinating enzymes importantly control intracellular thyroid state by removing iodine moieties from iodothyronines [8]. In general, the type 1 and type 2 deiodinases (D1 and D2) are regarded as TH-activating enzymes as they catalyze T4 to T3 conversion, while the type 3 (D3) deiodinase (D3) is regarded as an TH-inactivating enzyme. The three deiodinases display a strict spatiotemporal expression. D1 is highly expressed in the liver, kidney, and thyroid and can catalyze outer-ring and inner-ring deiodination. Under physiological conditions, D1 has an important role in serum rT3 clearance [9]. D1 also contributes to serum T3 levels, in particular during thyrotoxicosis. The antithyroid drug propylthiouracil (PTU) inhibits D1 activity, which is of clinical relevance in the context of the management of thyroid storm. D2 catalyzes outer-ring deiodination and is important for the local generation of T3 in the brain, pituitary, brown adipose tissue, and skeletal muscle. The role of D3 is to degrade T3 and T4 to lesser iodothyronines. During fetal development D3 is widely expressed, but during adult life, D3 has a limited expression. D3 can be reactivated under certain pathological conditions [8]. A clinical relevant condition can be rarely observed in tumors expressing strongly elevated D3 activity. The consequent excessive inactivation of TH affects systemic TH levels, and, therefore, this condition is coined consumptive hypothyroidism [10, 11]. Consumptive hypothyroidism requires supraphysiological TH replacement doses, and only removal of the tumor provides definitive treatment.

The genomic actions of T3 are induced via binding to its nuclear T3 receptor (TR), which functions as a ligand-dependent transcription factor [12]. TRs are encoded by two genes, *THRA* and *THRB*, which generate different receptor isoforms. TRα1 is predominantly expressed in the brain, bone, and heart, whereas TRβ1 is considered the major isoform in the liver and kidney. TRβ2, which only differs from TRβ1 at the N-terminus, is present in the retina, cochlea, and pituitary. TRs interact with a number of co-activators and co-repressors to form functional transcriptional units [12]. Binding of T3 to the TR, located on T3 response elements (TREs) in the promoter region of target genes, induces a conformational change of the receptor with release of co-repressors and recruitment of co-activators. The subsequent recruitment of the basal transcription machinery to the gene promoters results in altered transcription of these genes. Change in expression of T3-target genes ultimately underlies the clinical features observed in hypothyroidism or thyrotoxicosis.

Defective Cellular TH Transport (Allan–Herndon–Dudley Syndrome)

To date, the Allan–Herndon–Dudley syndrome (AHDS) is the only known disorder caused by a defective TH transporter. Mutations in *MCT8*, which is located on the X-chromosome, cause the AHDS [13, 14]. It is estimated that mutations in MCT8 account for approximately 1–2% of X-linked mental retardation syndromes [15]. Although the clinical syndrome was reported in 1944, it took 60 years before the genetic basis was identified. Ever since, a large number of families harboring mutations in *MCT8* have been identified [16]. A wide variety of mutations have been reported ranging from large deletions and nonsense mutations to missense mutations that alter expression, subcellular localization, or substrate transport.

Clinical Phenotype

The clinical phenotype comprises a "neurocognitive" component and a "peripheral" component dominated by signs of thyrotoxicosis. Prominent neurological features include a severe intellectual disability with IQ scores mostly below 30 and a globally delayed neurodevelopment. Most patients are unable to talk and hence communicate through sounds and nonverbal expressions (e.g., smiling, crying).

Neurological examination displays hypotonia, which is distinct for the axial muscles and manifested by the inability to keep their head upright and to maintain postural balance without support. Peripheral dystonia is a noticeable extrapyramidal sign. Dystonic posturing can occur spontaneously but is commonly provoked by passive movement of the body. Also, intentional and purposeful grasping (e.g., for toys) nearly always produces dystonia of the used limb and fingers. Dystonia has long been confused with spasticity, which only becomes slowly apparent with increasing age and is more pronounced in the lower versus upper extremities as is evidenced by the occurrence of hyperreflexia, tightening of the heel cords, and a positive Babinski sign [17]. Swallowing difficulties may limit adequate dietary intake and pose an increased risk to aspiration pneumonia.

Seizures have been reported in up to one quarter of AHDS patients [18]. It is important to document seizures with electroencephalogram (EEG), as repetitive movements due to extrapyramidal signs can be mistakenly interpreted as epilepsia.

Body weight, height, and head circumference are unremarkable at birth. Low body weight and profound low muscle mass evolve with advancing age. Since adequate dietary intake is already compromised by the severe neurological phenotype, the progressive decline in body weight can result in life-threatening cachexia. Increased perspiration and tachycardia are frequently noted.

Some patients are less severely affected. They are able to walk with support, have significant verbal comprehension, produce meaningful words, and are well able to communicate using assist devices (e.g., typing words on computers). Such patients are also able to keep their head upright and display less pronounced dystonic features.

Biochemical Studies

The AHDS is associated with abnormal TFTs. Serum T3 concentrations are strongly raised, which is particularly pronounced in childhood. Serum (F)T4 levels are low or in the low-normal range. Also, serum rT3 levels are generally decreased. In particular the T3:T4 ratio and T3:rT3 ratio are strongly elevated. Serum TSH levels are usually within the reference range but deemed inappropriate in view of both the low FT4 and high T3 levels. Little is known about TFTs at birth. Retrospective analysis of neonatal screening records has indicated that T4 levels are low and TSH levels are normal in AHDS patients shortly after birth [19].

Serum markers reflecting peripheral thyroid state can be abnormal due to the peripheral thyrotoxicosis. Illustrative examples include high sex hormone-binding globulin (SHBG) and ferritin levels and low creatinine, creatine kinase (CK), and cholesterol serum levels.

Imaging Studies

Virtually all AHDS patients have undergone brain imaging. A vast array of abnormalities noticed on brain MRI scans have been described in various case reports, with a delayed myelination pattern being a consistent finding [17]. Some parts of the brain ultimately show myelination (e.g., corpus callosum and cortical spinal tracts), whereas other areas remain delayed in myelination (e.g., subcortical U-fibers and periventricular white matter). Diffusion tensor imaging (DTI) can highlight the microstructural changes of white matter tracts [17]. Magnetic resonance spectroscopy (MRS) studies indicate increased brain choline levels, consistent with abnormal myelination [20].

Carriers

Female carriers harboring a heterozygous *MCT8* mutation do not show overt neurological features. Compared to noncarriers, females have somewhat lower serum FT4 levels [21]. One female with AHDS has been described, caused by a chromosomal translocation resulting in disruption of the MCT8 accompanied by a complete nonrandom inactivation of the normal X-chromosome [22].

Mechanisms of Disease

Defective TH transport by mutant MCT8 is the pathogenic cause of the AHDS. The clinical features are best explained by a mix of hypothyroid and thyrotoxic tissues. Depending on the expression of MCT8 and other TH transporters, tissues are either deprived of TH (e.g., brain) or exposed to toxic TH levels (e.g., liver and muscle). The current dogma holds that MCT8 is importantly expressed at the blood-brain barrier (BBB) [23–25]. Therefore, defective MCT8 precludes entry of TH into the brain resulting in inadequate cerebral TH levels. In view of this crucial relevance of TH for normal development, it is conceivable that MCT8 deficiency causes abnormal neurodevelopment. The concept of cerebral hypothyroidism in AHDS patients is supported by MRI scans showing delayed myelination, which is a T3-dependent process as well as postmortem histological studies in brains of AHDS patients [26].

The peripheral thyrotoxicosis is caused by the strongly elevated serum T3 levels. The origins of the typical TFTs in AHDS patients are not clarified yet. Likely, increased D1 activity largely contributes to elevated T3 levels in serum [27]. The low T4 levels are possibly explained by a combination of impaired thyroidal secretion and renal trapping of T4 [28–30].

Treatment

Treatment options for patients with the AHDS are limited and based on expert opinion or small case series.

Referral to rehabilitation physicians early in life is important to advice on measures anticipating contractures and scoliosis. In a multidisciplinary team including physiotherapists and occupational therapists, appropriate therapies, devices, and assistive technology can be individualized. Physiotherapists should acknowledge the prominence of the movement disorder in childhood compared with a stronger spastic component later in life.

All patients should be offered empirical symptomatic treatment. Seizures may warrant antiepileptic drugs. Empirical treatment with drugs to alleviate dystonia and drooling (e.g., anticholinergic drugs) can provide relief, but usually to a limited extent.

Feeding problems arise from difficulties in swallowing. Dieticians can provide advice on food intake, taking into account the catabolic state caused by the thyrotoxicosis in untreated patients. Nevertheless, percutaneous endoscopic gastrostomy (PEG) feeding is often required to meet daily calorie requirements.

Thyroxine has been prescribed to many patients, often before the diagnosis was genetically confirmed. Usually, thyroxine replacement therapy has been empirically initiated because of suspected central hypothyroidism in the context of normal serum TSH and low serum FT4 levels.

This approach is hardly effective in normalizing FT4 levels, but further worsens the elevated serum T3 levels, probably due to immediate conversion of T4 to T3 [15]. Therefore, thyroxine supplementation cannot be recommended for AHDS patients.

To reduce the peripheral thyrotoxicosis, a block-and-replace regimen has been applied in a few patients [15, 31, 32]. Patients were treated with PTU given its inhibitory effects on D1 activity together with a replacement dose of thyroxine. This treatment strategy was able to improve serum T3 levels along with other markers of thyrotoxicosis, although expectedly no neurocognitive improvement was noticed. Other antithyroid drugs (e.g., methimazole) were not effective in reducing serum T3 levels [15]. Given the rare but potentially severe and life-threatening side effects of PTU, the risks and benefits should be carefully balanced when initiating this therapy, in particular the lifelong need of PTU.

Effective therapy should not only normalize toxic TH effects in peripheral tissues but also normalize the decreased TH signaling in the brain. Theoretically, compounds that mimic T3 action but rely on other transporters than MCT8 for cellular entry are suited to reverse or prevent the neurological phenotype in AHDS patients. Also, such analogs expectedly negatively regulate TSH levels, thereby reducing endogenous TH production and secretion. After beneficial effects in Mct8 KO mice were noted, the T3 analog diiodothyropropionic acid (DITPA) has reportedly been applied in four patients [32, 33]. Serum T3 and SHBG concentrations improved in all patients, although no consistent effect on body weight was observed. The absence of beneficial effects on the neurological phenotype can be explained by different possibilities including the irreversibility of brain damage, age beyond therapeutic window, or insufficient access to the brain with the used dose.

Positive effects of triiodothyroacetic acid (Triac) have been observed on neuromotor parameters in an AHDS mouse model [34]. Studies are underway if Triac has potential as treatment in AHDS patients. Future trials should investigate if early initiation of T3 analog therapy can ameliorate the clinical phenotype.

Resistance to Thyroid Hormone Due to Mutations in TRβ (RTH-β)

Resistance to thyroid hormone (RTH-β) is caused by heterozygous mutations in TRβ. The first clinical description of RTH-β was reported over 50 years ago before the underlying genetic defect was identified [35]. Before the causative role of TRβ mutations was known, RTH-β was subclassified as generalized RTH (GRTH) or pituitary RTH (PRTH) based on the constellation of clinical signs and symptoms [36]. This classification was abandoned when genetic studies revealed that patients (within families) harboring identical mutations were assigned to different categories. Careful studies documented that clinical and biochemical markers did not differ between GRTH and PRTH patients [36].

Over 3000 individuals are known with RTH-β, and the incidence of RTH-β is estimated at 1:40,000 [36]. The vast majority is caused by heterozygous mutations, with a few individuals harboring homozygous TRβ mutations or deletions. Most patients have single nucleotide changes in the ligand-binding domain or the adjacent hinge domain, and three different clusters of hotspots have been identified. Between 10 and 15% of patients with features reminiscent to RTH-β do not harbor mutations in TRβ [36]. The molecular basis for this phenomenon is still elusive.

Clinical Phenotype

The clinical presentation is highly variable, ranging from isolated biochemical abnormalities to a mixture of hypothyroid and thyrotoxic features.

The vast majority of patients with heterozygous mutations have a goiter and sinus tachycardia. Approximately half of the RTH-β patients have neurocognitive symptoms. These can include anxiety and emotional disturbances, attention deficit hyperactivity disorder (ADHD), learning disabilities, and frank intellectual disability. Recurrent ear and throat infections are also common, in particular during childhood. A low body weight and height can be noted in children, although most patients reach a normal stature.

A few patients have been reported with homozygous TRβ mutations. Their clinical phenotype is more severe and includes apart from goiter and tachycardia also intellectual disability, delayed speech development and hearing loss, and severe and persistent growth retardation if left untreated [37].

Biochemical Studies

Patients with RTH-β display a typical endocrine fingerprint in serum. Serum FT4 and TT4 levels are elevated accompanied by non-suppressed or elevated TSH levels. T3 levels and rT3 levels are above the reference range as well. In patients with this constellation of biochemical results, RTH-β should be differentiated from a TSH-oma after assay interference has been ruled out. Additional tests include alpha subunits, SHBG (as a marker of T3 action in the liver—normal in RTH-β), and a TRH test (flat in TSH-oma). Markers of TH action in peripheral tissues are usually within the reference range.

Mechanisms of Disease

Most patients with RTH-β have heterozygous mutations. Several mechanisms may explain why heterozygous mutations cause RTH-β. Mutants can exhibit absent or diminished T3 binding and/or display abnormal binding with co-activators or co-repressors. Dominant negative activity of mutant over wild-type receptor explains why heterozygous mutations produce their clinical effects. Only homozygous TRβ deletion results in RTH-β, as wild-type TRβ expressed from one allele is sufficient for function. Therefore, RTH-β is dominantly inherited in heterozygous TRβ mutations, but recessively inherited in TRβ deletions.

The clinical features of RTH-β result from a combination of hypothyroid and thyrotoxic tissues, dependent on the predominant TR isoform expression. In general, mutations in TRβ result in decreased T3 action in predominantly TRβ-expressing tissues, whereas T3 action is increased in predominantly TRα-expressing tissues. As the pituitary solely expresses TRβ, the pituitary is less sensitive in detecting serum TH levels, explaining the non-suppressed TSH levels in the context of elevated circulating TH levels. The tachycardia is explained by the effects of toxic TH levels on the TRα-expressing heart. The growth problems are best explained by advanced ossification and increased mineralization mediated via supraphysiological TH levels acting on bone where TRα is the principal isoform [38]. Likewise, the hyperactivity, anxiety, and learning disabilities can be explained by elevated TH levels in the brain, which mainly expresses TRα. Alternatively, it has been proposed that mutant TRβ interferes with normal TRα function in tissues where both isoforms are expressed.

Imaging Studies

Ultrasound evaluation of the neck may be helpful to document and evaluate progression of a goiter. X-ray studies may reveal delayed bone maturation in children with RTH-β [39].

Treatment

There is no standard treatment for RTH-β. Given the large variability in symptoms, therapy should be individually tailored to alleviate symptoms. Beta-blockers may be helpful to reduce tachycardia.

In a subset of patients, administration of the T3 analog Triac, which has a higher affinity for TRβ than TRα, can be useful. Triac is still able to bind and modulate most of the mutated TRβ receptors. Triac interferes with TSH production in the pituitary, thereby reducing endogenous TH production and secretion, while simultaneously Triac mimics T3 action in other tissues. Case series indicate that Triac may normalize TFTs, alleviate symptoms of thyrotoxicosis, and improve nervousness, attention deficit, and hyperkinetic behavior and restlessness.

Monotherapy with antithyroid drugs has no standard place in the treatment of RTH-β. Antithyroid drugs lower serum TH levels and, consequently, increase serum TSH levels, thereby further stimulating goitrogenesis in both children and adults. Under certain circumstances, such as

children with failure to thrive and uncontrolled hypermetabolism, cautious use of antithyroid drugs might be prescribed [36].

Resistance to Thyroid Hormone Due to Mutations in TRα (RTH-α)

Although TRα was cloned 30 years ago [40] and its importance clearly established by transgenic mouse models [41–43], the first patients with inactivating mutations in TRα were identified in 2012 [44, 45]. Although the prevalence of RTH-α is unknown, it might be anticipated that it is similar to RTH-β. Mutations in TRα are either nonsense or missense mutations affecting the LBD.

Clinical Phenotype

Many patients display features of abnormal bone development. Growth retardation is noticed in childhood and, if left untreated, results in a short stature. In particular, the lower limbs seem disproportionally small [46]. In infancy, delayed closure of the fontanel and skull sutures has been reported. Macrocephaly is commonly observed. Clinical examination may reveal a broad face, flattened nose, macroglossia, and thick lips. An excessive number of skin tags are present in most adult cases, predominantly localized in the face, neck, and upper chest [46].

Patients may present with delayed motor and mental milestones in childhood. In particular, abnormalities in fine and gross motor skills manifested as dyspraxia, difficulty ("clumsiness") climbing stairs, ataxic gait, and dysarthria were noted. Frank intellectual disability including moderately low IQ has been reported in the minority of cases. Due to delayed bowel movements, constipation is a frequently encountered problem.

Biochemical Studies

Typical TFTs show normal serum TSH levels in the presence of low or low-normal FT4 and TT4 levels accompanied by raised FT3 and TT3 levels. Very low serum rT3 levels can be present, producing markedly elevated T3:rT3 ratios. However, many RTH-α patients do not display these characteristic hallmarks and TFTs, when tested, are within the normal range [47].

Mild normocytic, normochromic anemia is present in virtually all cases. Other hematological indices (iron, ferritin, folate, vitamin B12) are reportedly normal. CK levels can be elevated. IGF1 levels are reportedly low or low-normal.

Imaging Studies

X-ray studies are useful to detail delayed bone maturation. X-ray of the skull may reveal wormian bones, delayed fontanelle closure, and a thickening of the skull.

Delayed tooth eruption can be documented by dental radiographs. MRI studies of the brain may show microcephaly and smaller cerebellar size.

Mechanisms of Disease

At the molecular level, the pathogenic mechanisms are similar to those in RTH-β. TRα mutants bind T3 with variably affinities, and binding to co-activator or co-repressor proteins can be disturbed [46]. Dominant negative effects represent the interference of mutant TRα with wild-type TRα, a phenomenon also observed in patients with TRβ mutations.

The clinical features of RTH-α are best explained by diminished TH signaling in tissues that express the TRα isoform. The delayed bone maturation and growth are in keeping with the prominent role of TRα in bone through which T3 exerts its effects [38]. As TRα is the main isoform in the brain, the neurocognitive phenotype is best understood in the context of abnormal TH signaling in the developing brain. Also, constipation is explained by diminished TH signaling in the gut, as it has a predominant TRα expression.

The abnormal constellation of thyroid parameters are not completely understood, but likely caused by abnormal deiodination. Possibly, D3 activity is dysregulated as it is under the control of TRα in physiological conditions [48].

Treatment

Beneficial effects of thyroxine have been described [44, 45]. Administration during childhood reportedly increased height [44] and constipation [45]. Although positive effects of thyroxine initiation in infancy were associated with improved neurocognitive development [49], the neurocognitive phenotype may become less obvious during the natural course of this disease. Possibly, thyroxine treatment in adulthood might improve certain neurological features such as dyspraxia and social interaction [46].

A remarkably consistent finding is the absence of any thyroxine effect on anemia. The consequences of thyroxine treatment in RTH-a on the longer term are unknown. Given that TSH is readily suppressed, TRβ-expressing tissues might be exposed to elevated TH levels, possibly resulting in undesired thyrotoxic effects.

Theoretically, compounds that modify mutant TRα or interfere with the dominant negative activity of mutant TRα by preventing or altering aberrant co-repressor binding or histone deacetylase enzymatic activity could improve the phenotype [46]. In an RTH-α mouse model, the HDAC inhibitor suberoylanilide hydroxamic acid ameliorated some phenotypic abnormalities [50].

Thyroid Hormone Metabolism Defect

No disorders caused by inactivating mutations in any of the deiodinases have been reported yet. Deiodinases are selenoproteins as they contain the rare amino acid selenocysteine (Sec). Sec is crucially required for normal enzyme function, since it is located in the catalytic domain of the deiodinases. The genes encoding selenoproteins form a distinct group of 25 proteins in the human proteome and recode the UGA codon into a Sec. This requires the presence of an RNA stem loop structure called the Sec insertion sequence (SECIS) element, which is located in the 3'-UTR of the deiodinase mRNA. SECIS-binding protein 2 (SECISBP2 or SBP2) binds to the SECIS element and subsequently recruits selenocysteine transfer RNA ($tRNA^{[Ser]Sec}$) to insert Sec at the UGA position.

Three disorders involving defective processing of selenoprotein synthesis have been reported: mutations in SBP2, in SEPSECS, and in $tRNA^{[Ser]Sec}$.

Mutations in SBP2 affect selenoprotein synthesis, including the deiodinases. Since the discovery of the first patients with homozygous or compound heterozygous mutations in SBP2 in 2005, eight families have been reported [16]. SBP2 deficiency is a multisystem selenoprotein disorder with a typical thyroid fingerprint: serum (F)T4 and rT3 levels are raised, while T3 levels are low or low-normal, causing an elevated T4:T3 ratio, whereas TSH levels are either in the upper normal range or slightly elevated. All patients reportedly have delayed growth, but normal final height is reached in all patients. Most patients have myopathy, particularly of the proximal lower limbs and sensorineural hearing loss. Also, increased fat mass has been observed. Other reported (adult) features include central obesity, primary infertility, delayed developmental milestones, and enhanced skin photosensitivity [51, 52]. Measurements of selenoproteins in serum

such as glutathione peroxidase (GPx) and selenoprotein P (SePP) are decreased. Likewise, serum levels of selenium are typically low. X-ray studies can demonstrate delayed bone growth during childhood, while MRI scans can document abnormalities of specific muscle groups and an elevated ratio of subcutaneous fat to visceral fat [52]. Pulmonary function testing may indicate decreased pulmonary capacity likely due to myopathy.

Reduction in SBP2 activity principally affects the whole selenoproteome. The variation in clinical features between patients and compared to other selenoprotein deficiency syndromes is likely explained by residual activity of the SBP2 mutants and the biological hierarchy of selenoprotein synthesis. The clinical features result from deficiency of tissue-specific selenoproteins or accumulation of reactive oxygen species due to impaired antioxidant function (glutathione peroxidases and thioredoxin reductases). The exact mechanisms underlying the abnormal TFTs are not well understood, but the elevated T4 and rT3 levels over T3 levels indicate that outer-ring deiodination is predominantly affected.

There is no standard treatment for patients with SBP2 deficiency. Although growth is delayed in SBP2 deficiency, patients likely reach a normal final height spontaneously. Therefore, standard triiodothyronine administration in SBP2-deficient patients is not recommended. Selenium supplementation has been reported, but beneficial effects were not observed [53]. Vitamin E treatment can be considered to reduce elevated peroxidation products [54].

One patient has been reported with defective $tRNA^{[Ser]Sec}$ who bears partial similarity to SBP2-deficient cases [55]. Patients with SEPSECS deficiency have a severe neuromotor phenotype, but no obvious TFT abnormalities [56].

Conclusion

Disorders in TH signaling represent a growing group of clinical distinct entities. An overview of most common observations is summarized in Table 1. Although the molecular mechanisms are being increasingly elucidated, the need remains to cross-reference molecular studies with clinical observations to fully comprehend the underlying pathophysiology. Systematical clinical studies will help to further characterize and refine the clinical features of these disorders. As TH signaling disorders are rare diseases, treatment options should be explored in well-controlled studies through global joint efforts.

Table 1 Clinical, biochemical, and imaging studies and treatment in thyroid hormone signaling disorders

	Transport	Metabolism	Nuclear receptors	
Gene name	*MCT8*	*SBP2*	*THRB*	*THRA*
Clinical manifestations				
Neurocognitive	Severe intellectual disability and delayed motor development; dystonia	Mild delayed mental and motor development	ADHD; anxiety; intellectual disability in minority	Mild to severe delayed motor and mental development
Muscle	Hypotonia	Muscle weakness	Normal	Hypotonia can be present in early childhood
Growth	Decline in body weight during childhood	Delayed bone age and growth retardation in childhood	Delayed growth in minority during childhood	Delayed bone age and growth retardation
Heart	Tachycardia	Normal	Tachycardia	Normal or bradycardia
Other	Seizures	Skin photosensitivity; primary infertility		Skin tags; constipation
Laboratory studies				
TSH	Normal	Normal or slightly elevated	Normal or elevated	Normal
FT4	Low; low-normal	High	High	Normal or low-normal
T3	High	Low or low-normal	Normal or elevated	Normal or slightly elevated
rT3	Low	High	High	Low or normal
SHBG	High	High	Normal or high	Normal or high
Other	CK, creatinine low	Low selenium levels; low GPX activity	Lipids slightly elevated	Mild normocytic anemia
Imaging studies				
	Delayed myelination (MRI-brain)	Fatty infiltration in paraspinal/adductor muscle (MRI)	Delayed bone age (X-ray); goiter (thyroid ultrasound)	Delayed bone age and skull wormian bones (X-ray); microcephaly (MRI)
Histological studies				
	Low expression of T3-dependent genes (brain)	Myopathy (muscle)	Follicular hyperplasia (thyroid)	
Treatment				
	Supportive (anticholinergics; antiepileptic drugs); PTU/ thyroxine for thyrotoxicosis; T3 analogs (study)	Vitamin E can be considered	Supportive (beta-blockers)	Thyroxine

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Deiodination and Peripheral Metabolism of Thyroid Hormone

Monica Dentice and Domenico Salvatore

Introduction

Canonical thyroid hormone (TH) signaling results from the interaction of T3 with nuclear receptors and stimulation or repression of target genes. Ligand (T3) availability is under tight control of several intracellular checkpoints, which enable target cells to modify their own T3 fingerprint. A crucial step of intracellular T3 metabolism is catalyzed by the deiodinases. These enzymes can, within the single cell, enhance (D1 and D2) or reduce (D3) T3 concentrations. Thyroid hormone transport within the target cells is also a limiting step of thyroid hormone action. Various specific transporters have been isolated for the entrance and the clearance of the iodothyronines and constitute a complex system of active transport of THs inside and outside the cells. Concerted modulation of the different TH regulating factors is responsible for a spatiotemporal precise adaptation of the hormonal signal to the different cell-specific requirements.

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Peripheral Metabolism of Thyroid Hormones

The thyroid gland accumulates iodide from the circulation to produce the thyroid hormones T4 and T3. These are iodinated molecules that exert diffuse and pleiotropic effects in vertebrates. Although both molecules are biologically active, T3 is the most active thyroid hormone that can bind to thyroid hormone receptors and regulate the expression of thyroid hormone-regulated genes [1]. T4 (levothyroxine) is a pro-hormone that must be converted into T3 (triiodothyronine) to be active (Fig. 1). A consequence of this process is that there are two sources of T3, one directly produced and secreted by the thyroid gland and one derived from tissue conversion of T4 into T3. Of note, the human thyroid produces less than 20% of the body's T3 (about 30 μg/die) and peripheral conversion is responsible for 80% of it [2]. Thyroid-generated T3 (about 5 μg/die) is obtained partly via intracellular thyroglobulin digestion and partly via intrathyroidal T4-to-T3 conversion. These two processes produce thyroidal secretion of both T4 and T3 in a molar range of approximately15–11:1; this ratio may change depending on such conditions as iodine supplementation or thyroid disease. The conversion of T4 to T3 occurs consequent to the removal of an outer-ring iodine on T4 (known as "5′-deiodination") which is catalyzed by two enzymes, deiodinases type 1 and type 2 (D1 and D2) [3].

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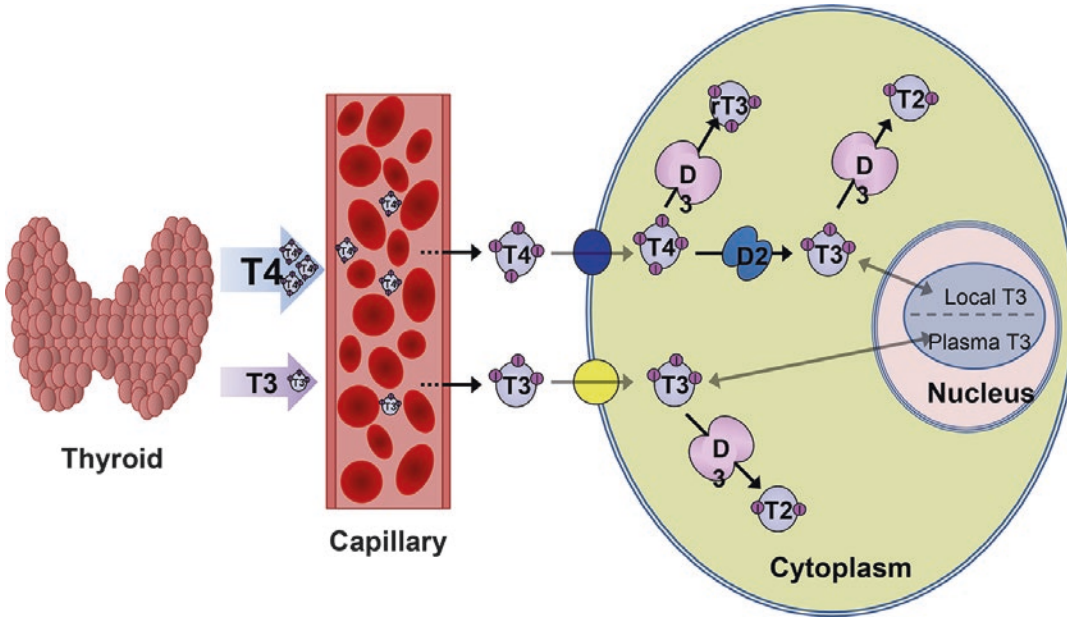

Fig. 1 *Thyroid hormones landscape.* Thyroid hormones T3 and T4, produced by the thyroid gland, are transported by the plasma to each target tissue where they can be activated or inactivated by deiodinases. Only the active form, T3, can bind receptors thereby activating or repressing the expression of target genes

Importantly, these enzymes are expressed in a tissue-specific fashion and are independently regulated to enable different tissues to modulate their "thyroid status" (i.e., to regulate the percentage of saturation of thyroid hormone receptor in the single cell) irrespective of serum thyroid hormone levels. 5′-Deiodination by D1 and D2 can increase intracellular T3 levels thereby potentially leading to intracellular thyrotoxicosis. In the opposite direction, type 3 deiodinase (D3) is considered the physiological inactivator of thyroid hormones because it catalyzes the conversion of T4 and T3 into inactive products (rT3 and T2) via a 5-deiodination reaction. This process locally produces a state of relative hypothyroidism.

Deiodination and Deiodinases

Every cell in the body is a potential target of thyroid hormones, which regulate the metabolism, growth, and differentiation. In healthy conditions, the thyroid gland produces about 80% of the body's T4 and about 20% of the body's T3. However, T3 is considered the only bioactive thyroid hormone because it has a much stronger affinity for thyroid receptors than the other iodothyronines.

Conversion of T4 to T3 by deiodination is the first step of thyroid hormone action and not only is regulated by the thyroid but is also catalyzed within target cells by a family of three selenoproteins, the iodothyronine deiodinases, that catalyze the reductive dehalogenation of iodothyronines, which is the major metabolic pathway regulating thyroid hormone action at pre-receptoral level [1, 3]. Depending on whether deiodination occurs on the inner (IDR) or outer ring (ODR) of the iodothyronine substrate, deiodination results in an activating pathway, or inactivating pathway, respectively. The rare amino acid selenocysteine (Sec) is essential for the dehalogenation reaction [4], as demonstrated by the finding that replacement of selenocysteine-cysteine drastically reduces the affinity of all three deiodinases for their substrates [3]. The main features of the deiodinases are shown in Table 1.

Type 1 deiodinase (D1) catalyzes both inner- and outer-ring deiodination mainly in the liver, kidney, and thyroid. D1-dependent deiodination is the only deiodination highly sensitive to inhibi-

Table 1 Main biochemical features and physiologic relevance of iodothyronine deiodinase

Human Iodothyronine Selenodeiodinases

	D1	D2	D3
Biochemical properties			
Molecular weight of monomer (Da)	29000	30500	31500
Half-life	Several hours	Approximately 20 minutes	Several hours
Subcellular location	Plasma membrane	Endoplasmic reticulum	Plasma membrane
Tissue with high activity	Liver, kidney	CNS, pituitary, brown adipose tissue, placenta	Placenta, CNS, hemangiomas
Physiological role	Clearance of rT3 and T3S	Thermogenesis, development, provides intracellular T3, major source of plasma T3	Development, clearence of T3 and T4, avoidance of intracellular T3 production
Role in diseases	Main source of plasma T3 in hyperthyroid patients	???	Consumptive hypothyroidism, increased T4/T3 clearence in pregnant women, illness

tion by PTU [5]—a finding that led to the first demonstration of the specificity of the T4-to-T3 conversion [6]. The human *Dio1* gene consists of four exons. The selenocysteine codon is located in exon 2 and the selenocysteine insertion sequence (SECIS) element is located in the fourth exon [7]. The *Dio1* gene encodes a protein of 27 kDa, and the molecular mass of the solubilized wild-type enzymes is about 50–60 kDa, which suggested that, like the other two enzymes, D1 forms homodimers in its native form thanks to integral membrane residues [8].

Thanks to its tissue-specific expression, D1 contributes to the regulation of systemic T3 levels by providing a significant portion of the circulating plasma T3 in euthyroid vertebrates, including humans [9–11]. In hyperthyroid conditions, increased thyroidal D1 activity is the first cause of the elevated T3 concentrations observed in hyperthyroid patients. Apart from hyperthyroidism, D1 plays critical roles in the non-thyroidal illness syndrome (NTIS) and in several human neoplasias [7]. Although the pathogenesis of NTIS is still controversial and some observations seem to question the effective roles played by deiodinases in the low T3 levels in illness [12, 13], it has been demonstrated that the peripheral T4-to-T3 conversion is reduced in illness due to a decrease in both hepatic/renal D1 activity and skeletal muscle D2 activity, which in turn increases the levels of rT3 and T2 [14, 15].

Besides its canonic deiodination activity, D1 modulates the clearance of TH [16]. Indeed, D1 has a remarkable substrate preference for reverse T3 (rT3) as well as for sulfated iodothyronines and could act as a scavenger to recycle iodine to enable the thyroid homeostatic production of THs [16]. Accordingly, in D1KO mice, iodothyronines escape deiodination and are massively excreted with a marked loss of the associated iodine, which demonstrates that D1 serves to recycle iodine within the organism and might be particularly important in an iodine deficiency setting [16].

Type 2 deiodinase (D2) mediates primarily outer-ring deiodination, and given its high affinity for T4, it is considered the main activator of T3 at peripheral level [17]. The human D2 protein is approximately 31 kDa and contains a

hydrophobic NH2 terminus and two selenocysteine residues, one in the active center and the other located close to the 3′ end of D2 mRNA [3, 18].

The activity of D2 has been extensively studied in brain and brown adipose tissues (BATs), where the D2-mediated T4-to-T3 conversion is an essential component of thyroid hormone action. In the brain, D2 is expressed in astrocytes, which convert T4 into T3 for the brain requirement by producing more than 75% of the nuclear T3 in the cerebral cortex in rat [2]. In the fetus, the brain almost entirely relies on the T3 generated locally by D2, while systemic T3 plays a critical role in the late postnatal and adult stages [19]. Such a finely tuned regulation of D2 activity correlates with T3's highly sensitive requirement of TH in the developmental period and shows how deiodinases can activate or inactivate TH in specific extraglandular tissues, in a spatial and temporal regulated fashion.

In brown adipose tissue (BAT), D2 is barely expressed in normal conditions but increases 10-fold–50-fold during cold exposure [20]. In vivo, BAT-specific D2 expression is essential for adaptive thermogenesis. Indeed, despite normal plasma T3 concentrations, cold-exposed D2KO mice are hypothermic and cold-intolerant due to impaired BAT thermogenesis, and they survive only by compensatory shivering which results in acute weight loss [20]. Accordingly, in vitro, brown adipocytes from D2KO mice have a markedly attenuated susceptibility to sympathetic stimuli by norepinephrine and forskolin, which induced only modest lipolysis, UCP1 mRNA, and O2 consumption due to impaired cAMP generation [20].

Recent findings have shown that D2 plays a role also in the skeletal muscle. Although it has long been known that D2 is expressed in the muscle [21], its biological role in the muscle has only recently been demonstrated because of its low levels [22, 23]. We recently demonstrated that D2 is required for normal mouse skeletal muscle differentiation of muscle stem cells (satellite cells) and regeneration (Fig. 2). The regenerative ability of mice lacking D2 and D2-null satellite cells is sharply delayed and can be rescued in vitro by T3 treatment. Furthermore, more sensitive assays of muscle D2 activity have shown that D2 is present and higher in slow than in fast muscles [24, 25].

Type 3 deiodinase (D3) has only inner-ring deiodination ability, thus preventing T4 activation and terminating T3 action, which is the main physiological inactivation of thyroid hormone action [26]. Human and mouse *Dio3* genes comprise a single exon, coding for a protein of 278 residues,

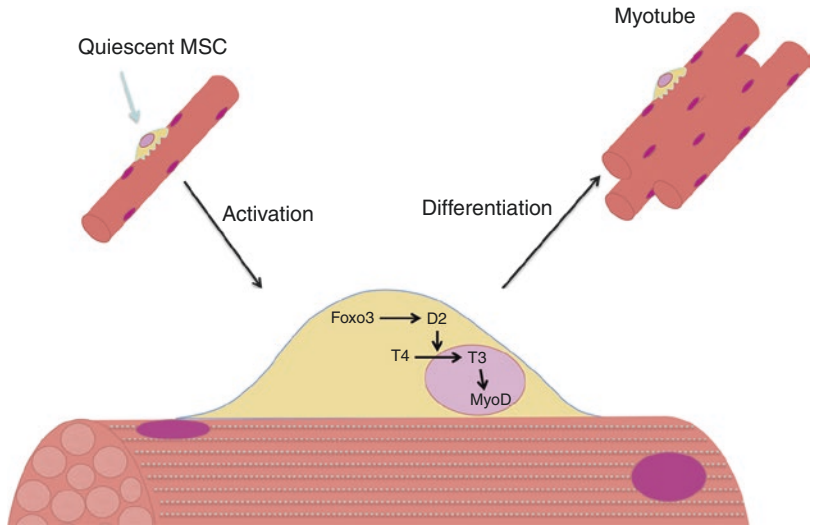

Fig. 2 *Role of D2 and the local production of T3 in myogenesis and satellite cell differentiation.* Quiescent satellite cells (MSC) can be activated by a stimulus such as a trauma and, once activated, undergo a program of regeneration-differentiation which is tightly regulated by T3. Type 2 deiodinase is positively regulated by the transcriptional factor FoxO3a and is upregulated during muscle differentiation. T3 activation by D2 increases MyoD expression and enables proper differentiation of myofibers

with a molecular mass of about 32 kDa, including a selenocysteine-encoding TGA in the catalytic pocket and an SECIS element in the 3'UTR [27]. A unique aspect of D3 is its high expression in developmental tissues and its absence in almost all adult tissues (with the exception of the skin and brain). Therefore, studies on D3 have focused on its role during development and have shown that D3 action is critical to protect the fetus from excessive exposure to active thyroid hormone [28]. However, studies conducted in the last two decades have shown that D3 expression is reactivated in specific pathophysiological contexts correlated with hyperproliferation conditions, such as tissue repair [29–31], inflammation [32], and cancer [33, 34]. Akin to fetal growth, most of these conditions are characterized by an elevated proliferation rate and cell growth, thereby introducing the new concept of D3 as oncofetal protein [26]. One of the most robust indications that many cancer cells reactivate D3 expression came from the discovery of the impressively low serum levels of THs found in patients with juvenile and adult hemangiomas, which was classified as "consumptive hypothyroidism" since it was discovered that by producing high levels of D3, the inactivation rate of thyroid hormone in the tumor exceeded the secretory capacity even of the thyroid gland, thus resulting in hypothyroidism [35, 36]. Later, two similar examples in basal cell carcinomas (BCC) of the skin and in colon cancer showed that D3 expression and activity were much higher in these two epithelial tumors than in the relative normal tissue. D3 inactivation in BCC and in colon cancer drastically reduces tumorigenesis both in vivo and in vitro. Importantly, the Shh/Gli2 pathway, miR21 pathway, and the Wnt-/β-catenin pathway are positive regulators of D3 expression in these two neoplasias, which highlight the relevance of D3 in oncogenic networks [26, 37].

Heart failure and several cardiac disorders are accompanied by alterations in TH levels and D3 expression [38, 39]. D3 is significantly induced during severe heart failure, and the fetal program is reactivated [40]. The finding of direct regulation of D3 transcription by the hypoxia-inducible factor (HIF-1α) reinforced the concept of D3 as a critical TH regulator in the cardiac-specific hypothyroid condition that follows myocardial infarct [38].

D3 also participates in the innate immune response by reducing T3 bioavailability and probably by supplying iodine required by myeloperoxidase to enable inflammatory cells to kill microbes [41]. Extensive studies carried out by Boelen and Fliers have shown that inflammation is often associated with low serum TH levels and with changes in the expression of liver D1 and D3 and of muscle D2 and D3 [32]. Both acute and chronic inflammation are marked by high D3 expression in neutrophils that infiltrate infected organs, predominantly polymorphonuclear cells and granulocytes, suggesting that enhanced degradation of T3 during inflammation may contribute to such a process, although the underlying mechanism is not yet known [42–44]. Similarly, D3 has been suggested as one of the effectors contributing to the pathogenesis of NTIS by reducing circulating TH levels during chronic illness [45]. Indeed, reactivation of D3 activity has been reported in the liver and skeletal muscle of critically ill patients, and moreover, high D3 levels were found in skeletal muscle biopsies from patients with septic shock and NTIS [46]. Importantly, D3 expression in these contexts positively correlates with serum rT3 [47] and negatively correlates with the serum T3/rT3 ratio [45].

Liver regeneration is a classic example of D3 reactivation associated with cellular proliferation. After keratectomy of 70% of the liver, quiescent liver cells reenter the cell cycle (G1 phase) and start a proliferation phase as the first step of liver regeneration [48]. These early regeneration events are characterized by reactivation of many fetal genes, which are not expressed in normal adult liver [48, 49]. D3 is among these genes [29]. D3 activity indeed was increased tenfold 20 h after partial hepatectomy in mice and was associated with a decrease in D1; BrdU levels, marker of proliferation, correlate with D3 activity. Importantly, serum T3 and T4 levels decreased and reached minimum levels 36 h after partial hepatectomy, thereby coinciding with the peak in D3 activity and BrdU incorporation [29].

The elucidation of the role played by deiodinases in all the above-reported biological processes revealed the importance of the deiodinases in the homeostasis of thyroid hormone action and opened the possibility of exploiting deiodination

to specifically modulate TH action in local circumstances and to overcome alterations of circulating hormone levels.

Transporters

The lipophilic biochemical nature of thyroid hormones has long supported the notion that passive diffusion across the plasma membrane is sufficient to enable thyroid hormones to bypass the bilayer of the eukaryotic plasma membrane. Only three decades ago did it emerge that specific carrier-mediated mechanisms of active transport are necessary for thyroid hormones [50]. Subsequently, many transporter families were identified, among which, monocarboxylate transporters (MCT 1–10), organic anion-transporting polypeptides (OATP), the L-type amino acid transporters LAT1 and LAT2, and bile acid transporters [51, 52]. These transporters mediate TH uptake in an energy- and Na+-dependent manner [53], and together with deiodinase action, this represents a critical pre-receptoral step in the control of TH availability in the cell.

Evidence of the physiological relevance of the MCT8 transporter came from the discovery of patients carrying the MCT8 mutation who had elevated serum T3 levels and severe psychomotor retardation, thereby indicating that MCT8 plays a pivotal role in brain development [54, 55]. Male MCT8-null patients are affected by the Allan-Herndon-Dudley syndrome (AHDS), which is an X-linked inherited brain disorder that causes severe neurologic dysfunctions [54, 55]. It also causes central hypotonia, muscular hypoplasia, spastic tetraplegia, and a global delayed development and myelination [56]. The observation that fibroblasts from AHDS-affected patients, associated with intracellular hypothyroidism, increased D2 activity and elevated TSH levels demonstrates that the absence of MCT8 generates a state of relative insensitivity to thyroid hormone. The clinical symptoms associated with AHDS indicate that the MCT8-expressing neurons are in a hypothyroid state consequent to the inactivating mutations in MCT8 [56–59].

Mice models of MCT8 knockout only partially recapitulate the TH-associated abnormalities of MCT8-mutant patients [59–61]. Indeed, unlike the severe neurological phenotype of patients with MCT8 mutations, both male and female MCT8 KO mice have a very mild neurologic phenotype. Nevertheless, MCT8 knockout animals fully recapitulate the alterations in circulating TH levels of AHDS patients, namely, impaired brain T3 uptake and decreased brain levels of T4 and T3. Unexpectedly, the liver and kidneys were in a thyrotoxic situation: increased T4 and T3 uptake and elevated T4 and T3 levels [59]. A second mouse model, lacking both MCT8 and OATP1C1 transporters, was much more similar to AHDS in terms of brain abnormalities [62], thus indicating partial overlapping of the functions of thyroid hormone transporters in the brain. MCT8/OATP1C1 KO mice showed similar alterations in peripheral TH homeostasis to those of MCT8 KO mice. Importantly, the uptake of T3 and T4 was much lower in the double KO mice than in the single mutant MCT8 KO and OATP1C1 KO mice, and, moreover, the double KO mice displayed pronounced abnormalities and compromised differentiation of GABAergic interneurons in the cerebral cortex [62].

Studies by Heuer [63] and Bernal [64] elegantly illustrated that the brain regulates its T3 supply by differentially expressing specific transporters and deiodinases (Fig. 3). Their model starts with the passage of T4 by OATP1C1 through the blood-brain barrier and the uptake of T4 in astrocytes. Subsequently, astrocytes activate a D2-mediated T4-to-T3 conversion and release T3, which is available for the uptake into the neurons by MCT8. Finally, neurons can degrade T3 by the action of type 3 deiodinase. Overall, this complex regulation exemplifies how the finely tuned expression of TH modulators can closely control TH action in a spatial- and time-dependent manner and reveals the unique function of MCT8 and OATP1C1 in mediating the passage of T3 into the central nervous system.

It is important to note that intracellular TH availability depends not only on its rate of uptake but also on the rate of TH efflux, which also occurs via active transport across the plasma membrane. All the transporters identified so far are capable of bidirectional transport, which involves both the intra- and extracellular exchanges [52].

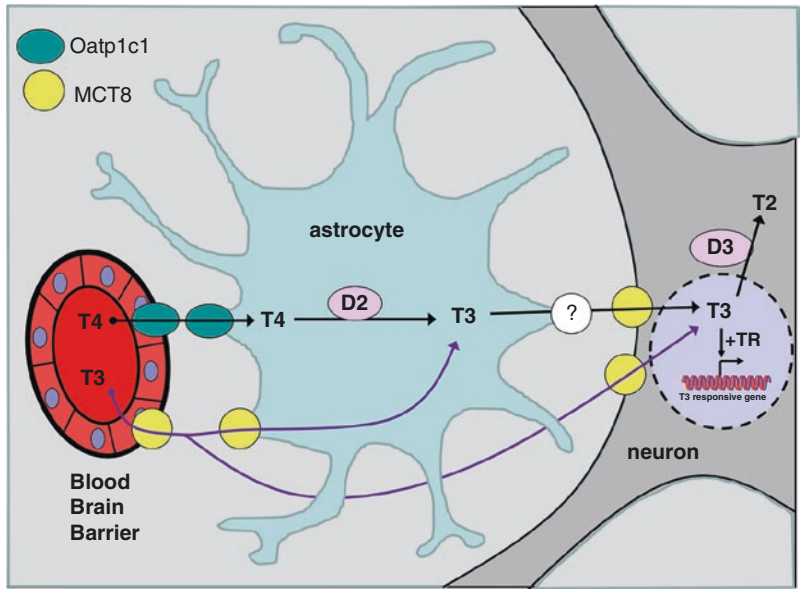

Fig. 3 *Complex role of transporters and deiodinases in thyroid hormone metabolism in the central nervous system.* T3 and T4 from the circulation can pass the blood-brain barrier. Upon entering the brain, T4 reaches the astrocytes via Oatp1c1-mediated transport. In the astrocytes, D2-mediated deiodination produces the active hormone T3, which can enter the neurons via MCT8-mediated transport

Role of Deiodinases and Regulation of Local TH Metabolism

Local Thyroid Hormone Metabolism

Appropriate levels of thyroid hormones are required in all tissues in many pathophysiological conditions. Consequently, it is not surprising that correct spatial and temporal expression of the three deiodinases, receptors, and transporters is an essential condition in healthy humans. The wide-ranging actions of local modulation of TH must therefore be exquisitely regulated at different levels. Many regulators of deiodinase expression and activity have been discovered in the last two decades. The first regulator of deiodinase expression and activities are the same TH substrates, as classical homeostatic feedback mechanism. T3 levels positively regulate D1 expression at a transcriptional level (the D1 promoter comprises two thyroid hormone-responsive regions [TREs]) [65, 66]. D2 expression is closely controlled by T4 at both transcriptional and posttranscriptional level [67]. D3 expression is also T3- dependent being positively regulated by TH and thus represents a powerful homeostatic mechanism of TH inactivation in thyrotoxic states [68]. Besides T3, other endocrine factors regulate D1 transcription, namely, GH [69], TSH [70], and glucocorticoids [71, 72].

D2 expression is cAMP-dependent as elegantly demonstrated in BAT [20]. CREB controls D2 transcription via a canonic CRE binding site located in the human Dio2 promoter [73]. Tissue-specific D2 expression in the thyroid and heart is under the control of tissue-specific transcriptional factors TTF-1 (in thyroid cells) [74], Nkx-2.5, and GATA4 in cardiomyocytes [75]. Furthermore, NF-kB increases D2 expression, which highlights the involvement of deiodinases in pathological contexts [76]. Unlike D1 and D3, D2 mRNA expression thus not strictly correlates with protein synthesis due to a prominent posttranscriptional machinery that regulates D2 stability [77]. Proteasomal degradation tightly controls D2 protein degradation, thereby considerably shortening D2 half-life to only 120 min [67]. Extensive studies have revealed a specific degradation machinery constituted by ubiquitination-deubiquitination enzymes that govern this type of regulation of D2 stability [33, 67, 78–80]. A more recent study showed that D2 and D3 activity, and thereby thyroid hormone signaling, can be modulated by the Sonic hedgehog (Shh) protein [79], which is a highly potent proliferation promoting morphogen [81].

There is a close correlation between changes in the level of D3 mRNA and changes in D3 activity, which indicates that D3 is primarily modulated at transcriptional level. Various agents are able to regulate D3 expression both in vitro and in vivo. First, the different growth factors EGF, FGF, TPA, serum, and phorbol esters modulate D3 expression [1, 82]. Importantly, D3 has been demonstrated to be under the control of critical morphogens controlling development and tumorigenesis. The first such demonstration was the discovery by Huang et al. that TGF-β stimulates D3 transcription via a Smad2/4- or 3/4-dependent pathway [83]. Subsequently, the Shh and Wnt pathways were found to be critical mediators of D3 and TH action in BCC and colon cancer [33, 34].

Plasmatic Thyroid Hormone Metabolism

At the plasma level, the deiodinases represent an important homeostatic mechanism that acts as the first line of defense when thyroid function is impaired or when the supply of iodine is not sufficient to maintain plasma T3 (and T4) level constant. In this context, during hypothyroidism or iodine deficiency, the T3-producing enzyme D2 is upregulated, while the levels of the inactivating D3 are reduced.

Under normal conditions, D2 is the major producer of plasmatic T3, whereas D1 contributes a minimal amount. Kinetics studies indicate that the T3 generated by D1 (mostly in the liver and kidney) can rapidly exit the cell and equilibrate with plasma probably because of the subcellular plasmatic localization of the enzyme. Vice versa, T3 generated by D2 is located in the nucleus and perhaps, for this reason, remains within the cell for a longer time (approximately 8 h). D1–D2 induce a positive flow of T3 that exits the cells and enters the circulation, while D3 reduces the amount of thyroid hormones that, being intracellularly degraded, do not return to the circulation. Type 2 deiodinase is a highly processive enzyme, with a very short half-life (about 30 min). It is expressed in the skeletal muscle, pituitary, brain, BAT, and reproductive tract.

Given the diffuse D2 expression, it is likely that multiple tissues collectively contribute to the daily T3 production via the D2 pathway. A mouse strain with a targeted deletion (Dio2 knockout mouse) had no gross phenotypic abnormalities, and development and reproductive function appeared normal, except for mild growth retardation (9%). Serum T4 and TSH levels were both significantly elevated (40% and 100%, respectively), which suggests that the pituitary gland is resistant to the feedback effect of plasma T4. Human diseases due to mutations in the *Dio*2 gene have not been identified. However, three single nucleotide polymorphisms (SNPs) have been identified in the human population. The best characterized of these (Thr92Ala) at codon 92 (rs225014) is quite common in various ethnic groups. The D2-Ala mutant isoform results from a nonconservative A→G variation in position 274 of the DIO2 gene coding region [16] that determines the presence of an alanine in position 92 of the D2 protein, thus altering the first amino acid of an 18 amino acid loop critical for D2 recognition by its ubiquitinating complex [17]. The prevalence of the homozygous expression of D2-Ala in the general population ranges between 12.7 and 16.4% [15]. Although the threonine residue in position 92 is not phylogenetically conserved, D2-Ala mutants have been related to various clinical conditions all suggestive of impaired TH action, i.e., insulin resistance and type 2 diabetes mellitus, mental retardation and low IQ [18], and altered bone metabolism [19]. Although extensive data indicate that the action of D2-Ala is defective in a clinical setting, the few studies that have investigated its enzymatic activity reported conflicting results.

The serum active thyroid hormones (T3 and FT3) are quite stable in the circulation over time, apart from a nocturnal peak in TSH secretion, despite a relatively short half-life (approximately 12–18 h). The combined potent homeostatic control mediated by the hypothalamus-pituitary-thyroid axis and the deiodinases ensures that T3 levels remain stable in humans for days, weeks, or months. Notably, the deiodinases represent an efficient tool with which to preserve serum T3 levels during disease or adverse conditions given the inverse relationship between D2 and D3 observed during hypo-/hyperthyroidism. While D2 (the T3-producing enzyme) is negatively regulated by T3, the opposite applies to D3. This

condition results in enhanced T3 production during hypothyroidism (and also during iodine deficiency) helped by a decreased TH clearance due to reduced D3 activity. The net effect is a synergic action mediated by deiodinase which help, together with the HPT axis, to maintain serum T3 levels within normal range.

Animal Models

Genetic mouse models of deiodinase deficiency have been instrumental in understanding the role of these enzymes in terms of developmental processes and of the complexes processes governing the systemic local control of TH action in adult tissues. However, paradigms of deiodinase actions have been challenged by the complex and somewhat unexpected phenotypes of D1, D2, and double D1–D2KO mice (reviewed by Galton et al. [84]). Surprisingly, both D1KO and D2KO but also double D1–D2KO mice have normal serum T3 levels and general health, growth, and reproductive capacity, thus challenging the generally accepted concept that the T4-to-T3 conversion induced by double D1–D2 deiodination is the major pathway to maintain normal T3 levels in the thyroid and extra-thyroidal districts. However, studies of D2KO mice reveal that peripheral control of the T4-to-T3 conversion is altered in genetic D2 depletion as demonstrated by abnormal TSH regulation [85], impaired adaptive thermogenesis [20], auditory dysfunction [86], and altered muscle regeneration [22]. D3KO mice had impaired fertility, significant perinatal mortality, and impaired growth [87]. The most prominent feature of this mouse model is a greatly altered thyroid status and physiology, which highlights the pivotal role of the D3 enzyme in this setting and in the maintenance of the HPT axis. Indeed, overexposure of the D3KO mouse to excessive levels of TH in utero and during the first weeks of perinatal life disrupts the HPT axis, with a subsequent hypothyroidism in the adult life. Strikingly, the HPT axis alterations in this mouse model resemble those observed in children born to mothers affected by hyperthyroidism during pregnancy [88].

All the complexities observed in the these animals might be explained by the alterations of the central control of thyroid hormones consequent to altered levels of deiodinases during the developmental period, which complicate the assessment of the functional roles of these enzymes in the adulthood. Consequently, investigations are now focusing on the use of conditional and tissue-specific knockout animals, which enable targeting of deiodinase expression to specific tissues at specific times. It is important to recall that the thyroid physiology of rodents differs considerably from that of humans, which partially explains the differences in phenotype between animal models and humans.

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Part II

Basis of Thyroid Disease

Epidemiology of Thyroid Disorders

Mark P. J. Vanderpump

Introduction

Thyroid diseases are amongst the most prevalent of medical conditions. Their manifestations vary considerably from area to area and are determined principally by the dietary availability of iodine which is an essential component of the thyroid hormones thyroxine (T4) and triiodothyronine (T3) produced by the thyroid gland. Thyroid dysfunction can be classified according to the severity of clinical findings, serum hormone levels, the presence or absence of thyroid antibodies, or the biochemical or physiological effect in the target tissues. The problems encountered in epidemiological studies of thyroid disorders are those of definition, for example, overt hypothyroidism and subclinical hypothyroidism; the selection criteria of the sample used; the influence of age, sex, genetic and environmental factors; and the different techniques used for the measurement of thyroid hormones. The limitations of epidemiological studies of thyroid disorders should therefore be borne in mind when considering the purported frequency of thyroid diseases in different communities [1].

Almost one-third of the world's population live in areas of iodine deficiency despite major national and international efforts to increase iodine intake, primarily through the voluntary or mandatory iodisation of salt [2]. Most of these people are in developing countries, but many in large industrialised countries of Europe are also affected. The ideal dietary allowance of iodine recommended by the World Health Organization (WHO) in adults is 150 μg of iodine per day which increases to 250 μg per day in pregnancy and lactation. International efforts to control iodine deficiency are slowing, and reaching the third of the worldwide population that remains deficient poses major challenges. Iodine deficiency impairs thyroid hormone production and has adverse effects throughout life, particularly early in life as it impairs cognition and growth. Recent epidemiological data suggest that iodine deficiency is an emerging issue in industrialised countries, previously thought of as iodine-sufficient [3].

In iodine-replete areas, most persons with thyroid disorders have autoimmune disease, ranging from primary atrophic hypothyroidism to Hashimoto's thyroiditis to thyrotoxicosis caused by Graves' disease. Cross-sectional studies in Europe and the United States (USA) have determined the prevalence of hyperthyroidism and hypothyroidism and the frequency and distribution of thyroid autoantibodies in different, mainly Caucasian, communities [1]. Data from screening large US population samples [4, 5] have revealed differences in the frequency of thyroid dysfunction and serum thyroid antibody

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concentrations in different ethnic groups, whereas studies from Europe [6] and China [7] have revealed the influence of dietary iodine intake on the epidemiology of thyroid dysfunction. Studies of incidence of autoimmune thyroid disease have only been conducted in a small number of developed countries [8]. Incidence rates provide a direct measure of the rate at which individuals in a given population develop a disease and thus provide a basis for statements about probability or risk of disease. By comparing incidence rates of a disease among population groups varying in one or more identified factors, analytic studies can detect whether a factor affects the risk of acquiring a disease and provide an estimate of the magnitude of the effect. Longitudinal studies are necessary to determine incidence rates, aetiological risk factors and the natural history of the disease process. The logistical and administrative difficulties of such studies explain their relative paucity [1].

Iodine Deficiency Disorders

Iodine deficiency occurs in an environment where the soil has been deprived of iodine from past glaciation, compounded by the leaching effects of snow, water and heavy rainfall, which removes iodine from the soil. The term iodine deficiency disorders (IDD) refers to all the ill-effects of iodine deficiency in a population that can be prevented by insuring that the population has an adequate intake of iodine (Table 1) [9]. The development of a goitre is the most visible effect of iodine deficiency, and endemic goitre exists in a population when more than 5% of the preadolescent (6–12 years) school-age children have an enlarged thyroid gland as assessed by clinical criteria. Iodine deficiency is defined by the WHO as a population median urinary iodine (UI) excretion of less than 100 μg per litre (Table 2).

Iodine deficiency is the most common cause of preventable mental impairment worldwide, and iodine supplementation pre-pregnancy may prevent this mild retardation in the intellectual development of future infants and children. In areas where the daily iodine intake is below 50 μg, goitre is usually endemic, and when the daily intake falls below 25 μg, congenital hypothyroidism is seen, which is a condition associated

Table 1 The spectrum of iodine deficiency disorders

Foetus	Abortions
	Stillbirths
	Congenital anomalies
	Increased perinatal mortality
	Endemic cretinism
Neonate	Neonatal goitre
	Neonatal hypothyroidism
	Endemic mental retardation
	Increased susceptibility of the thyroid gland to nuclear radiation
Child and adolescent	Goitre
	(Subclinical) hypothyroidism
	Impaired mental function
	Retarded physical development
	Increased susceptibility of the thyroid gland to nuclear radiation
Adult	Goitre with its complications
	Hypothyroidism
	Impaired mental function
	Spontaneous hyperthyroidism in the elderly
	Iodine-induced hyperthyroidism
	Increased susceptibility of the thyroid gland to nuclear radiation

Table 2 Epidemiological criteria presently recommended for assessing iodine nutrition based on median urinary iodine concentrations in school-age children

Median urinary iodine μg/L	Iodine intake	Iodine nutrition
<20	Insufficient	Severe iodine deficiency
20–49	Insufficient	Moderate iodine deficiency
50–99	Insufficient	Mild iodine deficiency
100–199	Adequate	Optimal
200–299	More than adequate	Risk of iodine-induced hyperthyroidism within 5–10 years following introduction of iodised salt in susceptible
≥300	Excessive	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid diseases)

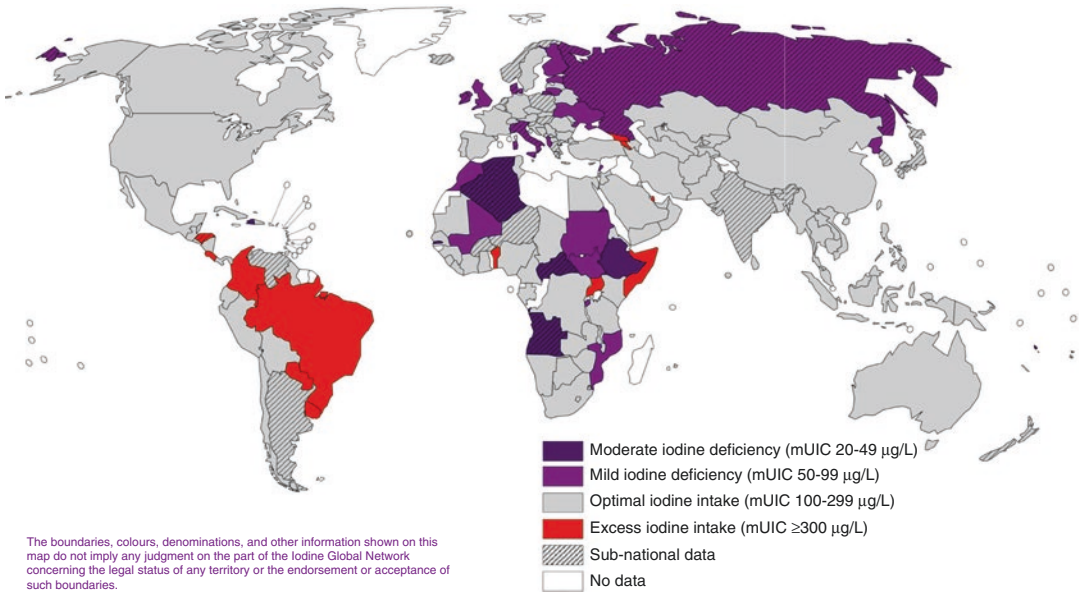

Fig. 1 Global scorecard of iodine nutrition, 2014–2015. Based on median UI concentration in school-age children [10]

with severe learning disabilities, deafness and impaired motor development. Epidemiological studies have demonstrated that reduced iodine intake during pregnancy leads to goitrogenesis, lower free T4 concentrations and increased serum TSH in pregnant women [2]. The prevalence of goitre in areas of severe iodine deficiency can be as high as 80%. Populations at particular risk tend to be remote and live in mountainous areas in Southeast Asia, Latin America and Central Africa. Controlled studies performed in iodine-deficient regions have confirmed that iodine supplementation eliminated new cases of congenital hypothyroidism, reduced infant mortality and improved cognitive function in the general population [8]. Goitrogens in the diet, such as thiocyanate in incompletely cooked cassava or thioglucosides in *Brassica* vegetables, can explain some of the differences in the prevalence of endemic goitre in areas with similar degrees of iodine deficiency. Autonomy can develop in nodular goitres leading occasionally to thyrotoxicosis, and iodisation programmes can also induce thyrotoxicosis, especially in those aged over 40 years with nodular goitres.

National (n = 121) or large subnational (n = 31) UI surveys have been done in 152 countries, representing 98% of the world's population (Fig. 1) [10]. In 2014, iodine intake was adequate in 112 countries, deficient in 29 countries and excessive in 11 countries [2]. During the past decade, the number of iodine-sufficient countries has increased from 67 to 112. Large countries that are still iodine deficient include developing countries (e.g. Ethiopia, Morocco and Mozambique) and countries in transition (e.g. Russia and Ukraine), but also several high-income countries (e.g. Denmark, Italy and the UK). Moreover, in several high-income countries, including the USA and Australia, iodine intakes have decreased in the past 30 years. Results of surveys suggest that many pregnant women in both developing and high-income countries, including the UK and the USA, have deficient iodine intakes. Of the European countries that have assessed iodine nutrition during pregnancy, two-thirds have reported inadequate iodine intakes [11].

The effects of mild-to-moderate iodine deficiency on cognition are less well known than those of moderate-to-severe deficiency, but it is assumed that there is a continuum of disability with more subtle impairments of intelligence quotient (IQ) and motor ability associated with

less severe deficiency. A systematic review of available published studies from 1980 to 2011 examined the relationship between iodine and mental development of children 5 years old and under and found that, regardless of study design, iodine deficiency had a substantial impact on mental development which translated into 6.9–10.2 IQ points lower in iodine-deficient children compared with iodine-replete children [12]. Methodological concerns included weak study designs, the omission of important confounders, small sample sizes, the lack of cluster analyses and the lack of separate analyses of verbal and non-verbal subtests. No large trials have been done in pregnant women with mild-to-moderate iodine deficiency to assess the effects of iodine repletion on infant development or post-partum maternal outcomes. As maternal T4 is crucial to foetal nervous system maturation, even modest states of iodine deficiency could be deleterious. Data from the USA, the Netherlands and Tasmania suggest that the children of women with hypothyroxinaemia may have psychoneurological deficits and delayed mental and motor function when compared with controls [3]. This correlates with the studies in classic areas of iodine deficiency where a range of psychological and neurological deficits in children has been described, but it is maternal hypothyroxinaemia rather than high serum TSH that is the clear biochemical abnormality.

Although the introduction of iodised salt has considerably improved the situation globally in the developing world, iodine deficiency remains an issue in continental Europe where it is estimated that up to 50% of children live in iodine-deficient communities [2]. The iodine intake may vary markedly within a country because of significant variations in the natural iodine content of food and water. Unless iodised salt is available, the main source of iodine in typical diets in North America and Europe is dairy products, supplying up to 50% of intakes, and this was confirmed in a survey of iodine status in 737 UK schoolgirls aged 12–14 years in whom the median UIC was 80 μg/L [13]. Milk intake was positively associated with UI, and a reduced milk intake was responsible for the decline in the UK iodine status. A study using samples and data from the UK-based Avon Longitudinal Study of Parents and Children (ALSPAC) found an association between low iodine status in early pregnancy (urinary iodine-to-creatinine ratio < 150 μg/g) and lower verbal IQ and reading scores in the offspring aged 8 years [14].

Despite the clear benefits in correcting iodine deficiency, a fear of iodine-induced thyroid dysfunction has at times delayed or limited the implementation of iodine supplementation in regions with iodine deficiency. In adults, mild iodine deficiency is associated with a decreased risk of overt and subclinical hypothyroidism, as well as autoimmune thyroiditis and an increased risk of non-toxic nodular goitre [2]. A sudden increase in iodine supply to those in an iodine-deficient region may enhance thyroid autoimmunity through both a cellular and humoral immune response and may result in hypothyroidism in those with damaged thyroid glands and hyperthyroidism in those with an underlying multinodular goitre or Graves' disease, although it is unlikely to do so if the deficiency is not severe and if the increase is relatively small. In developed countries there is a strong public health objective to lower salt intake to reduce the risk of hypertension. Salt iodisation is safe, equitable, largely self-financing and extremely cost-effective in an industrialised country. Iodisation methods can fortify salt to provide recommended iodine intakes even if salt intakes per head are reduced to less than 5 g per day. The alternative strategy is daily oral potassium iodide supplements to target the most susceptible groups, such as women if possible at least 3 months pre-pregnancy [2, 3].

Goitre and Thyroid Nodules

The most common thyroid disease in the community is simple (diffuse) physiological goitre [1]. Ultrasonography has been used in epidemiological studies to assess thyroid size, leading to much higher estimates of goitre prevalence than in studies in which goitre size was assessed by physical examination. In cross-sectional surveys, the prevalence of diffuse goitre declines with age,

the greatest prevalence is in pre-menopausal women, and the ratio of women to men is at least 4:1. In the Whickham survey, among the women 26% had a goitre; the frequency ranged from 31% in those aged less than 45 years (mostly diffuse) to 12% in those aged over 75 years (who had a higher proportion of nodular goitre).

Epidemiological studies suggest that 1% of men and 5% of women have thyroid nodules detected clinically and that the frequency increases with age and in iodine-deficient populations. With the increasing use of sensitive imaging techniques, an increasing proportion of thyroid nodules are detected incidentally. Many nodules are detected because of their size or anterior position in the neck, or the skill of the physician performing the examination, but most thyroid nodules will not be clinically recognised. Up to 50% of nodules greater than 1 cm detected by ultrasound are undetected by clinical examination. The prevalence of thyroid incidentaloma as an unexpected, asymptomatic thyroid nodule discovered during the investigation of an unrelated condition is 67% with ultrasonography (US) imaging, 15% with computed tomography (CT) or magnetic resonance imaging (MRI) of the neck and 1–2% with fluorodeoxyglucose (FDG) positron emission tomography [15].

Congenital Hypothyroidism

In iodine-replete areas, congenital hypothyroidism affects about one newborn in 3500–4000 births and is the most treatable cause of mental retardation [1]. There is an inverse relationship between age at diagnosis and IQ in later life. Eighty-five percent of the cases are due to sporadic developmental defects of the thyroid gland (thyroid dysgenesis) such as the arrested migration of the embryonic thyroid (ectopic thyroid) or a complete absence of thyroid tissue (athyreosis). The remaining 15% have thyroid dyshormonogenesis defects transmitted by an autosomal recessive mode of inheritance. Clinical diagnosis occurs in less than 5% of newborns with hypothyroidism because symptoms and signs are often minimal. Without prompt diagnosis and treatment, most affected children gradually develop growth failure, irreversible mental retardation and a variety of neuropsychological deficits. The value of screening for congenital hypothyroidism in heel-prick blood specimens is unquestioned, and it is now done routinely in many countries.

The apparent incidence of congenital hypothyroidism has more than doubled in recent years because of several factors, including more inclusive diagnostic criteria, shifting demographics and increasing survival of preterm infants [16]. The greatest increase has occurred in mildly affected children. Congenital hypothyroidism may be transient or persistent, but the natural history cannot be predicted by severity at diagnosis. In premature infants, who are especially vulnerable to hypothyroidism, the rise in serum TSH may be delayed and therefore detected only by routine follow-up screening.

Asymptomatic Autoimmune Thyroiditis

Raised serum concentrations of thyroid antibodies (antithyroid peroxidase (microsomal) (TPOAb) and anti-thyroglobulin (TGAb)) correlate with the presence of focal thyroiditis in thyroid tissue obtained by biopsy and at autopsy from patients with no evidence of hypothyroidism during life. Early post-mortem studies confirmed histological evidence of chronic autoimmune thyroiditis in 27% of adult women, with a rise in frequency over 50 years, and 7% of adult men and diffuse changes in 5% of women and 1% of men [1]. Patients with hypothyroidism caused by either atrophic or goitrous autoimmune thyroiditis usually have high serum concentrations of these same antibodies. These antibodies also are often detected in serum of patients with Graves' disease and other thyroid diseases, but the concentrations are usually lower.

The percentage of subjects with high serum TPOAb and TGAb concentrations increases with age in both men and women, and high concentrations are more prevalent in women than in men and less prevalent in blacks than in other

ethnic groups [5]. Using a competitive immunoassay procedure, the reported prevalence of detectable TGAb and TPOAb levels was 10% and 12% of the healthy population. A hypoechoic ultrasound pattern or an irregular echo pattern may precede TPOAb positivity in autoimmune thyroid disease, and TPOAb may not be detected in more than 20% of individuals with ultrasound evidence of thyroid autoimmunity [17].

Hypothyroidism

Primary hypothyroidism is an insidious condition with a significant morbidity and often subtle and nonspecific symptoms and clinical signs. The earliest biochemical abnormality is an increase in serum TSH concentration associated with normal serum free T4 and triiodothyronine (T3) concentrations (subclinical hypothyroidism), followed by a decrease in serum free T4 concentration, at which stage most patients have symptoms and benefit from treatment (overt hypothyroidism). The cause is either chronic autoimmune disease (atrophic autoimmune thyroiditis or goitrous autoimmune thyroiditis (Hashimoto's thyroiditis)) or destructive treatment for hyperthyroidism with either radioiodine or surgery which may account for up to one-third of cases of hypothyroidism in the community. Less frequent causes include surgery and radioiodine ablation for benign nodular thyroid disease and thyroid cancer, external beam irradiation of malignant tumours of the head and neck and drugs including lithium, amiodarone, interferon and checkpoint inhibitor therapies.

In iodine-replete communities, the prevalence of spontaneous hypothyroidism is between 1% and 2%, and it is more common in older women and ten times more common in women than in men [1]. Studies in Northern Europe, Japan and the USA have found the prevalence to range between 0.6 and 12 per 1000 women and between 1.3 and 4.0 per 1000 in men investigated. The prevalence is higher in surveys of the elderly in the community. A lower prevalence is seen in areas of iodine deficiency (Table 3).

Table 3 The effect of environmental iodine intake on the prevalence of subclinical thyroid disease

Iodine status	Subclinical hypothyroidism (%)	Subclinical hyperthyroidism (%)
Deficient	1–4	6–10
Replete	4–9	1–2
Excess	18–14	<1

Subclinical Hypothyroidism

In epidemiological studies, the term subclinical hypothyroidism (or mild thyroid failure) is used to describe the finding of a raised serum thyrotropin TSH but a normal free T4. It represents a compensated state in which increased TSH output is required to maintain normal circulating thyroid hormone levels. An elevated serum TSH is a sensitive indicator of some degree of thyroid failure, and there is a clear inverse relationship with free T4 levels. The term implies that patients should be asymptomatic, although symptoms are difficult to assess, especially in patients in whom thyroid function tests have been checked because of nonspecific complaints such as tiredness.

In the community, the most common aetiology is chronic autoimmune thyroiditis [1]. In the Whickham survey, 8% of women (10% of women over 55 years of age) and 3% of men had subclinical hypothyroidism. In the Colorado study, 9.4% of the subjects had a high serum TSH concentration (Fig. 2) [4]. Among those with a high serum TSH concentration, 74% had a value between 5.1 and 10 mU/L, and 26% had a value greater than 10 mU/L. The percentage of subjects with a high serum TSH concentration was higher for women than men in each decade of age and ranged from 4 to 21% in women and 3 to 16% in men. In the National Health and Nutrition Examination Survey (NHANES III), serum TSH concentrations increased with age in both men and women and were higher in whites than blacks, independent of serum antithyroid antibody concentrations (Fig. 3) [5]. Approximately 2% of adolescents aged 12–19 years had a serum TSH greater than 4.5 mU/L. Subclinical hypothyroidism is found at higher frequency in areas where iodine intake is high, but most cases are not of autoimmune origin (Table 3).

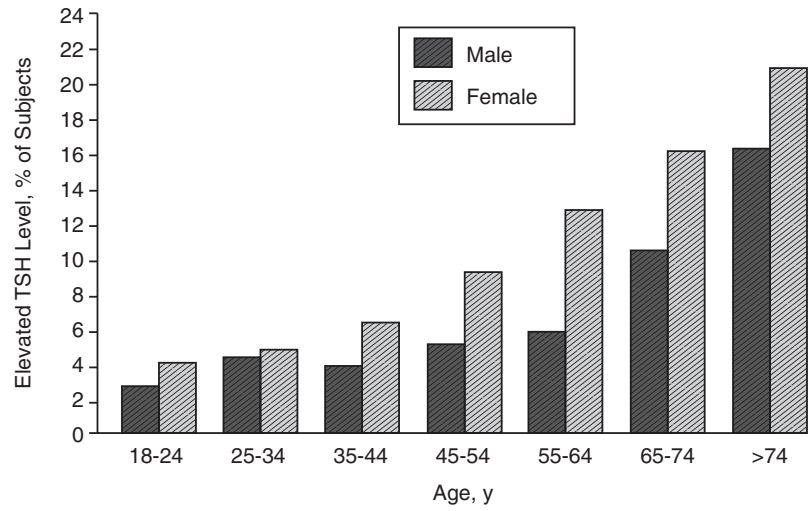

Fig. 2 The percentage of 25,682 subjects with a high serum TSH concentration, by sex and decade of age, in the Colorado thyroid disease prevalence study [4]

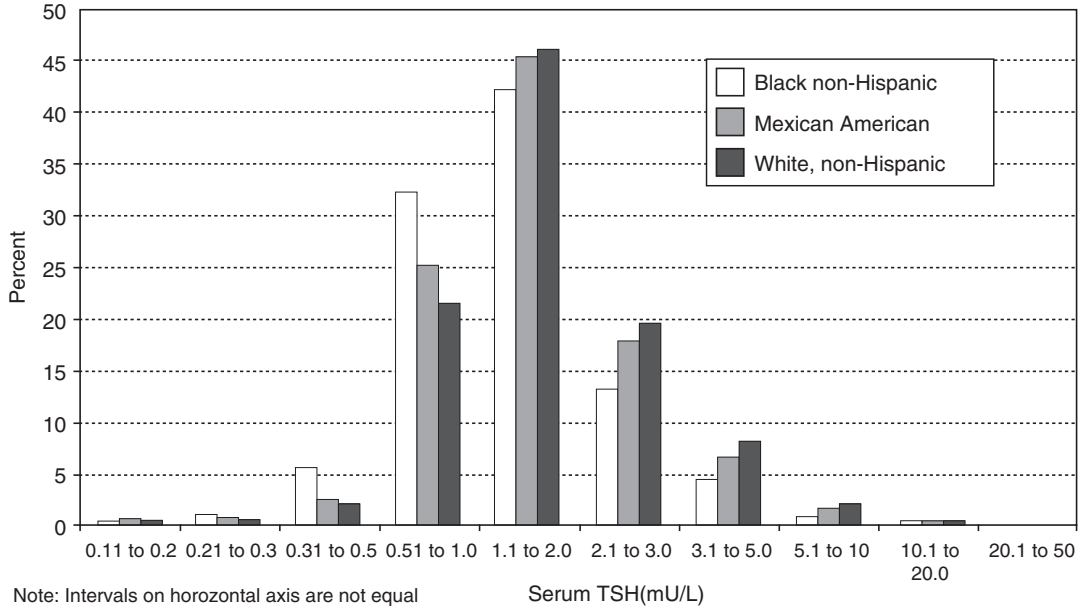

Fig. 3 Serum TSH distribution in US reference population by ethnicity [5]

There has been a growing controversy about the upper limit of the reference range for serum TSH [18, 19]. Reference ranges are derived from a reference population that comprises a large group of subjects who do not have thyroid disease and are otherwise well. By convention, a reference range usually only comprises 95% of a reference population. Thus, 2.5% of 'normal' individuals will fall above the reference range and 2.5% will fall below the range. For serum TSH, the reference population shows a log normal distribution and has a diurnal variation with the reference range in thyroid disease-free individuals typically cited as between 0·4 and 4·0 mU/L. The serum TSH reference range varies in different ethnic communities and trimesters of pregnancy and progressively shifts towards higher concentration with age. A further

analysis of the NHANES III data suggests that the reference range for serum TSH rises with age as the 97.5 centile for those subjects aged greater than 80 years was 7.49 mU/L, and 70% had a serum TSH greater than the population-defined upper limit of the reference range of 4.5 mU/L of whom only 40% were antithyroid antibody positive [20].

Spontaneous recovery has also been described in subjects with subclinical hypothyroidism, although the frequency of this phenomenon is unclear. In one study, 37% of patients normalised their serum TSH levels over a mean follow-up time of 32 months [21]. Normalisation of serum TSH concentrations is more likely to occur in patients with negative antithyroid antibodies and serum TSH levels less than 10 mU/L and within the first 2 years after diagnosis [17].

Incidence of Hypothyroidism

The 20-year follow-up of the Whickham cohort provided incidence data and allowed the determination of risk factors for spontaneous hypothyroidism in this period [22]. The mean annual incidence of spontaneous hypothyroidism during the 20-year follow-up period was 3.5 per 1000 and 0.6 per 1000 in surviving women and men, respectively (Fig. 4). Either raised serum TSH or positive thyroid antibodies alone or in combination were associated with a significantly increased risk of developing hypothyroidism. In the surviving women, the annual risk of spontaneous overt hypothyroidism was 4% in those who had both high serum TSH and antithyroid antibody concentrations, 3% if only their serum TSH concentrations was high and 2% if only their serum thyroid antibody concentration was high; at the time of follow-up, the respective rates of hypothyroidism were 55%, 33% and 27%. The probability of developing hypothyroidism was higher in those women who had serum TSH concentrations greater than 2.0 mU/L and high serum titres of antithyroid microsomal antibodies during the first survey (Fig. 5). All studies indicate that the higher the serum TSH value, the greater the likelihood of development of overt hypothyroidism in subjects with chronic autoimmune thyroiditis.

The other incidence data for hypothyroidism are from short (and often small) follow-up studies [8]. In elderly subjects, the annual incidence rate of hypothyroidism varies widely between 0.2 and 7% in the available studies. Data from the large population study in Tayside, UK, have demonstrated that the standardised incidence of primary hypothyroidism varied between 3.90 and 4.89 per 1000 women per year between 1993 and 2001. The incidence of hypothyroidism in men significantly increased from 0.65 to 1.01 per 1000 per year (P = 0.0017). The mean age at diagnosis of primary hypothyroidism decreased in women from 1994 to 2001 [23, 24].

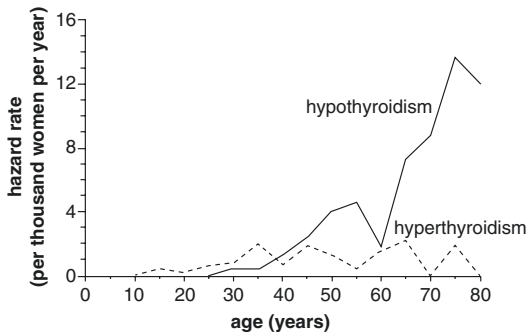

Fig. 4 Age-specific hazard rates for the development of overt hyperthyroidism and hypothyroidism in women at 20-year follow-up of the Whickham survey [22]

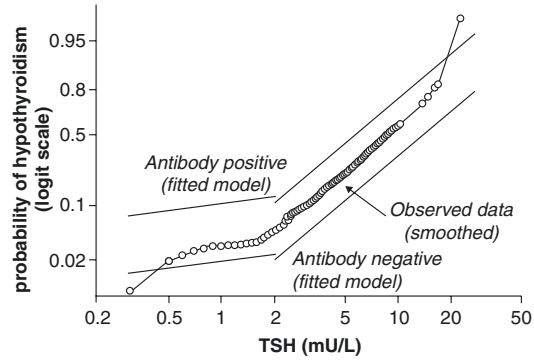

Fig. 5 Probability for development of hypothyroidism within 20 years with increasing values of serum TSH at first Whickham survey in 912 survivors. The coefficients for the fitted model are shown in the figure [22]

Hyperthyroidism

The most common causes of hyperthyroidism are Graves' disease, followed by toxic multinodular goitre, whilst rarer causes include an autonomously functioning thyroid adenoma, thyroiditis (viral or autoimmune) and drugs including iodine and amiodarone. In epidemiological studies, however, the aetiology is rarely ascertained. The prevalence of hyperthyroidism in women is between 0.5 and 2% and is ten times more common in women than in men in iodine-replete communities [1]. In NHANES III, in those subjects who were neither taking thyroid medication nor reported histories of thyroid disease, 2 per 1000 had 'clinically significant' hyperthyroidism, defined as a serum TSH concentration less than 0.1 mU/L and a serum total T4 concentration greater than 170 nmol/L [5]. The prevalence data in elderly persons show a wide range between 0.4 and 2.0%, and a higher prevalence is seen in iodine-deficient areas [1].

Subclinical Hyperthyroidism

Subclinical hyperthyroidism is defined as a low serum TSH concentration and normal serum T4 and T3 concentrations, in the absence of hypothalamic or pituitary disease, non-thyroidal illness or ingestion of drugs that inhibit TSH secretion such as glucocorticoids or dopamine [1]. Epidemiological studies differ in the definition of a low serum TSH concentration and whether the subjects included were receiving levothyroxine (L-T4) therapy. The reported overall prevalence is approximately 3%, with men and women over 65 years having the highest prevalence with approximately 50% taking L-T4. In the NHANES III study, the prevalence was highest in those subjects aged 20–39 years and those aged greater than 79 years [5]. In this study the percentage of subjects with serum TSH concentrations less than 0.4 mU/L was significantly higher in women than men, and black subjects had significantly lower mean serum TSH concentrations and therefore a higher prevalence of subclinical hyperthyroidism (0.4%) than whites (0.1%) or Mexican Americans (0.3%). The prevalence of subnormal serum TSH concentrations is higher in iodine-deficient populations (6–10%), due to functional autonomy from nodular goitres (Table 3) [2].

Among subjects with subclinical hyperthyroidism, those with low but detectable serum TSH values may recover spontaneously when retested. Non-thyroidal illness is an important cause of false-positive TSH test results. Data on the risk of progression of subclinical hyperthyroidism to overt hyperthyroidism are limited. In the majority of subjects, a detectable below normal serum TSH will eventually rise towards normal. In those subjects with an undetectable serum TSH and a confirmed aetiology as determined by thyroid scintigraphy due to Graves' disease or nodular disease, it has been calculated that the annual incidence is approximately 5–8% [25]. A large population study in Tayside, Scotland, followed 2024 subjects with at least two serum TSH measurements below the reference range for at least 4 months for up to 7 years [26]. Few subjects developed hyperthyroidism (0.5–0.7%), and the percentage of those reverting to normal increased with time, and this was more common in those with a baseline serum TSH between 0.1 and 0.4 mU/L.

Incidence of Hyperthyroidism

The incidence data available for overt hyperthyroidism in men and women from large population studies are comparable, at 0.4 per 1000 women and 0.1 per 1000 men, but the age-specific incidence varies considerably [1, 8]. The peak age-specific incidence of Graves' disease was between 20 and 49 years in two studies but increased with age in Iceland and peaked at 60–69 years in Malmö, Sweden [1]. The peak age-specific incidence of hyperthyroidism caused by toxic nodular goitre and autonomously functioning thyroid adenomas in the Malmö study was greater than 80 years.

In the Whickham survey cohort, the mean annual incidence of hyperthyroidism in women was 0.8 per 1000 with no new cases detected in

men (Fig. 4) [22]. In a large population study in Tayside, Scotland, 620 incident cases of hyperthyroidism were identified with an incidence rate of 0.77 per 1000 per year (95% confidence interval (CI), 0.70–0.84) in women and 0.14 per 1000 per year (95% CI, 0.12–0.18) in men [23]. The incidence increased with age, and women were affected two to eight times more than men across the age range. Further analysis suggested that the incidence was increasing in women but not in men between 1997 and 2001 [24].

Post-partum Thyroiditis

Thyroid antibodies, particularly TPOAb, occur in 10% of women at 14 weeks of gestation, which is compatible with the prevalence of thyroid antibodies in community surveys [1]. A proportion of these women will have subclinical hypothyroidism with a high serum TSH, but most will be euthyroid. However, after delivery a transient, destructive autoimmune thyroiditis that occurs between the 12th and 16th week post-partum will develop in 50% of TPOAb-positive women, as ascertained in early gestation, clinically apparent as post-partum thyroiditis (PPT). It presents as a temporary, usually painless, episode of hypothyroidism, occasionally preceded by a short episode of hyperthyroidism. Up to about a quarter of women progress to permanent hypothyroidism within approximately 5 years following an episode of PPT, particularly those with high antibody titres [27].

Screening for Thyroid Disease

Controversy exists as to whether healthy adults living in an area of iodine sufficiency benefit from screening for thyroid disease. The benefit from a screening programme must outweigh the physical and psychological harm caused by the test, diagnostic procedures and treatment [28]. The prevalence of unsuspected overt thyroid disease is low, but a substantial proportion of subjects tested will have evidence of thyroid dysfunction, with approximately 10% with subclinical hypothyroidism and 1% with subclinical hyperthyroidism. No appropriately powered prospective, randomised, controlled, double-blinded interventional trial of either L-T4 therapy for subclinical hypothyroidism or antithyroid therapy for subclinical hyperthyroidism exists [1].

Although epidemiological studies have shown an association between subclinical hypothyroidism and coronary heart disease in younger people (less than 65 years) or those with high serum TSH (greater than 10 mU/L) [29], recent evidence suggests that in older people, higher serum TSH and lower free T4 concentrations within the euthyroid range are associated with lower risk of multiple adverse events including mortality [30]. Treatment in those who are symptomatic, pregnant or preconception aged less than 65 years appears justified [31].

A meta-analysis demonstrated that endogenous subclinical hyperthyroidism was associated with increased risk of total, coronary heart disease (CHD) mortality and incident atrial fibrillation (AF) [32]. The highest risk of CHD mortality and AF is noted when the serum TSH is less than 0.10 mU/L. Subclinical hyperthyroidism might be associated with an increased risk for hip and non-spine fractures, but additional large, high-quality studies are needed [33]. Treatment may be indicated in patients older than 65 years with serum TSH less than 0.1 mU/L to potentially avoid these serious cardiovascular events, fractures and the risk of progression to overt hyperthyroidism [34]. Any potential benefits of therapy in subclinical hyperthyroidism must be weighed against the morbidity associated with the treatment of hyperthyroidism.

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Classification of Thyroid Diseases

Leonidas H. Duntas and Sofia Tseleni-Balafouta

Introduction

Classification of disease is a cardinal step toward the organization of its pathogenesis and symptomatology, the aim being the implementation of the optimal diagnostic and therapeutic approach. The last systematic classification of thyroid diseases was performed in 1969 by the American Thyroid Association and was mainly based on thyroid function and clinical aspects [1]. However, a new classification is required to incorporate the numerous advances achieved over the past half century in the genetics, molecular biology, and pathogenesis of thyroid diseases, the many results of studies, and the new therapeutics. In other words, there is today a need for a thoroughly updated platform upon which diagnostic and therapeutic management of thyroid diseases may be created and firmly established.

Valid suggestions have been made that a new classification should consider thyroid function, the evolution of disease, and various environmental factors as well as determine the parameters that identify real disease [2]. For instance, the widely used ICD-8 and ICD-10 classification systems are not uniform regarding the causes of hyperthyroidism, this being of importance when therapeutic guidelines should be implemented, while in cases of hyperthyroidism, cardiovascular risk likely depends on the degree of disease severity. Graves' disease is accompanied by higher FT4 levels in contrast to autonomous functioning nodule(s) that are marked by higher T3 levels.

Acute and Chronic Thyroiditis

The term thyroiditis includes a complex group of acute, subacute, and chronic inflammatory disorders of the thyroid, with a wide spectrum of etiologies ranging from autoimmune to infectious causes. While the classification of acute and subacute thyroiditis has been fairly effortlessly agreed upon and implemented, a number of tentative classifications of autoimmune thyroiditis are as yet controversial. This is mainly due to the complex character of the disease and the variable immune status and clinical picture of patients. The term "autoimmune thyroiditis" covers both Hashimoto's disease (HD) and Graves' disease (GD), as some patients with HD may initially present with or progress to hypothyroidism and those with GD may regress to hypothyroidism

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[3]. In both the above cases, patients are likely to exhibit autoantibodies against thyroglobulin (TgAB) or against thyroid peroxidase (TPOAB) or else both autoantibodies and antibodies against the TSH receptor that possibly act by stimulating or, less frequently, inhibiting the receptor [4]. It is thus evident that the inflammatory background in these two disorders is both similar and persistently present, as revealed through clinical and immune system laboratory tests.

In the past few years, the discovery has been made that HT can be differentiated into IgG4-positive and non-IgG4 thyroiditis [5], HT IgG4-positive being a new disease category involving a large number of organ systems, including in particular the thyroid [6]. Patients with HT IgG4-positive tend to be of younger age by comparison with the IgG4-negative group, though no major differences were observed as concerns sex or thyroid function status distribution or duration of disease between the two categories. The degree of fibrosis appears to be higher in the IgG4-positive than in the IgG4-negative group, and histology via IgG4 immunostaining is the mainstay of diagnosis [6]. A classification based on positivity or none of IgG4 could well be highly useful in determining immune-phenotype. Management of IgG4-RTD is both medical and surgical, with steroids, which usually induce a swift response, constituting first-line treatment [6].

Interferon-alpha (IFNα)-induced thyroid dysfunction is observed in up to 20% of patients with hepatitis C receiving the drug [7], while as many as 40% of patients developed thyroid antibodies [8]. It is thus clear that interferon-induced thyroiditis (IIT) is a major clinical problem among patients receiving interferon therapy. IIT can be classified into the autoimmune type and the non-autoimmune type, both of which can manifest as destructive thyroiditis or as hypothyroidism. Moreover, autoimmune IIT may manifest through the development of thyroid antibodies with no clinical disease; it can however develop into clinical disease, including both autoimmune hypothyroidism (Hashimoto's thyroiditis) and autoimmune thyrotoxicosis (Graves' disease). In a randomized international clinical trial, administration for up to 24 weeks of IFNα2a every 2 weeks in 869 patients with hepatitis C induced biphasic thyroiditis, with extreme values for the nadir and/or peak TSH being observed in 58% of the patients and hypothyroidism with TSH above 10 mU/L in 6.1% of subjects. It was additionally noted that pretreatment serum TSH levels were higher in females and that being Asian and a current smoker were negative predictive values [9].

Postpartum thyroiditis (PPT) is the occurrence of de novo autoimmune thyroid disease, excluding Graves' disease, in the first year postpartum. The incidence of PPT is 5.4% in the general population, and it is increased in individuals with other autoimmune diseases such as type 1 diabetes mellitus. The classic presentation of PPT of hyperthyroidism followed by hypothyroidism is seen in 22% of cases. The majority of women with PPT experience an isolated hypothyroid phase (48%), with the remainder experiencing isolated thyrotoxicosis (30%). Up to 50% of women who are thyroid antibody positive (thyroid peroxidase antibody and/or thyroglobulin antibody) in the first trimester will develop PPT [10].

Riedel's thyroiditis (RT) is a rare chronic fibrosing disorder characterized by a hard, infiltrative lesion in the thyroid gland; this is often associated with multifocal fibrosclerosis, as well as by local restrictive symptoms that are disproportionate to a palpable mass, and biochemically low serum calcium levels [11]. Interestingly, the clinicopathological features of RT suggest that IgG4 is often the underlying condition [12]. Once the diagnosis is established, an early initiation of anti-inflammatory agents positively influences the clinical outcome.

Classification of Thyroid Neoplasms

Most neoplasms located in the thyroid gland represent a primary neoplastic focus. Clinically detected metastatic foci in the thyroid are exceptionally rare, with the most frequent being renal cell carcinoma, breast and lung carcinoma, and metastatic melanoma [13]. Table 1 depicts the WHO classification of thyroid tumors published in 2004 [14]. Epithelial tumors are by far the

Table 1 Classification of thyroid diseases

1. Goiter (a) Nontoxic diffuse (b) Nontoxic single nodule (c) Nontoxic multinodular
2. Tumors (a) Benign neoplasms (adenomas) (b) Malignant (carcinomas) • Differentiated (papillary and follicular) • Poorly differentiated • Undifferentiated (anaplastic) • Medullary – Nonhereditary (sporadic) – Hereditary (familial)
3. Thyroiditis (a) Acute thyroiditis (b) Subacute thyroiditis (De Quervain) (c) Chronic autoimmune thyroiditis or Hashimoto's thyroiditis (HT) • Typical (goiter) • Nontypical (atrophic thyroiditis) (d) HT IgG4-positive HT IgG4-negative (e) Postpartum and silent thyroiditis (f) Riedel's thyroiditis
Primary hyperthyroidism
1. Diffuse hyperthyroid goiter with thyroid-associated orbitopathy or Basedow-Graves' disease 2. Multinodular hyperthyroid goiter or Plummer's disease 3. Autonomous single nodule *Other forms*: Hyperthyroidism due to Hashimoto's disease (Hashitoxicosis), pituitary resistance to thyroid hormones, TSH-secreting pituitary adenoma (secondary form), chorionic gonadotropin-secreting tumor, adenoma or carcinoma (follicular) of the thyroid, excessive exogenous thyroid hormones, amiodarone- or iodine-induced, postinflammatory, or from destruction of thyroid
1. Subclinical hyperthyroidism
2. Transient hyperthyroidism
3. Thyrotoxic storm
Thyroid-associated orbitopathy (TAO)
Primary hypothyroidism
(a) Primary hypothyroidism • Adult; diffuse and nodular goiter; iodine deficiency • Neonatal congenital (ectopia, agenesis, dyshormonogenesis) (b) Secondary: hypothalamic-pituitary or central hypothyroidism (c) Dyshormonogenetic congenital goiter (d) Generalized and peripheral resistance to thyroid hormones
1. Subclinical hypothyroidism
2. Transient hypothyroidism
3. Hypothyroid coma

most frequent primary neoplastic lesions, while primary lymphomas of the thyroid are the second most frequent malignancy, accounting for approximately 2% of extranodal lymphomas. Most of these are non-Hodgkin large B-cell lymphomas arising against a background of Hashimoto's thyroiditis [15]. Occasionally, neoplasms with mesenchymal differentiation are encountered, such as angiosarcomas or smooth muscle tumors as well as other rare tumors [16]. Primary thyroid epithelial tumors include benign lesions (adenomas) and a large heterogeneous group of malignancies (carcinomas). Thyroid epithelial malignancies (carcinomas) are principally divided according to their histogenesis into three main groups: (1) carcinomas originating from (or, more precisely, differentiating toward) the follicular cell epithelium, exhibiting some degree of thyroglobulin expression, (2) carcinomas from the parafollicular (C) cells of the thyroid gland with calcitonin expression, and (3) rare carcinomas with mixed follicular cell and parafollicular cell differentiation (mixed carcinomas) with co-expression of thyroglobulin and calcitonin (either by the same neoplastic cells or by different cell populations).

The vast majority of thyroid carcinomas are follicular cell carcinomas and are frequently grouped under the general term "thyroid cancer." Follicular cell carcinoma (FCC) represents a heterogeneous group of malignant neoplasms characterized by variable histologic appearance, molecular profiles, and largely indolent behavior, though they include certain subtypes with aggressive behavior. Since the management of cancer is invariably based on a histological classification of the tumor, while the biology of tumors correlates to a varying degree with the histological phenotype, it is evident that an accurate histological classification is essential for the follow-up of such tumors and for the validation and standardization of treatment strategies [17]. However, the above classification currently in use poses considerable difficulties for attending physicians, while in addition interobserver variability, even among "thyroid experts," further complicates the situation.

Table 2 WHO histological classification of thyroid tumors (2004)

Primary	
1. Epithelial (a) Follicular cell origin • Benign – Follicular adenoma Conventional type Oncocytic type • Uncertain malignant potential – Hyalinizing trabecular tumor • Malignant – Papillary carcinoma – Follicular carcinoma Conventional type Oncocytic type – Poorly differentiated carcinoma – Anaplastic (undifferentiated carcinoma (b) C-cell origin – Medullary carcinoma (c) Mixed follicular and C-cell origin – Mixed medullary and papillary carcinoma – Mixed medullary and follicular carcinoma (d) Epithelial tumors of different or uncertain cell origin – Mucoepidermoid carcinoma – Sclerosing mucoepidermoid carcinoma with eosinophilia – Squamous cell carcinoma – Mucinous carcinoma – Spindle cell tumor with thymus-like differentiation (SETTLE) – Carcinoma showing thymus-like differentiation (CASTLE) – Ectopic thymoma	2. Nonepithelial (a) Primary lymphoma/plasmacytoma (b) Angiosarcoma (c) Teratoma (d) Smooth muscle tumors (e) Peripheral nerve sheath tumors (f) Paraganglioma (g) Solitary fibrous tumor (h) Follicular dendritic tumors (i) Langerhans cell histiocytosis (j) Rosai-Dorfman disease Secondary (metastatic)

Among tumors, FCC exhibits an excellent correlation between histotype, genotype, and behavior. FCC may undergo multidirectional differentiation or progress to dedifferentiation, this resulting in phenotypes with mixed patterns: however, when the criteria in use differ, once again, classification is impeded. In the WHO classification, follicular cell carcinomas fall into three major groups according to the degree of phenotypic differentiation: well-differentiated thyroid carcinoma (WDTC), poorly differentiated (PDTC), and undifferentiated (anaplastic) carcinoma (ATC) (Table 2).

Well-differentiated carcinomas are in fact the majority of follicular cell thyroid carcinomas and are broadly categorized as either papillary or follicular carcinomas, which are further subdivided into particular variants presenting various histologic appearances and molecular profiles. Sometimes it is difficult to classify an individual tumor into a particular type (either papillary or follicular) since mixed features may be present.

(a) *Papillary thyroid carcinoma (PTC)*, the predominant histologic form of thyroid cancer, is, as noted above, by far the most common malignant neoplasm of the thyroid, which accounts for approximately 80% of all cases. The reported increasing incidence of thyroid cancer worldwide over the last four decades [18, 19] is in fact due in part to the improved detection of papillary thyroid carcinoma. Defining its frequency depends heavily on the diagnostic criteria performed, which show a large degree of inconsistency, leading to significant interobserver variation.

PTC derives its name from its distinctive arborizing papillary structure which was once utilized for the purposes of diagnosis. This is no longer the case, the diagnosis today mainly relying on distinctive nuclear morphology, including enlargement of the nuclei, optical clearing, overlapping, and membrane alterations such as grooves and pseudoinclusions (“papillary carcinoma

nuclei"). Thus, the diagnosis of PTC has become a cytological evaluation. This new diagnostic approach has had two important outcomes. First, it is partly responsible for the dramatic increase in detection of papillary thyroid carcinoma and therefore for establishing this tumor type as being predominant over all other types of thyroid cancer. Second, it has contributed to follicular thyroid carcinoma having become a relatively rare entity, since many follicular-patterned lesions previously diagnosed as follicular neoplasms, i.e., either carcinomas or even adenomas, are now designated as a follicular variant of papillary carcinoma (FVPTC) [20].

(b) *Follicular carcinoma (FTC)* itself represents a follicular-patterned lesion without the presence of PTC nuclei and demonstrates by definition some features of invasion, exhibiting either a widely invasive periphery (widely invasive FTC) or peripheral encapsulation with some degree of invasion (minimally invasive FTC). Angioinvasion is a significant criterion for malignancy and is suggested by some authors as being crucial for a definitive diagnosis of FTC. Follicular tumors without demonstrable signs of invasive growth are designated as adenomas and are expected to behave in a benign fashion. Since the diagnosis of malignancy is based on such a "microstaging system," the definition and degree of invasive behavior are of extreme importance in follicular neoplasms [21]. Once again, it is important to note the significant interobserver variability in evaluating signs of invasion, the many controversies that exist among thyroid experts, and the inconsistency of invasion as a major criterion of malignancy. Therefore, for all follicular tumors exhibiting a questionable capsular invasion, the term "follicular tumor of uncertain malignant potential" (FT UMP) has been introduced [22].

The majority of reported FTCs are encapsulated and are minimally invasive, with slight tumor capsular invasion alone without simultaneous vascular invasion. Such minimally invasive carcinomas have an appearance similar to that of follicular adenomas and rarely cause distant metastases. Widely invasive follicular carcinomas are much less common; however, 80% of these tumors cause distant metastases leading to a high mortality rate of around 20%.

(c) *Poorly differentiated thyroid carcinoma (PDTC)*. The term "poorly differentiated carcinoma" (PDC) was first introduced in 1983 by Sakamoto [23], while later Rosai named it "insular carcinoma" [24]. Representing a variant of a very heterogeneous group of neoplasms, it was initially defined as an epithelial cell neoplasm exhibiting some evidence of follicular cell differentiation, lying morphologically and behaviorally between the indolent well-differentiated thyroid carcinoma, either of the papillary or the follicular type, and the highly aggressive anaplastic carcinoma.

PDTC was classified as a separate entity in the current histological classification (WHO 2004), without universally established diagnostic criteria, this resulting in a controversial diagnosis, with significant interobserver variability among pathologists. Since it includes different phenotypes of variable aggressiveness and variable iodine accumulation (with to date conflicting reported data), the treatment strategies have not as yet been standardized.

At an international consensus conference held in Turin in 2006, uniform histologic diagnostic criteria for PDC were defined (the "Turin proposal") [25]. This conference also confirmed the presence of geographical differences among reported classical PDTC forms. However, there is still an ongoing debate as to the nature, the diagnostic criteria, the clinical significance, and the optimal therapeutic approach of PDC [26].

(d) *Anaplastic thyroid carcinoma (ATC)*. While differentiated thyroid carcinoma usually carries an excellent prognosis, in sharp contrast to this, undifferentiated (anaplastic) carcinoma is an extremely aggressive tumor, having by definition lost any evidence of phenotypic follicular cell differentiation and

exhibiting 100% disease-specific mortality and a median survival from diagnosis of around 6 months. ATC is a severe, locally invasive disease, infiltrating the neck structures and causing extensive necroses and hemorrhage, and is characterized by absolute refractoriness to radioiodine treatment. Most anaplastic carcinomas arise after dedifferentiation of longstanding differentiated carcinomas within a context of tumor progression. Therefore, the presence of differentiated elements in an otherwise anaplastic carcinoma is common, as are also anaplastic areas in differentiated tumors.

Approximately 10% of patients with well-differentiated carcinomas suffer recurrences, and some cases progress to highly aggressive PDC or, still worse, to lethal anaplastic Ca. Identification of this subset of tumors is essential because of their variable response to therapy, leading to significant morbidity and mortality. Selecting these high-risk patients with what are known as "real thyroid carcinomas" is the most important challenge for the therapeutic algorithm [27]. Besides clinicopathological adverse factors, such as age >45 years, large tumor size, extrathyroidal invasion, clinical lymph node metastasis, vascular invasion, and distant metastases, the so-called aggressive histology of well-differentiated carcinoma plays an important role in risk stratification. Unlike other human cancers, no grading system has so far been widely accepted for differentiated thyroid carcinoma, and subtyping into particular tumor variants seems more accurate. Follicular carcinomas are as already mentioned subtyped according to their invasiveness. Specifically concerning papillary carcinomas, the latter are subdivided into several subtypes, these based on tumor periphery (encapsulation vs. invasive borders), growth pattern (follicular, macrofollicular, cribriform-morular, solid), and cell morphology and cohesiveness (tall cell, oncocytic, clear cell, columnar cell, hobnail cell) or a combination creating specific morphology (diffuse sclerosing variant, Warthin-like variant) (Table 3). Some variants present a particularly difficult diagnostic challenge for the pathologist or else may require clinical investigation into a possible accompanying condition. For example, the cribriform-morular variant is mostly a hereditary disease caused by mutations of the oncosuppressor APC gene frequently associated with familial colonic polyposis (FAP) or colon carcinoma. The variant exhibits multicentricity and a generally excellent outcome.

Table 3 Classification of follicular cell Ca (FCC) (WHO 2004)

• Papillary Ca (PTC) – Conventional type – Variants
• Follicular Ca (FTC) (conventional or oncocytic) – Minimally invasive (encapsulated without angioinvasion or with angioinvasion) – Widely invasive Ca
• Poorly differentiated Ca (PDTC)
• Anaplastic Ca (ATC)

The most frequent subtypes of PTC are the conventional type, the follicular cell variant, and the tall cell variant. Some variants (e.g., tall cell, diffuse sclerosing, columnar cell, and hobnail cell variant) are considered to be more aggressive. They are reported to be associated with frequent extrathyroidal extension, multifocality or multidissemination, increased rates of lymph node and distant metastases, recurrences, resistance to radioiodine therapy, and tumor-related mortality [28–35].

However, there is a general lack of consensus regarding the issue of what diagnostic criteria should be applied along with the impact of the specific diagnosis, as well as the matter of the presence of simply an aggressive component in the clinical course [36]. A large multicenter study has demonstrated the differential prognostic risks of the three major variants (TCV-Common-FV), providing clinical implications for the specific variant-based individualized management [37].

Sometimes a tumor will include more than one variant, making the usefulness of subtyping debatable. For example, the tall cell variant may exhibit extensive tumor areas with hobnail cell morphology indicating loss of cohesiveness. Even more frequent is the presence of large areas with "squamoid" cells (large polyhedral cells containing abundant eosinophilic or pale cyto-

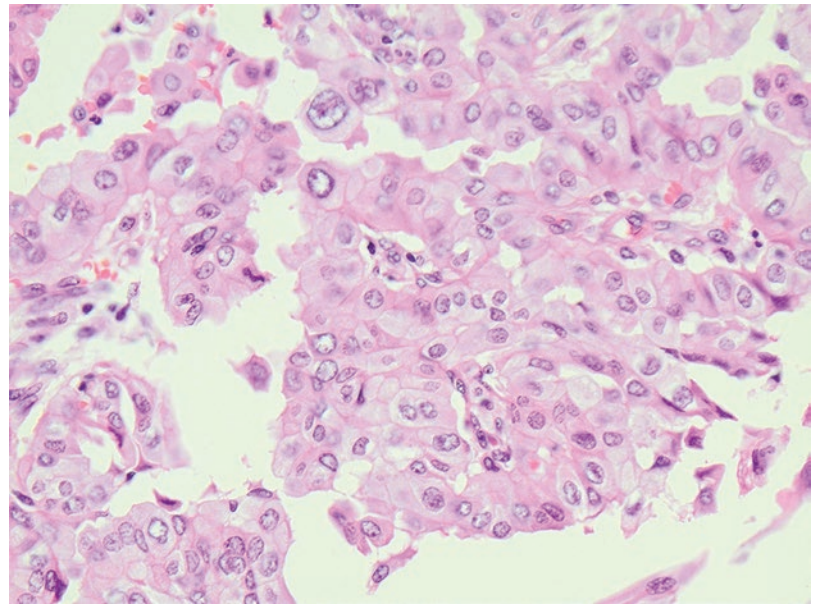

Fig. 1 Papillary Ca tall cell variant with areas of large, polyhedral, "squamoid" cells. This type of tumor mainly has an adverse outcome

Table 4 Subtyping of papillary carcinoma

1. Conventional (common) type
2. Variants
(a) Follicular
(b) Solid
(c) Oncocytic
(d) Warthin-like
(e) Cribriform-morular
(f) Tall cell
(g) Diffuse sclerosing
(h) Columnar cell
(i) Hobnail cell

plasm) in PTC of tall cells, these mostly recorded as "squamous metaplasia" having no correlation with the adverse outcome of the highly aggressive squamous carcinoma [14]. However, in our large series the presence of such squamoid areas was mainly correlated with aggressive tumors with extrathyroidal extension or with tumor recurrences (unpublished data) (Fig. 1).

The follicular variant of PTC includes two different entities, namely, the demarcated/encapsulated and the diffuse/invasive nonencapsulated ("infiltrative") variant (Table 4). The encapsulated variant tends to be, phenotypically and molecularly, a hybrid neoplasm possessing many similarities to follicular carcinoma, including the follicular pattern and frequent RAS mutations ("RAS-like genotype") [38]. It may show some degree of capsular invasion ("invasive RAS-like genotype") or no evident invasion ("noninvasive RAS-like genotype"). The diagnosis relies on the nuclear features of PTC, and, in cases without any signs of invasive potential, the encapsulated follicular variant remains a debatable entity, especially due to incomplete nuclear features. Biologically it is associated with very low to borderline malignancy, especially when there is absence of invasion of the tumor capsule ("noninvasive encapsulated follicular variant"). The increased number of PTC cases has mainly been attributed to the RAS-mutated follicular variant of PTC, suggesting the possible role of environmental factors [39].

The infiltrative follicular variant exhibits irregular borders and may display BRAF mutations ("BRAF-like genotype") [40]. From the biological point of view, it behaves like the conventional type of PTC, being a low-grade malignant tumor.

The encapsulated noninvasive follicular variant of PTC is at present the hottest topic in thyroid pathology. This is mainly due to the high interobserver variability among pathologists in interpreting the nuclear morphology, their determination of the "threshold" varying. Inevitably, the uneven use

of the criteria (either too liberally or too strictly) results in inconsistent histological diagnoses. Moreover, this variant has dramatically increased over the past two to three decades, today constituting 10–20% of all thyroid cancers diagnosed in Europe and North America [41]. Different follow-up studies have attributed this increase to an overtly liberal and subjective assessment of the variants in question, since the crucial issues of how many nuclear features and to what extent they should be present are not as yet established. As a result tumors not fulfilling the major nuclear criteria are overdiagnosed as papillary carcinoma.

To overcome these difficulties in classifying questionable cases demonstrating minor or focal nuclear features and not justifying a diagnosis of malignancy, Williams et al. have proposed the term "well-differentiated tumor of uncertain malignant potential" (WDT UMP) [22]. However, this terminology, while highly convenient for pathologists, does nothing to aid clinicians in their choice of therapy and follow-up. Recently, Nikiforov et al. [41] introduced the term "non-invasive follicular thyroid neoplasm with PTC nuclei" (NIFTP) to classify all follicular-patterned lesions (with <1% papillary structures) exhibiting nuclei with features of PTC nuclei of varying degrees and extent, lacking any sign of invasion [42]. Lying morphologically and molecularly "between" benign and malignant lesions, this entity may represent a precursor lesion to an invasive tumor. It includes all cases previously termed WDT-UMP together with cases of encapsulated follicular variants of PTV without any invasion of the tumor capsule. Both entities share the same excellent prognosis, and, since they have minimal risk for adverse outcome, no further treatment seems to be necessary. Furthermore, the use of this terminology for the cases of noninvasive encapsulated follicular variant of PTC reduces the psychological consequences associated with a diagnosis of cancer [41]. It is obvious that since the detection of invasive foci is crucial for the diagnosis of malignancy, the reliability of such a diagnosis depends largely on the pathologist's experience and on the thoroughness in sampling and slides evaluation as does the diagnosis of malignancy in follicular tumors.

Among thyroid experts there is disagreement about the usefulness of subtyping vs. grading for prognostic stratification. Akslen and Livolsi [43] reported that subclassification of PTC had only a minor prognostic impact, whereas histological grade, based on nuclear atypia, tumor necrosis and vascular invasion, was a strong independent prognostic marker. The so-called high-grade features (such as nuclear atypia-polymorphism, mitoses, necroses) have also recently been reported to be indicative of aggressive tumor behavior similar to that of poorly differentiated Ca [42–44]. Peripheral invasiveness has likewise been reported to be more relevant to the prognosis than growth pattern or cell morphology [45]. The manner of growth (invasive versus encapsulated) is obviously indicative of the aggressiveness of a neoplasm against the host parenchyma and might be relevant for a classification scheme. However, both the presence of high-grade features as well as the invasiveness of a tumor are as yet not fully taken into account and therefore not included in the WHO (2004) classification.

Extrathyroidal extension beyond the thyroid and extensive vascular invasion are significantly correlated with an adverse outcome [46]. Encapsulated follicular cell carcinomas (papillary, follicular, or Hurthle cell) are generally regarded as low-grade tumors, especially in the absence of any vascular invasion [47].

The placing of oncocytic tumors in the WHO (2004) Classification is still a debatable issue. No thyroid neoplasm has created more confusion or debate than oncocytic neoplasms. Some consider all of them as malignant, but most cite 80% or more as benign [48, 49]. The WHO (2004) classification defines oncocytic Ca as a variant of PTC or of FTC and not as a separate group. However, this may change in the future. See Addendum Section with the most recent classification.

Another limitation of the WHO (2004) Classification is the lack of a group with "borderline" morphology between benign and malignant (a tumor with uncertain malignant potential) as well as a noninvasive carcinoma, this constituting a missing link. These entities are essential in our perception of the multistep process of

carcinogenesis from normal-looking tissue to clear-cut malignant tissue. See Addendum Section with the most recent classification.

Despite the increased number of efforts, classifications have not significantly improved over the past decade or so, and a pathologic reclassification of follicular-patterned thyroid lesions is most evidently justified. In an effort to overcome some limitations as well as the difficulties in classifying follicular cell thyroid carcinomas consisting of more than one phenotypic component, Kakudo et al. [50, 51] have made a new classification proposal (Table 5), replacing the traditional terms "papillary" and "follicular" with the term "adenocarcinoma" of some degree of differentiation, as is standard in other organs. Furthermore, they have attempted to define the entities included in each group in order to stratify the tumors according to the prognosis (Table 6). Recently, the authors made an effort to stratify tumor aggressiveness of follicular cell tumors by applying the Ki-67 labeling index, a marker for cell proliferation reported to be significant for the prognosis of thyroid carcinomas as is the case in other human tumors [45, 52].

Table 5 Types of the follicular variant of PTC (FVPTC)

1. Invasive type
(a) BRAF mutation frequent
(b) Biological behavior of low aggressiveness
(c) Safe histological diagnosis
2. Encapsulated ("hybrid neoplasm")-EnFvPTC
(a) RAS mutations frequent
(b) Borderline biological behavior
(c) Inconsistent histological diagnosis based on the PTC nuclei

Table 6 Classification proposal [51]

FA	Follicular adenoma (benign)
WDTUB	Well-differentiated tumor of uncertain behavior (borderline)
WDA	Well-differentiated adenocarcinoma (low risk)
MDA	Moderately differentiated adenocarcinoma (moderate risk)
PDC	Poorly differentiated carcinoma (high risk)
UC	Undifferentiated carcinoma (lethal)

The major challenge that the pathological classification must address in the near future is the establishment of criteria leading to a reliable, observer-independent stratification of thyroid cancer for a more rational planning of disease management. Still lacking are widely accepted diagnostic and prognostic histological markers, with the result that morphology remains the gold standard for the classification of thyroid carcinoma. However, phenotypic differences between the tumors according to differences in signaling are obvious, while the genetic diversity of differentiated follicular cell-derived Ca is likely to reflect the complex histology of these tumors and the inconsistency of histological classification. In this context, it is evident that the implementation of molecular techniques will enable us to deepen our understanding of the impact of the diverse phenotypes and eventually to develop a combined molecular-pathological classification [53, 54].

Studies conducted in multiple research labs as well as genomic sequencing data from The Cancer Genome Atlas (TCGA) for PTC have resulted in the identification of genetic alterations in more than 90% of cases, making PTC one of the genetically best characterized of human cancers [55–64]. The above developments together with multiplatform molecular data provided in large sample sizes from a wide array of institutions today present us with the opportunity to refine the classification of PTC by differentiating it into molecular subtypes and to associate them with clinically relevant parameters [65, 66]. For example, PTC is a MAP kinase-driven cancer that has two mutually exclusive drivers with different signaling consequences: BRAF v600 with high MAPK signaling [67, 68] and mutated RAS with lower MAPK signaling. The expression of genes responsible for iodine uptake and metabolism is greatly reduced in BRAF v600-driven carcinomas, whereas it is largely preserved in RAS-mutated tumors [69]. A subdivision into BRAF-mutated and RAS-mutated tumors could very possibly show a good correlation with the outcome. It seems clear that the implementation of molecular techniques will result in enrichment of our knowledge on the impact of the diverse phenotypes and eventually provide a combined molecular-pathological classification [41, 42].

Table 7 Kakudo et al. classification

WDT-UB	"UMP"-tumors, EnFvPTC, micro-Ca
WDA	Common type PTC and FTC minimally invasive with <4 foci angioinvasion
MDA	Aggressive PTC variants, FTC with >4 foci of angioinvasion and encapsulated Ca with high-grade features
PDC	PDC of WHO, tumors with minor anaplastic transformation and tumors with distant metastases at presentation

(e) *Medullary carcinoma*, a malignant neuroendocrine tumor, also occurs in the thyroid differentiating toward the parafollicular (C) cells and expressing calcitonin, the common neuroendocrine marker, and several polypeptides. According to its genetic background, it is classified as nonhereditary (sporadic) or less frequently as hereditary (familial) (Table 7) The latter may occur in isolation (familial medullary Ca) [70] or be part of an autosomal dominantly inherited cancer syndrome in coexistence with pheochromocytoma and hyperparathyroidism designated as multiple endocrine neoplasia type II [71, 72]. Ninety percent of cases concern MEN IIa. MEN IIb is an aggressive variant of MTC in coexistence with a marfanoid habitus and multiple ganglioneuromas.

(f) The *phenotype of MTC* may exhibit diverse morphological features; however, subtyping does not significantly affect the prognosis. The genetic background of hereditary MTC includes several possible germline mutations in the RET proto-oncogene with variable impact on the behavior of the tumor. According to current data, a significant genotype-phenotype correlation might lead to a possible classification on the basis of the genotype, allowing a more individualized therapeutic approach [73].

Addendum

The WHO Classification of thyroid tumors was revised while the present chapter was in press. The revised classification is as follows.

Revised WHO Classification of thyroid tumors (2017)

Follicular adenoma
Hyalinizing trabecular tumour
Other encapsulated follicular patterned thyroid tumours
Follicular tumour of uncertain malignant potential
Well differentiated tumour of uncertain malignant potential
Non-invasive follicular thyroid neoplasm with papillary-like nuclear features
Papillary thyroid carcinoma (PTC)
Papillary carcinoma
Follicular variant of PTC
Papillary microcarcinoma
Columnar cell variant of PTC
Oncocytic variant of PTC
Follicular thyroid carcinoma (FTC), NOS
FTC, minimally invasive
FTC, encapsulated angioinvasive
FTC, widely invasive
Huerthle (oncocytic)cell tumours
Huerthle cell adenoma
Huerthle cell carcinoma
Poorly differentiated thyroid carcinoma
Anaplastic thyroid carcinoma
Squamous cell carcinoma
Medullary thyroid carcinoma
Mixed medullary and follicular thyroid carcinoma
Mucoepidermoid carcinoma
Sclerosing mucoepidermoid carcinoma with eosinophilia
Mucinous carcinoma
Ectopic thymoma
Spindle epithelial tumour with thyrmus-like differentiation
Intrathyroid carcinoma
Paraganglioma and mesenchymal/stromal tumours
Paraganglioma
Peripheral nerve sheath tumours (PNSTs)
Schwannoma
Malignant PNST
Benign vascular tumours
Hemangioma
Cavernous hemangioma
Lymphangioma
Angiosarcoma
Smooth muscle tumors
Leiomyoma
Leiomyosarcoma
Solitary fibrous tumour
Hematolymphoid tumours

	Langerhans cell histiocytosis
	Rosai Dorfman disease
	Follicular dendritic cell sarcoma
	Primary thyroid lymphoma
Germ cell tumours	
	Benign teratoma (grade 0 or 1)
	Immature teratoma (grade 2)
	Malignant teratoma (grade3)
Secondary tumours	

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Iodine Deficiency

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Assessment and Epidemiology of Iodine Deficiency

Iodine deficiency (ID) impairs thyroid hormone production and has many adverse effects in humans collectively termed the iodine deficiency disorders (IDD) [1, 2]. The most serious effect of iodine deficiency is cognitive impairment; a recent WHO review concluded that providing iodized salt to populations resulted in a significant 72–76% reduction in risk for low intelligence (defined as IQ<70) and an 8.2–10.5 point overall increase in IQ [3]. The WHO has recommended nutrient intakes for iodine (Table 1) [2]. Iodine status of populations can be assessed by using a biomarker of exposure, urinary iodine concentration (UIC), and biomarkers of function, goiter and thyroid function tests [4]. In populations, UIC is the recommended biomarker and reflects recent iodine intake, because the kidney excretes >90% of dietary iodine in the subsequent 24–48 h [4]. The WHO recommends the use of the median UIC to classify population iodine status, expressed as μg/L (Table 2) [2]: a median <20 μg/L suggests severe iodine deficiency, 20–49 μg/L moderate iodine deficiency, 50–99 μg/L mild iodine deficiency, 100–299 μg/L optimal iodine intake, and >300 μg/L excessive intake [2]. These criteria are used in this chapter. Although it does not directly assess thyroid function, a deficient median UIC in a population predicts a higher risk of developing thyroid disorders. ID, unlike most micronutrient deficiencies, is not limited to developing countries with poor diets. Iodine-deficient soils are responsible for the historic "goiter belts" of Midwestern North America, southern Australia, the Alps and Apennines in Europe, and inland areas of England and Wales [5]. Diets will be deficient in iodine in these areas unless iodine enters the food chain through addition of iodine to foods or dietary diversification introduces foods produced in iodine-sufficient regions. Based on the median UIC, in 2017, iodine intake is adequate in 111 countries, deficient in 19 countries, and excessive in 10 countries (54 countries have no data) [6]. Large populous countries that remain iodine deficient include developing countries (e.g., Mozambique, Morocco) and middle-income countries (e.g.,

Table 1 Recommendations for iodine intake (μg/day) by age or population group (data from [2])

Age or population group	Recommended nutrient intake (WHO)
Children 0–5 years	90
Children 6–12 years	120
Adults >12 years	150
Pregnancy	250
Lactation	250

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Table 2 Epidemiological criteria for assessing iodine nutrition based on median urinary iodine concentrations of school-age (6–12-year-old) children and adults, as well as pregnant and lactating women

	Iodine intake	Iodine nutrition
School-age children and adults		
<20 μg/L	Insufficient	Severe iodine deficiency
20–49 μg/L	Insufficient	Moderate iodine deficiency
50–99 μg/L	Insufficient	Mild iodine deficiency
100–299 μg/L	Adequate	Optimal
>300 μg/L	Excessive	Risk of iodine-induced hyperthyroidism, autoimmune thyroid disease
Pregnant women		
<150 μg/L	Insufficient	
150–249 μg/L	Adequate	
250–499 μg/L	More than adequate	
≥500 μg/L	Excessive	
Lactating women		
<100 μg/L	Insufficient	
≥100 μg/L	Adequate	

Adapted from [2]

Russia, Ukraine) and industrialized countries (e.g., Italy, Denmark) [6]. In the USA and Australia, iodine intakes have fallen in recent decades [5].

Thyroid Adaptation to Iodine Deficiency

The relationship between iodine intake and thyroid disorders in populations is U-shaped: both deficient and excessive iodine intakes may impair thyroid function. If dietary iodine intakes are low, the thyroid increases clearance of circulating iodine. Deficient iodine intake increases TSH secretion from the pituitary and increases the expression of sodium/iodide symporter (NIS) to maximize the thyroidal iodine uptake [7]. The thyroid accrues a larger percentage of ingested iodine, reuses the iodine from the turnover of thyroid hormones more efficiently, and reduces renal iodine clearance. In mild to moderate ID, overall serum thyroglobulin (Tg) and thyroid size usually increase, while serum TSH, T3, and T4 often remain in the normal range [8–10]. There are typically no, or weak, associations between UIC and thyroid hormones, but UIC correlates with Tg and thyroid size [9]. Tg is higher in adults with moderate ID compared to those with mild ID [10]. In a population with mild ID, many will develop simple goiter, and some will develop nodules [11]. Mean TSH is usually not elevated in populations with mild ID, so the etiology of diffuse goiter in mild ID remains uncertain.

Populations with mild to moderate ID may have lower mean TSH than sufficient populations because of an increase in thyroid nodularity and multinodular toxic goiter (MNTG), particularly in older adults, and the higher frequency of MNTG lowers overall TSH [12]. There appears to be a U-shaped relationship between UIC and TSH, with the lowest TSH in the UIC range of 250–350 μg/L [13]. In moderate to severe ID, there is a modest increase in TSH, while T4 remains in the normal range, and many people develop subclinical hypothyroidism [14, 15]. As ID becomes more severe, TSH increases further, while T3 increases or remains unchanged, and T4 decreases due to preferential thyroidal secretion of T3 [16]; this conserves iodine because the activity of T3 is four times that of T4, but T3 contains only 75% as much iodine for its synthesis [7]. TSH is usually inversely correlated with T4 but not with T3, suggesting tighter control of TSH secretion by T4 than by T3 [17, 18]. In chronic severe ID, most individuals have increased TSH concentrations, and nearly all develop goiter [19]. Finally, when thyroidal iodine is exhausted, T4 and T3 fall, TSH sharply increases, and there is an increase in overt hypothyroidism [20].

Goiter and Nodules

The relationship between iodine and risk for diffuse goiter shows a U-shaped curve, with higher rates at deficient and excess intakes, while risk for nodular goiter is increased only at deficient intakes. In a 5-year, prospective study in three populations with mild ID, more-than-adequate iodine intake, or excessive iodine intake (median

UICs of 88, 214, and 634 μg/L, respectively) in China, the cumulative incidence of diffuse and nodular goiter was 7.1, 4.4, and 6.9% and 5.0, 2.4, and 0.8%, respectively [21]. Another study in China found those consuming uniodized salt had a 25–36% higher risk of thyroid nodules compared to those consuming iodized salt [22]. In a Danish study, among older women in an area where iodine intake was moderately low, 33% had an enlarged thyroid, 24% had palpable goiter, and 6% had undergone goiter surgery [23]. After 4 years of salt iodization in Denmark, in two regions with mild and moderate ID, thyroid size decreased in all age groups with the greatest decrease in the area with moderate ID [24, 25]. In cross-sectional surveys in Italy before and after introduction of iodized salt, goiter rate was lower after iodization due mainly to less diffuse goiter (10.3% vs. 34.0%), while nodular goiter fell in individuals aged 35 years or younger (3.8% vs. 11.3%) but was not changed at older ages [26].

Hyperthyroidism

Populations with mild to moderate ID typically have higher rates of hyperthyroidism and lower TSH compared to populations with sufficient intakes. Danish adults with deficient iodine intakes (≈40–70 μg iodine/day) had a 2.3 higher lifetime risk for hyperthyroidism compared to Icelanders with excessive intakes (≈400 μg/day): in Denmark the most common cause was MNTG in older adults, while in Iceland most cases were Graves' disease in younger adults [27]. MNTG develops in areas of ID because ID promotes growth and mutagenesis of autonomous thyrocytes that produce high amounts of thyroid hormone [28]. Comparing Danish adults with mild versus moderate ID before iodization, there was more hyperthyroidism in those with moderate ID (96.7 vs. 60.0 per 100,000 person-years) mainly due to an 87% higher rate of MNTG in those with moderate ID, while the incidence of Graves' disease was comparable [29]. In populations with moderate ID, there are higher incidences of solitary toxic adenoma [30] and amiodarone-associated hyperthyroidism [31].

Increasing iodine intakes in populations with ID typically increases the incidence of hyperthyroidism, particularly if the level of iodine fortification is high and the ID was severe [32, 33]. Older adults with nodular thyroid disease are at greatest risk; although most are euthyroid pre-iodization, they may have radioactive iodine uptakes that are not suppressible and low serum TSH which does not respond to thyrotropin-releasing hormone [34]. However, the increase in hyperthyroidism after introduction of iodine is transient because iodine sufficiency lowers the future risk of developing autonomous thyroid nodules. In Switzerland, in the first 2 years after the iodine content of salt was raised from 7.5 to 15 ppm, the incidence of toxic nodular goiter rose by 12% but then fell to a stable level of only 25% of the initial incidence [35]. A Danish study compared hyperthyroidism before and 4 years after salt iodization and reported 50% lower rates of subclinical hyperthyroidism post-iodization and a trend toward lower rates of overt hyperthyroidism [36].

Hypothyroidism

The prevalence of hypothyroidism is higher in areas with severe ID than in areas of optimal iodine intake. In contrast, in mild to moderate ID, prevalence of subclinical and overt hypothyroidism is typically lower than in areas of sufficient iodine intake [27]. In two regions of Denmark with mild versus moderate ID before salt iodization, the incidence of autoimmune hypothyroidism was ≈50% lower in moderate ID [23]. In the 5-year prospective Chinese study described above in cohorts with deficient, sufficient, and excessive iodine intake, the cumulative incidence of overt hypothyroidism did not differ (0.2, 0.5, and 0.3%), but there was an increase in subclinical hypothyroidism with sufficient and excessive intakes (0.2, 2.6, and 2.9%) [37]. In Danish adults before and 4 years after iodized salt was introduced in two regions with previous mild and moderate ID, TSH was 16% higher in both regions across all ages post-iodization, and there was a modest overall increase in overt hypothy-

roidism only in the region with previous mild ID [38]. Over the first 7 years after introduction of salt iodization in Denmark, there was an increase from 38.3 to 47.2/100,000/year in the incidence rate of hypothyroidism, mainly in young and middle-aged adults [39]. It is uncertain if the higher TSH in populations with excessive iodine intake is explained by an increase in TSH only in people with some degree of thyroid autoimmunity (see next section) or if it is a more general phenomenon of iodine-impaired thyroid function [40]. Although individuals with autoimmune thyroiditis may develop hypothyroidism when exposed to iodine [41], many individuals living in sufficient iodine intake areas with increased TSH do not have thyroid antibodies [42].

Thyroid Autoimmunity

Cross-sectional studies have shown that thyroid autoimmunity is higher in populations with deficient iodine intakes [43, 44] possibly because individuals with nodular goiter, which is more common in ID, often have circulating thyroid antibodies. However, an increase in iodine intake may also increase thyroid autoimmunity [45, 46] possibly through increasing antigenicity of Tg [47]. In Danish adults before (median UIC 61 μg/L) and 4–5 years after salt iodization (median UIC 101 μg/L), the rate of TPO antibodies > 30 U/mL increased from 14 to 24%, and the rate of Tg antibodies>20 U/mL increased from 14 to 20%; the strongest increase was in young women and at low titers [48]. In the 5 year prospective Chinese study described above that compared cohorts with deficient, moderate, and excessive iodine intake, the cumulative incidence of autoimmune thyroiditis was 0.2, 1.0, and 1.3%; however, there was no significant difference in the incidence of TPO antibodies or in Graves' disease [37, 49].

Thyroid Cancer

The overall incidence of thyroid cancer in populations does not appear to be influenced by the usual range of iodine intakes from dietary sources [50]. Recent systematic reviews have found no association of dietary iodine intake with thyroid cancer [50, 51]. Although ecological studies have suggested an increase in papillary cancer after the introduction of iodized salt to populations [50], there are many confounding factors that could account for this association, including increasing diagnostic intensity and other environmental factors [50]. Differences in iodine intake between regions may affect the distribution of thyroid cancer subtypes: in areas of optimal iodine intake, there appear to be fewer follicular thyroid cancers (FTC) but more papillary thyroid cancers (PTC) [52–54]. A review reported the ratio of PTC to FTC was 3.4:1–6.5:1 in areas of sufficient iodine intake versus 0.19:1–1.7:1 in ID [54]. Chronic severe ID is a risk factor for follicular thyroid cancer and anaplastic thyroid cancer [55]. This may be explained by the fact that goiter and nodules are major risk factors for thyroid cancer in both men and women [56]: a pooled analysis found a relative risk of 5.9 (95% CI 4.2–8.1) in individuals with a history of goiter [57].

Conclusions

In populations, as iodine intakes increase from severe ID to mild ID and then to iodine sufficiency, there is a shift from excess hypothyroidism to excess hyperthyroidism, which is transient, and then a small shift back toward excess mild hypothyroidism. In severe ID there is more hypothyroidism because, despite an increase in thyroid activity to maximize iodine uptake and recycling, there is not enough iodine available to maintain thyroid hormone synthesis. In mild to moderate ID, the thyroid gland is able to compensate for the low iodine intake by increasing thyroid activity, and this maintains euthyroidism but at a price: in some individuals, chronic thyroid stimulation will lead to thyroid nodularity and autonomy. This increase in nodularity subsequently increases risk of hyperthyroidism if iodine intakes increase. However, this is transient, and when iodine sufficiency normalizes thyroid activity, this results, in the long term, in reduced nodularity and autonomy. The modest

increase in mild hypothyroidism that occurs with sufficient iodine intakes may be due to thyroid autoimmunity and may also be transient, but more studies are needed.

Research Needs

Future research needs include (1) prospective cohort studies of population iodine intake and long-term risk of thyroid disorders, (2) a better definition of the ranges of deficient iodine intakes that increase risk of thyroid disease in populations, and (3) the potential modifying role of genetic and environmental factors (pollutants, other micronutrient deficiencies).

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Selenium Deficiency and Thyroid Disease

Margaret P. Rayman and Leonidas H. Duntas

Introduction

Selenium (Se) is an essential nutrient for human health [1]. As selenocysteine (Sec), in humans it is incorporated into 25 selenoproteins that have a wide range of functions, ranging from antioxidant and anti-inflammatory agents to the production of active thyroid hormone [1, 2]. An indication of the importance of Se to the thyroid is the fact that it contains the highest concentration of Se in the human body and is able to retain that level of Se under conditions of severe deficiency which cause its depletion from many other tissues [3].

Selenoproteins in the Thyroid

A number of selenoproteins are expressed in thyrocytes, e.g. the deiodinase isozymes (DIO1, DIO2, though not DIO3), members of the glutathione peroxidase family (GPX1, GPX3, GPX4), the thioredoxin reductases (TXNRD1 and TXNRD2), SEP15, selenoprotein P (SELENOP) and selenoproteins M and S [4]. The selenoproteins listed below play particularly important roles.

The deiodinases: DIO1 and DIO2 can activate thyroxine (T4) by transforming it into tri-iodothyronine (T3) through removal of the 5′-iodine, while DIO1 and DIO3 are able to prevent T4 from being activated by converting it to inactive, reverse T3 [5] (Fig. 1). DIO3 can also inactivate T3 by 5-deiodination to thyronamines (T2). Outside the thyroid, DIO1 is predominantly expressed in the liver, kidney and pituitary, while DIO2 is expressed in the central nervous system, pituitary, heart, bone and brown adipose tissue and is largely responsible for local conversion of T4 to T3 in target tissues [2]. DIO3 is not present in the thyroid but is found in foetal tissue and in the placenta and central nervous system in adults; it protects sensitive cells from thyrotoxic concentrations of active T3 [2, 6].

It is of interest that a common genetic variation in *DIO1* is associated with serum concentration of free T4, though the effect is modest [7, 8]. Of greater relevance to clinicians is the rs225014 polymorphism in *DIO2*. The rarer CC genotype of this polymorphism, present in 16% of the Weston Area T4 T3 Study (WATTS) population, was associated with worse baseline general health questionnaire scores in patients on T4 (CC vs. TT genotype, 14.1 vs. 12.8, $P = 0.03$) [9].

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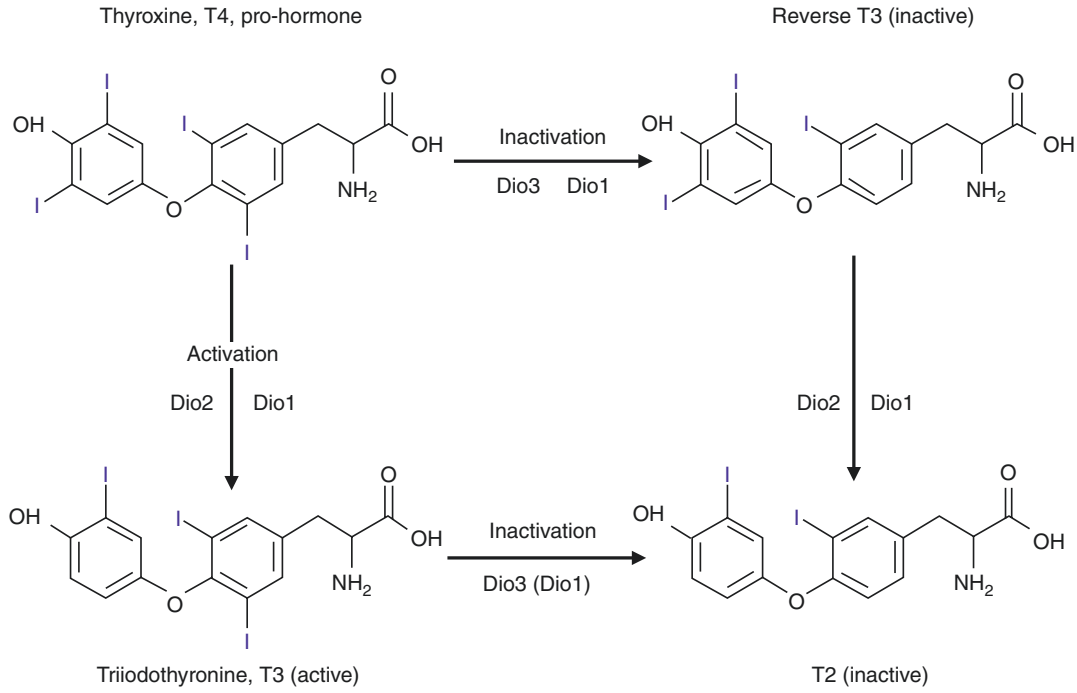

Fig. 1 Major pathways of thyroid hormone deiodination [5]

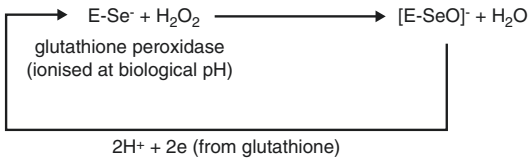

Fig. 2 GPXs catalyse the removal of H_2O_2 (and lipid hydroperoxides) converting it to harmless water, thus protecting the thyroid from excessive exposure to H_2O_2

Furthermore, patients with this genotype showed greater improvement on combined T4/T3 than on T4 therapy. The lack of effect on serum thyroid hormone levels implies that this is a local effect in the brain.

The glutathione peroxidases: GPX3 is secreted at the apical side of the thyrocyte membrane where it degrades excess hydrogen peroxide (H_2O_2) that has not been used by thyroid peroxidase (TPO) for the iodination of tyrosyl residues of thyroglobulin or for iodotyrosine coupling [10] (Fig. 2). GPX1 protects the intracellular compartment from excessive H_2O_2 that may diffuse into the thyrocytes, while GPX4 can deal with lipid hydroperoxides in the mitochondria [6, 10]. Low expression and genetic variance in GPX3 have been linked to differentiated thyroid cancer [4, 11], as discussed in more detail below.

The thioredoxin reductases: TXNRD1 and TXNRD2 help with intracellular redox control and antioxidant defence within the thyrocyte [10]. Recent data also suggest a potential role for TXNRD1 in increasing TSH-dependent sodium-iodide symporter (NIS) expression, which may be particularly important during recovery from iodide excess [12, 13].

Selenoprotein S (SELENOS): SELENOS is among a number of selenoproteins located in the endoplasmic reticulum (ER); it is involved in the control of the inflammatory response in the ER [14]. It protects against transcription of several genes encoding pro-inflammatory cytokines that are involved in the pathogenesis of Hashimoto's thyroiditis [14]. A polymorphism in SELENOS that affects the risk of Hashimoto's thyroiditis will be briefly discussed in the relevant section below.

Effects of Se (Selenoprotein) Deficiency

Deficiency of Se (or selenoproteins) has a number of adverse effects on the thyroid, as shown in Table 1. Each of these thyroid conditions will be discussed separately, but we will start with a recent study that illustrates the effect of Se deficiency on multiple thyroid conditions simultaneously.

Higher Prevalence of a Number of Thyroid Diseases Associated with Low Population Se Status

Se status in China varies from high to low with a band of Se deficiency ranging from the north-east to the south-west of the country [15]. However, remarkably, areas of very different Se content in soil and foodstuffs coexist in Shaanxi Province, Western China, to the extent that both Se deficiency diseases and selenosis have been observed [16]. Ziyang county is an area of high-soil-Se concentration, while by contrast, Ningshan county is a low-soil-Se area, these differences being reflected in the Se content of crops. As these populations had high genetic, environmental and lifestyle similarities and comparable iodine status, a large-scale, population-based, cross-sectional investigation was carried out in these two counties to investigate the effect of Se status on thyroid disease [16]. Ziyang county was defined as "adequate Se" and Ningshan county as "low Se", in line with earlier findings on Se status in different locations within Shaanxi province.

Table 1 Effects of Se (selenoprotein) deficiency

Thyroid condition/s	Numbered references
Multiple conditions: enlarged thyroid, hypothyroidism, subclinical hypothyroidism, autoimmune thyroiditis	[15, 16, 18, 19]
Myxoedematous cretinism	[2, 10, 20]
Effects of selenoprotein deficiency resulting from SECIS-binding protein 2 (SBP2) mutations	[21–28]
Enlarged thyroid/goitre	[30–39]
Autoimmune thyroid disease/ Graves' disease	[40–54]
Autoimmune thyroid disease/ Hashimoto's thyroiditis	[40, 42, 55–62, 66–70]
Thyroid cancer	[4, 11, 71–75, 78–81]

A total of 6152 participants from the adequate- and low-Se counties were recruited to the study. They completed demographic and dietary questionnaires and underwent physical and thyroid ultrasound examinations. Serum samples were analysed for thyroid function parameters and Se concentration. Serum Se was compared between different demographic, dietary and lifestyle categories in the two counties. The relationship between Se status, dietary factors and pathological thyroid conditions was explored by logistic regression adjusted for potential confounders, i.e. occupation, education, sex, age, smoking and alcohol intake.

Complete data sets were available from 3038 adequate-Se and 3114 low-Se participants in whom median (IQR) Se concentrations differed almost two-fold [103.6 (79.7, 135.9) vs. 57.4 (39.4, 82.1) μg/L; $P = 0.001$]. The prevalence of pathological thyroid conditions was significantly lower in the adequate-Se than in the low-Se county (18.0% vs. 30.5%; $P < 0.001$). Higher serum Se was associated with lower odds [OR (95% CI)] of enlarged thyroid [0.75 (0.59, 0.97)], hypothyroidism [0.75 (0.63, 0.90)], subclinical hypothyroidism [0.68 (0.58, 0.93)] and autoimmune thyroiditis [0.47 (0.35, 0.65) (raised thyroid peroxidase antibodies, TPO-Abs)] [16].

Readers may be surprised by the high level of pathological thyroid conditions in this study. However, it should be noted that 69% of the participants were female and that the iodine status was "more-than-adequate" [17] in both counties (urinary iodine concentration in urban residents of both counties was 224 μg/L, while in rural residents it was 271 μg/L in Ziyang and 240 μg/L in Ningshan) [16]. More-than-adequate iodine status has been associated in previous studies with a higher risk of autoimmune thyroiditis, hypothyroidism and goitre [18, 19].

This study clearly shows an association between low-Se status and increased risk of a number of different thyroid pathologies, most

notably autoimmune thyroiditis, where the risk was more than double in the low-Se than in the adequate-Se county. It also suggests that having an adequate-Se status possibly protects the thyroid in a situation of more-than-adequate iodine intake. Increasing Se intake may have the potential to reduce risk in other areas of low-Se status which exist not only in China but in many other parts of the world.

Myxoedematous Cretinism

The myxoedematous form of cretinism, which presents with severe hypothyroidism, mental retardation, developmental retardation and myxoedema, was first characterised in Northern Zaïre (now the Democratic Republic of Congo) [2]. Numerous studies have shown that combined iodine and Se deficiencies in conjunction with exposure to goitrogens precipitate this condition in which an inadequate intake of iodine and reduced thyroid hormone biosynthesis lead to enhanced thyroid stimulation by TSH [10]. Enhanced thyroid-stimulating (TSH) receptor activation stimulates the production of H_2O_2 by the dual oxidases (Duox) [10]. In the absence of adequate iodide for thyroid hormone synthesis, H_2O_2 accumulates, causing damage to thyrocytes and necrosis and fibrosis of thyroid tissue [10]. The severe deficiency of Se in this region means that GPX activity is low, inhibiting the degradation of excess H_2O_2, which results in inadequate protection of thyrocytes and follicular structure [10]. Simultaneous goitrogen exposure may well exacerbate this situation by (1) inhibiting iodide uptake via the NIS (in the case of thiocyanate from improperly prepared cassava, a staple in this region) and/or (2) reducing TPO activity via flavonoids present in soy and millet [20].

Though this is an extreme example of severe deficiency of both iodine and Se and is unlikely to be encountered in clinical practice outside Africa, there is a lesson here that is relevant to any situation of iodine deficiency, i.e. that an adequate intake of Se is needed to protect an iodine-deficient thyroid from excessive levels of reactive oxygen species.

Mutations in the SECIS-Binding Protein 2 (SBP2)

Mutations in selenocysteine-binding protein 2 (SBP2), though rather rare, interfere with the synthesis of the selenoproteins, leading to adverse health effects as described below. SBP2 is a trans-acting factor crucial for the insertion of Se, as Sec, at the active centre of the 25 selenoproteins [21]. Cis-acting sequences in the mRNA and novel trans-acting factors exclusive to Sec incorporation are required for the translation of selenoprotein mRNAs [22]. Selenoprotein synthesis is highly dependent on Se availability, and selenoprotein expression obeys a strict hierarchy, particularly when Se intake is limited. Sec incorporation necessitates UGA codons in selenoprotein mRNAs being decoded as Sec; however, the decoding of UGA as Sec involves the reprogramming of translation, since UGA is normally read as a stop codon. By contrast, the Sec insertion machinery depends on additional factors, such as nuclear genome-encoded transfer RNA (tRNA[Ser]Sec), cis-acting sequences in the mRNA of each selenoprotein and, among several other proteins, ribosomal protein L30 and soluble liver antigen protein [22].

The human SBP2 gene, which is located on chromosome 9, contains 17 exons, encodes 854 amino acids and plays a crucial role in selenoprotein synthesis [23]. Deficiency of SBP2 results in decreased concentrations of selenoenzymes, including antioxidant selenoenzymes and SELENOP, causing increased reactive oxygen species (ROS) in peripheral blood cells and immune deficits. In patients with global selenoprotein deficiency due to defective SBP2, many features, among them photosensitivity and age-dependent hearing changes, are attributed to ROS-mediated damage following the loss of antioxidant defence [24]. A reduction in selenoproteins in peripheral blood cells is associated with abnormal mononuclear cell cytokine secretion, T-lymphocyte proliferation and telomere shortening. Of note, however, is the observation made in mice lacking the antioxidant selenoenzyme GPX1 that elevated ROS led to enhanced systemic and cellular insulin sensitivity [25].

Inherited defects in humans have been identified in six families having mutations in SBP2, with three boys of the first three families, ranging in age from 6 to 14.5 years, presenting characteristics of growth retardation and thyroid dysfunction with low serum T3, elevated T4 and marginally higher levels of TSH [1, 25]. The picture of SBP2 gene mutations among these identified families is variable, with a 12-year-old girl of the fourth family presenting with delayed maturation, congenital myopathy and bilateral sensorineural loss, while a 2-year-old child of the fifth family had global development delay together with multiple gastrointestinal symptoms, such as eosinophilic colitis, fasting non-ketotic hypoglycemia with low insulin levels, muscle weakness and slight bilateral high-frequency hearing loss [26]. The proband of the sixth family, aged 35 years, presented with primary infertility, skin photosensitivity, fatigue, muscle weakness, severe Raynaud's disease (digital vasospasm), impaired hearing and rotatory vertigo.

Hence we see that mutations of SBP2 result in a multisystem disorder with impaired biosynthesis of various selenoproteins, while the different manifestations of symptoms, even among members of the same family harbouring the same mutation, highlight the hierarchical regulation in individual selenoprotein synthesis and expression that is differentially affected by the cellular Se content [25, 27] (Table 2).

Table 2 Typical laboratory and clinical findings caused by mutations in the SBP2 gene affecting selenoprotein synthesis in humans and mice

Selenoprotein	Peripheral tissues
DIO	T4 ↑, T3 ↓, rT3 ↑, TSH n (↑)
SELENON	Muscular dystrophy
GPX	Increased cellular ROS generation
TXNRD	Reduced redox activities
SELENOP	Reduced transport and availability of selenium
SELENOF	Protein folding and endoplasmic reticulum stress
SELENOM	
SELENOS	
SectRNA ([Ser]Sec)	SELENOP ↓, Se ↓
	Glutathione s-transferase ↓
	Hepatocellular necrosis

The adapted selenoprotein gene nomenclature is according to Gladyshev et al. [99]

Se supplementation in either an organic or an inorganic Se form was conducted in SBP2 deficient subjects of the same family, three affected and two unaffected siblings, for the period of 1 month. It was observed that Se-rich yeast elevated serum Se concentrations in all subjects regardless of genotype; however, while sodium selenite clearly raised SELENOP concentrations in the unaffected subjects, this occurred to a lesser extent among the affected subjects. Notably, in the SBP2-deficient individuals, neither of these two Se forms was able to elevate GPX activity nor to rectify abnormalities in thyroid function. The latter result suggests that there was no positive impact on impaired deiodinase expression and that when there is a regular adequate daily Se intake, Se is not a limiting factor in SBP2-deficient individuals [28].

Recently, a human tRNA[Ser] Sec mutation was identified in a proband presenting with low plasma Se concentration and a variety of symptoms, including abdominal pain, fatigue and muscle weakness [29]. The mutation, mediated by reduced expression and diminished 2′-O-methylribosylation at uridine 34 in mutant tRNA[Ser]Sec, caused a marked reduction in expression of stress-related selenoproteins, but not of the antioxidant selenoenzymes. The study of this mutation underscores the importance of tRNA modification for the synthesis of selenoproteins and indicates that a human selenocysteine tRNA defect selectively disrupts selenoprotein synthesis; it moreover identifies tRNA[Ser]Sec as a potential therapeutic target in these cases [29].

Selenium and Goitre

Chronic iodine deficiency (ID) constitutes the main environmental factor in the aetiology of goitre, since it causes a compensatory increase of TSH following the decreased production of T4 and T3, which results in thyroid gland enlargement. By contrast, the pathogenesis of nodular goitre is linked to a large number of environmental non-iodine-related factors, in conjunction with genetics [30]. Among the envi-

ronmental factors associated with incidence of goitre, Se deficiency is prominent, as it has been strongly implicated in the genesis of the disease because of its exacerbation of iodide toxicity [2]. The associations between serum Se and thyroid volume as well as the association between serum Se concentration and risk for goitre have been analysed in participants of two cross-sectional studies carried out before (n = 405) and after (n = 400) the introduction of iodine fortification in Denmark [31]. Se was found to be significantly negatively associated with thyroid volume (P = 0.006), while a low-Se status significantly heightened the risk for thyroid enlargement (P = 0.007) and was also likely to elevate the potential for development of multiple nodules (P = 0.087) [31].

A clinical study in Africa (Uganda) revealed that Se deficiency, despite extensive iodised salt coverage of about 95%, was associated with goitre persistence [32]. Serum Se concentration was significantly higher in non-goitrous controls than in goitrous patients, and serum Se greater than 102.8 μg/L had a statistically significant protective effect against goitre.

In Isfahan, Iran, urinary iodine concentration (UIC) and plasma Se were measured in 2331 schoolchildren [33]. Overall, 32.9% of the children had goitre. Children who were goitrous had lower plasma Se than non-goitrous children (mean ± SD, 66.86 ± 21.82 and 76.67 ± 23.33 μg/L, respectively, P = 0.006). This may be linked to decreased defence mechanisms due to reduced or inactivated antioxidant enzyme expression, aggravated oxidative stress and inflammation, which, when coinciding with severe iodine deficiency, lead to necrosis. The latter has been corroborated by other studies showing no association of serum Se with goitre in borderline iodine-sufficient areas. Meanwhile, the fact that in one iodine-sufficient area, there was no association between serum Se and either thyroid volume or goitre [34, 35] further underlines the predominant role of iodine deficiency in goitrogenesis and the important role that Se status may have in various parts of the world in states of iodine deficiency and excess.

In an experimental study in rats, it was demonstrated that when both iodine and Se are deficient, iodine overload exerts diffuse toxicity in the thyroid gland with necrosis or inflammatory reaction. In iodine-deficient, Se-adequate thyroids, the tissue resumed its normal appearance, while in Se deficiency, the inflammation evolved to fibrotic tissue: 15 days after the toxic iodine overload, the connective tissue volume was twice the control value [36]. These results imply that Se deficiency combined with iodine deficiency may increase necrosis, induce fibrosis and impede restorative mechanisms. Furthermore, while in Se-deficient and control groups, epithelial cells and fibroblast-proliferation indices were comparable [37], in Se-deficient thyroids the inflammatory reaction was more prominent and was induced by macrophages in which transforming growth factor beta (TGF-β) immunostaining was strongly positive.

A lack of iodine induces oxidative stress which, depending on duration and intensity, can result in DNA damage and mutagenesis, thereby providing a platform for the frequent nodular transformation of endemic goitre [38]. Se inadequacy aggravates oxidative stress and limits H_2O_2 degradation, since it reduces the concentration of the enzyme that degrades excessive H_2O_2 during thyroid hormone synthesis, i.e. GPX3 [39]. Though some H_2O_2 is essential for T4 synthesis, uncontrolled generation of H_2O_2 may result in its being released into the thyrocytes, leading to destruction of the parenchyma and necrosis [39] (Fig. 3). However, it has been postulated that Se, in the form of GPX, is capable of breaking down H_2O_2 excess and, as TXNRD, is able to prevent cell damage and necrosis of thyrocytes.

Selenium and Autoimmune Thyroid Disease (AITD)

Se and iodine are essential cofactors in the development of autoimmune thyroiditis (AIT), a pathological condition that includes Graves' disease (GD), Graves' ophthalmopathy (GO), Hashimoto's thyroiditis (HT) and post-partum and painless thyroiditis [40, 41]. GD, which is characterised by autoantibodies directed against

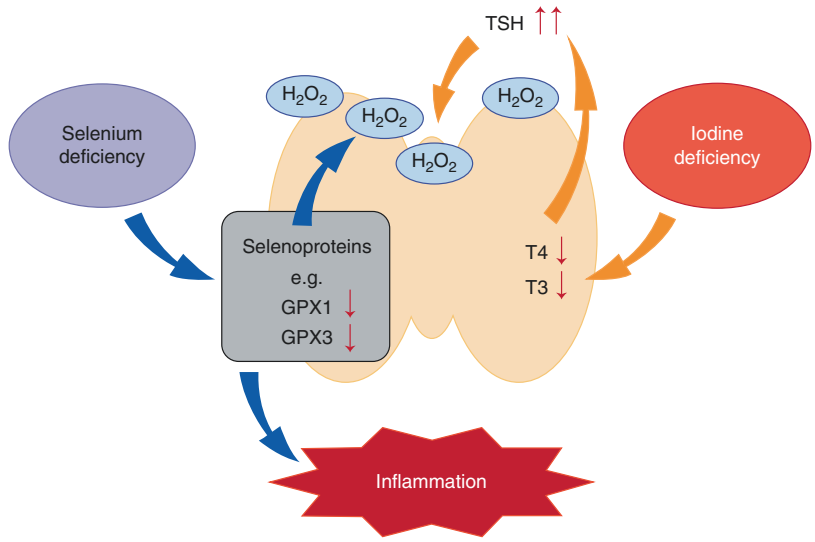

Fig. 3 Iodine deficiency results in lower T4 and T3 and increased TSH, which stimulates hydrogen peroxide (H_2O_2) generation, the substrate for the synthesis of thyroxine. In the state of selenium deficiency, depending also on the degree of deficiency, levels of selenoproteins, e.g. GPX1 and GPX3, gradually decrease, followed by massive diffusion of H_2O_2 into the thyroid parenchyma, inflammation and consequently its destruction (see also [39])

the TSH receptor (TRAb), is the most common form of hyperthyroidism in younger patients.

Selenium and Graves' Disease

It has been suggested that the mechanism by which Se deficiency leads to the exacerbation of GD might be linked to a lack of selenoprotein antioxidant-defence mechanisms, in particular those of the GPXs and TXNRDs, which, in Se sufficiency, protect the thyroid gland from the deleterious effect of peroxides produced during the synthesis of thyroid hormones [42]. There is evidence that Se supplementation in GD, which is characterised by considerable oxidative stress, decreases that stress owing to the ability of GPX to remove hydrogen peroxide and lipid hydroperoxides. It is of special note that TXNRD1 has been reported increased in GD, revealing its involvement in the pathogenesis of the disease [43]. Based on the above observations, a study was conducted in New Zealand with the aim of investigating the effects of excess iodine intake, as iodate, on thyroid function and Se status [44]. The results showed that excess iodate induced hypothyroidism in a number of participants and hyperthyroidism in several others for about 4 weeks. Furthermore, while excess iodate reduced whole-blood GPX (WBGPX) activity, Se supplementation increased plasma Se and slightly elevated WBGPX [44].

Serum Se was found to be lower in patients with newly diagnosed GD than in controls (mean ± SD: GD, 89·9 ± 18·4 μg/L; controls, 98·8 ± 19·7 μg/L; $P < 0{\cdot}01$); this was subsequently confirmed in a multivariate logistic regression analysis model [45]. By contrast, in a linear model, Se was similar in patients with autoimmune hypothyroidism (AIH) and controls ($P = 0{\cdot}86$). It is moreover noteworthy that in the multivariate analysis, Se was marginally decreased in patients with AIH compared to controls [45]. Concomitantly, in 47 patients with GD and GO, markers of oxidative stress, such as H_2O_2, lipid hydroperoxides (ROOH), thiobarbituric acid-reacting substances (TBARS) and ceruloplasmin (CP), superoxide dismutase (SOD) and catalase (CAT), were found to be increased, whereas GPX and glutathione reductase activities were decreased [46]. These findings clearly point to the presence of oxidative metabolism and excessive ROS generation in both GD

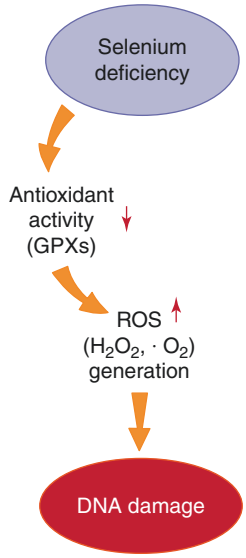

Fig. 4 Selenium deficiency decreases antioxidant activity of glutathione peroxidases (GPXs), facilitating the generation of reactive oxygen species (ROS) that may induce DNA damage

and GO and are supported by other studies showing that superoxide radical production stimulates proliferation of orbital fibroblasts and promotes the synthesis of glycosaminoglycans which, by attracting water, induce periorbital edema [47]. Importantly, Se deficiency increases oxidative stress, which may cause DNA damage (Fig. 4).

Smoking has been strongly associated with GO, which, by increasing the production of ROS, induces oxidative stress and is thus substantially involved, along with other factors, in occurrence and maintenance of the disease. In a retrospective analysis to determine the impact of disease severity on Se status in 84 consecutive GO patients, Se and SELENOP concentrations were measured before treatment commencement and were compared with a clinical activity score (CAS), the severity of eye changes (NOSPECS) status and the concentrations of TRAb [48]. Serum Se and SELENOP levels did not differ between GO patients with active versus inactive or mild versus severe disease activity, indicating that, despite Se status being relatively low, disease severity or activity did not seem to directly affect Se or SELENOP concentrations. Se supplementation decreases the formation of pro-inflammatory cytokines; while acting in synergy with antithyroid drugs, it contributes to stabilising the autoimmune process in GD and alleviating the symptoms in GO [49]. Better disease control has been reported by administering a combination treatment of antithyroid drugs with a fixed combination of antioxidants in GD patients [50]. Recently, a small prospective study performed in China in 41 recurrent GD patients documented a positive effect of Se supplementation combined with methimazole (MMI) treatment for 6 months in terms of rate of remission and TRAb decrease [51]. By contrast, in another study carried out in Italy in 30 GD patients over a period of 3 months, no effect of co-adjuvant administration of Se with MMI was observed [52].

More importantly, encouraging results were obtained from a recent large, multicentre, randomised, placebo-controlled clinical trial in patients with mild GO. This clearly demonstrated the beneficial effects of Se supplementation, in the form of selenite, on the patients' quality of life and overall ophthalmic involvement, while progression to more severe forms of GO was diminished [53].

In an effort to elucidate which cellular mechanisms may be affected by Se in GO, cultured orbital fibroblasts from GO patients were treated with H_2O_2 to induce oxidative stress, following pre-incubation with selenomethylselenocysteine (SeMCys) [54]. Incubation with SeMeCys reduced hyaluronic acid (HA) production and led to inhibition of the increase in endogenous cytokines, namely, tumour necrosis factor-alpha (TNFα), interleukin-1 beta (IL1β) and interferon gamma (IFNγ), all of which rise with oxidative stress and are implicated in the pathogenesis of GO [54]. Thus there is evidence suggesting that Se supplementation may be beneficial in GD, especially in cases of mild-moderate GO, owing to its associated antioxidant and anti-inflammatory effects.

Selenium and Hashimoto's Thyroiditis (HT)

AITD, including its most common form HT, is on the increase worldwide. It is considered to be caused by multiple environmental factors trigger-

ing autoimmune response in genetically susceptible individuals, though the exact mechanisms are as yet not well characterised [55, 56]. Meanwhile, it has been determined that at least seven genes are involved in the aetiology of AITD [56]; the first AITD gene discovered, HLA-DR3, is associated with both GD and HT. Nevertheless, there is mounting evidence that nutritional factors and environmental pollution by metals and chemicals (e.g. organochlorines, pesticides) could be important factors in the disturbing increase in this disease.

In the context of nutritional factors and HT, apart from iodine, Se is the most important for several reasons: (a) selenoenzymes are indispensable for thyroid hormone metabolism, (b) the redox and antioxidant properties of the selenoproteins protect the thyroid gland from toxic derivatives of intrathyroid hormone metabolism and, (c) it possesses extensive anti-inflammatory effects through the selenoproteins, notably SELENOS which is involved in the control of the inflammatory response in the ER [57]. In a Portuguese study, the *SELENOS* −105G/A promoter polymorphism (rs28665122) was strongly associated with circulating levels of cytokines, such as IL-1β, IL-6 and TNF-α [14], that are known to be involved in HT pathogenesis. A-allele carriers of this polymorphism were more than twice as likely as GG-homozygotes to have HT; in male carriers, the risk was fourfold higher [14].

This awareness of the importance of Se has resulted in studies being carried out in numerous countries over the last 15 years supplementing Se, combined or not with LT4, in patients with HT (for reviews, see [58, 59]). The results are, however, inconclusive and several questions remain unresolved. In particular, one meta-analysis concluded that Se supplementation is associated with a significant decrease in TPOAb titres at 3 months, that it improves mood and/or general well-being and that the different patterns of response that have been observed in the various studies on Se supplementation in HT might be linked to baseline TPOAb titres [60]. By contrast, another meta-analysis observed that though the changes from baseline were statistically significant in several studies, the studies were at high risk of bias; hence the evidence for or against the efficacy of Se supplementation in patients with HT remains incomplete [61]. In a more recent meta-analysis of an HT population, though statistically significant reduction of serum TPOAb levels after 3, 6 and 12 months in the LT4-treated group and after 3 months in the untreated HT group was detected, there was no correlation with clinical measures [62].

These discrepancies in results could be attributed to the inhomogeneity of the studies as regards form and dose of Se applied, baseline serum Se status, unknown iodine status in many areas, the latter possibly being of cardinal importance, and single nuclear polymorphisms (SNPs) of selenoproteins, which, to our knowledge, have not thus far been addressed in any study.

The presence of thyroid autoantibodies is relatively high in women of childbearing age [63]. One notable RCT has been carried out in pregnant women positive for TPOAbs. Up to 50% of such women develop post-partum thyroiditis of whom 20–40% subsequently become hypothyroid [64]. In an Italian study, 151 TPO-Ab-positive women were randomly assigned to supplementation with 200 μg (microgrammes) Se/d (as selenomethionine) or placebo during pregnancy and the post-partum period [65]. TPOAb titre fell significantly during gestation in both groups but the reduction was significantly greater in the selenium-supplemented group ($P = 0.01$) and remained so in the post-partum period ($P = 0.01$) (see Fig. 5). Compared to women on placebo, those on selenium had a significantly lower incidence of post-partum thyroid disease (28.6% vs. 48.6%; $P < 0.01$) and permanent hypothyroidism (11.7% vs. 20.3%; $P < 0.01$). In contrast to women on placebo, ultra-sound echogenicity did not fall in those supplemented with selenium. At the end of the post-partum period, grade 2–3 thyroiditis had developed in 44.3% of women on placebo but only in 27.3% of women on selenium ($P < 0.01$) (105).

Though there is clear evidence that Se is involved in the pathogenesis of AIT and that Se supplementation may affect the natural course of the disease [66], the mechanisms are not yet well

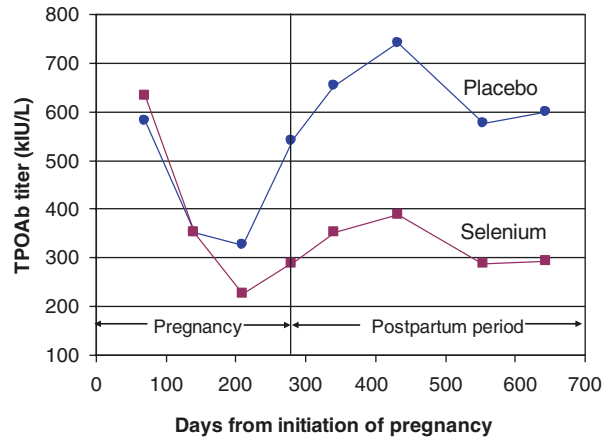

Fig. 5 Se Protects against post-partum autoimmune thyroid disease (adapted from [65])

defined. Experimental studies using a NOD.H-2(h4) mouse model to induce iodine AIT demonstrated reduced Treg cells and Foxp3 mRNA expression in splenocytes in the AIT group than in the controls ($P < 0.01$) [67]. Moreover, Se administration, as sodium selenite, significantly increased the number of Treg cells and the expression of Foxp3 mRNA compared to the untreated AIT group ($P < 0.05$), decreased serum thyroglobulin antibody (TgAb) titres and reduced lymphocytic infiltration into the thyroid. It was therefore postulated that Se supplementation may restore normal levels of CD4(+)CD25(+) T cells by upregulating the expression of Foxp3 mRNA in mice with AIT [67].

In another experimental study using Lewis AIT rats with adequate iodine intake to investigate the effects of different Se doses on the expression of Fas/FasL, an apoptosis protein, it was shown that high Se intake decreased the expression of Fas on thyrocytes and impeded the development of AIT [68].

Se plays an important role in thyroid pathology, including AIT, it having firmly been established over the past couple of decades that in areas with severe, and even mild, Se deficiency, an elevated incidence of thyroid disease is observed. This is due to reduced activity of Se-dependent GPX activity within thyroid cells, with Se-dependent enzymes also exerting modulating effects on the immune system [69]. This fact has once more been confirmed in a recent large cross-sectional observational study in China described in the section "Higher Prevalence of a Number of Thyroid Diseases Associated with Low Population Se Status" [16]. A large number of studies presently exist demonstrating that the supplementation of this key trace element can have a significant effect on inflammatory activity in thyroid-specific autoimmune disease.

The above report and numerous others, as well as the apparent efficacy of Se administration in many cases of autoimmune thyroiditis, whether hypo- or hyper-, is reflected in a recent survey conducted by the Italian Associazione Medici Endocrinologi [70] regarding the clinical use in medical practice of Se. However, there is a caveat, since the results of the survey showed that while Se supplementation is often considered and used for clinical conditions, it is also sometimes prescribed for conditions that may be beyond the recommendations of evidence-based medicine. It is therefore clear that more evidence from well-organised studies and clinical trials is urgently required to determine precisely what role Se can play in the treatment of thyroid disease and particularly of AIT.

Selenium and Thyroid Cancer

Though higher Se status has been associated with lower cancer risk and Se supplementation has reduced cancer incidence in some, but not all, studies [1, 71], the association of Se intake with thyroid cancer (TC) is uncertain, despite a strong

rationale for its effect. In a recent meta-analysis including eight eligible articles involving 1291 subjects, patients with TC exhibited lower serum Se and magnesium concentrations but higher levels of copper than the healthy controls [72]. Moreover, in a subgroup analysis, trans-regional differences of low Se in TC patients were detected in Norway and Austria but not in Poland, suggesting that racial and other local factors might be involved in the development of TC [72]. On the other hand, in another study in Poland, Se and zinc levels were lower, whereas copper/Se and zinc/Se ratios were found to be higher in the tissue of patients with TC than in patients with other thyroid diseases [73]. In addition, high levels of copper and zinc as well as of copper/Se and zinc/Se ratios in the blood of those with TC may reflect tumour progression [73]. These results remain to be confirmed, while investigations are required to determine conclusively whether Se and/or zinc are implicated in carcinogenesis but also if they can be biomarkers of proliferation of disease.

The associations between Se concentrations and the diagnosis of TC were explored in an area of Se adequacy in the USA [74]. Sixty-five euthyroid patients were identified who were scheduled for thyroidectomy because of TC or suspicion of TC nodular disease. Se concentrations were not significantly lower in those with TC; however, serum Se concentration was inversely correlated with disease stage ($p = 0.011$), suggesting a potential association. In a large prospective cohort of 566,398 men and women aged 50–71 years in the National Institutes of Health-American Association of Retired Persons Diet and Health Study, no association was found between dietary intake of Se and TC risk nor was there any evidence of an association between quintile of selenium intake and TC [75].

In relation to the above studies, it must be mentioned that finding a correlation of low Se with disease or disease stage is only to be expected as plasma Se will fall in inflammatory conditions owing to a reduction in SELENOP expression [76, 77].

There are a number of mechanisms by which Se could affect TC. Antioxidant selenoenzymes in the thyroid may protect thyrocytes from oxidative damage from H_2O_2 generated there. The antioxidant selenoenzyme, GPX3, appears to be particularly important in thyroid cancer. GPX3 mRNA is highly expressed in thyrocytes, and its expression was found to be downregulated in five of six thyroid cancer samples but only in one of six matched normal controls [4]. Three SNPs in GPX3 were significantly associated with the risk of differentiated thyroid cancer in a study of 268 cases and 378 controls from the Chinese population in Taiwan [11].

Se, through the selenoproteins, may also be implicated as an antimutagenic agent in the prevention of DNA damage and the malignant transformation of normal cells by upregulating the activity of repair enzymes such as DNA glycosylases [78]. This has been observed in cultured cells and clinical studies, where Se supplementation reduces the frequency of DNA adducts and chromosome breaks and thereby likely decreases the occurrence of detrimental mutations that ultimately contribute to carcinogenesis. Se can act via the selenoproteins, such as GPX and TXNRD, which play crucial roles in antioxidant defence and in maintaining the cellular reducing environment. Enhanced TNXRD activity could have a beneficial impact on oxidative stress, although possible adverse effects must be taken into account. Other functions of TXNRD may be relevant to cell signalling pathways. However, the functional status of the TXNRD system during in vivo chemoprevention with Se needs to be better defined, since in vitro studies have shown inhibitory effects of Se on the TNXRD system correlated with growth inhibition by Se [79].

The first report showing that Se induced inhibition of TC cell growth was reported in a study with follicular cell lines (FRO) [80]. FRO cells were treated with 150 μM seleno-L-methionine (SeMet) to assess viability over 3 days and examined for effects on the cell cycle. The treated cells exhibited an overexpression of growth arrest and DNA damage-inducible GADD34 and GADD153 gene expression which was confirmed with RT-PCR and Western blot. It is therefore apparent that SeMet inhibits thyroid cancer-cell proliferation through a time-dependent upregulation of

the GADD family of genes and induces arrest in the S and G2/M phases of the cell cycle. Hence interventions with Se may have the potential to reduce the risk of TC incidence and mortality, particularly in patients with inadequate-Se status [81].

Se Intake and Status Around the World

The intake of Se shows tremendous variability from one part of the world to another ranging from deficient (7 μg/d) to toxic (4990 μg/d) levels [1]. Thus, intake of Se is high in Venezuela, Canada, the USA and Japan, and much lower in Europe, particularly in Eastern Europe. China has areas of both selenium deficiency and excess. New Zealand intake, formerly recognised as low, has improved as a result of greater importation of higher-Se Australian wheat [1].

This geographical variability in intake and status relates not only to the Se content of the soil on which crops and fodder are grown but to many other factors that determine the availability of Se to the food chain such as Se speciation, soil pH and organic matter content. Mean intake is some 40 μg/d in Europe and 93 (F) to 134 (M) μg/d in the USA [1]. Supplements of Se contribute to intake and are quite commonly consumed, particularly in the USA, where some 50% of the population take dietary supplements [82]. Though recommended Se intake varies by authority and averages 60 μg/d for men and 53 μg/d for women [82], single Se supplements generally contain much more Se than that, i.e. 100 μg per tablet or, more often, 200 μg.

Food Sources of Se

In terms of concentration in foods, Brazil nuts are the richest Se source though they are generally not a commonly eaten food, and in any case, the content is very variable, ranging from 0·03 to 512 mg/kg fresh weight [83]. After Brazil nuts come organ meats and seafoods, followed by muscle meats, cereals and grains [1]. However the Se content of cereals and grains varies widely, ranging from extremely low (mean values of 0·025–0·033 mg/kg dry weight in the UK) to as much as 30 mg/kg in high-Se areas of the USA [83]. Thus, in the USA, grains such as wheat are excellent Se sources and provide some 37% of dietary Se [84] but only provide 26% of UK intake [85].

Measurement of Se Status

Methods of Measuring Se Status

Measurement of Se in blood plasma or serum is the commonest way of measuring Se status [86]. Most often nowadays the measurement is carried out by inductively coupled plasma mass spectrometry (ICP-MS). These measures reflect recent Se intake, i.e. over the last few days. Typical values in the Europe are from 70 to 100 μg/L and in the USA, from 120 to 150 μg/L [1].

Whole-blood Se is a longer-term measure of Se status as erythrocyte Se is included, and erythrocyte turnover is around 120 days. The value is some 25% higher than that of plasma Se [87].

However, it needs to be appreciated that the concentration of Se in plasma or serum falls independently of selenium status in the presence of a systemic inflammatory response [86], thus measurement of Se in plasma/serum or even in whole blood can give a false picture of Se status.

Erythrocyte selenium can be measured by ICP-MS and is unaffected by the systemic inflammatory response [86]. It can be used to assess selenium status across a wide range of selenium intakes, but there are few other published data for comparison.

Toenail Se (less subject to contamination than fingernail Se) is an excellent way of measuring Se status and is an even longer-term measurement as clippings from all ten toes are laid down over a period of months. Furthermore, it is a way of measuring Se status at an earlier time point as toenails are clipped up to 1 year from when they were laid down [88]. Measurement is often carried out by neutron activation analysis.

Functional measures of Se status measure concentrations or activities of selenoenzymes such as GPX (plasma, erythrocyte, platelet), SELENOP or TXNRD are also used, though there can be analytical problems [86] and the values obtained often vary from laboratory to laboratory.

Relevance of These Measurements of Se Status to Production of Thyroid Hormones

Comparison with other organs shows that the thyroid gland is at the very top of the hierarchical Se supply; hence Se status is not a factor that normally determines thyroid hormone concentration [6]. This means that in healthy people, mild Se deficiency will not alter the serum concentration of thyroid hormones, nor is there any evidence of an impaired or altered activity level of the deiodinases in regions with moderate or low Se intake [10]. This conclusion is reinforced by the finding in a number of studies of a minimal effect of Se supplementation on thyroid hormone concentrations or the T3:T4 ratio or in critical-care patients [6, 89]. Only severely or chronically reduced Se availability is likely to impair deiodinase expression with resultant adverse effects on function [10]. However, as pointed out by Schomburg, "the extent to which Se modulates peripheral thyroid hormone action is not yet known" and, importantly, low Se supply may limit local thyroid hormone activation [6] resulting in adverse effects. Clinicians need to bear in mind the possible lack of relationship between local tissue Se concentrations and Se status measurements in circulating blood components.

Relevance of These Measurements of Se Status to Thyroid Protection

Though there is little evidence of low Se status affecting thyroid hormone production through the deiodinases, the same is not true of the glutathione peroxidases. In particular, GPX1 and GPX3 function largely as stress-related selenoproteins [90], meaning that they are not high in the selenoprotein hierarchy and their synthesis is sensitive to the Se supply. Hence in a state of low-Se status (as normally measured, see section "Methods of Measuring Se Status"), these selenoproteins that can protect an oxidatively stressed thyroid by removing excessive amounts of H_2O_2 (and lipid hydroperoxides) will not be formed in adequate amounts.

Under What Circumstances Does the Thyroid Particularly Need Protection by Se/Selenoenzymes?

The thyroid is dependent on protection by selenoenzymes under two main sets of circumstances:

1. *Iodine deficiency*: As explained in section "Myxoedematous Cretinism", this leads to excessive accumulation of H_2O_2 that needs to be removed by GPXs. Deficiency can be exacerbated by goitrogen exposure which inhibits iodine uptake [20]. Examples of goitrogens are:

(a) Thiocyanate formed from glucosinolates in cruciferous vegetables
(b) Thiocyanate formed from cyanogenic glucosides from cassava, lima beans, linseed, sorghum, sweet potato
(c) Thiocyanate from cigarette smoking
(d) Perchlorate ingested from food or water [91]
(e) Nitrate from high-nitrate drinking water or other sources [92, 93]

2. *Iodine excess*: As explained in section "Higher Prevalence of a Number of Thyroid Diseases Associated with Low Population Se Status", excessive intake of iodine causes oxidative stress in the thyroid that can be ameliorated by adequate-Se intake. Chronic high nutritional intake of iodine (> 500 μg/day) or more-than-adequate iodine intake is associated with a higher risk of autoimmune thyroiditis, hypothy-

roidism and goitre [10, 18, 19]. Not only long-term iodine excess but the introduction of a universal salt iodisation programme can have a deleterious effect—at least in the short term—on a thyroid that has adapted to iodine deficiency [10, 94]. An enhanced Se supply ensures that the thyrocytes and colloidal lumen will have adequate amounts of GPX, TXNRD, SELENOS and other relevant selenoproteins to protect them from excessive H_2O_2, other reactive oxygen species or inflammation resulting from increased iodising activity [10].

Recommendations

As explained above, Se requirements may vary according to the iodine status of the region. Deciding on whether additional intake of Se is needed in a particular patient requires taking into account a number of factors. Ideally, you would measure your patient's plasma/serum Se. If it is 120 μg/L or above (though see section "Methods of Measuring Se Status" for caveats relating to the inflammatory response), the patient should be adequately well protected and may have adverse effects if exposed to additional Se. Without measuring Se status, consideration of the following factors should help you decide.

Location [1]: People living in the UK, Europe, especially Eastern Europe, and parts of China may well be Se deficient, while those in N America and Japan are Se replete. Those living in Venezuela may already be ingesting toxic levels. The Australian situation is mixed, but New Zealand Se intakes are on the low side despite recently increased imports of Se-rich wheat. Only consider recommending a Se supplement in a low-Se-status country.

Diet [1]: Enquire into the dietary habits of your patient, and see if he/she is eating the Se-rich foods mentioned in section "Food Sources of Se". If there appear to be few, or no, Se-rich sources, suggest a dietary modification (taking into account your location) or a low-dose supplement (say 50–100 μg Se/d).

Age: The elderly are more likely to need extra Se as they may have more evidence of oxidative stress and inflammation [95].

Sex: Women are at greater risk of thyroid disorders so may benefit more from additional Se. This is particularly the case in pregnancy where women positive for TPO antibodies who were supplemented with 200 μg Se/d had a reduced risk of post-partum thyroid disease and permanent hypothyroidism [65].

State of health: If your patient has subclinical hypothyroidism or thyroid antibody positivity, you should consider whether to increase his/her Se status to reduce the risk of developing autoimmune thyroiditis.

Polymorphisms in selenoproteins: It is unlikely that you will have your patients genotyped, but if you do, there are two polymorphisms that may affect your treatment. Hypothyroid patients with the rarer CC genotype of the rs225014 *Dio2* polymorphism showed greater improvement on combined T4/T3 than on T4 therapy so you might see if this is the case for your patient. Patients carrying the A allele of the *SEPS1* −105G/A promoter polymorphism (rs28665122) are at significantly greater risk of Hashimoto's thyroiditis, particularly if male [14]. In both cases, these patients may benefit from an increased Se intake, unless their dietary intake is already adequate to good, i.e. 75–100 μg Se/d.

Supplement dose: If recommending a supplement, in general, do not recommend one with more than 100 μg Se/d; women will be fine with 50 μg Se/d, a dose that can be found in multivitamin/mineral tablets. A dose of 100 μg Se/d (as Se yeast) given to someone in the UK will raise plasma Se to around 140 μg/L which is more than enough to optimise the synthesis of all the selenoproteins [89].

Supplement form: Either Se yeast (which behaves in the body like wheat-Se) or sodium selenite (the latter is not non-specifically incorporated into body proteins in place of methionine) is fine [83].

Risk of Se toxicity: Bear in mind that though Se is essential, excessive intake of Se is toxic, and supplements of Se of 200 μg/d, generally considered to be quite safe, had toxic effects (alopecia, dermatitis, squamous cell carcinoma,

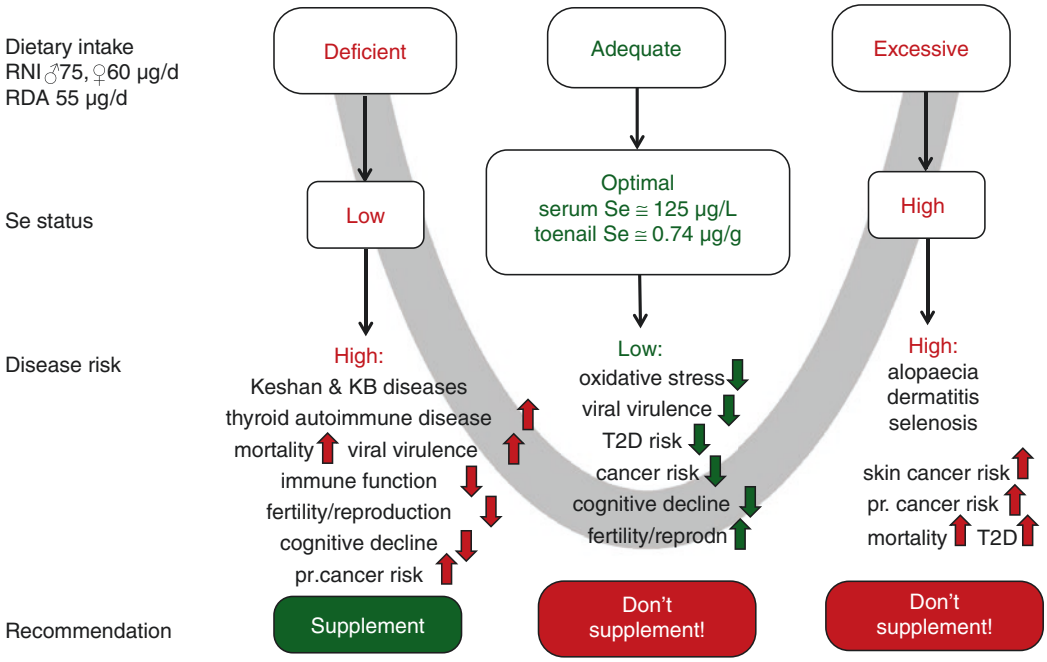

Fig. 6 U-shaped relationship between Se status and disease risk (for references, see [1])

type-2 diabetes) in North American men [96–98], though these men had a higher Se status than European men. As for many nutrients, there is a U-shaped relationship between Se status and disease risk (Fig. 6), so you should aim for an adequate, safe intake [1].

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Part III

Diagnostic and Therapeutic Approaches

Laboratory Testing in Thyroid Disorders

Stefan K. G. Grebe

Introduction

Laboratory testing is an indispensable part of the diagnosis of functional and structural thyroid diseases, assessment of disease severity, and of response to therapy. In this chapter we will review tests of thyroid function and discuss thyroid tumor marker assays and testing for thyroid autoantibodies. We will cover (1) the physiological basis of thyroid function testing, (2) available thyroid tests including their technology and their strength and weaknesses, (3) how to determine whether test results are normal or abnormal or whether they have changed significantly over time, and (4) how to select tests for different clinical applications and interpret the results.

Physiological Basis of Thyroid Laboratory Testing

The hypothalamic-pituitary-thyroid feedback axis forms the basis of clinical thyroid function testing, facilitating diagnostic categorization of thyroid hormone test results (Fig. 1).

At the level of the thyroid gland, thyrotropin (TSH) regulates cellular activity, stimulating thyrocytes to express proteins necessary for thyroid hormone production and to increase thyroid hormone synthesis and secretion. Under normal metabolic conditions, the gland secretes ~90% thyroxine (T4), ~8–10% triiodothyronine (T3), and <2% reverse T3 (rT3) [2, 3]. During intense TSH receptor (TSHR) stimulation, or in case of iodine deficiency, the proportion of T3 formation might increase [4] (Fig. 2).

Upon secretion, transport of the hydrophobic thyroid hormones in blood is facilitated by the thyroxine transport proteins, thyroxine-binding globulin (TBG), transthyretin (TTR), and albumin (ALB) (Table 1). These proteins collectively have exceedingly high binding capacity (>99.7% of T4 and T3 are protein bound), but show significant differences in binding affinity, and consequently on and off rates of thyroid hormone in the capillary vasculature (from a few seconds to over half a minute) [2, 6–8]. This facilitates nuanced delivery of hormone to target tissues, even if demand exceeds the circulating free thyroid hormone pool. Transport into target cells is also highly regulated, via expression and activity of specific thyroid hormone transporters and promiscuous organic amino acid transporters [9].

Within cells, there is yet another layer of regulation through expression and activity of deiodinases that convert the much less active T4 into either T3 (type II), which has 10–30 times higher affinity for the thyroid hormone receptor (THR) than T4 [10, 11], or into inactive rT3

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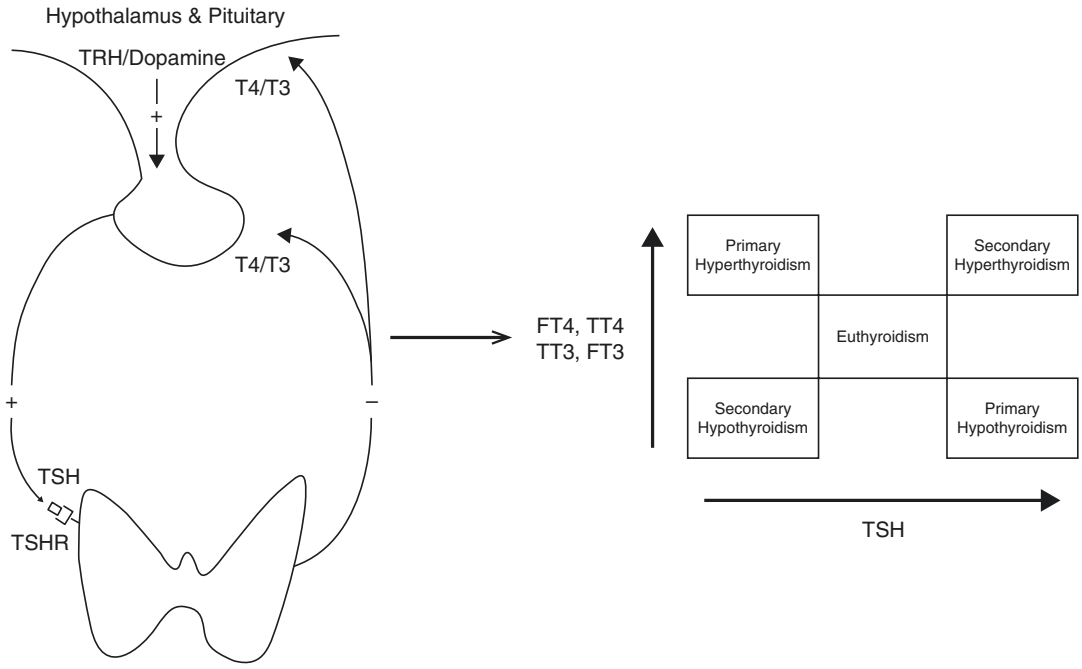

Fig. 1 High-level picture of the hypothalamic-pituitary-thyroid axis (*left panel*) and how it can be leveraged for thyroid function diagnosis by simultaneous measurement of peripheral thyroid hormones and thyrotropin (TSH) (*right panel*). Modified from Grebe 2012 [1] with permission from publisher

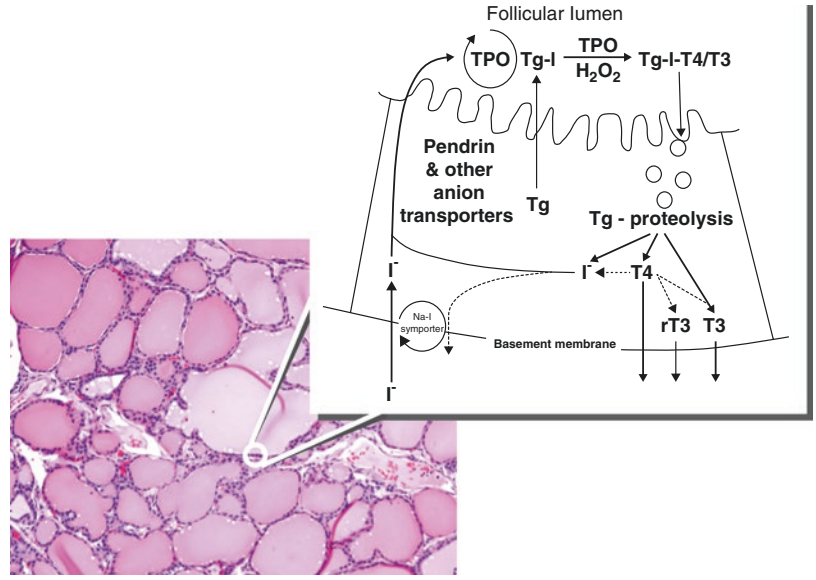

Fig. 2 Photomicrograph of normal thyroid tissue with a cartoon insert depicting the process of thyroid hormone production in follicular thyrocytes. From Grebe 2013 [5] (I own the copyright)

(type III). However, most circulating T3 and rT3 are derived from membrane-bound type I deiodinase, which can generate either T3 or rT3 and which is primarily expressed in liver and kidney [12–15].

The final level of regulation occurs at the gene transcription level. Of the hundreds to thousands of genes that are regulated by thyroid hormone, only a subset, determined by tissue context and metabolic state of the tissue, will be accessible for

Table 1 Serum thyroid hormone-binding/transport proteins

	TBG	TTR	Albumin
Serum concentration (g/L)	~0.01–0.03[a]	~0.2–0.4	~35–50
Half-life (days)	~5[b]	~2	~20
K_d (M)			
T4	10^{-10}	10^{-8}	10^{-6}
T3	10^{-9}	10^{-6}	10^{-5}
Percent serum T4/T3 protein-bound[c]			
T4	~40–75	~20–40	~5–30
T3	~40–75	<5	~20–40
Total across all binding proteins (T4/T3)	99.9/99.7		

[a]May rise 2–5-fold in pregnancy (peak: ~weeks 24–34) and also in other high estrogen states
[b]Prolonged in high estrogen states
[c]Varies with method used for measurement. Thyroxine-binding protein electrophoresis gives lower values for TBG T4/T3 binding than other methods

thyroid hormone-induced gene expression regulation. On the level of the thyroid hormone receptor, the relative expression levels of the four thyroid hormone receptor isoforms, the availability of the RxR co-receptor and its ligand 9-cis-retinoic acid, and the availability of various other cofactors will further modify a cell's response to thyroid hormone [11].

In addition to follicular cells, the thyroid also contains nests of parafollicular neuroendocrine cells, C-cells. These cells, like parathyroid cells, sense serum calcium levels and secrete calcitonin, a peptide hormone that lowers serum calcium by inhibiting bone resorption. Its calcium homeostatic role in humans is minor compared to parathyroid hormone or the vitamin D-related hormones. In addition to calcitonin, C-cells produce small quantities of various other neuropeptides and biogenic amines [16–18].

Thyroid Laboratory Tests

Basic Assay Performance Parameters

A brief review of basic assay performance terminology, definition, and parameters is useful, before discussing individual thyroid tests in this section (Box 1).

Box 1 Assay Performance Parameters

1. *Accuracy* (aka bias): Deviation from the "true" value expressed as a fraction or percentage deviation from "true." "True" is the expected value when spiking a defined amount of reference grade material into a blank sample and measuring it with the best established methodology. Comparing results of a large number of patient samples with a lab that uses reference methodology is also often used.
2. *Imprecision* (aka precision; aka repeatability): range of results obtained upon repeat testing of the same sample (usually ≥20 replicates). Expressed as percentage coefficient of variation (SD/Mean × 100). Intra-assay: replicates all run in the same assay on a single run. Inter-assay: run with the same assay over several runs.
3. *Limit of blank* (LOB; aka upper limit of blank, ULOB; aka critical limit): the upper boundary of the central +/−2SD of the signal distribution (a normal distribution is assumed or data are transformed to fit a normal distribution) that is obtained upon replicate measurement of samples that contain no analyte (usually ≥20 replicates).

$$\text{LOB} = \text{Mean}_{\text{blank}} + \text{unidirectional } Z\text{-score for 95\% probability} \quad (=1.645) \times \text{SD}_{\text{blank}}$$

4. *Limit of detection* (LOD; aka lower limit of detection, LLOD; aka "analytical sensitivity"): The lowest concentration that can be distinguished from the LOB with ≥95% certainty (a normal distribution is assumed or data are transformed to fit a normal distribution). It is

obtained by replicate testing (usually ≥20 replicates) of a sample with a known low concentration that is >LOB. The experiment might have to be repeated if the chosen concentration was too high or too low.

LOD = LOB + unidirectional *Z*-score for 95% probability (=1.645) × $SD_{low\text{-}sample}$

5. *Limit of quantitation* (LOQ; aka lower limit of quantitation, LLOQ; aka "functional sensitivity"): lowest concentration that can be reliably measured based on predefined goals of inter-assay accuracy (bias) and inter-assay repeatability (precision/imprecision). These goals should ideally be selected based on the anticipated ability to maintain them in day-to-day testing. In addition, one should take into account the clinical impact that these bias and imprecision thresholds would have. However, in endocrine testing, these goals are commonly arbitrarily defined as bias and imprecision of <20% each. Recommendations are that at least 20 different runs should be evaluated to generate the accuracy and precision data.

   The LOQ is usually the lowest concentration that is reported for a given test. It cannot be lower than the LOD.
6. *Linear measurement range* (aka dynamic range; aka analytical measurement range): the range of values between lowest and highest analyte concentration, which falls on the linear portion of the assay's calibration curve.
7. *Dilution linearity*: ability to perform sample dilution and obtain the result that would have been predicted based on the analyte concentration that was present in the undiluted sample. Tolerated deviations from the expected result are generally ±10–20%. Laboratories have to establish dilution linearity if they wish to report results of dilution testing of samples that contain analyte concentrations above the upper limit of the linear measurement range.
8. *Cross-reactivity*: interference in the measurement process by compounds that are not the analyte, but are sufficiently similar to it, to be mistaken for analyte during the measurement process. Expressed as fraction or percentage of signal generated by the cross-reacting substance compared to an equal concentration of analyte (i.e., 5% cross-reactivity means that 1 unit of cross-reacting substance produces 5% of the signal of the 1 unit of the target analyte).
9. *Interferences*: anything that can impair the accuracy or repeatability of the measurement process. Cross-reactivity can be seen as one form of interference.

Other interferences include substances that interfere with the detection process. Since nearly all immunoassays (IAs), except gamma-counting radioimmunoassays, use light detection, optically active substances such as hemoglobin or bilirubin fall into this category. Interferences that affect antigen-antibody immune-complex capture, such as biotin that prevents the binding of biotin-labeled antigen-antibody complexes to streptavidin, also fall into this category. All interferences in this group lead to low assay signal, which in a competitive IAs results in false high results, while immunometric IAs give false low results.

The next big group of IA interferences are substances, such as high concentrations of lipids or immunoglobulins, which might disrupt the antigen-antibody reaction. Mostly, but not always, a low assay signal is produced.

Finally, interferences by autoantibodies, directed against the analyte, and by heterophile antibodies, directed against assay antibodies, can result in false high or false low results (see Figs. 11 and 12).

Core Thyroid Function Tests

Assays Used for Core Thyroid Function Testing

The core thyroid function tests are TSH and T4. For the latter, mostly the free fraction, free T4 (FT4), is measured. T3 is also frequently measured, again mostly in its free form (FT3).

TSH is usually the starting point for testing, because the exponential response of TSH secretion to changes in peripheral thyroid hormone levels allows earlier and more confident detection of disturbances of thyroid function than measurement of peripheral thyroid hormones, at least if the hypothalamic-pituitary-thyroid feedback loop is intact (Fig. 3). However, this profound response of TSH makes it a suboptimal measure of severity of thyroid dysfunction, and in most cases, FT4 or FT3 are also tested.

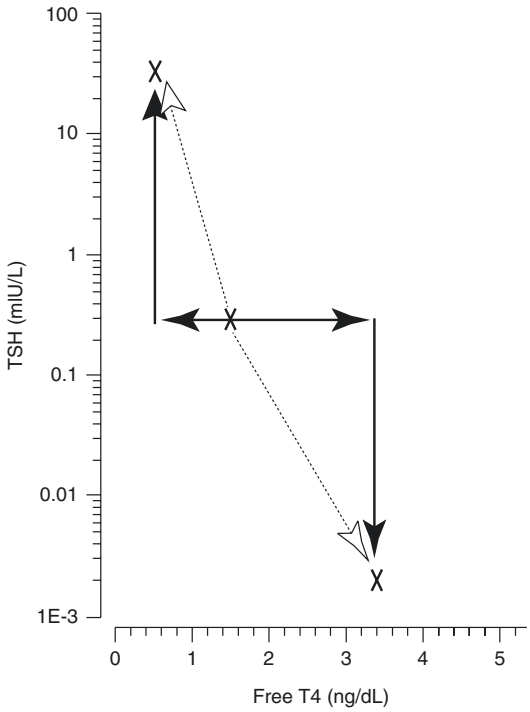

Fig. 3 Schematic depiction of the exponential response of serum thyrotropin (TSH) to changing peripheral thyroid hormone levels. In this example, a halving or doubling of serum free thyroxine (FT4) concentrations leads to a ~60-fold upward or downward change, respectively, in serum TSH concentration. The magnitude of this response varies between individuals, and for changes that do not exceed the FT4 normal reference range, the response is more muted [19]. From Grebe 2012 [1] with permission from publisher

Performance Characteristics of the Assays Used

The vast majority of core thyroid function tests are performed by IAs, mostly on automated platforms. The IAs for T4, FT4, T3, and FT3 are competitive IAs (Fig. 4), because the small size of T4 and T3 precludes the use of sandwich immunometric IAs. By contrast, TSH assays are almost without exception sandwich immunometric IAs (Fig. 4). This assay format offers advantages over competitive IAs with regard to reduced cross-reactivity, better detection sensitivity, and a wider dynamic range of measurement (Table 2), and all modern TSH assays have essentially no cross-reactivity with LH, FSH, or hCG, detection sensitivities of <0.02 mIU/L, and a > 4 Log_{10} dynamic range. By contrast, competitive thyroid hormone assays continue to be occasionally plagued by cross-reactivities, rT3 immunoassays might cross-react with T4 [20], while the dynamic range limitations force manufacturers to design their assays to be either very accurate at low concentrations or at high concentrations; to achieve both is impossible (Fig. 5). For total T4 and T3 assays, this can be easily addressed by sample dilution. However, because of the vast binding capacity of thyroid hormone-binding proteins, free thyroid hormone assays do not give reliable results upon sample dilution.

A reference, or at least candidate reference, technology is available for T4, FT4, T3, and FT3 assays in the form of liquid chromatography, tandem mass spectrometry (LC-MS/MS, Fig. 6). LC-MS/MS largely avoids the inherent shortcomings of competitive IAs (Table 2); in the case of the free hormone measurements, LC-MS/MS is coupled with physicochemical separation of free hormones from protein-bound hormones, either by equilibrium dialysis or by centrifugal filtration (Fig. 7). LC-MS/MS allows highly accurate hormone measurements, because T4 and T3 can be purchased as chemicals of defined and certified purity. LC-MS/MS can therefore be used to check, and possibly adjust, the calibration and the accuracy of T4, FT4, T3, and FT3 immunoassays.

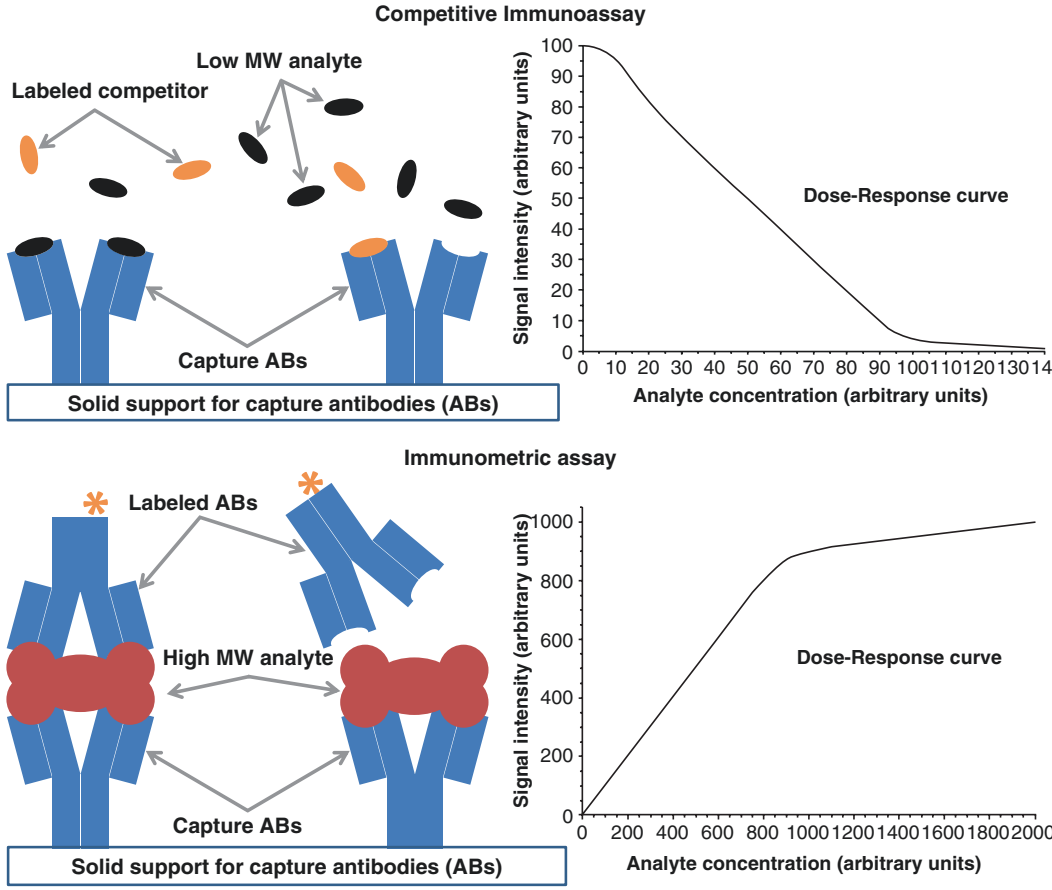

Fig. 4 Cartoon of the assay configurations of competitive (top panel) and immunometric (bottom panel) immunoassays (IAs). In a competitive IA, analyte in a patient sample competes with added labeled analyte for binding to a limited number of assay antibodies (left of upper panel). The more labeled analyte is bound, the higher the generated signal. The dose-response curve is inversely proportional to the concentration of analyte present in the patient sample (right of upper panel). Since the assay antibody concentration has to be smaller than the sum of patient analyte and added labeled analyte, the dynamic range of this type of assay is narrow, with the example dose-response curve being only linear between ~10 and ~95 arbitrary units. In an immunometric IA, analyte is typically sandwiched between two assay antibodies (at least one of which is labeled), which are directed against different analyte epitopes (left of lower panel). The more analyte is present, the higher the signal. The dose-response curve is directly proportional to the concentration of analyte present in the patient sample (right of lower panel). The dynamic range is only limited by the amount of assay antibodies in the assay, with the example dose-response curve being linear between ~10 and ~900. Immunometric IAs only work for analytes that are large enough to allow simultaneous binding of two antibodies (>1500–3000 Da)

By contrast, there is no reference—nor a candidate reference—methodology for TSH measurement. Moreover, the current international standard for TSH (WHO 81/565), as all previous preparations, is pituitary sourced, and its exact composition, purity, and isoform mix have not been defined. The units were assigned based on consensus immunoassay and bioassay results. The data of the validation of this standard material showed the possibility of gross errors in assay performance, and geometric means had to be used to assign values to the international standard. Even then, geometric coefficients of variation of 10–20% were observed in the measured values [21]. This is not surprising, as different assays might recognize different variants of natural TSH [22]. Based on high-resolution mass spectrometry studies of bovine pituitary TSH

Table 2 Performance characteristics of thyroid-relevant assay methodologies

	Competitive immunoassay	Immunometric immuno assay	LC-MS/MS[a]
Thyroid relevant analytes	TT4, TT3, FT4, FT3, rT3, TPO-AB, TgAB, TRAB	TSH, TBG, TTY, Alb, TPO-AB, TgAB, Tg	TT4, TT3, FT4, FT3, rT3, Tg
Candidate reference methodology	No	No	Yes
Analytical characteristics			
Limit of detection Dynamic range General accuracy High concentration accuracy Imprecision Turnaround time	Medium to low[b] Narrow (≤2 $\log_{10}$) Average Poor to average[b] Medium (CV 5–15%) Slow(≥3 days to quick (same day)	Lowest Wide (≥3 $\log_{10}$) Poor to high Poor to average[c] Low (CV 3–10%) Quick	Low Widest (≥4 $\log_{10}$) High High Medium (CV 5–15%) Slow to medium (1–3 days)
Analytical interferences			
Cross-reactivity Autoantibodies Heterophile ABs Other reagent-related interferences	Low to modest Modestly affected Rarely affected Rarely to modestly affected	Low Highly affected Highly affected Rarely to modestly affected	Lowest Not affected Not affected Not affected
Comparability between different assays for the same analyte	Poor to average	Poor to average	Average to good
Skill level required for testing	Low to high	Low	Medium to high
Cost of testing	Low to high	Low to medium	Low to high

[a]With equilibrium dialysis or centrifugal filtration for free hormones
[b]The limited dynamic range forces assay designers to configure the assay either for low or for high concentrations
[c]At very high analyte concentrations, the assay antibodies of immunometric assays all become occupied with analyte. In a two-step assay, where excess antigen is washed off after capture, this leads to detection of signal plateau. In a one-step assay, the analyte capture and detection antibodies are mostly individually occupied by analyte, with few sandwiches being formed. The detection "hooks" downward (falls off proportionally with the amount of excessive antigen), in extreme cases down to "normal" levels or below

preparations, prepared in a similar fashion, one can expect that the current international standard preparations also contain dozens of other proteins, including other pituitary hormones, albumin, hemoglobin, and various tissue proteins [23]. Consistent with this scenario is that parallel testing of a reference recombinant human TSH showed substantial deviation of measurements from expected values, when run as unknown against the WHO 81/565 International Standard [21]. Until this situation is resolved, most likely with a move to recombinant TSH standard material, standardization or harmonization of TSH assays remains difficult.

However, the peripheral thyroid hormone assays do not fare much better. Total T4 is the only peripheral thyroid hormone that shows acceptable agreement between different IAs

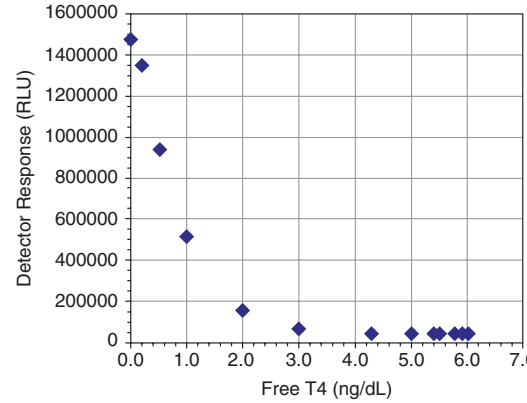

Fig. 5 Example of an actual dose-response curve of a competitive IA for FT4. The signal response is linear from ~0.2 to ~1.5 ng/mL. With data transformation (typically log-logit) that can be extended to ~4 ng/dL, but any higher concentrations of FT4 cannot be distinguished from each other. This particular assay would therefore not be well suited for monitoring patients with very high FT4 levels

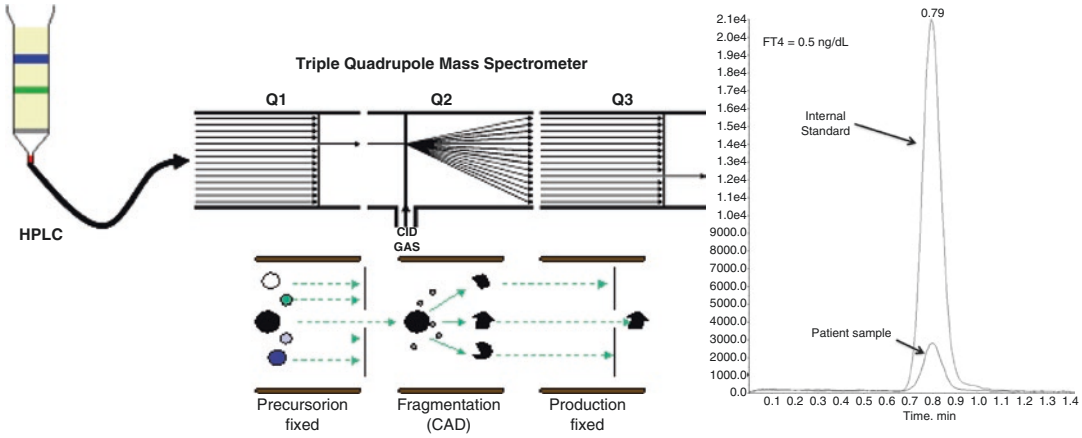

Fig. 6 Simplified diagram of a liquid chromatography, tandem mass spectrometry (LC-MS/MS, aka LC triple quadrupole MS) measurement system. A HPLC system (leftmost display item) separates the sample into different components based on their physicochemical properties (e.g., hydrophilic versus hydrophobic). The HPLC effluent is continuously injected into the near vacuum of the mass spectrometer, where its constituent molecules are ionized and then accelerated toward the detector. They pass into the first quadrupole mass filter (Q1), which in this example is set to only let pass through ionized molecules of a specific mass-to-charge ratio (*M*/*Z*; for low molecular weight compounds, this is usually equal to their molecular weight). The selected molecular ions then enter Q2, where they collide with nitrogen gas, fragmenting them into smaller parts, which then enter another mass filter (Q3), again set to only allow passage of fragment ions of a specific *M*/*Z*. These in turn hit an impact detector, which generates an ion chromatogram (HPLC time on *x*-axis, signal strength on *y*-axis). In this example, a representative chromatogram of FT4 detection in dialysate buffer is shown. A nonradioactive isotopic T4 internal standard has been included, to correct for analytical mishaps

(defined as results being within about +/−20% of each other for the same sample), and despite some improvement over time, results of thyroid hormone testing continue to differ between platforms [24–28], as shown in Fig. 8. In addition, some combinations of different FT4 (or FT3) assays might display relatively poor correlation with each other (correlation coefficients of between ~0.2 and ~0.8) [25].

The measurement of free thyroid hormones also continues to be problematic with regard to its accuracy and its validity in some patient groups. As mentioned above, physicochemical separation of free hormones from protein-bound hormones before measurement is considered the gold standard for FT4 and FT3 testing. However, the vast majority of free thyroid hormone assays are performed by competitive IAs. The available IAs use three strategies (two of which are conceptually related to each other) to selectively measure just the small free fraction of T4 or T3 (Fig. 9). However, at best, these approaches come close to reference methodology when used in normal individuals [27], but at worst, they fail in a significant subgroup of patients with disturbed thyroid function, in particular those with very high or very low binding protein concentrations, many patients with abnormal binding proteins, and those with very high or very low FT4 or FT3 levels [29]. If one, again, uses +/−20% deviation from the reference methodology as the yardstick and looks at different commercial FT4 and FT3 assays, this can be predicted to result in inaccurate measurements in 10–20% of outpatients and probably a larger proportion of inpatients [30]. When one holds clinical diagnostic sensitivity constant at 100%, the clinical impact of these inaccuracies for the diagnosis of hyperthyroidism or hypothyroidism ranges from a mild drop in specificity to 97–99% in some assays to a marked decrement to 80–90% specificity in others [25]. Notably, the optimal thresholds for diagnoses in these cases, derived by receiver operator curve analysis, are not necessarily identical to the upper or lower reference limits of these tests, suggesting real-life performance might be worse.

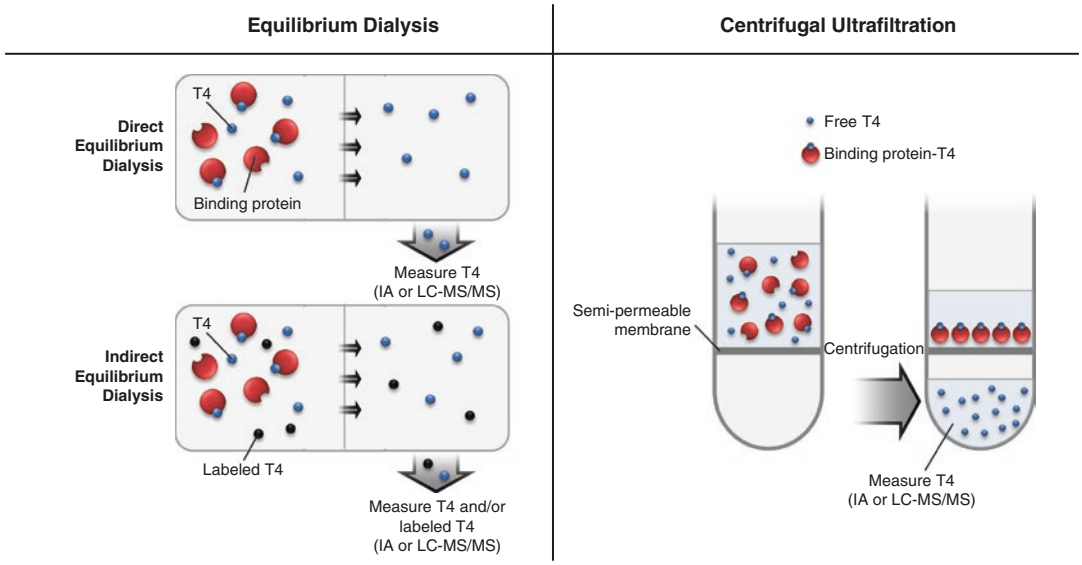

Fig. 7 Schematic depiction of the two gold standard methods used for free thyroid hormone measurement, equilibrium dialysis, and centrifugal ultrafiltration. In equilibrium dialysis, a patient serum sample is dialyzed against an isotonic buffer solution. Free thyroid hormone (in this example T4) can cross the semipermeable membrane between sample and buffer freely, but thyroid hormone bound to binding proteins can't. Over a period of 8–12 h, the concentration of free thyroid hormone in the sample and in the buffer reaches equilibrium. Measurement of T4 or T3 in the buffer then allows back-calculation of the FT4 or FT3 concentration in the sample, based on the total volume of patient sample and buffer. The measurement can either be a direct measurement of T4 or T3 in the buffer, or an indirect measurement (labeled T4 or T3 that was added at the beginning of dialysis is measured), or a combination of the two (in this case, the labeled fraction can be used to normalize results and correct for testing mishaps). In centrifugal filtration, the high G-forces push liquid and low molecular weight compounds, such as free thyroid hormones, through a small pore filter membrane into a separate compartment, while protein-bound thyroid hormone remains behind. The separation is performed so rapidly that there is no time for the binding equilibrium forces to pull significant amounts of thyroid hormone off the binding proteins. T4 or T3 are then measured in the filtrate

Unfortunately, the physicochemical reference methods also have some problems, notably higher complexity of testing, slower turn-around time, higher cost, and imprecision that is almost twice that of automated IAs (Table 2). Due to their core reliance on a physical separation step, the physicochemical methods also suffer from a higher rate of measurement failures than automated immunoassays, mostly due to faults (holes) in dialysis membranes or filtration devices.

All methods for free hormone measurements are vulnerable to false high results in patients who are receiving heparins (including low molecular weight heparins). These anticoagulants liberate free fatty acids in vivo and in vitro, which in turn displace thyroid hormones from binding proteins. This leads to false high measurements in all free thyroid hormone assays, but because of the long duration of equilibrium dialysis, the effects might be more marked when this method is used.

Total T4 and total T3 measurements are typically more accurate than FT4 and FT3 measurements, when results are compared to LC-MS/MS. Their lack of current popularity is largely due to the fact that females of reproductive age, as well as all individuals, who are taking estrogen preparations, have high levels of thyroid hormone-binding proteins, in particular TBG. Moreover, TBG levels vary during the menstrual cycle, resulting in fluctuating total thyroid hormone levels. In addition, in extremely high estrogen states, such as pregnancy or during fertility treatments, total T4 and total T3 assays might give erroneously low results, due to

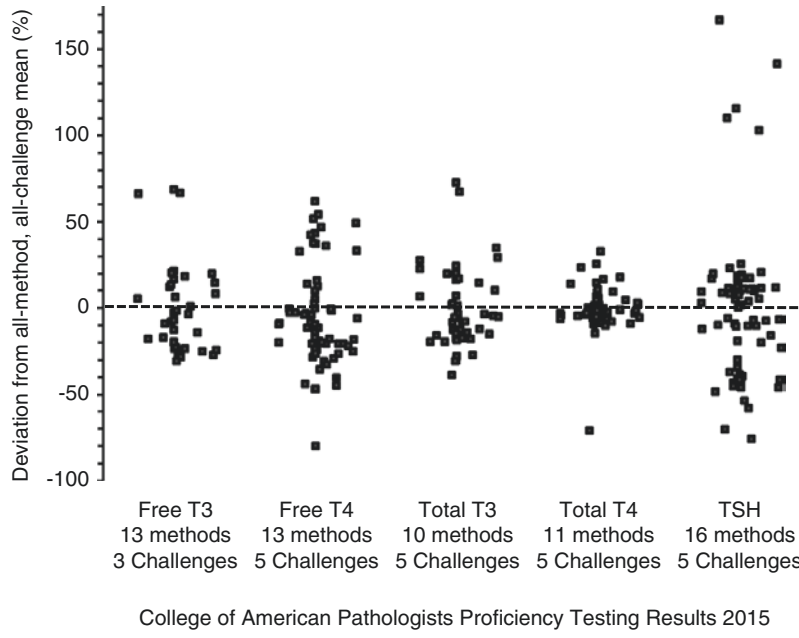

Fig. 8 Result agreement of different immunoassays systems for the measurement of free and total T3 andT4 and of TSH. The results of mandatory US proficiency testing for thyroid hormones for the year 2015 are plotted. Individual challenges typically cover low, medium, and high concentrations over the period of a year. Each method is used by at least 20 different laboratories (most by several hundred laboratories). The results of all challenges and methods for each analyte (FT3, FT4, total T3, total T4, and TSH, respectively) have been normalized to their respective all-methods, all-challenges mean. The individual results have been plotted as percentage deviation of their respective all-methods, all-challenges mean. If all assays were in perfect agreement, then all data points would lie on the dotted line that runs through 0% deviation

incomplete displacement of thyroid hormones from the binding proteins during the assay procedure (Fig. 10). While these diagnostic challenges are somewhat addressed by free thyroid hormone assays, a case can be made that in many patients total thyroid hormone assays are not significantly inferior to the free hormone assays in diagnostic accuracy (with the exceptions noted above). Moreover, total T4 assays are the best harmonized of all the thyroid hormone assays, and in situations where patients undergo repeated testing and this testing cannot be, or is not, performed with the same assay throughout, total T4 assays might be preferable to FT4 assays.

Assay Interferences

Most IAs can be affected by so-called "matrix-related" interferences, namely, hemolysis and hyperbilirubinemia, hyperlipidemia, and hyperproteinemia, as well as potential interferences by autoantibodies, heterophile antibodies, and assorted other interferences directed against assay reagents (e.g., biotin interference in assays that utilize streptavidin-biotin interactions for assay antibody anchoring or capture) (Box 1, Table 2, Figs. 11 and 12) [32–37]. As a rule, LC-MS/MS assays are not affected by any of these interferences.

Noncore Thyroid Function Tests

Assays Used for Noncore Thyroid Function Testing

Reverse T3 and thyroid hormone-binding proteins (TBG, transthyretin, albumin) are sometimes measured, when core thyroid function tests give inconclusive or confusing results. The various patterns of T4, T3, FT4, FT3, and TSH that are seen in these situations are discussed in more

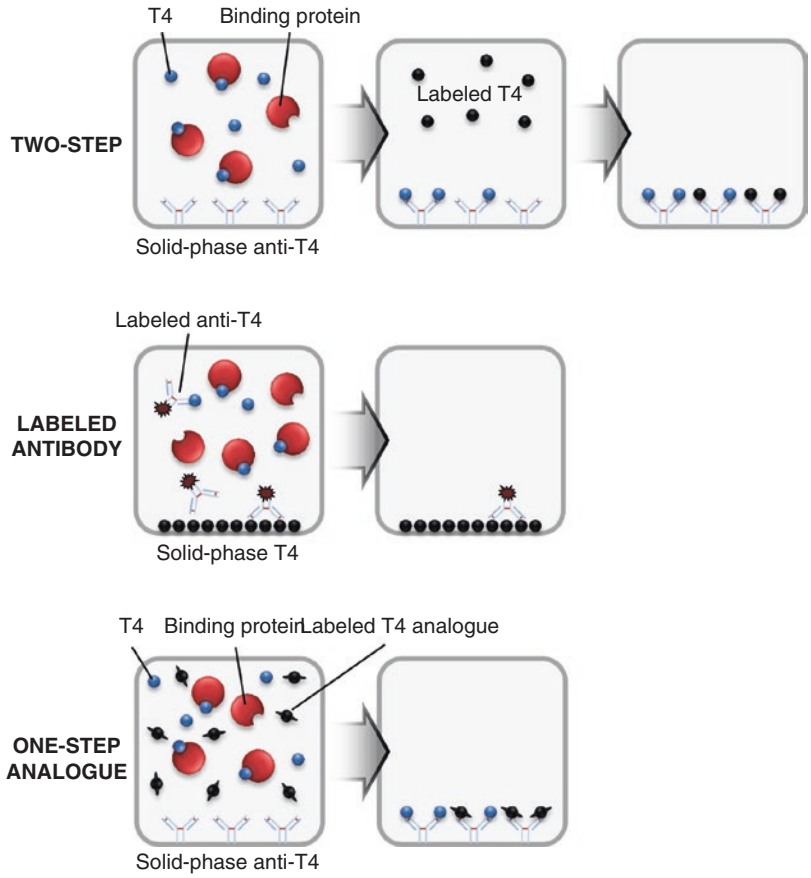

Fig. 9 Schematic depiction of the three commonly used immunoassay methods for free thyroid hormone measurements, using FT4 as the example. The two-step method relies on a very short and precisely timed incubation of assay reagents with patient serum. This favors binding of just the free T4 or T3 fraction to the assay antibodies. There is not enough time for significant amounts of protein-bound T4 or T3 to dissociate from the binding proteins and bind to the assay's anti-T4 or anti-T3 antibodies. Next there is a vigorous washing step to remove all thyroid hormone-binding proteins. This is followed by addition of labeled T4 or T3 and measurement of the signal. In practice, it has proven impossible to achieve a perfect washing step. The labeled antibody approach is a recent variation on the two-step method. Labeled T4 or T3 antibodies bind to either solid phase-bound T4/T3 or to the free T4/T3 in the patient serum. Following a short incubation, binding proteins and antibodies that have bound patient T4 or T3 are washed off, and the signal is measured. These assays have shown themselves more robust than the original two-step assays, but, again, their theoretical advantages have not been entirely translated into practice. The one-step analogue approach takes a completely different tack. These assays use an artificial T4 or T3 analogue that has been designed to have equal affinity as T4 or T3 to the assay antibodies, but negligible affinity to thyroid hormone-binding proteins. This should result in a perfectly accurate FT4 or FT3 measurement without the need for fickle incubation times or extremely vigorous washing steps. In reality, the creation of such a perfect competitor is, of course, impossible, and these "analogue" FT4 and FT3 measure something in between free and total T4

detail in this chapter's final section. The underlying conditions include (1) non-thyroidal illness, where measurement of rT3 and thyroid hormone-binding protein analysis might be indicated [38–40]; (2) suspected primary or acquired thyroid hormone transport protein abnormalities, which is investigated with thyroid hormone-binding protein analysis [7, 8]; and (3) suspected deiodinase anomalies, including primary genetic deiodinase defects and consumptive hypothyroidism due to tumors expressing high levels of type 3 deiodinase, which benefit from rT3 testing [15, 41–43].

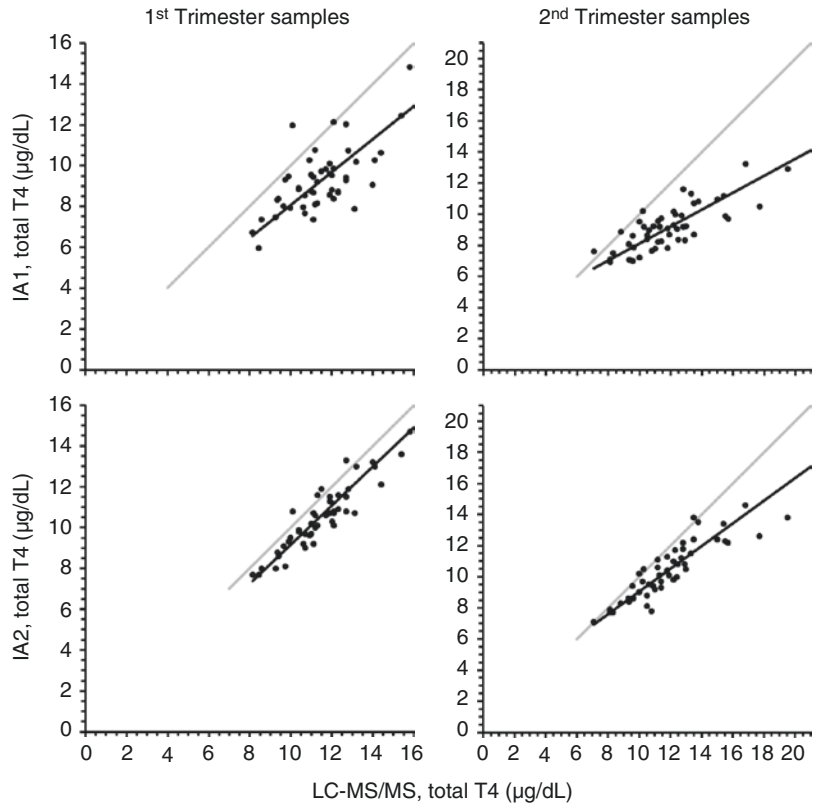

Fig. 10 Measurement bias of two different total T4 immunoassays against LC-MS/MS measurement for samples from women in the first and second trimester of pregnancy. The LC-MS/MS method completely denatures all binding proteins before measurement and should represent the most accurate T4 result. The results of the immunoassays are plotted against LC-MS/MS. The gray lines represent hypothetical identity of measurements. Immunoassay 1 (IA1) shows a constant (high) and a proportional (low) bias against LC-MS/MS. The latter is dramatically worsened when second trimester samples are measured. Immunoassay 2 (IA2) displays the same pattern, but both constant and proportional bias are much smaller than what is seen with IA1. Total T3 and total T4 assays have to dissociate thyroxine from its binding proteins before measurement. Unlike older manual immunoassays, which used protein denaturation, or pH manipulation, followed by adsorption of T3 or T4 to charcoal or resins before the actual competitive IA was performed, modern total T3 and T4 assays use displacement reagents. These displacement methods work fine in most situations, but when TBG concentrations are extremely high, as during pregnancy, they might fail

Performance Characteristics of the Assays Used

Reverse T3 is mostly measured by competitive immunoassays, although LC-MS/MS assays are also used and provide a candidate reference methodology. All available assays are either research kits or laboratory-developed assays. There are, to my knowledge, no published studies comparing multiple rT3 IAs with each other. There has been a recent study comparing a radioimmunoassay (RIA) rT3 kit (RIAZEN reverse T3, ZenTech, Anleur, Belgium) with a rT3 LC-MS/MS method. This comparison showed a reasonable correlation (r = 0.928), but a substantial regression slope, with the RIA measuring about 2.5 times higher than the LC-MS/MS method, suggesting either different calibration or cross-reactivity of the RIA, most likely with T4, as has been previously reported [20, 44]. Therefore, rigorously established assay-specific reference intervals are mandatory for rT3 testing, and the same assay should always be used for serial testing.

Thyroid hormone transport proteins are either measured individually, almost always with immunometric IAs, or they are electrophoretically separated from each other after incubation with

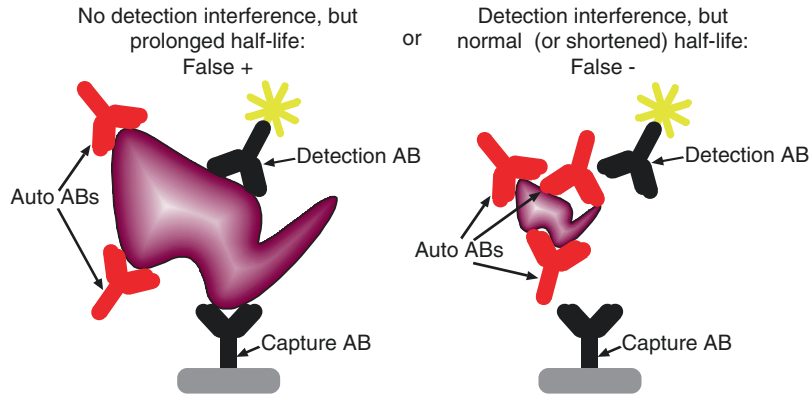

Fig. 11 Example of autoantibody interferences in immunoassay. The left panel shows a case where the antibodies do not interfere with analyte detection by IA. However, in many of these situations, autoantibodies will increase the half-life of their antigenic target. Since this is usually rendered biologically inert by the bound antibodies, this results in an analytically and biologically false high result. The right panel shows autoantibodies that interfere with analyte detection by IA. In this case, the measurement result will be false low. Modified from Grebe 2009 [31] with permission by publisher

radiolabeled T4 (thyroxine-binding protein electrophoresis, TBPE). The two approaches are complimentary. The former allows quantitation of the binding proteins, while the latter assesses their ability to bind T4, which is important, because some inherited or acquired binding protein abnormalities do not reduce serum-binding protein concentrations but lead to abnormal binding proteins or displacement of thyroid hormone from the binding proteins because of endogenous or exogenous competitors. In addition, TBPE can detect anti-T4 autoantibodies, which might occur in a significant minority of patients with autoimmune thyroid disease. In most of these cases, there are very high total T4 concentrations and, in instances when the autoantibodies have relatively low avidity, also very high FT4 levels. The TSH is typically normal; the autoantibody-bound T4 is not bioactive. In the TBPE assay, these autoantibodies are detected by high T4 concentrations at the origin of the gel, where immunoglobulins largely remain during the electrophoresis. Of the binding protein measurements, serum ALB is fairly well standardized, while TTR and TBG are poorly standardized, with all the consequences for assay-to-assay comparability discussed before. TBPE is very reproducible, provided similar amounts of patient serum, similar doses of labeled T4, and comparable electrophoretic conditions are used.

Assay Limitations and Interferences

The assay strengths/weaknesses and interference discussed before apply to all the noncore thyroid function testing IAs.

Tumor Marker Testing in Thyroid Disease

Tumor Marker Assays Used in Thyroid Disease

Thyroglobulin (Tg), calcitonin (Ct), and carcinoembryonic antigen (CEA) are three soluble tumor markers frequently used in thyroid disease, mainly in follow-up.

Tg is highly specific for thyroid follicular cells. It is therefore extremely well suited for follow-up of thyroid cancer patients who have undergone complete ablation of all thyroid tissue (total thyroidectomy with or without radioactive iodine remnant ablation). In successfully treated patients, serum Tg levels should be extremely low or undetectable. Tg is less useful for diagnosis of thyroid cancer, despite the fact that many thyroid cancer patients at diagnosis will have higher than average serum Tg levels; the overlap with the normal population is too large [31].

Ct is fairly specific to thyroid C-cells, albeit not quite to the same degree as Tg is for follicu-

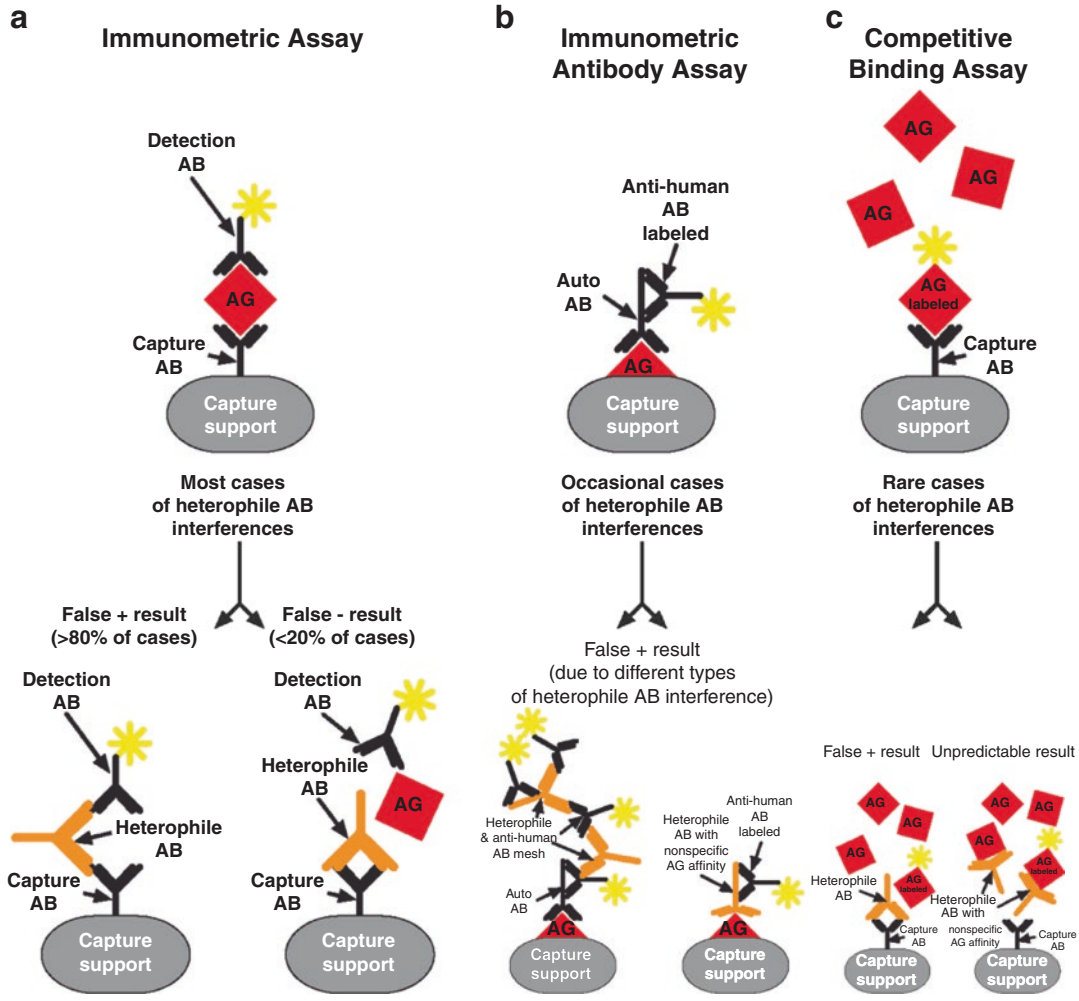

Fig. 12 Effect of heterophile antibodies on immunoassay measurements. Heterophile antibodies (HAB) are antibodies found in some patient samples that can bind to the antibodies used in immunoassays, thereby potentially leading to inaccurate results. Immunometric IAs are most vulnerable to HAB interferences, but immunometric antibody IAs can also be affected, and rarely competitive IAs might be affected. In immunometric IAs (panel A), the most common interference pattern is a false positive/high result due to the HAB bridging capture and detection antibody in the absence of actual analyte. In a much lower percentage of cases, the HAB will bind only to one of the assay antibodies, thus leading to a false low result. In immunometric antibody IAs (panel B), the interference is typically false positive. In competitive IAs, the interference is mostly false positive, but in some cases, it can be either false positive or false negative. Modified from Grebe 2009 [31] with permission by publisher

lar cells; some Ct might be produced by non-thyroidal tumors, in particular those containing neuroendocrine components, some hemato-lymphatic malignancies, during systemic inflammatory conditions, including severe infections, during pregnancy and lactation, and during the first few weeks of life. Nonetheless, Ct is the key tumor marker used for medullary thyroid carcinoma, based on the same rationale as outlined above for Tg. However, unlike Tg, circulating serum Ct levels in patients with intact thyroid glands are very low (with the exception of some of the scenarios outlined above), and serum Ct measurement is therefore also widely used for diagnosis of medullary thyroid carcinoma [45, 46].

CEA is a malignancy-associated, rather than organ-specific, tumor marker. It is elevated in a subset of medullary thyroid carcinomas, in particular those that are poorly differentiated. If it is

elevated at diagnosis in a patient, it can be used in parallel with Ct for subsequent disease monitoring or, instead of it, if Ct is negative, as is sometimes observed in poorly differentiated tumors. By token of its linkage to less well-differentiated tumors, CEA is also a prognostic marker [47–49].

Performance Characteristics of Thyroid Tumor Marker Assays

The assays used for Tg, Ct, and CEA are mostly immunometric IAs, although use of mass spectrometry is increasing for Tg measurements, and some RIAs continue to be used for Tg and Ct. International standard preparations are available for all three analytes. The combination of this fact with the oncology-focused use of these analytes has, over time, led to much lesser result difference between assays from different manufacturers than what is observed in thyroid hormone and TSH testing. For example, most of today's commercial Tg assays correlate with each other with r values >0.95 in patient samples and display systematic biases to each other of <20%. Tg LC-MS/MS assays agree equally well with each other and also quite well with Tg IAs, at least in patients without detectable TgAB in their serum (see below) [50, 51]. Nonetheless, because of the critical nature of comparable results in tumor follow-up, use of the same assay over time for a given patient is still recommended, in particular if tumor marker doubling (or halving) times are calculated, as has become popular for Ct and, more recently, Tg [48, 52, 53].

A low limit of quantification is crucial for all Tg assays. Ideally, it should be ≤0.1 ng/mL. Less than 1% of patients with serum Tg levels below this threshold will have persistent or recurrent disease, even if the measurement is performed without any stimulation [53, 54]. Some Tg immunometric IAs achieve this cutoff, some come close (0.2–0.3 ng/mL), but several other Tg assays have higher lower limits of quantitation, including some of the current immunometric IAs (≥0.9 ng/mL), as well as the available competitive IAs and Tg-MS assays (both ≥0.5 ng/mL; although some Tg-MS assays now achieve 0.1 ng/mL). For several of these assays, stimulation testing might still be necessary in some patients, either through T4 withdrawal or by means of recombinant human TSH injection. Finally, while Tg is primarily used as a tumor marker, its measurement might also assist in diagnosing nonneoplastic thyroid tissue destruction. In particular, subacute thyroiditis, silent thyroiditis, and postpartum thyroiditis often show substantial serum Tg elevations due to follicular destruction.

Assay Limitations and Interferences

The IAs for Tg, Ct, and CEA are all subject to the well-known interferences that can affect immunometric or competitive IAs (Table 2). However, it should be noted that hooking of immunometric IAs is more commonly encountered in tumor marker testing than in other applications, because patients with a large tumor burden might occasionally have astronomically high tumor marker levels.

Another important point of note is that autoantibody assay interferences are particularly common in Tg assays. Between 15% and 30% of thyroid cancer patients have detectable anti-Tg autoantibodies (TgAB), a rate at least twice that of the general population. Interference with Tg measurements by IAs is a possibility in each of these cases [31, 55]. In immunometric IAs, such interference results in a false low measurement bias, whereas competitive IAs (mostly RIAs) usually show the opposite (false high). Finally, some RIAs might give false high results in TgAB-positive samples with low Tg concentrations (≤1 ng/mL) but false low results, if Tg concentrations in the sample are higher (≥25 ng/mL) [50]. Since TgAB interferences can occur even at very low TgAB concentrations, well below of what is considered diagnostic for autoimmune thyroid disease, this represents a substantial problem. LC-MS/MS assays have been developed to overcome this problem. Tryptic digestion of patient serum cleaves all serum proteins, including TgAB and Tg, into predictable fragments, which can then be measured by MS, in theory overcoming the TgAB interference problem (Fig. 13). Indeed, in TgAB-positive cases, Tg-MS detects higher serum concentrations of Tg than immunometric IAs; by contrast, in TgAB-negative patients, immunometric IAs and Tg-MS assays agree much closer with each other [50]. In addition, Tg-MS detects Tg in a

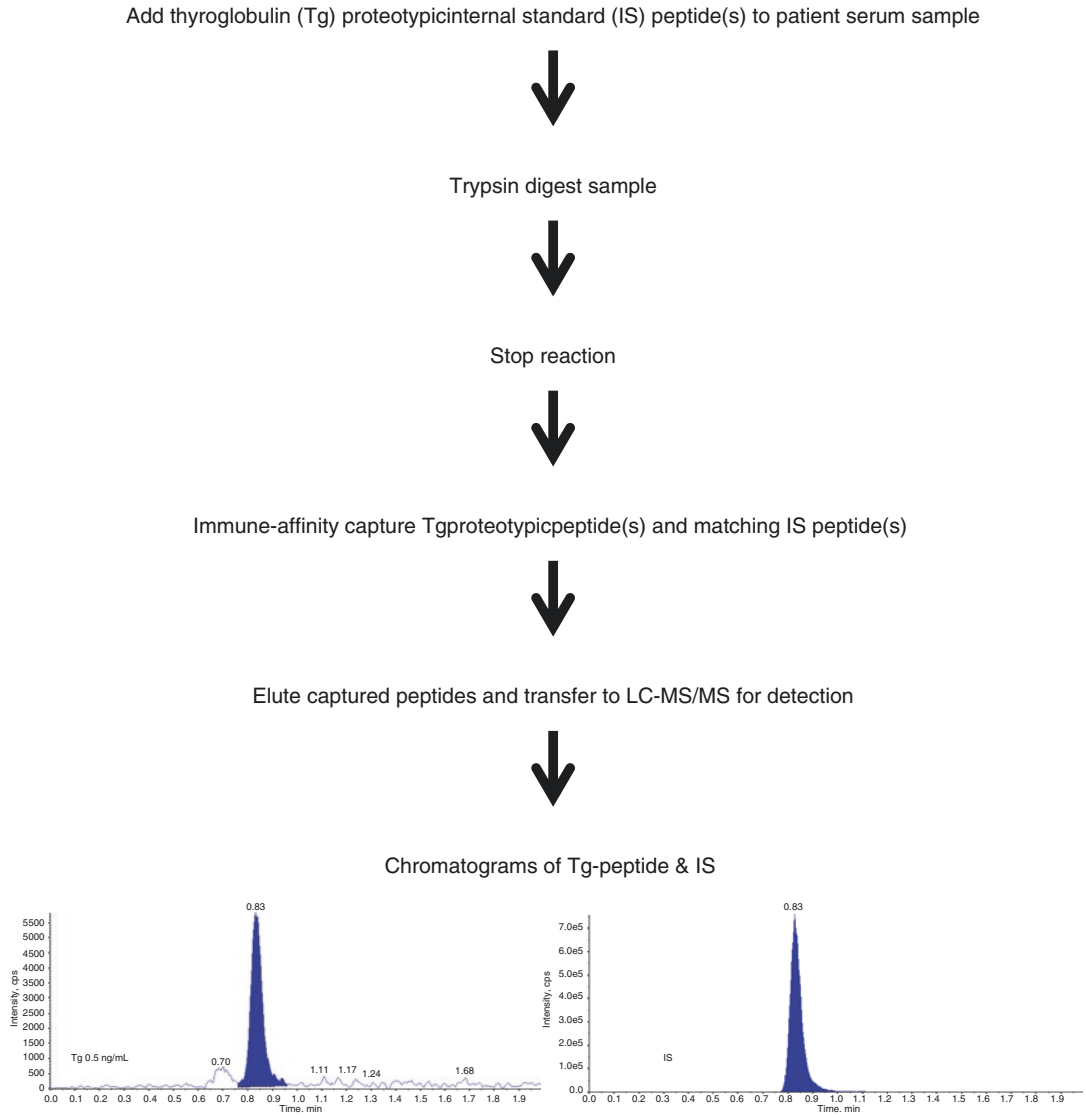

Fig. 13 Workflow diagram for thyroglobulin measurement by LC-MS/MS. Extensive sample preparation, trypsin digestion, and post-digestion immune affinity capture of Tg proteotypic peptides are necessary to achieve acceptable detection sensitivity. Interference by Tg autoantibodies is eliminated, due to the trypsin digestion step, which destroys these antibodies (along with all other proteins in the sample)

significant subset of patients with TgAB, who have no measurable circulating Tg by immunometric IA [50, 56]. However, the current Tg-MS assays are not infallible. In patients with confirmed clinical disease, who are TgAB-positive and Tg-negative by IA, about 40% have no detectable Tg by LC-MS/MS. It remains to be determined, whether this is due to low levels of circulating Tg, which most of the current Tg-MS assays cannot detect due to their ~5-fold higher detection limit compared to the latest generation Tg IAs, or absence of Tg secretion by the tumor, or secretion of Tg with an altered amino acid sequence, which causes it being missed by the current Tg-MS assays.

Ct and CEA have not been reported to have high rates of autoantibody interferences. However, Ct measurements are sometimes compromised by

the poor stability of this analyte. Like many peptide hormones, it is readily cleaved by serum- and cellular peptidases. Delays in sample processing and freezing of samples can lead to false low results. Another issue is interference from Ct fragments and abnormal isoforms, which can result in nonlinear assay responses. This is seen primarily in tumor patients with elevated Ct levels. Serial dilution of the sample might allow determination of the true result, but in some instances, no valid answer can be given. Based on these limitations, we and others have investigated procalcitonin (PCT), calcitonin's precursor, which is clinically used a sepsis marker, as an alternative analyte. PCT shows similar clinical performance as Ct when used as a medullary thyroid carcinoma tumor marker, while being free of the stability and fragment problems that affect Ct [57, 58]. Unfortunately, in case of systemic inflammation or infection, PCT is similarly unreliable as a thyroid tumor marker as Ct.

Thyroid Autoantibody Testing

Autoantibody Assays Used in Thyroid Disease

The majority of cases of hypo- and hyperthyroidism in developed countries are due to autoimmune disease. Detection of autoantibodies against thyroid antigens is frequently helpful in (1) confirming or ruling out an autoimmune etiology, (2) estimating the risk of progression of subclinical disease to clinical disease, (3) monitoring ongoing autoimmune disease activity, and (4) predicting disease relapse.

The widely available thyroid autoantibody tests are TgAB, antithyroid peroxidase autoantibodies (TPO-AB), and anti-TSH receptor autoantibodies (TSHR-AB). All these assays are quantitative assays.

Performance Characteristics of Thyroid Autoantibody Assays

Modern TgAB and TPO-AB assays are either competitive IAs or immunometric IAs (Fig. 14). TSHR-ABs are also frequently measured by conventional competitive autoantibody assays, in which the TSHR-ABs in patient serum compete either with labeled TSH or labeled standardized TSHR-AB preparations for binding to intact (or partial) TSHRs. These assays are commonly called thyrotropin receptor-binding antibody (TRAB) assays or thyrotropin-binding inhibitory immunoglobulin (TBII) assays. Alternatively, or in addition, functional assays can be used, which are typically denoted as thyroid-stimulating immunoglobulin (TSI) assays or as thyroid-stimulating autoantibody (TSAb) assays. These assays measure the ability of patient serum, compared to reference serum, to stimulate cAMP production in cells that express the TSHR. Most commonly, Chinese hamster ovary (CHO) cells are used, which have been double transfected with a (sometimes modified) human TSHR and with a luciferase construct, the expression of which is driven by a cAMP-dependent promoter. Cells are lysed after incubation, substrate is added, and glow chemiluminescence is measured, with the signal being directly proportional to the amount of intracellular cAMP that was generated during incubation. The advantage of the TSI/TSAb assay over TRAB/TBII assays is that the stimulating (or not) nature of TSHR-ABs can be unequivocally determined and that it has better detection sensitivity for stimulating TSHR-AB (Fig. 15) [59, 60]. The disadvantages are greater assay variability and more labor-intensive, costly and slower, workflows. In addition, at extremely high concentrations of stimulating TSHR-AB concentrations, TSI/TSAb assays might show hooking. The TSH receptor, like many G-protein receptors other than adrenergic receptors, multimerizes for signaling [61]. At very high concentrations of stimulating TSHR-AB, all the receptors at the cell surface are individually occupied by TSHR-AB, with little opportunity to multimerization and, hence, diminished downstream cAMP production, resulting in low luciferase transcription and low chemiluminescent signal (Fig. 15).

Different TPO-AB assays compare poorly with each other. The same is true for TgAB assays. The relationship between measurements by different assays is only linear when data are log-transformed. Even then slopes of greater than

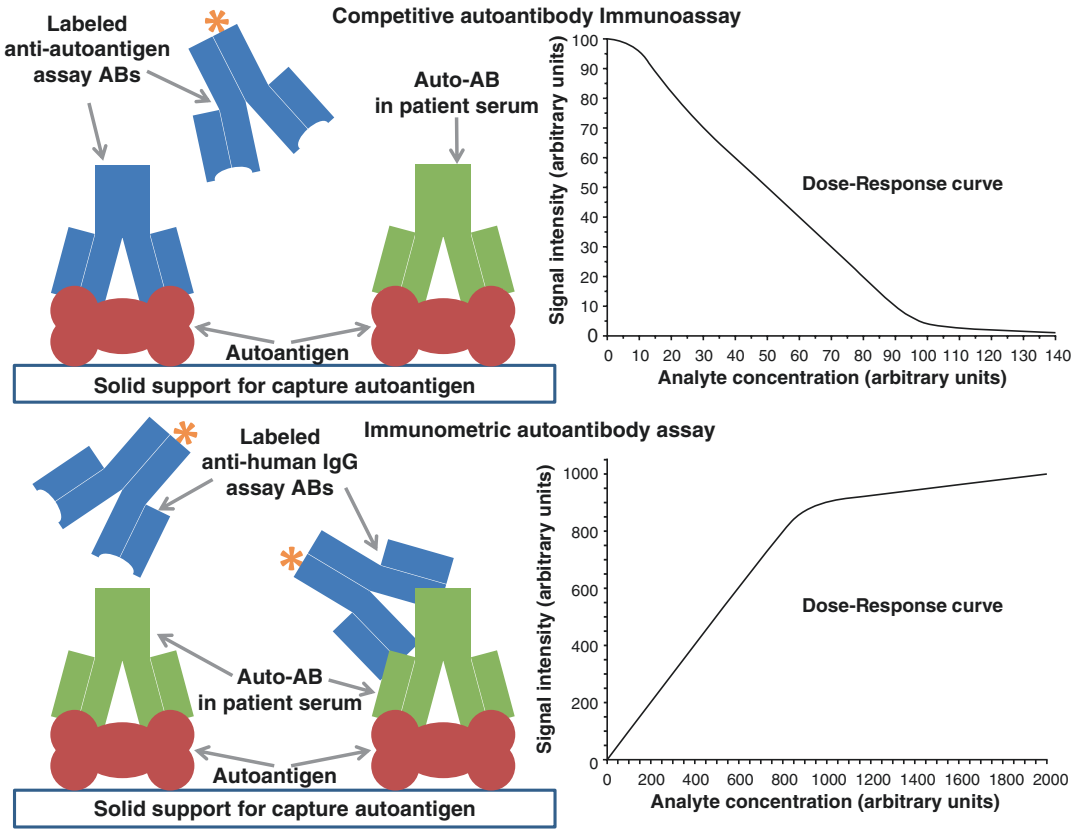

Fig. 14 Cartoon of competitive and immunometric antibody assays. These assays are very similar to ordinary competitive and immunometric IAs, except that they use defined amounts of antigen to capture auto-ABs present in patient serum, either in competition with labeled assay-ABs against the antigen (top panel, left) or in an immunometric design (bottom panel, left), by detecting auto-AB that have bound to the antigenic target by using labeled anti-human IgG ABs (generally generated in mice or rabbits). The dose-response curves (right side of both panels) are analogous to those in ordinary competitive and immunometric IAs, respectively

1.5× log10 (15-fold) are not uncommon, and up to 10× log10 (100-fold) are observed for some combinations of assays. Moreover, correlation coefficients rarely exceed 0.9 and might be lower than 0.7 in many cases (Fig. 16) [62]. The main reasons for this are (1) that the reference materials used (NIBSC preparations 66/387—TPO-AB and 65/93—TgAB) are pooled serum preparations that are more than 40 years old and (2) that Tg and TPO are both large molecules, which makes it likely that different assays use different portions of these molecules as the antigen. Therefore, while manufacturers might succeed in creating pooled serum calibrators that agree reasonably well with the pooled standard preparations, an individual patient's range of autoantibodies might not. For serial measurements, as opposed to diagnosis-only applications, the same assay must always be used, or when the assay is changed, re-baselining should be performed.

The situation is slightly better for TSHR-AB assays. A human monoclonal autoantibody reference material is used, which allows highly reproducible calibration. Almost all TRAB/TBII assays now report in international units (IUs). There is modest agreement between different TRAB/TBII assays, with mostly acceptable

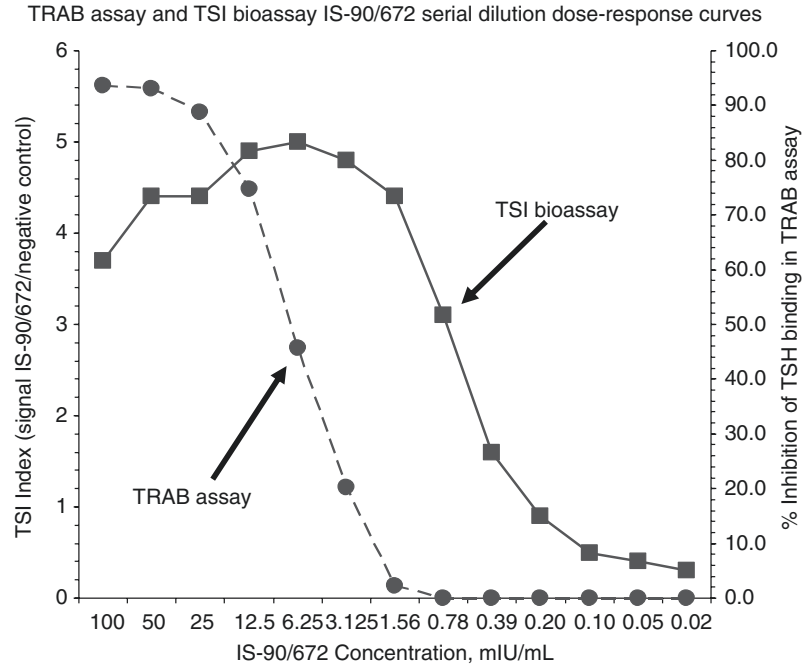

Fig. 15 Dilution series (high to low from left to right) of international standard material for thyroid-stimulating immunoglobulins (human monoclonal TSHR antibody preparation). The squares/solid line represent the dose-response curve for a commercial TSHR-AB bioassay (TSI), while the circles/dashed line shows the dose-response curve of an automated competitive TSHR-AB binding assay (TRAB). The x-axis lists the IS-90/672 concentration. The left y-axis gives the TSI assay response expressed as a fraction of the response of a control sample without TSHR-AB (to convert into percent multiply by 100). The right y-axis shows the percentage of inhibition of TSH binding in the TRAB assay. The response curves are parallel but shifted to relative each other. It is apparent that the TSI assay is several orders of magnitude more sensitive to stimulating TSHR-AB than the TRAB assay; however, at very high concentrations, the TSI assay shows "hooking" (see main text). From Grebe 2012 [1] with permission from publisher

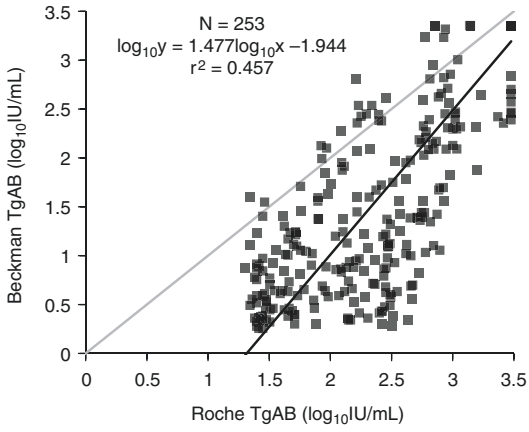

Fig. 16 Example of an assay comparison of two thyroglobulin autoantibody (TgAB) assays. Data had to be log-transformed to achieve reasonable correlation of results, but even then, it is apparent that assay agreement is poor, with a substantial slope and mediocre correlation

slopes and correlation coefficients >0.9. However, some assay combinations continue to show poor agreement [63], and the numerical agreement between TRAB/TBII assays and the functional TSI/TSAb assays is also mediocre (after log transformation of data r values of ~0.7). TSI/TSAb assays at the moment still report the ratios (or percentage) of stimulatory activity in patient samples compared to a reference, rather than in IUs.

Assay Limitations and Interferences

Heterophile interferences can occasionally affect immunometric autoantibody IAs, leading to false high results (Fig. 12). Biotin interference should also be mentioned, as the market-leading TRAB assay (Roche Diagnostics) is streptavidin-biotin capture based.

Determining Whether Test Results Are Abnormal or Different from Previous Results

Understanding the assays used for thyroid-related blood testing is one prerequisite for optimal test selection and result interpretation. The second important foundation is how to classify test results in an optimal fashion into "normal" or "abnormal" results and how to determine whether a measured analyte concentration has truly changed significantly upon repeat testing. The concepts used for these purposes are "reference ranges" and "minimal significant change."

Reference Ranges

Reference ranges, or more precisely healthy population reference intervals, are used throughout nearly all of laboratory testing in order to classify test result in patients as "normal" or "abnormal." Reference intervals typically reflect the central 95% of the result distribution that is seen in healthy subject for a given laboratory test. However, for a significant minority of laboratory tests, either only low or only high values of laboratory test will denote possible pathology. In these cases, reference ranges are "asymmetrical," showing either a high or a low cutoff, but not both.

In some instances, when there are data, or strongly held medical beliefs, which suggest that a healthy population-based approach is suboptimal for identifying individuals at risk of disease, medical decision intervals are used instead. An example are the periodically updated lipid guidelines, which set upper cutoffs at levels that are deemed to reflect a low disease risk, rather than the 97.5th percentile of the population.

A healthy population is also not necessarily the best reference point for tumor marker reference ranges. Tumor marker measurements are primarily used for follow-up, and should be as low as possible in case of successfully treated neoplasia, in particular if one is dealing with organ-specific markers (e.g., Tg or Ct). In this case, the lower limit of quantitation of the assay might become the upper limit of the reference interval.

However, most laboratory tests in thyroidology use healthy population-based reference intervals. As it turns out, this is problematic. TSH, total T4, free T4, total T3, and free T3 all display low indices of individuality (<0.5), with the lowest being observed for TSH [64–69]. The index of individuality is calculated by dividing the within-subject variation upon repeat testing by the between-subject variability. A low index of variability therefore means that an analyte shows marked individuality, with a given individual's range of results being narrower than the population reference range, and, consequently, disease might be detected at a much later stage than optimal (Fig. 17). In an ideal world, patients would have their thyroid function tests always performed with the same assay, in the same laboratory, to minimize between-assay and between-laboratory imprecision (see below), and the serial results would be compared with each other, rather than to a population reference range, with flagging of potentially abnormal results based on a predetermined likelihood threshold that disease is present (for calculation of this threshold, see below).

Unfortunately, this individualized approach continues to be used sparsely, for a variety of reasons (see below). The focus in the thyroid field has therefore been on refining population-based reference ranges. The strategies used have centered on (1) studying larger populations, allowing improved coverage of different age groups across both genders, (2) performing studies in different populations or countries, and (3) performing studies in populations screened in more detail for the absence of thyroid disease (e.g., extensive screening questionnaire, ultrasound of thyroid gland, measurement of various antithyroid autoantibodies, etc.) [70–77]. Most of the studies have focused on TSH, because of its largely undisputed role as the front-line marker of thyroid function. These studies have shown little difference in the lower TSH end of the TSH reference range but ethnic and regional differences in the upper cutoff (between ~3.5 mIU/L and > 5 mIU/L). There is also some evidence that the elderly might have a higher upper TSH limit

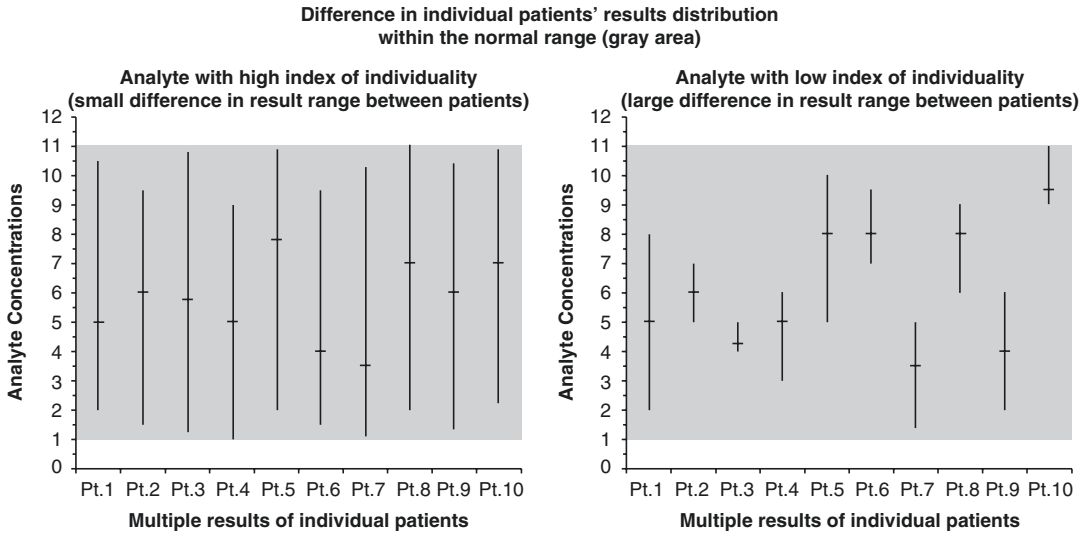

Fig. 17 Example of two hypothetical analytes with high (left) and low (right) indices of individuality. Ten hypothetical subjects are depicted who underwent repeated testing for two hypothetical analytes, one with a high and one with a low index of individuality. The error bars display mean and range of results from repeated testing over time. The gray backgrounds represent the healthy population reference intervals. For the hypothetical analyte with a high index of individuality, there are only small differences in the range of results that are obtained for each subject, and the results of all subjects span almost the entire healthy population reference range. Serum iron measurement in male subjects would be an example of a real analyte following this pattern. For the hypothetical analyte with a low index of individuality, there are large differences in the range of results that are obtained for each subject, and the results of none of the subjects come even close to covering the entire healthy population reference range. Measurement of any of the thyroid hormones would be an example of real analytes following this pattern

(>5 mIU/L) than younger individuals, while children have very high TSH levels during the first year of life (at birth up to ~15 mIU/L at 1 year still ~8 mIU/L). The upper limit of the TSH reference range in children does not reach adult levels until ~10 years of age. It was also found that populations, which were extensively screened for the absence of thyroid disease or risk factors of thyroid disease, often, but not always, have lower upper limits of their TSH reference range (≤2.5 mIU/L) than members of the same population that had not been prescreened to the same extent. Within each study, such observed trends are likely real. However, comparison of different reference range studies is problematic, and clinical guidelines that list fixed cutoff levels for either upper of lower reference ranges are probably misguided. The reason for this is that significant differences in TSH results remain between different assays and that none of the studies undertook any harmonization efforts that might have mitigated the impact of these assay discrepancies. For the time being, TSH population-based reference ranges remain method specific, and age-, gender-, or ethnicity-specific cutoffs may not be transferable from one method to another. Similar considerations apply to peripheral thyroid hormone population-based reference ranges.

Minimal Significant Change

Minimal significant change, also known as critical difference or reference change value, is an important concept in serial laboratory testing. It is based on combining inter-assay variability and biological variability to arrive at thresholds for significant changes. A normal distribution of analytical and biological variability is usually assumed, and for each standard deviation score (*Z*-score, Table 3), corresponding to a probability that the change is not due to chance, the critical

Table 3 *Z*-scores for probabilities from 0.99 to 0.5

Probability	Unidirectional *Z*-score	Bidirectional *Z*-score
0.99	2.33	2.58
0.95	1.65	1.96
0.90	1.28	1.64
0.85	1.04	1.42
0.80	0.84	1.28
0.75	0.68	1.11
0.70	0.52	0.99
0.65	0.39	0.87
0.60	0.25	0.76
0.55	0.13	0.65
0.50	0 (mean)	0 (mean)

difference between two test results obtained from the same subject can be calculated: $\sqrt{2} \times Z \times \sqrt{CV_A^2 + CV_{IB}^2}$, where CV_A and CV_{IB} are the coefficients of variation of analytical variability and intra-subject biological variability, respectively. For example, if we have decided that we want to be 95% certain that two serial results with CV_A and CV_{IB} of 10% each are different from each other, and we use the bidirectional *Z*-score for a 95% probability, which is 1.96, we get $1.41 \times 1.96 \times 14.14 = 39.1\%$. Any change larger than that will have a > 95% likelihood of being "real."

As shown in this example, the required change is often larger than most clinicians would intuitively expect. However, for many laboratory tests, including most thyroid-related tests, this change is much smaller than what would be required for the result to fall outside the population reference range (see above). In addition, in a monitoring situation, one is often primarily interested in unidirectional changes, e.g., a fall in FT4 concentrations following treatment of Graves' disease or a rise in thyroglobulin values in a patient suspected of suffering a thyroid cancer recurrence. In this case, a unidirectional *Z*-score can be used (Table 3), which for the above example would result in a critical difference of 32.9%, rather than 39.1%. Depending on the perceived importance to flag small changes, one can also select *Z*-scores that correspond to lower probabilities than 95% for a change to be not due to chance, accepting that this might lead to more false-positives. If, for example, we were to apply unidirectional and bidirectional *Z*-scores for an 80% probability (Table 3) in our example, the critical differences would shrink to 16.7% and 25.5%, respectively.

A significant hindrance to the widespread use of the minimal significant change concept is that the biological variability for a specific analyte in a specific patient is usually unknown. However, there are published intra-subject biological variability data for most thyroid-relevant tests [64–69, 78–80]. Substituting these values for the unknown ones in a specific patient is still likely to allow earlier detection of significant changes than the use of population reference intervals and is likely to be more reliable than a physician's hunch. Furthermore, once a large number of serial measurements are available for a patient, a variety of statistical tests can be used (time series analysis, ANOVA for repeated measures with post hoc *T*-tests, Tukey Cramer multi-comparison *T*-test with connecting letter report, etc.), to discern significant changes, assuming that the assay(s) used is/are not changed and that the patient is not exposed to drugs or diseases that are likely to affect the biological variability of the analyte(s) in question. Finally, as point of patient testing increases and becomes less invasive, or noninvasive, we can anticipate that increasing proportions of the population will actually have data on their own biological variability for several common analytes, including some thyroid function tests.

Despite all its advantages, the critical difference approach is currently sparsely used. Doubling time of serial tumor marker measurements is a minimalistic example of its real-life application that has gained traction, including in thyroid cancer follow-up, but there is little use beyond this in a formal way. Laboratories are failing the clinicians by not providing these data. In part this failure is due to laboratory testing being increasingly decoupled from clinical practice and fragmented across many providers, making it difficult to match patients and results in a serial fashion. In addition, there is little control about what assay is used to test a specific patient, and not infrequently patients are tested for a

given analyte by different assays over time. Due to the persistent lack of harmonization between different assays, this effectively precludes the use of the minimal significant change approach in such patients.

Testing Approach and Result Interpretation

Thyroid Function Assessment

Initial Diagnosis

As mentioned before, TSH is the best single initial test. However, unless testing is performed solely for the purpose of screening of asymptomatic individuals, it is preferable to also measure peripheral thyroid hormones. FT4 is usually the analyte of choice. In adult outpatients, who are relatively well, FT4 immunoassays are the most convenient and cost-effective choice. In very young children (<2 years of age), in multimorbid patients on multiple medications, in pregnant women, and in seriously ill inpatients, consideration should be given to use a FT4 assay that is based on dialysis or centrifugal filtration, unless an urgent result is required.

Total T4 is a valid, and perhaps superior, alternative to FT4 in adult male patients, prepubertal children over the age of 2, and postmenopausal women who are not on female hormone replacements.

Measuring total T3 or FT3 for initial diagnosis is only indicated if there is a high index of suspicion that the patient has early Graves' disease or an autonomous adenoma.

If only borderline abnormalities are observed, or if TSH and FT4 results are incongruent, measuring total T3 or FT3 is indicated. Alternatively, testing can be repeated after a few weeks.

Result Patterns and Their Interpretation

Once a definitive result pattern has been established, further testing should proceed based on the differential diagnoses suggested by the result patterns. The following result patterns might be observed:

I. Normal TSH and normal FT4 and normal FT3

These patients are most likely euthyroid. Further testing is usually not indicated. However, depending on the clinical presentation, transient thyroiditis in the transition phase from thyrotoxic to hypothyroid phase is a possibility. Finding a significantly elevated Tg (as judged by population references intervals for individuals with an intact thyroid gland) might point into this direction.

Additional considerations apply to patients with known, and treated, primary hypothyroidism. Some individuals will have biochemical euthyroidism, as judged by population reference intervals, but might be mildly under- or over-treated based on their individual reference ranges (see above). If historical data are available, these individual ranges can be taken into account to adjust T4 dosing. Adding T3 has also been advocated if such individuals suffer from hypothyroid symptoms, based on the assumption that patients with ongoing symptoms on T4 treatment might have polymorphisms in their type II deiodinase gene, resulting in diminished T4 to T3 conversion. Type II deiodinase genotypes indeed correlate with thyroid hormone parameters [15, 42]. Reverse T3 measurement is therefore advocated by proponents of this theory in order to identify individuals that might benefit from T3 treatment in addition to T4, with high rT3 levels been viewed as evidence of inadequate T4 to T3 conversion. However, based on the basic physiology of autoregulation of T4 to T3 conversion [14], one would expect that adding T3 to T4 treatment would simply downregulate peripheral T4 to T3 conversion at the expense of creating additional rT3, i.e., an infinite treatment-test-treatment cycle is initiated. For T3 replacement to work as intended, the patients' treatment might have to consist entirely of T3, or T3 might have to be added to T4 in a novel way.

Another biochemically euthyroid group are patients (T4 treated or not), who have a past history of Graves' disease and have received thyroid ablative therapy (radioiodine or surgery). These individuals might still have active thyroid auto-immunity, which puts them at risk of worsening extrathyroidal manifestations of Graves' disease, such as ophthalmopathy [81, 82]. Pregnant females that fall into this category might also pass TSRH-ABs to their fetus. In the case of pregnant women with a past history of Graves' disease, TRAB or TSI measurement in the first trimester is therefore considered standard of care. If results are within the reference range, further testing is unnecessary, while an elevated TRAB or TSI should prompt repeat testing and, in case of >3.5-fold elevations, referral to high-risk obstetric care [83–85].

II. Low TSH (<0.1 mIU/L) and elevated FT4 or FT3

This constellation is typical for primary hyperthyroidism, and the majority of patients with this result pattern will fall into this category. The key to management is to determine the cause of thyrotoxicosis, with the chief contenders in order of likelihood being Graves's disease, toxic nodular thyroid disease (single or multiple nodules), and the thyrotoxic phase of transient thyroiditis. Less common causes include exposure to large amounts of iodine on the background of a large nodular goiter, ectopic thyroid tissue (e.g., struma ovarii), and, extremely rarely, activating germline mutations of the TSHR.

In addition, this pattern can occur when there is no primary thyroid disorder. Uncommon, but not rare, examples of this include T4 or T3 overdoses, amiodarone treatment, and gestational thyrotoxicosis, usually on a background of hyperemesis gravidarum or molar pregnancy (extremely high concentrations of abnormally glycosylated isoforms of hCG cross-react with the TSHR) [86, 87]) or extremely rarely due to familial gestational thyrotoxicosis (germline mutant TSHR with increased cross-reactivity with hCG) [88].

The most common differential diagnostic problems are between Graves' disease and (1) toxic nodular goiter (in particular if the patient has no extrathyroidal stigmata of Graves' disease and a somewhat nodular goiter), (2) transient thyroiditis, and (3) gestational thyrotoxicosis (which has a similar prevalence as first trimester onset Graves' disease: 0.1–0.4% of pregnancies) [87, 89]. TSHR-AB measurement is the key test to distinguish between these possibilities. Elevated TSHR-AB, detected by TRAB or TSI assay, are highly sensitive and specific (>95%) for recent onset Graves' disease [1] and can confirm or exclude the diagnosis.

One should also consider the possibility of a laboratory error or artifact, in particular if clinical symptoms and signs are at odds with the laboratory results. While this constellation of laboratory results is not usually seen with antibody interferences in IAs, it can be observed due to high biotin intake by the patient. At least half of all IAs used in thyroid function testing rely on streptavidin-biotin interactions to capture assay antibodies. Biotin, which is not infrequently taken by patients for a variety of unproven indications at doses of 300–2000-fold excess of the recommend daily intake [33, 37], will prevent this interaction, thus leading to low assay signals. In an immunometric IA, i.e., TSH, this leads to a false low result, while in competitive IAs, i.e., total T4, FT4, total T3, FT3, and TRAB/TBII, it will lead to a false high result, thus mimicking Graves' disease result pattern perfectly. However, neither TSI/TSAb bioassays nor FT4 assays by dialysis/centrifugal filtration and LC-MS/MS will be affected, thus providing an important clue to this interference. Similarly, repeating thyroid function testing with assays that do not use biotin-streptavidin interaction will give correct results. Alternatively, testing can be repeated a few days after discontinuing biotin, because biotin is a water-soluble vitamin with a short half-life.

III. Low TSH (usually >0.1 mIU/L but <lower limit reference range) and normal FT4 and FT3

The most common causes of this set of results are subclinical hyperthyroidism and mild thyroid hormone overtreatment, followed by abnormalities in thyroid hormone binding to transport proteins and metabolism of thyroid hormone. The latter two are usually due to non-thyroidal illness or a variety of drugs. Reverse T3 is often elevated in this situation, but simply repeating thyroid function tests after some time might also suffice.

IV. Normal or low (usually <0.1 mIU/L) TSH and low FT4 or FT3

This pattern is more often seen in non-thyroidal illness than the one described above, but it is also common during, or immediately after, treatment of hyperthyroidism. TSH can remain suppressed for several weeks in this situation, even when peripheral thyroid hormone levels have fallen below the reference range.

This pattern is also not infrequently seen in malnourished patients, and it can sometimes be seen with a variety of medical drugs (e.g., many antiepileptic medications) [90, 91].

Finally, secondary hypothyroidism, i.e., pituitary or hypothalamic disease, needs to be considered in this scenario.

V. Elevated TSH (usually >10 mIU/L) and low FT4 or low FT4 and low FT3

This is the classical pattern seen in full-blown primary hypothyroidism. T4/FT4 is always low, albeit sometimes only marginally. However, T3/FT3 is only low in severe hypothyroidism, because peripheral conversion of T4 to T3 is upregulated in this situation, while rT3 production is downregulated. Patients with significantly low T3/FT3 are at risk of myxedema coma if this state has persisted for a prolonged period of time. Other markers of severity of hypothyroidism include serum cholesterol, creatine kinase, and corticosteroid-binding globulin, which might be substantially elevated in full-blown hypothyroidism, while sex hormone-binding globulin levels might be low.

The most common reason for primary hypothyroidism is chronic autoimmune thyroiditis, followed by iatrogenic causes, in rough order of frequency radioactive iodine treatment, thyroidectomy, antithyroid drug treatment, other drugs (Table 4) [92], iodine deficiency, external beam neck irradiation, environmental goitrogens, infiltrative disorders [e.g., amyloidosis, sarcoidosis, Riedel's thyroiditis], thyroid developmental abnormalities (thyroid absent), and thyroid hormone dysgenesis (thyroid present, inborn errors of thyroid cell function).

The medical history and thyroid autoantibody testing are the cornerstones of the initial diagnosis of the etiology of primary hypothyroidism. For the rare genetic disorders, genetic testing provides the answer for known disorders and, through whole exome sequencing, increasingly for unknown genetic causes.

VI. Elevated TSH (> upper limit of reference range but <10 mIU/L in most cases) and normal FT4 and FT3

Table 4 Some drugs other than antithyrotoxic drugs that might cause hypothyroidism[a]

Mechanism of induction of hypothyroidism	Drugs
Interference with thyroid hormone synthesis or release	Iodine Iodine-containing drugs Various contrast reagents Amiodarone Kelp tablets Some expectorants Perchlorate Aminoglutethimide Thalidomide Lithium
Immune dysregulation/ thyroiditis	Interferon-alpha Interleukin-2 Amiodarone Various tyrosine kinase inhibitors

[a]Some of these drugs, most notably amiodarone, might also cause hyperthyroidism

This is the typical picture in subclinical hypothyroidism. This entity is often, more or less arbitrarily, further subdivided into patients with TSH values above the upper limit of the population reference interval, but below 10 mIU/L (≥80% of cases), and those with TSH values above 10 mIU/L (≤20% of cases). While the latter group is generally considered to be in need of thyroid hormone replacement therapy, there is no consensus as to the former [93, 94].

At least 30% in the former group have an autoimmune etiology, which can often be confirmed by thyroid autoantibody testing. In the >10 mIU/L group, the majority have an autoimmune etiology.

Other causes for this pattern include intermittent T4 therapy for hypothyroidism and the recovery phase of non-thyroidal illness.

Heterophile antibody interference in immunometric IAs should also be considered as a cause of a false high TSH in this group of patients. In this case, patients are actually euthyroid.

VII. Normal or elevated TSH (usually <10 mIU/L) and elevated FT4 or FT3

This is a rare pattern with a relatively limited number of causes.

T4 or T3 autoantibodies with or without coexisting hypothyroidism is a possibility. Usually other thyroid autoantibodies are also detectable in these patients. T4 antibodies can be confirmed by TBPE assay.

Familial dysalbuminemic hyperthyroxinemia can also present this way. Many FT4 and FT3 IAs will give false high results in these patients (although less elevated than total T4 or T3 assays) [95], but retesting FT4 or FT3 by dialysis or centrifugal filtration followed by LC-MS/MS nearly always gives correct results in this situation. If the TSH is elevated, then there is usually coexisting hypothyroidism.

Amiodarone can also cause this result pattern. In this case, the TSH result is the most reliable indicator of the patient's thyroid function status.

Finally, thyroid hormone resistance and TSH-secreting pituitary tumors (or, even rarer, other neuroendocrine tumors that secrete TSH) have to be considered once the other possibilities are excluded. Finding a significant molar excess of the common alpha subunit of pituitary glycoprotein hormones (LH, FSH, TSH, hCG), αPGH, is considered helpful [96]. However, the usefulness and reliability of this αPGH/TSH ratio have to be questioned. There is no assay standardization/harmonization, there are virtually no published data comparing different αPGH assays, and clinical validation is scarce. Pituitary imaging will usually have to be performed, as well as genetic testing for thyroid hormone receptor mutations, deiodinase defects, and thyroid hormone transporter mutations.

Follow-Up Testing/Monitoring

Long-term treatment for hypothyroidism is a stable follow-up situation. Once an optimal thyroxine dose has been established, TSH testing every 6–12 months should suffice, unless there is intervening illness, significant change in body weight, or recurrent symptoms, when more frequent testing might be necessary, and FT4 might also have to be measured.

For most other situations, TSH plus FT4 (or FT3) testing is usually indicated, and the time intervals vary with the clinical situation. Given the T4's half-life (5–8 days), test intervals should be >1 week. T3 with its half-life of about 1 day allows more frequent testing, but its usefulness in monitoring is limited, except in recurrence of Graves' disease, where T3/FT3 might become elevated slightly before T4/FT4, or in patients who are taking a mixture of T4 and T3 or just T3. Result patterns in patients on mixed replacement, or on T3-only replacement, can be difficult to interpret, because T3 has a more rapid effect on serum TSH levels than T4, and in pure T3 replacement, no T4 would be detectable. Timing of blood draws needs to be standardized, with medication to be taken after the draw.

In patients with Graves' disease, who are undergoing treatment, TSH might remain suppressed for prolonged periods of time, and there is little point monitoring it more often than fortnightly. FT4 testing is the mainstay of monitoring in this situation.

As to monitoring thyroid autoantibody levels, TSHR-AB monitoring might be useful for assessing ongoing thyroid autoimmune activity. However, it remains uncertain, whether its predictive value is sufficient to guide treatment decisions.

Tumor Marker Testing

Thyroglobulin

Tg testing at regular intervals is a key part of thyroid cancer follow-up. The 2015 ATA management guidelines for adult patient with thyroid nodules and differentiated thyroid cancer do not recommend preoperative Tg or TgAB measurements, because their predictive value is unproven. The ATA guidelines recommend performing the first post-op Tg measurement 3–4 weeks post-thyroidectomy, which should be adequate with or without knowledge of pre-op Tg levels (Tg serum half-life is ~30 h [97]). The testing can be performed on thyroxine, if a highly sensitive Tg assay (lower limit of quantitation ≤0.1 ng/mL) is used. A Tg on thyroxine of ≤0.2 ng/mL is associated with ~1% risk of persistent or recurrent disease [31, 53, 54]. Stimulated Tg measurements (thyroid hormone withdrawal or rhTSH stimulation) are optional, with a rise of serum Tg to <1 ng/mL being considered consistent with the absence of active disease [53].

If patients are TgAB-positive, using the TgAB assay's limit of quantitation as the cutoff, a Tg result by IA is not trustworthy. Remeasurement by a Tg-MS assay addresses this problem in part, but as discussed before, while this gives an accurate result for patients above the lower limit of quantitation of the MS assay (usually 0.5 ng/mL), only 60% of the TgAB-positive cases with undetectable Tg by IA (<0.1 ng/mL) and recurrent/residual disease will be detected with current Tg-MS assays [50, 56].

Depending on whether the patient is deemed to fall into the low-risk, intermediate-risk, or high-risk category, based on clinical _presentation, imaging, and histopathology, Tg is remeasured at 6–24-month intervals, again, unstimulated or stimulated. TgABs should always be measured alongside and will become undetectable over time in many cured patients [53].

The concept of doubling times of Tg is considered in the guidelines and is matched to three tiers of recurrence risk: >3 years low, 1–3 years intermediate, and <1 year high; a rise of ≥0.3 ng/mL per year is endorsed as an alternative to identify an increased risk of recurrence [52, 53].

While the guidelines acknowledge the importance of the differences between different Tg assays, they don't seem to consider the role of consistency of assay performance over time for judging changes in Tg levels. The minimal significant change concept, if it incorporates the inter-assay imprecision over multiple reagent lots and time periods of ≥1 year, would be eminently applicable in this scenario and would likely deliver much improved diagnostic accuracy and earlier detection of recurrence.

Finally, for all post-op Tg testing, the recommendations in the ATA document assume the patient has undergone total thyroidectomy +/− radioiodine remnant ablation. Remnant thyroid tissue produces serum Tg levels of 0.5–1 ng/mL per gram tissue, depending on serum TSH levels [98, 99]. Consequently the recommendations on how to handle Tg testing in these patients are limited to the statement that "… rising Tg values over time are suspicious for growing thyroid tissue or cancer," which as a recommendation has essentially no value. Again, the minimal significant change concept might help here, although TSH fluctuations might have to be factored in.

Calcitonin and CEA

Considerations for calcitonin and CEA are very similar as those listed for Tg. A difference is that Ct is also frequently used diagnostically, with levels above 50 pg/mL considered as suspicious, while serum concentrations within the reference range make MTC very unlikely. For levels above the reference range, but below 50 pg/mL, penta-

gastrin or calcium infusion might be used to stimulate Ct secretion. A stimulation >50 pg/mL is considered suspicious of C-cell hyperplasia, and > 100 pg/ml denotes a likely MTC. There are numerous variations of stimulation protocols that are used, and, of course, Ct assay standardization is not perfect, so these cutoffs should be treated as recommendations, rather than gospel [46, 100].

Throughout Ct testing, the potential pitfalls of assay interferences, which have been discussed before, need to be considered.

Preoperative Ct levels are correlated with tumor size and therefore indirectly with the risk or persistent disease, which rises from 2% for patients with <50 pg/mL to 37% for those with levels of >500 pg/mL [48, 100].

In follow-up, biochemical cure is considered when Ct levels are at or below the lower limit of quantitation of the assay used. Levels within the reference interval are considered suspicious, and those above it strongly suggest persistent or recurrent disease. In doubtful cases, stimulation testing might be used, with the same criteria as for initial diagnosis. The concept of Ct doubling time is firmly established in MTC follow-up, and a doubling time of <2 years is associated with a higher risk of bad outcomes [47, 48].

CEA as a tumor-specific rather than organ-specific marker can be used diagnostically, but it will not be specific for medullary thyroid carcinoma. As indicated before, it might be positive in some Ct-negative cases and often denotes a more aggressive tumor. Doubling time is used in follow-up, and for tumors that express both Ct and CEA, both should be measured. If the doubling time of one is <2 years, this denotes a worse prognosis, even if the other maker has a longer doubling time [47]. Again for both CEA and Ct, the concept of minimal significant change might offer improved detection accuracy of recurrence and should be considered.

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Ultrasonography of the Thyroid and Cervical Lymph Nodes

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Basic Ultrasound Principles

Ultrasounds (US) are acoustic waves that are characterized by a greater than 20 kHz frequency and propagate through physical media by means of a sequence of compressions and rarefactions. These pressure waves move longitudinally through the tissues at a velocity that is influenced by the elastic and density properties of the medium [1]. The frequency (defined as the number of pressure peaks per second) and the wavelength (defined as the distance between pressure peaks) of these US waves may widely change according to the characteristics of the emitting US probe [2, 3].

The energy of the US pulses and of the reflected echoes is decreased ("attenuated") during their propagation through the tissue. US attenuation increases with the distance of the target from the transducer and the frequency of the US wave. Thus, US examination of close targets, as the superficial structures of the neck, may be performed with high-frequency probes (range, 10–15 MHz) that provide an elevated "axial resolution" (the capacity to distinguish two adjacent small-sized structures from an apparently single greater entity) for the optimal imaging of thyroid gland and cervical lymph nodes [2, 3].

When, inside the medium, the US waves collide with structures with a different elastic quality, part of their energy is reflected toward the emitting probe. The intensity of the echoes that are generated by this interaction is determined by the difference of acoustic impedance between contiguous tissues. Negligible US echoes are generated by the interface of tissues with similar impedance, while the whole wave energy is reflected in case of adjacent structures with marked difference of acoustic impedance. Usually, the interface between different tissues generates low-intensity echoes. In case of calcifications, however, all US waves are reflected with a nearly complete absence of imaging behind the interface ("acoustic shadowing"). In contrast, the almost complete transmission of US waves through fluid collections is followed by a stronger signal behind the cyst ("acoustic enhancement") [4].

At B-mode (bidimensional) examination, the speckled signals that provide the texture of the thyroid gland and lymph nodes are due to the interference between multiple scattered echoes. The intensity and type of these reflected signals depend on the abovementioned parameters and also on the ratio between the size of the interface and the wavelength. Echoes reflected from a large area of interface, such as the border between

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the liver and diaphragm, are "specular" (the echoes travel directly back toward the transducer). Intermediate-sized interfaces "diffract" the US beam, while small-sized structures, such as erythrocytes, "scatter" the acoustic waves in several directions [1–3]. A frequent artifact caused by US reverberation is the "comet tail sign." Solid colloid crystals within the fluid content of a cyst may shake under the stimulus of US waves and produce additional echoes that go back to the transducer after the first reflected signal. This usually benign finding should be carefully distinguished from the tiny echoes reflected from intranodular microcalcifications of papillary thyroid carcinoma [4].

US wave attenuation is due not only to the reflection and scattering but also to the friction-like losses due to the oscillatory motion induced by the pulse progression within the tissue. This energy loss, due to the conversion of mechanical energy into heat, is defined as "absorption" and represents the most relevant component of US attenuation [1–3]. During diagnostic examination, the US waves are generated by an emitting transducer (or US probe). The US probe is an electronic device that converts electric energy into the mechanical energy of US waves by means of piezoelectric elements [5]. The activation of these rods of lead zirconate titanate ceramic, placed in epoxy matrix, results in their vibration and in the generation of US waves. Subsequently, when the reflected sound waves get in touch with the crystals of the receiving probe, the same piezoelectric elements convert the vibration in electric signals [6].

Neck US examination is usually performed with linear array transducers that sequentially stimulate the thin lines of piezoelectric elements with the emission of perpendicular US pulses [6]. The echo signals detected by the transducer are preamplified, and the similarly reflective structures are displayed in the B-mode image with the same brightness, regardless of their depth. The difference in the amplitude of reflected echoes generates a gray-scale image, while the different timing of their detection provides the information about the depth from which the echoes are reverberating. The real-time fusion of a consecutive sequence of one-dimensional images (A-mode) permits their organization in a two-dimensional picture on the US display [4, 6].

Rapidly moving targets, such as blood erythrocytes, generate very low-intensity echoes that are not displayed in gray-scale US images. However, the Doppler US signal processing may demonstrate the echoes of moving small-sized targets because they change in frequency and amplitude according to the velocity and direction of their movement [7, 8]. Movements toward the transducer generate positive frequency shifts, while movements away from the transducer produce a negative frequency shift. So, color Doppler (CD) imaging provides a graphic representation, with different colors, of the direction and speed of blood flow within the body. A different signal processing, defined as power Doppler (PW) imaging, is employed for the detection of low-velocity blood flow within small-sized vessels [9]. The assigned color represents the total amount of flow, irrespective of its velocity and direction, with a high sensitivity for the slow flows that are inadequately imaged by conventional CD. This fine detailing of small and irregular vessels may depict thyroid tissue vascularization and may provide additional information about the risk of malignancy of thyroid nodules and lymph nodes [10].

How to Perform Thyroid Ultrasound Examination

Thyroid US examination is performed on patients in the supine position and with their neck in hyperextension over a pillow [11]. The US machine is generally placed on the right side of the examining bed, while the operator, if right-handed, is on the left side (on the right side of the patient). Thyroid gland is preliminarily scanned along the longitudinal and transverse planes for a general evaluation. Subsequently, the entire region is systematically explored in the longitudinal plane starting from the midline to explore thyroid isthmus and then laterally on each side to view the medial, central, and lateral aspects of each lobe and of the adjacent cervical

region. Each longitudinal scan is performed from the sternal notch toward the hyoid bone region [12]. The vascular landmarks of the lateral borders of the thyroid gland are the common carotid artery and the jugular vein. US examination should be extended cranially beyond the thyroid cartilage to assess the presence of the pyramidal lobe, thyroglossal duct cysts, or masses of different origin. Subsequently, the whole thyroid should be carefully evaluated along transverse planes of the upper, middle, and lower part of each lobe [4, 12]. In the presence of thyroid or cervical lesions, the detected nodules or lymph nodes should be examined in several planes by carefully rotating the probe over the area of interest [4].

Thyroid US Findings

Sonographic data that may be of use for the clinical management of diffuse or focal thyroid disease should be carefully assessed [12]. The normal thyroid gland is composed by two pear-shaped lobes connected by a central isthmus. Usually, the thyroid gland size is 20 mm or less in both the transverse and anteroposterior diameter and is 55 mm or less in its longitudinal diameter [4]. Thyroid volume may be estimated with the ellipsoid formula (see below) or with a dedicated tridimensional software. At B-mode US examination, thyroid echo-texture appears fairly homogeneous with a slight ground-glass appearance [4, 12].

In Hashimoto's thyroiditis and in Graves' disease, the lymphocytic infiltration and the damage of tissue architecture result in a variable decrease of thyroid gland echogenicity, the loss of the normal ground-glass pattern of thyroid tissue, and the formation of several hypoechoic pseudo-nodules. These areas of deep lymphocytic infiltration may be encircled by hyperechoic bands of fibrosis and are usually incompletely demarcated [13]. The use of color or power Doppler imaging demonstrates a striking and diffuse increase in blood flow in Graves' disease, while in Hashimoto's thyroiditis tissue vascularization may be either moderately increased or nearly completely absent [14]. The heterogeneous texture of Hashimoto's thyroiditis should be carefully assessed because the presence of small-sized neoplastic foci might be concealed. Finally, the less frequent De Quervain's granulomatous thyroiditis is characterized by multiple ill-defined hypoechoic areas that may change in shape and position during the evolution of the disease [12, 14].

Besides the US volume and appearance of the thyroid gland, attention should be dedicated to the presence of thyroid nodules due to their elevated prevalence and their non-negligible risk of malignancy. B-mode, color Doppler, and sonoelastographic features are reported to have varying abilities to predict the risk of thyroid carcinoma [15–18]. The predictive value of US assessment of thyroid nodules is partly decreased by the overlap of the US features of benign and malignant thyroid lesions. However, a few well-defined US prognostic patterns are presently recognized [19, 20] and may be summarized as follows:

(a) US findings suggestive of a benign lesion:

- Simple cyst (fluid collection with thin regular margins)
- Spongiform nodule (isoechoic appearance with microcystic spaces comprising >50% of the nodule)
- Mostly cystic (>80%) nodule containing colloid fluid (comet tail signs) with regular margins devoid of vascular signals

(b) US findings suggestive of a malignant lesion:

- Marked hypoechogenicity (in comparison with pre-thyroid muscles)
- Marginal abnormalities (lobulated, irregular, or spiculated margins)
- Taller-than-wide shape (AP > TR diameter when imaged in the transverse plane)
- Intranodular microcalcifications (hyperechoic foci <2 mm with no posterior shadowing)
- Broken calcified rim with extension of hypoechoic tissue beyond the calcified margin

- Evidence of aggressive growth (extension of the lesion beyond the thyroid capsule, invasion of the strap muscles, or infiltration of the tracheal cartilage)
- Presence of suspicious cervical lymphadenopathy (see paragraph 6)
- Of note [19, 20]:
  - Follicular neoplasms (either follicular adenoma or follicular carcinoma) may be visualized as isoechoic or mildly hypoechoic nodules with intranodular ("spokes and hub") vascularization and well-defined, but usually irregular, peripheral halo.
  - Medullary thyroid carcinomas may present with variable shape and echogenicity but most of them are hypoechoic and lobulated. Intranodular globular calcifications are specific but inconstant features of this thyroid tumor.
  - The coexistence of two or more suspicious US criteria greatly increases the risk of thyroid cancer.

(c) Borderline US findings that are inconstantly associated with thyroid malignancy:

- Mild hypoechogenicity (in comparison with the surrounding thyroid tissue)
- Intranodular macrocalcifications (hyperechoic foci >2 mm that are associated with posterior shadowing)
- Indeterminate hyperechoic spots (images that are suspicious for but cannot be clearly defined as microcalcifications)
- Centrally predominant or chaotic vascularity
- Elevated stiffness at sonoelastography

The diagnostic accuracy for malignancy of suspicious findings is partially blunted by their low sensitivity and by the relevant interobserver variation [21]. Thus, in most thyroid nodules, US signs are not clearly predictive of a malignant lesion, whereas the absence of clearly suspicious features is not fully diagnostic for a benign nodule [22, 23].

A more comprehensive evaluation of the major US patterns of thyroid nodules is treated in the following paragraphs.

Size and Number

The size of a thyroid lesion is not a predictive factor for malignancy [24, 25]. Thus, a large size nodule is of interest not because of the risk of carcinoma but because differentiated thyroid carcinomas greater than 4 cm may be associated with a more advanced disease [26].

Follow-up of thyroid nodules is usually based on the assessment of their volume and US features [19, 20]. The increase in size is not predictive for malignancy because hyperplastic nodules frequently show a slow but progressive growth [27, 28], whereas papillary carcinomas may be rather steady over time [29]. The infrequent aggressive tumors (mostly poorly differentiated or anaplastic carcinoma and thyroid lymphoma) show a rapid growth but are clearly characterized by major US features of malignancy [20, 30]. Due to the sonographic interobserver variability, quantified in about 20% for any nodule diameter [31], nodule volume should be calculated with the ellipsoid formula (longitudinal diameter x transverse (or left to right) diameter × anteroposterior diameter × p/6) [17]. A 50% volume increase is usually considered as the threshold for a definite assessment of nodule growth [20].

The presence of either solitary or multiple thyroid nodules has a low influence on the risk of malignancy, at least in areas of borderline iodine deficiency [16, 24]. In goiters with several sonographically similar nodules and in diffuse thyroid hyperplasia with multiple anechoic or mixed lesions, the number of nodules is of limited clinical usefulness.

Echogenicity and Structure

The vast majority of thyroid carcinomas show a hypoechoic appearance, but many benign thyroid nodules are hypoechoic as well. Thus, mild hypoechogenicity represents a sensitive but poorly specific predictor of malignancy [17, 18, 32]. Conversely, marked hypoechogenicity (a sonographic appearance that is darker than that of pre-thyroid muscles) is associated with a relevant risk of malignancy with a positive predic-

tive value up to 94% [15, 32]. The accurate assessment of nodule echogenicity may be partially hampered by the interobserver variability or by the coexistence of chronic autoimmune thyroiditis [33].

Nodules with a predominant cystic component only rarely represent a thyroid carcinoma. Yet, thyroid malignancy cannot be completely ruled out because a prevalence from 4 to 6% of cystic (mostly papillary) carcinomas is reported in a few surgically controlled series of thyroid lesions [34, 35]. The risk of malignancy increases with the amount of the solid component and the coexistence of suspicious US signs [34]. Thus partially cystic thyroid lesions with a <80% fluid component and irregular or eccentric solid areas should be carefully evaluated.

The isoechoic thyroid nodules that are characterized by the aggregation of multiple tiny fluid areas that involve more than 50% of the volume (defined as "spongiform nodules") are associated with an elevated probability of benign nature [20, 34].

Margins and Shape

The majority of benign nodules show a round to oval shape [23, 36] with a <1 ratio between their anteroposterior and transverse diameters when measured in a transverse plane. Thus, a more tall-than-wide shape, defined as a ratio ≥1, is suspicious for malignancy because of its expression of a centrifugal modality of growth [18]. This feature is highly specific for malignancy but, unfortunately, is characterized by a low sensitivity [19].

Ill-defined margins, due to the absence of a clear differentiation of the nodule perimeter from the surrounding thyroid tissue, may be associated with malignancy [16]. Conversely, the presence of irregular margins, either spiculated or lobulated, is highly suspicious for carcinoma [15, 16].

A hypoechoic halo associated with a regular smooth profile is a typical feature of benign hyperplastic nodules and is due to the peripheral arrangement of nodular vessels, as demonstrated by the vascular signals at color or power Doppler imaging [4]. A peripheral halo may be revealed in a minority of papillary thyroid carcinomas and in part of follicular carcinomas, as well. In these cases, however, the hypoechoic halo is generally thick and irregular due to peripheral fibrosis and degenerative changes [37, 38].

Calcifications

Three types of calcifications may be distinguished: microcalcifications, macrocalcifications, and peripheral (or "rim") calcifications [39, 40].

Microcalcifications are defined as tiny (<2 mm) intranodular hyperechoic spots that are devoid of posterior shadowing unless densely crowded [41]. These bright echoes are generated by psammoma bodies and are highly suggestive of papillary thyroid carcinoma [16, 18]. Their specificity for malignancy is high, but their sensitivity is rather low [24]. Microcalcifications should be differentiated from the hyperechoic spots defined as "comet tail" signs [4, 42]. These last US findings are due either to solid drops of colloid or to the interfaces present in complex lesions and are usually associated with a benign nodule [4]. When the intranodular hyperechoic spots cannot be recognized with certainty as microcalcifications, these uncertain US findings should be reported as "indeterminate hyperechoic spots" in order to prevent an overdiagnosis of papillary thyroid carcinoma [43].

The majority of macrocalcifications are due to degenerative changes and may be demonstrated in long-standing benign nodular goiters. The presence of macrocalcifications in solitary nodules, however, should be considered as a potential sign of malignancy, especially if associated with necrotic changes. The peculiar globular calcifications are a frequent US feature in medullary thyroid carcinoma [42–44]. Macrocalcifications in nodules previously treated by percutaneous ethanol injection or laser ablation do not correspond to worrisome findings [45].

Peripheral rim calcifications are sometimes observed in long-standing benign nodules. However, a focal discontinuity of the eggshell

rim associated with the extrusive growth of hypoechoic tissue should be considered as suspicious for malignancy [20, 46].

Vascular Signals

Color and power Doppler examination offer a clear demonstration of the vascular architecture of the thyroid gland and of its focal lesions [12]. Three patterns of vascular signals may be demonstrated with Doppler evaluation [12, 47, 48]:

- Peripheral signals. Vascular signals are detected along the periphery of the nodule. Intranodular vascular images are absent or scarce.
- Intranodular pattern. A marked vascularity is revealed in the central part of the nodule and is more relevant than in the surrounding thyroid gland.
- Absence of vascularization. No blood flow is demonstrated in either the peripheral or central part of the lesion.

The predictive value for malignancy of nodule vascularization is hampered by the lack of unambiguous differences between benign nodules and carcinomas [36]. Many carcinomas (specifically follicular and Hürthle cell thyroid carcinomas) show a dominant intranodular vascularization, but this finding may be observed in benign lesions as well (particularly in follicular adenomas and in hyperfunctioning hyperplastic nodules) [49–51]. A sparse vascularity is frequently associated with benign nodules, but small-sized papillary carcinomas may show a complete absence of vascular signals, as well [36]. Thus, color and power Doppler examination provide only complementary data for the risk of malignancy of thyroid nodules [19, 20].

Of note, the abovementioned suspicious US signs are established on the basis of the sonographic features of papillary thyroid carcinoma, the most frequent thyroid tumor [16]. These findings, however, may be different in the less common follicular and Hürthle cell tumors and in medullary thyroid carcinoma. In follicular thyroid carcinomas, hypoechogenicity is observed in a minority of tumors, while a halo is present in the majority of these tumors [49–51]. Additionally, microcalcifications are rare in follicular neoplasms, while macrocalcifications are more frequent. These features are present in benign and malignant follicular neoplasms, but the irregular thickness of the peripheral halo, the inhomogeneous solid content, and the large size are in favor of a follicular carcinoma [49–51]. The US features of medullary thyroid carcinoma are quite variable and may be ambiguous. The sonographic findings of the majority of these tumors are similar to those of papillary carcinomas and are classified as suspicious at US examination, but part of medullary carcinomas may demonstrate an indeterminate or even a deceitful benign appearance [52, 53].

Elastographic Pattern

Elastography evaluates the nodule stiffness with the appliance of an external force that is generated by the US probe. The grade of tissue displacement is analyzed and represented on a color scale in comparison with the stiffness of perinodular tissue [54]. The sensitivity for malignancy of elastography is reported as high with a remarkable negative predictive value [54, 55]. In a multicenter series of thyroid nodules, the presence of one suspicious US sign (hypoechogenicity, microcalcifications, irregular margins, intranodular vascular signals, and taller-than-wide shape) had an 85% sensitivity and 91% NPV. When elastography was combined with B-mode sonographic features, the sensitivity for malignancy increased up to 97% and the NPV to 97% [56]. As these results were substantially confirmed by subsequent studies and by a meta-analysis [57], the combined use of elastography with B-mode US and color Doppler evaluation seems to be a reliable approach for the selection of those nodules that do not deserve fine needle cytologic assessment [58]. Real-time elastography, however, is markedly operator-dependent, and a consistent methodology for data reporting is still missing [57]. Nodules with a large fluid component or with macrocalcifications are not appropriate for elastographic evaluation, and multinodular

goiters with coalescent nodules or chronic thyroiditis are not effectively assessed [56].

Quantitative elastographic techniques, such as the determination of strain index, the acoustic radiation force impulse [59], and the supersonic shear wave [60, 61], demonstrated an improvement in the diagnostic performance, in the applicability to lesions in areas difficult to scan, and in case of coexistent chronic thyroiditis. Thus, in selected cases which are ambiguous at gray-scale examination, the sensitivity and the negative predictive value for malignancy of B-mode US features may be improved with the complementary use of elastographic examination.

Ultrasound Classification Systems for Thyroid Nodules

Data obtained from US evaluation of thyroid nodules may be organized in a standardized reporting system to provide a stratified risk of malignancy, similar to the imaging reporting system developed for breast lesions [62]. This approach could better differentiate the nodules that should be evaluated with FNA from those that need only a low-intensity clinical surveillance [63]. Standardized US reporting systems may improve communication between clinicians and pathologists and the audit procedures for the management of thyroid nodules. In general, the accuracy of these categorizations parallels their complexity. TIRADS, the first of these classification systems, offers a rating of the risk of malignancy that increases with the presence of suspicious US features and the absence of benign findings. The TIRADS scheme is based on ten US patterns combined into categories with increasing risk of malignancy and demonstrates a good correlation with cytologic findings. [64]. The BTA system classifies the thyroid US features in five categories at increasing risk of malignancy, from U1 (normal thyroid gland) to U5 (very suspicious lesion) [43]. The ATA system is based, with some differences, on a five-class categorization, as well, that is associated with an increasing risk of malignancy [20]. On the basis of US findings, nodules are rated from the class 1 (benign) to the class 5 (high suspicion) (Table 1). Finally, the recent AACE-ACE-AME system is based on a less articulated three-class rating of the US features, with a risk of malignancy that ranges from <1% for the US class 1 to >50–70% for the US class 3 [19] (Table 2).

Each of these US classification systems, and others as well [36, 65], permits a rapid and reliable communication of the expected risk of cancer and guides the decision for FNA [58]. In our opinion, the TIRADS, BTA, and ATA systems are best suited for thyroid referral centers for the analysis and comparison of clinical data, while the simple AACE-ACE-AME three-class US rating system may be more practical for the use in routine clinical assistance. The US images representative of the different risk categories of this last classification are summarized in Figs. 1, 2, and 3.

Table 1 American Thyroid Association Thyroid Nodule and Cancer Guidelines

Benign (risk <1%)
Purely cystic nodules (no solid component)
Very low suspicion (risk<3%)
Spongiform or partially cystic nodules without any of the US features described in low, intermediate, or high suspicion patterns
Low suspicion (risk 5–10%)
Isoechoic or hyperechoic solid nodule or partially cystic nodule with eccentric solid area without
• Microcalcifications • Irregular margin • Extrathyroidal extension • Taller-than-wide shape
Intermediate suspicion (risk 10–20%)
Hypoechoic solid nodule with smooth margins *without*
• Microcalcifications • Extrathyroidal extension • Or taller-than-wide shape
High suspicion (risk>70–90%)
Solid hypoechoic nodule or solid hypoechoic component of partially cystic nodule with one or more of the following features
• Irregular margins (infiltrative, microlobulated) • Microcalcifications • Taller-than-wide shape • Rim calcifications with small extrusive soft tissue component • Evidence of extrathyroidal extension

Sonographic patterns and estimated risk of malignancy [20]

Table 2 AACE, ACE, AME 2016 clinical practice guidelines

US class 1. Low-risk ultrasound features
• Thyroid cyst • Mostly cystic nodule with reverberating artifacts • Spongiform nodule
► The expected risk of malignancy is about 1%
US class 2. Intermediate-risk ultrasound features
• Isoechoic or slightly hypoechoic nodule • Complex nodule without suspicious features
May be present: • Central vascularity • Macrocalcifications • Indeterminate hyperechoic spots • Elevated stiffness at elastography
►The expected risk of malignancy is from 5 to 15%
US class 3. High-risk ultrasound features
Nodules with at least one of the following suspicious features:
• Marked hypoechogenicity • Spiculated or microlobulated margins • Microcalcifications • Taller-than-wide shape • Extrathyroid growth or pathologic adenopathy
►The expected risk of malignancy is from 50 to 90% in accordance with the presence of one or more suspicious findings

Thyroid ultrasound features and risk of malignancy [19]

Cervical Lymph Node Topography

The topography of neck lymph nodes is defined according to the scheme originally proposed by the American Joint Committee on Cancer and the American Academy of Otolaryngology—Head and Neck Surgery [66–68], currently included in the pTNM classification of thyroid cancer [69, 70]. This classification identifies a central neck compartment and two (right and left) lateral compartments [66–69, 71, 72]. The central neck compartment includes levels VI–VII and extends craniocaudally from the hyoid bone to the horizontal plane of the innominate artery, with the carotid arteries and the trachea as its lateral and medial limits, respectively. Instead, the lateral neck compartments, which comprise levels II–V, are limited medially by the carotid arteries and laterally by the anterior margin of the trapezius muscle [66–68, 71, 72] (Fig. 4).

At US evaluation, the lymph nodes of the lateral neck compartments (levels II–V) may be detected in the triangular area covered anteriorly by the sternocleidomastoid muscle, while the

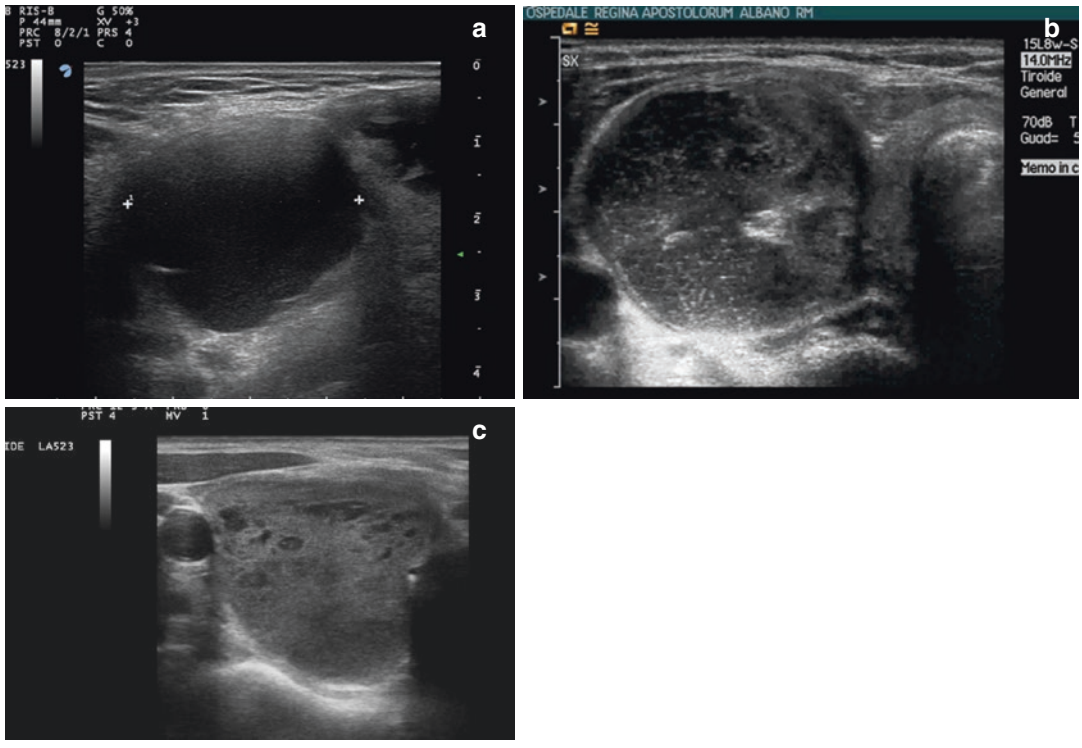

Fig. 1 (**a**) US low-risk lesion: pure cyst; (**b**) US low-risk lesion: mostly cystic nodule with reverberating artifacts and no suspicious content; (**c**) US low-risk lesion: isoechoic spongiform nodule (2016 AACE/ACE/AME classification of US Risk Categories)

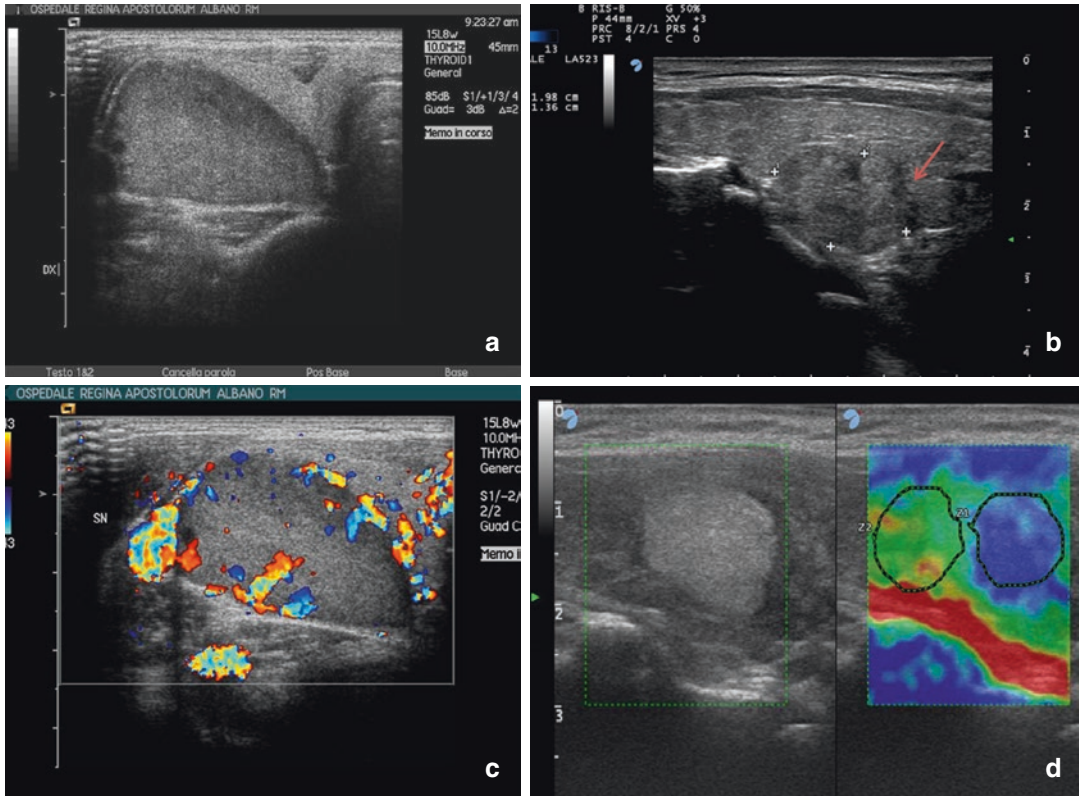

Fig. 2 (**a**) US intermediate-risk lesion, slightly hypoechoic nodule with regular margins 2016 AACE/ACE/AME classification; (**b**) US intermediate-risk lesion, isoechoic nodule, ovoid shape, regular rim calcification 2016 AACE/ACE/AME classification; (**c**) US intermediate-risk lesion, intranodular vascularization 2016 AACE/ACE/AME classification; (**d**) US intermediate-risk lesion: elevated stiffness at elastography (blue color)

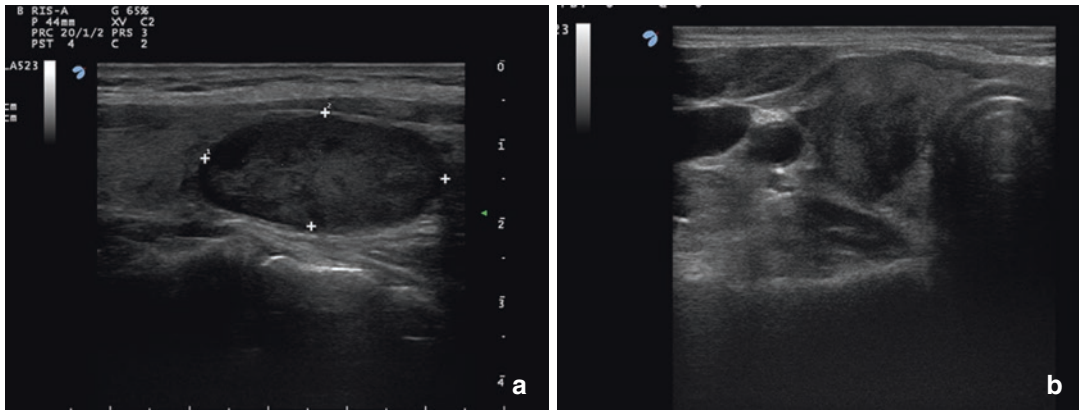

Fig. 3 (**a**) US high-risk lesion, marked hypoechogenicity 2016 AACE/ACE/AME classification; (**b**) US high-risk lesion, more tall-than-wide shape. Irregular margins are also present 2016 AACE/ACE/AME classification; (**c**) US high-risk lesion, intranodular microcalcifications (posterior shadowing due to cluster arrangement) 2016 AACE/ACE/AME classification; (**d**) US high-risk lesion: extrathyroidal growth and coexistent suspicious adenopathy 2016 AACE/ACE/AME classification

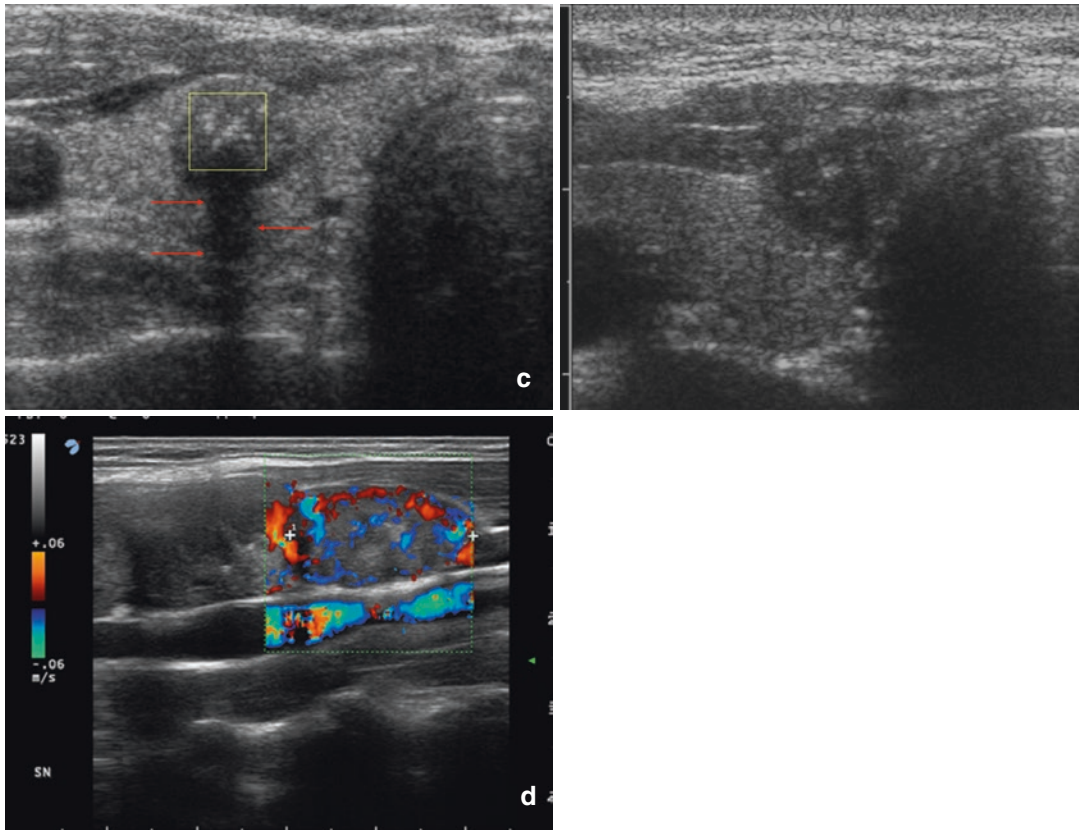

Fig. 3 (continued)

lymph nodes of the central neck compartment (levels VI–VII) are contiguous to the thyroid gland, the trachea, and the esophagus [67, 73]. Level VI is limited by the carotid arteries laterally, the hyoid bone superiorly, and the suprasternal notch inferiorly, while level VII corresponds to the upper portion of the anterosuperior mediastinum, above the innominate artery [66–68, 71, 72].

Within the lateral neck compartments, level II encompasses the area from the base of the skull to the hyoid bone, having the stylohyoid muscle and the posterior margin of the sternocleidomastoid muscle as the anterior and posterior limits, respectively [66–68, 71, 72]. The lymph nodes in this area may be involved by metastases from carcinomas either located in the upper third of the thyroid gland lobes or arising from the oral and nasal cavity, pharynx, larynx, and parotid glands. Levels III and IV extend from the hyoid bone to the cricoid cartilage and from the cricoid to the clavicle, respectively, with the sternohyoid and the sternocleidomastoid muscles as their anterior and posterior limits [66–72]. Level III and IV lymph nodes are the most frequently affected by thyroid cancer metastasis spreading to the lateral compartments [74–82]; yet, they may as well harbor metastasis from tumors originating in the oral cavity, pharynx, larynx, and esophagus [83]. Level V, defined anteriorly by the posterior margin of the sternocleidomastoid muscle, posteriorly by the trapezius muscle, and inferiorly by the clavicle, is more rarely involved by metastasis from thyroid carcinoma [84, 85]. Level I stretches from the mandible to the anterior belly of the digastric muscle and is quite exceptionally affected by thyroid cancer metastases [84–86].

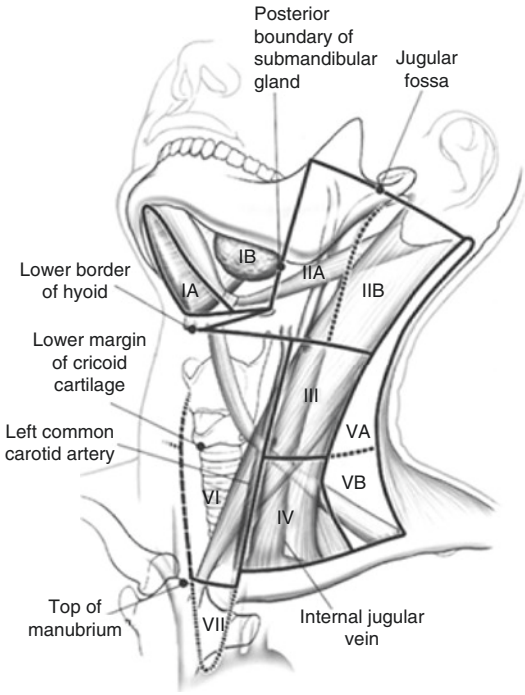

Fig. 4 Topographic classification of neck lymph nodes. From: Som PM, Curtin HD, Mancuso AA. Imaging-based nodal classification for evaluation of neck metastatic adenopathy. Am J Roentgenol. 2000;174:837–44

Neck Lymph Node US Findings

Cervical lymph node evaluation is centered on the assessment of their dimension, shape, echotexture, vascularization, and presence of hilum. Unfortunately, in analogy with thyroid nodules, no single finding is both highly sensitive and highly specific. Thus, the risk of malignancy should be established on the basis of a comprehensive evaluation of multiple US features [67, 83, 87–94].

Sonographic anatomy of benign lymph nodes is characterized by oval shape, regular margins, homogeneous hypoechoic texture, and the presence of a hyperechoic hilum [95] (Fig. 5). The size of lymph nodes is a frequently deceitful feature; although malignant lymph nodes are frequently increased in volume, also benign inflammatory lymph nodes (particularly in young patients and in the lateral cervical compartments) may be conspicuously enlarged [87–93, 95]. Conversely, small (e.g., <1 cm diameter) metastatic lymph nodes are frequently detected in the central neck compartment (Fig. 6). If the longest diameter of lymph nodes cannot be con-

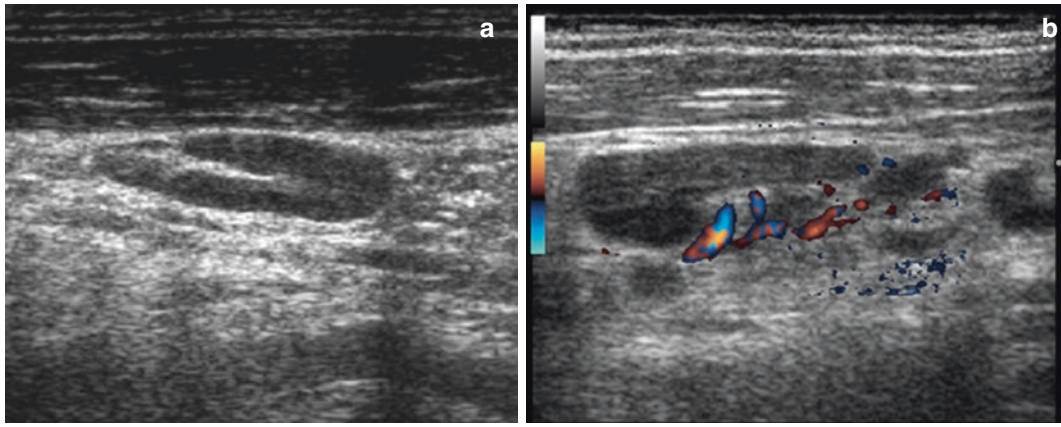

Fig. 5 (**a** and **b**) Representative appearance of a benign lymph node in the lateral neck compartment. (**a**) The shape of the lymph node is oval and elongated and its hilum is well distinct as a central linear hyperechoic zone; (**b**) power Doppler examination confirms the presence of the hilum with a central, "branch of a treelike" vascular architecture

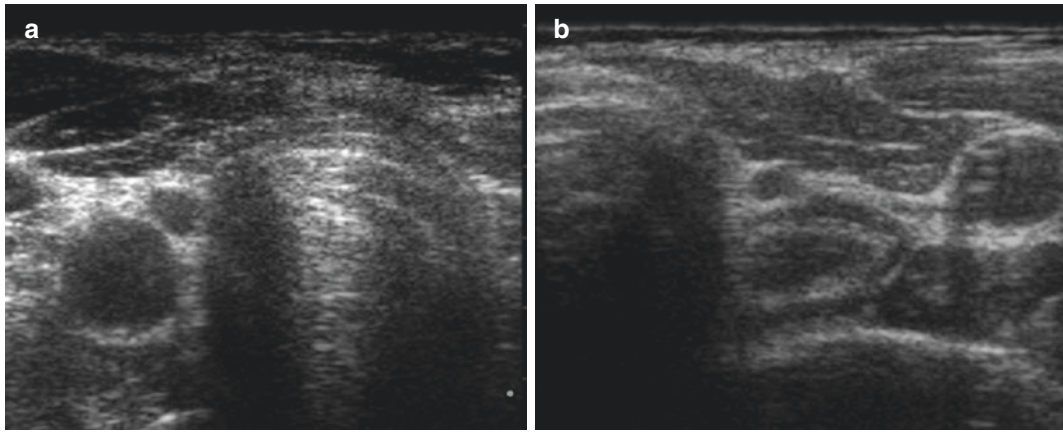

Fig. 6 (**a** and **b**) Former total thyroidectomy for papillary thyroid carcinoma. Persistence of disease in the central neck compartment at neck US examination. (**a**) A level VI suspicious lymph node is revealed as 5-mm hypoechoic lesion with fairly irregular margins between the right carotid artery and the trachea wall. (**b**) US examination of the left thyroid bed reveals a small round-shaped hypoechoic lesion close to the trachea and just above the esophageal wall

sidered per se a reliable index of malignancy, the short axis (i.e., the minimum diameter) may provide a more consistent clue for predicting malignancy [87–93]. A short axis greater than 7 mm for lymph nodes in level II and greater than 6 mm in the other cervical areas was described as an important risk factor (88.5% accuracy) [89] for malignant involvement. These data were confirmed in a large series of patients with thyroid carcinoma submitted to lymph node resection [90].

Changes in the lymph node shape, with a rounded instead of elongated profile, are usually regarded as a suspicious US sign (Fig. 7). For practical purposes, a shortest to longest (S:L) axis ratio ≥0.5 (or a longitudinal to transverse diameter ratio≤2 L:T) is considered a reliable index of malignancy [83, 87–93]. Nevertheless, the diagnostic accuracy of this parameter varies across the different neck compartments. In a series of 94 patients undergoing surgery for PTC, the specificity and PPV of the "round shape" were 90.2% and 66.7%, for lymph nodes of levels II–V, and 11.3% and 30.9% for level VI lymph nodes, respectively [96]. The lower diagnostic accuracy of this shape change in the central cervical compartment is due to the coexistence of inflammatory conditions and, most frequently, to the presence of chronic autoimmune thyroiditis

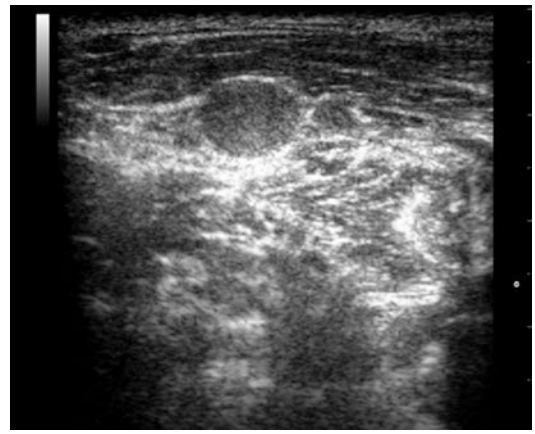

Fig. 7 Former total thyroidectomy for medullary carcinoma. Small lymph node metastasis in the left lateral neck. At US examination the lesion appears solid and with a round-shaped, "bumpy" appearance. Notably, the hilum image is absent

[88, 97]. So, lymph node location is a relevant factor in the assessment of a potential malignancy. In patients with thyroid carcinoma, the majority of metastatic lymph nodes are detected in the mid to low jugular area (defined as levels III–IV), while the lymph nodes detected at levels I–II are more likely to be benign [75–81, 96–101].

A clearly detectable hilum is as a reliable sign of benignity (Fig. 4); yet, its absence has

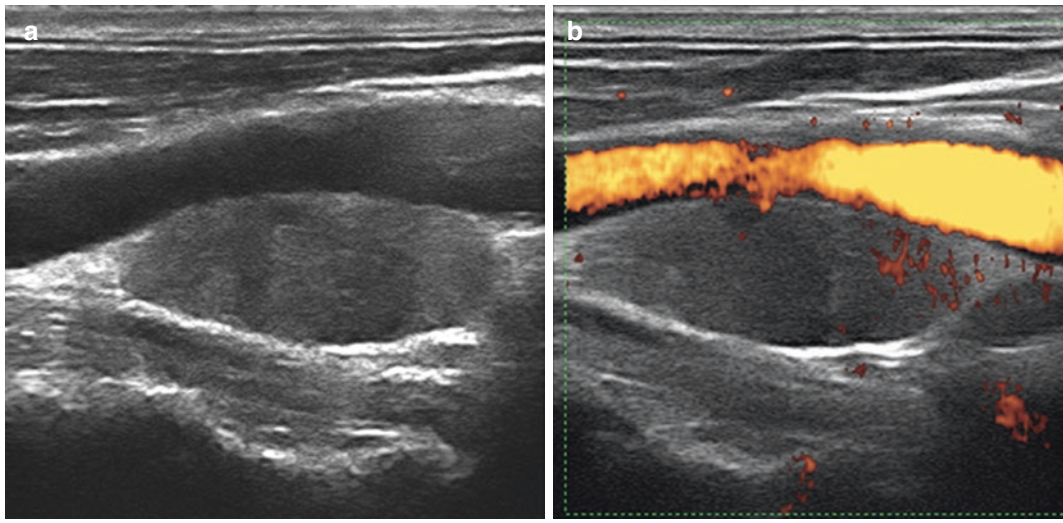

Fig. 8 (**a** and **b**) Former total thyroidectomy for papillary thyroid carcinoma. Persistence of disease in the lateral neck compartment at neck US examination. A large lymph node metastasis is located posteriorly to the carotid artery. This hypoechoic lesion shows well-depicted hyperechoic margins and compresses without infiltration the vessel wall

been reported in up to 40% of benign lymph nodes [90]. Furthermore, the absence of lymph node hilum represents a more predictive sign of malignancy in the lateral than in the central neck compartment [96] (Figs. 8 and 9). In uncertain cases, color Doppler evaluation may demonstrate a vascular hilum that is not visible in B-mode images [102]. Thus, according to some evidence, the positive predictive value of the true absence of the lymph node hilum may be as high as 92% [89].

Lymph nodes with hyperechoic, "thyroid-like" texture may be observed in patients with metastatic disease from differentiated thyroid cancer, prevalently in the lateral neck compartment (Fig. 10, panel A) [90, 93, 103].

Intranodal microcalcifications, appearing as hyperechoic spots without (or less frequently with) posterior shadowing, have an elevated predictive value for malignancy but are detected in a small fraction of metastatic lymph nodes. Thus, as for thyroid nodules, this finding has a high specificity but a low sensitivity for malignancy [90, 93, 103].

Lymph nodes characterized by a fluid component or by a cystic appearance are highly suspicious for metastatic disease and may be the only US evidence of a clinically occult papillary microcarcinoma [104–110] (Fig. 10). Cystic changes are predictive of metastatic lymph nodes from thyroid carcinoma with a 95% specificity,

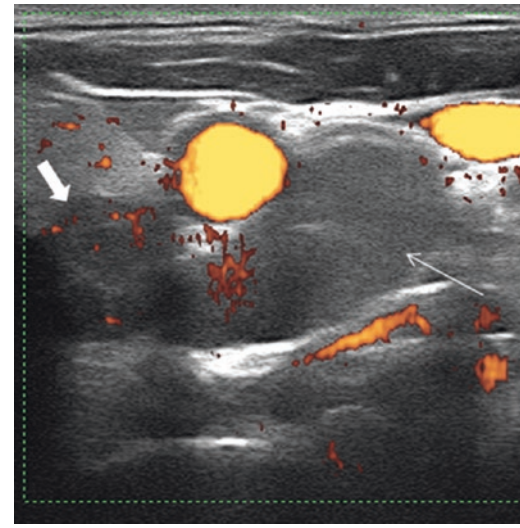

Fig. 9 Medullary thyroid carcinoma. Coexistence of disease in the lateral neck compartment at neck US examination. The primary lesion is visible as a large hypoechoic mass in the lateral portion of the left thyroid lobe (thick arrow); the metastatic lymph node (thin arrow) appears as a similarly hypoechoic lesion, with no evidence of hilum and ill-defined margins, between the carotid artery and the jugular vein

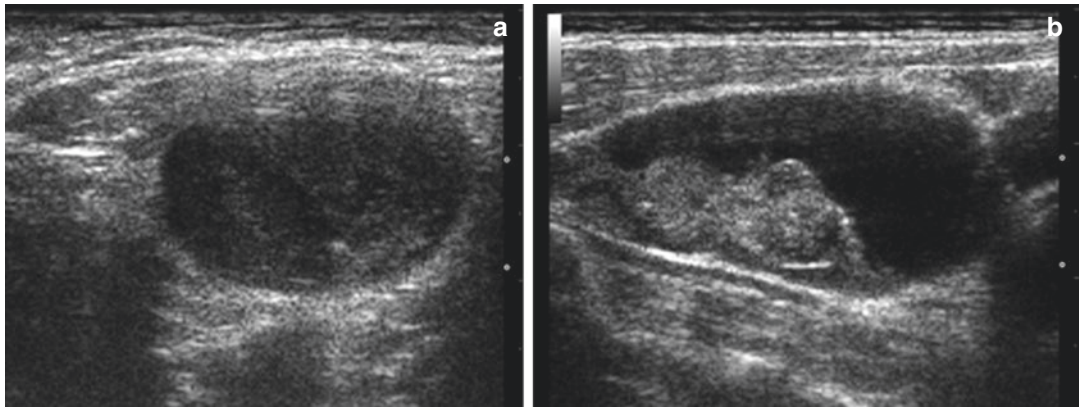

Fig. 10 (**a**, **b**) Former thyroidectomy for papillary thyroid carcinoma. Cystic metastatic lymph nodes. (**a**) Lymph node metastasis in the left lateral neck compartment: the lymph node presents as a large (30 mm in its major diameter) inhomogeneously hypoechoic, "balloon," cystic lesion. (**b**) Large lymph node metastasis in the right lateral neck compartment. The lesion shows a complex structure, with a prevalent anechoic fluid component and an irregularly shaped eccentric solid portion. Indeterminate hyperechoic spots are present

but a tuberculosis infection should be always ruled out in absence of coexistent suspicious thyroid nodules [111]. Of note, lymphomatous cervical lymph nodes may assume a pseudocystic, balloon appearance, mimicking a cystic metastasis from thyroid cancer [112]. Finally, the evidence of ill-defined borders in metastatic lymph nodes may indicate the presence extracapsular spread [91].

When the results from gray-scale US examination are equivocal, color Doppler examination may provide useful information [113–115]. The majority of normal cervical lymph nodes demonstrate a well-recognizable vascular architecture, with predominant signals at the hilum level (Fig. 5) [102, 113, 114]. The vascular hilum may appear as a centrally located, longitudinally oriented structure or, alternatively, as a dot-like polar vessel, with minor, symmetric radial branches [113, 114]. Although vascular mapping in benign lymph nodes may be scanty, if not completely absent, inflammatory lymph nodes sometimes exhibit a quite rich and diffuse vascular arborization [93, 113]. Conversely, highly vascularized malignant lymph nodes show a chaotic, multifocal vascular pattern, with both peripheral and central color flow mapping (Fig. 8) [113–116]. In particular, peripheral vascularity, seemingly due to neo-angiogenetic events in tumoral nests that alter the normal lymph node architecture, has been described as a specific sign of malignancy (Fig. 11) [93, 113, 114]. The measurement of Doppler waveform parameters (e.g., the resistance and pulsatility indexes) does not provide any additional relevant information in the clinical practice [93].

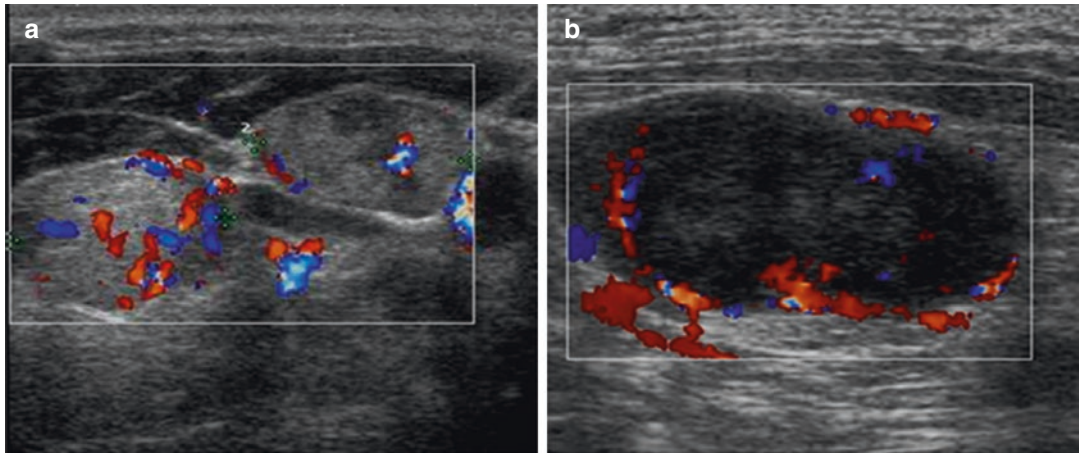

Fig. 11 (**a** and **b**) Color Doppler examination of suspicious cervical lymph nodes in papillary thyroid carcinoma. (**a**) A pair of lymph nodes with an echo-texture that mimics thyroid parenchyma. The vascular pattern is irregular and both central and peripheral; (**b**) large solid, deeply hypoechoic lymph node metastasis that shows a central and peripheral vascularization, as well

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The Role of Elastography in the Management of Thyroid Nodules

Hervé Monpeyssen and Jean Tramalloni

The discovery in a soft tissue area of a stiffer area has always been subject to suspicion. Nodules that are stiff at palpation often suggest malignancy. An increase in tissue stiffness is related to a loss of elasticity, meaning its ability to recover its initial shape after deformation.

Malignant neoplasms are often characterized by the desmoplastic transformation of their stroma which is responsible for the presence of collagen and myofibroblasts. This tumor stroma promotes the proliferation of malignant cells (and could even initiate them) [1]. However, some benign fibrous tumors such as fibrous histiocytomas can nevertheless be very stiff.

By studying the deformability/stiffness couple, elastography reproduces the palpable feeling of stiffness.

The concept of stiffness measurement was first reported in 1980 by a French researcher named Eisencher. The TM mode was the first modality, and he named his technique "echo-sismography," also designated "rhythmed ultrasonic palpation" (speech in Congress) [2].

Two years later, Dickinson published a paper on the measurement of soft tissue motion using a combination with A-Scan [3].

In 1987, Krouskop used a pulsed Doppler ultrasonic system to carry out noninvasive measurements of the mechanical properties of soft tissues in order to adjust a prosthesis for the management of amputation stump rigidity [4].

However, the term "elastography" was first introduced by Johnathan Ophir et al. in 1991 [5] to describe a quantitative method for the assessment of the elasticity of biological tissues. In 1993, his team published the preliminary results of in vivo elasticity imaging: "Elasticity imaging using ultrasound with application to muscle and breast" [6].

Elastography: Technical Approach

Numerous ultrasound elastographic methods are currently available, all of them measuring tissue displacement. The deformation may be represented in an elasticity image (elastogram) or as a local measurement using three techniques: [7]

- Direct measurement (acoustic radiation force impulse—ARFI)
- Calculation of the tissue strain
- Record of the propagation of the shear waves

Quasi-static Elastography (QSE): Strain Imaging

The first technique developed uses external compression, i.e., decompression cycles applied by

H. Monpeyssen (✉) · J. Tramalloni
American Hospital of Paris,
Neuilly-sur-Seine, France

M. Luster et al. (eds.), *The Thyroid and Its Diseases*, https://doi.org/10.1007/978-3-319-72102-6_12

the transducer, and is called quasi-static elastography (or strain elastography—SE). It is mostly a qualitative technique utilized when the deformation of the tissue of interest is assumed to be uniform.

It depends on **Young's modulus** of stiffness/elasticity, which reflects the relationship between the deformation of a solid structure and the constraint applied to it. This force was first induced by the ultrasound transducer and later by the arterial pulsations [8]. This vibration is so slow that it is considered quasi-static. The transducer will collect data in real time, thus enabling identification of the nodule and normal tissue deformation. Color (or gray-scale) encoding allows differentiation between tissues based upon their intrinsic deformation and, hence, information on their stiffness (inverse of the deformation). The viscoelasticity of the tissue remains a problem [9] (Fig. 1).

Strain elastography is widely available from many US manufacturers.

Because the colorimetric analysis is not always easy, to avoid the subjectivity of this analysis, studies using quantification of the map color were conducted, with inconstant results (Fig. 2).

Semiquantitative analysis provides numerical values that correspond to the deformation ratios. The machine calculates a ratio between the regions of interest (ROI) localized by the operator on the nodule and the healthy tissue. The calculation can thus be made using the rates of deformation of the structure (strain rate) (Fig. 3).

The appearance of a nodule according to color mapping must be compared with the appearance of normal surrounding tissue. In some cases, this comparison is not possible due to a lack of available normal tissue.

- Huge nodule
- Nodule in empty thyroid bed
- Abnormal neighboring tissue (autoimmune disease)

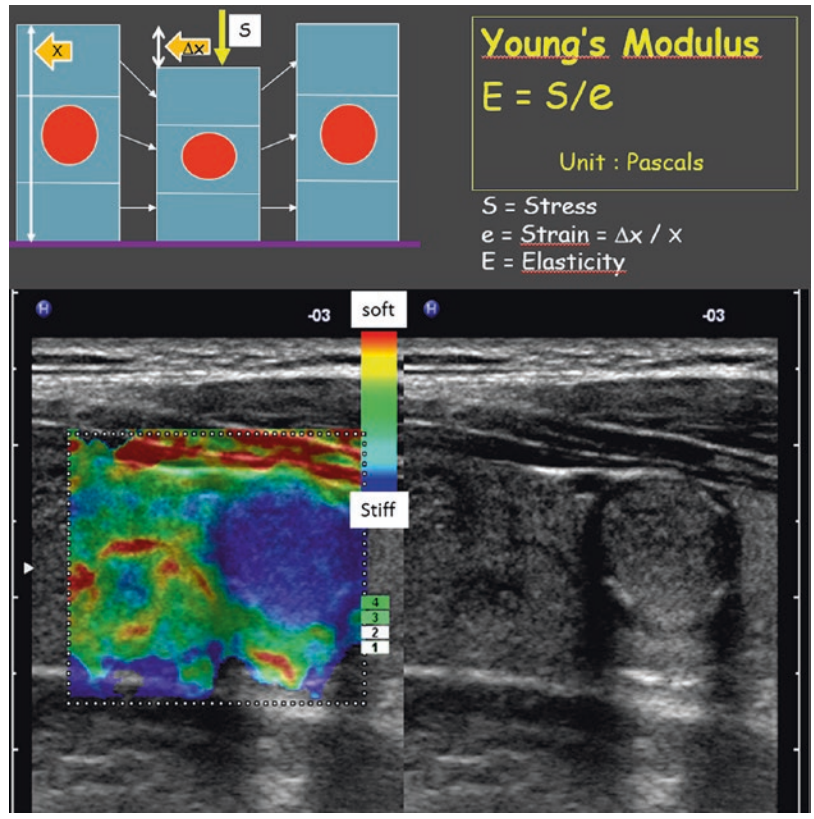

Fig. 1 Strain elastography: schema of compression/relaxation and stiffness calculated by Young's modulus. Elastogram of a stiff nodule (papillary cancer) (Hitachi)

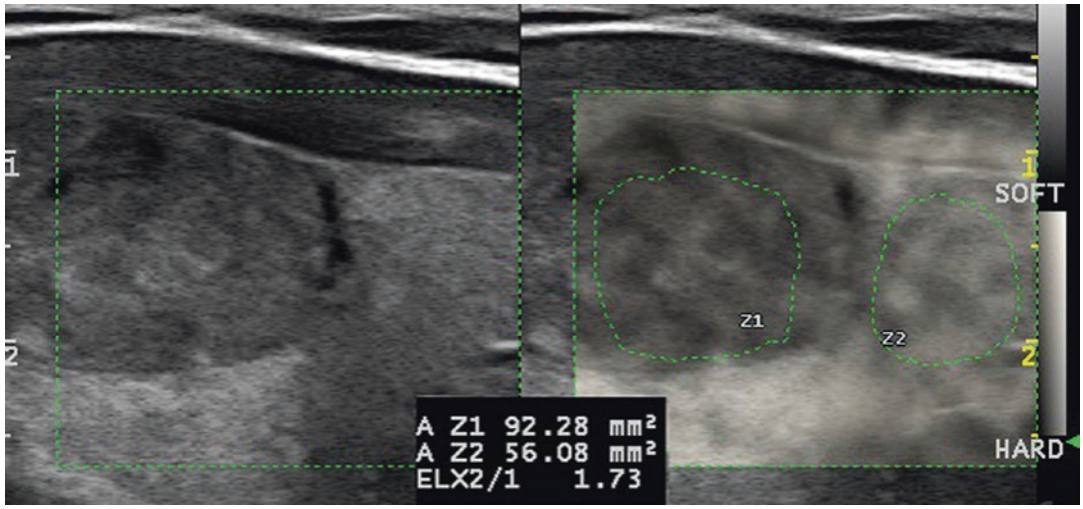

Fig. 2 Strain elastography map color quantification. Benign nodule with intermediate ratio (Esaote)

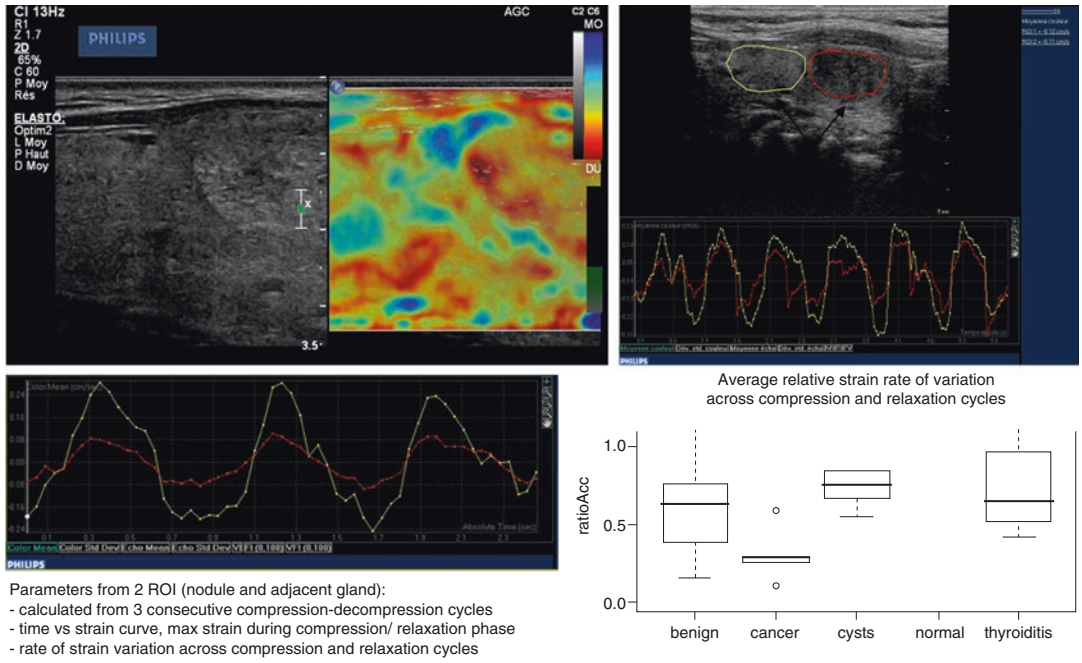

Fig. 3 Strain elastography with semiquantitative analysis. Two ROIs are localized on the strain image. Calculation of stiffness index is realized with Q-Lab software using the strain rate (Philips)

Without semiquantitative analysis, the comparison of map color is frequently subjective.

Dynamic Methods

The probe creates a focused ultrasonic beam (cone beam) with a finite duration. The energy of the beam is converted into a force, namely, acoustic radiation force impulse (ARFI). It generates a brief localized displacement characterizing the viscoelastic property of the tissue. A shear wave (SW) (or transverse wave) is created, perpendicular to the wave propagation direction. It spreads parallel to the skin plan. The speed of the shear waves increases in accordance with tissue stiff-

ness, and the elasticity measurement can be expressed in m/sec or converted into kilopascals (ARFI-shear wave velocity (ARFI-SWV)) (shear modulus and then Young's modulus). In the case of a stiff nodule, the velocity increases and thereby the stiff value (in kilopascal-kPa) [10, 11].

Shear Wave Speed Measurement

1. Transient elastography (TE) allows quantitative evaluation of tissue stiffness based upon the measurement of the shear wave velocities propagating perpendicular to the US beam direction. It provides a single point measurement without imaging capabilities and has been validated for the diagnosis of liver fibrosis (Fibroscan®, Echosens, Paris, France).
2. Point shear wave elastography (pSWE)—ARFI quantification. The measurement is performed in a single small region of interest (ROI) less than 1 cm^3 that can be moved upon an anatomical B-mode image (point quantification) (Siemens. Philips).

Shear Wave Speed Imaging (SWE)

Shear velocity information, which is quantitative and is displayed for imaging, provides a real-time map of elasticity. The elastogram is overlaid with the B-mode image or with side-by-side modality. As in semiquantitative QSE, the ROIs are positioned in the nodule and in the surrounding healthy tissue. The screen is refreshed each second (SuperSonic Imagine, Aix-en-Provence, France; Toshiba MS, Nasu, Japan; General Electric Healthcare).

Supersonic Imaging (Aixplorer): This device is able to monitor shear wave propagation at frame rates above 10,000 Hz (due to an ultrafast beam former) and to keep the dynamics of the acquisition thanks to UltraFast imaging processing. It eliminates many of the limitations of conventional shear wave elasticity imaging techniques by avoiding the repetition of successive shear wave tracking sequences and tracking in real time the propagation of the shear waves in a single ultrafast acquisition (Fig. 4). Multiple ROIs with variable shapes can be positioned upon the area of interest. For each ROI, the software instantly calculates the mean, minimum, and maximum stiffness as well as the standard deviation (the latter increases with increasing tissue elasticity heterogeneity). It also provides an elasticity ratio between the two ROIs (Fig. 5).

Toshiba (Applio Platinium): The acquisition modality is different, using a "line-by-line" emission technique. The shear wave propagation is displayed, enabling the choice of the best frame for calculation of the stiff value (Fig. 6).

General Electric (LogisE9 XDclear): The system uses the same technique as that of Toshiba.

Siemens (ACUSON S3000): The new device provides a 2D SWE image with Virtual Touch™ Quantification (VTq) (Fig. 7). The VTIQ image is a color-coded display of shear wave velocity within the ROIs. The shear wave speed may be quantified in these ROIs.

Reproducibility

It is suitable for quasi-static elastography, with a correlation coefficient ranging from 0.73 to 0.79 for inter-operator reproducibility and 0.73 to 0.84 for intra-operator reproducibility [11, 12].

Regarding shear wave, all the studies attest of the high level of reproducibility [13–15].

Elastography of the Thyroid

Elastography was first used in thyroid imaging in 2005.

Andreas Lyshchik et al., in an in vitro study, [16] described the "Elastic moduli of thyroid tissues under compression." He found a very significant difference between papillary cancers and healthy tissue (Table 1).

A few months later he published the first in vivo study, "Thyroid gland tumor diagnosis at ultrasound elastography" [17].

Practical Examination [18]

One must however bear in mind that, while elastography can provide useful additional information for nodule characterization, it should not be

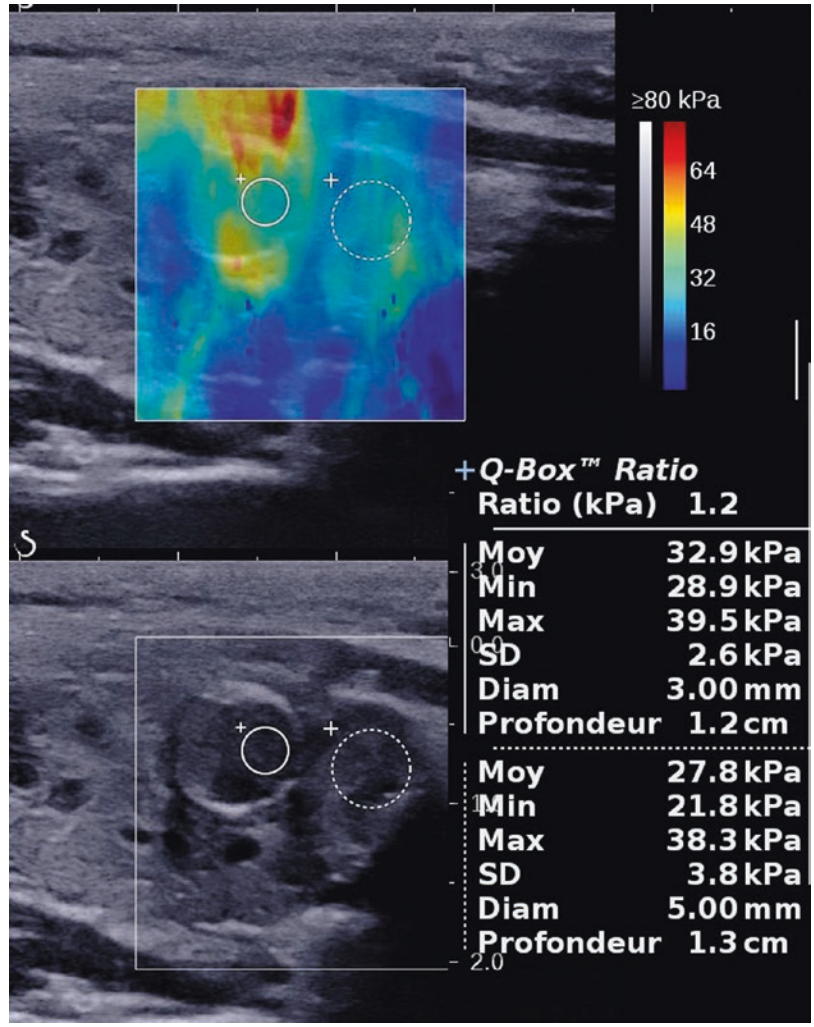

Fig. 4 Shear wave elastography. Discontinued egg-shell nodule generates an anterior artifact. Nevertheless, the stiffness analysis is possible. The ratio between nodule and surrounding healthy tissue is normal. The elastographic scale and the ROI location are correct (Supersonic Imagine)

considered as a substitute for conventional ultrasound examination [19].

In routine clinical practice, each nodule must be stratified according to a TIRADS score (Table 2) and accurately located within the gland based on a highly sensitive color map. At least two elastographic acquisitions are used for each nodule. The examination is painless; the patient may be asked to hold his breath for a very short period. The ultrasound probe is positioned in front of the nodule (sterile water can be used as connecting gel if FNAC is provided in the same procedure). Regions of interest are determined after each screenshot, but postprocessing studies are often carried out. Although the acquisition does not take long, it increases the total duration of the US examination (that do not exceed 10 min in routine practice). The stiff score noted in the report for each nodule has the potential to modify the basic TIRADS score [20].

Quasi-static Elastography

The carotid beats generate a sufficient deformation to create a quantitative map image on which the regions of interest are located. The difference in color encoding between the nodule and the surrounding tissue is analyzed, the score classification having been established by Rago et al. [21], Tranquart et al. [22], and Asteria et al. [23]. The nodules that show low stiffness with a homogeneous or predominantly homogeneous pattern are consistent with benign lesions. On the other

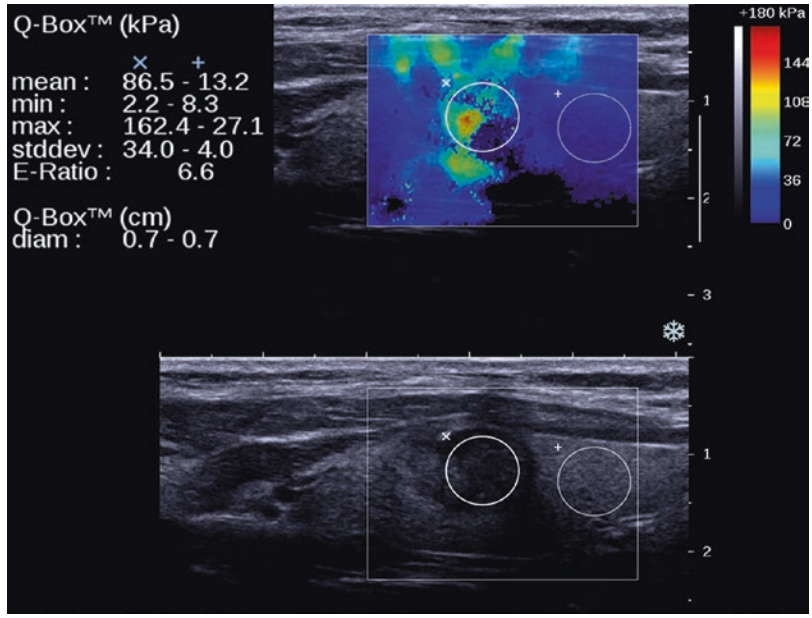

Fig. 5 Shear wave elastography. Papillary thyroid cancer. The nodule is very stiff (maximum 162 kPa), heterogeneous due to necrotic areas (standard deviation = 34) with a very high stiffness ratio (6.6)

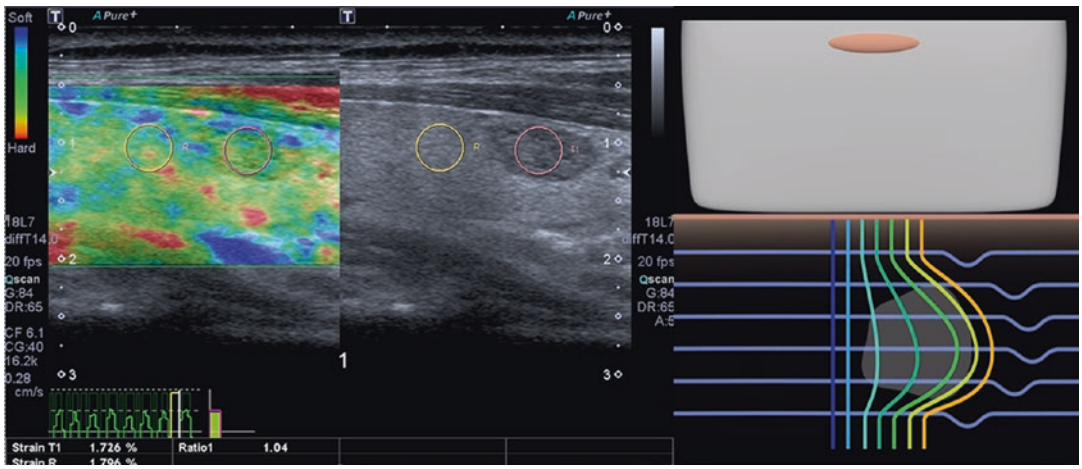

Fig. 6 Shear wave elastography: real-time map of elasticity and shear wave propagation. Benign nodule with normal stiff ratio (Toshiba)

hand, stiff nodules are considered malignant (Fig. 8).

The best approach is to add a semiquantitative analysis to the colorimetric study providing a good level of reproducibility, as mentioned in some preliminary studies.

Shear Wave Elastography

The ultrasound pulse is generated by the probe. The pressure on the patient's skin must be very light (because of the risk of creating a "push" effect artifact). The dual screen displays (B-mode and B-mode with elastogram) precise the location of the ROIs. It is important to move the first (small) ROI in the nodule; once the area with the higher signal is found, the diameter of the ROI should be magnified until the standard deviation remains low (analysis of a homogenous sample). Ideally, the second ROI must be located at the same first deep level. The stiffness

value with its strain ratio appears automatically in the Q-Box.

Some studies have proposed thresholds beyond which cancer should be suspected [15].

- 35–90 kPa for maximum stiffness
- 3.7 for maximum ratio

Other publications using the ARFI system have produced the same results.

Setting principles for the Aixplorer (no major difference from the Applio-Toshiba setting):

- The thyroid presetting is too high (180 kPa); thus the stiffness measurement of a nodule will be wrong due to the mislocation of the ROI. The maximum value of the elastography scale must definitely be lowered, to around 80 kPa. If the tumor exhibits a very high stiffness value, this value will be increased. The value recorded in numerous publications has been reduced due to the conservation of the original presetting (Fig. 9).
- The intensity of the signal must be sufficient to conduct the examination. In the event of a weak signal, the deep area should be colorless or uniformly low. Next, the "pen" presetting must be used for penetration, or the probe must be replaced by a linear probe with lower frequency (SL10).
- The gain can be increased until the elastographic sound appears.
- Artifacts must be avoided: due to excess pressure with the probe ("push" effect) and in the deep area due to stiffer organs—the trachea and carotid. This explains the difficulties for the analysis of an isthmic nodule. In this case, the coronal approach is useful.

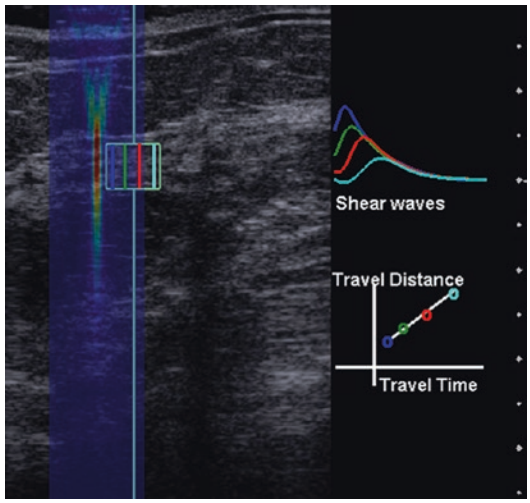

Fig. 7 ARFI system: Virtual Touch™ Quantification (VTq). The focused push pulse generates shear waves. The tracking beams detect SW peak, and shear velocity is computed using linear regression (Siemens)

Summary of the Literature

The preliminary report of Lyshchik in 2005 was followed by numerous papers on quasi-static elastography with manual compression of the thyroid or carotid shear stress. They all showed a high rate of malignancy associated with stiff nodules, whereas benign nodules were soft in most cases.

Rago et al. reported in 2007 the clinical application of thyroid elastography using a five-point scale. Using this criterion, Rago's study includes 92 consecutive patients with a single nodule. The sensitivity was calculated to be 97% and the specificity 100% [21].

Asteria et al. described a classification using a four-point scale: in 86 nodules, sensitivity and specificity were calculated at 94.1% and 81%, respectively [23].

Table 1 Rigidity of different thyroid tissues compared with normal tissue: in vitro measurement [16]

Histology	Stiffness / normal
Colloid adenoma	x 1.7 – 2.4
Papillary cancer (mild compression)	x 5
Papillary cancer (heavy compression)	x 17
Follicular cancer	x 1

Table 2 French TIRADS classification (Courtesy G. RUSS) [20]

TI-RADS SCORE	MEANING
1	NORMAL EXAMINATION
2	BENIGN
3	VERY PROBABLY BENIGN
4A	LOW SUSPICION
4B	HIGH SUSPICION
5	PRACTICALLY CERTAINLY MALIGNANT

In 2010, the meta-analysis carried out by Bojunga et al. concerned 8 studies with surgery as the gold standard and 639 studied nodules [24]. Quasi-static elastography has a sensibility of 92% and a specificity of 90% for the diagnosis of thyroid cancer. However, there was an important selection bias, since the prevalence of cancer was 24%, which is very different from that of a normal ultrasound report.

Concerning semiquantitative QSE, three studies confirmed the good results of colorimetric analysis.

The first used is Q-Lab software (Philips US, Bothell, WA, USA) [25]. The calculation of the deformation slope ratio between the nodule and healthy tissue shows significant differences depending on the nature of the lesion (Fig). Cantisani et al.'s study included 97 patients referred for thyroid surgery [26]. An elasticity ratio greater than 2 made it possible to obtain the following results: sensitivity of 97.3, specificity of 91%, PPV of 87.8%, and NPV of 98.2%. Elastography was more sensitive and specific than all the other ultrasound data. Vorlander's study, involving a large number of patients (309), found a NPV of 100% for a ratio of 3.2 and a PPV of 42% for a ratio of 6.7 [27].

Regarding quasi-static elastography, a number of publications question the value of the technique [28, 29]. This could possibly be due to the absence of semi-quantification and the lack of reproducibility during the learning curve *with/* due to compression by the probe (devices without indication of stress level).

SWE was first reported for diagnosing thyroid nodules in 2010 by Sebag et al. [13]. The author shows that the combination of B-mode US and SWE provides enhanced sensibility and specificity. The emphasis was on certain specificities of the technique: quantitative, operator-independent, and reproducible. These results were confirmed 2 years later by the same team, with a threshold stiffness value of 65 kPa [30].

In 2013, the first SWE meta-analysis by Zhang et al. concerned 5 publications and 698 nodules [15]. The author concluded that SWE is a highly reproductive procedure, applicable to all type of nodules. The heterogeneity for the specificity and positive LR is principally due to the lower results of one of the studies [31]. The explanation is probably the wrong setting of the machine (the maximum value of the elastography scale was 180 kPa on the figures included in the publication). The same problem appeared in Szczepanek-Parulska et al.'s publication and Tian's meta-analysis [32, 33] and probably accounts for the disappointing result of SWE in comparison with QSE.

Liu's meta-analysis shows the high sensitivity and specificity of ARFI for differential diagnosis between benign and malignant nodules while also confirming the current interest in the combination with conventional ultrasound [34].

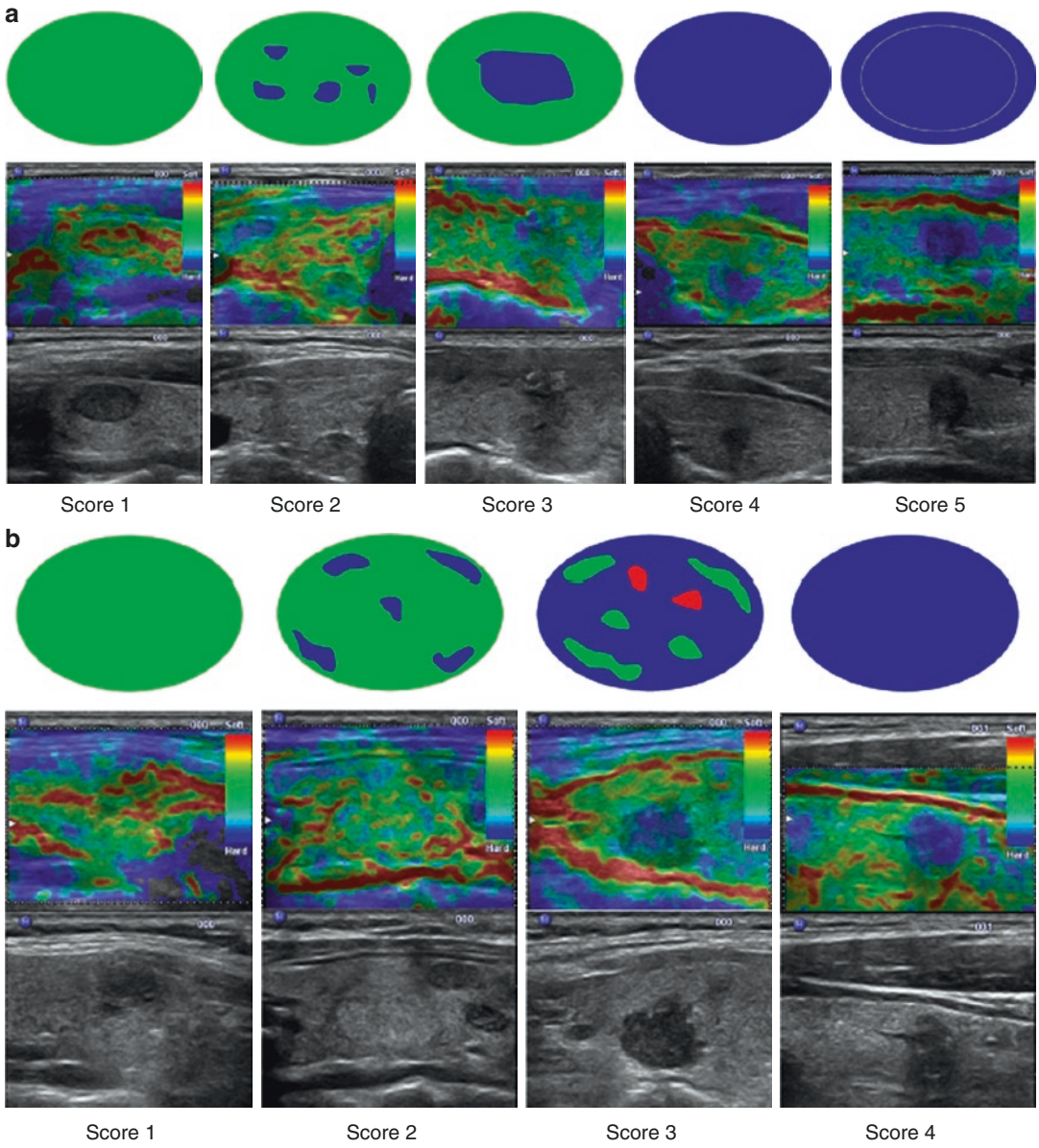

Fig. 8 Qualitative assessment of strain elastography. Scores by Rago (**a**) [21] and Asteria (**b**) [23]. Homogenous green pattern indicates soft nodule and homogenous blue pattern indicates stiff nodule

Moreover, Park et al. in 2015 showed that quantitative parameters of SWE are an independent predictor of thyroid malignancy [35].

In 2013, the European Federation of Societies for Ultrasound in Medicine and Biology published EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography [36]. With regard to the thyroid, two of the recommendations were:

- Elastography is an additional tool for thyroid lesion differentiation.
- Based on expert opinion, elastography may be used to guide the follow-up of lesions negative for malignancy at FNA.

Some recent developments are not preconized by these recommendations: the authors of a recent meta-analysis propose the omission of

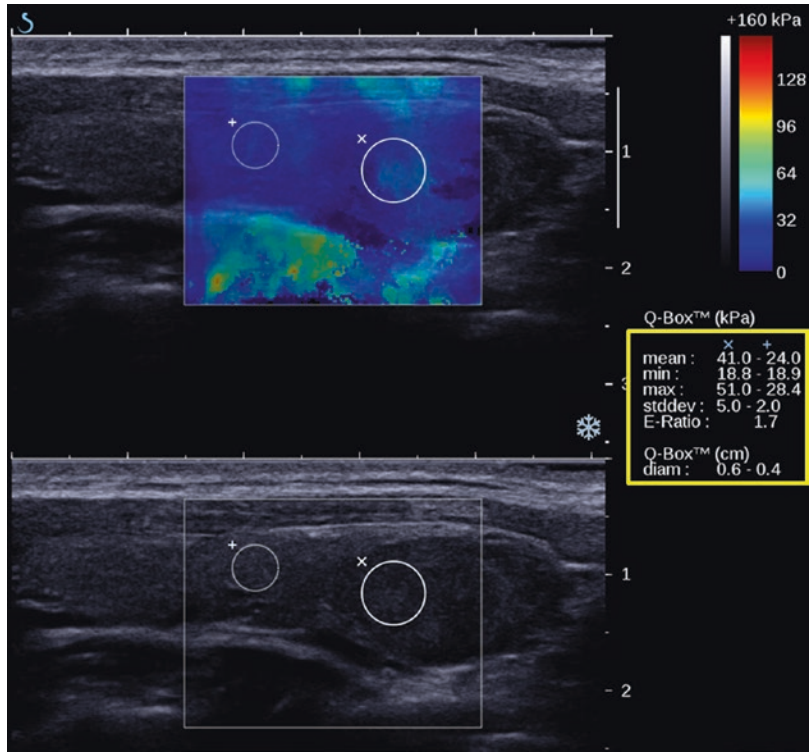

Fig. 9 Shear wave elastography: wrong setting of the machine. High maximum value of elastography scale in case of soft nodule (Supersonic Imagine)

FNA cytology in the case of a completely soft nodule (Asteria score 1) [37].

The World Federation for Ultrasound in Medicine and Biology (WFUMB) recently published the WFUMB Guidelines and Recommendations on the Clinical Use of Ultrasound Elastography: Part 4. Thyroid [38]. The 25 recommendations are issued on the level of evidence of the published literature and on expert group consensus. They compared strain and SWE elastography.

A recent meta-analysis (Hu) points to better specificity of strain elastography [39]. The He et al. study shows *an* equality of efficacy between the Aixplorer and Applio-Toshiba systems [40].

Thyroid Pathology: Information/Data Provided by Elastography

Nodular Analysis

According to most of the reported series, elastography enables enhancement of the positive predictive value (PPV) and the negative predictive value (NPV) [41, 42] of malignancy obtained via conventional ultrasound studies. It is therefore predicted to become the eighth parameter for thyroid nodule characterization. It was included in the French TIRADS classification. This point was recommended by domestic society (French Endocrine Society) [43] and ultrasound international societies (Fig. 10) [44]. The recent EU-TIRADS score does not confirm this proposition [45].

It is however of note that the above observed improvement in PPV and NPV in nodule characterization seems to be closely related to operator experience and skill. When the two scores are high, information obtained from elastography provides less benefit than that of the conventional US. This probably accounts for the disappointment felt among certain expert colleagues possessing great expertise in thyroid ultrasound analysis [29].

Notably, elastography is useful for the location of cystic nodules (Fig. 11) with viscous component (resembling hypoechoic solid nodules) and pseudonodules. In these cases, it is a time-saving application.

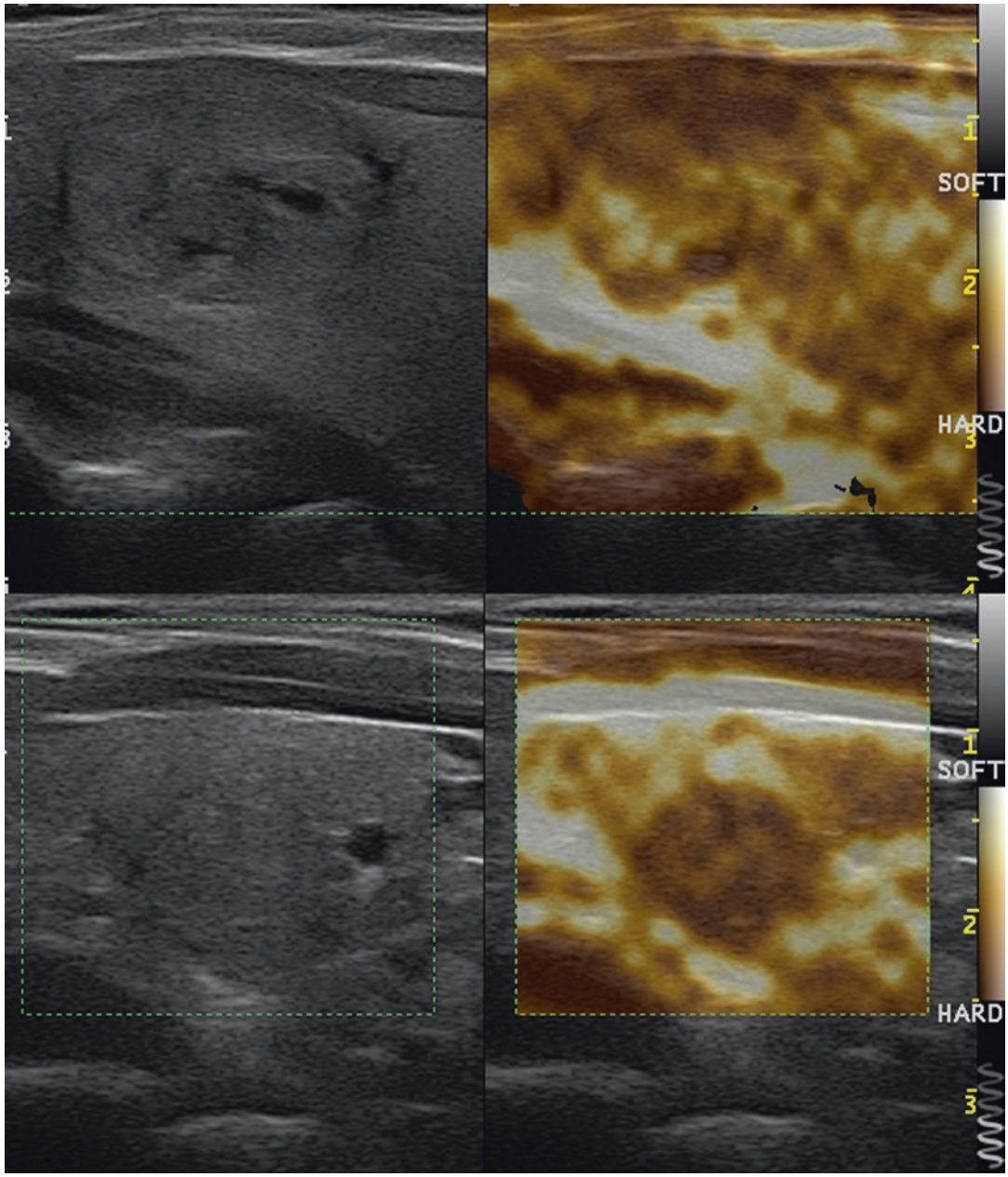

Fig. 10 Improvement of TIRADS by QSE elastography: two nodules TIRADS 3. Top image, the nodule is probably soft; bottom image, suspicious of malignancy (TIRADS 4b)

Other Thyroid Diseases

Shear wave elastography enables objective tissue stiffness quantification by providing a numerical value varying between 10 and 40 kPa for healthy tissue with some studies proposing the use of SWE for the characterization of non-nodular thyroid disease.

- Autoimmune thyroiditis (AIT). In the event of nodule(s) occurring during AIT, the stiffness ratio will likely be artificially low and thus falsely reassuring. The numerical stiffness value therefore needs to be considered [46, 47]. On the other hand, SWE was recently proposed to select patients with a higher

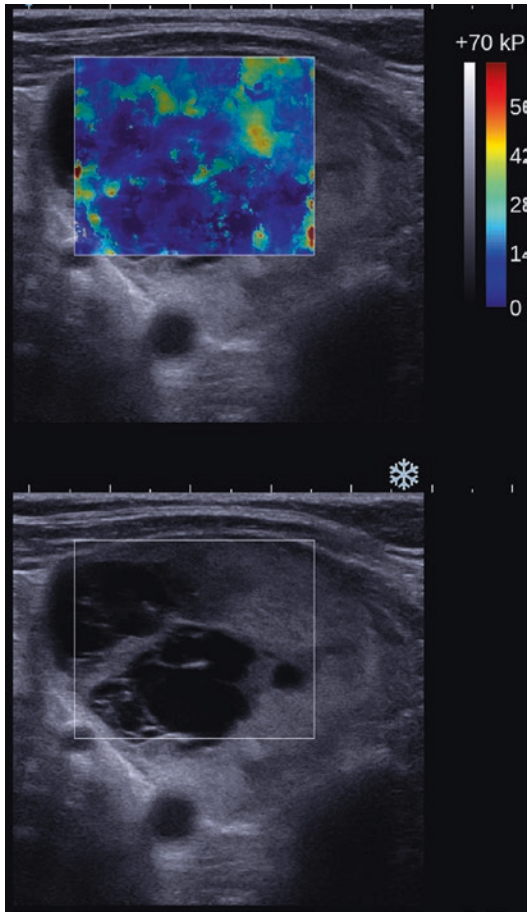

Fig. 11 Nodule with important fluid component the dark blue color of the cystic area corresponds with lack of shear wave propagation in fluid

stiffness value requiring biological investigations [48, 49].

- Riedel's thyroiditis. This rare form of thyroiditis due to IgG4 exhibits a very high stiffness value, and SWE is a good method to unmask the possibility of this diagnosis (Fig. 12) [50].
- Thyroid bed after surgery. In the case of thyroidectomy for cancer, the emphasis of solid hypoechoic vascularized tissue in the thyroid bed is suspicious for recurrence. The possibility of parathyroid adenoma is not rare, but its ultrasound data are the same as those for cancer recurrence. SWE may provide the solution.

Other Cervical Diseases

- Lymph nodes. Elastography can also be useful to investigate cervical lymph nodes. In QSE, metastatic thyroid adenopathy has a very different appearance compared with normal lymph node. Without any surrounding healthy tissue, the comparison is impossible. In this case, SWE seems to be easier to select lymph nodes for fine needle aspiration cytology [51]. In addition, the detection of cystic component (lack of SWE signal) is an important sign of malignancy (Fig. 13).
- Parathyroid. A recent study described the high sensitivity and specificity of SWE (ARFI) to differentiate parathyroid adenoma from benign and malignant thyroid nodules (especially in case of posterior nodule(s)) [52].

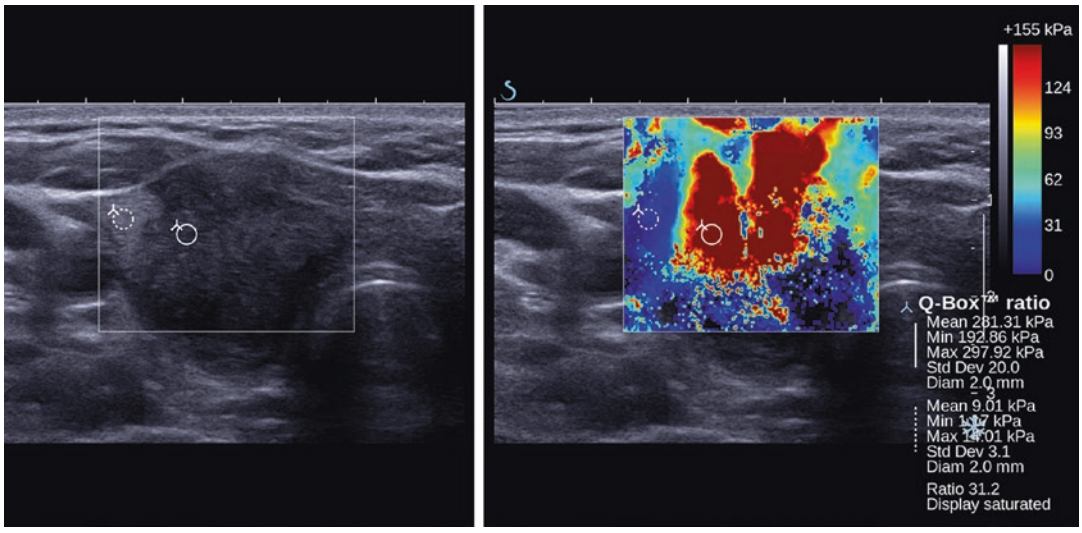

Fig. 12 Riedel's thyroiditis. Hypoechoic nodule, with very high maximum stiffness value and ratio (Supersonic Imagine)

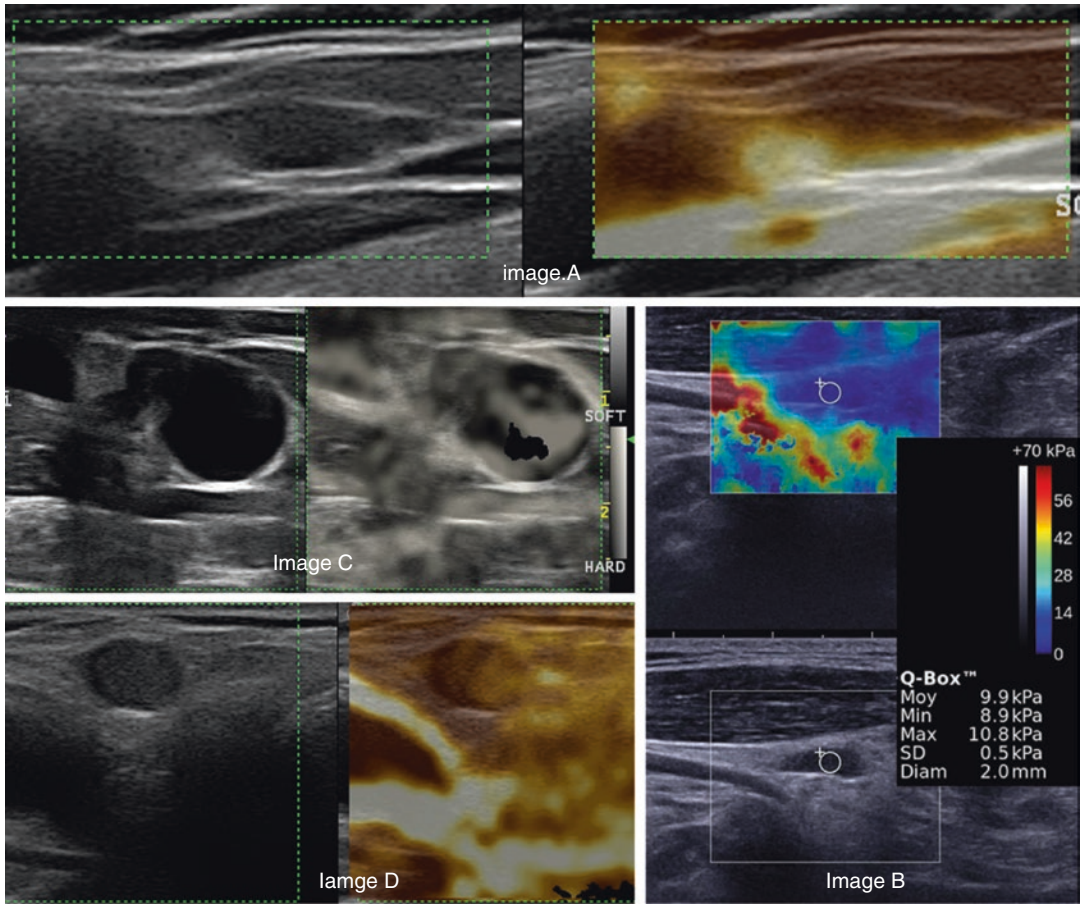

Fig. 13 Lymph node elastography. Normal lymph node. QSE (image **a**) SWE (image **b**) Lymph node metastasis: QSE: Cystic (image **c**) and stiff (image **d**)

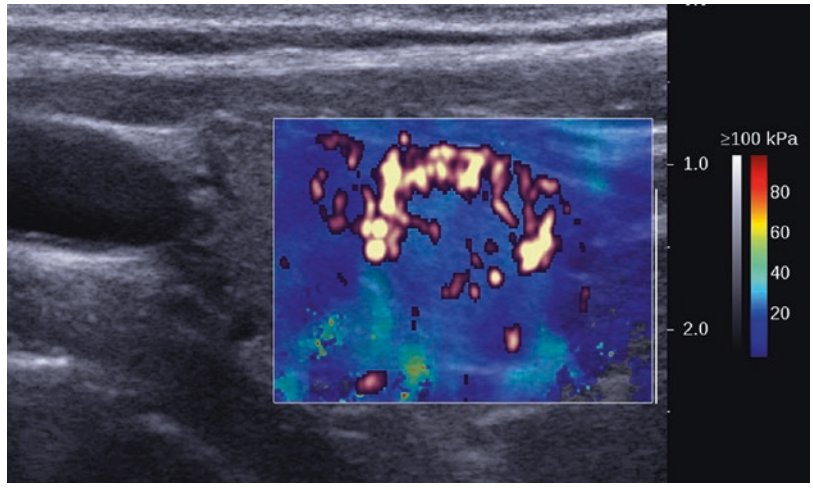

Fig. 14 Shear wave elastography with power Doppler encoding (Supersonic Imagine)

Thyroid Elastography: Today and Tomorrow

Such an Important Technique Is Always in Research and Development (R&D)

- In the case of a deep nodule, the problem of the loss of signal is now resolved, thanks to new adapted probes.
- Some hyper-vascularized nodules may behave like stiff nodules. Supersonic imaging is used to develop a new application incorporating SWE and Doppler on the same screen (Fig. 14).
- The future may be twin procedures (shear wave elastography + contrast-enhanced ultrasound (CEUS)).

So Today, What Does Elastography Bring to the Thyroidologist?

The elastographic score is the eighth data component of TIRADS (Table 2), providing as it does an enhancement of ultrasound nodule characterization together with an increase in PPV and NPV and an improvement of TIRADS (negatively correlated with the expertise of the operator). It thus represents a reduction in the number of cytologies [53] and probably of surgeries. It cuts down the time for the examination of multinodular goiter, cystic nodules, and pseudonodules of thyroiditis (Fig. 15).

But What Additional Information Do We Need to Obtain?

Indeterminate cytologies (15% of samples) remain a major problem for thyroidologists. According to the Bethesda score [54], type 3 (AUS) and type 4 (follicular neoplasm) characterize, respectively, 10% and 25% of follicular cancers. Could elastography shed more light on these ambiguous cases (Table 3)?

The results of publications reporting on QSE devices are not unanimous [55–58].

SWE alone seems to be a valuable tool for determination of the preoperative malignancy risk of follicular-pattern nodules [59, 60]. The first part of the French SWEETMAC study has not, meanwhile, confirmed these results [61]. It is one of several ongoing studies, coupled with other investigations, using SWE (e.g., molecular biology, miRNA).

Concerning the nodules with non-diagnostic cytologies, Capelli's study [57], confirming Rago et al.'s study [62], shows that QSE alone is able (after two FNAC Bethesda one) to diagnose 12/15 cancers and all the cancers in association with conventional sonographic features.

What Information Will We (Probably) Never Obtain?

Elastography will never be substituted for histology. We know that tissue stiffness increases with stroma proportion and numerous malignant tumor cancers are devoid of stroma (follicular cancers, follicular variant of papillary cancers, poorly differentiated cancers). The aim of elastography is not to explain why a particular cancer is soft and why some benign lesions are stiff.

Conclusions in the Form of Reflections

Thanks to the relatively recent development of elastography, we have had the privilege of living a particularly exciting page of scientific history, whose progressive advances are typical in the field of innovative technology.

Thirteen years after the first description of the technique, endocrinologists discovered its application to the thyroid. While the very first response was one of simple curiosity about this discovery, more enterprising colleagues quickly realized its considerable potential and proceeded to serious studies and investigations. *It was the age of pioneers*.

Their publications produced *a period of ever-increasing interest and enthusiasm* in the medical community and numerous teams set about using QS elastography, reporting their finds in a lot of papers, the first meta-analysis being published in 2010. SWE confirmed the QSE studies, and, today, elastography is on its way to becoming fully established in technical procedures.

On the other hand, some eminent voices have raised doubts about the true significance of the technique. In fact, during this period, the value of nodule characterization was considerably enhanced while OR though meanwhile TIRADS has revolutionized our practices, with or without

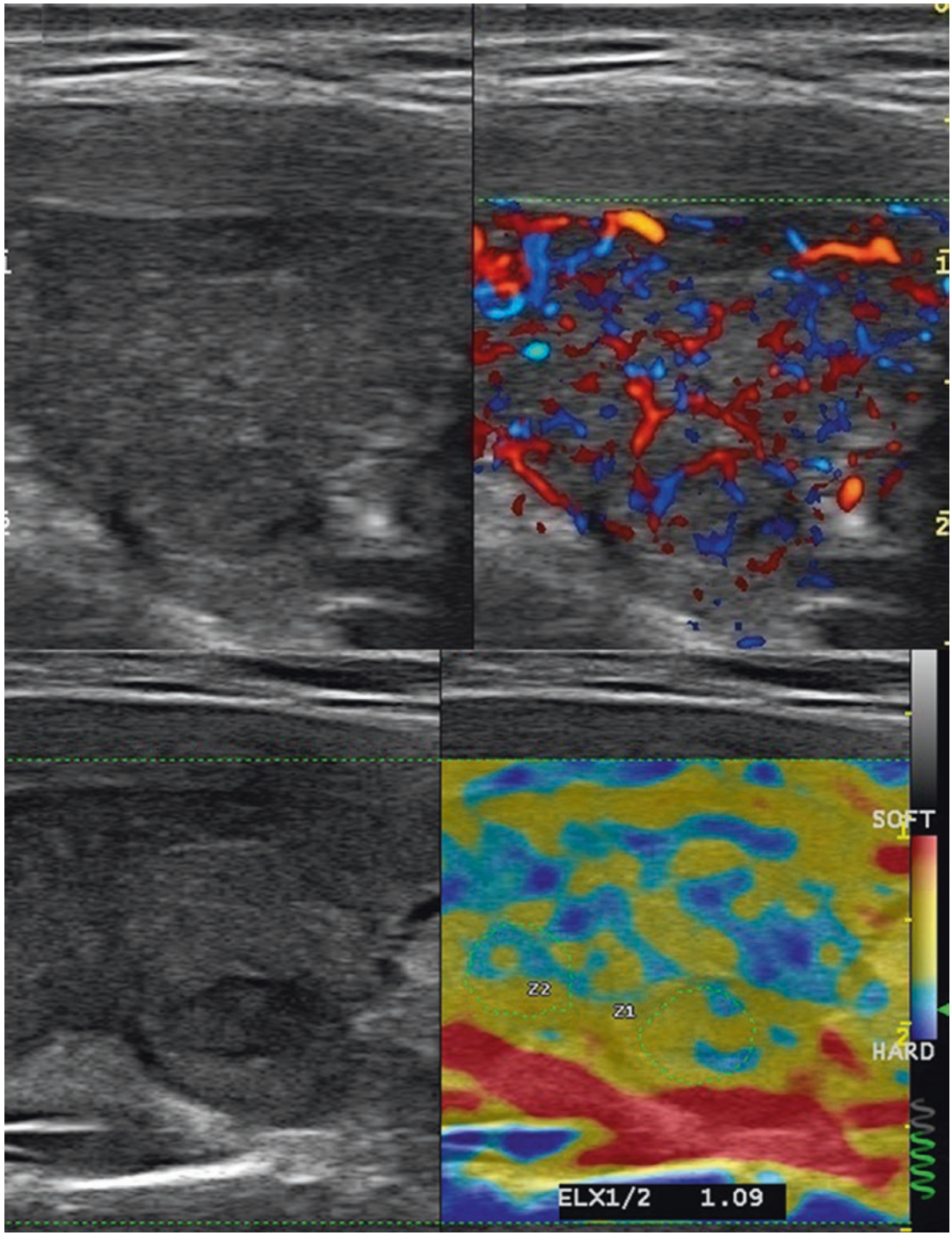

Fig. 15 Pseudonodular appearance during autoimmune thyroiditis: lesion without precise shapes in gray scale, without ring vessels in color Doppler, without stiffness ratio in strain elastography

elastography. Thus, *a period of loss of confidence has come about*. This however has not stemmed the flow of papers on the subject (280 between 2012 and 2016), though it has generated *the present time of reflection* in correlation with the huge technical developments brought about by R&D.

Table 3 Bethesda system [49]

• Cytological aspect	Cancer risk
• 2 Benign	<1%
• 3 Follicular lesions	5-15%
• 4 Follicular Neoplasm +/- oncocyts	15-30%
• 5 Suspect of malignancy	50-75%
• 6 Cancer	90%

The next step will probably be *the age of reason*, in other words, the realization that elastography constitutes an essential element of nodule analysis in conjunction with—and not in place of—classical ultrasound characterization [63], also incorporating CEUS, scintigraphy, molecular biology, and miRNA in the event of difficult cases.

Until the advent of a new technique……???

Glossary

AIT Autoimmune thyroiditis
ARFI Acoustic radiation force impulse
AUS Atypia of undetermined significance
CEUS Contrast-enhanced ultrasound
ETA European Thyroid Association
EFSUMB European Federation of Societies for Ultrasound in Medicine and Biology
FNA Fine needle aspiration cytology
IgG4 Immunoglobulin G4
kPa Kilopascal
miRNA Micro-RNA
NPV Negative predicting value
PPV Positive predicting value
QSE Quasi-static elastography
RD Research and development
ROI Region of interest
SE Strain elastography
SWE Shear wave elastography
TE Transient elastography
TIRADS Thyroid imaging reporting and data system
TM Time motion
VTq Virtual touch quantification
2D Two dimensional = B-mode = Gray scale
US Ultrasound

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Imaging of Differentiated Thyroid Cancer with Iodine-124 and F-18-FDG

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^{124}I PET/CT

^{124}I is a positron emitter with a half-life of 4.2 days, a positron yield of about 22% with relatively high energies, and a complicated decay scheme with high-energy gamma and X-rays [1]. Even though this tracer has been used since the early 1960s for thyroid imaging, the clinical application of this tracer is limited, mainly due to availability and cost. Therefore, most published studies using ^{124}I PET or PET/CT are of retrospective nature with only a small number of patients. However, some centers use ^{124}I PET/CT routinely in high-risk DTC patients as well as in clinical trials, and the increasing number of publications in the last decade underlines the importance of this tracer in thyroid cancer imaging. It has been shown convincingly that ^{124}I PET/CT is more sensitive in detecting metastatic thyroid cancer than gamma camera imaging with ^{131}I [2, 3]. For instance, in a study in 25 patients, ^{124}I PET identified 50% more sites of disease than the pretreatment ("diagnostic") scan with ^{131}I [3]. Besides higher sensitivity of the PET scanner, the main strength of ^{124}I PET/CT is a precise quantification of lesion doses, the so-called lesion dosimetry. The main goal of a clinically applied ^{124}I PET/CT dosimetry protocol is not only to quantify lesion doses but also to calculate the blood dose as a surrogate parameter for radiation-associated bone marrow toxicity (Fig. 1). The results derived from this dosimetry approach enable the physicians to calculate an individual activity to treat the target lesions without overcoming the threshold for bone marrow toxicity [4, 5]. Particularly for high-risk DTC patients with repeated ^{131}I treatments (RAI), this approach becomes crucial. For instance, in a retrospective study of 34 patients, ^{124}I PET allowed for reliable volume estimation (>0.80 mL) in 59 lesions in 17 patients [2]. In another study, ^{124}I PET lesional dosimetry was used to calculate the amount of RAI needed to achieve doses of ≥ 100 Gy to all metastases without exceeding dose of 2 Gy to the blood; ^{124}I PET led to a change in management in 25% of patients [6]. In another study, 15 of 30

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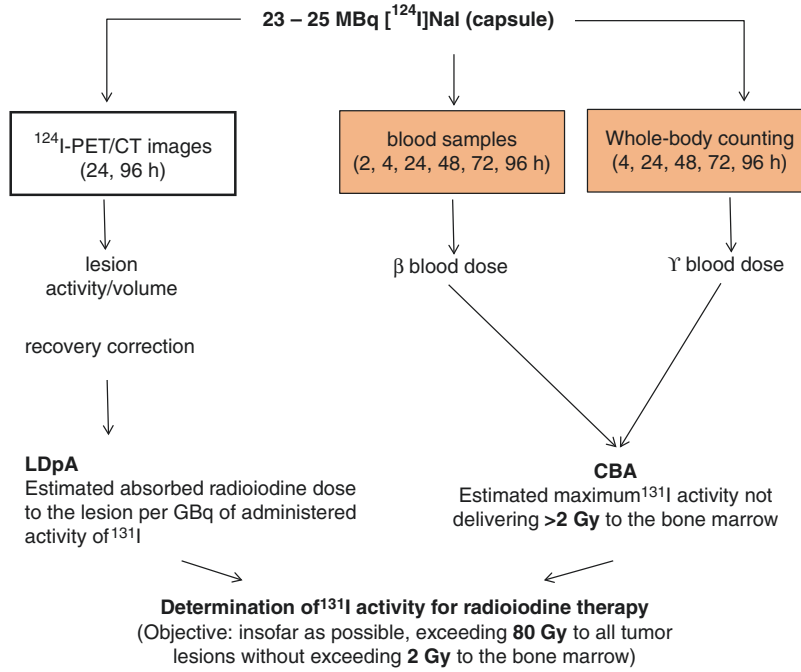

Fig. 1 This is an example for a ^{124}I dosimetry protocol. This protocol is curently applied in an university hospital for routine patients

patients with known metastatic disease showed no ^{124}I uptake on serial scans (4, 24, 48, and 72 h), despite sufficiently high TSH levels, suggesting that these lesions would also be refractory to ^{131}I treatment [7]. A smaller study in 12 patients disagreed with this conclusion and suggested that ^{124}I PET should not be used in isolation to prevent treatment with ^{131}I [8]. However, it should be realized that uptake of ^{131}I alone on posttreatment scans is not sufficient for response as proposed in this study, unless the local dose from accumulated iodine is tumoricidal. A recently published study showed a high level of agreement of pretherapeutic ^{124}I PET and intra-therapeutic ^{131}I SPECT lesion detection [9].

Despite the debate about the role of ^{124}I PET or PET/CT in the clinical setting of DTC treatment, its role is crucial in the evolving field of redifferentiation treatment of radioiodine refractory (RAIR) thyroid cancer through inhibition of MAPK signaling. Ho et al. nicely showed that inhibition of MEK1/MEK2 in RAIR thyroid cancer is effective to increase iodine accumulation in tumor lesions significantly [10]. Even though this was a pilot study with 22 patients, this approach is a change of paradigm in the treatment of RAIR thyroid cancer, not only because of the positive results but moreover because of understanding the underlying mechanism behind this effect [11]. To assess the effect of the used MEK inhibitor in this trial, Ho et al. applied a pre- and post-drug treatment ^{124}I PET/CT (Fig. 2). They defined that patients with a lesion dose higher than 20 Gy would benefit from an ^{131}I treatment, and their results correlated nicely with this assumption, showing the predictive value of ^{124}I PET/CT dosimetry. There are currently trials ongoing with different MAPK inhibitors in which ^{124}I PET/CT is included for dosimetry purposes. The use of ^{124}I PET/CT in this setting is unavoidable since the redifferentiation effect of the investigated drug has to be quantified to select the patients carefully for a RAI. Giving standard treatment activities of ^{131}I without knowing the calculated lesion dose can result in less radiation dose and therefore insufficient treatment of the target lesion. In the authors' view, a careful dosimetry approach in this setting is unavoidable, and an ^{124}I PET/CT is the preferred tool for this purpose.

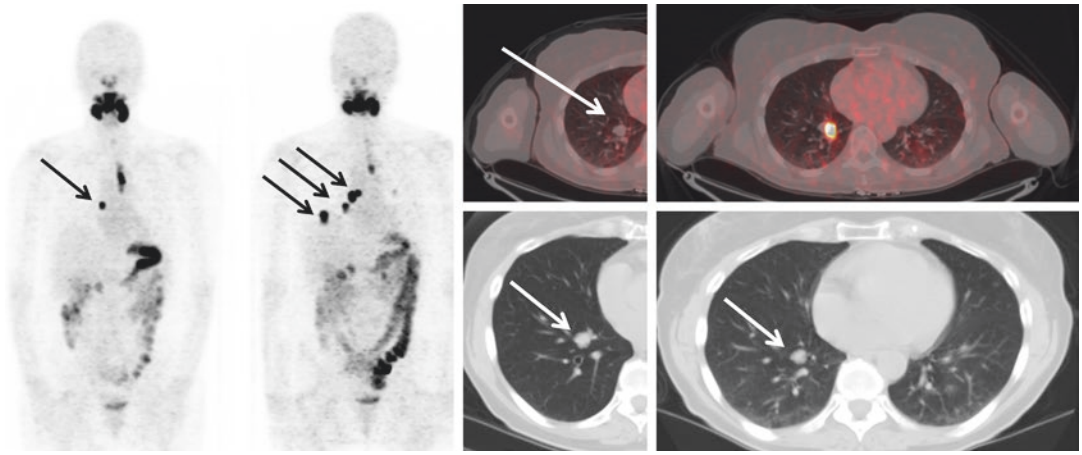

Fig. 2 Left panel shows MIPs of two ^{124}I PET scans before (left image) and after treatment with selumetinib for 4 weeks (right image) and axial sections of selected locations from a patient who was treated with selumetinib for about 4 weeks. The right panel shows axial images of I-PET/CT (upper row) and CT images (lower row) of this patient. The CT images represent one target lesion 3 months after treatment. Lesions which showed a higher uptake after redifferentiation treatment with selumetinib showed a response to ^{131}I treatment

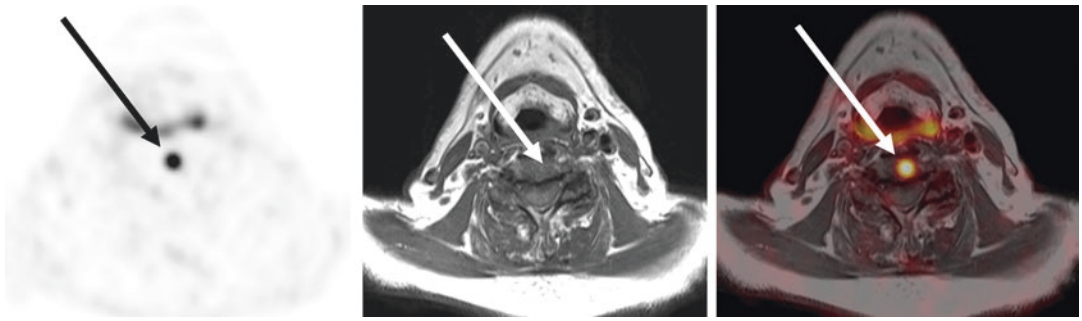

Fig. 3 Left panel shows a ^{124}I PET-positive lesions of a thyroid cancer patient outside the thyroid bed. The middle panel shows the corresponding MRI scan and the right panel the fusion of both scanning devices

^{124}I PET/MRI

PET/MRI is an evolving technique not only but also for imaging in cancer. Even though it is not as successful as PET/CT as it was introduced, there is a high number of studies published in the last years revealing the role of PET/MRI in cancer imaging. Despite the fact that the quantification properties of simultaneous PET/MRI scanner are currently investigated, there are two major reasons why PET/MRI may play a role in the future, when it comes to thyroid cancer imaging. The first reason is that an ^{124}I PET/MRI has less radiation exposure compared to PET/CT, particularly, if the PET/CT includes a full diagnostic CT scan. Therefore, ^{124}I PET/CT imaging or dosimetry is rarely performed in pediatric patients. A PET/MRI may overcome this shortcoming of PET/CT.

The second reason has to do with the dosimetry itself. To perform a precise dose calculation, the volume of the target lesion has to be determined as precise as possible. Even though this can be estimated to some extent using the PET images itself, for very small lesions (below the resolution of the PET scanner), it can't be done in a reliable manner. Due to high soft tissue contrast, MRI detects even very small lesions, which has implications to lesion dosimetry (example shown in Fig. 3) [12]. Unpublished current data also show that the quanti-

fication properties of a PET/MRI scanner compared to a PET/CT scanner (both scanners from one company) show a high correlation, which enable a reliable dosimetry with an ^{124}I PET/MRI.

^{18}F FDG PET/CT

An inverse relationship between FDG avidity and radioiodine uptake (originally described by Feine as the "flip-flop phenomenon" [13]) is often seen in metastatic thyroid cancer lesions; increased FDG uptake is generally associated with decreased disease-specific survival [14]. Despite the ongoing debate of this phenomenon, whether, for instance, the "flip-flop" occurs in the same lesion (for instance, due to selection of clones with more aggressive features after radioiodine treatment), it's widely accepted that thyroid cancer lesions with increasing FDG uptake in the course of the disease show refractoriness to RAI therapy and poorer prognosis (Fig. 4) [15]. A histopathologic study also showed that the majority of metastases in patients with radioiodine-refractory FDG-positive metastases were of histologically aggressive subtypes [16]. In another study, Grabellus et al. showed that lesions refractory to radioiodine (this was correlated with ^{124}I PET/CT) showed a higher expression of GLUT-1 and higher FDG uptake [17]. The DTCs however in this study did not show an upregulation of GLUT-1, suggesting that GLUT-1 is a molecular marker for increased aggressiveness of thyroid cancer, and their expression can be monitored in vivo through FDG PET/CT. Another interesting study by Grabellus et al. showed a higher GLUT-1 expression in $BRAF^{V600E}$-positive thyroid cancer compared to *BRAF* wild-type patients. It's believed that patients harboring a $BRAF^{V600E}$ mutation show a poorer prognosis compared to *BRAF* wild-type patients [18]. Since it's rather impossible to biopsy every tumor lesion in patients to identify the BRAF mutation status, a FDG PET/CT scan can point to the presence of a mutation due to the fact that these lesions show a higher FDG uptake [19]. Therefore a FDG PET/CT scan is useful not only to stratify patients based on the risk of recurrence or poorer prognosis but also for therapy management. In this line,

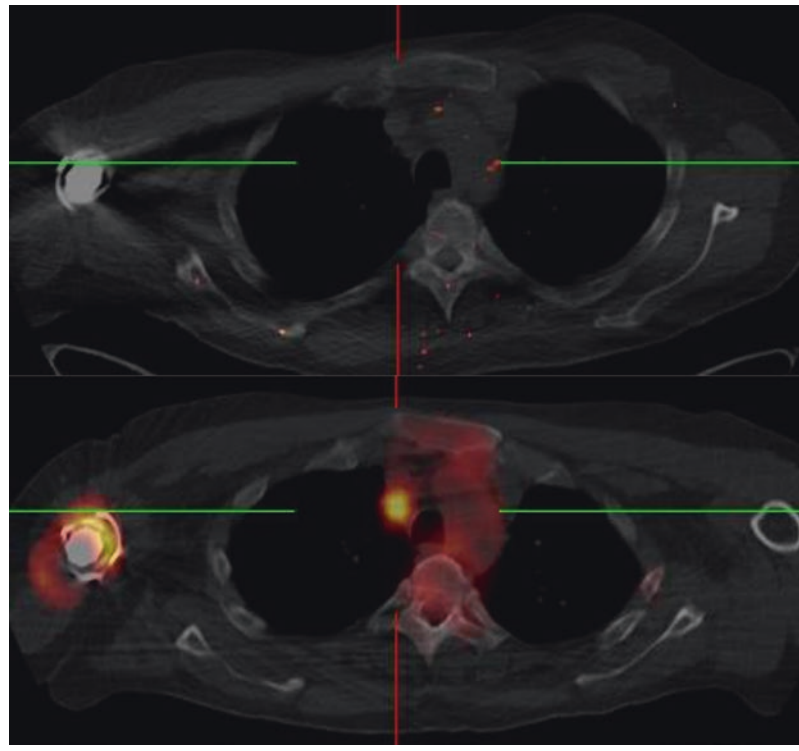

Fig. 4 Upper panel shows axial image of a lymph node metastasis which is negative on ^{124}I PET. The same lesion is positive on ^{18}F-FDG-PET scan. Both scans were performed within 1 week on the same patient

a study with 90 consecutively included patients showed that in high-risk situation based on FDG PET/CT results, the TNM status was changed for about 10% and the therapy management for 21% of the patients [20].

The prognostic value of FDG PET in advanced thyroid cancer was well documented in a classic study with 125 patients in whom survival was lower among patients with FDG-positive disease as compared to patients without FDG avid lesions: Patients with a volume of FDG avid disease >125 mL had a significantly shorter survival [21]. FDG PET may also help in the management of patients with differentiated thyroid cancer presenting with elevated Tg levels but a negative ^{131}I scan. According to current guidelines, a FDG PET/CT scan may therefore be useful in radioiodine refractory thyroid cancer patients for risk assessment and to verify recurrence. However, it is recommended to perform a careful patient selection before a FDG PET/CT scan to locate tumor recurrence. Since the tumor marker Tg is very sensitive, an FDG PET/CT scan may be false negative for very low Tg levels (due to very low tumor burden). Therefore, it is recommended to perform a FDG PET/CT scans for patients with a Tg level higher than 10 μg/L [22, 23]. Additionally, the probability of detection of FDG-positive lesions is increased if the PET/CT scan is performed under TSH-stimulated condition. There seems to be a mechanistic correlation between the activation of the TSH receptor and PI3K/AKT/mTOR pathway upregulation, which leads to a higher translocation of GLUT1 to the membrane and consecutively to a higher FDG uptake [24]. Even though this mechanism is not fully understood, many clinical studies show a significant higher detection rate of FDG-positive tumor lesion, if the FDG PET/CT scan is performed under TSH stimulation [25].

Summary

PET is a powerful tool in thyroid cancer imaging. Besides the here discussed two tracers, there are many other tracers for imaging of specific receptors and intracellular targets, which enables PET to visualize tumor biology in real time in patients.

^{124}I PET/CT is increasingly used for imaging and dosimetry purposes in thyroid cancer and will play a major role in the future, particularly for treatment evaluation of new drugs for redifferentiation of RAIR thyroid cancer patients. ^{124}I PET/MRI is a new tool with promising applications in particular settings.

^{18}F-FDG-PET/CT is a useful technique for imaging dedifferentiation of metastatic thyroid cancer and is also valuable for risk stratification and prediction of survival in high-risk thyroid cancer patients and has therefor impact in patient management.

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Surgery for Benign Goiter

Kerstin Lorenz

Indication and Extent of Thyroid Surgery

Recognition of the pathogenesis involved in multinodular goiter (MNG) development influenced surgical strategy in the way to refrain from safeguarded standard bilateral subtotal resections to a more selective approach, morphological-functional resection, aiming to resect all autonomous or cold nodules, more often resulting in lobectomy than in tissue-preserving resections [1–6]. In solitary thyroid nodule surgery is indicated when malignancy cannot be excluded otherwise, or in functional autonomy at the patient's preference, and after therapeutical alternatives have been evaluated and discussed with the patient [7–10]. Indication to surgery in multinodular goiter is mainly influenced by the size and extension of goiter, objective or subjective compression symptoms, and suspected malignancy. Various types of resection may be adequate for benign euthyroid nodular goiter ranging from enucleation-excision to subtotal thyroidectomy to lobectomy, unilaterally or bilaterally [1, 4, 11–13]. Contrary, in malignancy and in Graves' disease, bilateral thyroidectomy without thyroid tissue remnants should be sought [8, 10, 13]. There is a validated association of progressive complication rates with more radical thyroid resection [3, 14]. Recurrent laryngeal nerve palsy rate and hypoparathyroidism are elevated in lobectomy compared to subtotal thyroid resection [12, 15, 16]. However, modern technical adjuncts like magnifying loops, bipolar diathermy, and intraoperative neuromonitoring have been added to enhance meticulous surgical maneuvers in proximity to the RLN and PG, leading to further decrease of unfavorable results despite the prevalence of more radical resection, resulting in over all very low permanent rates of vocal cord palsy and permanent hypoparathyroidism in the hands of specialized experienced thyroid surgeons [12, 14–23].

Preoperative Assessment

Routine patient history and physical exam are required. Clinical investigation should further include specific family history of thyroid disorders, time of development in prevalent symptoms or thyroid growth, and actual size. Associated typical clinical signs of thyroid disease and underlying thyroid functional status referring to signs of hyper- or hypothyroid status should be recognized.

In preparation for thyroid surgery, a minimum of laboratory tests are necessary to assess besides routine blood tests. Euthyroid functional status is

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determined by basal TSH value in the normal range; normal serum calcium level excludes underlying overt hypercalcemic hyperparathyroidism. In autoimmune thyroid disease and Graves' disease, thyroid antibodies may be representative for present disease activity. Basal calcitonin level may indicate underlying c-cell hyperplasia or medullary thyroid carcinoma [10, 24].

Thyroid imaging comprises ultrasonography, thyroid scintigraphy, and CT or MRI. Sonography of the suprasternal thyroid gland evaluates the size, nodularity with localization, number and quality of nodules, as well as neighboring structures and cervical lymph nodes [7, 25–30]. Elastography may provide further information regarding nature of the lesion [31]. There are a number of useful classifications available to classify ultrasonographic findings with regard to pathology, assisting in guidance of surgery [28, 32]. Sonography also enables fine needle aspiration cytology (FNAC) of suspicious lesions. FNAC may provide evidence of malignancy with Bethesda classification V and IV; however a considerable number of results will not provide reliable evidence, especially in the setting of multinodular goiter, and in FNAC results of follicular neoplasm [32–37].

Thyroid scintigraphy may be performed with different tracers and usually aims to assess functional status of sonographically detected lesions. In autonomous nodules, histopathology is predominantly benign, whereas cold nodules or ^{99m}Tc-MIBI (methoxyisobutylisonitrile)-positive nodules may harbor malignancy in up to 10–20% [30, 38].

Sectional studies with CT or MRI help to assess extent of large goiters with substernal extension into the mediastinum and thorax and identify possible ectopic manifestations as well as adjacent structures [39–46] (Fig. 1a–d). With this information, compromised or difficult airway settings and optional surgical approach can be adequately anticipated, and necessary preparation is ensured. Thus, intubation in severely compressed trachea may be only performed in the patient awake with endoscopy, and small-sized tube and potential conversion to sternotomy or thoracotomy may be considered and prepared for [10, 47].

Direct or indirect laryngoscopy is essential to determine the functional status of vocal cords preoperatively and is recommended in all cases as dysfunctions. RLNP are found in up to 1% without previous cervical surgery and may be clinically asymptomatic [17–21, 48, 49]. Prevalent unilateral RLNP will directly influence surgical decision making in reassessment of indication to surgery at a certain time, reconsideration of the planned extent of resection, as well as the surgical strategy with regard to definition of the dominant side to start with. Application of intraoperative continuous neuromonitoring (CIONM) is highly recommended for thyroid surgery in patients with preexisting contralateral RLNP. Moreover, postoperative airway management will be optimized when preoperative vocal cord dysfunction and intraoperative RLN functional assessment are recognized and measures to handle expected airway problems can be timely initiated [20].

Surgical Procedure of Thyroidectomy

Surgical Approach

Surgery for benign euthyroid MNG in suprasternal extension is traditionally performed by a Kocher incision. Minimal access thyroidectomy procedures are generally not recommended in typically larger-sized bilateral MNG and are not described here. In case of substernal extended MNG, CT or MRI imaging may be helpful to estimate the chance of a successful transcervical procedure [11, 50] (Fig. 1b–d). Partial unilateral extension below the sternal notch or clavicle in primary surgery will rarely require extension of the transcervical approach as patient's positioning with adequate reclination will already elevate part of the substernal portion of the thyroid. In larger proportions of substernal extension, measurement of the maximum diameter of the mediastinal or intrathoracic goiter and space in the narrowest

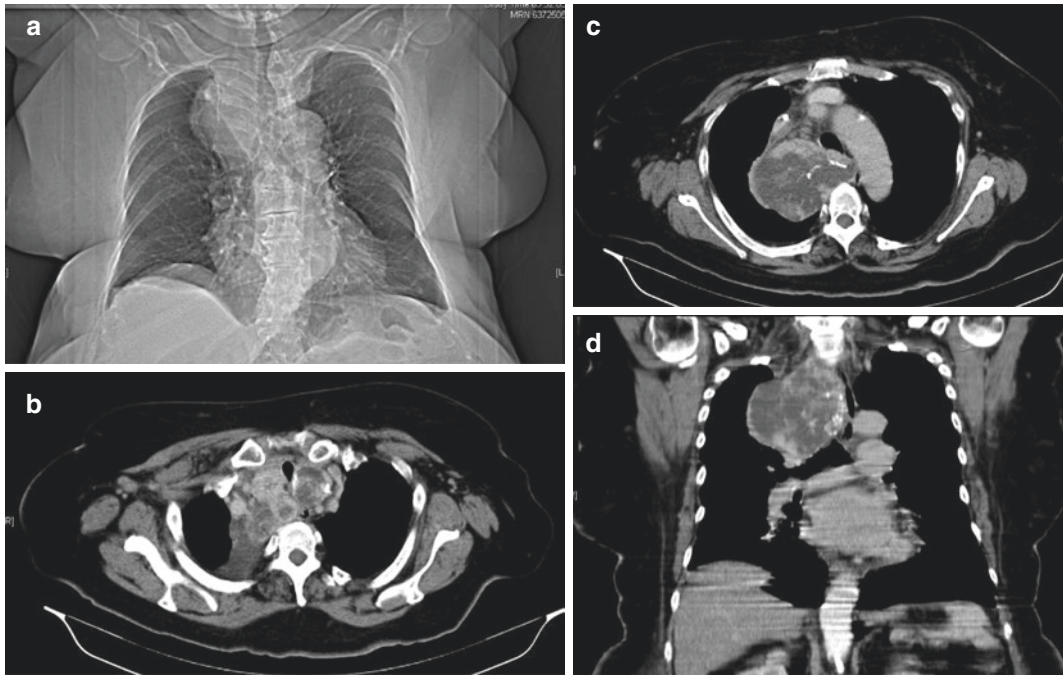

Fig. 1 Bilateral recurrent multinodular goiter with intrathoracic extension (female; 82 years; previous bilateral subtotal resection 37 years earlier) (**a**) X-ray of bilateral recurrent multinodular goiter with intrathoracic extension (**b**) CT of bilateral recurrent multinodular goiter with intrathoracic extension (**c**) CT of bilateral recurrent multinodular goiter with intrathoracic extension (**d**) CT of bilateral recurrent multinodular goiter with intrathoracic extension

area of thoracic outlet in the lateral view assist in estimation of success of the transcervical option [40, 42–44].

In MNG with bilateral multiple nodules, total thyroidectomy is recommended to eliminate all prevalent nodules as well as to preclude recurrence that is reported to be as high as 20–50% in MNG when subtotal thyroidectomy is performed [51, 52].

Technique of Thyroidectomy

Following induction and intubation, the patient is positioned on the table in the operating room with slight neck extension and surgical site disinfection and dressing. Incision for primary surgery with a Kocher incision is in length correlated with the goiter size and patient's individual constitutional factors, mostly between 4 and 5 cm, ideally placed in a natural neck groove and about 1–2 cm, superior to the sternal notch. The platysma is divided and subplatysmal preparation performed to mobilize the skin-platysma flap, usually extending cranially up to the larynx and caudally down to the sternal notch. Elevation fixation of the flap and a retractor installed below provide accessibility throughout surgery. Incision in the midline in full length with careful preservation of the anterior jugular veins whenever possible is performed. Retracting the strap muscles, approach to the dominant thyroid lobe is gained by careful dissection between the sternothyroid muscle and the thyroid capsule toward the carotid sheath. In IONM-guided surgery, identification of the vagus nerve (VN) with intermittent IONM or placement of an electrode to the VN for CIONM precedes further dissection [17–21]. Intact VN ION parameters prior to dissection of the ipsilateral thyroid lobe confirm intact RLN function and functional technique of the ION system in all cases with preoperative intact vocal cord function. VN ION

parameters represent the reference parameters for any ION changes or events throughout the preparation [22, 23]. The anatomical variation of a nonrecurrent laryngeal nerve (non-RLN) must always be expected when first operating on the right side, except in a full situs inversus where it may be encountered on the left side. Numerous variations of non-RLN courses may be found with different levels of joining the VN exposing the non-RLN at preparative risk in an early phase of the dissection (Fig. 2a). In application of ION, this variant becomes identifiable very early by the failure to retrieve an ION signal at the VN distal to the junction of the non-RLN with the VN. Thus, on the right side, early distal VN stimulation close to the thoracic outlet receiving no signal and positive VN signal at any level superior to this may be helpful to alert the surgeon to look for a non-RLN. A non-RLN is always associated with a lusorian artery that may be visualized with angio-CT or angio-MRI [53] (Fig. 2b). Proficient sonographers may also be able to identify the associated vascular variant preoperatively. Retraction of the thyroid lobe medially facilitates division of the middle thyroid vein, preceding identification of the RLN in the central compartment in caudal or cranial proximity of the inferior thyroid artery. Sometimes division of the inferior or superior thyroid pole vessels or even the isthmus may become necessary in order to identify and visualize the RLN for dissection in its course within the tracheoesophageal groove. It is of note that the course of the RLN differs significantly in angulation from the left and right side due to the course behind the subclavian artery on the right side and longer course behind the aorta on the left. Identification of the midline with the trachea offers ideal orientation to guide dissection in direction from the inferior to the superior pole as this will in the majority of cases allow the RLN to be visualized and kept unperturbed in its original position throughout the dissection. Careful dis-

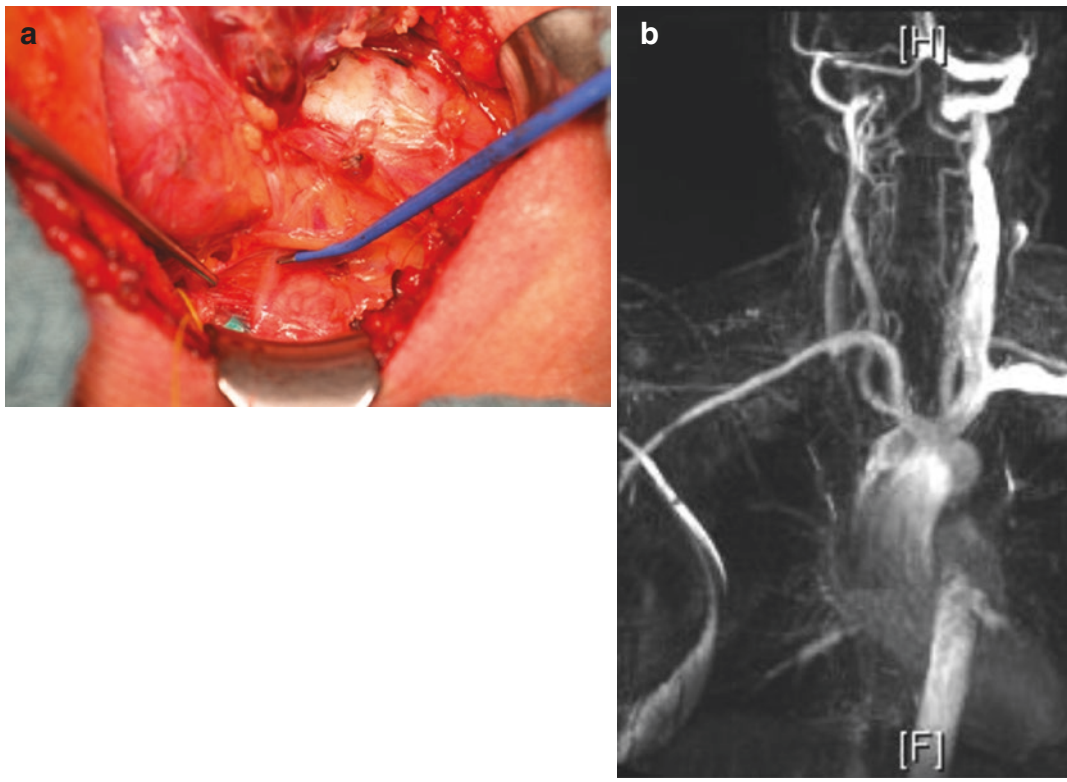

Fig. 2 Nonrecurrent inferior laryngeal nerve (**a**) Intraoperative steep course of nonrecurrent inferior laryngeal nerve (**b**) Corresponding angio-MRI of lusorian artery in nonrecurrent inferior laryngeal nerve

section precluding any bleeding provides identification of inferior and superior PG in their typical positions and safeguarding their delicate blood supply. Optional adjunctive fluorescent PG imaging is presently under investigation to enhance identification of PG and its blood supply to improve results of intact PG function in thyroid surgery [54]. Devascularized PG should be autotransplanted. This is best performed immediately by dissection of the affected PG whose particles are then positioned in a bloodless pocket, e.g. in the sternocleidoid muscle. Permanent sutures are used to close and mark the autotransplantation site. Recognition of thyroid tissue extending below the clavicle or sternal notch, possibly associated with the thyrothymic ligament, a tuberculum Zuckerkandl, as well as any pyramidal lobe, should be taken care of to prevent unnecessary sites of recurrence. At the junction of the RLN with the inferior thyroid artery, special care is taken to identify its course above or below the artery. In the presence of a tuberculum Zuckerkandl, the course of the RLN is oftentimes obscured, and ION with the handheld stimulation probe may assist identification of the course prior to dissection that is necessary to further visualize the RLN here. At this point, identification of the superior PG should be taken care of before proceeding cranially in order to preserve its vascularization that is mostly in close proximity to the thyroid gland. At the level of Berry's ligament, the RLN course may be exposed very close to the fibers or even in between; thus dissection in this area must also be carried out with clear recognition of the nerve's course. Prelaryngeal branching of the RLN into two or even three fascicles is frequent, and ION will predominantly only be positive for the anterior fascicle; however, all fascicles identified should be preserved as they may be important for the posticus muscle innervation affecting voice outcome [19, 20, 55] (Fig. 3). Division of the superior thyroid vessels in close proximity to the gland ensures preservation of the superior PG that should be previously identified. In this area, the external branch of the superior laryngeal nerve (EBSLN), important for voice projection, should be identified. Again, visual identification of the EBSLN may be assisted by

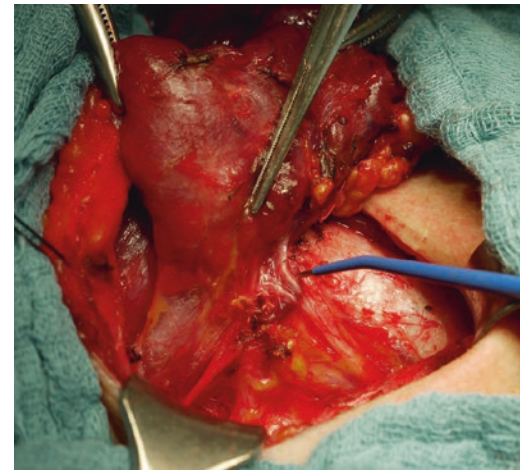

Fig. 3 Prelaryngeal bifascicular branching of the recurrent laryngeal nerve

ION that enables to find the nerve's course also when it is hidden from visualization within the cricothyroid musculature. Whenever the EBSLN courses exposed at or in between the superior thyroid vessels, dissecting clamps or sealing instruments must be taken care of positioning without affecting the EBSLN [56]. In case of preserved isthmus, the last step in thyroid lobectomy as described is mobilization and resection of the pyramidal lobe whenever present and dissection of the isthmus in proximity to the contralateral lobe. Closure after thyroidectomy is performed by readapting the midline of the strap muscles, the platysma, and skin and wound dressing.

In planned bilateral thyroid resection, preference of en bloc resection of the whole thyroid to lobectomy and optional frozen section evaluation of the first side resected must be made with regard to surgical consequence and underlying disease. In IONM-guided thyroid surgery, it is obligatory to validate intact VN function by ION or CIONM prior to proceeding to the contralateral side. Nonusers must be sure that functional integrity of the RLN was provided and need to rely on their experience in evaluation of RLN functional integrity before continuing to the contralateral side. In case of compromised RLN function identified by severe events or loss of signal (LOS) in ION or CIONM after the first side, it is highly recommended to refrain from operating the contralateral

side at this time. LOS and severe event in ION and CIONM are strongly associated with postoperative vocal cord dysfunction and at least transient RLNP [19–23]. In order to prevent potential bilateral vocal cord palsy (VCP) that oftentimes necessitates tracheostomy, a staged procedure is preferred allowing for safe surgery of the contralateral side when vocal cord function is fully restored. In case of permanent VCP and pressing indication to surgery of the contralateral side, this type of high-risk procedure is best planned in expert centers with application of CIONM and preparation of the postoperative phase, expecting possible airway management problems with reintubation, vocal cord lateralization, or tracheostomy [20].

Surgery for Recurrent Goiter

Surgery for recurrent goiter may be technically demanding and is associated with higher morbidity compared with primary surgery [1, 4, 51, 52]. Therefore, evaluation should assess indication to surgery especially carefully as not all recurrent goiters may require surgery. Mainly RLNP rate increases in series up to 3.6%, and permanent hypoparathyroidism rate increases to 3–10% [15, 16]. Postoperative hemorrhage and surgical site infections may be elevated due to generally prolonged duration of surgery in recurrent thyroid surgery [50, 57–59]. Essential difference is the preferred lateral approach to the thyroid, thereby avoiding the scarred plane of the midline approach between the sternothyroid muscle and the thyroid capsule where the course of the RLN may considerably be altered and obscured. In the lateral approach, the medial border of the sternocleidoid muscle is identified, and dissection starts here to identify the VN with or without ION or CIONM and proceeds to dissect toward the central compartment with the strap muscles. These are divided usually in the middle portion in oblique direction, and the thyroid capsule is then exposed. Stepwise dissection of the adjacent strap muscles from the thyroid capsule is often necessary to access the plane in which the RLN courses. ION or CIONM may help to identify any RLN running in the scar capsule alongside the thyroid lobe or obscured within scar tissue objectivizing its identification prior to dissective maneuvers. This may be enhanced by elevation of stimulation intensity up to 2 mA while looking for the RLN. Once the RLN is identified and visualized, return to stimulation with 1 mA is generally advised and sufficient. Identification of PG in recurrent thyroid surgery may also be challenging and sometimes be negative. In these cases, early postoperative determination of serum calcium and parathyroid hormone (PTH) enables timely and adequate substitution to prevent hypocalcemic symptoms.

Surgery for Hyperthyroid Thyroid Disease

Treatment of choice for hyperthyroidism is predominantly conservative. However, when medical treatment fails or hyperthyroidism needs to be corrected timely, surgery may become necessary. Mainly two forms of hyperthyroidism are referred to surgery, Graves' disease and autonomously functioning thyroid tissue without immunologic origin. While Graves's disease may be responsive to antithyroid drug (ATD) treatment and antibody production can be self-limited in the course, autonomously functioning follicular thyroid tissue is refractory, i.e., ATD is not curative, and may only serve to transiently convert a patient perioperatively into a euthyroid stage [38, 60, 61]. In autonomous follicular thyroid tissue, radioiodine therapy or surgery remains the curative treatment options. Generally, both forms of hyperthyroidism may present subclinical, defined by suppressed TSH, possibly elevated peripheral thyroid hormones, and without clinical signs of hyperthyroidism, or they may transform into overt hyperthyroidism revealing clinical symptoms like tachycardia, arrhythmia, tremor, nervousness, and hyperthermia besides the hyperthyroid laboratory. Degrees of severity may transform progressively from subclinical stage to thyrotoxicosis up to thyrotoxic crisis in which patients are vitally endangered and require intensive care treatment. Accordingly, therapeutic options are tailored alongside the urgency of elimination of the hyperthyroid state, with the

lightest stages of hyperthyroidism being treated mostly conservatively with ATD, to radioiodine ablation up to thyroidectomy, and ultimatively emergency thyroidectomy in case of thyrotoxic crisis.

Today, most surgical society's guidelines recommend total thyroidectomy as the preferred procedure to safely eliminate all hyperfunctioning thyroid tissue in multifocal autonomous follicular hyperthyroidism and to reliably eliminate all antibody triggering thyroid tissue in Graves' disease [5, 8–10]. Contrary, in case of localized areas of autonomous follicular hyperthyroidism, selective excision may be curative [51].

Although surgery in the euthyroid state is preferable, this may in instances not be achievable in a certain time frame, and surgery must then be performed in (overt) hyperthyroidism. The technique of thyroidectomy does not differ from the above detailed. However, complication rates of thyroid surgery for hyperthyroidism are reportedly elevated compared to procedures in euthyroid benign goiter. Pronouncedly permanent hypoparathyroidism in Graves' disease prevails at about 1.2–6% and 1–3.5% in autoimmune follicular hyperthyroidism when total thyroidectomy is performed. Permanent RLNP rates are reported to lie between 0.6 and 3.4%, while rates of postoperative hemorrhage are comparable with surgery for benign goiter [3, 16, 50, 57–59, 61].

Surgery for Thyroiditis

Various inflammatory thyroid diseases are integrated in the collective entity. Irrespective of the specific type of thyroiditis, surgery is infrequently indicated. These are most often development of thyroid nodules in underlying Hashimoto's thyroiditis for exclusion of malignancy and abscess formation in subacute de Quervain's thyroiditis or Riedel's thyroiditis [62, 63]. In active thyroiditis, thyroid tissue is mainly very firm, adjacent tissue may barely be separable, and planes may be obscured, posing patients potentially at an elevated surgical risk. In Hashimoto's thyroiditis, the number and distribution of suspective nodules determine the extent of surgery, ranging from selective nodule excision to total thyroidectomy. In subacute de Quervain's thyroiditis, surgery may only be indicated in abscess formation that is often associated with prior anti-inflammatory medical treatment and/or fine needle aspiration cytology [56] (Fig. 4a, b).

In case conservative treatment fails, open drainage of the abscess without aiming at extended thyroid tissue resection will be sufficient [62]. Surgery for Riedel's thyroiditis is only exceptionally indicated when conservative

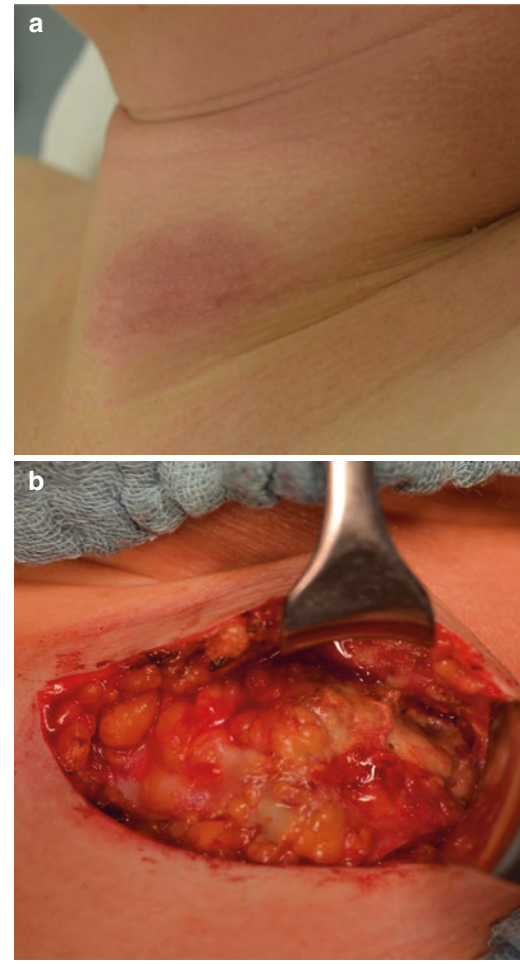

Fig. 4 Abscess in de Quervain's thyroiditis following corticoid medication and fine needle aspiration cytology (**a**) Clinical presentation of abscess in de Quervain's thyroiditis (**b**) Purulent finding at surgery of abscess in de Quervain's thyroiditis

treatment fails as it may impose the highest technical demand to a thyroid surgeon due to the multiple infiltrations and growth irrespective of neighboring structures or planes and with the highest risk of surgical complications. Moreover, even radical surgical treatment cannot reliably exclude local recurrence with bothersome scarce reserve of treatment options. However, in very experienced and specialized centers, successful surgical treatment may be achieved for Riedel's thyroiditis [63].

Surgery for Retrosternal Goiter

In addition to the above-stated aspects of assessment and planning of surgery in retrosternal goiter, there are some differences in strategic and technical aspects of thyroidectomy. In case the retrosternal extension of the goiter is not considerably compensated with patient positioning and a transcervical approach is estimated feasible, dissection should begin with the upper pole. The aim is to fully liberate the upper pole in order to pull up the retrosternal portion into the cervical region without having to extend the access [42, 44–46]. Dissection of the upper pole vessels with identification and preservation of the superior PG, and potentially the EBSLN, is followed by identification of the RLN at its entrance point into the larynx and/or the junction with the inferior thyroid artery. This is important as the course of the RLN may be endangered when pulling the retrosternal part of the goiter at the upper pole into the cervical region as this may stretch the RLN when it courses across or medially, while lateral RLN course rarely exposes the nerve to traction or stretching. This is crucial in recurrent goiter with retrosternal extension as the course of the RLN may be further altered by scarring or become fixed to the scarred tissue. In these cases, pulling may not be tolerated, and extension of the approach to partial or full sternotomy may become necessary [41, 43]. Again, this scenario of an altered nerve course is superiorly identified by CIONM when repetitive events as there are a decreasing of amplitude and increase in latency are observed. Whenever transcervical approach is unsuccessful or appears to be compromising RLN integrity, partial or full sternotomy is performed. Finalized cervical dissection performed prior to the sternal split allows for limited exposure time of the mediastinum during sternotomy and transsternal dissection time. Partial sternotomy may be sufficient to mobilize goiters that require more space in the narrowest space between the sternal notch and the trachea and is performed with an oscillating saw in close communication with the anesthesiologist to momentarily retain the lung and pause ventilation. Full-length sternotomy may be necessary for intrathoracic and mediastinal MNG with broader extension. Closure after sternotomy may be facilitated with firm resorbable sutures or metal. Thoracic drainage is obligatory whenever the pleura is opened in the procedure.

Surgical Complications of Thyroidectomy and Follow-Up

General surgical complications involve hemorrhage, wound infection, and thrombosis or embolism. Specifically postoperative hemorrhage after thyroid surgery is a high urgency event necessitating immediate surgical revision because of the imposing airway problem. Trained personnel and clear procedural instructions are essential in the postoperative observation. Hemorrhage after thyroidectomy occurs in the majority of cases within 4–6 h postoperatively; however, it may occur as late as 72 h postoperatively [64]. Surgical site infection after thyroidectomy is infrequent; however, conservative treatment with antibiotics may not be sufficient, and surgical revision with optional open wound treatment preceding secondary closure may be necessary. Thrombosis and embolism in association with thyroid surgery are rare events. Patients at risk need to be identified and prophylaxis initiated accordingly. Procedure-specific complications in surgery for benign MNG are RLNP and hypoparathyroidism. Both are clearly associated with the extent of resection; however, permanent rates of RLNP and hypoparathyroidism are low in the hands of

experienced specialized thyroid surgeons. Recent data from a major health-care insurance in Germany covering a broad spectrum of departments performing thyroid surgery reported permanent rates of RLNP of 1–3%, and 10–12% of hypoparathyroidism. RLN damage during thyroid surgery may be caused by direct injury with nerve transection, clamping, suture placement or clips, or thermal lesion, whereas indirect injury is mainly caused by traction of the nerve during mobilization of the thyroid lobe or thyroid mass. In expert hands, direct RLN injury is a rare event and indirect traction lesions dominate. In postoperative RLN dysfunction and anatomically preserved nerve integrity, potential of full recovery within 6 months up to 1 year is high. Permanent RLNP is defined when vocal cord dysfunction persists beyond 6 months, although exceptionally late recovery over 12 months or more postoperatively was reported. Besides various degrees of voice alteration, unilateral RLNP may also be coupled with swallowing dysfunction and symptomatic or silent aspiration [3, 15, 16, 50]. Laryngological evaluation of indication to specific therapy should be offered in these cases. In permanent RLNP, a variety of conservative, interventional, and surgical therapeutic measures are available in order to improve voice or respiration.

In postoperative hypoparathyroidism, severity of hypocalcaemia and of clinical symptoms may require prolonged hospitalization with intravenous calcium substitution or can be managed by oral calcium and/or vitamin D intake [15, 16]. In all cases, follow-up with control of calcium and PTH blood levels is recommended to enable weaning off substitution in time and to prevent overtreatment with risk of hypercalcemia as well as documenting the persisting need for substitution. Any hypoparathyroidism requiring calcium and/or vitamin D medication beyond 6 months postoperatively is defined as permanent, and perspective of potential functional PG restitution declines with the duration of treatment required.

Besides the potential complications, follow-up after thyroid surgery assesses remaining thyroid function or adequacy of thyroid substitution. In case less than total thyroidectomy was performed, recurrent goiter or remedial specific thyroid disease may also need to be included in follow-up assessment [51, 52].

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Part IV

Hypothyroidism

The Thyroid and Its Regulation by the TSHR: Evolution, Development, and Congenital Defects

Heiko Krude and Heike Biebermann

Background

Congenital hypothyroidism (CH) is the most frequent inborn defect of the endocrine system occurring in approx. 1 in 3000 newborns. In 90% the affected children suffer from developmental defects that lead either to the complete absence of the thyroid gland, to an ectopic gland somewhere between the normal position in front of the trachea and the origin at the base of the tongue, or to a hypoplastic thyroid tissue. In the other 10%, inborn defects of thyroid hormone synthesis and metabolism result in CH despite the presence of a morphologically normal gland. In all cases of primary congenital hypothyroidism, the lack of appropriate functional thyroid tissue leads to fetal and neonatal hypothyroidism that results in severe elevation of TSH, which is nowadays diagnosed within the first days of life by neonatal screening programs [1, 2]. Of utmost importance is the successful treatability of CH with L-thyroxine that started already in the 1880 – at that time with thyroid extracts – and was optimized since the 1970s by the introduction of newborn screening programs based on the description of better outcome in patients treated early after birth [3]. The implementation of TSH screening in 1974 [4] enabled a normal cognitive and somatic development and quality of life [5]. During the last three decades, the efforts to understand the pathogenesis of CH resulted mainly in the discovery of genetic defects only in the smaller group of patients suffering from thyroid hormone synthesis defects. Here, mutations in genes that are important for iodine transport, organification, storage, and recycling were described and allow now in 10% of patients with a genetic diagnosis that most recently can be done based on disease gene panel approaches [6]. At the same time, although the basic mechanisms of thyroid development were discovered, the molecular causes of the larger group of children with thyroid developmental defects remained elusive. Mutations in the TSH receptor (TSHR) [7–9] and subsequently in three different transcription factors, e.g., PAX8 [10], FOXE1 [11], and NKX2.1 gene [12, 13], that coordinate the complex steps of thyroid development were found in thyroid hypoplasia, athyrosis, and ectopy. Only in 5% of patients with thyroid dysgenesis mutations in these genes can be detected [14]. Due to the very particular finding of almost complete discordance of thyroid dysgenesis in monocygotic twins [15], it is expected that rather epigenetic then genetic defects represent the major molecular mechanism in thyroid dysgenesis, which need to be confirmed.

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One rare variant of CH is the central isolated absence of TSH, which in turn can be the result of a mutation in the TSH-beta gene itself or of a defect in normal pituitary development [16, 17]. While those very rare cases (app. 1 in 25,000) of central congenital hypothyroidism compromise the normal development of the affected newborn in the same way as the primary CH cases, their fate is in most cases depended on an early clinical diagnosis, because the lack of TSH is not detected in most newborn screening programs [18]. Severe hypothyroidism and the apparent lack of functional thyroid tissues in some patients with TSH-beta gene mutations, however, reflect the important role of TSH for the proper development and function of the human thyroid.

The Evolution of the Thyroid Gland

In evolution, the appearance of the first thyroid-like features goes back to the very first cells that reach the capability to incorporate and organify iodine. One of these still living species are the brown algal kelps that are very efficient to accumulate iodine from seawater and to store iodine in a tyrosine-bound simple structure, the monoiodotyrosine, MIT, and diiodotyrosine DIT [19, 20]. Still it is not entirely clear what physiological role iodinated tyrosines play in these algae. However, in the course of further evolution, thyroid hormones can be generated already in phyloplankton, and these iodinated tyrosines gain function as exogenous hormones that act on the metamorphosis of echinoderm species like sea urchins, which already express a functional receptor that interacts with thyroid hormones (TH) [21]. At this level of thyroid evolution, we can observe the transformation of an exogenous iodinated substrate into a hormone that fits into a receptor molecule that coevolved in parallel.

Besides this coevolution of TH and their receptor, the first obvious morphological example of thyroid precursor tissue is the so-called endostyle in the chordate species amphioxus and *Ciona intestinalis* [22] (Fig. 1). The endostyle represents just a group of cells in the endodermal inflow tract of these fascinating prevertebrate species. Here, we can already observe the main features of thyroid function: iodine accumulation by a transporter, organification by peroxidase, and transcriptional organization of this process by those genes that are known to play a pivotal role in thyroid cells through all animal species, pax and nkx genes [22]. One important function of TH in those species with an endostyle is – as also later in evolution in amphibians – the regulation of the maturation during metamorphosis (Fig. 1).

While the cells in the endostyle still accumulate iodine and perform the organification in the cell body, the next step during thyroid evolution was the development of thyroid follicles with the follicular lumen as the extracellular space for iodine storage and organification. One early example is the thyroid equivalent in the Agnatha species “sea lamprey” with the particular finding that before metamorphosis of this early vertebrate, an endostyle is built that changes after metamorphosis into a true thyroid follicle. All later fish species are living with single thyroid follicles lined along the anterior arteries as the anatomic equivalent of the thyroid in the pre-mammal phyla (Fig. 1). The thyroid as a gland developed late in evolution of tetrapods and can be found now in, e.g., frogs. The final step of positioning of the thyroid gland in the anterior neck occurred only in the mammalian development since in all pre-mammalian animals like the echidna or also birds the thyroid is positioned in the mediastinum close to the heart outflow tract [22]. Nevertheless, in all species with different levels of cellular organization of TH synthesis, the hormone itself and its biosynthesis with iodine accumulation via the sodium/iodide transporter (NIS) and peroxidase activity at tyrosine residues of the thyroglobulin matrix protein remained the same as well as the transcriptional regulation of thyroid cell development and function by pax and nkx transcription factors.

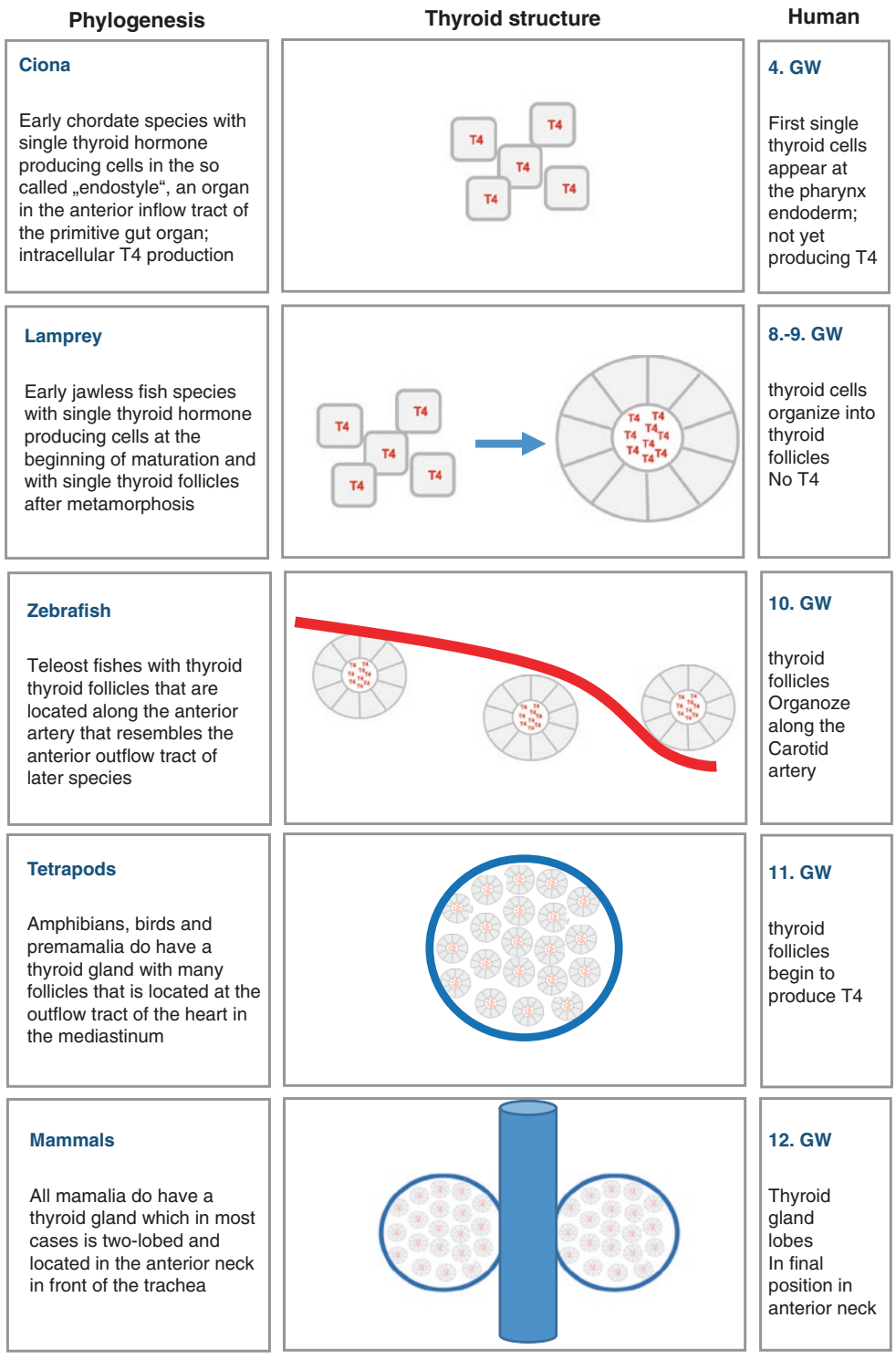

Fig. 1 *Overview of thyroid development during evolution and human organogenesis*. The different steps of thyroid development are shown in schematic drawings beginning with the single thyroid hormone producing cells in *Ciona intestinalis* via the occurrence of thyroid follicles in lamprey, thyroid follicles in fish, and the final thyroid gland in tetrapods and mammals. On the left side, the corresponding steps of human thyroid development during embryogenesis are depicted. However, the two processes are not completely in parallel but the major steps are the same. For more details and references, see main text

The Coevolution of TSH and the TSHR

In vertebrates the three glycoprotein hormones (GPH) FSH, LH, and TSH and their respective receptors are remarkably conserved. Their evolutional origin dates back to the first pair of glycoprotein hormone and its receptors in drosophila and continued via several rounds of gene duplication and gene losses. Today the living jawed vertebrates separated from a jawless vertebrate ancestor already 550 million years ago and the study of the GPHs in the jawless, agnathan species living still today, the sea lamprey and the hagfish, show that already in these species a pituitary GPH is present. However, this GPH is particular in that it contains an ancestral alpha subunit, the GPA2 ortholog of the mammal thyrostimulin A2 subunit. Together with the finding that in lamprey already two different GPH receptors are present, one with homology to the LH/FSH receptor and the other with homology to the TSHR, it seems possible that already in the jawless vertebrate precursor species an overlapping pituitary-gonadal and pituitary-thyroid axis existed [23]. Obviously, because in more distant vertebrates no pituitary equivalent is present, the lamprey is the first example of a functional interplay of a pituitary that is activated by brain releasing factors and transmits the brain signal into the body. Here the lamprey GPA2-GPHB heterodimeric pituitary GPH can in principal already act on a TSHR precursor on thyroid follicular cells and therefore represent the first active pituitary-thyroid axis [24].

Now, with the development of a TSH-like regulatory hormone, thyroid function could be integrated via the central nervous system (CNS) into higher physiological organization. In amphibians TSH is essential to regulate metamorphosis which is induced by, e.g., external seasonal signals that are recognized in the CNS. The two key developmental features of many species, the metamorphosis and reproductive maturation, are both controlled by thyroid hormones that are triggered by TSH [25]. Thus, TSH-regulated thyroid function gained already very early during evolution a central role for the main functions of survival.

While thyroid cells are active in many species without the stimulatory effect of TSH, later during evolution, thyroid function became more and more dependent on TSH action. Mice with a complete loss-of-function mutation in the TSHR are extremely hypothyroid and cannot survive [26], and human patients who are born without functional TSH due to a TSH-beta subunit mutation suffer from a severe form of hypothyroidism [16].

Interestingly, the appearance of a TSH-like molecule in jawless fishes coincides not only with the regulated function of the thyroid but in addition also with the appearance of the follicular structure of thyroid cells. For example, in lamprey a follicular structure of the thyroid cells is first seen only after metamorphosis. This structure is typical for all following species during evolution. In all more ancestral species, thyroid cells are not organized in follicles – and no pituitary-thyroid axis with a TSH-like GPH is present. Therefore, one can speculate that the TSH signal is one important prerequisite for the formation of thyroid follicles. This hypothesis of a key function of TSH for thyroid follicle development is underlined by recent findings in stem cell-based experiments. Several groups succeeded in triggering thyroid development from embryonic stem cells by the use of different growth factors or by expressing thyroid-specific transcription factors in stem cells. However, only the substitution of TSH into the cell media allows the formation of functional follicular structures [27, 28].

Development of the Human Thyroid Gland, TSH/TSHR System, and T4

During human embryogenesis, the development of the thyroid remarkably resembles the different steps of thyroid evolution. Like the endostyle of the early vertebrate *Ciona intestinalis*, the very first precursor cells of the human embryo occur at the anterior endoderm of the pharynx as a budding of endodermal cells. This first thyroid precursor develops already in the 4th week of gestation during gastrulation (Fig. 1). While in *Ciona intestinalis* thyroid development stops in that stage and these single endodermal cells of

the endostyle start to produce T4, in human development several weeks of additional organogenesis are following. From the 4th to 7th week of gestation, the thyroid precursor cells divide and aggregate in two lobes that are located to the final position in the anterior neck in front of the trachea. During these steps of early development, the transcription factors PAX8 and NKX2.1 are already expressed [29]. However, the thyroid precursor cells are still single cells that are not organized in follicular structures at these first weeks of organogenesis. In the final position which is reached in week 7, genes for thyroid hormone synthesis are already expressed like the peroxidase (TPO) and thyroglobulin (TG)-mRNA as well as the TSHR [30]. In accordance with the detailed work of Szinnai et al. [30], it seems to be the later expression of the sodium/iodide transporter (NIS) gene that enables the thyroid around week 11 to start with T4 production. At this time histological studies reveal the presence of thyroid follicles that express the TPO and TG at their luminal membranes [30]. It can be speculated that these rather late follicle developments coincide with rising TSH levels that are expressed and secreted around the same time from the fetal pituitary.

Fetal TSH and T4 values were measured in cord blood from 12th week of gestation. Interestingly already in the 12th week, TSH levels reached concentration of 4 mU/mL that is higher compared to the respective maternal values. Fetal TSH levels increase constantly until birth and reach the known higher levels of newborns compared to adults. T4 is at the beginning of week 12 which is still very low and develops with a time shift compared to TSH until birth. Together, the histological findings of Szinnai et al. [30] and the cord blood measurements of free and total T4 as well as free and total T3 by Thorpe-Beeston et al. [31] are consistent in the finding that after reaching its final position in the anterior neck in week 7, the increasing TSH is stimulating a follicular functional structure at week 11 that subsequently starts to produce T4 after week 12. Therefore, it seems obvious that during embryogenesis until week 12, the fetus development is influenced by very low T4 levels that are supplied by the maternal circulation (Figs. 1 and 2). Only after week 12 T4 levels increase significantly and are measurable in the cord blood [31] and those only from this time point on, an influence of the fetal T4 itself on the further development can be expected (Fig. 2).

Concerning further morphological thyroid maturation, fetal ultrasound data are available [32] that demonstrate the growth from gestational weeks 20–36. At week 20, when the overall histological structure, gene expression, and T4 pro-

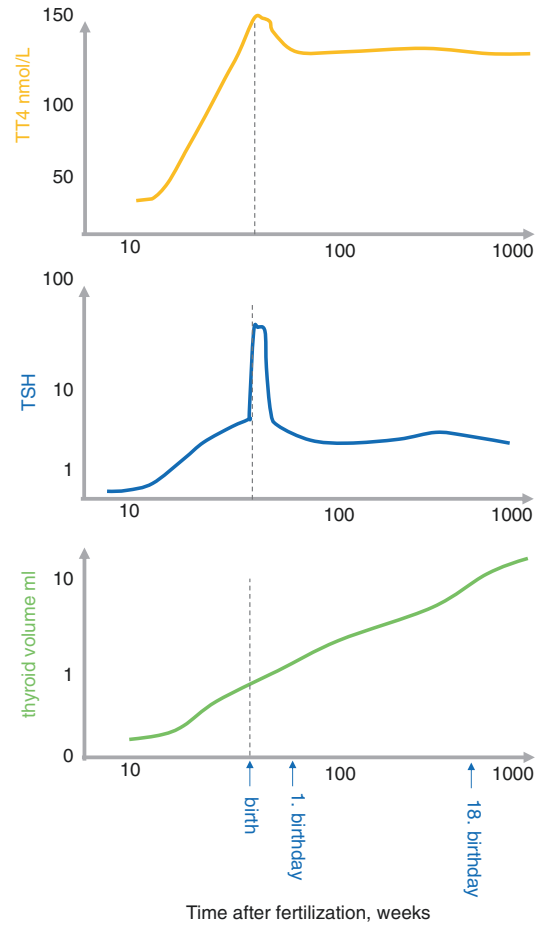

Fig. 2 *Functional and structural fetal development of human thyroid*. The human thyroid gland reaches its final organ structure and position already after the first trimester at the end of the 12th week of gestation. Thereafter T4 synthesis increases continuously in parallel with the increased TSH secretion from the pituitary and a constant increase of the thyroid volume. For more details and references, see main text

duction are already mature [30], the thyroid volume is still very small with only 0.08 mL (Fig. 2). Until birth the thyroid increases by a factor of 10 with the most significant increase in the last weeks of gestation to reach a newborn volume of 0.8 mL on average. The two data sets of fetal thyroid volume and fetal thyroid function very nicely correlate in that this documented increase of thyroid volume by Ho et al. [32] is in parallel with the increase of T4 production as shown by Thorpe-Beeston et al. [31].

However, after birth this parallel development does not pursue, and despite a further ten times increase of thyroid volume until adulthood, the T4 values decline after birth and are then kept constant until adulthood (Fig. 2). The postnatal development of TSH is particular because delivery leads to a sharp increase for 2 days up to 50 mU/L [33] (Fig. 2), and thereafter TSH declines to the normal levels of childhood which are still higher compared to adults with an upper normal value of 4.5–5.0 mU/L. These higher TSH normal values need to be kept in mind when interpreting thyroid function in childhood. Together these findings suggest that during fetal life thyroid function increases in parallel with thyroid volume but that after birth thyroid function in terms of serum T4 and T3 levels is regulated in a constant, individual range that is independent of thyroid volume and is most likely regulated by the TSH/TSHR system (Fig. 2). However, to reach the constant T4 and T3 concentration in the blood, the thyroid has to increase the daily secretion according to the increasing blood volume of the growing child.

Inherited Defects of T4 Synthesis (Dyshormonogenesis)

Following the first descriptions of "sporadic cretinism" by Osler in the late 1880s [34], it took more than 100 years until the molecular cause of the first patients with congenital hypothyroidism (CH) was discovered. Already in the nineteenth century, Osler included in a review several CH cases with severe goiter. In those children with an enlarged thyroid, researchers already expected a defect of thyroid hormone synthesis based on the biochemical studies of thyroid goitrous tissue in those patients who had surgery because of their goiter. After sequencing the human TG gene and TPO gene in the beginning of the 1990s [35, 36], it was in 1991 that in a goitrous CH patient a first mutation in TG was discovered [36] (Fig. 3). Here the familial occurrence of a goitrous CH in a consanguineous family was very suggestive for a recessive Mendelian inheritance of thyroid dyshormonogenesis. The availability of goitrous tissue in one family member enabled the investigation of the TG mRNA that led to the finding of a splice defect that causes the loss of exon 4 and subsequently to the discovery of a genomic C to G mutation affecting the exon 4 3′ splice site [36]. Only 1 year later, the same group reported about the first mutation in the human TPO that resulted in severe goitrous congenital hypothyroidism [35] (Fig. 3). Again this breakthrough was based on goitrous thyroid tissue from a CH patient after thyroidectomy in that biochemical studies strongly suggested a TPO defect and sequencing of the tissue-derived TPO mRNA revealed a homozygous frameshift mutation in exon 8 [35].

In the following 15 years, all further genetic defects of thyroid hormone synthesis known so far were discovered (Fig. 3): the defect of iodine transport in terms of the NIS gene (or SLC5A5) (three groups in 1997 [37–39]) and pendrin gene (or SLC26A4) mutations [40], defects of hydrogen peroxide generation [41] and DUOXA2 [42], and the defect of iodine recycling by the iodotyrosine deiodinase (IYD) (or DEHAL1) [43] (Fig. 3). While the complete loss of TPO or TG function in homozygous patients results in severe CH, the phenotype of the other defects of T4 synthesis is more variable and can be even transient in the case of DUOX2, DUOXA2, and IYD mutations. Due to the variable phenotypes of dyshormonogenesis and the low predictability which gene might be affected, more recently the molecular diagnosis is based on next-generation sequencing techniques that allow the parallel sequencing of all known candidate genes for CH. As demonstrated most recently by those "gene panel" diagnostic studies, up to 60% of CH

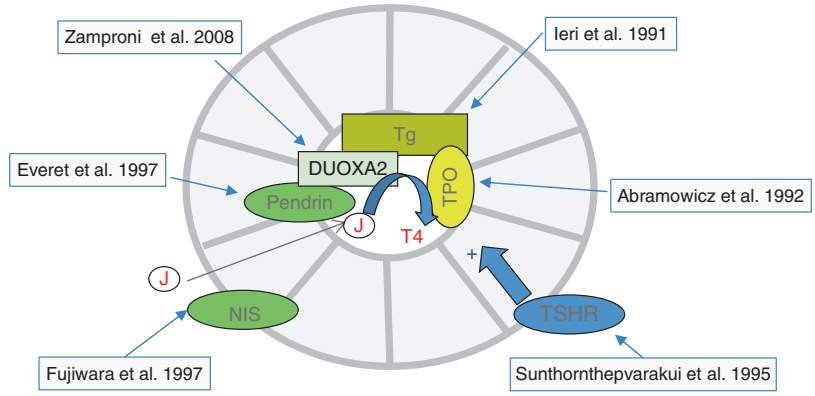

Fig. 3 *Gene defects of thyroid dyshormonogenesis*. Most mutations in the key proteins of human thyroid hormone synthesis leading to inherited thyroid dysfunction were identified already in the 1990s and which were depicted here in its sequence of discovery in clockwise order

patients with a normal or goitrous gland can be diagnosed in terms of a clear genetic defect [44]. In another 20% a likely but not proven defect can be diagnosed. Most mutations are present in the TG and TPO genes with some variations in the mutation rate depending on the genetic background [45]. The search for a causative mutation in CH cases with a thyroid gland in place is recommended by the most current guidelines [46] because the likely recurrence of another case in the affected families should guide the obligatory diagnostic procedure to detect early after birth a hypothyroid state especially in regions where a routine screening program is not available.

Nevertheless, it remains challenging to improve the genetic diagnostics of the presently unsolved 20% of cases of CH patients with a gland in place but no conclusive genetic defect. In those cases only one coding sequencing mutation or even no mutation was found so far. Given the likely genetic cause of CH in this patient group, genome sequencing strategies will detect noncoding sequence alterations in the TPO or TG gene as likely additional defects.

Defects of Thyroid Development (Thyroid Dysgenesis)

In contrast to the smaller group of CH patients with a thyroid in place – either goitrous or with a normal volume – the majority of 80–90% of CH newborns are diagnosed with alterations of thyroid morphology in terms of complete absence, hypoplastic or ectopic glands [14]. In those patients with "thyroid dysgenesis" (TD), very particular epidemiological features argue against a classical Mendelian inheritance: First, large cohort studies revealed sporadic occurrence in that only very few familial cases of TD (3–5%) were identified [47]. Second, the search for an environmental impact on the rate of TD – either geographical or climate or infectious disease factors – failed despite a very good epidemiological coverage in newborn screening programs [48]. And third, and most striking, the meta-analysis of available twin data revealed a complete discordance of TD in monocygotic twins in that only one child is affected, despite the same genome and the same maternal "environment" of both twins [49]. Together these data argue for a non-Mendelian and rather epigenetic than genetic defect in the majority of TD cases. So far, such an epigenetic molecular defect, either on the level of DNA methylation or chromatin modification, was not discovered yet.

Nevertheless, in a few children with CH due to TD, mutations could be identified on the basis of candidate gene approaches. In these studies transcription factor genes were investigated that were initially identified in mice to be expressed in the thyroid and be involved in the normal organogenesis of the thyroid gland. Consistent findings with an obvious phenotype of TD were diagnosed so far in three of those "thyroid transcription factors": NKX2.1, PAX 8, and FOXE1; remarkably all three defects were discovered in the same year, 1998 [10–12].

Patients with mutations in these three thyroid transcription factors are frequently affected by

additional symptoms and other malformations due to fact that the mutated transcription factors are not only relevant for the thyroid but also for other organ development, like the brain, the lung, and the kidney. Those more complex and severe phenotypes make the genetic diagnosis of this transcription factor mutations in terms of genetic counselling most relevant.

The NKX2.1 gene was already discovered in 1990 [50], and knockout mouse studies had revealed severe defects of the thyroid, lung, and brain morphology in the absence of the gene product [50]. The first mutation affecting a thyroid transcription factor was reported in April 1998 as a deletion in the gene locus of the NKX2.1 gene (former names TTF1 and TITF1) [12]. Four years later, the first missense mutation in Nkx2.1 was reported [13]. Accordingly, in patients with a heterozygous NKX2.1 deletions [51], severe pulmonary problems can be present after birth with only mild thyroid dysfunction and later on developmental delay, representing alterations of the lung, thyroid, and brain function, respectively. Further description of a growing number of affected children [51] depicted the detailed phenotype of this new disease: In all patients a movement defect is present, starting with muscular hypotonia in the first year of life and then followed by uncontrolled movements (reflecting the central role in the basal ganglia of the brain that coordinates movements). In around 50% a functional – in some patients only mild elevation of TSH – or structural thyroid defect is present, and in only 30% also the lung is affected, which can be very severe and lethal in the first days of life [51]. Therefore, it turned out that the NKX2.1 gene that already plays a central role in thyroid cell function in the endostyle of the very early species *Ciona intestinalis* – where no brain is evolved in evolution yet – turned out to be most important for the brain in humans and that the heterozygous loss of NKX2.1 function results in patients in a mainly neurological disease, rather than a thyroid disease.

However, the variable manifestation of heterozygous NKX2.1 loss-of-function mutations such as only minor symptoms in affected parents and severe phenotype in the offspring hampers an appropriate genetic counselling. Genetic diagnosis is mandatory in those families with a developmental delay in CH patients despite an optimal treatment with LT4 since nowadays treatment of CH leads to normal motor and mental development and a delay should point to a search for unusual genetic defects like the NKX2.1 mutations.

The PAX8 gene became a candidate gene for TD after its discovery as part of the pax gene family involved in the organogenesis of a variety of embryonic structures and the thyroid absence phenotype in Pax8 knockout mice [52]. The second thyroid transcription factor defect in TD patients was diagnosed in the PAX8 gene in May 1998 [52]. Identical to NKX2.1 patients, also in PAX8-deficient patients already, the loss of one allele leads to a phenotype with mild to severe thyroid dysgenesis that can be a lack of only one thyroid lobe with still normal thyroid function or an almost absence of functional thyroid tissue [10, 53, 54]. In a few patients, additional defects of the kidney were observed that were always unilateral with one still functional kidney and no clinical symptoms [55]. Although the PAX8 gene is also expressed in the midbrain-hindbrain region, so far no neurological symptoms in PAX8 heterozygous patients were diagnosed yet [54]. Therefore, due to the lack of renal and CNS symptoms, patients affected by PAX8 mutations can expect a normal life given that LT4 is started early and with an appropriate dose.

The third molecular defect in TD patients was discovered in patients with a syndrome that was clinically described already in 1989 by Bamforth and Lazarus [56]. Patients with this very rare "Bamforth-Lazarus syndrome" are affected by TD in association with cleft palate, curiously spiky hairs, and severe mental retardation despite treatment of CH. After cloning the second "thyroid transcription factor" by DiLauros group in 1997 [57], it turned out that this transcription factor belongs to the FOX gene group (FOXE1) and that knockout mice are affected by a cleft palate in addition to thyroid malformations [58]. Based on this particular association, the first FOXE1 mutation in patients with the Bamforth-Lazarus syndrome was reported in August 1998 [11], and it turned out that all other patients with this par-

ticular phenotype of TD, cleft palate, mental retardation, and spiky hairs harbor complete loss-of-function mutations in FOXE1 with a recessive inheritance [54]. Although the severity of the cleft palate phenotype is somehow variable, the clinical picture of FOXE1 deficiency is very consistent, and the clinical diagnosis should lead to an immediate genetic diagnosis of the FOXE1 gene. Not all patients with association of TD and cleft palate are harboring a FOXE1 mutation (personal observation); however, those patients had normal hairs.

TSHR and Its Inherited Defects

Besides the two so far described CH groups of "hormonodysgenesis" – due to mutations in genes that are important for T4 synthesis – and "thyroid dysgenesis," due to transcription factor mutations, a third group of patients with a defined defect in the regulation of thyroid development by loss-of-function mutations of the TSHR can be separated which are also affected by primary CH with elevated TSH and variable degrees of thyroid dysgenesis.

Consistent with the relatively late appearance of TSH during evolution – as described before in the lamprey – several animal studies in zebrafish and mice revealed a role for TSH during thyroid development rather late in the embryonic stages of follicle generation and not during early steps of thyroid initiation. The inactivation of the TSHR in zebrafish embryos resulted, for example, in a reduced number and size of the fish thyroid follicles but let the first budding and maturation intact [59, 60]. The results were very comparable in different mouse models of TSHR inactivation, either by the targeted knockout or in a spontaneous TSHR mutation mouse line (hyt/hyt). Detailed studies of thyroid development in these models did clearly show an unaltered initial organogenesis with a defect of later folliculogenesis, like in fish. However, in newborn mice without a functional TSHR, severe hypothyroidism is present that is lethal due to sucking weakness if the affected mice are not immediately substituted with T4 [26].

The first patients with inactivating TSH gene mutations were described already in 1995 [9]. Most interestingly and surprising in the light of the mice data, the phenotype was not CH but just an elevation of TSH with normal T4 and T3 [9]. The underlying TSHR gene mutations were functionally tested, and it turned out that one allele – inherited from the mother – still exhibited a partial function [9], those explaining consistently the difference to the more severe phenotype in mice without any TSHR function.

The first CH patient with TSHR mutations was reported 2 years later in 1997. At that time a girl with CH with reduced, but measurable T4 and a mildly hypoplastic thyroid gland was found to inherit two different loss-of-function mutations that were both differently affected in their functional capability to induce cAMP generation, one with complete loss of function and the other one with a 100 times reduced activity [61]. These two cases argued for an important role of the TSHR in thyroid function also in human physiology, but at the same time suggested that at least a minimal residual activity of one allele can compensate for a more severe hypothyroid state. A more severe thyroid phenotype with low T4 and an "apparent" athyreosis – but measurable thyroglobulin – was then reported in 1998 [62], again with two different TSHR mutations that both were argued to be functionally inactive due to an exon skipping and a frameshift but were not tested in vitro. The further more comprehensive screening for TSHR mutations in larger cohorts of CH patients revealed a very low rate of patients, who were mainly affected by thyroid hypoplasia or had a normal gland. An overview of all published inactivating TSHR mutations can be found at http://www.ssfa-gphr.de [63, 64]. The most recent mutation screening by next-generation sequencing in a large cohort of 384 Chinese patients with CH and isolated elevated TSH detected in 1.6% of CH and 4.2% of elevated TSH likely causative TSHR mutations [6], a number that is comparable to the mutation frequency in thyroid transcription factor genes in CH patients.

However, as already depicted in the Chinese study, so far more patients were diagnosed with

TSHR mutations that result in an isolated TSH elevation (with normal thyroid hormone levels and normal morphology) than in CH. Most likely, this more frequent "hyperthyrotropinemia" can be explained by the observation that in a heterozygous state loss of TSHR function can be compensated by elevated TSH levels with eventually normal T4 and T3 levels. More recently a large cohort of those individuals with "hyperthyrotropinemia" due to TSHR mutations was reported that exhibited over a long follow-up period of several years normal and stable T4 and T3 levels despite a very high TSH of more than 100 mU/L in some patients [65, 66]. Most important in terms of clinical relevance, in those individuals with a stable elevated TSH and normal T4 and T3, the authors argued against a treatment with levothyroxine but rather voted for a tight follow-up with yearly serum controls.

Most likely, the higher mutation frequency is related to the also dominant expression of the mutations as the Italian cohort has also presented patients with only one affected allele [67]. Here the phenotype of the patients is explained by a dominant-negative effect of the mutated TSHR allele on wild-type TSHR function [67].

To estimate the effect of identified mutations that cause CH or hyperthyrotropinemia, a general view on TSHR function is necessary (Fig. 4). The TSHR belongs to the large superfamily of G-protein-coupled receptors and to the subfamily of glycoprotein hormone receptors. The overall structure of the TSHR resembles a typical class A GPCR structure with exception of the large extracellular domain. The extracellular domain is important for ligand binding and is subdivided in different regions (Fig. 4). Thyrotropin binds to the extracellular N-terminal "leucine-rich repeat domain" (LRRD) constituted by around 250 amino acids and shows a typical and common structural fold for LRRDs [68], which was evidenced by solved TSHR LRRD crystal structures [69, 70]. In the concave site of the scythe blade-like-shaped LRRD [71], a complementary pattern of amino acid side-chain properties for hormone recognition and binding is provided (Fig. 4) [72]. The second extracellular N-terminal part is additionally important for hormone binding and signal transformation to the transmembrane serpentine domain (SD) [73]. Approximately 130 amino acids, this extracellular part connects the LRRD and the SD. This so far called hinge region can be subdivided into subregions according to the occurrence of two cysteine-rich fragments which are connected to each other by cysteine bridges [74]. These cysteine boxes are localized N- and C-terminally, and they are of high structural and functional importance at all glycoprotein hormone receptors (details reviewed in [75]).

The TSHR can activate different G-protein subtypes [76–79] and signaling pathways [80–82], whereby Gαs-induced signaling was long time thought to be the major signaling pathway [83]. However, recent few hints from pathogenic mutations and G-protein deletion studies in mice demonstrated that activation of the Gq/11 phospholipase C pathway is also important for thyroid growth and thyroid hormone synthesis [65, 84–86].

To date, more than 30 naturally occurring inactivating mutations are known. Despite several diverse mechanisms of inactivation, these mutations are often characterized by impaired basal constitutive signaling which is a key feature of the wild-type TSHR [87, 88]. Several structural regions and amino acids have been reported that might influence the level of endogenous basal signaling activity in glycoprotein hormone receptors. These were mainly identified by design and functional characterization of chimeric receptors or by testing pathogenic and site-directed side chain substitutions [74]. Within the intracellular loop 2 of the TSHR, mutations have been identified at several amino acids that significantly decrease the level of basal Gαs-related signaling, such as Phe525, Met527, Leu529, Asp530, and Arg531 [89, 90]. These mutations probably directly interrupt specific intracellular loop 2 interactions with the G-protein molecule. This finding of TSHR intracellular loop 2 is of special importance, because such a high sensitivity for regulation of basal activity was not found in systematic mutagenesis studies at the intracellular loop 1 or intracellular loop 3 [89, 91]. The specific contribution of the intracellular loop 2

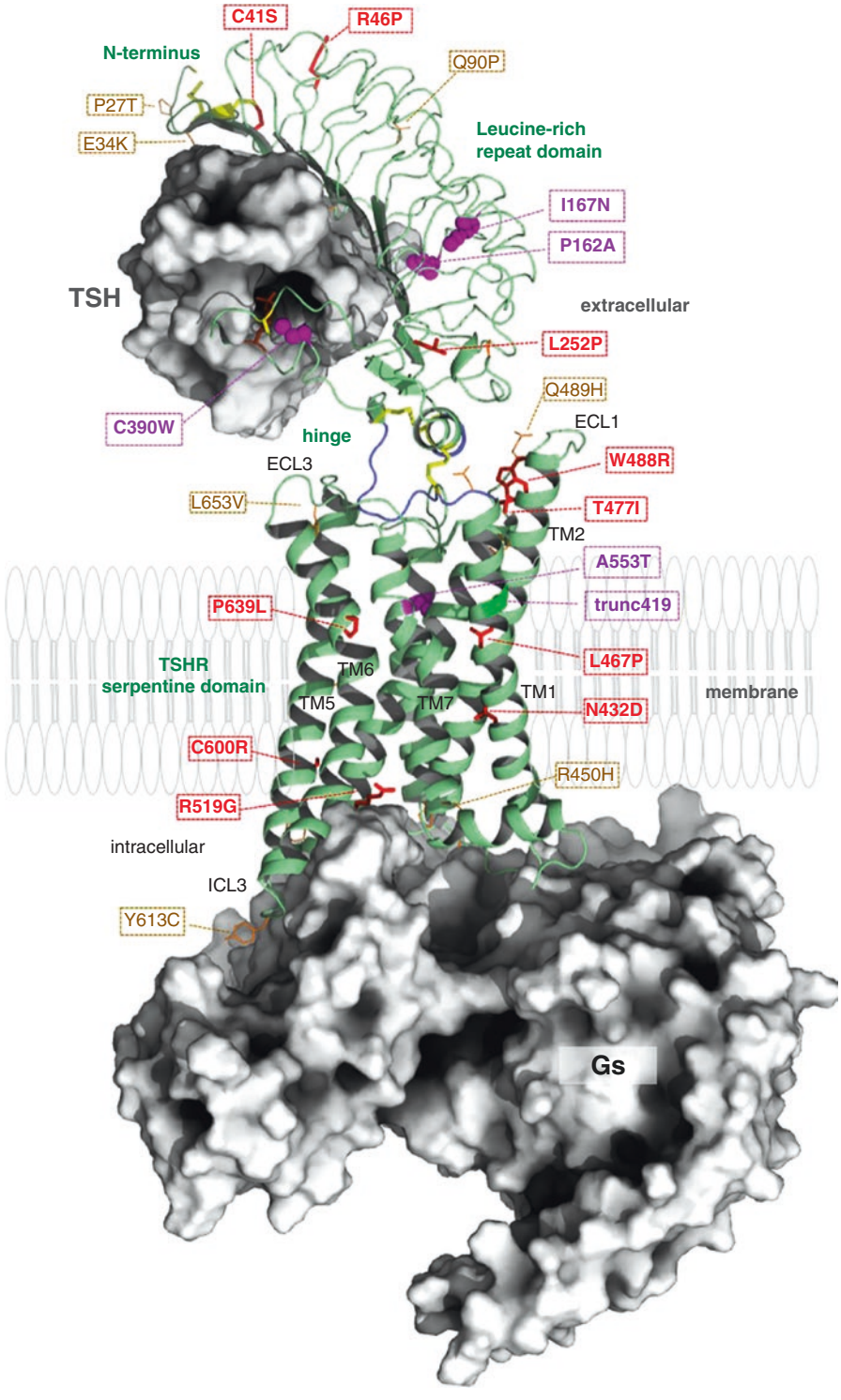

Fig. 4 *Structural active state model of the complex between TSHR, TSH, and Gs*. The leucine-rich repeat domain (LRRD) together with the hinge region constituting the extracellular receptor part of the TSHR, where the heterodimeric TSH (gray surface) binds. A tethered intramolecular agonistic fragment (blue backbone ribbon) is

(continued)

located between the extracellular loops and is comprised by amino acids from both C-terminal ends of the LRRD and the hinge region. Several disulfide bridges (yellow sticks) in the extracellular region stabilize the conformations and are additionally important for spatial adjustment of the receptor components relative to each other. Seven helices and their connecting loops arrange the serpentine domain, which spans the membrane from the extra- to the intracellular side. Here intracellular effectors can bind in an active receptor signaling state, like the heterotrimeric Gs protein (gray surface). Pathogenic inactivating mutations (orange lines, magenta spheres, red sticks) were identified at each receptor part (examples are shown and labeled) and in consequence can have differential functional impact in dependency on their particular spatial localization. They either interrupt the signal transduction path inside the protein, lead to protein misfolding, or hamper ligand binding or G-protein activation. Several of these substitutions lead to a complete loss of signaling (red sticks), whereby others (orange lines) are partially inactivating. Specific mutations are leading to truncated receptor variants, like at position 419 (green). All information were received from the GPHR information platform *SSFA-GPHR* (http://www.ssfa-gphr.de [63, 97]). This figure was kindly provided by Dr. Gunnar Kleinau, Institute of Experimental Pediatric Endocrinology, Charité University Medicine Berlin

for coupling of Gαs has been strongly supported by the β_2-adrenergic receptor structure determination (ADRB2) in complex with Gαs [92].

Furthermore, a naturally occurring mutation at TMH6, Pro639Leu, was recently identified [93]. This was the first described inactivating mutation at TSHR TMH6. Transmembrane helix 6 of TSHR is a hot spot for naturally occurring mutations, but only for such mutations leading to TSHR activation (see http://www.ssfa-gphr.de [64, 94]). It is well confirmed that the basal state of the TSHR is constrained by polar contacts between TMHs 2, 3, 6, and 7. Amino acids Asp460, Asn674, and Asp633 at these helices interacting by hydrophilic contacts to each other and several CAMs have been identified by mutagenesis studies or by naturally occurring mutations at these positions [95, 96].

Conclusion

In summary, the available data on the genetics of CH due to thyroid dysgenesis argue for a role of thyroid transcription factor mutations as well as of the TSHR gene that could in principle result in a wide spectrum of morphological alterations of the gland including the complete absence in, e.g., FOXE1-deficient patients. However, the frequency of all mutations together does not exceed 5% ([6] and own data), which leads again to the initially mentioned hypothesis that TD is rather an epigenetic than a genetic disease given the striking discordance of TD in monocygotic twins.

Nevertheless, irrespective of the underlying genetic or epigenetic cause, CH is nowadays a very efficient treatable disease, and in all patients – at least those who are not affected by the very rare syndromic variants – a normal development and life can be offered given that they are treated already within the first days of life and with an appropriate high dose of levothyroxine [46]. In light of the disastrous outcome of untreated CH patients – as documented in Osler's review in 1887 – this favorable outcome of CH patients makes clinical research for CH a big success story, although so far the molecular cause of the majority of patients remains elusive.

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Hypothyroidism

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Hypothyroidism is a state of decreased circulating thyroid hormones. As thyroid function is controlled by the hypothalamic-pituitary-thyroidal system, hypothyroidism may occur due to malfunction at any of these levels [1, 2]. When the production and secretion of hormones by the thyroid gland is impaired, the condition is *primary hypothyroidism*. When the defect is located on hypothalamic neurons or in the pituitary, it is *central hypothyroidism*.

Depending on its severity, hypothyroidism can be either subclinical or overt, ranging from mild to severe cases. In subclinical hypothyroidism, the serum TSH (thyroid-stimulating hormone) is above the normal reference range, but FT4 (free thyroxin) and T3 (triiodothyronine) are normal. Overt hypothyroidism is defined as a serum FT4/TT4 that is low in conjunction with an elevated TSH. The aim of this chapter is the discussion of overt hypothyroidism; subclinical hypothyroidism is discussed in a separate chapter.

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Epidemiology

Hypothyroidism is a common clinical situation in endocrine practice. Its prevalence varies among different surveys. The most extensive study that aimed to evaluate the spectrum of thyroid disease in a community was the Whickham Survey, a 20-year follow-up cohort of 2779 adults of Great Britain. In this cohort, the mean incidence of spontaneous hypothyroidism in women was 3.5/1000 survivors/year (2.8–4.5) and in men was 0.6/1000 survivors/year (0.3–1.2). Risk factors for the development of hypothyroidism were serum TSH at baseline (TSH >2.0 mIU/L), especially in the presence of antithyroid antibodies [3, 4]. A 13-year period cohort in Australia corroborates these data [5]. In this study individuals with serum TSH between 2.5 and 4 mIU/L had an increased risk of hypothyroidism. In the presence of thyroid peroxidase antibodies (TPOAb), the risk was higher in subjects with the highest TPOAb titers. In the United States, according to the National Health and Nutrition Examination Survey (NHANES III), the prevalence of hypothyroidism was 4.6% (0.3% overt and 4.3% subclinical) [6]. This national survey also demonstrated that hypothyroidism is more prevalent among women, increases with age, and is higher in whites and Mexican Americans than in blacks [6].

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Pathophysiology

The thyroid gland is connected to the central nervous system through a feedback control loop [2]. Within the hypothalamus and the pituitary, T4 is converted to T3, which acts on thyroid hormone receptors (TRs), located in the nucleus of the cells. In the paraventricular nucleus of the hypothalamus, T3 induces TRH (thyrotropin-releasing hormone) gene expression and synthesis of TRH, which binds to specific cell membrane receptors on the surface of thyrotrophs to stimulate the production and secretion of TSH (thyroid-stimulating hormone). T3 also induces directly TSH gene expression in the pituitary. TSH, in turn, acts via TSH receptors (TSHR) on the surface of thyroid cells to stimulate thyroid hormone synthesis and release.

The hypothalamic-pituitary-thyroidal system is complex and under the influence of many neurotransmitters and other hormones. Its main objective is to keep thyroid hormones in an optimal range. T4 is also converted to T3 peripherally in multiple tissues, including the liver, brain, and muscle.

When serum T4/T3 concentration is reduced due to a primary thyroid disease, TSH production is increased to reestablish the equilibrium; this is the basis for the diagnosis of *primary hypothyroidism*. If the hypothalamus or the pituitary is not able to respond, thyroid hormones will continue to be low, along with low or inappropriately normal levels of TSH; this condition is known as *central hypothyroidism*. It may occur as a consequence of anatomic or functional disorder of the pituitary gland (*secondary hypothyroidism*) or the hypothalamus (*tertiary hypothyroidism*) [7].

Etiology

Primary hypothyroidism is responsible for most of the cases of hypothyroidism in clinical practice. In iodine-sufficient geographical areas, the main cause of primary hypothyroidism in adults is chronic autoimmune thyroiditis (Hashimoto's thyroiditis), which causes progressive autoimmune infiltration of the thyroid gland and destruction of the thyrocytes. Clinically, it correlates with the presence of high titers of antiperoxidase antibodies (TPOAb) [8].

Post-thyroidectomy and post-ablation therapy with radioiodine for the treatment of Graves' disease are both common causes of hypothyroidism. External irradiation of the head and neck may lead to hypothyroidism typically after several years.

Iodine deficiency is a global public health problem that is being successfully overcome with World Health Organization strategies to improve iodine intake around the world. The recommended minimum dietary intake of iodine for adults is 150 μg/d [9]. The most universally used measure to control iodine deficiency is salt iodization. The overall global status of iodine deficiency has improved in the last decades, but iodine deficiency is still the most common cause of hypothyroidism on a global basis [10].

Many medications used for nonthyroidal diseases are known to cause hypothyroidism [11].

Amiodarone, a class III antiarrhythmic drug, is a classical example of a drug that impairs thyroid function [11, 12]. Its chemical structure is similar to thyroid hormones structure, containing 37% of iodine by weight. Hence, an average daily dose of 200 mg provides 74 mg of iodine, markedly higher than the required daily allowances of inorganic iodine. Amiodarone is an amphophilic drug with a large distribution volume and a half-life of at least 40–60 days. Additionally, it inhibits its type I deiodinase significantly. It can induce either hypo- or hyperfunction of the thyroid. In iodine-repleted countries, it more commonly causes hypothyroidism. Amiodarone-induced hypothyroidism is observed in 5–15% of patients on the medication.

Lithium, used in the long-term management of bipolar disorder, has many effects on thyroid physiology, but the underlying molecular mechanisms are not completely understood [13]. Lithium is concentrated by the thyroid 3–4 times more than in the plasma and may cause hypothyroidism due to inhibition of thyroid hormone synthesis and secretion. The prevalence of lithium-induced hypothyroidism ranges from 3 to

52%, depending on the population studied, duration of lithium therapy, and the laboratory evaluation [13].

The increasing use of tyrosine kinase inhibitors (TKI) for the treatment of malignant diseases has drawn attention to their effect on thyroid function [11, 14]. Since the first reports of hypothyroidism during sunitinib treatment, in 2006 [15], many cohort studies with different TKIs have been conducted. Sunitinib appears to induce hypothyroidism more frequently than other TKIs, probably because it targets a broad spectrum of tyrosine kinases; this action theoretically may induce thyroid ischemia via capillary regression and constriction. In addition, sunitinib and other TKIs may increase the metabolism of levothyroxine via an increased activity of type 3 deiodinase in peripheral tissue; additional mechanisms are also proposed. Consequently, patients receiving exogenous thyroid hormone replacement may need adjustments of levothyroxine dosages when using TKIs [14]. ANOTHER PARAGRAPH: Immune checkpoint-blocking antibodies are a promising class of drugs for cancer treatments. These drugs increase antitumor immunity by blocking intrinsic down-regulators of immunity, such as cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed cell death 1 (PD-1) and programmed cell death ligand 1 (PD-L1). However, they have been associated with several immune-related adverse events. In the endocrine system both hypothyroidism and hyperthyroidism have been reported, as well as other endocrinopathies, and these abnormalities may be permanent, or last at least as long as the patient is taking the medication. The underlying mechanisms are still unknown, but may include increased T-cell activity against antigens present in tumors and healthy tissues, increased levels of preexisting antibodies, increased levels of inflammatory cytokines, and enhanced complement-mediated inflammation. Examples of these agents include ipilimumab, nivolumab, pembrolizumab, atezolizumab, avelumab, durvalumab (PLEASE ADD A NEW CITATION HERE: Postow MA, Sidlow R, Hellmann MD. Immune-Related Adverse Events Associated with Immune Checkpoint Blockade. New England Journal of Medicine. 2018 Jan 11;378(2):158-68).

Interferon α (IFNα) and other cytokines may cause thyroid dysfunction through their effect on the immune system, such as the development of Hashimoto's thyroiditis, or the production of thyroid autoantibodies [16]. However, there are also non-autoimmune mechanisms that can lead to destructive thyroiditis and hypothyroidism.

Other causes are listed on Table 1.

Central hypothyroidism is characterized by a defect of thyroid hormone production due to insufficient stimulation of an otherwise normal thyroid gland [7]. Classically, this condition can be subdivided into *secondary*, due to pituitary diseases that affect the production or secretion of thyroid-stimulating hormone (TSH), or *tertiary*, due to presumed reduced release of thyrotropin-releasing hormone (TRH) from the hypothalamus. In prac-

Table 1 Causes of hypothyroidism

Primary
Chronic autoimmune thyroiditis (Hashimoto's thyroiditis)
Surgical excision of the thyroid gland
Radioiodine ablation
External irradiation of head and neck
Iodine deficiency (endemic goiter)
Drugs (amiodarone, lithium, tyrosine kinase inhibitors, immune checkpoint-blocking antibodies)
Interferons and cytokines
Thyroid infiltration (amyloidosis, hemochromatosis, sarcoidosis, Riedel struma)
Congenital causes
Central
Invasive or compressive lesions (pituitary macroadenomas, craniopharyngiomas)
Iatrogenic factors (cranial surgery or irradiation)
Injuries (head trauma, traumatic delivery)
Vascular accidents (pituitary apoplexy, postpartum pituitary necrosis)
Autoimmune diseases (lymphocytic hypophysitis, poliglandular autoimmune diseases)
Infiltrative lesions (hemochromatosis, sarcoidosis, histiocytosis X)
Inherited diseases (pituitary transcription factors defects, TSHβ or TRHR mutations)
Infected diseases (tuberculosis, mycosis, syphilis)
Resistance to thyroid hormone

tice, however, the result is a reduction in the release of biological active TSH. Central hypothyroidism is a rare clinical condition, accounting for only 0.1% of the cases of hypothyroid patients. Although isolated deficiency of TSH can occur, more often the patient will present other pituitary hormone deficiencies. The main causes are listed in Table 1, in order of frequency [7].

Resistance to thyroid hormone was first described in 1967 [17] and is characterized by reduced responsiveness of target tissues to thyroid hormone. In fact, it is not a state of decreased circulating thyroid hormones; serum T4 andT3 are usually normal or elevated, but the T3 receptor does not function normally [18]. A variety of molecular mechanisms are involved to explain the phenotypes already identified [19–21]. A new classification and nomenclature was proposed for inherited defects of thyroid hormone action, cell transport, and metabolism [22]. Resistance to thyroid hormone is in the differential diagnosis of inconsistent clinical and laboratorial findings.

Clinical Presentation

The clinical presentation of hypothyroidism is broad and reflects the lack of thyroid hormones at the tissue level. The natural history of the progression from euthyroidism to overt autoimmune hypothyroidism is a process that may take several years.

The magnitude of the symptoms depends on the pace that the hypothyroidism was established and the degree of biochemical hypothyroidism. After total thyroidectomy, a patient is generally more symptomatic than a patient that had been evolving with a chronic thyroiditis over years. Moreover, there is significant individual variation, so that some patients present few symptoms in spite of low levels of thyroid hormones, while others are highly symptomatic with less pronounced laboratory findings.

Hypothyroidism affects different organs in a variable manner. The clinical manifestation at each body system depends upon the level of thyroid hormone deficiency [23].

General and Psychological Symptoms

Fatigue, general weakness, and somnolence are nonspecific symptoms not readily recognizable as hypothyroidism [23]. Impairment of memory and attention worsens as thyroid hormone levels decrease. In moderate to severe deficiency, cognitive tests reveal recent memory loss and difficulties in performing calculations as well as reduced attention and slow reaction time [24]. Depression is frequently associated to hypothyroidism [25], but acute mania episodes have also been described [26].

Nervous System

Headache and paresthesias are common symptoms, especially in hypothyroidism after surgery [27]. In this case it is important to distinguish paresthesia due to hypocalcemia. Deafness is a very characteristic symptom, as well as vertigo.

An interesting sign of hypothyroidism is the delay in the relaxation phase of deep tendon reflexes, such as the ankle reflex [28]. It is easily performed during physical examination and correlates well with the degree of hypothyroidism.

Nutrition and Metabolism

In hypothyroidism, there is a slowing down of body metabolism, which can explain many symptoms and signs of this disease. Reduced thermogenesis and low metabolic rate correlates with cold intolerance. Appetite is decreased, but patients may observe a modest weight gain due to water and salt retention; obesity, however, is not caused by hypothyroidism. The turnover of protein, biosynthesis of fatty acids, and lipolysis is reduced [23]. Since thyroid hormone regulates cholesterol synthesis and degradation, total and LDL-cholesterol levels are increased, while HDL and triglycerides are normal or slightly increased [29]. There are usually no clinically relevant changes on fasting plasma glucose and fasting insulin levels.

Skin and Appendages

There is accumulation of glycosaminoglycans and proteins in the subcutaneous tissue that leads to non-pitting edema, so-called myxedema [27]. Eyelids are often edematous, and the eyebrows are sparse, especially in the lateral margin. The tongue is large and the lips are thick. Taken together, these signs, which are observed in severe hypothyroidism, were described as myxedematous facies.

The skin is thick, dry, and pale. The hair is dry and sparse, lacks shine, and grows slowly. The nails are thickened and brittle. Sweating is reduced [27].

Cardiovascular System

There is a vast list of cardiovascular manifestations of the hypothyroidism [23]. Pulse rate and cardiac output are decreased. Peripheral resistance is increased, leading to diastolic hypertension. In severe cases, congestive heart failure and cardiac hypertrophy may occur, as well as pleural and pericardial infusions. These abnormalities result in increased heart shadow and electrocardiographic changes, such as low voltage with conduction disturbances. Overall, there is decreased exercise tolerance. Angina may occur before or after thyroid hormone replacement therapy, and is related to structural lesion in the coronary arteries [30].

Respiratory System

Dyspnea is a frequent but nonspecific complaint in hypothyroid patients. Hypoventilation and hypercapnia are observed only in severe cases of hypothyroidism. Obstructive sleep apnea may coexist and be worsened by hypothyroidism due to soft tissue infiltration of the pharynx and altered regulatory control of pharyngeal muscles [31].

Gastrointestinal System

Constipation is the most common gastrointestinal symptom. Patients often complain of dyspepsia, gaseous distention, and nonspecific abdominal pain as a result of reduced gastrointestinal motility.

Hepatic metabolism is decreased and liver function tests are mildly deranged. Gallbladder motility is reduced, as well as bilirubin excretion, accounting for an increased risk of gallstones [32].

Renal Function

Serum creatinine levels are increased in about half of patients with hypothyroidism as a consequence of decreased renal plasma flow and decreased glomerular filtration rate. There is impaired renal excretion of water, increased total body water, and, occasionally, hyponatremia [33]. Renal manifestations, when observed, may be reversible with the treatment of hypothyroidism.

Musculoskeletal System

Muscle weakness, myalgia, and cramps are common features of hypothyroidism [27]. In adults, frequently there is no impact on bone health. In children, however, hypothyroidism leads to a characteristic epiphyseal dysgenesis, delayed linear bone growth, and short stature [23].

Arthralgias and joint stiffness may be present. Hypothyroidism must be ruled out in patients with carpal tunnel syndrome.

Hematopoietic System

Normocytic normochromic anemia often occurs in the setting of hypothyroidism [27]. Conversely, anemia is an important differential diagnosis of hypothyroidism because of fatigue and general weakness complaints.

Reproductive System

Hypothyroidism affects significantly the spermatogenesis, mainly the morphological parame-

ters [34]. Some men present with erectile dysfunction that is restored with the treatment of hypothyroidism [35].

In women, hypothyroidism is associated with different patterns of menstrual disorders that vary from oligo-amenorrhea to hypermenorrhea-menorrhagia, with reduced fertility [36]. When spontaneous pregnancy occurs, there is high risk of abortion and gestational complications.

In severe hypothyroidism, hyperprolactinemia may occur due to chronic TRH stimulation [23]. The resultant galactorrhea-amenorrhea syndrome contributes to further menstrual and fertile disorders.

In both genders, libido is reduced during hypothyroidism.

Endocrine System

In severe hypothyroidism, the adrenal cortex function is substantially compromised. The hypothalamic-pituitary-adrenal axis is impaired. Adrenal glands are often atrophic [37].

Primary autoimmune adrenal insufficiency may be found in association with primary autoimmune hypothyroidism, as part of polyglandular autoimmune syndromes.

Diagnosis

The diagnosis of hypothyroidism relies strongly on thyroid function tests that corroborate the symptoms and signs observed. On physical examination the thyroid may be normal, atrophic, or enlarged.

The single best test for the diagnosis of hypothyroidism is the TSH assay [38]. Combined with the clinical picture, this test defines the diagnosis and guides other complementary tests.

In primary hypothyroidism, the response of the hypothalamic-pituitary-thyroid axis is an increase in TSH secretion in order to stimulate thyroid function. In overt hypothyroidism TSH levels are elevated, usually over 10 mIU/L [39]. A simultaneous free T4 (FT4) test is decreased and further supports the diagnosis.

Healthcare providers must be aware of some pitfalls in the interpretation of TSH assays. There is a diurnal variation of up to 40% on specimens performed during the same time of the day [40]. This is not usually an issue as the TSH rise occurs mainly during the evening or night time hours. During the recovery phase from nonthyroidal illness, TSH levels may increase up to 20 mIU/L [41]. Heterophilic or interfering antibodies can falsely elevate serum TSH values [42].

In hypothyroidism, thyroid hormones are below the normal reference range or in its lower portion. Both T4 and T3 circulate bound to specific proteins in serum, mainly thyroxin-binding globulin (TBG). Many physiological, pathological, and pharmacological conditions alter T4 and T3 binding in serum. To avoid the interference of these factors, the assessment of FT4 has largely replaced the use of total hormones. The measurement of T3, either total or free, is less accurate for the diagnosis of hypothyroidism because of compensating mechanisms in peripheral tissues [43].

Anti-thyroglobulin antibodies (TgAb) and antiperoxidase antibodies (TPOAb) help to define the etiology. Higher TPOAb concentrations show higher risk of progression to overt hypothyroidism [5].

Special Situations

Pregnancy

During pregnancy, the signs and symptoms of hypothyroidism may be confusing. Some patients are asymptomatic or attribute their symptoms to normal pregnancy. According to a retrospective US cohort, hypothyroidism, especially following postsurgical excision or post-radioiodine ablation, is associated with obstetrical, labor, and delivery complications [44].

In the first half of gestation, overt hypothyroidism is associated with increased fetal loss, low birth weight, and congenital circulatory system and musculoskeletal malformations [45]. Severe maternal hypothyroidism, especially in the third trimester of pregnancy, may

be associated with intellectual impairment in the offspring [46]. Further studies are warranted.

Depression

Hypothyroidism must be ruled out in patients with depression. With the widespread use of TSH measurements, patients are treated while in the subclinical stage, but eventually overt hypothyroidism may develop. If unrecognized, hypothyroidism will complicate the course of depression and the efficacy of the specific treatment.

The treatment of hypothyroidism in depressed patients is essentially the same, with levothyroxine (LT4) replacement. However, recent studies have suggested that some patients may utilize combined T4/T3 therapy in order to restore mood and to achieve psychological well-being [47, 48]. This area is controversial and further studies are needed to clarify this subject.

Elderly

The prevalence of hypothyroidism increases with age [6]. In the elderly age group, the symptoms of hypothyroidism may be more subtle and confounded by the presence of comorbidities. Elderly individuals are more prone to myxedematous coma, a major complication of severe hypothyroidism [49].

The diagnosis of primary hypothyroidism relies on elevated TSH levels, but one must consider normal age related changes in hypothalamic-pituitary function, mainly for individuals over 80 years old. For every 10-year age increase after 30–39 years, the 97.5th centile of TSH increases by 0.3 mIU/L [50]. This means that some disease-free population may present TSH levels above the normal reference range derived from a younger population. It is important to recognize and to avoid mistreating this subpopulation.

Treatment, when appropriate, should begin with small doses of levothyroxine, adjusted gradually. Elderly are more susceptible to the adverse effects of thyroid hormone excess, such as atrial fibrillation and osteoporotic fractures. Although controversial at present, the target serum TSH should be between 4-6 mIU/L in persons greater than age 70-80 years [51].

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Central Hypothyroidism

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Introduction

A defect in thyroid hormone secretion due to an insufficient stimulation by thyrotropin (TSH) of a normal thyroid gland leads to the so-called central hypothyroidism (CH). This rare and heterogeneous disease is caused by anatomical and/or functional abnormalities of either pituitary gland (secondary hypothyroidism) or hypothalamus (tertiary hypothyroidism), and it may be congenital or acquired. The hypothyroid state is usually mild and diagnosis is made on the basis of the coexistence of defective thyroid hormone circulating levels and low/normal TSH levels. Similarly to what occurs in patient with primary hypothyroidism, CH treatment is based on L-thyroxine (L-T4) supplementation. Since TSH secretion is suppressed even during low-dose L-T4 treatment, circulating free thyroxine (FT4) levels should be measured to evaluate the adequacy of L-T4 treatment. This chapter will analyze our current understanding of the causes of CH as well as highlighting pitfalls in treatment and diagnosis.

Epidemiology

CH accounts for no more than 1 of 1000 hypothyroid patients, its prevalence being estimated to range from 1:20,000 to 1:80,000 in the general population (Prince et al. 2001). It can affect patients of all ages with no female prevalence, an observation in contrast to what is observed in primary hypothyroidism. As far as congenital CH is concerned, its prevalence depends on the screening strategy. In fact, TSH-based protocol used by most neonatal CH screening programs is effective in diagnosing only the primary hypothyroidism since CH is usually associated with inappropriately normal/low TSH in the presence of low FT4 circulating levels. When neonatal screening program for congenital hypothyroidism is based on the contemporary measurement of TSH and FT4, the prevalence is 1:160,000 [1, 2].

Finally, a study from the Netherlands demonstrated that a screening algorithm based on combined measurement of TSH, T4, and thyroxine-binding globulin results in a diagnosis of congenital CH in 1 in 16,000 newborns [3].

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Pathogenesis

CH is caused by anatomical and/or functional abnormalities of either pituitary gland (secondary hypothyroidism) or hypothalamus (tertiary hypothyroidism) though, in many instances, both pituitary and hypothalamus may be concomitantly affected. Moreover, CH may be congenital or acquired (Table 1). Defects in TSH secretion may be quantitative and/or qualitative according to the cause of the disease. For example, in patients with mutations of TSH *beta* gene, CH is caused by "abnormal" TSH molecules that lack part of the C-terminal amino acid sequence. Some of these TSH beta mutants are unable to heterodimerize with the *alpha* subunit and are therefore inactive [4]. Other mutations may form an incomplete heterodimer with preserved immunoreactivity in some TSH measurement methods but are completely devoid of normal bioactivity [5]. Conversely, in the majority of the acquired CH forms, TSH deficiency is related to a combined reduction in TSH-secreting cell number and an impaired secretion of bioactive TSH [6]. In this setting, immunoreactive TSH circulating levels may be normal or even slightly increased [7]. It is worth noting that the secretion of bioinactive TSH is often related to CH associate to an impairment of hypothalamic function (i.e., tertiary hypothyroidism). Indeed, previous studies from our laboratory showed that changes in TSH carbohydrate structures support the view that glycosylation modulates the expression of TSH biological activity [8, 9].

Congenital CH

Congenital CH may be classified as isolated or combined. The isolated CH is characterized by mutations affecting genes coding for TSH beta, TRH receptor, or immunoglobulin superfamily member 1 (IGSF1) [10].

In the majority of patients, congenital CH is associated to different pituitary hormone deficiencies, and some additional syndromic features may be present according to the genes coding for pituitary transcription factors, such as HESX1, LHX3, LHX4, SOX3, OTX2, PROP1, and POU1F1 [11] (Tables 1 and 2).

Table 1 Causes of central hypothyroidism

Acquired	
Invasive	Pituitary macroadenomas, craniopharyngiomas, meningiomas, gliomas, metastases, carotid aneurysms
Iatrogenic	Cranial surgery or irradiation, drugs (e.g., bexarotene)
Injury	Head traumas, traumatic delivery
Immunologic lesions	Lymphocytic hypophysitis
Infarction	Postpartum necrosis (Sheehan), pituitary apoplexy
Infiltrative lesions	Sarcoidosis, hemochromatosis, histiocytosis X
Infective lesions	Tuberculosis, syphilis, mycoses
Congenital	
Isolated	TSHB, TRHR, IGSF1
Combined	HESX1, LHX3, LHX4, SOX3, OTX2, PROP1, POU1F1

TSHB thyroid stimulating hormone β-subunit, *TRHR* thyrotropin-releasing hormone receptor, *IGSF1* immunoglobulin superfamily member 1 gene

Acquired

Neoplasia affecting the hypothalamus-pituitary region as well as therapeutic interventions on sellar and extrasellar tumor masses (i.e., surgery and radiotherapy) represents the most frequent cause of acquired CH. In particular, pituitary macroadenomas may induce hypopituitarism by affecting either pituitary cells or pituitary stalk. In this respect, nonfunctioning pituitary adenomas, PRL-secreting pituitary adenomas, and GH-secreting pituitary adenomas are the tumors more frequently involved. It has been demonstrated that at presentation, isolated or multiple pituitary deficits are diagnosed in 62% of patients with pituitary nonfunctioning macroadenomas, CH being found in 27% of them [12, 13]. Craniopharyngiomas are in general slowly growing extrasellar tumors, and the most common presenting clinical symptoms are visual field deficits and hypopituitarism. At presentation, GH deficiency is the most common pituitary deficit

Table 2 Clinical presentation of congenital forms of CH

Gene mutated	Pituitary hormone deficiencies	Other clinical features	Pituitary at MRI
TSHB	TSH	None	Enlarged/normal
TRHR	TSH, PRL	None	Normal
IGSF1	TSH, GH	Macroorchidism, increased body weight, hypoprolactinemia, and transient growth hormone deficiency	Normal
POUF1	GH, TSH, PRL	None	Variable hypoplasia
PROP1	GH, TSH, LH, FSH; ACTH (late)	None	Enlarged/normal/hypoplasia
HESX1	GH; TSH, LH/FSH, ACTH (late)	Septo-optic dysplasia	Hypoplasia
LHX3	GH, TSH, LH, FSH, PRL	Limited neck rotation, short cervical spine, sensorineural deafness	Enlarged/normal/hypoplasia
LHX4	GH, TSH, ACTH; LH/FSH (variable)	Cerebellar abnormalities	Hypoplasia
SOX3	GH, TSH, ACTH, LH, FSH	Mental retardation	Hypoplasia
OTX2	GH, TSH, ACTH, LH, FSH	Anophthalmy, retinal abnormalities	Normal/hypoplasia

diagnosed (up to 100% of patients), followed by TSH (up to 25% of patients) deficiency in children. In adults, growth hormone deficiency is reported in 80–90% of cases, and gonadotropin deficiency is present in 70% of patients, followed by TSH and ACTH deficiency (40% of patients each) [14–16]. According to published series, postsurgical hypopituitarism is diagnosed in the majority of patients with craniopharyngiomas, CH being reported in 40–95% of cases [15, 16].

Hypopituitarism can also result from direct and indirect irradiation of the hypothalamic-pituitary axis, the risk of the development of CH being related to both the biological effective dose given to the area and the total radiation dose delivered [17, 18]. Radiation-induced CH occurs in patients who undergo radiotherapy for pituitary tumors and craniopharyngiomas, but also in 10–50% of patients irradiated for nasopharyngeal or paranasal sinus tumors [19, 20] and in 12–65% of patients irradiated for brain tumors [21, 22]. Data on the long-term effect on pituitary function of newer methods of delivery of radiation (e.g., proton beam therapy, Leksell gamma knife, and stereotactic linear accelerator) are still scarce. However, recent findings suggest that hypopituitarism (including CH) occurs even after these new irradiation methods, the prevalence of this complication being possibly reduced [23].

Traumatic brain injuries represent another cause of hypopituitarism, the prevalence of anterior pituitary dysfunction ranging from 15 to 68% [24]. Interestingly, in this setting CH is diagnosed in up to 29% of patients [24]. Hypopituitarism may be also a rare consequence of cerebrovascular accidents (i.e., subarachnoid hemorrhage or infarcts) that have been found to be associated to CH in less than 2% of cases [25].

Granulomatous diseases (i.e., sarcoidosis, tuberculosis, and histiocytosis X) can induce CH by directly acting on the pituitary stalk. Hypopituitarism and CH are described also in all iron overload states (i.e., hemochromatosis, patients with β-thalassemia who need several blood transfusions) [26, 27].

Hypophysitis is a condition that is characterized by lymphocytic infiltration of the pituitary gland. On the basis of the histopathological picture, it can be classified as lymphocytic or granulomatous hypophysitis, xanthomatous, IgG4-related, and necrotizing hypophysitis being considered as rare variants [28]. Hypopituitarism is the most prevalent symptom of lymphocytic hypophysitis, CH being the pituitary hormone deficiency most frequently diagnosed after central hypoadrenalism [28]. The increasing use of anti-CTLA-4 antibody treatment (i.e., ipilimumab and tremelimumab) has resulted in a sig-

nificant increase in hypophysitis, reaching 10% of patients with cancer [29].

Finally, CH has been found in adult patients characterized by the development of GH, PRL, and TSH deficiencies and the presence of detectable circulating anti-PIT-1 antibodies, the so-called anti-PIT-1 antibody syndrome [30].

Clinical and Biochemical Presentation

The clinical features of acquired CH are usually milder than that observed in primary hypothyroidism, this discrepancy being mainly due to both the presence of a residual TSH pituitary reserve and the physiological constitutive activity of TSH receptor [31, 32]. Moreover, clinical manifestations of acquired CH are often masked by other coexistent pituitary deficiencies.

In congenital CH, various syndromic clinical features may be present depending on the gene involved (Table 2) [11]. Patients with loss-of-function TSH *beta* mutations are characterized by a severe central hypothyroidism, clinically undetectable at birth, biochemically associated with elevated glycoprotein hormone alpha subunit and an impaired TSH response to TRH administration. The prolactin secretion is normal and is fully responsive to TRH test [5]. Inactivating TRH receptor mutations lead to a CH characterized by the complete absence of TSH and PRL responses to TRH [33–35]. In first reported cases with biallelic *TRHR* mutations, associated clinical manifestations were mild (growth retardation, delayed bone age) despite biochemical evidence of CCH, with T_4 levels ranging from 40 to 88% of the lower limit of normal. Some bioactive TSH was produced, and there was apparently no attributable neurological deficit despite the late treatment initiation, suggesting sufficient childhood thyroid hormone production to prevent severe developmental delay. However, T_4 replacement did improve growth and quality of life in these individuals [33, 34]. Although TRHR is expressed on lactotrophs and mediates prolactin secretion in response to exogenous TRH, a female homozygote for p.R17* *TRHR* underwent two pregnancies and lactated normally, suggesting that TRHR is not obligatory for these functions in humans [33]. IGSF1 was recently identified as an X-linked cause of CH deficiency syndrome characterized by central hypothyroidism, increased body weight, macroorchidism, and in some cases, hypoprolactinemia and/or transient growth hormone (GH) deficiency [10, 36]. A subset of female carriers (about 18%) also exhibits CH. A delayed adrenarche, as a consequence of PRL deficiency, seems to be part of the clinical phenotype of patients with IGSF1 deficiency [37]. Adult male patients with IGSF1 deficiency exhibit mild deficits in attentional control on formal testing [38].

Biochemically, the diagnosis of CH is based on the finding of low FT4 in the presence of low/normal/slightly elevated TSH circulating levels [39], provided that factors interfering in the measurement methods have been ruled out (i.e., thyroid autoantibodies or abnormal binding proteins) [40]. Interestingly, a slight increase in immunoreactive TSH may be observed in patients with hypothalamic CH, this condition possibly misdiagnosing a condition of primary subclinical hypothyroidism [40]. The lack of a nocturnal TSH rise related to the presence of abnormalities in circadian TSH secretion may confirm the diagnosis of CH, though this evaluation can be performed in hospitalized patients only [41]. TRH testing may be useful to differentiate pituitary from hypothalamic CH, the first being characterized by an exaggerated/delayed and/or prolonged TSH response that is blunted in the latter [39]. However, since in acquired CH both pituitary and hypothalamus may be involved, the practical utility of TRH test is limited. However, an absent or impaired FT4 and FT3 responses, as measured at 120 and 180 min after TRH injection, indirectly indicate the secretion of TSH bioinactive. Since in normal subjects FT4 levels are characterized by a 10% variation over time, it has been suggested that a decrease in circulating FT4 larger than 20% is suggestive for CH in patients followed for pituitary diseases, even if FT4 concentrations still remain into the normal range [42].

Once that the biochemical diagnosis has been confirmed, a family history of CH, a suggestive clinical history (e.g., head trauma, subarachnoideal hemorrhage, previous brain irradiation, or surgery), or specific symptoms (e.g., headaches or visual field defects) should lead to the execution of a pituitary MRI and to a further evaluation of the other hypothalamic-pituitary axes.

Nonthyroidal illness is a relatively common finding following any acute or chronic illness (e.g., poor nutrition/starvation, sepsis, burns, malignancy, myocardial infarction, post-surgery, chronic liver, and renal disease) that leads to a condition biochemically similar to CH. This condition seems to be related to downregulation of TRH neurons in the paraventricular nucleus, to a reduction in TSH secretion, and to modifications in thyroid hormone metabolism in several target tissues [40]. It is important to be aware of this transient phenomenon and to correlate biochemical data to the clinical status of each patient in order to avoid inappropriate treatment.

Treatment and Follow-Up

Similarly to what occurs in patients with primary hypothyroidism, CH treatment should lead to the restoration and maintenance of euthyroidism. In this respect, standard L-thyroxine (L-T4) therapy is used in the majority of patients with CH since no evidences support the superiority of a combined treatment with L-T4 plus triiodothyronine in both adults and children [43–46].

Unlike for primary hypothyroidism, serum TSH levels cannot be used in the monitoring L-T4 therapy. In fact, it has been demonstrated that TSH secretion is suppressed even during low-dose L-T4 treatment, this finding being possibly related to the negative feedback of circulating hormones on the few residual thyrotropes [47, 48]. Interestingly, Ferretti et al. demonstrated that in 80% of CH patients, TSH is suppressed during LT4 treatment even though serum FT4 levels were still in the hypothyroid range, thus suggesting that the finding of normal serum TSH levels during LT4 treatment suggests a possible CH under treatment. Subsequently, it was clearly demonstrated that TSH levels above 1.0 mU/L might be considered as a sign of insufficient replacement in CH patients [48]. Nonetheless, several recent papers dealing with substitutive L-T4 therapy in patients with CH have underlined the pitfalls in achieving optimal replacement [49]. Koulouri and collaborators have approached the problem by using their Department's clinical information system to identify all patients with hypothalamic-pituitary lesions and divided them into high risk and low risk of CH [50]. They then compared FT4 values in these groups of patients with patients with primary hypothyroidism adequately treated with L-T4, i.e., those with normal levels of circulating TSH during replacement therapy. These authors concluded that CH patients are generally undertreated. Moreover, they suggest that levels of FT4 around 16 pmol/L (their laboratory reference range being 9–25 pmol/L) might represent an appropriate target in treated CH patients. Interestingly enough, this conclusion is quite similar to the one we reached in the past, i.e., to target FT4 values at the middle of the laboratory range of normal values.

In CH, free thyroid hormones should be measured to evaluate the adequacy of L-T4 treatment, low FT4 values suggesting undertreatment, and high FT3 levels possibly indicating a condition of overtreatment. Attention should be payed during the follow-up that blood for FT4/FT3 measurement is withdrawn before ingestion of daily L-T4 tablets. Serum FT4 levels laying in the middle-upper part of the normal range might represent an appropriate target in L-T4-treated CH patients [45, 47, 50, 51]. In this respect, it has been suggested that in the majority of treated CH patients, circulating levels of FT4 within the normal range are reached with a mean LT4 daily dose of 1.5 ± 0.3 and 1.6 ± 0.5 μg/kg bw, these doses being similar to those reported for primary hypothyroidism [42, 47]. Finally, the usefulness of biochemical indexes of thyroid hormone action at the tissue level (e.g., SHBG, cholesterol, Gla protein, BGP, and carboxyterminal telopeptide of type 1 collagen) in monitoring LT4 treatment in CH is limited by the fact that these parameters may be affected by alterations in adrenal, somatotrope, gonadal, or adrenal functions [42].

Table 3 Age-dependent L-T4 replacement therapy in children

Age	L-T4 dose
0–3 months	10–15 μg/kg/day
4–6 months	8–10 μg/kg/day
1–5 years	5–6 μg/kg/day
6–12 years	4–5 μg/kg/day
>12 years/puberty incomplete	2–3 μg/kg/day
>12 years/puberty complete	1.5–1.7 μg/kg/day

In adults L-T4 treatment should be started at low daily dosage and then gradually increased by 25 μg every 2–3 weeks in order to reach full replacement dose, the majority of patients reaching normal FT4 and FT3 levels with L-T4 daily dose ranging from 1.5 to 1.6 μg/kg bw, i.e., doses similar to those used in the treatment of primary hypothyroidism [39]. As commonly done in the presence of primary hypothyroidism, children with CH require L-T4 doses that are higher than those used in adults, progressively lower doses being required according to age (Table 3). It is worth noting that treatment should be started as early as possible and at full replacement doses in order to prevent serious damage of brain system.

In CH patients, concomitant estrogens or GH replacement therapies may require a significant increase in LT-4 doses to reach normal FT4 circulating levels [39]. The increase in L-T4 requirement observed during estrogen therapy [52] is possibly related to the transient increase of thyroxine-binding globulin levels that induces a reduction in FT4 bioavailability [53]. Subsequently, it is advisable to evaluate FT4 and FT3 circulating levels 6–8 weeks after the initiation of estrogen replacement therapy [52].

It has been demonstrated that GH deficiency per se may mask subclinical forms of CH that are diagnosable once rhGH has been initiated [54–58]. GH administration has been shown to enhance peripheral deiodination of T4 to T3 [59], and this effect on T4 metabolism is biologically relevant only in patients with combined pituitary hormone deficiencies and a partial impairment of thyrotrope function [55, 56, 58].

Finally, it is mandatory to exclude a concomitant condition of central adrenal insufficiency prior to L-T4 therapy initiation. In fact, euthyroidism restoration might precipitate an adrenal crisis in unrecognized central hypoadrenalism by increasing cortisol metabolism and glucocorticoid requirement. If adrenal function cannot be evaluated prior to L-T4 start, a prophylactic treatment with steroids (i.e., hydrocortisone or cortone acetate) should be started.

Summary

CH is a rare and heterogeneous disease caused by anatomical and/or functional abnormalities of either pituitary gland or hypothalamus, and it may be congenital or acquired. Despite the increase in the knowledge of CH causes, several CH cases classified as idiopathic remain unexplained. This is true for some familial CH forms as well as for CH-acquired cases possibly related to specific antithyrotrope antibodies.

Clinical presentation is usually mild, and diagnosis is made on the basis of the coexistence of low thyroid hormone circulating levels and low/normal/slightly elevated TSH levels. CH treatment is based on L-T4 supplementation, free thyroxine levels being measured before blood withdrawal in order to evaluate the adequacy of the treatment. In this respect, it is advisable to reach FT4 levels laying in the middle-upper part of the normal range. However, further studies are needed to better understand thyroid hormone metabolism and action at the tissue level, whose results will provide more specific markers for a more precise tailoring of replacement therapy.

In managing CH patients, it should be taken into consideration the possible interplay between CH treatment and possibly coexistent pituitary hormone deficiencies. In particular, it is mandatory to exclude a concomitant condition of central adrenal insufficiency prior to L-T4 therapy initiation.

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Subclinical Hypothyroidism

Bernadette Biondi and Leonidas H. Duntas

Introduction

The term "subclinical hypothyroidism" (SCH) has classically been used to define that condition in which increased serum thyroid-stimulating hormone (TSH) levels are accompanied by thyroid hormone serum concentrations within the normal population-based reference range [1]. However, SCH (as is also subclinical hyperthyroidism for analogous reasons) is today considered to be a misnomer. This is because, inter alia, it does not include instances of thyroid hormone deficiency, nor does it account for the observed phenomenon of slow disease shifting by degrees from minimal to mild and eventually to manifested disease that peaks with aging and in which there is a clear predominance of women compared with men [2, 3]. Thus, SCH is usually stratified according to the serum TSH level into mild, moderate, or severe [Table 1]. Such distinctions are critical since adults, especially the elderly, suffering from SCH are at greater risk of coronary heart disease, heart failure, and cardiovascular mortality, while conflicting data have been reported as to the possible association between SCH and cognitive impairment, depression, and the risk of fractures [4].

Table 1 Stratification of subclinical hypothyroidism according to serum TSH

Condition	TSH mIU/L
Mild	4–6
Moderate	6–10
Severe	Above 10

This review aims to assess the current evidence on the clinical aspects of the disorder, associations with other clinical conditions and diseases, as well as more recent developments in treatment recommendations in adults with SCH.

Prevalence, Diagnosis, and Etiology

The population prevalence of SCH is about 10%, rising to 18%–22% in the elderly [5, 6]. In the classic Whickham study including 2779 persons conducted in the United Kingdom in 1977, SCH, defined as having a TSH > 6 mU/L, was reported in 5.0% of individuals older than 18 years [7]. The diagnosis of SCH is mainly based on a TSH measurement, a laboratory test that has been considerably refined over the last 25 years, achieving ever greater sensitivity and reliability in measuring very low as well as high TSH levels [8].

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Importantly, TSH concentration constitutes the most powerful predictor for the outcome of spontaneous SCH in patients over 55 years, as these subjects, who present with low serum TSH, exhibit a low incidence of overt hypothyroidism (OH) [9]. The Cardiovascular Health Study performed at the University Clinic of Pennsylvania estimated the persistence and progression of SCH over a four-year period in 459 individuals aged 65 years and older not taking thyroid medication [10]. A TSH of above 10 mIU/L was independently associated with progression to OH, while transitions between euthyroidism and SCH were common but not affected by age and gender at 2 and 4 years [10]. It is hence evident that since a high serum TSH may often be spontaneously reversed, a confirmation of TSH values 6–8 weeks later, which is necessary also to exclude laboratory fluctuations, is mandatory prior to treatment decision. Serum TSH and free T4 (FT4) exhibit a substantial variability among healthy persons, although the range of variability within an individual healthy person is relatively narrow: this is an important observation pointing to a unique set point of the hypothalamic-pituitary-thyroid axis for each individual [11, 12]. Moreover, the latter finding explains why a TSH level of 10 mIU/L can be accompanied by a normal FT4 level in one person but declined in another.

The most common cause of SCH is chronic autoimmune thyroiditis (CAIT) associated with antithyroid peroxidase (TPOAb) and/or anti-thyroglobulin antibodies (TgAB) (Hashimoto's thyroiditis) [11]. A distinct ethnic difference has been reported, with higher prevalence found among Caucasians and Mexican Americans, while particularly black and mulatto people are seen to be less prone to develop SCH [13, 14].

It is of note that an inverse, statistically significant association between current alcohol consumption and progression of SCH, due to CAIT, to overt hypothyroidism has been reported [15]. It was thus proposed that alcohol intake (about 11–20 units a week) has a protective effect on progression of SCH. There was no association with the type of alcohol consumed, e.g., wine vs. beer, gender, or region of residence [15]. The mechanisms of interaction between alcohol and the immune system are complicated, involving a diversity of immune responses, such as loss of natural killer cell activity, changes in cytokine production, and even alterations, in cases of large alcohol intake, in both Th1- and Th2-mediated immunity [16]. Among modifiable factors that may have an impact on thyroid autoimmunity, smoking appears to be negatively associated with both thyroid autoimmunity and hypothyroidism and positively associated with mild TSH decreases [17]. Smoke exposure was associated with 200% greater odds of low normal TSH 0.1–0.4 mU/L.

Excess iodine intake, i.e., ≥ 300 μg/daily, may unmask CAIT and promote hypothyroidism, particularly in areas with mild and moderate iodine deficiency [18].

Cognition, Depression, and Quality of Life

There have been conflicting results regarding the association between SCH and cognition and quality of life (QOL), though recent naturalistic studies did not observe any significant relationship [19]. Nevertheless, a reduction in QOL is frequently reported in patients with thyroid autoimmune diseases regardless of thyroid dysfunction and despite the fact that thyroid peroxidase (TPO) antibodies have, moreover, been positively associated with trait markers of depression [19]. Hypothyroidism has been linked to depression, as it may trigger affective disease and psychotic disorders. Meanwhile, depressive patients have a higher frequency of hypothyroidism, and patients with hypothyroidism have a higher occurrence of depressive syndrome [20]. Hypothyroidism can cause structural abnormalities of the hippocampus through altering (sometimes seriously) blood flow and glucose metabolism in the brain, changes which can affect memory performance. On the other hand, the HUNT study reported a protective effect of SCH for anxiety disorder, i.e., up to 19% fewer anxiety cases per 1 mU/L increase in TSH in men and a trend toward less anxiety in women [21]. By contrast, in females

on LT4 treatment, TSH was positively associated with both depression and anxiety [21]. It is hence evident that the relationship between thyroid function and depression is not as yet well defined [22], nor has it been definitively established whether thyroid hormone replacement therapy is effective in treating SCH-associated neurobehavioral impairments [23].

In the elderly SCH does not tend to be associated with depression, although advanced SCH may elevate the risk of depressive syndrome [24, 25].

Clinical Manifestations and Associations with Cardiovascular Disease

Individuals with SCH are often asymptomatic, although clinical manifestations including nonspecific complaints or symptoms similar to those registered in overt hypothyroidism, such as fatigue, weakness, weight gain, cold intolerance, and constipation, may be revealed following a careful clinical examination. Furthermore, despite the fact that SCH has been associated with atherosclerotic cardiovascular disease (CVD), it remains uncertain whether SCH represents a higher risk for CVD, since dyslipidemia in elderly people is considered a common biochemical condition. Importantly, though a 20-year follow-up of 2779 participants in the Whickham study did not reveal any association between thyroid autoimmunity and CVD [26], a reanalysis of these study data exclusively defining SCH by TSH measurement did disclose an adverse effect of SCH on CVD rate and mortality [27], while an increase in CVD, including both major forms of heart disease and coronary heart disease (CHD), has been reported in various longitudinal and cohort studies [28]. In the Busselton Health Study, Western Australia, serum TSH and FT4 concentrations were measured in 2108 archived serum samples [29]. In this cross-sectional study in which the prevalence of CHD was observed in persons with and without subclinical thyroid disease, it was found that subjects with SCH had a significantly higher prevalence of CHD than euthyroid subjects. In the longitudinal part of the study, the risk of cardiovascular mortality and CHD events, both fatal and nonfatal combined, were examined: here it was found that individuals with SCH had 21 cardiovascular deaths compared with 9.5 expected (age- and sex-adjusted hazard ratio, 1.5:95%) and 33 CHD events compared with 14.7 expected [29]. The heightened risk for CHD events remained significant after adjustment for standard CV risk factors, suggesting that SCH may be an independent risk factor for CHD [29]. However, in a meta-analysis, no difference was detected in CHD prevalence in SCH patients over 70 years old when compared with euthyroid persons, although a higher incidence of congestive heart failure [HF] was recorded in those patients with SCH having a TSH >7.0 mU/L [30]. In another meta-analysis investigating whether SCH is associated with both prevalence and incidence of augmented ischemic heart disease (IHD) and cardiovascular mortality, a higher IHD incidence and prevalence as well as cardiovascular mortality were clearly observed in SCH subjects compared with euthyroid individuals younger than 65 years, but not in subjects older than 65 years [31]. These data suggest that increased vascular risk may be present only in younger individuals with SCH. Partially in line with these findings was a classical longitudinal study conducted in Leiden, the Netherlands, with the participation of 85-year-olds, which revealed that a higher TSH may well be protective against CVD in the very old [32]. Of note, CHD risk associated with SCH did not differ by TPOAb status, indicating that biomarkers of thyroid autoimmunity do not have any impact on CHD outcome [33]. The importance of age in the relationship between SCH and CHD as well as in the cardiovascular outcome of patients with SCH is extensively discussed in a recent analysis by Cooper and Biondi [34].

Recently it was demonstrated that thyroid-stimulating hormone receptors (TSHRs) are expressed in cardiomyocytes and that TSH may downregulate sarcoplasmic reticulum calcium ATPase (SERCA2a) activity and expression in neonatal rat cardiomyocytes [35]. It is hypothesized that TSH, by binding to TSHRs in cardiomyocyte membranes, possibly inhibits the

protein kinase A/phospholamban (PKA/PLN) signaling pathway in these cells [35], this denoting that high serum TSH levels could well influence cardiovascular system function and act as an independent risk factor in systolic and diastolic heart dysfunction in hypothyroidism.

SCH and Associations with Lipids

Serum lipid levels are influenced by thyroid status, and there is evidence pointing to a link between SCH and an unfavorable lipid profile [36]. Among SCH patients, considerably altered cholesterol and lipoprotein metabolism have been noted, especially in those cases where serum TSH levels are over 10 mIU/L. The irregularities include elevated plasma levels of total cholesterol (TC) as well as of low-density lipoprotein cholesterol (LDL-C); meanwhile, the observed altered ratios of TC/high-density lipoprotein cholesterol (HDL-C) and LDL-C/HDL-C strongly indicate a potentially greater risk for CVD [37]. SCH impact on lipids has been shown to be inconsistent: it appears to be directly related to the degree of TSH elevation, becoming more pronounced as SCH progresses to OH, this leading to enhanced propensity to atherosclerosis. The elevated LDL-C levels characterizing hypothyroidism decrease the expression of LDL mRNA and LDL receptors numerically while reducing the binding of LDL-C to its receptor, resulting in increased half-life of LDL-C and diminished degradation of LDL in the fibroblasts, thus modifying its residence time in serum and its susceptibility to oxidation [38]. Finally, other alterations observed in SCH involve serum triglycerides, apolipoprotein B (ApoB), lipid subparticle size, and LDL-C oxidizability, though the results are inconsistent [39, 40].

Although it is estimated that 1–11% of all patients with dyslipidemia have SCH, the effects of SCH on serum lipid values in these subjects are not clear [41]. There is also a significant difference in the prevalence of SCH and OH after adjusting for age and sex between nondyslipidemic and dyslipidemic subjects, as the presence of dyslipidemia does not predict the presence of hypothyroidism [41]: it is well established that insulin resistance may modify the effects of SCH on serum lipid levels [42]. Notably, morbidly obese patients exhibit significantly lower mean levels of TC and a significantly lower prevalence of hypercholesterolemia (50.9 vs. 72.7%, $p < 0.01$) when compared with nonobese patients [43], while they also exhibit lower mean serum HDL-C and higher serum triglycerides. Thus, the impact of elevated serum TSH on the lipid profile varies in morbidly obese compared to nonobese patients, suggesting that the obese might not be truly hypothyroid. Accordingly, measuring TC could be a helpful tool to determine whether a morbidly obese patient presenting high TSH requires levothyroxine treatment or not [43].

In another prospective population-based study, high TSH levels within the reference range have been associated with modestly increased blood pressure levels, both systolic and diastolic, and adverse serum lipids. It is therefore essential to bear in mind that TSH levels may covary with blood pressure and lipid levels among people with obviously normal thyroid function [44].

In a recent study aimed to quantify remnant-like lipoproteins (RLPs), small dense LDL (sdLDL), and hepatic lipase (HL) activity in women with SCH (TSH > 4.5 mIU/L) before and after levothyroxine replacement treatment, RLP levels were found to be elevated and HL activity reduced, both parameters being reversed by levothyroxine treatment [45].

Regarding the effects of LT4 on lipids in patients with SCH, some longitudinal studies found a nonsignificant reduction of TC via LT4 treatment [46–48]. Of interest, a two-arm study including (a) short-term investigation of 11 postmenopausal females with SCH examined before and 6 weeks after LT4 at incremental doses (50, 100, 150 μg/day) and (b) a long-term controlled study with LT4 treatment in 105 females, matched for age and menopausal status, for at least 1 year [49]. Long-term T4 treatment was associated with a reduction in total and LDL cholesterol measurements only in those over 55 years receiving suppressive doses of T4; however, there were no significant difference in lipids in those with

normal serum TSH compared with non-T4-treated controls [49]. On the other hand, when stratifying SCH according to its severity, a statistically significant decrease in TC and LDL-C was shown by means of LT4 only in the more advanced forms of SCH [50].

The assessment of carotid artery intima-media thickness (IMT) (by high-resolution ultrasonography) and the lipoprotein profile were assessed in 45 SCH patients (aged 37 ± 11 year) at baseline and after 6 months of randomized, placebo-controlled LT4 treatment [51]. A significant reduction of both TC and LDL-C and mean IMT (by 11%, $P < 0.0001$) by LT4 therapy was observed. Moreover, the decrement in IMT was directly related to the decrease of TC and TSH, indicating that early carotid artery wall changes are present in SCH patients [51]. Although these studies involved a small group of homogenous patients, the highly accurate diagnostic technique that was employed to assess the cardiovascular risk detected an improvement in associated cardiovascular risk factors in those under treatment with replacement doses of LT4 [51].

Another study evaluating the effects of LT4 replacement on non-HDL-C levels in SCH and OH reported significant decrements of the serum concentrations of TC, non-HDL-C, RLPs, and ApoB, whereas no significant changes in the serum concentrations of LDL-C, HDL-C, triglycerides, apolipoprotein A-I, and Lp(a) were observed [52]. In all 39 patients, the reduction in the non-HDL-C levels correlated with the reduction in the LDL-C, RLPs, and ApoB levels. The decrease of non-HDL was related to the decrease of LDL-C, ApoB, and RLPs. Importantly, this study, which indicated a role of non-HDL in the altered metabolism of LDL-C in SCH, could comprise an indirect method to estimate ApoB. The above results are usefully integrated in the information evaluating the cardiovascular risk presenting a more qualitative assessment and a more precise target of therapy [53].

In contrast, a study in which 110 hypercholesterolemic patients with high or low normal TSH were recruited and assigned to treatment with LT4, only those with a TSH between 2.0 and 4.0 mIU/L exhibited a significant reduction of lipids [54]. The discrepancy among the various studies might be due to a number of confounders that likely influence the lipids, such as smoking, obesity, drugs, wide individual and interindividual variations in metabolic and therapeutic responses, as well as insufficient statistical power [54].

Newly emerging CVD risk factors, such as serum C-reactive protein and retinol-binding protein 4 levels as well as hemostatic parameters, mainly underscored by the increased activity of factor VII, have recently been associated with SCH [55] .

Recently it was investigated whether LT4 treatment influences the plasma levels of fibroblast growth factor 21 (FGF21), an endogenous regulator of energy metabolism [56]. Treatment of 107 patients with overt hypothyroidism and of 116 with SCH with LT4 restored the decreased circulating levels of FGF21 in both patient groups and increased FGF21 plasma levels: this observation most probably accounts for the concomitant improvement of metabolic disorders such as hypercholesterolemia and insulin resistance [56]. Importantly, the increased FGF21 levels were correlated with the increase of FT4 and FT3 levels.

Treatment

The recently issued international guidelines recommend that SCH patients should be considered for treatment while scrupulously taking into consideration age, sex, peripheral targets, and lipid metabolism. In other words, the most advisable approach is a carefully individualized one to accurately identify those patients who should be treated [57, 58].

There is a rationale for thyroid hormone replacement therapy with LT4 only in individuals with TSH levels >10 mIU/L, as the available data indicate an increased risk for CVD in patients with more severe SCH [59]. Two important meta-analyses have linked SCH to the risk of HF and CHD events and mortality [60, 61]; the pooled analysis of the individual participant data from the available prospective studies showed that the

risk of CHD events and mortality increased with higher TSH concentrations and was significantly increased in patients with TSH levels ≥10.0 mIU/L. In the absence of large randomized trials, the results of these meta-analyses offer good evidence that treating SCH in adults with serum TSH ≥10 mU/L will improve the cardiovascular risk (Fig. 1). Serum TSH levels are anticipated to normalize during LT4 therapy, this in correspondence to the age of the patients.

LT4 treatment of mild and/or progressively rising TSH levels in young and middle-aged people, a condition that is typically associated with such cardiovascular risk factors as hypertension, hypercholesterolemia, insulin resistance or diabetes, kidney failure, or isolated diastolic dysfunction), is still controversial. Nevertheless, recent data suggest that treatment of mild SH in the latter context may improve the cardiovascular outcome in these patients, especially younger

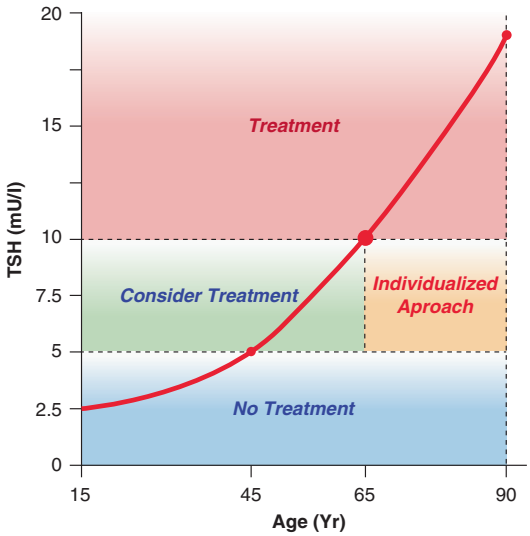

Fig. 1 Young and middle-aged patients below 65 years old with SCH having confirmed TSH above 5 mIU/L should be treated with levothyroxine, while treatment should be considered in patients with a serum TSH concentration between 5 and 10 mU/L, taking into account the positivity of antibodies against thyroid peroxidase (TPOAb) or thyroglobulin (TgAb), comorbidities, gender, and clinical status. Patients older than 65 years should be treated when the serum TSH level is higher than 10 mUI/L, while treatment should be carried out employing an individualized approach when TSH is between 5 mU/L and 10 mIU/L

persons, by reducing the risk of fatal and nonfatal IHD events and mortality [62].

More careful consideration is required as regards the anticipated outcome of thyroid hormone replacement in elderly persons suffering from mildly increased TSH, since this population frequently manifests higher TSH levels as compared to younger subjects, which the increases, however, merely represent a physiological process. Thus, given that older age is likely to affect TSH levels, several studies have proposed the use of modified reference limits for elderly populations in the diagnosis of mild thyroid failure [63]. Interestingly, it has been reported that treatment appears to improve several other markers associated with CVD, including carotid intima-media thickness (cIMT) and other predictors of vascular risk. Meanwhile, studies assessing biochemical, functional, and structural variables associated with an elevated risk of vascular events in older individuals have produced variable results, although this could be attributable to the very complex interactions between SCH and predictors of vascular disease [64].

Also of considerable importance are the results of a recent double-blind, randomized, placebo-controlled trial including 737 adults 65 years of age (Thyroid hormone Replacement for Untreated older adults with Subclinical hypothyroidism(TRUST) + the Institute for Evidence-based Medicine in Old age (IEMO) collaboration trial) with persistent SCH [65]. A total of 368 patients received LT4 (at a starting dose of 50 μg/day, or 25 μg if the body weight was <50 kg or the patient had CHD), with dose adjustment according to TSH level, while 369 patients received placebo. There was no apparent positive effect of LT4 therapy on the symptoms and quality of life of the patients with persistent SCH [65]. However, due to the fact that the study was underpowered, it was not possible to determine any effect on cardiovascular risk and mortality.

Despite the fact that the recommendations on screening are discordant, it has been convincingly advocated that thyroid function testing should be undertaken in patients who are at risk for hypothyroidism, in subjects over the age of 60 years, and in individuals with known CHD and HF [66].

In conclusion, management strategies, including screening and treatment of SCH, still remain controversial in patients with mild TSH increase. Since large, randomized, controlled studies to assess the benefit/risk of treatment of mild SCH in both young and elderly patients have not been conducted, the most reasonable strategy is to base treatment on the guidelines while also prudently adopting a tailored therapeutic approach.

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Treatment of Hypothyroidism

Jacqueline Jonklaas

Introduction

Replacing a deficient hormone is a relatively simple concept. As with most other hormone deficiencies, thyroid hormone deficiency is usually a lifelong condition that cannot be cured. It is therefore incumbent upon the treating physician to be sure that the replacement supplied fully reverses the symptoms and signs of the deficiency without causing any untoward side effects [1]. Tracing the history of the treatment of hypothyroidism shows that there have been alterations in treatment practices. Early treatment of hypothyroidism relied on the use of desiccated animal thyroid extracts [2, 3]. Thyroid extracts were subsequently largely replaced by synthetic thyroid hormone preparations, and levothyroxine (LT4) became the standard of care for treating hypothyroidism [1–3]. Currently, despite the efficacy of synthetic LT4, there is a growing concern that its use may not recapitulate normal euthyroid physiology and may not fully restore the health of hypothyroid individuals [1, 4]. This has spurred an interest in synthetic combination therapy with LT4 and liothyronine (LT3) [4, 5]. In addition to continuing to explore how best to completely reverse the symptoms and signs of hypothyroidism, it is necessary to fully appreciate the benefits and risks of both LT4 monotherapy and LT4/LT3 combination therapy.

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Hypothyroidism: The Basics

Once hypothyroidism has been diagnosed and treatment is being pursued, the tenets of successful treatment are to select an appropriate LT4 treatment dose, administer the dose in a manner that will produce adequate and stable levels of thyroid hormones, and then adjust the LT4 dose for ongoing stability [1, 3]. Concomitant with stabilization of biochemical parameters is the paramount need to return the treated patient to health and avoid the adverse health consequences of overtreatment or undertreatment [1].

Dosing

Once hypothyroidism has been diagnosed, the majority of patients can be started immediately with a full replacement dose of LT4 [6]. For an individual without residual thyroid function, this dose is approximately 1.6 μg/kg/day [1]. Patients who have undergone thyroidectomy may require 1.8 μg/kg/day. For patients who retain some endogenous function, a lower dose may restore a normal serum TSH value. Other algorithms for estimating a reasonable replacement dose take

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into account the serum TSH value at the time of initial diagnosis of the patient [7]. When initiating LT4 treatment, there should be consideration of the duration of the hypothyroidism, the patient's age, and other medical conditions. Older age and patient frailty may necessitate the use of a lower LT4 dose of 25–50 μg that is gradually titrated upward.

Timing

Selecting a regular schedule of LT4 administration will likely facilitate adherence to the regimen. A consistent time of administration would also be anticipated to minimize any fluctuations in absorption associated with food, beverages, and other medications. Absorption may be maximized and most consistent when LT4 is taken on an empty stomach 1 h before breakfast [8]. However, it is also important to choose a schedule that a patient can maintain without forgetting to take his/her medication. Several timing options are available to patients and have been studied in comparison with each other (see Table 1) [9–13]. The least ideal time, if LT4 is being taken in tablet form, is with breakfast, as this is associated with the most impaired and variable absorption [8]. Overall, 1 hour before breakfast and at bedtime may be the regimens associated with the lowest and least variable TSH values. However, patient preference and ability to maintain the regimen must also be taken into account. As long as a particular regimen is not associated with unacceptable variation in TSH values, the LT4 can be increased if the TSH value is higher than the desired target.

It is possible that if mealtime consumption is most convenient for the patient, then a liquid LT4 preparation may best normalize serum TSH. However, head-to-head randomized trials of LT4 tablets compared to LT4 liquid have not yet been conducted. A recent randomized trial of LT4 liquid taken either with breakfast or 30 min before breakfast showed similar TSH values in both circumstances [14]. However, the liquid preparation used was not one that is available in the United States. A number of case series favor the concept that liquid preparations are better absorbed under various circumstances [15–18], including proximity to breakfast, concomitant enteral feeding [19], coffee consumption [20, 21], proton pump inhibitor use [22], and malabsorption syndromes [23, 24] (see Table 2). If the

Table 1 Effect of LT4 timing on serum TSH

Study	Design[a]	Serum TSH values				
		With breakfast	0.5 h before breakfast	1 h before breakfast	Bedtime/3 h after dinner	Others
Bolk et al. [9]	Randomized crossover	–	5.1	n/a	1.2↓	–
Bolk et al. [10]	Randomized crossover	–	2.66, 3.86	–	1.74, 2.36↓	–
Bach-Huynh et al. [8]	Randomized crossover	2.94	–	1.06↓	2.19	–
Elliot et al. [11]	Nonrandomized crossover	–	–	–	1.77	2.06 (1 h after breakfast)
Seechurn (2012)	Nonrandomized crossover	12.6	–	3.14↓	–	–
Rajput et al. [12]	Randomized, parallel	–	5.13	–	3.27	–
Ala et al. [13]	Randomized crossover	–	2.03↓	–	–	3.35 (1 h before dinner)
Cappelli et al. [14]	Randomized crossover	2.58		2.69		

↓ = lower TSH value

[a]All trials involve the tablet form of LT4, except Cappelli trial which utilized liquid LT4

Table 2 Situations in which liquid levothyroxine may be associated with improved absorption

Study	Design	Situation	LT4 intervention	Results
Vita et al. [21]	Nonrandomized single crossover	Elevated TSH with coffee consumption with LT4	Eight patients switched from tablets to gel capsules either with or 1 h before coffee consumption	TSH less affected by timing of coffee with gel capsule use
Cappelli et al. [14]	Nonrandomized single crossover	Coffee consumption with LT4	54 switched from liquid LT4 with coffee to 30 min before coffee	No change in TSH seen
Pirola et al. [23]	Retrospective review	Elevated TSH following gastric bypass	Four patients switched from tablets to liquid	TSH improved with liquid
Giusti et al. [15]	Retrospective review	Patients with thyroid cancer taking LT4	59 patients switched from tablet to liquid each taken 30 min before breakfast	Same TSH with tablets and liquids
Negro et al. [17]	Retrospective review	Hypothyroid patients taking LT4	100 patients taking liquid compared with 100 patients taking tablets	Less variable TSH values with liquid
Vita et al. [22]	Nonrandomized single crossover	Patients with elevated TSH during LT4 and PPI therapy	24 patients switched from tablet to liquid	TSH improved with liquid
Cappelli et al. [14]	Retrospective review	Hypothyroid patients taking LT4	Five-year records of patients aged >65 years taking either LT4 tablet of liquid were examined	TSH values more stable with liquid LT4 than tablet
Pirola et al. [19]	Randomized parallel	Patients requiring enteral feeding	Ten patients taking LT4 tablets compared with ten patients taking liquid. Enteral feeding stopped for 60 min in tablet group only	TSH not increased when liquid given with enteral feeding
Brancato et al. [16]	Retrospective review	Patients taking LT4 tablets within 1 h before breakfast	54 patients switched from tablet to liquid	TSH lower with liquid
Santaguida et al. [15]	Nonrandomized single crossover	Patients with malabsorption	31 patients switched from tablet to lower dose of gel capsule	TSH maintained with lower gel capsule dose
Cappelli et al. [14]	Randomized crossover	Hypothyroid patients taking LT4	77 patients switched from liquid LT4 30 min before breakfast to with breakfast or vice versa	TSH not increased when LT4 liquid is taken with food

results from these case series and nonrandomized trials are confirmed, it appears that liquid LT4 preparations may be an excellent means to overcome many causes of impaired absorption [1].

Adjusting

A particular TSH target can be achieved by serial adjustment of a patient's LT4 dose. Maintenance of that TSH should then be possible with continuation of that same dose, if no other influences act to perturb the equilibrium. After initiating or changing a patient's LT4 dosage, further adjustment is best performed after repeating the laboratory evaluation 6 weeks later. This allows the serum concentration of free thyroxine (FT4) to reach steady-state levels [1]. The serum TSH is presumed to have had sufficient time to reflect these steady levels. In order to not miss the need for LT4 dose adjustment during the first half of pregnancy, repeat testing of TSH every 4 weeks is recommended [25]. Repeat testing of TSH and FT4 at sooner, nonsteady-state time points such as 3 weeks may be reasonable during pregnancy under special circumstances such as when a

serum TSH value has been well out of the target range, making it desirable to verify that the trajectory of abnormal thyroid analytes toward reversal is adequate.

If the patient's TSH is mildly elevated to less than 10 mIU/L, or subnormal but not undetectable, an increase or decrease to the next available dose may be sufficient to normalize the TSH concentration. Significantly out-of-range TSH values may require larger dosage changes. However, there is a considerable variation in individual patient sensitivity to dosage changes, so that the most important principle of dose titration is to monitor the patient's response. Some patients may be under-replaced or over-replaced on the available doses and may require other adjustments such as alternating their prescribed doses. However, complex replacement regimens should be avoided if possible. Once the goal TSH is achieved, the serum TSH can be repeated in another 3–6 months to ensure continued adherence to therapy and euthyroidism. Clinical euthyroidism should be manifest as biochemical euthyroidism is maintained, although several months may be necessary for patients to feel well. Patients with stable TSH values can be monitored annually. It has even been suggested that less frequent monitoring is adequate in certain groups of patients [26].

Avoiding Fluctuations

Numerous factors may potentially contribute to serum TSH levels not remaining within the targeted parameters during therapy (see Table 3). These include, but are not limited to, altered timing of administration, omitted dosages, interfering medications, timing with respect to food and medications, altered body weight, development of gastrointestinal conditions, and pregnancy [1, 3]. Treatment with higher doses of LT4 also seems to predict a greater likelihood of a future abnormal TSH value, possibly indicating less endogenous thyroid reserve to protect from fluctuation [27]. It should also be remembered that there is a circadian in serum TSH values, even in LT4-treated patients, such that some natural variation with TSH values of the order of 2 mIU/L is anticipated [3, 9].

Table 3 Factors that may alter a patient's LT4 dose requirement or alter TSH

Factors	Increased TSH and increased requirement	Decreased TSH and decreased requirement
Weight increase	√	
Weight decrease		√
Pregnancy	√	
Drugs		
Impaired absorption LT4 (e.g., calcium carbonate, phosphate binders)	√	
Increased protein binding/transport of LT4 (e.g., estrogen)	√	
Decreased protein binding/transport of LT4 (e.g., androgens)		√
Increased metabolism of LT4 (e.g., antiepileptic drugs, tyrosine kinase inhibitors)	√	
Altered gastric pH (e.g., proton pump inhibitors)	√	
Malabsorption	√	
Gastritis	√	
Reversal of gastritis		√
Close proximity to food	√	
Separation from food		√
Factors	Increased TSH	Lowered TSH
Omitted doses	√	
Additional doses		√
LT4 dose >150 μg	√	√
Drugs		
Metformin		√
Statins		√
Vitamin C		√
Glucocorticoids		√
Bexarotene		√

Improving Health Outcomes

It is clear from studies of treatment of myxedema coma and reversal of the euthyroid state after withdrawal from thyroid hormone for scanning and treatment of thyroid cancer that euthyroidism is associated with less hypothyroid symptoms

and improved quality of life [1, 28, 29]. Symptom questionnaires indicate an increased symptom burden in patients with hypothyroidism [30], and several different validated questionnaires are available [31]. Reversal of hypothyroidism is typically associated with weight loss. One study documented a mean weight loss of 4.3 kg over a year, along with increased resting energy expenditure and increased activity. However, the weight loss was primarily loss of lean body mass, not fat mass, and the investigators suggested that excretion of excess body water was responsible [32]. Although improved outcomes are anticipated after treatment of hypothyroidism, as overt hypothyroidism is not left untreated, there are no controlled studies of long-term outcomes of the treated versus untreated state.

Avoiding Risks

Laboratory monitoring indicates that despite ease of LT4 dose adjustment, many patients with hypothyroidism have out-of-range TSH values. Thus, treatment of hypothyroidism is associated with risks of both overtreatment and undertreatment. Biochemical evaluation in several populations of individuals taking LT4 shows that up to 20% of individuals may be overtreated or undertreated (Fig. 1) [33–37]. For example, of a population attending a health fair, 18% were receiving inadequate LT4 therapy, and 22% were receiving excessive LT4 therapy

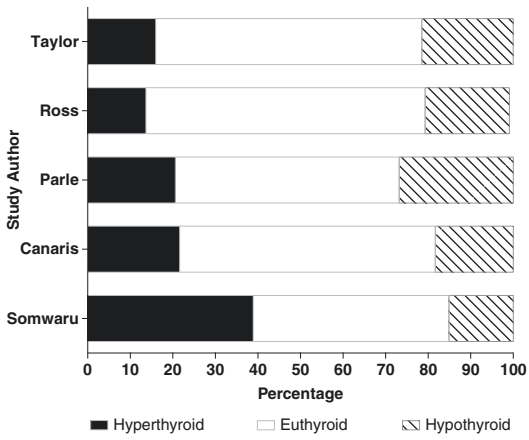

Fig. 1 Thyroid status in patients taking levothyroxine

[37]. This is even a problem in those over 65 years of age [33]. These studies were conducted during period between 1990 and 2014, suggesting that this is an ongoing issue. Whenever possible, therapy should be adjusted to avoidance of iatrogenic hypothyroidism or hyperthyroidism. Risks of overtreatment include decreased bone mineral density and increased cardiac arrhythmias [38, 39]. These data suggest more rigorous monitoring, or attention to factors associated with altered requirement for LT4 is needed. Some data suggests that the rate of LT4 initiation is highest in older populations [33, 40]. These may be the very populations that are most susceptible to the side effects of iatrogenic hyperthyroidism. If age-adjusted reference ranges are taken into account [1], it is possible that a subset of these individuals could have been monitored without LT4 therapy.

Hypothyroidism: Debates and Controversies

Although many patients feel well on LT4 therapy, a proportion of patients who are adequately treated based on their normal serum TSH levels do not feel restored to their baseline health. The percentage of patients with symptoms such as inability to think clearly, putting on weight, and difficulty remembering things was 32–46% in one study [41]. Even if recent thyroid blood tests indicated biochemical euthyroidism, based on the local laboratory reference ranges of 0.1–5.5 or 0.2–6.0 mU/L, symptoms were reported in 34–48%. This compares with symptoms being reported in 25–35% of control participants. Fatigue also appears to be more prevalent in euthyroid patients compared with euthyroid controls [42] and also compared with patients being treated for thyroid cancer [43]. Another study, in which subjects were recruited by letter and then subsequently took part in cognitive testing and completed questionnaires assessing well-being, also showed worse scores in both these areas in the euthyroid patients taking LT4, compared with standard reference values [44]. Patients with treated hypothyroidism also appear to be more likely to be diagnosed with depression and other psychiatric disorders [45].

This decreased quality of life in biochemically euthyroid patients with hypothyroidism could be hypothesized to be due to several different causes. First and foremost, it is possible that there is some aspect of the hypothyroid state that is not adequately reversed with traditional LT4 treatment. Alternatively, there may be an ascertainment bias because patients who do not feel well are more likely to be screened for and diagnosed with hypothyroidism. Being categorized as having a chronic condition could also be detrimental to a patient's well-being. Some support for this explanation is provided by a study showing that being aware of diagnoses such as diabetes, hypertension, and hypothyroidism was linked with worse self-reported health [46]. Finally, there may be some autoimmune or genetic condition, either associated with hypothyroidism or underpinning the development of hypothyroidism, which causes morbidity. Examples of such conditions could be autoimmunity or genetic variations.

Targeting TSH Values

The laboratory parameter that is used to adjust LT4 therapy is the serum TSH. TSH concentrations are associated with serum FT4 concentrations in a well-described log-linear relationship. However, this relationship is complex and may be different in individuals without thyroid disease compared with those being treated with LT4. The relationship may also be affected by patient age and sex and body mass index [47–49]. Reported laboratory reference intervals for commercial clinical laboratories are, by definition, based on 120 healthy individuals. Reference intervals for TSH vary but are approximately 0.4–4 mIU/L. Based on large population-based studies, individuals without thyroid disease typically have mean TSH values of 1–2 mIU/L. This has led to suggestions to lower the upper limit of the TSH serum interval to 2.5–3 mIU/L [50, 51]. There are arguments both supporting and not supporting this suggestion [50, 52]. If a lower upper limit of the TSH reference interval was adopted, logically lower TSH values would be targeted during the treatment of hypothyroidism.

The ideal TSH value for a particular patient may depend on the patient's age, coexistent medical conditions, and any benefits that may be associated with specific TSH values. When considering whether the normal reference interval should be narrowed, a key question is whether there are proven benefits of lowering a patient's TSH from, for example, 3.6–1.1 mIU/L. This same question would also be relevant if one were considering whether a specific part of the normal range should be the goal of treatment in a particular individual. Interestingly, a recent meta-analysis suggested that normal TSH values within the upper part of the normal reference range were associated with adverse cardiovascular and metabolic outcomes, whereas TSH values within the lower part of the normal range were associated with reduced bone mineral density and fracture risk [53].

In theory, some potential advantages of lowering a patient's serum TSH within the boundaries of the normal range include a more favorable lipid profile, reduced body weight, and improved patient satisfaction or well-being. Theoretical advantages of raising a patient's TSH within the normal range might include less development of osteoporosis. There seems to be a trend for improvement in total cholesterol when modest lowering of TSH values is achieved [54, 55]. In a crossover trial of 56 patients who were treated for 8-week periods with three doses of LT_4 in a random order to achieve TSH values of 2.8, 1, and 0.3 mIU/L, there was progressive lowering of total cholesterol with lowering of TSH values [54]. However, most of the reduction may have been due to lowering of cholesterol in the group that included many patients with subclinical hyperthyroidism. The available data do not seem to support a relationship between weight and altered TSH values within the normal range [54, 55]. A study in which patients were maintained at low-normal or high-normal TSH values for a year showed no decrement in body mass index or percentage body fat associated with the lower TSH, despite an increase in resting energy expenditure [55]. Based on such studies, there is insufficient data to support targeting a particular TSH value within the normal range.

Monitoring Other Thyroid Analytes

While monitoring LT4 treatment, it is essential to check a serum TSH. Following FT4 concentrations may also be helpful. In general, targeting triiodothyronine (T3) values has not been endorsed [1]. If T3 or free T3 (FT3) concentrations are checked during the course of monitoring LT4 therapy, they are usually in the low normal or low range compared with values seen in individuals with endogenous thyroid function [1]. Altered FT4/FT3 ratios in patients receiving LT4 therapy have been proposed as a measure of impaired type 2 deiodinase activity and impaired T3 homeostasis [56] (see Section "How Would Combination Therapy Be Monitored?").

When Levothyroxine Does not Restore Quality of Life

As discussed above, not all LT4-treated patients feel well. It is an appealing concept that this may be due to not replicating the TSH values seen in healthy individuals without thyroid disease. However, dissatisfaction with therapy does not appear to be ameliorated by targeting a TSH that approximates the lower half of the normal range (see Section "Targeting TSH Values").

It is well known that LT4-treated individuals have higher FT4 levels, lower T3 levels, and higher FT4/T3 ratios than individuals with intact thyroid function [57–59] [reviewed in [1]]. It has been suggested that the biochemical signature of reduced FT3/FT4 ratios and the clinical finding of lack of well-being in patients can be paired together as possible cause and effect. This has led to trials of "combination therapy," mainly involving synthetic thyroid hormones (LT4 and LT3) but also recently involving porcine thyroid extract.

A genetic basic for the failure of LT4 to restore well-being has also been sought. A secondary analysis of the largest combination therapy trial [60] suggested that patients with a particular genetic variation, the Thr92Ala variant of the type 2 deiodinase, respond differently to treatment of their hypothyroidism [61]. Approximately 16% of the populations are homozygous for this substitution. Not only did these individuals have worse scores on the general health questionnaire while taking LT4, but they also had a better response to combination therapy in which 50 μg LT4 was replaced by 10 μg LT3 [61]. This genetic variation may potentially lead to altered type 2 deiodinase enzyme velocity and to a decrease in conversion of T4 to T3 [62] [discussed in [1]]. It is possible that if patients with this particular variant had been specifically targeted in combination therapy trials, a greater benefit of combination therapy would have been documented.

Recently it has been shown that expression of the Thr92Ala D2 polymorphism in cell lines is associated with enzyme accumulation in the Golgi apparatus and disruption of cellular functions [63]. Microarray studies of the cerebral cortex of Thr92Ala carriers have shown transcription of genes involved in inflammation and apoptosis [63]. These two findings combined could suggest a reason for the neurocognitive symptoms in Thr92Ala carriers. However, the effects of genetic variations in the deiodinases on human health appear to be complex and extend beyond thyroid function to osteoarthritis and insulin resistance [64]. The results of the human microarray studies do not directly explain why individuals homozygous for Thr92Ala might prefer combination therapy that includes LT3, especially as the expression of T3-responsive genes was unaltered [63].

A recent animal study examined three different therapies in hypothyroid rats [65]. These therapies were subcutaneous LT4 administration, subcutaneous LT4 administration and LT3 injection, and continuous administration of both LT4 and LT3 via subcutaneous pellets. Greater inactivation of type 2 deiodinase in tissues other than the hypothalamus, lower serum T3 levels, and higher T4/T3 ratios were seen in these rats during both monotherapy and intermittent therapy with T3, compared with the combination therapy employing a subcutaneous slow release T3 pellet. The continuous delivery of thyroid hormones was also associated with closer replication of measures of skeletal muscle functioning, liver functioning, and expression of T3-responsive

genes, as compared with that seen in the control animals [65]. It is possible, if a similar phenomenon is seen in humans, that benefits of combination therapy might only be seen with a sustained-release T3 preparation (see Section "Are Additional Trials of Combination Therapy Needed?").

Evidence About Combined Levothyroxine/Liothyronine Therapy

Apart from a single trial of combination therapy conducted in 1970 and a recent abstract reported in 2015 at the annual meeting of the American Thyroid Association, the trials comparing synthetic combination therapy to LT4 monotherapy have been conducted between 1999 and 2010 [60, 66–77]. A trial comparing thyroid extract therapy with LT4 therapy was reported in 2013 [78].

Why Consider Combination Therapy?

Combination therapy has been explored because of the documented patient dissatisfaction with LT4 monotherapy. It is possible that failure to replace the component of T3 that comes directly from the thyroid gland is the cause of this dissatisfaction. In humans with native thyroid function, the thyroidal production of T4 is 85 μg and that of T3 is 6.5 μg daily [discussed in [1]]. The ratio of thyroid hormones produced directly from the thyroid gland is thus a T4/T3 ratio of 14:1. A further 26.5 μg T3 is made daily by peripheral conversion from T4 [discussed in [1]]. Individuals without their native thyroid function are thus missing the 6.5 μg T3 that would normally be produced within the thyroid gland. If the 6.5 μg of T3 made by the thyroid is provided by exogenous LT3 instead, 6–7 μg of LT3 could be provided daily, depending on the absorption of an oral dose of LT3. LT3 absorption is better than that of LT4 and is of the order of 95% [79]. If inactivation of the type 2 deiodinase also occurs in humans during monotherapy, as has been shown in rats [65], there may also be less than expected production of T3 from T4 by peripheral deiodination during LT4 monotherapy, and additional LT3 may theoretically be needed to compensate for this.

Combination Therapy Thus Far

Thirteen trials comparing synthetic combination therapy to LT4 have been conducted [60, 66–77] [reviewed in [1]]. Despite this extensive body of research into therapy with both LT4 and LT3, clear benefit from this combination therapy has not yet been shown. These trials have not shown improvement in a variety of outcome measures, including symptoms, with the implementation of combination therapy. However, most of these trials had fewer than 100 patients. Sample sizes were too small to detect differences in analyses stratified by baseline symptoms. The length of the trials has generally been short, with most being 5–16 weeks in duration [66–68, 70, 72–75, 77]. Four of the trials were 4–12 months long [60, 69, 71, 76] (see Table 4). Another concern is that in most of these trials, participants were given LT3 once daily, which would have increased their serum T3 levels only for part of the day. There were nine trials that used once-daily LT3 dosing [60, 67, 68, 70–73, 75, 77], four trials that employed twice-daily LT3 dosing [66, 69, 74, 76], and none that used LT3 given three times daily (see Table 4).

The trials were heterogeneous with respect to the achieved serum TSH, FT4, and T3 levels in the monotherapy versus combination therapy groups [reviewed in [1]]. Some studies resulted in different TSH concentrations between groups (two lower and three higher in the combination therapy group), potentially confounding the study results or reducing the ability to demonstrate differences between the groups. Another issue with the trials performed so far relates to the populations studied. Older individuals, those with comorbidities, and males have been understudied. The predominance of healthy middle-aged females in the current studies (see Table 4) leads to potential problems with generalizing results to the entire population with hypothyroidism. Additional considerations with these trials are that patients with residual symptoms were not specifically targeted and that some

Table 4 Characteristics and outcomes of combination therapy trials

Authors	No. of participants	Mean age (years)	Sex (% F)	Daily dosing frequency	Duration	Psychologic benefit?	Patient preference for T4/T3	Weight	Lipid profile
Appelhof	130	46–49	83–89	Twice	15 weeks	↑ combo 5:1	Yes	↓ combo 5:1	cholesterol↓ combo
Bunevicius	33	46	94	Once	5 weeks	↑ combo	Yes	n/a	No diff
Bunevicius	10	34	100	Once	5 weeks	↑ (tendency only) combo	Yes	No diff	n/a
Clyde	44	43–45	77–86	Twice	4 months	No diff	n/a	No diff	No diff
Escobar-Morreale	26	48	100	Once	8 weeks	No diff	Yes	No diff (BMI)	No diff
Fadeyev	36	40–43	100	Once	6 months	n/a	No	No diff	LDL chol ↓ combo
Nygaard	59	46–47	93	Once	12 weeks	↑combo	Yes	No diff	n/a
Rodriguez	27	47	83	Once	6 weeks	No diff	No	No diff	n/a
Saravanan	573	57	83–84	Once	12 months	No diff	No	No diff	No diff
Sawka	33	49	90	Twice	15 weeks	No diff	n/a	n/a	n/a
Siegmund	23	23–69	81	Once	12 weeks	No diff	n/a	n/a	No diff
Valizadeh	60	38–39	80	Twice	4 months	No diff	n/a	No diff	No diff
Walsh	101	48	92	Once	10 weeks	No diff	No	No diff	↑ combo
Hoang[a]	70	51	76	Once	16 weeks	No diff	Yes	↓ combo	No diff

↓ = decreased, ↑ = increased

[a]Hoang trial employed thyroid extract

patients may have had some residual thyroid function based on having Hashimoto's hypothyroidism (see Section "Have the Correct Groups Been Studied?").

The heterogeneity of the trials makes it difficult to assess outcomes with the use of meta-analyses. Psychologic benefit, or a tendency toward benefit, was seen in four trials. A preference for synthetic combination therapy was seen in four out of the five crossover trials in which preference was assessed (see Table 4). Overall, including a trial of thyroid extract therapy, approximately 66% of patients in the trials with a crossover design preferred combination therapy. Patients preferred combination therapy in one of the trials with a parallel design. However, the largest trial, which had a parallel design, was not associated with patient preference for combination therapy [60].

Other outcomes documented in these trials do not appear to differ between the monotherapy and combination therapy groups (see Table 4). However, the range of parameters monitored has been quite limited. Most studies showed no impact on body weight or blood pressure, two showed a favorable effect on the lipid profile, and two showed an increase in bone turnover markers. Heart rate was increased in two studies but decreased in two others. When monitored, echocardiographic parameters were unaffected [67]. Bone mineral density was only assessed in one study and was not altered [71]. Eleven patients from the WATTS study [60] underwent 24-h monitoring of blood pressure and pulse [80]. There were no significant differences in these parameters in the patients treated with combination therapy, compared with those treated with LT4 monotherapy. Atrial fibrillation in association with a low serum TSH was reported in one study [75]. In another study, an individual with a history of atrial premature beats who had no arrhythmia present at enrollment was noted to have atrial premature beats prior to adjustment of the study medication at 5 weeks [66]. In summary, there are no apparent differences in outcomes in these trials, but no cardiovascular event or fracture data are available and long-term data are lacking.

Thyroid Extract Therapy

There is a single 16-week duration crossover trial of thyroid extract therapy compared to LT4 therapy [78]. In this trial, 1.667 μg LT4 was substituted for 1 mg of thyroid extract, and the mean dose of thyroid extract was 80 mg daily. This trial was associated with a small but significant weight loss and patient preference for the extract therapy. However, there were no changes in multiple psychologic measures. The biochemical profiles of the patients were notably different during the two therapies. Mean serum T3 concentrations were much higher during extract therapy, 136 ng/dL compared with 89 ng/dL during LT4 therapy. Post-dosing concentrations of serum T3 were reported for two patients, and although these remained within the reference interval, there is a theoretical concern about "T3 thyrotoxicosis" with thyroid extract therapy. Older literature [reviewed in [1]] demonstrates high levels of serum T3 after taking thyroid extract, but higher doses and different preparations of thyroid extract were used. Similar to the situation with synthetic combination therapy, and in contrast to the situation with LT4 monotherapy, thyroid extract therapy is characterized by low concentrations of FT4. For example, in the crossover trial above [78], the mean FT4 concentration during LT4 therapy was 1.36 ng/dL compared with 0.85 ng/dL during extract therapy. Thus, a potential disadvantage of thyroid extract therapy is that it also, like LT4 therapy, does not replicate the normal ratio of circulating T4 and T3 found in humans with endogenous thyroid function.

Have the Correct Groups Been Studied?

It is possible that particular subgroups of patients may benefit from combination therapy. Potential subgroups could include those who are dissatisfied or unhappy while taking LT4 therapy and those with deiodinase polymorphisms affecting conversion of T4 to T3. It could also be speculated that those who are completely athyreotic or those with low serum T3 levels during monotherapy may respond better to combination therapy.

These subgroups have not been specifically targeted before in the previously reported trials, although patients with depression and fatigue were included in some trials [see Table 8 in [1]]. When considering the use of combination therapy, the benefits and risks need to be carefully weighed. If specific subgroups of patients experience measurable benefits from combination therapy, some risks may be acceptable, and combination therapy could be targeted to those who are most expected to benefit.

How Would Combination Therapy Be Monitored?

Combination therapy could potentially include synthetic LT4 and LT3, with both selection of the ratio of T4 to T3 and choice of the frequency of the T3 administration (daily, twice daily, three times daily). Alternatively thyroid extracts could be considered to be a fixed-dose combination therapy with a T4/T3 of approximately 4.2:1 with T3 given once daily. A pharmaco-equivalence study has shown that the LT4/LT3 equivalence ratio is approximately 3:1. For example, approximately 150 μg LT4 is equivalent to 50 μg LT3 [81]. Therefore, if a patient were being converted from LT4 monotherapy to combination therapy, with maintenance of the same dose, there would need to be a reduction in the LT4 dose depending on the LT3 dose being added, according to the 3:1 conversion factor. Recommendations for converting a patient who is not doing well while taking LT4 from LT4 monotherapy to combination therapy have been published [82]. There are other important considerations if rational combination therapy is to be safely undertaken. If steady serum levels of T3 are both physiologic and desirable, either multiple small doses of LT3 (e.g., 2 μg four times daily or 2–3 μg three times a day) or a sustained-release T3 preparation would be needed. These examples employ small doses of LT3 that are not available as single tablets. Currently, LT3 is available as 5hcontrolled community-based and 25 μg tablets.

An important parameter that could be targeted during combination therapy is patient symptoms. This is particularly important, because unresolved symptoms are what typically lead a patient to request combination therapy. The associated question is which symptoms or quality of life measures would best reflect successful combination therapy. Symptoms can be assessed using validated quality of life questionnaires. Since changes in thyroid symptoms might be as apparent in general quality of life questionnaires, it is important that standardized and/or validated thyroid-related quality of life questionnaires be used. Examples of such questionnaires include ThyPRO, Chronic Thyroid Questionnaire, and Underactive Thyroid-Dependent Quality of Life Questionnaire [83–85] [reviewed in [1]]. However, such questionnaires have not typically been employed in a routine clinical setting but have been used primarily in the research setting, so this is an untested and potentially time-consuming approach.

The optimal biochemical monitoring of patients receiving combination therapy has not been elucidated. It is not clear whether serum TSH is as helpful a marker of euthyroidism in a patient receiving combination therapy. Potential targets, in addition to serum TSH, include FT4, T3, FT3, and the FT4/FT3 ratio. The concept of considering serum T3 as a target has been quite controversial [1, 86]. An additional concern presents itself if serum FT3 is a therapeutic target. Assays for FT3 may be less accurate, in part due to the low concentrations of hormone being measured [87, 88]. The FT3/FT4 ratio has been proposed as a simple estimate of deiodinase activity during LT4 monotherapy [56]. It is possible that a FT3/FT4 ratio would reflect a combination of peripheral conversion plus the exogenous LT3 being supplied during combination therapy. However, even if the FT4/FT3 or FT3/FT4 ratio is a meaningful target, the desired target value has not been defined. It is likely that any monitoring strategy that includes a combination of several laboratory analytes would necessitate additional costs for the patient. These costs would be in addition to the extra costs of the LT3 therapy itself.

In addition to selecting the best thyroid analytes to measure during combination therapy,

another consideration is the timing of testing and whether a peak or trough thyroid hormone level is being sampled. Depending on the timing of phlebotomy, high serum T3 levels may be encountered. Only two trials of synthetic combination therapy assessed post-dosing T3 concentrations and did so in a small subset of 10–12 patients [75, 80]. In one study, patients were taking 10 μg LT3 once daily combined with LT4. A 42% increase in serum FT3 concentration was seen within 4 h after the administration of 10 μg LT3 [80]. Three of the ten patients had serum T3 levels that were above the upper limit of the laboratory reference range for part of the day. In the other study, in which 5% of the patient's dose of LT4 was replaced by a single dose of LT3 that was prepared in-house, there was a 54% increase in FT3 concentrations approximately 2 h after LT3 administration [75].

Consideration of the timing of phlebotomy is particularly important if LT3 is given only once daily. Serum T3 and FT3 levels peak approximately 2.5 h after dose administration [75, 80, 89, 90]. A trough serum T3 level is clearly both lower and more predictable than a post-dose T3 level, as illustrated in three studies of once-daily LT3 dosing [75, 80, 90]. The 24-h profile of serum TSH concentrations following once-daily LT4/LT3 administration appears to show more fluctuation than serum TSH levels following daily LT4 administration [80]. In patients receiving combination therapy, the TSH nadir was at 6 h following LT4/LT3 dosing, before returning to the pre-dosing value about 10 h later [80]. If a trough T3 concentration, and its associated TSH level, was being targeted because of the predictability of analyte values prior to the next LT3 dosing, phlebotomy in the early morning prior to any of that day's LT3 administration might be particularly useful for monitoring therapy. This might also be most inconvenience and costly for patients.

Are Additional Trials of Combination Therapy Needed?

The number of patients who have participated in the trials of combination therapy already completed totals 1225. It is possible that a bigger trial needs to be conducted in which a large number of patients are studied in a single trial in which standardized outcomes and validated measures of quality of life are utilized. Alternatively, it is possible that any future trials may best focus on a particular subgroup of patients: those who do not feel well; those with type 2 deiodinase polymorphisms or other genetic variations, athyreotic patients; or those who have particularly low serum T3 levels during monotherapy (see Section "Have the Correct Groups Been Studied?"). It would also be important to include a population that is representative of the hypothyroid population in general, including those with other medical conditions, and to ensure that parameters such as serum TSH are not different in the treatment groups. Other considerations in future trials would be using more frequent administration of LT3 in order to achieve more stable serum T3 concentrations. Three times-daily LT3 therapy (as monotherapy) has been successfully achieved [81, 91], but such frequent medication administration is challenging to maintain during long-term therapy. A sustained-release T3 preparation would clearly circumvent this issue. The spectrum of medications that are available in sustained-release formulations has not yet extended to LT3.

Conclusion and Future Challenges

Clinicians who are treating patients with hypothyroidism have at their disposal an excellent therapy that rapidly and effectively reverses the stigmata of hypothyroidism. This is indeed a significant advance since Bettencourt and Serrano treated hypothyroidism by grafting half a sheep's thyroid gland into a myxedematous patient [92]. Levothyroxine is easy to administer and monitor and results in excellent quality of life for most patients. However, despite the successes in treating hypothyroidism, there are clearly nuances of treating hypothyroidism that have not yet been elucidated. There is a need to fine-tune thyroid hormone replacement so that it provides satisfactory treatment to all those with hypothyroidism. Development of a sustained-release T3 prepara-

tion and harnessing thyroid follicles generated from stem cells [93, 94] are potential avenues through which this might be achieved.

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Myxedema Coma

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Myxedema coma is a relatively rare life-threatening condition resulting from severe deficiency of circulating thyroid hormones. Understanding the pathogenesis of the condition, appropriate recognition of the clinical signs and symptoms, and rendering prompt and accurate diagnosis and treatment are crucial to optimize survival. It was probably first reported in 1879 by Ord in a report of the Clinical Society of London in 1888 in which two of 12 patients with fatal hypothyroidism appeared to have died in coma [1]. The entity remained unreported in the literature until 1953 [2, 3], and by now there have been perhaps 300 cases reported and, of course, many that have not been reported. Most patients with myxedema coma have had symptoms of hypothyroidism for many months, and the onset of stupor or coma is precipitated by cold exposure, by infection or other systemic disease, or by drugs, as noted below. There may be a past history of antecedent thyroid disease, thyroid hormone therapy that was discontinued for no apparent reason, or radioiodine therapy for hyperthyroidism. Examination of the neck may reveal a surgical scar and no palpable thyroid tissue or goiter. Once the diagnosis is brought to mind, its confirmation should be relatively easy based upon the presenting clinical and laboratory findings. Unfortunately, unless there is early diagnosis and implementation of appropriately vigorous therapy, the outcome is often dismal [4].

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Epidemiology and Precipitating Events

The incidence rate of myxedema coma is 0.22/1,000,000 per year [5]. A typical and common presentation is that of a hospitalized elderly woman with a history of long-standing hypothyroidism who for various reasons had not been taking replacement hormone. Although 80% of cases occur in women >60 years of age, myxedema coma does occur in younger patients as well, with 36 documented cases reported during pregnancy [6, 7]. Often the patient may have previously undiagnosed hypothyroidism and the descent into coma is facilitated by the development of a systemic illness such as a pulmonary or urinary tract infection, congestive heart failure, or a cerebrovascular accident (Table 1). In other cases, there is a past history of antecedent thyroid disease, thyroidectomy, or treatment with radioactive iodine, e.g., for Graves' thyrotoxicosis, or

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Table 1 Factors reported to precipitate myxedema coma

Drugs
Withdrawal of L-thyroxine
Anesthetics
Sedatives, hypnotics
Tranquilizers
Narcotics
Amiodarone
Lithium carbonate
Infections, sepsis
Cerebrovascular accidents
Congestive heart failure
Cold; winter temperatures
Surgery; trauma
Metabolic disturbances
Hypercapnia
Acidosis
Hypoglycemia
Hyponatremia
Others:
Gastrointestinal bleeding
Ingestion of raw bok choy

of thyroid hormone replacement therapy that was discontinued for no apparent reason. The so-called secondary or tertiary hypothyroidism on the basis of pituitary or hypothalamic disease, respectively, is relatively rare, being seen in about 5–15% of patients [8]. It is probably no coincidence that patients with myxedema coma tend to present with greater frequency in the winter months, suggesting that external cold may be an aggravating factor. It is not always clear whether some of the associated abnormalities such as hypoglycemia, hypercalcemia, hyponatremia, hypercapnia, and hypoxemia were conditions that actually precipitated the coma or were secondary consequences of myxedema coma. Pulmonary infection may occur as a secondary event because of hypoventilation due to somnolence, and the somnolent or comatose state predisposes to a risk of aspiration pneumonia and sepsis. In other patients, sedative, analgesic, antidepressant, hypnotic, antipsychotic, and anesthetic drugs were likely to have precipitated myxedema coma because of their ability to depress respiration. Drug-induced myxedema coma is particularly likely to occur in hospitalized patients because the latter types of drugs are typically dispensed in hospital, and their physicians may not be aware that the patient has hypothyroidism. The risks associated with these drugs are real, and their use in a hypothyroid patient sets up a chain of events that can lead to coma. A case described by Church et al. is typical in this regard; they reported a 41 years old male patient with no known history of thyroid disease who developed myxedema coma after being administered combined therapy with aripiprazole and sertraline [9]. There is also a report of myxedema coma induced by chronic ingestion of large amounts of raw bok choy. This Chinese white cabbage contains glucosinolates, which have breakdown products such as thiocyanates, nitriles, and oxazolidines that inhibit iodine uptake and the subsequent synthesis of thyroid hormones by the thyroid gland. When eaten raw, digestion of the vegetable releases the enzyme myrosinase which accelerates production of the abovementioned thyroid disruptors [10].

Clinical Signs and Symptoms

Two of the cardinal features of myxedema coma are hypothermia (often profound to 80 °F (26.7 °C)) and unconsciousness [11, 12]. Coincident infection may be masked by the presence of hypothyroidism such that the patient presents as afebrile in spite of an underlying severe infection. In view of the latter and the fact that undiagnosed infection might lead inexorably to vascular collapse and death, the recommendation for empiric use of broad spectrum antibiotics in patients with myxedema coma has some merit. Underlying hypoglycemia may further compound the decrement in body temperature. Although coma is the predominant clinical presentation, signs or symptoms of disorientation, depression, paranoia, or hallucinations (“myxedema madness”) may often be elicited. The course often is one of lethargy progressing to stupor and then coma, with respiratory failure and hypothermia, all of which may be hastened by the administration of drugs that depress respiration and other central nervous system (CNS) functions. Most patients have the characteristic

features of severe hypothyroidism, such as dry, coarse, and scaly skin; sparse or coarse hair; nonpitting edema (myxedema) of the periorbital regions, hands, and feet; macroglossia; voice hoarseness; and delayed deep-tendon reflexes [13]. Moderate-to-profound hypothermia is common. The neurological findings may also include cerebellar signs, e.g., poorly coordinated purposeful movements of the hands and feet, ataxia, adiadochokinesia, poor memory and recall, or even frank amnesia. Associated abnormal findings may be documented on electroencephalography such as low amplitude and a decreased rate of α-wave activity. Status epilepticus has been also described [14], and up to 25% of patients may experience seizures possibly related to hyponatremia, hypoglycemia, or hypoxemia.

Respiratory System

It is believed that it is a combination of a depressed hypoxic respiratory drive and a depressed ventilatory response to hypercapnia that constitutes the mechanism for hypoventilation in profound myxedema [15, 16]. In addition, upper airway partial obstruction caused by enlargement and edema of the tongue or vocal cords may also play a role. Tidal volume also may be reduced by other factors such as pleural effusion or ascites. Hypothyroid patients may be predisposed to pneumonitis as a precipitating factor because of airway hyperresponsiveness and chronic inflammation [17], and effective pulmonary function in myxedema coma is often not achieved without prolonged mechanically assisted ventilation. Recovery from respiratory failure may be slow despite apparently adequate therapy.

Cardiovascular Manifestations

The findings considered typical of hypothyroid heart disease also are found in myxedema coma and include cardiac enlargement, decreased cardiac contractility, and nonspecific electrocardiographic abnormalities. Patients diagnosed with myxedema coma are at increased risk for shock and potentially fatal arrhythmias. Typical ECG findings include bradycardia; varying degrees of block, low-voltage, flattened or inverted T waves; and prolonged Q–T interval which can result in *torsades de pointes* ventricular tachycardia [18]. Myocardial infarction should also be ruled out by the usual diagnostic procedures, because aggressive or injudicious T4 replacement may increase ischemia and the risk of infarction. However, myocardial infarction may be somewhat more difficult to rule out due to low voltage on EKG, particularly in the presence of pericardial effusion. In addition, hypothyroidism alters the LDH isoenzyme pattern and elevates total CPK levels although most of the elevation is in the MM band (from skeletal muscle). Patients with myxedema coma may have hypotension because of decreased intravascular volume, and cardiovascular collapse and shock may occur late in the course. If at all, the latter characteristically responds only when both a vasopressor drug such as dopamine and thyroid hormone are given. Moreover, cardiac contractility is impaired, leading to reduced stroke volume and cardiac output, but frank congestive heart failure is rare. Reduced stroke volume in severe cases may also be due to the cardiac tamponade due to pericardial effusion. The cardiac enlargement may be due to either ventricular dilatation or pericardial effusion. Rarely, the pericardial fluid is rich in mucopolysaccharides and/or lipids and on aspiration may present a colloidal gold appearance.

Gastrointestinal Manifestations

The gastrointestinal tract in myxedema may be marked by mucopolysaccharide infiltration and edema of the muscularis as well as neuropathic changes leading to gastric atony, impaired peristalsis, and even paralytic ileus. The presence of gastric atony [19] may be problematic for the absorption of oral medications. A neurogenic oropharyngeal dysphagia has been described that is associated with delayed swallowing and may account for the predisposition to aspiration and risk of aspiration pneumonia [20]. Ascites is not uncommon and has been documented in one report of 51 cases [21]. Another potential compli-

cation is gastrointestinal bleeding, secondary to an associated coagulopathy [22]. It is important to recognize the underlying mechanisms of these acute gastrointestinal complications in order to avoid unnecessary surgeries for an mistakenly apparent "acute abdomen" [23].

Renal and Electrolyte Manifestations

Hyponatremia and a decreased glomerular filtration rate and renal plasma flow are rather consistent findings among patients with myxedema coma. The hyponatremia is due to the inability to excrete a water load, caused both by increased serum antidiuretic hormone [24] and impaired water diuresis caused by reduced delivery of water to the distal nephron [25]. Urinary sodium excretion is normal or increased, urinary osmolality is high relative to plasma osmolality, and there may be bladder atony, with retention of large residual urine volumes. Hyponatremia is a common finding observed in patients with myxedema coma and it alone may cause lethargy and confusion, and depending upon its duration and severity, hyponatremia will add to altered mental status, and if severe, could be responsible for the coma. Renal failure may occur due to underlying rhabdomyolysis with extremely high creatine kinase levels [26–29].

Hematological Manifestations

Patients with myxedema coma are predisposed to severe infections, including sepsis, due to granulocytopenia and a decreased cell-mediated immune response. They may also present with a microcytic anemia secondary to hemorrhage, or a macrocytic anemia due to vitamin B12 deficiency, which may also worsen the neurological state. In contrast to the tendency to thrombosis seen in mild hypothyroidism, severe hypothyroidism is associated with a higher risk of bleeding due to coagulopathy related to an acquired von Willebrand syndrome (type 1) and decreases in factors V, VII, VIII, IX, and X [30]. The von Willebrand syndrome is reversible with T4 therapy [31]. Another cause of bleeding may be disseminated intravascular coagulation associated with sepsis.

Hypothermia

Hypothermia may be dramatic (<80 °F) but may be absent in about one-fourth of patients, possibly because of underlying infection masking the hypothermia. Indeed, because hypothermia may be the first clue to the diagnosis, the possibility of myxedema coma should be entertained in any unconscious patient without fever in spite of an infection. The presence of hypoglycemia is likely to decrease body temperature further. Hypothermia has prognostic significance, with survival correlating with the presenting body temperature, and those patients with a core temperature below 90 °F are likely to do poorly [32, 33].

Neuropsychiatric Manifestations

Patients with myxedema coma may have a history of lethargy, depression, slowed mentation, poor memory, cognitive dysfunction, or even psychosis ("myxedema madness"). Focal or generalized seizures caused by CNS dysfunction, hyponatremia, or hypoxemia due to reduced cerebral blood flow may be seen in one-fourth of patients [34].

Infections

An impaired host response to infection may be in part responsible for the high mortality rate in myxedema coma. As mentioned above, detection of infection is often clouded by the presence of hypothermia. Rather, the presence of normothermia in a myxedematous patient should alert the physician to the possibility of an underlying infection. Similarly, the patients often perspire little, and their pulse rate tends to be slow, both of which may cause the physician to overlook the possibility of underlying infection. Pulmonary infections may aggravate or even cause hypoventilation, and susceptibility to these infections may be increased

because of aspiration caused by neurogenic dysphagia or seizures [34]. In one retrospective series, 12/23 (52%) patients died of sepsis, and sepsis was highly correlated with mortality [32]. Given that an infection may be difficult to recognize, initiation of empiric broad spectrum antibiotic therapy may be justified. Alternatively, a very thorough search should be undertaken with cultures of all body fluids with antibiotics only given when evidence of infection is present.

Diagnosis

It should be feasible to make this diagnosis on purely clinical grounds. In sum, the typical myxedema coma patient is a woman in the later decades of her life who may have a past history of thyroid disease and who is admitted to hospital typically in winter with pneumonia. Physical findings could include bradycardia, macroglossia, hoarseness, delayed reflexes, dry skin, general cachexia, hypoventilation, and hypothermia, commonly without shivering. Laboratory evaluation may indicate hypoxemia, hypercapnia, anemia, hyponatremia, hypercholesterolemia, and increased serum lactate dehydrogenase and creatine kinase. On lumbar puncture there is increased pressure and the cerebrospinal fluid will have high protein content. Although an elevated serum TSH concentration is the most important laboratory evidence of the diagnosis, the presence of severe complicating systemic illness or treatment with drugs such as dopamine, dobutamine, or corticosteroids may serve to reduce the elevation in TSH levels [35, 36]. Moreover, there may also be a pituitary cause for the hypothyroidism, in which case an increased TSH would not be found, but treatment should not be delayed while awaiting the results of TSH measurement. A lumbar puncture done for the evaluation of coma will reveal little other than high protein content in the cerebrospinal fluid. Nearly all patients with myxedema coma have very low serum total and free T4 and triiodothyronine (T3) concentrations, and associated nonthyroidal illness will contribute to the observed reduction in serum T3 concentration. In an attempt to objectify the diagnostic process, Popoveniuc et al. [37] developed a scoring system based upon signs and symptoms at presentation, but it has yet to be validated (Figs. 1 and 2).

Fig. 1

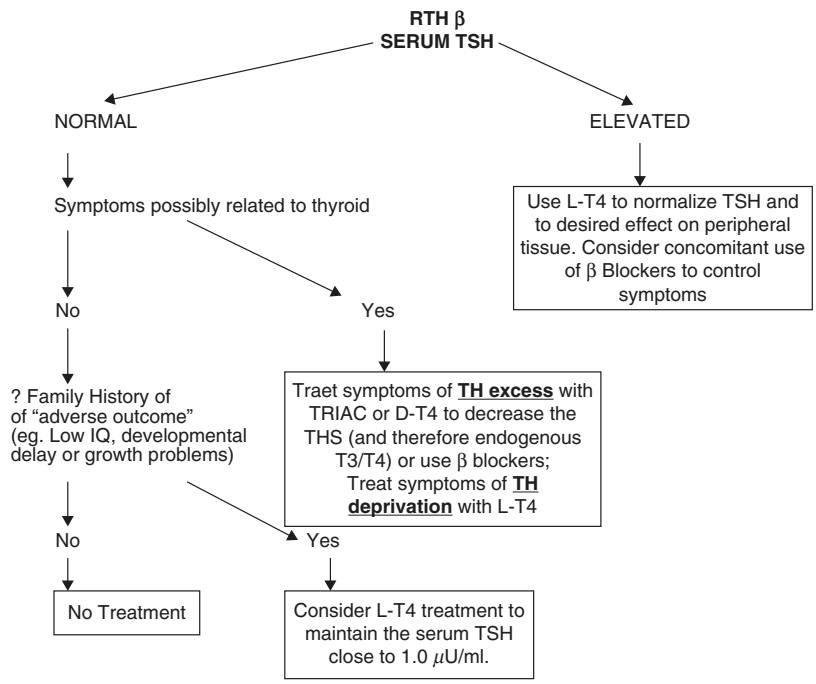
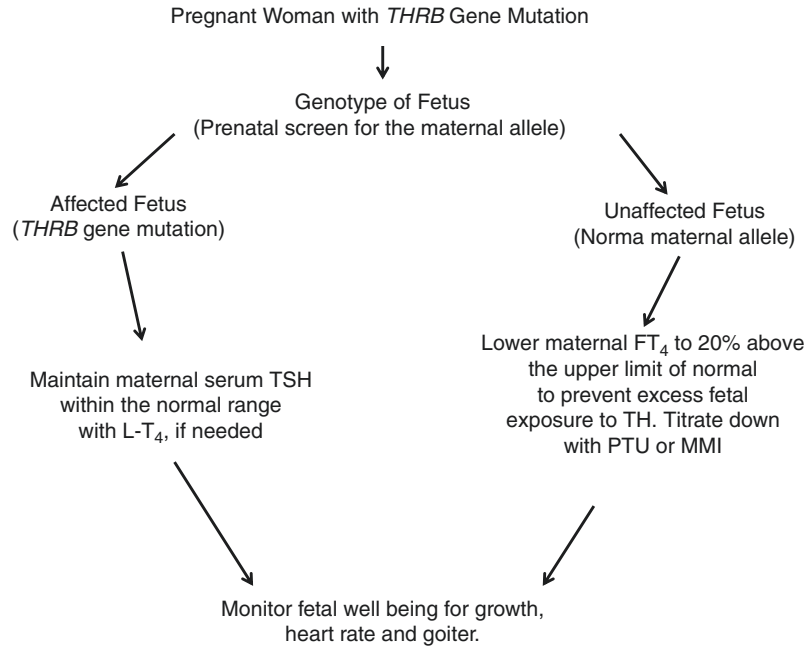


Fig. 2 Caption



Treatment

In view of the high mortality rate among patients with myxedema coma, treatment should be instituted promptly as soon as the diagnosis is strongly suspected. Treatment with thyroid hormone alone without addressing all of the physiologic and metabolic derangements described herein is inadequate therapy, and will likely contribute to a poor prognosis. All patients should be admitted to an intensive care unit so that their pulmonary and cardiac status can be monitored continuously. A central venous pressure line should be used to monitor volume repletion therapy, particularly in elderly patients or those with cardiac disease.

Ventilatory Support

The patient's comatose state is perpetuated by hypoventilation, with CO_2 retention and respiratory acidosis. The single most important supportive measure is the maintenance of an adequate airway because of the high mortality rate associated with the inexorable respiratory failure. As indicated above, hypoventilation is an important component of myxedema coma and is a common cause of death in these patients. Respiratory function should be evaluated by assessment of both pulmonary functions (blood gas measurements), ruling out the possibility of pulmonary infection and ensuring that there is no airway obstruction by macroglossia or myxedema (edema) of the larynx. In comatose patient, mechanical ventilation is usually required during the first 36–48 h (especially those in whom drugs were the precipitant of the hypoventilation), but in some patients it may be necessary to continue assisted ventilation for as long as 2–3 weeks [34]. During the period of ventilatory support, arterial blood gases should be measured frequently, and it may be necessary to insert an endotracheal tube or even perform tracheostomy to ensure adequate oxygenation. The endotracheal tube should not be removed until the patient is fully conscious and there is evidence that the removal will be successful. The hypercapnia may be rapidly relieved with mechanical ventilation, but the hypoxia tends to persist possibly due to shunting in non-aerated lung areas [38]. It is obviously important to maintain an open upper airway for ventilatory support in order to relieve or prevent

hypoxemia and hypercapnia, and it is advisable not to extubate the patients prematurely and to wait until the full consciousness is attained.

Hyponatremia

Low serum sodium may cause a semicomatose state or seizures even in euthyroid patients, and the very severe hyponatremia (105–120 mmol/L) in profound myxedema is likely to contribute substantially to the coma in these patients. While comatose patients must be given some saline (and glucose) intravenously to replace daily losses, the volume should be limited in those patients with mild-to-moderate hyponatremia. Mortality rates in critically ill patients with symptomatic hyponatremia have been reported to be 60-fold higher than in patients without hyponatremia [39]. The appropriate management of severe hyponatremia often requires administration of a small amount of hypertonic saline (50–100 mL 3% sodium chloride), enough to increase sodium concentration by about 2 mmol/L early in the course of treatment, followed by an intravenous bolus dose of 40–120 mg furosemide to promote a water diuresis [40]. A small quick increase in the serum sodium concentration (2–4 mmol/L) is effective in acute hyponatremia because even a slight reduction in brain swelling results in a substantial decrease in intracerebral pressure [41]. On the other hand, too rapid correction of hyponatremia can cause a very dangerous complication, the osmotic demyelination syndrome. In patients with chronic hyponatremia this complication is avoided by limiting the sodium correction to less than 10–12 mmol/L in 24 h and less than 18 mmol/L in 48 h. After achieving a serum sodium level of >120 mmol/L, restriction of fluids may be all that is necessary to correct hyponatremia. The administration of fluid or saline therapy requires careful monitoring of volume status based on clinical parameters and central venous pressure measurements, especially in patients with significant cardiovascular decompensation. Therapy with a vasopressin antagonist seems reasonable because vasopressin levels are typically elevated in myxedema. The available agents, conivaptan [42, 43] and tolvaptan, have been successfully employed to treat euvolemic and hypervolemic hyponatremia. Conivaptan has been approved for this purpose by the US FDA. Recommendations for conivaptan administration include first administering a loading dose of 20 mg by i.v. infusion over 25–30 min followed by continuous i.v. infusion at a rate of 20 mg per day for an additional 2–4 days. Tolvaptan is initially administered as an oral dose of 15 mg for the treatment of hospitalized patients with euvolemic and hypervolemic hyponatremia in a setting of the syndrome of inappropriate antidiuretic hormone secretion (SIADH), hypothyroidism, adrenal insufficiency, or pulmonary disorders [42]. No data are available on the use of conivaptan in more severe hyponatremia (<115 mEq/L) in hypothyroid patients [43, 44].

Hypothermia

Thyroid hormone must be given to restore body temperature to normal, but its action is slow. Ultimately, treatment with T4 and/or T3 enables restoration of body temperature to normal. Simultaneously, blankets to prevent heat loss or increasing room temperature can be used as additional interventions to keep the patient warm until the thyroid hormone effect is achieved [4]. While very gentle warming of patients with hypothermia with an electric blanket at the lowest heat settings is advisable, too aggressive warming may cause peripheral vasodilatation, which may then lead to hypotension or shock.

Hypotension

Hypotension should also be correctable by treatment with T4 and/or T3. However a hypotensive patient may require additional volume repletion therapy. Fluids may be administered cautiously as 5–10% glucose in 0.5 N sodium chloride if hypoglycemia is present or as isotonic normal saline if hyponatremia is present. An agent such as dopamine might be employed to maintain coronary blood flow, but patients should be weaned

off the pressor as soon as possible because of the risk of a pressor-induced ischemic event. Theoretically, there could be an adverse interaction between vasopressor drugs and thyroid hormone, but this risk is countered by the potential high mortality rate when the hypotension remains refractory to less aggressive therapy. Administration of hydrocortisone (100 mg i.v. every 8 h) is indicated if there is any suspicion of adrenal insufficiency and should be continued until the hypotension is corrected. The typical dosage of hydrocortisone is 50–100 mg every 6–8 h during the first 7–10 days with tapering of the dosage thereafter based upon clinical response and any plans for further diagnostic evaluation. Decreased adrenal reserve has been found in 5–10% of patients on the basis of either hypopituitarism or primary adrenal failure accompanying Hashimoto's disease (Schmidt syndrome). The other rationale for the treatment with corticosteroids is the potential risk of precipitating acute adrenal insufficiency due to the accelerated metabolism of cortisol that follows T4 therapy. The clinician should be aware of signs and symptoms signaling coexisting adrenal insufficiency such as hypotension, hypothermia, hypoglycemia, hyperkalemia, and hyponatremia.

Glucocorticoid Therapy

As indicated above, one relatively uncommon cause of myxedema coma is on the basis of central hypothyroidism, and such patients may also manifest corticotropin (ACTH) deficiency and may therefore benefit from corticosteroid therapy. Similarly, some patients with primary hypothyroidism due to Hashimoto's thyroiditis may have concomitant primary adrenal insufficiency (Schmidt syndrome). Whether the hypoadrenalism is primary or secondary, it can aggravate clinical features in myxedema coma to include hypotension, hypoglycemia, hyponatremia, hyperkalemia, and azotemia. Nevertheless, while most patients reported have been covered with empiric corticosteroids, most patients with myxedema coma have normal serum cortisol concentrations, although their ACTH and cortisol responses to stress may be slightly impaired [45, 46]. Another oft stated rationale for administering corticosteroids is based on earlier observations that thyroid hormone therapy increases cortisol metabolism and clearance. In any case, there is no downside to short-term glucocorticoid therapy which can then be discontinued when the patient is alert and stable. Hydrocortisone usually is given in an i.v. dosage of 50 to 100 mg every 6 to 8 h for a few days, after which it is tapered and discontinued.

Thyroid Hormone Therapy

While it might seem quite basic and straightforward that patients with the profound hypothyroidism of myxedema coma need thyroid hormone, the method to provide that thyroid hormone remains controversial. The questions relate to which thyroid hormone preparation to give and how to give it (dose, frequency, and route of administration). The optimum treatment remains uncertain, because of the scarcity of clinical studies and obvious difficulties with performing controlled trials. There is a necessity to balance the need for quickly attaining physiologically effective thyroid hormone levels against the risk of precipitating a fatal tachyarrhythmia or myocardial infarction. The main uncertainty is whether to administer T4 alone, with conversion to T3 being dependent on the patient's endogenous deiodinase activity, or to directly administer both T4 and T3. Parenteral preparations of either T4 or T3 are available for intravenous administration. Although oral forms of either T3 or T4 can be given by nasogastric tube in the comatose patient, this route is fraught with risks of aspiration and uncertain absorption, particularly in the presence of gastric atony or ileus. Different approaches to treatment are based on balancing concerns for the high mortality of untreated myxedema coma and the obvious need for attaining effective thyroid hormone concentrations in different tissues fairly rapidly, against the risks of high-dose thyroid hormone therapy, which may include atrial tachyarrhythmias or myocardial infarction. Because of the rarity of myxedema coma and the

paucity of studies of the effects of treatment, the optimal approach to thyroid hormone therapy remains uncertain and several different approaches have been used.

Advocates of administering T4 alone point out that it provides a steady and smooth, but rather slow, onset of action with low risk for adverse effects. Conversely, the onset of action of T3 is more rapid, but its serum (and probably tissue) concentrations fluctuate more between doses. In the very sick patient with a "low T3 syndrome" superimposed upon true hypothyroidism, serum T3 levels may not be very readily restored to the reference range, making the use of T4 therapy less problematic, because serum measurements of free T4 are easier to interpret as the values vary less between doses. In either case, serum TSH values should provide information about the impact of treatment at the tissue level, with the caveat that the critically ill patient may exhibit TSH suppression as part of the nonthyroidal illness syndrome. Preparations of T4 for parenteral administration are available in vials containing 100 and 500 μg. A high dose, given as a single intravenous bolus dose, was popularized by a report suggesting that replacement of the entire extrathyroidal pool of T4 (usually 300 to 600 μg) was desirable to restore near-normal hormonal status as rapidly as possible, with the pool then maintained by administration of 50–100 μg daily given either i.v. or orally [47]. With this regimen, serum T4 concentrations increase abruptly to supranormal values and decrease to within normal range in 24 h. As the administered T4 is distributed throughout the extracellular and then intracellular spaces, serum T3 levels increase slightly, and serum TSH levels decrease substantially. In one of the prospective studies examining the treatment of myxedema coma, Rodriguez et al. employed a T4 loading dose in 11 patients as suggested by Holvey et al. [47]. Mortality correlated with APACHE score and the severity of underlying systemic illness with 4/11 patients dying, only one of whom had been administered high doses of T4 (500 μg loading dose). The best outcomes were in younger patients with lower APACHE and Glasgow Coma Scale scores. Although the importance of extrathyroidal T4 conversion to T3 was not known when this regimen was proposed, the approach of Holvey et al. has proved effective in other centers. Twenty-four patients with myxedema coma or severe hypothyroidism reported from Germany were treated initially with T4 in doses ranging from 25 to 500 μg with six deaths [49]. Dutta et al. [32] noted no difference in survival between patients given oral vs. i.v. T4.

The rate of conversion of T4 to T3 is reduced in many systemic illnesses (the euthyroid sick or low T3 syndrome) [36], and hence T3 generation may be reduced in myxedema coma as a consequence of any associated illness (hypothyroid sick syndrome). As a consequence, some clinicians suggest that small supplements of T3 should be given along with T4 during the initial few days of treatment, especially if obvious associated illness is present. When therapy is approached with T3 alone, it may be given as a 10 to 20 μg bolus followed by 10 μg every 4 h for the first 24 h, dropping to 10 μg every 6 h for days 2–3, by which time oral administration should be feasible. T3 has a much quicker onset of action than T4, and increases in body temperature and oxygen consumption may occur 2–3 h after i.v. T3, compared with 8–14 h after i.v. T4 [50]. The other advantage of T3 is the fact that it crosses the blood/brain barrier more rapidly than T4, which may be particularly important in patients with profound neuropsychological symptoms [51]. One possible benefit of T3 is exemplified by a case report of a patient with myxedema coma and cardiogenic shock who responded to T3 but not T4 therapy [52]. On the other hand, treatment with T3 alone is associated with large and unpredictable fluctuations in serum T3 levels, and high serum T3 levels during treatment have been associated with fatal outcomes [49]. Significant clinical improvement may be seen within 24 h with T3, but the more rapid action of T3 may be associated with a higher risk of adverse cardiovascular actions, and in one report high serum T3 concentrations during treatment with T4 alone were associated with fatal outcome in several patients [49]. In another series of eight patients, two of the three

patients treated with high-dose T4 died, whereas the other five who were treated with smaller doses of T4 or T3 survived [33]. The authors reviewed 82 cases from the literature and found that advanced age, high-dose T4 therapy, high-dose T3 therapy, and cardiac complications were associated with mortality. They concluded that a 500 g dose of T4 should be safe in younger patients, but lower doses are safer in the elderly.

Consequently, our approach to therapy attempts to take all of these issues into account and to prudently administer both T4 and T3 [53]. The T4 is given intravenously in a dose of 4 μg/kg lean body weight (or about 200–250 μg), followed by 100 μg 24 h later and then 50 μg daily, either i.v. or orally, as appropriate. The dose subsequently is adjusted on the basis of clinical and laboratory results, as in any other hypothyroid patient. With respect to T3, the initial intravenous dose is 10 μg, and the same dose is given every 8–12 h until the patient can take maintenance oral doses of T4. As little as 2.5 μg, T3 has been noted to result in clinical improvement [53]. Other experts prefer to give T4 alone, in a loading dose of 400–500 μg followed by 100 to 200 μg daily as described above, and give T3 in 24 to 48 h only if there is a suboptimal response to T4. No general guide to treatment can take into account all the factors that might affect sensitivity to thyroid hormone, such as age, intrinsic cardiovascular function, and neuropsychiatric status. Hence, patients should be monitored closely before each dose of thyroid hormone is administered. In addition to the specific treatments considered above, attention should be given to any comorbid conditions, recognizing that drug dosages may need to be modified because hypothyroidism can result in altered drug distribution and metabolism. With aggressive comprehensive treatment, most patients with myxedema coma should recover. Better, however, that it be prevented, by earlier recognition of hypothyroidism. Dutta et al. [32] noted more severe clinical manifestations in patients who had discontinued T4 therapy in contrast to de novo cases is another reason to ensure that patients with hypothyroidism do not discontinue therapy for whatever reason.

General Supportive Measures

In addition to the specific therapies outlined, other treatments will be indicated as in the management of any other elderly patient with multisystem problems. This might include the treatment of underlying problems such as infection, congestive heart failure, diabetes, or hypertension. The dosage of specific medications (e.g., digoxin for congestive heart failure) may need to be modified based on their altered distribution and slowed metabolism in myxedema.

Prognosis

Even with this vigorous therapy, the prognosis for myxedema coma remains grim, and patients with severe hypothermia and hypotension seem to do the worst. In the past, the mortality rate was as high as 60–70%, and it has been reduced to 20–25% with the advances in intensive care management (i). Several prognostic factors may be associated with a fatal outcome (ii, iii, iv) and include older age, persistent hypothermia or bradycardia, lower degree of consciousness by Glasgow Coma Scale, multi-organ impairment indicated by an APACHE II score of more than 20, or SOFA score more than 6. The most common causes of death are respiratory failure, sepsis, and gastrointestinal bleeding. In sum, myxedema coma is a true medical emergency that requires a multifaceted approach to treatment in a critical care setting. Early diagnosis and prompt treatment, with meticulous attention to the details of management during the first 48 h, remain critical for the therapy to result in an optimal outcome.

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Heart in Hypothyroidism

Bernadette Biondi and George J. Kahaly

Hemodynamic Alterations and Cardiovascular Complications in Overt Hypothyroidism

Hypothyroidism is characterized by a low cardiac output [1–6] (Table 1). In severe hypothyroidism, cardiac output is decreased as a result of a decrease in both stroke volume and heart rate [3–6]. Circulation time is increased in hypothyroid patients, and an enhanced arterial resistance and reduced venous resistance limits the return of blood to the heart [1–6]. Buccino reported that the cardiac papillary muscle obtained from animals with hypothyroidism showed a depression of the force velocity curve and reduced rate of tension development, indicating significant contractile abnormalities [7]. The first study to assess systolic function in patients with severe hypothyroid was performed by Crowley and Ridgway; the results proved that systolic time intervals are characterized by prolongation of the pre-ejection period and reduction of the left ventricular ejection time with a resultant increase in the pre-ejection period/left ventricular ejection period (PEP/LVET) ratio [8] (Fig. 1). These abnormalities were reversible with physiologic thyroxine replacement, and, during therapy, delta PEP was inversely correlated with serum thyroxine and directly correlated with serum thyrotropin [8] (Fig. 1).

Preload is largely determined by total blood volume and venous return as well as the contractile activity of the atrium and the filling property of the ventricle [3, 6]. Cardiac preload is decreased in hypothyroid patients due to the impaired diastolic function and the decreased blood volume [6, 9] (Table 1). A prolonged isovolumic relaxation time of the left ventricle was first reported by Manns in 1976; the index of relaxation of the cardiac muscle was identified by means of combined apex cardiography and phonocardiography [9]. Subsequently, an impaired left ventricular diastolic function, characterized by slowed myocardial relaxation and impaired early ventricular filling, has been confirmed in hypothyroid patients by Doppler echocardiography [10]. In addition, tissue Doppler imaging has revealed changes in myocardial time intervals in several segments in healthy hypothyroid patients [11].

Cardiac manifestations and clinical features in hypothyroid patients are dyspnea on exertion, easy fatigability, decreased exercise tolerance, and hypertension (Table 2) [3–6]. These findings may be explained by the reduced systolic and diastolic functions at rest and during exercise, as demonstrated by radionuclide ventriculography [12, 13]. This technique has provided evidence

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that the peak filling rate is significantly decreased in patients with lower FT4 levels; and the time to peak both emptying rate and filling rate is increasingly prolonged in patients with more severe hypothyroidism [12]. Additionally, left ventricular ejection fraction at rest and during exercise and cardiopulmonary exercise testing are reduced in hypothyroid patients and tend to improve when euthyroidism is reached [12–14]. These data could suggest that left ventricular function is reversibly depressed by thyroid hormone deficiency [12–14]. Thyroid hormones modulate the strength of both respiratory and skeletal muscles and affect regulatory mechanisms of adaptation to incremental effort. In hypothyroidism, cardiovascular exercise testing and analysis of respiratory gas exchange demonstrate low efficiency of cardiopulmonary function as well as impaired

Table 1 Hemodynamic changes in hypothyroidism

Myocardial contractility	↓
Systemic vascular resistance	↑
Circulation time	↑
Diastolic blood pressure	↑
Arterial stiffness	↑
Cardiac output	↓
Cardiac index	↓
LV stroke volume	↓
LV systolic function	↓
LV diastolic function	↓
Exercise tolerance	↓

Table 2 Cardiac manifestations in untreated persistent hypothyroidism

• Bradycardia
• Diastolic dysfunction
• Diastolic hypertension
• Dyspnea on effort
• Endothelial dysfunction
• Dyslipidemia
• Heart failure
• CHD events
• CHD mortality
• Pericardial and pleural effusion
• Ventricular arrhythmias

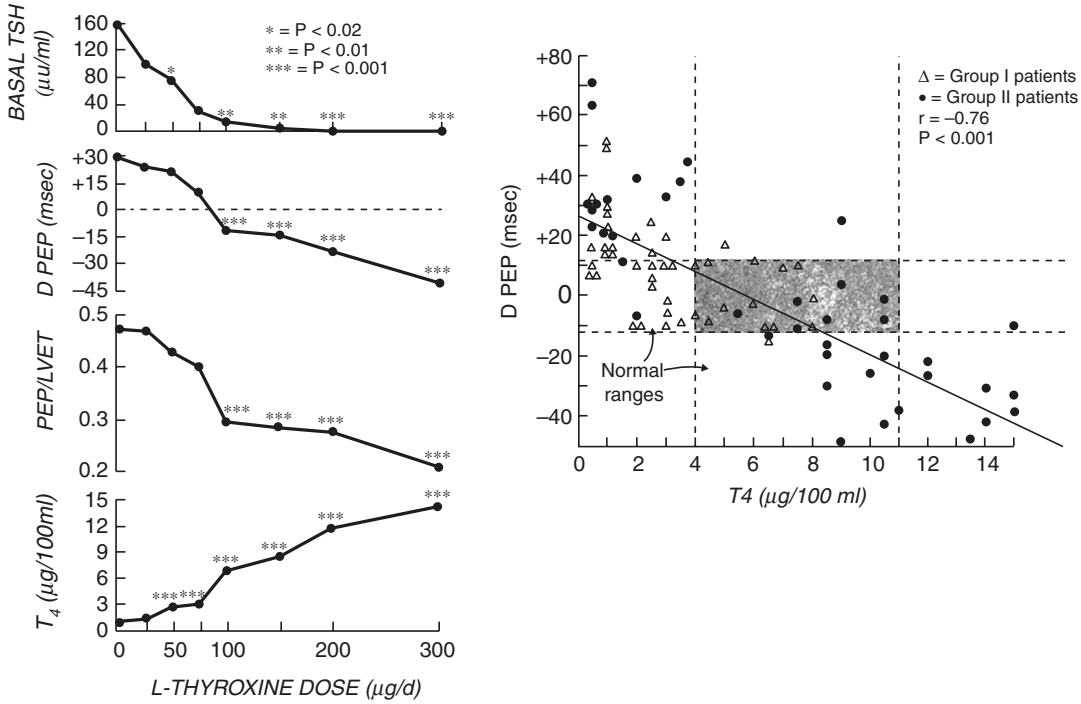

Fig. 1 Relation of L-thyroxine dose to △PEP, PEP/LVET, serum total thyroxine (T_4), and serum thyrotropin (TSH) concentration [8]

chronotropic, contractile, and vasodilator reserves, which are reversible when euthyroidism is restored [14]. During exercise, the increment of minute ventilation and oxygen pulse are significantly lower in thyroid dysfunction versus euthyroidism [14]. Especially in older patients with thyroid disease, markedly reduced workload, both at the anaerobic threshold and at maximal exercise, is observed [14]. In hypothyroidism, inadequate cardiovascular support and mitochondrial oxidative dysfunction during exercise mostly cause intracellular acidosis. These abnormalities partly explain why subjects with hypothyroidism are intolerant to exertion [14].

The peripheral circulation in hypothyroidism is characterized by an increased vascular resistance and a prolonged circulation time [3–5, 15–17]. Afterload is increased in patients with hypothyroidism as a result of increased systemic vascular resistance and arterial stiffness [15–17] and is one of the major factors determining myocardial oxygen consumption [18] (Table 1). The increase in cardiac afterload can account for the finding that the hypothyroid myocardium is energy inefficient despite the low level of overall oxygen consumption [18].

Renal perfusion, measured by glomerular filtration, is decreased in hypothyroidism [19–21]. Although sodium excretion is normal, free water clearance is impaired and can lead to hyponatremia [19–21]. Total-body albumin distribution is expanded in myxedema, in keeping with the development of high-protein-content effusions in many body cavities. Pericardial effusions can occur in severe hypothyroidism and occasionally may be so large to cause cardiomegaly on routine chest X-ray [3, 6]. Echocardiography demonstrates small-to-moderate effusions in about 30% of overtly hypothyroid patients. Cardiac tamponade is very rare, and the pericardial effusion usually disappears a few weeks or months after initiation of thyroid hormone replacement therapy [3–6].

Hypothyroidism may increase the risk for atherosclerosis by several mechanisms [22]. Diastolic hypertension in conjunction with dyslipidemia and increased arterial stiffness are well-recognized risk factors for the development of atherosclerosis in overt hypothyroidism [3–6, 22]. Severe hypothyroidism is associated with lipid abnormalities, especially increased total and LDL cholesterol, triglycerides, and lipoprotein (a) [22, 23]. Coagulation abnormalities might be additional risk factors for atherosclerosis in thyroid hormone deficiency [22].

Cardiovascular Function in Subclinical Hypothyroidism

Important changes in cardiac structure and function have been reported in patients with subclinical hypothyroidism, whose severity depends on the degree and duration of thyroid hormone deficiency [24–28]. The impairment of left ventricular diastolic function, which is characterized by slowed myocardial relaxation and impaired ventricular filling, represents the most consistent cardiac abnormality reported in patients with subclinical hypothyroidism [29, 30] (Fig. 2). Moreover, an impaired systolic function has been identified with sensitive techniques such as pulsed tissue Doppler echocardiography and cardiac magnetic resonance imaging [31, 32]. Left ventricular systolic and dia-

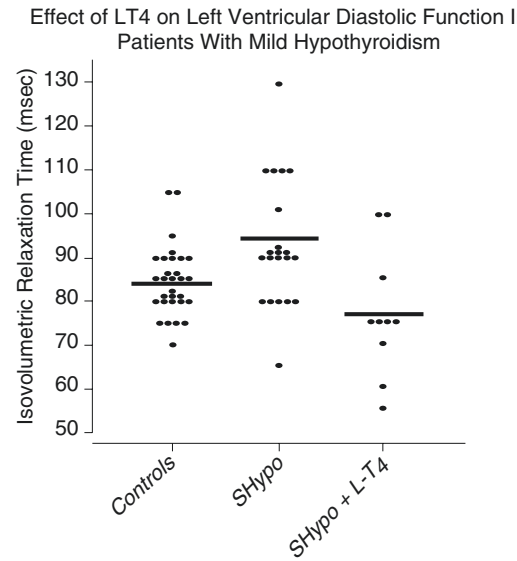

Fig. 2 Isovolumetric relaxation time (IRT) in controls and in patients with SHypo before (SHypo) and after L-T4 replacement therapy (SHypo + L-T4) [29]

stolic dysfunction on effort has been documented by radionuclide ventriculography in SHypo patients in comparison with euthyroid controls [32]. The finding of impaired systolic and diastolic function during exercise might have clinical implications in SHypo patients similar to those that occur in overt disease [33].

Similar to overt disease, SHypo can impair vascular function by inducing an increase in systemic vascular resistance and arterial stiffness and by altering endothelial function, thereby potentially increasing the risk of atherosclerosis and coronary artery disease (CHD) [34–37].

More severe cardiovascular and metabolic adverse effects have been reported in SHypo patients with serum TSH >10 mIU/L [24–28, 38]. The lipid pattern is particularly altered in smokers and in insulin-resistant SHypo subjects [38, 39]. Data on the link between SHypo and homocysteine, high-sensitivity C-reactive protein, and coagulation parameters are conflicting and require additional studies to clarify the potential role of these "nontraditional" cardiovascular risk factors in increasing the cardiovascular risk in SHypo [24–28].

Heart Failure and Overt and Subclinical Hypothyroidism

Cardiac symptoms are dominant in the clinical presentation of patients with severe and long-standing hypothyroidism with the occurrence of bradycardia, congestive heart failure, (HF) and pericardial and pleural effusion [3–6] (Table 2). Chronic hypothyroidism in adult rats induces maladaptive changes in the shape of myocytes with the development of HF [40]. Experimental studies have demonstrated that hypothyroidism causes cardiac atrophy, due to decreases of α-MHC expression and increases of β-MHC expression [41]. Moreover, it leads to chamber dilatation and impaired myocardial blood flow [40, 41]. However, the administration of replacement doses of L-thyroxine reduces myocyte apoptosis and can improve cardiovascular performance and ventricular remodeling in experimental hypothyroidism [1, 2].

In humans, significant changes in myocardial gene expression (α-MHC and phospholamban) were documented in hypothyroid patient with dilated cardiomyopathy, by measuring the mRNA extracted from endomyocardial biopsy specimens, before and after thyroxine replacement therapy [42]. In this patient, the administration of thyroid hormone and the restoration of euthyroidism produced an increase of alpha-myosin heavy chain gene expression with a trend toward the beta- to alpha-myosin heavy chain shift leading to an improvement in cardiac function [42]. This study represents the first evidence in humans that replacement therapy with L-thyroxine may reverse hypothyroid cardiomyopathy by affecting myocardial gene expression [42].

Recent data suggest that thyroid hormone deficiency may be responsible for an increased risk of heart failure events even in patients with subclinical hypothyroidism [43–45].

In the Cardiovascular Health Study, Rodondi and co-workers evaluated 3.065 adults of 65 years of age or older in order to identify the risk of HF over 12 years of follow-up [44]. They performed a routine echocardiography over 6 years and showed that participants with TSH levels of 10 mIU/L or greater had a higher peak early ventricular filling velocity (E) (after adjusting for age, gender, and systolic blood pressure) which was associated with incident HF (HR 1.14 for each 0.1 m/s increment, 95% CI 1.09–1.18 $p < 0.001$) [44]. During the follow-up, patients with TSH of 10 mIU/L or greater had a higher risk of HF events with low ejection fraction compared to euthyroid participants (80% vs. 45% $p = 0.08$; HR 1.88; CI 1.05–3.34) [44]. Conversely, the risk of CHF was not increased among older adults with TSH levels between 4.5 and 9.9 mIU/L [44]. These results argue that subclinical hypothyroidism with TSH ≥10 mIU/L represents an important risk factor for HF in older adults. These findings have been recently confirmed by a meta-analysis performed on six prospective cohort studies including 2068 patients with subclinical hypothyroidism. In this study, the Thyroid Studies Collaboration group performed a pooled analysis of individual participant data with available thyroid function tests

and subsequent follow-up of HF events [45]. The pooled data were stratified according to age, sex, gender, race, TSH levels, and preexisting cardiovascular disease and HF. The final risk of HF events was significantly increased in patients with TSH levels ≥10 mIU/L (HR 1.86; CI 1.27–2.72) compared to euthyroid controls [45]. Interestingly, the increased risk of HF in adults persisted after excluding patients with preexisting HF or atrial fibrillation (AF) [45]. Further adjustments for cardiovascular risk factors and other potentially confounding risk factors for HF did not significantly change the association with HF events [45]. Moreover, the risk of HF was increased after excluding participants using thyroid medications (mainly T4 replacement) at baseline and during periods of follow-up [45]. Other studies have even suggested that SHypo may be a risk factor for cardiac death in patients with preexisting chronic heart failure [46]. Hypothyroidism may frequently coexist in patients with chronic HF, with a prevalence of about 18% in all patients with HF. The onset of hypothyroidism may exacerbate progression of HF in cardiac patients [47, 48]; even mild or SHypo was independently associated with a greater likelihood of HF progression in patients with chronic HF [47, 48].

These results may explain why the American College of Cardiology guidelines for HF published in 2010 recommended screening with serum TSH of all newly diagnosed cases of HF [49].

Coronary Artery Disease Events and Mortality in Overt and Subclinical Hypothyroidism

Clinical and autopsy studies have shown that coronary artery disease frequently occurs and may progress more rapidly in hypothyroid patients [22, 50]. An increased risk of coronary heart disease events and mortality has been reported in young patients affected by SHypo [51]. A meta-analysis by Rodondi and the Thyroid Studies Collaboration assessed the risk of CHD events, CHD mortality, and total mortality in SHypo patients [52]. The risk of CHD events and CHD deaths was examined in 25.977 participants from seven prospective cohort studies. The results were analyzed in relation to age, sex, race, TSH concentrations, and preexisting cardiovascular diseases. The severity of SHypo was stratified according to three categories of TSH concentration (4.5–6.9, 7.0–9.9, and 10.0–19.9 mIU/L). The risk of CHD events and mortality from CHD increased with higher TSH concentrations. In age- and sex-adjusted analyses, the HR for CHD events was 1.89 (95% CI, 1.28–2.80) for a TSH level of 10–19.9 mIU/L [52]. The resulting HR for CHD mortality was 1.58. Risks did not significantly differ by age, sex, or preexisting cardiovascular diseases [52]. This analysis demonstrates a significant trend of increased risk of both CHD events and mortality at higher serum TSH concentrations, particularly in participants with a TSH level of 10 mIU/L or greater [52].

On the contrary, total mortality was not increased among participants with SHypo, and the results were similar after further adjustment for traditional cardiovascular risk factors [52].

Benefit of Treatment with Replacement Doses of L-T4 on Heart Failure and Coronary Heart Disease in Overt Hypothyroidism (Table 3)

The administration of thyroid hormone benefits the hypothyroid heart because it improves myocardial contractility and diastolic function and reduces cardiac afterload, which is the major determinant of oxygen consumption [6, 24–26]. The administration of replacement doses of L-thyroxine can improve cardiovascular performance and ventricular remodeling in experimental hypothyroidism [1, 2, 41, 42, 52, 53]. Some cases of myxedema and congestive heart failure have been reported in old studies, with an improvement in the manifestations of myxedema and heart failure during therapy even with desiccated thyroid [52]. The electrocardiogram in hypothyroidism is characterized by sinus brady-

Table 3 Recommendations to improve the prognosis of patients with hypothyroidism and cardiovascular complications

• Prompt diagnosis of cardiovascular complications in elderly patients and in those with underlying heart disease (ECG, Holter ECG, Doppler echocardiography)
• Restoration of a euthyroid state with L-thyroxine as soon as possible in young patients without underlying cardiac disease
• Hospitalization when heart function does not improve upon restoration of euthyroidism
• Start replacement therapy at a low dose and gradually increase while monitoring the patient's condition in elderly patients with known or suspected coronary artery disease and in patients with underlying heart disease
• Perform bypass procedures or angioplasty before L-T4 therapy in case of unstable angina or worsening or appearance of angina during L-T4 treatment
• Use lower doses of L-T4 in the elderly

cardia, low voltage, prolongation of the action potential duration, and the QT interval [3–6]. This latter condition may predispose hypothyroid patients to ventricular arrhythmias and rarely to acquired torsade de pointes [6]. However, even ventricular arrhythmias may improve or completely resolve after L-T_4 treatment [6].

The hypometabolic state associated with hypothyroidism benefits the ischemic myocardium by lowering oxygen demand [18]. This could explain data showing that thyroid hormone therapy improves myocardial efficiency and leads to regression of angina symptoms in hypothyroid patients [54]. In a large retrospective study, replacement therapy with thyroid hormone leads to a decrease in angina frequency more often than to a worsening (38% vs. 16%) [54]. However, L-T4 may trigger severe angina, myocardial infarction (MI), and arrhythmias in presence of asymptomatic underlying ischemic heart disease and severe hypothyroidism [55]. Coronary revascularization may be necessary to tolerate L-thyroxine in some patients with longstanding and untreated hypothyroidism associated with severe atherosclerosis [6, 55, 56].

Consequently, full replacement doses of L-thyroxine can be safely given to young patients with overt hypothyroidism without underlying cardiac disease [6, 56]. In contrast, in elderly patients with known or suspected coronary artery disease and in patients with underlying heart disease, replacement therapy should be started at a low dose and gradually increased while monitoring the patient's condition [6, 56]. Resting and exercise electrocardiograms often show ischemic-like ST segment and T-wave changes in hypothyroidism, but these alterations are not a useful test for the evaluation of an associated coronary artery disease and may disappear after replacement therapy [6]. Therefore, in case of unstable angina, or worsening or appearance of angina during L-T_4 treatment, the possibility of significant occlusive coronary artery disease should be accurately assessed [6]. If patients are not candidates for percutaneous intervention, coronary artery bypass grafting can be performed even in the setting of overt hypothyroidism [6]. Controlled studies have demonstrated that it is safe to perform bypass procedures or angioplasty before L-T4 therapy [6].

The optimal replacement dose of L-thyroxine should take into account the age of patients and the cause of hypothyroidism. Indeed, the L-thyroxine dosage should be lower in the elderly and higher in patients with overt hypothyroidism, particularly those who have undergone thyroidectomy or prior iodine treatment for Graves' disease [56].

Benefit of Treatment with Replacement Doses of L-T4 on Heart Failure and Coronary Heart Disease in Subclinical Hypothyroidism

A few randomized placebo-controlled studies have demonstrated an improvement of the cardiovascular function in patients with mild (TSH ≤10 mIU/L) and more severe subclinical hypothyroidism (TSH ≥ 10 mIU/L) after replacement doses of L-thyroxine [24–28]. Diastolic dysfunction is reversible after replacement therapy [29, 57, 58] Moreover, vascular function (SVR, arterial stiffness, endothelial function) may significantly improve when euthyroidism is restored [59–63].

In the reanalysis of the Wickham survey on 97 individuals (mean age, 49 years), during a 20-year follow-up, all-cause mortality was significantly lower in L-T4-treated patients with SHypo than in untreated individuals after adjusting for age, gender, and cholesterol levels [64]. The risk of heart failure was significantly lower in thyroxine-treated patients than in untreated patients during the follow-up in the Cardiovascular Health Study [45]. Patients with TSH > 10 mIU/L had an increased risk of HF during the period of L-thyroxine withdrawal than during its use [45]. This supports a potential reduction of the risk of HF with replacement doses of L-thyroxine.

Important meta-analyses [45, 52] provide sufficient evidence to justify the treatment of patients with SHypo having a serum TSH level above 10 mIU/L to reduce the risk of HF and CHD. Therefore, the evidence appears substantive for a beneficial effect of L-T4 replacement therapy in patients with ≥10 mI/L to prevent the risk of HF and CHD events. Prospective randomized controlled studies are necessary to evaluate the potential effects of L-T4 to prevent the risk of HF and CHD and their negative prognosis in patients with subclinical hypothyroidism. Whether or not to treat patients with mild SHypo remains controversial. Mild SHypo may be associated with a greater cardiovascular risk in young and middle-aged people [25–28]. Recently, Razvi and co-workers examined the outcomes of treated individuals with mild SHypo (serum TSH of 5.01–10.0 mIU/L) by analyzing data from the United Kingdom General Practitioner Research Database [65]. They stratified the analyses according to subsequent L-T4 treatment for younger (40–70 years) vs. older (>70 years) patients. For a median follow-up period of 7.6 years, 52.8% of younger and 49.9% of older patients with mild SHypo were treated with a median L-T4 dosage of L-T4 of 75 μg/d (range, 12.5–175 μ/d). After adjustment for baseline cardiovascular risk factors, age, sex, baseline serum TSH levels, and L-T4 use, the number of incident ischemic heart disease events was lower in the L-T4-treated younger group (adjusted HR, 0.61; 95% CI, 0.39–0.95). All-cause mortality was lower in the treated younger group (multivariate-adjusted HR, 0.36; 95% CI, 0.19–0.66), primarily because of a reduction in circulatory and cancer-related deaths [65]. This study clearly indicated that treatment of mild SHypo with L-T4 was associated with better outcomes in ≤70-year-old people with respect to incident fatal and nonfatal ischemic heart disease events and mortality. On the other hand, treatment of older people with SHypo was not associated with similar benefits. However, the major limit of this study is the retrospective design [65].

Two meta-analyses reported that an association between SHypo and CHD existed only in patients younger than 60 years [66, 67] with no evidence of greater risks of CHD events, CHD mortality, and total mortality among pooled participants over 80 years of age [67].

On the other hand, modestly increased serum TSH levels have been associated with longevity in several cross-sectional studies in elderly patients vs. younger controls and in nonagenarians with reported familial longevity [68]. A pattern of decreased mortality in SHypo was observed in the Leiden 85-Plus Study of 558 individuals aged 85 years who had been monitored for 4 years [69]. There was no link between persistent SHypo and cardiovascular mortality (HR, 1.07; 95% CI, 0.87–1.31) in 679 patients with SHypo of at least 65 years of age, enrolled in the recent Cardiovascular Health Study and not taking thyroid preparations [70].

Therefore, although some studies have demonstrated the potential beneficial effects of L-T4 therapy to improve cardiovascular risk in patients with mild SHypo, large randomized controlled studies will be required to assess the importance of this treatment in presence of minimal TSH elevation especially in elderly patients. About 20% of patients with hypothyroidism may be overtreated during replacement therapy, and this suggests that a higher TSH level should be reached during replacement treatment with L-T4, especially in elderly subjects compared to younger people [71]. There is an increased risk of AF in overtreated patients with iatrogenic subclinical hyperthyroidism; this risk may be avoided by normalizing serum TSH according to the age [72].

Conclusion

An early detection and an effective treatment of patients with thyroid dysfunction are essential to improve their cardiovascular prognosis.

Clinical studies have demonstrated that replacement doses of L-thyroxine may improve cardiovascular remodeling and function in patients with hypothyroidism. Replacement doses of L-T4 should be considered in patients with SHypo and TSH > 10 mIU/L to prevent the risk of HF and CHD. However, large, randomized, controlled clinical trials are necessary to assess the benefits of treatment to improve cardiovascular mortality and morbidity in patients with subclinical hypothyroidism and serum TSH ≥ 10 mIU/L.

All of the available trials concur that replacement therapy may improve systolic, diastolic, and vascular function and hence cardiovascular hemodynamic in patients with mild SHypo. These results should be verified in larger randomized trials and longitudinal studies, assessing cardiac morbidity and mortality.

The aim of treatment with L-thyroxine should be to normalize serum TSH levels according to the age of the patients, the etiology of hypothyroidism, and the associated comorbidities. This treatment does not have any adverse effect when the same L-thyroxine product is used and thyroid function is regularly monitored. Overtreatment with L-T4 should be avoided because of the adverse cardiovascular and skeletal consequences of iatrogenic hyperthyroidism in elderly people. Existing evidence suggests that treatment of mild SHypo should probably be avoided in patients older than 60 years of age because there is no definitive evidence that L-T4 treatment will reduce cardiovascular mortality in these patients.

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Part V

Hyperthyroidism

Graves' Disease

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Introduction to Thyrotoxicosis

Traditionally, the term *thyrotoxicosis* is used to signify the clinical syndrome resulting from an increase in circulating thyroid hormone levels, regardless of the source of excess thyroid hormone [1]. On the other hand, *hyperthyroidism* refers to increased biosynthesis and secretion of thyroid hormones. Thus, thyrotoxicosis can be further classified as hyperthyroidism. For instance, thyrotoxicosis can result from increased secretion of thyroid hormone from the thyroid gland, such as seen in Graves' disease. Alternatively, thyrotoxicosis can result from destruction of the gland with release of thyroid hormone or exogenous intake of thyroid hormone, neither of which is associated with increased thyroid hormone synthesis. Proper identification of the causes of thyrotoxicosis is crucial for both diagnostic and treatment purposes.

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Clinical Signs and Symptoms

The typical symptoms of thyrotoxicosis are indicative of the effect of excess thyroid hormone throughout the body and enhanced beta-adrenergic activity. The most commonly reported symptoms are fatigue and weight loss, which occur in 70 and 60% of patients, respectively [2, 3]. About 50% of patients report heat intolerance, tremor, or palpitations. Common physical examination findings include tachycardia, a palpable goiter, and tremor. Table 1 summarizes signs and symptoms of thyrotoxicosis by organ system.

Older patients tend to have fewer symptoms than younger patients, which makes the clinical diagnosis more difficult in the elderly [4]. Furthermore, the elderly are at a higher risk of significant cardiac arrhythmias such as atrial fibrillation, which is encountered in up to 20% of older patients [2, 5]. Cardiovascular manifestations have the most serious consequences, as thyrotoxicosis is associated with increased morbidity and mortality due to the development of heart failure and thromboembolism secondary to cardiac dysrhythmias [5, 6].

Thyroid storm or thyrotoxic crisis is an extreme and life-threatening state of severe thyrotoxicosis that occurs in less than 10% of patients [7]. It is usually precipitated by an acute event such as infection, sepsis, diabetic ketoacidosis, trauma, surgery, myocardial infarction,

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Table 1 Signs and symptoms of thyrotoxicosis by organ system

Organ system	Signs	Symptoms
Cardiovascular	Tachycardia, widened pulse pressure, atrial arrhythmia, congestive heart failure	Palpitations, shortness of breath, chest pain
Gastrointestinal	Weight loss, decreased gastric secretion, mild transaminitis	Hyperdefecation, increased appetite
Hematologic	Anemia, hypercoagulability	
Musculoskeletal	Myopathy, proximal weakness, increased bone turnover, decreased bone density, periodic paralysis (rare)	Weakness, tremor
Nervous/psych	Hyperactivity, tremor, brisk reflexes, seizure (rare)	Fatigue, anxiety, depression, restlessness, emotional lability, psychosis (rare)
Reproductive	Oligomenorrhea and decreased fertility (female), gynecomastia and decreased libido (male)	
Respiratory	Respiratory muscle weakness	Shortness of breath
Skin	Increased perspiration, hair loss and thinning, onycholysis, edema	Flushing, heat intolerance

pregnancy, or labor. The diagnosis is clinical and should be made when there is severe thyrotoxicosis on examination. In one case series, over 60% of patients had cardiac manifestations such as severe tachycardia or atrial fibrillation [8]. Other serious clinical manifestations include hyperpyrexia, psychosis, stupor, coma, and jaundice. Thyroid storm has a mortality of 10% and is considered a true endocrine emergency [9]. A detailed discussion of thyroid storm can be found later in this textbook.

Evaluation of Thyrotoxicosis

Identifying the source of excess thyroid hormone is essential for determining the appropriate therapeutic intervention. The evaluation should begin with a thorough history and physical examination. Focused inquiry can include questions about whether there was a precipitating viral illness, use of thyroid hormone supplements or iodine-containing drugs, a history of radioiodine exposure in the preceding 2–3 months, or high iodine content in the diet. With respect to the physical exam, it is important to evaluate for clinical signs such as tachycardia, elevated systolic blood pressure, irregularly irregular rhythm consistent with atrial fibrillation, tremor, a rapid relaxation phase of reflexes, and diaphoresis. The Means-Lerman scratch is an uncommon mid-systolic murmur best heard over the upper part of the sternum that results from the rubbing of a hyperdynamic pericardium against the pleura [10]. Examination of the thyroid gland should include an assessment of size (normal is under 20 mg, which generally corresponds to each lobe being the size of the end phalanx of the individual's thumb). Additionally, any tenderness to palpation, the presence of thyroid nodules, thyroid bruits, and/or cervical lymphadenopathy should be noted.

Suspected overt thyrotoxicosis should be confirmed by biochemical testing and is usually characterized by a suppressed thyroid-stimulating hormone (TSH) level (<0.01 mU/L) in addition to increased levels of free thyroxine (T4) and triiodothyronine (T3) [11]. Subclinical thyrotoxicosis is present when there is a reduced or low TSH while circulating concentrations of T3 and free T4 are normal. The etiology of thyrotoxicosis may often be apparent based on a clinical presentation consistent with Graves' disease, but when the diagnosis is not obvious, further diagnostic testing should also include measurement of radioactive iodine uptake (RAIU). While TSH is suppressed, the RAIU would be expected to be zero. Generally, values greater than 10% are consistent with hyperthyroidism in a thyrotoxic patient. Technetium scintigraphy (TcO_4) is an alternative imaging modality that provides less total body radiation exposure [11, 12]. While it is effective in detecting both functional and cold thyroid nodules, scintigraphy is less

sensitive than ultrasound for the identification of nodularity [13].

Thyroid ultrasound can measure thyroid volume and blood flow, especially in pregnant patients where nuclear studies are contraindicated [14]. Additionally, thyrotropin receptor antibodies (TRAb), antithyroid peroxidase (TPO) antibodies, and anti-thyroglobulin (Tg) antibodies may document the presence of autoimmune thyroid disease [15]. Figure 1 provides a systematic approach for the evaluation of thyrotoxicosis in the nonpregnant patient.

Differential Diagnosis

The most common cause of endogenous thyrotoxicosis is Graves' disease, which comprises approximately 80% of cases [1]. Other common etiologies include toxic multinodular goiter, toxic adenoma, and silent thyroiditis [16]. In this section, we will briefly discuss the broad differential of thyrotoxicosis and present clinical features that distinguish one diagnosis from another. Graves' disease will be discussed in significant detail later in this chapter.

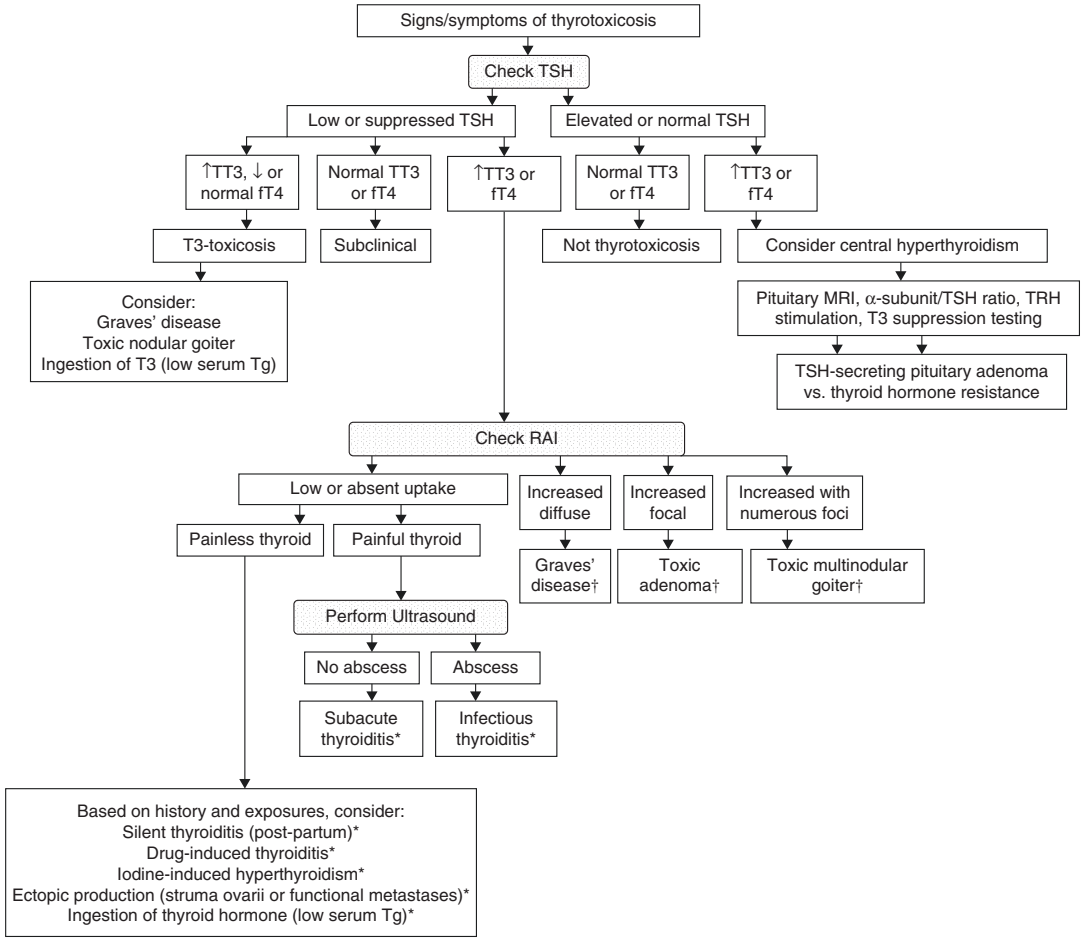

Fig. 1 A systematic approach for the evaluation of thyrotoxicosis. *TT3* total triiodothyronine, *fT4* free thyroxine, *TT4* total thyroxine, *TSH* thyroid-stimulating hormone, *TRH* thyrotropin-releasing hormone, *Tg* thyroglobulin, *RAI* radioactive iodine. *TT3/TT4 ratio is <20 ng/μg. †TT3/TT4 ratio is >20 ng/μg

Multinodular Goiter/Solitary Toxic Nodule

Toxic nodular goiter, which comprises multinodular goiter and solitary toxic nodule, results from diffuse or focal hyperplasia of thyroid follicular cells over time. While it is less common than Graves' disease, the prevalence of toxic nodular goiter is higher in regions of iodine deficiency and in older patients [17]. While the etiology is not entirely known, many cases are caused by TSH receptor G-protein defects that leave the cellular activation system of thyroid hormone synthesis in a constantly stimulated state [18–20]. Patients progress from subclinical hyperthyroidism to overt hyperthyroidism as these nodules grow and become autonomous, independent of regulation by TSH. The diagnosis should be suspected in hyperthyroid patients with physical examination findings or ultrasound evidence of a thyroid nodule or multinodular goiter. Thyroid scintigraphy can distinguish functional nodular thyroid disease from Graves' disease. A toxic adenoma will have increased radioactive iodine uptake confined to the nodule with corresponding uptake suppression in other areas of the thyroid. Toxic multinodular goiter will have more than one focal area of increased radioactive iodine uptake compared with the elevated diffuse homogenous uptake as seen in Graves' disease.

Thyroiditis

Thyroiditis refers to destruction of thyroid follicular cells with release of thyroid hormone, often resulting in thyrotoxicosis. In thyroiditis, the thyroid gland is not actively synthesizing and secreting thyroid hormone, so it is distinguished by a low radioactive iodine uptake. The thyrotoxic phase is usually followed by transient hypothyroidism and subsequent recovery. The most common etiologies of thyroiditis are painless (spontaneous), painful subacute (de Quervain's), infectious, and drug-induced.

Painless thyroiditis is autoimmune-mediated and usually self-limited. Patients with painless thyroiditis often have high serum concentrations of anti-TPO and anti-Tg antibodies [15]. Subacute or painful thyroiditis is thought to be caused by an inflammatory reaction to a viral infection and is characterized by a prodrome with fatigue, malaise, and myalgias [21]. The erythrocyte sedimentation rate (ESR) is usually greater than 50 mm/h and can be elevated over 100 mm/h [11]. Infectious (acute) thyroiditis is an uncommon condition, which presents as sudden onset neck pain, tenderness, fever, and a fluctuant unilateral neck mass. Over half of cases are caused by either *Staphylococcus* or *Streptococcus* species [22]. Ultrasound can be used to differentiate infectious from subacute thyroiditis, as infectious thyroiditis presents with an abscess. Thyroid function is usually normal in these cases but thyrotoxicosis can be observed transiently.

Drug-induced thyroiditis can occur in patients receiving amiodarone, interferon-alfa, lithium, and tyrosine kinase inhibitors [23]. Amiodarone-induced thyrotoxicosis (AIT) occurs by two mechanisms, iodine-induced hyperthyroidism (type 1) and destructive thyroiditis (type 2). Type 1 AIT occurs in patients with underlying Graves' disease or toxic nodular goiter, where the excess iodine load from amiodarone fuels thyroid hormone production [24]. In contrast, type 2 AIT is a destructive thyroiditis that destroys thyroid follicular epithelial cells due to a direct toxic effect of the drug. The two forms appear similar biochemically, but thyroid ultrasound reveals increased vascularity and a diffusely enlarged or nodular goiter in type 1 AIT [25, 26].

Less Common Causes of Thyrotoxicosis

Stimulation of the TSH receptor on thyroid follicular cells by human chorionic gonadotropin (hCG) at extremely elevated levels, such as in molar pregnancy or choriocarcinoma, can lead to clinical thyrotoxicosis or thyroid storm [14, 27]. This diagnosis can be made by measuring serum hCG levels. Additionally, thyrotoxicosis can result from central hyperthyroidism, in the form of either resistance to thyroid hormone (RTH) or a TSH-secreting pituitary adenoma. Both are

uniquely characterized by measurable levels of circulating TSH in the setting of elevated levels of free T4 [28]. A TSH-secreting pituitary adenoma usually presents with signs and symptoms milder than those in patients with hyperthyroidism of thyroid origin such as Graves', in addition to potential mass effects of the pituitary tumor [29]. This can be differentiated from RTH by a pituitary MRI revealing a mass, an elevated alpha-subunit/TSH ratio, and a blunted response to thyrotropin-releasing hormone (TRH) stimulation and T3 suppression testing [11, 30].

Hyperthyroidism associated with recent iodine exposure (Jod-Basedow phenomenon) occurs most frequently in iodine-deficient areas, especially in those with pre-existing underlying thyroid autonomy such as multinodular goiter [31]. With an excess iodine load, the autonomous areas of thyroid tissue can produce thyroid hormones independent of regulatory mechanisms [32]. Exposure to iodinated contrast media has also been reported to occasionally precipitate development of hyperthyroidism, especially in the elderly [33].

Other less common causes of thyrotoxicosis include exogenous and ectopic hyperthyroidism. Production of thyroid hormone from thyroid tissue in abnormal locations, such as in struma ovarii, lingual goiter, or metastatic thyroid carcinoma, may be localized by whole-body iodine scanning. Surreptitious ingestion of thyroid hormone can cause factitious thyrotoxicosis, which presents with a nonpalpable thyroid gland and with suppression of circulating thyroglobulin levels [3, 11]. Dietary health supplements marked for "thyroid support" have also been shown to contain clinically relevant amounts of T3 and T4, which can cause iatrogenic thyrotoxicosis in an unsuspecting patient [34].

Thyrotoxicosis in Pregnancy

To meet the increased metabolic needs during pregnancy, there are multiple changes in thyroid hormone physiology that occur which require special consideration. First, estrogen increases the production of thyroxine-binding globulin (TBG), which is the main thyroid hormone transport protein [14]. To sustain adequate free hormone concentrations, synthesis of T4 and T3 must increase. Moreover, degradation of T4 and T3 by uteroplacental-bound deiodinase type 3 further drives the need for this compensatory increase in thyroid hormone synthesis [35]. Increasing levels of hCG, which is structurally homologous to TSH, activates the TSH receptor on thyroid follicular cells, increasing total and free T4 concentrations, which results in a reciprocal physiologic decrease in TSH.

Thyrotoxicosis in pregnancy has a prevalence of between 0.1 and 0.4%, and the most common cause is Graves' disease, representing 85% of cases [36]. Gestational transient thyrotoxicosis (GTT), which results from excessive stimulatory action of hCG on the TSH receptor, has a prevalence ranging from 2 to 3% in the European population to 11% in Singapore [14]. GTT is typically seen with hyperemesis and multiple gestations [35]. The differential also includes those entities mentioned above, such as toxic nodular goiter and thyroiditis, but occurrences are rare.

Introduction to Graves' Disease

The most common cause of thyrotoxicosis is Graves' disease, an autoimmune disorder caused by stimulating antibodies to the thyrotropin, or TSH, receptor on thyroid follicular cells. The incidence of Graves' disease is slightly over 20 cases per 100,000 individuals each year [37]. Graves' disease predominantly affects patients ages 40–60 years with a female to male predominance ranging from 5:1 to 10:1 [38]. In one study, over 3% of women and 0.5% of men had a lifetime probability of developing Graves' disease [37].

Pathogenesis of Graves' Disease

In Graves' disease, the main autoantigen is the TSH receptor, which is a G-protein-linked receptor with seven transmembrane-spanning domains [18]. TSH acts via the TSH receptor to regulate thyroid growth as well as hormone production

and secretion. Patients with hyperthyroidism from Graves' disease have circulating stimulating autoantibodies that bind to the leucine-rich repeat region of the TSH receptor on follicular cells [39]. Binding of antibodies to the TSH receptor activates G-proteins and transmembrane adenylyl cyclases, leading to a rapid increase in cyclic adenosine monophosphate (cAMP) and unregulated production of thyroid hormone [40]. However, not all TRAb are stimulatory, as patients can also have blocking antibodies that inhibit the activity of TSH on the receptor. The balance between concentrations of stimulating and blocking antibodies may explain fluctuations in the clinical presentation of Graves' disease [41]. Moreover there exist neutral antibodies that do not impact TSH binding but are involved in cell signaling and oxidative stress-induced apoptosis [42].

Several factors predisposing individuals to develop Graves' disease have been proposed. Epidemiologic studies have revealed that genetic susceptibility plays a role in the pathogenesis of Graves'. In particular, there are strong associations between Graves' disease and variants in the class II major histocompatibility component (MHC) molecule HLA-DR3, which is involved in antigen presentation [43]. Additionally, single nucleotide polymorphisms in the TSH receptor and thyroglobulin genes have also been implicated in Graves' disease [43–45]. While 70–80% of susceptibility to autoimmune thyroid disease is attributed to genetic factors, the remaining 20–30% is from environmental exposures, including infections, stress, iodine intake, and radiation exposure [46]. Moreover, administration of interferon and other immunomodulating drugs can induce thyroid autoimmunity and precipitate hyperthyroidism [47, 48]. Smoking also appears to increase the risk of Graves' disease by twofold [49].

Clinical Manifestations of Graves' Disease

Patients with Graves' disease present with typical symptoms of thyrotoxicosis such as weight loss, fatigue, palpitations, tremor, anxiety, and tachycardia, as discussed earlier in this chapter. Additionally, patients can present with a characteristic ophthalmopathy, thyroid dermopathy, and thyroid acropachy, which are autoimmune manifestations of Graves' disease that will be discussed in further detail in a subsequent chapter of this textbook. Briefly, ophthalmopathy occurs in approximately 50% of Graves' patients. Approximately 20–30% of cases are clinically relevant, and 3–5% are severe cases with intense pain and sight-threatening compressive optic neuropathy or corneal breakdown [50]. The development of ophthalmopathy usually coincides with the onset of thyrotoxicosis, but it can precede or follow thyrotoxicosis [51]. Common clinical signs include upper eyelid retraction, exophthalmos, periorbital edema, and erythema [52]. Patients often report diplopia, blurry vision, photophobia, a dry gritty sensation in the eyes, or pressure behind the eyeballs. Graves' ophthalmopathy is usually bilateral but can present asymmetrically in 10–15% of cases or even unilaterally [53, 54].

Thyroid dermopathy is present in 4–13% of patients with Graves' disease and is almost always seen in conjunction with Graves' ophthalmopathy [55]. The presence of both dermopathy and ophthalmopathy is indicative of severe autoimmune disease. Thyroid dermopathy is characterized by non-pitting scaly thickening and induration of the skin and is also known as pretibial myxedema due to its frequent localization to the pretibial areas of the lower legs. However, lesions can occur in other regions as well, especially in areas of prior trauma [56]. The skin lesions typically are either flesh-colored or yellowish-brown but can have an orange peel texture [57]. Hyperpigmentation and hyperkeratosis can also be present. These regions are most often mild and bothersome only from a cosmetic perspective. However, in its most severe form, thyroid dermopathy can have features of elephantiasis causing severe pain and discomfort [58].

An even rarer extrathyroidal expression of Graves' disease is thyroid acropachy, or clubbing of the fingers and toes, which occurs approximately in 0.3% of Graves' patients [59]. It almost always occurs in the presence of both ophthalmopathy and thyroid dermopathy; approximately 20%

of dermopathy patients have acropachy [55]. Usually thyroid acropachy is painless but it can be associated with significant swelling causing pain and loss of function. The disease process is usually symmetric but can present in a single digit [55].

Thymic enlargement has been associated with Graves' disease, though the precise pathophysiology of thymic hyperplasia remains unclear [60]. Interestingly, thymic enlargement is reversible and tends to regress with successful treatment of hyperthyroidism with antithyroidal drugs, radioiodine, or thyroidectomy [61].

Diagnostic Evaluation of Graves' Disease

Graves' disease should be suspected in a patient with the clinical signs and symptoms of thyrotoxicosis in combination with extrathyroidal manifestations such as ophthalmopathy, dermopathy, or acropachy [12]. Physical examination can reveal a goiter with a thyroid bruit, which is highly characteristic of Graves' disease [62]. The initial biochemical evaluation should include assessment of the TSH, free T4, and total T3 levels. A total T3 to total T4 ratio of greater than 20 (ng/μg) is more suggestive of Graves' disease than other forms of thyrotoxicosis (such as subacute thyroiditis and exogenous levothyroxine thyrotoxicosis), due to a disproportionate increase in thyroidal T3 secretion [11, 63].

In clinical settings where the diagnosis is uncertain, measurement of TRAb can distinguish Graves' disease from other causes of thyrotoxicosis. In fact, third-generation TRAb assays have high sensitivity and specificity in the upper 90% [64, 65]. Currently there exist two methods for assessing TRAb. The thyroid-stimulating immunoglobulin (TSI) assay measures the ability of antibodies to bind to the TSH receptor and stimulate cAMP production, whereas the thyrotropin-binding inhibiting immunoglobulin (TBII) assay measures the capacity of antibodies to inhibit TSH binding to an in vitro TSH receptor preparation [64]. Notably, measurements of anti-Tg and anti-TPO antibodies can be elevated but are not unique to Graves' disease [12].

The evaluation of thyroid function in pregnancy must make use of trimester-specific reference ranges for TSH: 0.1–2.5 mIU/L in the first trimester, 0.2–3.0 mIU/L in the second, and 0.3–3.0 mIU/L in the third, if locally generated ranges are not available [11]. Instead of measuring free T4, which is highly variable and lacks standardization during pregnancy, total T4 measurements should be used to measure hormone concentrations during pregnancy [66]. However, pregnancy-adjusted reference ranges must be used because total T4 levels increase by approximately 50% above prepregnancy levels [14]. Measurement of TRAb is essential for diagnostic and prognostic purposes during pregnancy, as TRAb crosses the placenta and may also trigger neonatal hyperthyroidism [35].

Several imaging modalities are utilized in the diagnosis of Graves' disease in the nonpregnant patient. Thyroid uptake of radioactive iodine is typically diffuse and greater than 30–50% in Graves' disease [3]. In pregnant patients with Graves' for which nuclear studies are contraindicated, color flow Doppler sonography will reveal a diffusely enlarged and hypoechoic thyroid gland with increased extranodular and perithyroidal vascularity [12, 67]. "Thyroid inferno," a sonographic finding in which multiple areas of color flow are seen diffusely in both systole and diastole, may be noted in untreated patients with Graves' disease [62]. While one study showed no difference between scintigraphy and sonography in rates of diagnosis, ultrasound was found to be more cost-effective and had a higher sensitivity for detecting concomitant nodular lesions [13].

Treatment of Graves' Disease

The therapeutic goal in treating Graves' disease is either to attain biochemical euthyroidism with antithyroidal drugs or achieve hypothyroidism following radioactive iodine or thyroidectomy. Furthermore, it is important that treatment occurs in a timely manner to avoid complications of untreated Graves' such as atrial fibrillation and osteoporosis. There are three strategies for treatment: (1) normalization of thyroid hormone pro-

duction with antithyroidal drugs (ATDs), (2) surgical removal of the gland with total thyroidectomy, and (3) destruction of the gland with radioactive iodine. The treatment modality should be based on the clinical scenario as well as the patient's preference. Interestingly, in a study of 179 patients randomized to one of the three treatments, no significant differences in quality of life were observed among the treatment modalities [68].

Antithyroidal Drugs

Thionamides are the antithyroidal drugs utilized in Graves' disease and comprise carbimazole, its active metabolite methimazole (1-methyl-2-mercaptoimidazole), and propylthiouracil (6-propyl-2-thiouracil, PTU). These medications interfere with iodination of tyrosine residues in thyroglobulin, which is a key step in thyroid hormone synthesis [69]. PTU also inhibits the conversion of T4 to T3 within the thyroid and in peripheral tissues [1, 70]. ATDs are the preferred therapy for patients with mild hyperthyroidism and high likelihood of remission, multiple comorbidities carrying high surgical risk, an inability to follow radiation safety guidelines, or with moderate to severe active Graves' ophthalmopathy, as radioiodine therapy is associated with precipitating a worsening of this condition [11]. ATDs are also preferred over definitive therapies during pregnancy, which will be further discussed later in this section [71].

PTU and methimazole are available in the United States, whereas carbimazole, a third agent, is a precursor of methimazole and is available in many countries. Prior to ATD initiation, a complete blood count with differential and liver panel should be obtained due to the association of ATDs with agranulocytosis and hepatic toxicity. The titration method of ATD administration involves starting at a higher dose of ATD and then decreasing to a maintenance dose as a euthyroid state is restored [12]. An initial dose of methimazole can be initiated at 10–30 mg per day based on the degree of thyroid dysfunction and lowered to 5–10 mg daily for maintenance therapy. On the other hand, PTU requires administration 2–3 times per day with initial doses of 50–150 mg three times daily followed by maintenance therapy of 50 mg two to three times daily. After initiation, biochemical thyroid studies should be reassessed in 4 weeks and subsequent monitoring can occur every 2–3 months [11]. In the past, the block-replace method was popular and involved administering a high dose of ATD with levothyroxine to assist in permanent resolution of the hyperthyroid state. However, this method has fallen out of favor as it has been shown to result in a higher rate of adverse events [72].

Interestingly, there are geographic differences in prescribing patterns, as ATDs are more favored in Europe and Japan as compared to in the United States [1, 73]. Among the types of ATDs, carbimazole and methimazole are favored over PTU in most cases due to increased compliance with once-a-day dosing and more rapid normalization of serum T3 and T4 concentrations [69]. Side effect profile is also a strong consideration. Minor side effects include cutaneous reactions, arthralgias, and gastrointestinal upset, which occur in approximately 5% of patients on either drug [3]. In a recent systematic review of observational studies, the predominant adverse effect of methimazole was rash (6%) whereas it was hepatic involvement in 2.7% of patients on PTU [74]. While both methimazole and PTU are associated with hepatotoxicity, it is less severe in methimazole and usually causes cholestatic dysfunction, while PTU can cause hepatocellular inflammation [75]. Because PTU can cause fulminant hepatic necrosis and is one of the common etiologies of drug-related liver transplantation, the FDA issued a safety alert in 2010 regarding PTU's risk for causing severe liver injury and acute liver failure [76].

Agranulocytosis is a serious, life-threatening side effect that occurs in approximately 1 in 500 patients and presents with high fever and severe pharyngitis [3]. Patients should be counseled to stop the medication immediately and call their physician for a complete blood count if they experience these symptoms. Treatment usually involves hospitalization, administration of broad-spectrum antibiotics, and granulocyte colony-stimulating factor. Antineutrophilic cytoplasmic

antibody (ANCA)-positive vasculitis is another rare side effect related to PTU use but is even rarer with methimazole use [3].

Once euthyroid levels are achieved on an ATD, biochemical testing can be performed at 2–3-month intervals. There is insufficient evidence currently to recommend periodic monitoring of white blood cell counts or liver function for preventative purposes; however, assessment of these parameters is certainly reasonable in any patient who experiences fever, sore throat, pruritic rash, jaundice, acholic stools, nausea, fatigue, or dark urine [11]. Patients on methimazole are recommended to continue on therapy for 12–18 months to prevent recurrence. Furthermore, a TRAb level prior to discontinuing therapy can aid in predicting the patient's chances of remission. Those with persistently elevated TRAb may continue therapy for another 12–18 months or consider alternative definitive therapy [77]. After discontinuation of ATDs, thyroid function should be monitored every 1–3 months for 6 months and then at increasing intervals if normal.

Radioiodine Therapy

Radioiodine (RAI) is given orally as sodium iodide (^{131}I), which is rapidly absorbed and concentrated in the thyroid, causing necrosis of thyroid cells due to beta particle release [71]. The gradual loss of thyroid tissue results in hypothyroidism, which is the goal of this therapy. RAI should be considered in females planning pregnancy in the future but not for at least 6 months; patients with significant surgical risk, previous neck operations, or extensive external irradiation; or those with contraindications or side effects to ATD use [11]. RAI is absolutely contraindicated during pregnancy, nursing, or when planning for an upcoming pregnancy within 6 months [71]. In patients who have a concomitant thyroid nodule, further evaluation is indicated to determine if the nodule is benign or malignant. If a thyroid nodule is concerning for malignancy in the presence of Graves', the recommended treatment would be to pursue total thyroidectomy over RAI therapy after rendering the patient euthyroid with ATDs.

Biochemical hypothyroidism is usually achieved with administration of 10–15 mCi [11]. Alternatively, the dose can be estimated based on RAI uptake as well as thyroid gland size [12]. Because RAI can induce a transient increase in thyroid hormone levels, the elderly, those with severe hyperthyroidism, and those with a high risk of cardiovascular complications may benefit from beta-blockade or ATDs before and after RAI, though there is limited evidence to support this practice [11]. When pretreatment methimazole is given, it should be stopped 3–7 days prior to RAI and resumed at the same interval after treatment [73].

All reproductive aged women are required to have a negative pregnancy test before RAI. Women who are nursing should stop breastfeeding for at least 6 weeks prior to RAI administration. All patients should receive extensive counseling on radiation safety precautions as determined by the National Council on Radiation Protection and Measurement [78]. Pertinent topics include the duration and extent of contact avoidance with others, hygiene precautions to reduce exposure to bodily fluids, and avoidance of commonly shared household items. Posttreatment conception should not occur until at least 6 months in women to allow restoration of a euthyroid state at the time of conception and 3–4 months in men [11].

Adverse events posttreatment include radiation thyroiditis, which can manifest as neck tenderness and transient exacerbation of the thyrotoxicosis [73]. Furthermore, sialadenitis, neck swelling, and glossitis can be observed. To avoid development of sialadenitis, some suggest sucking on hard candy that does not contain iodinated red dyes or chewing on gum in the week following treatment [79]. Unfortunately, patients with mild ophthalmopathy and/or those with a history of tobacco use or positive TRAb may develop or experience progression of the associated eye symptoms following ^{131}I [80–82]. This complication can be prevented by prophylaxis with low-dose oral prednisone. Further discussion on the specific indications is beyond the scope of this section.

Generally, patients should have biochemical thyroid testing every 4–6 weeks after RAI to determine when thyroid hormone replacement will be required. Serum TSH can be suppressed for over a month following RAI so assessment should be based on free T4 and total T3 levels [11]. Any beta blockers or ATDs can be tapered once free T4 and total T3 have normalized. Levothyroxine should be started when the free T4 falls below the reference range. If hyperthyroidism is present 6 months after therapy, a repeat treatment may be considered [11]. Patients with Graves' ophthalmopathy should have follow-up with an ophthalmologist 6–12 months after RAI therapy [80].

Total Thyroidectomy

With the development of ATDs and RAI, thyroidectomy has become the least recommended treatment for Graves' disease [3]. However, thyroidectomy should be considered as the preferred therapy in select clinical scenarios, such as compressive symptoms from a significantly enlarged thyroid gland, low RAI uptake, in difficult to control type 1 AIT, concern for thyroid malignancy, moderate to severe ophthalmopathy, or coexisting hyperparathyroidism [11]. Thyroidectomy is also indicated for those planning for pregnancy in less than 6 months, those experiencing adverse effects of ATDs, or for patients with an inability to follow radiation safety guidelines.

In preparation for thyroidectomy, patients must be rendered euthyroid for 1–3 months with ATDs [3]. For patients who are intolerant to ATDs, treatment with beta-blockade, potassium iodide, steroids, and cholestyramine have been recommended in the preoperative period [83]. Iodide has been shown to be beneficial prior to thyroidectomy as it decreases the rate of blood flow, thyroid vascularity, and intraoperative blood loss [84]. ATDs should be stopped at the time of thyroidectomy and beta blockers should be weaned following surgery.

There exist several significant complications of thyroidectomy, which include transient or permanent hypoparathyroidism resulting in hypocalcemia, recurrent or superior laryngeal nerve damage, infection, and postoperative bleeding. Thyroidectomy should be performed by an experienced, high-volume thyroid surgeon, as the complication rates of thyroid surgery are inversely correlated with surgeon experience and case volume [85]. Near-total or total thyroidectomy is recommended as the complication rates are similar and the risk of relapse is higher with a subtotal thyroidectomy [86]. Patients should be informed postoperatively that they will require thyroid hormone replacement indefinitely usually at 1.6–1.7 μg/kg/day. Furthermore, parathyroid hormone, calcium, and albumin levels should be obtained postoperatively for monitoring. With modern surgical techniques, thyroidectomy may be considered to be an overall safe surgery for patients especially since there are high cure rates and negligible recurrence rates [86].

Beta-Adrenergic Blocking Agents

For symptomatic relief in the setting of palpitations, irritability, exercise intolerance, and tremor and/or to target heart rate less than 90 beats per minute in a patient with Graves' disease, a beta-adrenergic blocker can be initiated. Propranolol, in particular and in high doses, blocks the conversion of T4 to T3 through inhibition of 5′-monodeiodinase and appears to have a greater effect on tremor compared to β-1 selective agents [87]. However, those with relative contraindications to beta blockers may better tolerate β-1 selective agents. The various options for beta-blockade are as follows: propranolol (10–40 mg TID-QID), atenolol (25–100 mg daily or BID), metoprolol (25–50 mg QID), or nadolol (40–160 mg daily). An esmolol infusion is used only in the setting of thyroid storm [3, 11]. Please refer to the thyroid storm section of this text for complete management guidelines.

Treatment of Graves' Disease in Pregnancy

The ultimate goal of Graves' disease management during pregnancy is to keep the mother and fetus in a near euthyroid state with the lowest possible dose of antithyroidal medication. Recent

studies of ATD side effects in pregnancy have influenced changes in management guidelines. In a Danish population study, the use of ATDs in pregnancy was associated with birth defects in 3.4% of exposed children [88]. The frequency of birth defects after ATD exposure was 75 times higher than the risk of maternal agranulocytosis and liver failure [88].

For women with Graves' disease who are contemplating pregnancy, counseling should focus on treatment options (i.e., ATDs, RAI, or thyroidectomy) for attaining euthyroidism prior to the attempt to conceive. Those who are already on ATDs should contact their physicians immediately once they are pregnant. In a newly pregnant female who is euthyroid on low-dose methimazole (5–10 mg daily) or PTU (under 200 mg daily), discontinuation of ATD can be considered given the potential for teratogenic effects, and close monitoring should be performed every 1–2 weeks [89]. Once the patient remains euthyroid off ATDs, continued monitoring can be extended to 2–4 weeks.

In a pregnant female with a high risk of becoming thyrotoxic off ATDs, continuation of therapy may be necessary. PTU remains the recommended thionamide during the first trimester of pregnancy due to the risk of teratogenicity with methimazole, and individuals on methimazole should be switched as early as possible once pregnancy is confirmed [11]. A dose ratio of 1:20 should be utilized when converting from methimazole to PTU. In other words, 10 mg daily methimazole is equivalent to 100 mg twice daily PTU [3]. Previously it was recommended that patients return to methimazole in the second trimester; however, newer guidelines do not make any recommendations on switching the ATD [11, 89]. In unique situations such as intolerance to ATDs in which thyroidectomy is indicated, the optimal timing of surgery is during the second trimester [71]. Thyroidectomy should be avoided in the first and third trimesters due to teratogenic effects associated with general anesthesia, risk of fetal loss in the first trimester, and preterm labor in the third semester. Overall, thyroidectomy at any point in pregnancy may confer an increased risk of maternal complications from surgery [90].

Thyroid function tests should be checked every 4 weeks throughout pregnancy with the goal of keeping total T3 and T4 values at or slightly above pregnancy-adjusted reference ranges with a TSH below the corresponding reference range [89]. Approximately 30–50% of pregnant women may enter remission as pregnancy advances, in which case the ATD can be reduced or discontinued [3]. TRAb measurement during pregnancy can help assess the activity of thyroid autoimmunity and guide decisions on neonatal monitoring. Pregnant women with Graves' should have TRAb levels measured during the first trimester and repeated at 18–22 weeks gestation if elevated, as the antibodies can affect fetal thyroid function [89]. If a subsequent measurement at 18–22 weeks is positive and the patient remains on ATDs, another value should be repeated at 30–34 weeks. Fetal surveillance for neonatal hyperthyroidism may be indicated when maternal TRAb levels exceed three times the upper limit of normal [89]. In the first year postpartum, thyroid function tests should be measured every 2–3 months. In patients who discontinue ATDs in late pregnancy, there is a possibility for relapse but such a recurrence of thyrotoxicosis may also be caused by postpartum thyroiditis [91]. The differential diagnosis at this stage in a lactating mother may be difficult.

Prognosis of Graves' Disease

Management of Graves' disease involves one or more of three therapies: antithyroidal drugs, RAI, or thyroidectomy. Definitive treatment with RAI or thyroidectomy results in elimination of thyroid tissue at the expense of permanent hypothyroidism requiring lifelong thyroid hormone replacement. In a systematic review, relapse rates were low at 10–15% for definitive treatment, and no difference between rates was observed between RAI and thyroidectomy [74]. On the other hand, ATDs offer a conservative option for treatment, but patients have a higher rate of recurrence of Graves' disease, which varies between 30 and 70%, with highest rates in the immediate 3–6 months after discontinuation of ATDs [12].

Multiple factors have been associated with the risk of recurrence after ATDs and include male sex, young age, a larger thyroid size, the presence of persistent TRAb, a high serum T3/T4 ratio following ATD withdrawal, cigarette smoking, and severe ophthalmopathy [92–95]. Regardless of which treatment is chosen, lifelong monitoring of thyroid function is necessary, and patients must be counseled regarding long-term follow-up.

Conclusion

Thyrotoxicosis has a characteristic clinical syndrome, which must be recognized early to prevent serious consequences. The differential diagnosis of thyrotoxicosis is broad, and thus the evaluation of thyrotoxicosis must include a detailed history and physical, biochemical studies, and imaging tests if the etiology is unclear. Graves' disease is the most common cause of thyrotoxicosis. The mainstays of treatment are antithyroidal drugs, RAI, or thyroidectomy. Special considerations must be given in the diagnosis and treatment of Graves' patients in pregnancy due to the physiologic changes in pregnancy and concerns for the well-being of the fetus. A thorough understanding of the clinical scenario as well as patient preferences will help physicians guide therapy for their patients, as each treatment has its own benefits and limitations.

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Graves' Ophthalmopathy

Luigi Bartalena

Definition

The term Graves' ophthalmopathy (or orbitopathy, GO) defines the complex ocular or, rather, orbital changes that are found in patients affected with Graves' disease or, far less frequently, with chronic autoimmune thyroiditis [1]. Thus, GO is most commonly associated with hyperthyroidism but may occur also in patients who are euthyroid or hypothyroid (euthyroid or hypothyroid GO) [1]. GO represents the most frequent of the extrathyroidal manifestation of Graves' disease, which also includes the rare thyroid dermopathy (or pretibial myxedema) and the exceptional thyroid acropachy [1].

Epidemiology and Natural History (Table 1)

Although severe and even sight-threatening forms of GO may be observed, recent studies indicate that GO is present in only about 20–25% of patients with Graves' disease [5, 6] and is usually mild and rarely progressive [7]. Among patients with no GO at baseline, the large majority (87%) are still GO-free after an 18-month follow-up during antithyroid drug treatment, 10% develop mild GO, and less than 3% moderate-to-severe GO [5]. Conversely, many patients have a spontaneous remission of initially mild GO, although progression to more severe forms of the disease may infrequently also occur [7]. Moderate-to-severe forms account for approximately 5% of cases [1]. Information on the natural history of moderate-to-severe GO is not available, because these patients are promptly treated with disease-modifying therapies, such as immunosuppressive drugs and/or orbital radiotherapy. The decline in severity of GO is supported by the observation that the proportion of patients with moderate-to-severe and active GO referred to thyroid-eye clinic part of the European Group on Graves' Orbitopathy (EUGOGO) has decreased in the last 10 years [8]. This milder phenotype and more favorable course of GO observed nowadays are probably related to earlier diagnosis and treatment of both Graves' hyperthyroidism and GO, to a more effective actions on modifiable risk factors for GO [9] (see below), and to a more effective interaction between general practitioners and specialized centers through the development of referral pathways [10].

The age-adjusted incidence rate of GO (all degrees of severity) in the Olmsted County study was 16 cases per 100,000 per year for women and 2.9 cases per 100,000 per year for men [2]. The incidence of moderate-to-severe forms of GO

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Table 1 Epidemiology and natural history of Graves' ophthalmopathy

Female-to-male ratio	2:1
Peaks of incidence	Fifth and seventh decades of life
Overall prevalence in newly diagnosed Graves' disease	20–25%
Prevalence of moderate-to-severe forms	5%
Incidence rate (all degrees of severity)	16 per 100,000 per year (women)
	2.9 per 100,000 per year (men)
Incidence rate of moderate-to-severe forms	26.7 per million per year (women)
	5.4 per million per year (men)
Phases of the disease	Active phase, plateau phase, inactive phase

Derived from Bartley [2], Bartley et al. [3], Laurberg et al. [4], Tanda et al. [5], Bartalena et al. [6]

was reported to be 16.1 per million per year (women, 26.7; men, 5.4) in a recent Danish study [4]. GO is more prevalent in women, with a female-to-male ratio of 2:1 [11], and shows two age peaks, in the fifth and seventh decades of life [3]. Although rarely, GO may be present also in children [12]. Asians have a lower risk of developing GO than Caucasians [13]. In the majority of cases, the onset of GO coincides with the onset of hyperthyroidism, but GO may precede or develop long after diagnosis and treatment of hyperthyroidism [11, 14].

The course of GO is characterized by an initial phase of florid inflammation (active phase) associated with the appearance of clinical manifestations, followed by a short phase of stabilization (plateau phase), and then by a progressive remission of inflammation up to inactivation (burnt-out disease). Complete disappearance of clinical manifestations of the disease is unlikely when GO is full blown.

Pathogenesis

The pathogenic role of genetic factors in GO is not well defined, and no clear differences have been found between Graves' patients with or without GO [15–19]. An association between GO and major histocompatibility complex (MHC), cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4), or intercellular adhesion molecule 1 gene polymorphisms has been investigated [18], but results are conflicting [20, 21].

Most of the clinical manifestations of GO can be mechanistically explained by the increased orbital content (expansion of the retroocular adipose tissue, enlargement of the extraocular muscles) within a rigid body structure such as the orbit. This remodeling of the orbital tissues is the consequence of an autoimmune inflammatory disorder triggered by the migration of autoreactive T-helper cells into the orbital space [22]. The orbital space is infiltrated by CD4+ T cells and, to a lesser extent, CD8+ T cells, B cells, fibrocytes, mast cells, and macrophages. Orbital fibroblasts are the prime target and key effector cells in the pathogenesis of GO [23]. After recognition of one or more antigens (shared with the thyroid) on fibroblast surface, facilitated by HLA class II antigen expression on antigen-presenting cells (B cells, macrophages), CD4+ T cells secrete cytokines which activate CD8+ T cells and autoantibody-synthesizing B cells [Ajjan and Weetman 2004] and stimulate orbital fibroblasts [24]. The latter cells proliferate, may differentiate into myofibroblasts and adipocytes, accumulate and secrete hyaluronic acid (HA), and synthesize and secrete chemoattractants (interleukin-16, RANTES, CXCL10) and a number of cytokines (interleukin-1, interleukin-6, interferon-γ, tumor necrosis factor-α, interleukin-8, interleukin-10, platelet-derived growth factor, transforming growth factor-β), which automaintain the ongoing inflammatory process [25]. Accumulation of HA is fundamental, because it is hydrophilic and thereby attracts water and causes edema of the extraocular muscles and orbital tissue [22]. Orbital fibroblasts can be distinguished on the basis of expression or lack of expression of a cell surface glycoprotein (thymocyte antigen-1, thy-1). While thy-1-positive fibroblasts are mainly present in the extraocular muscles and produce HA, thus contributing to edema and enlargement of extraocular muscles, thy-1-negative fibroblasts are mostly represented in the connective tissue

and may differentiate into adipocytes, contributing to the expansion of fibroadipose tissue [22].

Evidence has accumulated suggesting that the link between the thyroid and the orbit, i.e., the shared antigen triggering the above cascade of events, might be represented by the TSH receptor (TSHR). The TSHR is expressed more in GO orbital fibroblasts than in control orbital fibroblasts and in orbital tissue from patients with active GO than from patients with inactive GO, and its expression is enhanced when preadipocyte fibroblasts differentiate into adipocytes [22]. TSHR antibodies (TRAb) activate the TSHR through the adenylyl cyclase/cAMP pathway and the phosphoinositide 3-kinase (PI3K) pathway [26] and stimulate HA production in orbital fibroblasts. In different experimental systems, this has translated into an increased adipogenesis [27, 28]. In animal models, BALB/c mice immunized by TSHR A-subunit by electroporation, orbital remodeling and adipogenesis reminiscent of that found in human GO could be observed [29, 30]. Other animal models seems to support the role of the TSHR in the pathogenesis of GO [31–33]. Another player in GO might be the insulin-like growth factor-1 (IGF-1) receptor (IGF-1R). An increased expression of IGF-1 and its receptor has been shown in orbital cells from GO patients [34]. HA synthesis from GO orbital fibroblasts could be induced by immunoglobulins (IgGs) from Graves' patients, but not by human recombinant TSH, and this effect could be blocked by an IGF-1 receptor blocking agent (1-H7) [35]. Other studies showed that TSHR-stimulating monoclonal antibody M22 can stimulate cAMP production and HA synthesis in GO fibroblasts [36], and this effect can be inhibited by both 1-H7 and a small molecule inhibiting TSHR activation [26, 36]. The two receptors (IGF-1R and TSHR) colocalize in orbital fibroblasts [34]. Locally synthesized IGF-1 acting through the IGF-1R might contribute to the effect of TRAb on the TSHR [37]. Interestingly, it was recently shown in primary cultures of GO fibroblasts that the stimulating effect of M22 or GO IgGs on HA synthesis could not by blocked by one anti-IGF-1R antibody and was only partially blocked by a second anti-IGF-1R antibody [38], suggesting that the cross talk between the TSHR and the IGF-1R, likely important in the pathogenesis of GO, might not involve direct binding to the IGF-1R.

Risk Factors and Prevention (Table 2)

While age, gender, and genetics are non-modifiable risk factors, several modifiable risk factors for the occurrence/progression of GO have been identified (Table 1).

Smoking

The prevalence of smokers is higher among Graves' patients with GO than in those without GO; smokers have a higher risk of developing more severe forms of GO and have a lower and slower response to high-dose glucocorticoids and orbital radiotherapy for moderate-to-severe and active GO [1, 40]. Smokers are more prone to have radioiodine-associated de novo occurrence or exacerbation of GO [41, 42]; conversely, as shown in a retrospective study, refrain from smoking might be associated with a lower risk for the occurrence of exophthalmos and diplopia [43]. HA synthesis is increased, and adipogenesis is enhanced

Table 2 Risk factors for Graves' ophthalmopathy and preventive measures

Risk factor	Preventive measure
Cigarette smoking	Refrain from smoking
Thyroid dysfunction	Correct both hyper- and hypothyroidism, and stably maintain euthyroidism
Radioiodine treatment	Use oral steroid prophylaxis in at-risk patients (mainly smokers) when giving radioiodine
Oxidative stress	6-month selenium supplementation in patients with mild Graves' ophthalmopathy
TSH receptor autoantibodies	Control hyperthyroidism with antithyroid drugs to reduce serum autoantibody concentration
Late diagnosis and management	Prompt referral to specialized centers, except for mildest cases

Derived from Bartalena et al. [39]

when human orbital cells in cultures are exposed to smoke extracts [44]. Although mechanisms whereby cigarette smoking exerts its negative effects on GO need to be fully clarified, evidence is sufficient to indicate quit smoking as an important preventive action in patients with GO [9].

Thyroid Dysfunction

GO is negatively affected by an abnormal thyroid status, likely through the activation of the TSHR, by TRAb in the hyperthyroid state and by TSH in the hypothyroid state, leading to an increased expression of thyroid antigens and an exacerbation of autoimmune reactions [9]. Prompt restoration and stable maintenance of euthyroidism are, therefore, of utmost importance in Graves' patients [39, 45].

Radioiodine Treatment for Hyperthyroidism

Radioiodine is associated with a small but definite risk of progression of GO [46, 47]. This is most likely to occur in smokers [42] or when post-radioiodine hypothyroidism is not promptly corrected [48, 49]. This untoward effect can be almost always prevented by a short concomitant course of relatively low doses of oral prednisone (steroid prophylaxis) [39, 47, 50]. Prednisone administration can be avoided in patients with inactive GO, particularly if they do not smoke [51].

Oxidative Stress

Graves' disease is associated with an increased oxidative stress [52]. Antioxidants might, therefore, play a positive role for both hyperthyroidism and GO. Selenium is known for its antioxidants and immune-regulating actions. In a randomized clinical trial, it was shown that a 6-month supplementation of selenium could improve mild and active GO and prevent its progression to more severe forms [53]. Whether selenium may be beneficial also for moderate-to-severe GO is unsettled. Recently published guidelines indicate that selenium supplementation should be offered to patients with mild GO [39].

TSHR Antibodies

As indicated above TSHR and TRAb are important players in the pathogenesis of GO [22]. TRAb are independent risk factors for the occurrence of severe GO [54]. There is no way to reduce directly TRAb, but antithyroid drug treatment is usually associated with a gradual reduction in serum TRAb concentration [55], possibly due to an amelioration of autoimmune reactions associated with restoration of euthyroidism. Thyroidectomy is also associated with a reduction, whereas radioiodine treatment causes an increase in serum TRAb levels that may last for a few years [55].

Taking into account the above risk factors, primary prevention measures include refrain from smoking; control of thyroid dysfunction; a 6-month selenium course, if mild GO is present; and a prompt referral to specialized centers under most circumstances [39]. The recent observation that the use of statins is associated with a 40% decreased hazard of developing GO [56] needs to be confirmed in prospective studies.

Clinical Features

Signs and Symptoms (Table 3)

GO is generally bilateral and symmetrical, but unilateral or asymmetrical forms are not uncommon and may be relatively more frequent in euthyroid or hypothyroid GO patients [57]. Upper lid retraction, responsible for the thyroid stare, is the most frequent sign, present in about 90% of GO patients [58], and associated with lid lag on downgaze. Proptosis or exophthalmos, i.e., protrusion of the eye globe(s) due to the increased orbital content (expanded fibroadipose tissue, enlarged extraocular muscles), is present in more than 70% of patients with GO [58]. Together with upper lid retraction, it is responsible for corneal exposure

Table 3 Clinical features of Graves' ophthalmopathy

Changes in the appearance of the eyes	Lid retraction, lid lag, stare
	Exophthalmos
	Swelling of eyelids
	Red yes
	Chemosis (swelling of conjunctiva)
	Strabismus
Ocular surface involvement	Lacrimation
	Photophobia
	Grittiness (sandy sensation)
Extraocular muscle involvement	Diplopia (intermittent, inconstant, constant)
	Strabismus
	Retrocular pain at rest
	Pain with eye movements
Optic nerve involvement	Impaired color sensitivity
	Blurring of vision

Table 4 Assessment of disease activity by the clinical activity score (CAS)

Swelling
• Eyelid swelling
• Swelling of caruncle or plica
• Swelling of conjunctiva (chemosis)
• Chemosis
Redness
• Redness of eyelids
• Conjunctival hyperemia
Pain
• Spontaneous retroocular pain
• Pain with eye movements
One point is given to each item, if present. CAS is the sum of points
CAS ≥3/7 = active Graves' ophthalmopathy

Derived from Mourits et al. [61]

and incomplete lid closure (lagophthalmos) at night. Severe exophthalmos may cause globe subluxation. Corneal exposure may lead to secondary corneal lesions (punctate keratitis, corneal ulcers, corneal breakdown). Lower lid retraction is related to the degree of exophthalmos. Extraocular muscle dysfunction is observed in about 40% of GO patients [58], causing diplopia. Diplopia can be intermittent (present when the patient is tired or in the morning when awakening), inconstant (at the extremes of gaze), or constant (present also in primary gaze, correctable or not correctable with prisms). In addition to diplopia, extraocular muscle inflammatory involvement is responsible for retro-orbital pain, either at rest or with eye movements. This is contributed to also by periorbital soft tissue inflammation and congestion, which manifest with lid edema and redness, conjunctiva hyperemia and chemosis, and caruncle edema. Patients often complain of lacrimation, gritty sensation, and photophobia. Dysthyroid optic neuropathy (DON) is the most serious manifestations of GO, because, like corneal breakdown, it is sight-threatening. DON may be due to optic nerve stretch, but is more commonly caused by optic nerve compression by the enlarged extraocular muscles, particularly posteriorly at the orbital apex. DON may be heralded by desaturation of colors, afferent pupil defect, blurring of central vision, and disk edema [59].

Activity and Severity

Activity identifies the phase in the course of GO (active phase vs. inactive phase), while severity indicates the degree of involvement of orbital structures. Both parameters are fundamental to define the general treatment plan for GO. Assessment of activity takes advantage of a color photographic atlas originally developed by Dickinson and Perros [60] and now freely downloadable from the EUGOGO website (http://www.eugogo.eu). A quick, although imperfect tool to assess the disease activity is the clinical activity score (CAS) [61] (Table 4). In its 7-item form, commonly used for measuring effects of immunosuppressive treatments, it includes eyelid edema, edema of caruncle, chemosis, eyelid redness, conjunctival hyperemia, ocular or retro-orbital pain at rest, and pain with ocular movements. One point is given for each item present; CAS is the sum of the points. GO is considered active if CAS is ≥3/7 [39, 45]. In its 10-item formulation, recent changes in proptosis, extraocular muscle function, and visual acuity are also considered useful at the time of the first visit to assess recent progression of GO. Assessment of severity is controversial, different protocols have been published [62, 63], but consensus has not reached yet. The NOSPECS classification of severity (Table 5) is

Table 5 Assessment of disease severity by the NOSPECS system (abridged)

*N*o signs or symptoms
*O*nly signs, no symptoms: lid aperture (mm) measured by a rule
*S*oft tissue involvement: swelling of periocular tissues, redness of the eyes
*P*roptosis: exophthalmos (mm) measured by exophthalmometer
*E*xtraocular muscle involvement: assessment of subjective diplopia (absent, intermittent, inconstant, constant) or objective impairment in motility assessed by eye muscle ductions
*C*orneal involvement: absent/present (punctuate keratitis, ulcers)
*S*ight loss (due to optic nerve involvement): assessed by best-corrected visual acuity, color vision, optic disk evaluation, relative afferent papillary defect, visual field defects)

a useful mnemonic tool. In the guidelines on the management of GO recently published by EUGOGO [39], GO has been classified into mild, moderate-to severe, or sight-threatening, according to the degree of involvement of different parameters (lid retraction, soft tissue changes, exophthalmos, diplopia, corneal damage, DON) (Table 6).

Quality of Life

GO may cause relevant changes in *appearance*, related to lid retraction and stare, exophthalmos, swelling of periocular soft tissues, and *functioning*, due to diplopia or decrease in visual acuity. Overall these changes may have a profound impact on psychological health and quality of life (QoL) of affected individuals [64, 65] which may be long-lasting and associated with important socioeconomic consequences [66]. Evaluation of changes in the quality of life can be done using a disease-specific validated questionnaire [67] downloadable from the EUGOGO website and translated into several languages. Assessment of GO-QoL is relevant for selecting therapeutic strategy and evaluating treatment outcome.

Table 6 Assessment of GO severity according to the European Group on Graves' Orbitopathy (EUGOGO)

Mild GO
Usually one or more of the following features are present: minor lid retraction (<2 mm), mild soft tissue involvement, exophthalmos <3 mm above upper limit of normal for age and gender, no or intermittent diplopia, no or mild corneal involvement responsive to lubricants. Impact on quality of life (QoL) is insufficient to justify immunosuppressive treatment
Moderate-to-severe GO
Usually two or more of the following features are present: lid retraction ≥2 mm, moderate or severe soft tissue involvement, exophthalmos ≥3 mm above upper limit of normal for age and gender, inconstant or constant diplopia, and no dysthyroid optic neuropathy (DON). Impact on quality of life (QoL) is sufficient to justify immunosuppressive treatment (if GO is active) or surgery (if GO is inactive)
Sight-threatening (very severe) GO
Patients with DON and/or corneal breakdown

Derived from Bartalena et al. [39]

Diagnosis

Diagnosis of bilateral and overt GO is easy on clinical grounds, particularly if associated with hyperthyroidism due to Graves' disease. Orbital imaging (MRI, CT) in overt cases shows typical extraocular muscle enlargement (with tendon sparing) and/or increased fat mass. The extraocular muscle enlargement might be a relatively early phenomenon, and the increase of the fat mass would come later [68]. All muscle can be involved, although the inferior rectus and the medial rectus are more frequently involved for unknown reasons. Diagnosis is more intriguing in asymmetrical or unilateral forms of GO, especially if the patient is euthyroid or hypothyroid, or there is no evidence of thyroid autoimmune phenomena. Under these circumstances, orbital imaging is of paramount importance to rule out a number of other GO-unrelated conditions enlisted in Table 7. Among the latter, an emerging condition which may be hardly distinguishable from GO is immunoglobulin G4-related disease [69, 70]. Tendons are spared also in this condition, but the lateral rectus muscle and the lacrimal glands are more commonly involved than in GO [69]. Biopsy is the clue for diagnosis.

Table 7 Main causes of enlarged extraocular muscles and/or exophthalmos other than Graves' orbitopathy

Cushing's syndrome
Obesity
Orbital pseudotumor
Idiopathic myositis
Orbital cellulitis
Immunoglobulin G4-related orbital disease
Erdheim–Chester disease
Orbital lymphoma
Orbital meningioma
Leukemia
Rhabdomyosarcoma
Metastases (breast cancer, melanoma, lung cancer, pancreatic cancer, seminoma, carcinoid)
Vascular causes (arteriovenous malformations, carotid-cavernous fistula, angioma)
Systemic manifestations of amyloidosis, sarcoidosis, vasculitis
Wegener's granulomatosis
Eosinophilic granuloma
Cysts

Management of Associated Thyroid Dysfunction

Management of Graves' hyperthyroidism still relies on imperfect treatments, because antithyroid drugs belonging to the class of thionamides are bound to a high relapse rate, whereas radioiodine treatment and thyroidectomy inevitably cause lifelong hypothyroidism [71–75].

Antithyroid Drug Treatment

Antithyroid drugs represent the first-line treatment in Europe, Asia, and South America [76], while radioiodine treatment is the preferred modality in North America [77]. Antithyroid drugs do not appear to have direct effects on GO. However, normalization of thyroid status and stable maintenance of euthyroidism associated with treatment may favor an amelioration of GO by blunting ongoing autoimmune reactions, as reflected by the usual, progressive decrease in serum TRAb concentration [55]. Very long treatment with thionamides has been reported to be associated with stable or improved ocular conditions [78].

Radioiodine Treatment

Radioiodine treatment may cause an exacerbation of GO in about 15% of cases [79], especially in smokers [42] and in patients with preexisting GO of recent onset [80, 81] or with high TRAb levels. The radioiodine-associated progression of GO can almost always be prevented by concomitant steroid prophylaxis with relatively low doses of oral prednisone [50, 80, 82], although few reports showed that GO could rarely progress despite this prophylactic treatment [81, 83]. An important factor that may concur to progression of GO after radioiodine treatment is late correction of post-radioiodine hypothyroidism [48, 49]. Steroid prophylaxis could be avoided in patients with long-standing inactive GO, who do not smoke and whose hypothyroidism is promptly corrected [51].

Thyroidectomy

Thyroidectomy is less commonly used than antithyroid drugs and radioiodine as first-line treatment for Graves' hyperthyroidism [71]. Its effect on GO is neutral [84]. However, in view of the hypothesis that GO may be triggered by autoimmune reactions directed toward autoantigen(s) shared by the thyroid and the orbit, surgical removal of autoreactive T cells and autoantigens might be beneficial for GO [11]. Two recent studies, one retrospective [85] and the other randomized and prospective [86], seem to support the idea that early thyroidectomy might improve the outcome of immunosuppressive drug treatment in patients with active GO.

Management of Hyperthyroidism in Patients with GO

Treatment of hyperthyroidism when GO is present remains a challenging dilemma [87] (Table 8). In patients with *mild and active GO*, treatment of hyperthyroidism is independent of GO and is based on established criteria (age, goiter size, first episode vs. relapse, patient's

Table 8 Treatment of Graves' hyperthyroidism in patients with associated orbitopathy

Severity of GO	Treatment of hyperthyroidism
Mild and active	Choice of treatment (antithyroid drugs, radioiodine treatment, thyroidectomy) is selected independently of GO and based on established criteria, including patient's choice
	If antithyroid drugs are used, a 6-month selenium supplementation should be given
	If radioiodine treatment is selected, steroid prophylaxis should be given in at-risk patients
Mild and inactive	Choice of thyroid treatment (antithyroid drugs, radioiodine treatment, thyroidectomy) is selected independently of GO and based on established criteria, including patient's choice.
	If radioiodine treatment is selected, steroid prophylaxis is not indicated, unless there are risk factors for radioiodine-associated progression of GO
Moderate-to-severe and active	Treatment of GO should be prioritized
	Patients can be maintained euthyroid with antithyroid drugs or receive definitive treatment, while GO is being cured. There is no current evidence on superiority of either approach
Moderate-to-severe and inactive	Choice of thyroid treatment (antithyroid drugs, radioiodine treatment, thyroidectomy) is selected independently of GO and based on established criteria, including patient's choice
	If radioiodine treatment is selected, steroid prophylaxis is not indicated, unless there are risk factors for radioiodine-associated progression of GO
Sight-threatening	Hyperthyroidism must be controlled with antithyroid drugs, and definitive treatment, if needed, must be postponed until DON and/or corneal breakdown has been cured and GO is inactive

Derived from Bartalena et al. [46]

choice) [46] or regional differences [77]. In these patients, if antithyroid drugs are used, a 6-month course of selenium supplementation is recommended [39, 46, 53]. The usefulness of selenium supplementation in patients treated with radioiodine or thyroidectomy remains to be established [88]. Steroid prophylaxis is indicated in patients who smoke and have high TRAb levels [50] after radioiodine, but not in patients undergoing antithyroid drug treatment of thyroidectomy [46]. In patients with *mild and inactive GO*, in principle any treatment is unlikely to affect GO, and steroid prophylaxis is not recommended [46] (Table 8). Patients with *moderate-to-severe and active GO* should be promptly treated with appropriate treatments (see below) for the orbital disease. Thyroid treatment in these patients is controversial. One line of thinking suggests that these patients should receive long-term antithyroid drug treatment while their GO is being cured, postponing possible ablative therapy thereafter [78, 89]. Other authors think that, even in these patients, thyroid ablation by thyroidectomy, radioiodine treatment, or both (total thyroid ablation) might be performed while curing GO [90–94]. Superiority of either approach in terms of outcome of GO remains to be demonstrated [46]. Patients with *moderate-to-severe and inactive GO* can be treated with any therapy for hyperthyroidism, and steroid prophylaxis is not needed if there are no risk factors for GO progression after radioiodine treatment, particularly smoking [46]. *Sight-threatening GO* is an emergency and needs to be treated with large doses of intravenous glucocorticoids and/or orbital decompression. In this case, hyperthyroidism should be managed with antithyroid drugs to maintain the patient euthyroid. Definitive treatment should be postponed until DON or corneal breakdown has been cured and GO is stably inactive [46].

Management of GO (Table 9)

Measures for All Patients with GO

All patients with GO of any degree should have risk factors for GO progression put under control. In particular, they should be urged to refrain from smoking. Hyperthyroidism and hypothyroidism should be corrected, and euthyroidism should be stably maintained. Ocular surface inflammation and dry eye should be treated with nonpreserved artificial tears, usually containing sodium hyaluronate, applied several times a day, or osmoprotective agents, such as carboxymethylcellulose. In case of corneal exposure (lagophthalmos), gel and ointments should be applied at nighttime [39]. Except for mildest cases which improve by normalizing thyroid dysfunction and using topical treatments, patients with GO should be referred to specialized centers or thyroid-eye clinics, because this is associated with a better outcome and patient's higher satisfaction [95].

Table 9 Management of Graves' orbitopathy

Measures for all patients with GO
Restore euthyroidism
Urge refrain from smoking
Local measures (artificial tears, ointments)
Refer to specialized centers or thyroid-eye clinics except for the mildest cases
Mild GO
Wait-and-see strategy
Selenium supplementation
Intravenous glucocorticoids only occasionally, if quality of life is severely affected
Rehabilitative surgery, if needed or required by the patient, when the disease is stably inactive
Moderate-to-severe and active GO
First-line treatment
Intravenous glucocorticoids [high-dose oral glucocorticoids are an option, with a less favorable profile of effectiveness and tolerability]
Second-line treatments (in the case of a partial response to first-line treatment)
Second course of intravenous glucocorticoids (not to exceed a cumulative dose of 8 g of methylprednisolone)
Orbital radiotherapy associated with oral glucocorticoids
Oral glucocorticoids associated with cyclosporine
Rituximab
Moderate-to-severe GO and inactive
Rehabilitative surgery, if needed or required by the patient, including one or more of the following procedures, in this order: orbital decompression, squint surgery, eyelid surgery
Sight-threatening GO
Very high doses of intravenous glucocorticoids
Urgent orbital decompression if response to intravenous glucocorticoids is absent or poor within 2 weeks

Derived from Bartalena et al. [39]

Mild Disease

In most cases of mild GO, a wait-and-see strategy is enough, because these cases may remit and only infrequently progress to more severe forms of disease. Selenium supplementation is useful, as discussed above [53]. Some patients with mild GO have, nevertheless, a profound deterioration of their QoL. In these cases, immunosuppressive treatment as for moderate-to-severe GO may be used. However, in principle this treatment should be avoided in patients with mild GO, because risks outweigh benefits [39].

Moderate-to Severe Disease

High-dose intravenous glucocorticoids are the first-line treatment for *moderate-to-severe and active GO* [96]. Intravenous glucocorticoids are more effective and better tolerated than oral glucocorticoids [64, 65, 96, 97]. Intravenous glucocorticoids are usually administered in 12 weekly, slow (2–3 h) infusions. The most common cumulative dose is 4.5 of methylprednisolone (6 infusions of 500 mg, followed by 6 infusions of 250 mg) [64, 65]. A shorter protocol (500 mg for 3 days for 2 weeks, followed by 250 mg for 3 days for 2 weeks) proved to be less effective [98]. In a large randomized clinical trial carried out by EUGOGO in which three different cumulative doses of methylprednisolone (2.25 g, 4.98 g, 7.47 g) were compared, the highest dose was slightly more effective but also associated with a higher rate of adverse events [99]. Therefore, also considering the potential serious side effects of

this treatment [100], EUGOGO recommended a medium dose (4.5 g) for most cases, reserving the higher dose (7.5 g) to most severe cases [6, 39, 73, 76]. In any case, the cumulative dose should not exceed 8 g to minimize the risk of liver toxicity [101, 102], and the single dose should not be higher than 0.75 g [103]. This treatment is contraindicated in patients with severe cardiovascular problems, uncontrolled hypertension, uncontrolled diabetes, liver dysfunction and recent viral hepatitis, and psychiatric disorders [96]. Glucocorticoids appear to be particularly effective on soft tissue changes and ocular motility, whereas exophthalmos is less responsive [45]. Effectiveness is greater when GO duration is less than 1 year. One problem observed with glucocorticoid therapy is represented by the not infrequent recurrence of the disease [99], making a second course of intravenous glucocorticoids or the use of a second-line treatment necessary. Indeed, what to do when the first course of intravenous glucocorticoids provide only a partial response remains a dilemma and a challenge [87].

Orbital radiotherapy is a valid second-line treatment, effective particularly on extraocular muscle dysfunction [104]. It is administered in ten daily fractions over a 2-week period, with a cumulative dose of 20 Gy per eye [104], although different protocols have been proposed [104]. Orbital radiotherapy and *oral* glucocorticoids in combination are more effective than either treatment alone [104]. No information from randomized clinical trial is available on whether adding orbital radiotherapy to *intravenous* glucocorticoids improves the treatment outcome. However, two recent retrospective studies showed the effectiveness of combination therapy, particularly in women [102], and the greater effectiveness of the combined therapy compared to intravenous glucocorticoids alone [105]. Randomized clinical trials are needed to clarify this issue. Data on long-term safety are reassuring, but orbital radiotherapy should be avoided when diabetic or hypertensive retinopathy is present [45].

Another second-line treatment for moderate-to-severe and active GO is the combination of cyclosporine and oral glucocorticoids. This is based on the results of two randomized clinical trials [106, 107], which showed that the combination of the two drugs was more effective than either drug alone. The starting dose of cyclosporine was 5 mg/kg body weight in one study [106] and 7.5 mg/kg body weight in the other one [107]. Cyclosporine treatment may be associated with adverse events, including dose-dependent liver and renal toxicity, infections, and gingival hyperplasia [39].

Rituximab is a CD20+ B cell-depleting monoclonal antibody, initially used for the treatment of non-Hodgkin lymphoma and then off-label in several autoimmune disorders. After few uncontrolled studies [108], two small randomized clinical trials have been recently published on its use in patients with moderate-to-severe GO. In one study rituximab was compared with placebo [109], while in the other one the drug was compared with intravenous glucocorticoids [110]. The two studies provided conflicting results. In the first study, the outcome in patients treated with rituximab was not different from that of patients receiving placebo [109]. Conversely, in the second study rituximab treatment was associated with a higher rate of inactivation of GO compared to intravenous glucocorticoids and was not followed by flare up of GO observed in 31% of glucocorticoid-treated patients [110]. The reasons for this discrepancy between the two studies remain elusive, but in the first study duration of disease was longer, possibly making the patients less responsive to the treatment [109]. In the absence of larger, multicenter randomized clinical trials, for the time being rituximab cannot be recommended as first-line treatment for GO. Minor and major adverse events may occur with rituximab. In the two above studies, progression of DON occurred in 4 of 25 patients (16%) during rituximab treatment [111]. Accordingly, rituximab should not be used in patients with impending or overt DON [39].

Because all of the available therapies for moderate-to-severe and active are not always effective [72], advantages and disadvantages of each treatment should be thoroughly discussed with the patient in a shared decision-making process which puts the patient at the center of healthcare [112].

In patients with *moderate-to-severe and inactive GO*, medical treatment has no role, and different types of rehabilitative surgery, including orbital decompression, squint surgery, and eyelid surgery, may be required for residual cosmetic and functional reasons. Should all of the above procedures be needed, they should be performed in the given order [39].

Sight-Threatening Disease

Sight-threatening GO may be due to DON and/or corneal breakdown and constitutes an emergent situation. Corneal breakdown should be treated by different measures aimed at protecting the cornea, such as lubricants, moisturized chambers, blepharorrhaphy, and tarsorrhaphy. DON requires immediate treatment with high doses of intravenous glucocorticoids (500–1000 mg of methylprednisolone for 3 consecutive days or on alternate days) [113, 114]. This treatment can be repeated on the next week, but if the recovery of visual acuity is absent or poor, the patient should be submitted to urgent decompression surgery to preserve his/her sight [39].

Conclusions and Perspectives

GO is a rare disease, particular in its severe expressions. This likely derives from earlier diagnosis and treatment of the orbital disease and associated thyroid dysfunction. Prompt referral to specialized centers plays a major role in this regard. Overt GO is usually easy to diagnose, but asymmetrical or unilateral forms require a careful diagnostic work-up, particularly if autoimmune phenomena and/or thyroid dysfunction is absent. Management of moderate-to-severe and active forms of GO still relies on imperfect treatments [71, 72]: intravenous glucocorticoids remain, for the time being, the first-line treatment. This and other treatments are not targeting pathogenic pathways of the disease. However, recent studies have improved our understanding of the pathogenesis of the disease [37, 115, 116], and it is expected that the results of ongoing and future trials using drugs targeting different actors involved in the cascade of events occurring in the GO orbit (orbital fibroblasts, B cells, T cells, cytokines) will be available in the near future.

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Subclinical Hyperthyroidism

Gabriela Brenta and José Sgarbi

Definition

Subclinical hyperthyroidism has been biochemically defined by values of serum TSH below the lower range of reference with thyroid hormones within the normal range [1].

Subclinical hyperthyroidism is also known as subclinical thyrotoxicosis, a broader term that refers to inappropriately high thyroid hormone action in tissues. Subclinical thyrotoxicosis therefore includes both the "exogenous" and the "endogenous" forms. Exogenous subclinical thyrotoxicosis is due to administration of thyroid hormones, while the endogenous form can be explained either by the "release of stored thyroid hormones" or by a "true form of hyperthyroidism with increased synthesis and secretion of thyroid hormones by the thyroid gland" [2].

Endogenous subclinical thyrotoxicosis due to the release of stored thyroid hormones is usually transient, while the endogenous subclinical thyrotoxicosis due to the "true form of hyperthyroidism with increased synthesis and secretion of thyroid hormones by the thyroid gland" is usually of a permanent nature. From now on we shall refer to permanent endogenous subclinical thyrotoxicosis with the term "Shyper."

Two categories of Shyper can be defined according to levels of TSH below the lower normal limit [1]:

Grade 1 Shyper: the one that is between the functional sensitivity of the second TSH generation methods, 0.1 mIU/L, and the lower limit of the reference range of TSH, usually considered as 0.39 mIU/L

Grade 2 Shyper: is the category defined by TSH levels below 0.1 mIU/L

Etiology

As stated endogenous subclinical thyrotoxicosis can be transient or persistent [3]. The causes of Shyper are the same as those of overt hyperthyroidism: Graves' disease and autonomously functioning thyroid nodules (AFTN). AFTN include the solitary toxic adenoma (TA) and toxic multinodular goiter (TMNG).

Graves' disease has an autoimmune origin where thyrotropin receptor antibodies (TRAbs) stimulate the thyroid gland to produce thyroid hormone [4], while AFTN is mainly caused by the gradual progression of hormone secretion from autonomous nodules with somatic gain-of-function mutations in the TSH receptor or the stimulatory Gs alpha subunit [5, 6].

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Table 1 Differential diagnosis of Shyper with other causes of low TSH [1]

1. Delay in the recovery of thyrotrophs after treatment for hyperthyroidism (delayed readjustment of the thyroid axis)
2. Pregnancy (in 1° trimester)
3. Non-thyroidal illness (NTI)
4. Drugs (dopamine, corticoids, somatostatin analogues, dobutamine, amphetamine, bexarotene, bromocriptine)
5. Central hypothyroidism (in general with low or normal T4)
6. Psychiatric diseases
7. Age-related reduced thyroid hormone clearance
8. Presence of heterophile antibodies (HAMA)

Transient endogenous subclinical thyrotoxicosis, on the other hand, is mainly due to different types of thyroiditis, including subacute, (viral or DeQuervain's), silent, and postpartum thyroiditis [7, 8], and recent excess iodine intake, such as in type 2 amiodarone-induced thyrotoxicosis [9], or other drugs such as interferon-alpha [10]. Treatment of overt hyperthyroidism with antithyroid drugs or radioiodine can also origin transient endogenous subclinical thyrotoxicosis [3].

Exogenous subclinical thyrotoxicosis can result from an unintentional over-replacement of thyroid hormones in hypothyroid patients [11, 12], the surreptitious intake of thyroid hormones in non-approved indications such as obesity [13], or intentional TSH suppression therapy in differentiated thyroid cancer [14] or in patients with nontoxic multinodular goiters although this procedure is no longer recommended [15].

The differential diagnosis of Shyper with other causes of low TSH levels is described in Table 1.

Epidemiology

Although the prevalence of Shyper might be estimated about 4.2% [16], it really depends upon the considered levels of TSH, the iodine intake, and the age of the analyzed population. Population studies in iodine-sufficient areas show a prevalence of Shyper that spans from 0.7% in case of Shyper 2 and up to 1.8% for Shyper 1 [17], while in elderly subjects living in an iodine-deficient area, the proportion of Shyper might increase up to 15% [18].

With regard to age differences, it has to be considered that TMNG is more prevalent in aged patients, while Graves' disease is more frequent in younger populations [19].

Unfortunately, the exogenous form is by far the most frequent cause of subclinical thyrotoxicosis. It has been reported that up to 40% of hypothyroid patients under levothyroxine therapy are over-replaced and have TSH below the lower limit of TSH [11]. In a more recent communication, however, a lower prevalence (9.6%) of iatrogenic thyrotoxicosis was found for those patients on thyroid hormone participating in the Baltimore Longitudinal Study of Aging. Exogenous thyrotoxicosis accounted for approximately half of both prevalent and incident low TSH events [12].

Natural History (Progression to Overt Hyperthyroidism)

One important aspect to consider for the management of Shyper is the possibility of progression to an overt form of hyperthyroidism. However this depends mainly on the cause of Shyper and on the initial level of TSH.

In Graves' disease, TSH values have better chance of reverting to normal values or to progress rapidly to clinical hyperthyroidism unlike TMNG that usually has a more indolent course [20]. Nevertheless, caution should be taken in these patients because despite their low progression to overt disease, certain situations such as an iodine load in a contrast study may precipitate severe hyperthyroidism [21]. Furthermore, the size of the hot nodule is also related to overt thyrotoxicosis. A nodule with a diameter of 3 cm or larger has been associated to 20% of overt thyrotoxicosis in 6 years [22].

With regard to the initial level of TSH suppression, some reports suggest that Shyper may spontaneously resolve, especially if the levels of TSH are low but detectable [23–25]. Likewise, in aged patients with TSH between 0.1 and 0.4 mIU/L and in whom AFTN was the main

cause of Shyper, the progression toward clinical hyperthyroidism was described to be unfrequent (approximately 1% per year) [26]. Furthermore, in another study performed in patients above 60 years old followed for 10 years, only 4.3% developed overt hyperthyroidism [27]. On the other hand, in patients with TSH <0.1 mIU/L or grade 2 Shyper, a higher rate of progression to overt hyperthyroidism (hazard ratio 3.4, confidence interval 1.6–7.0) was reported [28].

According to the variable evolution that Shyper may have, in untreated patients it has been recommended to monitor with TSH and free T4 or T3 every 6–12 months or sooner if there is a change of the clinical picture [1]. This procedure will inform about persistence, progression, or disappearance of the disease.

Biochemical and Morphologic Diagnosis

TSH and Thyroid Hormones Determination

The determinations of TSH and total and/or free thyroid hormones are used for the diagnosis of Shyper.

Serum TSH is used as the first-line diagnostic test for Shyper because even a small elevation in serum free T4, that is still within the normal range, will cause a decrease in serum TSH outside its reference range. This is explained by an inverse log-linear relationship between TSH and the concentrations of free thyroxine (T4). This relationship determines that small linear increases in free T4 concentrations are associated with an exponential decrease in TSH concentrations [29].

Although TSH is a very robust assay, given the inherent biological variability of TSH and potential episodes of silent thyroiditis and systemic illness, several authoritative guidelines advice to assure the diagnosis of Shyper with 1 second determination of TSH after 2–3 months [1] or 3–6 months [2]. Although an extended interval to reassess TSH is optimal, certain clinical circumstances such as atrial fibrillation, cardiac disease, or other serious medical conditions may compel physicians to repeat the TSH determination in a shorter lapse of time.

In the second hormone assay, it has been recommended to measure free T4 and free triiodothyronine (T3) or total T3 to discard overt hyperthyroidism, central hypothyroidism, or non-thyroidal illness (NTI) [1]. Moreover, in T3 toxicosis, free T4 might be normal, while high levels of free T3 might discriminate Shyper from overt free T3 toxicosis [30].

TRAbs can also be included in the second determination for cases in which it is deemed necessary to distinguish between Graves' disease and TMNG [31]. They are especially useful when a thyroid scan and uptake are unavailable or contraindicated (e.g., during pregnancy and nursing). In iodine-deficient areas, however, the differential diagnosis might be difficult since approximately 17% of patients with scintigraphic criteria for TMNG may be positive for TRAb reflecting an overlap between both diseases [32].

In order to distinguish Graves' disease or AFTN from thyroiditis, the ratio of total T3 to total T4 can be useful. In true hyperthyroidism more T3 is synthesized than T4 with a ratio (ng/mcg) that is usually >20, while it is <20 in painless or postpartum thyroiditis [33].

In hospitalized patients, diagnosis of Shyper can be a challenge, because a suppressed TSH is less specific than in ambulatory patients and because free T4 assays are not reliable in that setting. Considering that TSH levels can become transiently subnormal in the acute phase of NTI, the degree of TSH suppression might be of aid for diagnosis. TSH levels <0.01 mIU/L may indicate true hyperthyroidism, while a low but detectable TSH may imply a transient TSH reduction or the result of the use of dopamine and steroids [34].

Several biochemical markers such as alkaline phosphatase, sex hormone-binding globulin (SHBG), liver enzymes, osteocalcin, cholesterol, etc. can be employed to study the peripheral action of thyroid hormones [35, 36]. However, their use is not recommended in Shyper due to their lack of accuracy.

Nuclear and Imaging Studies

Scintigraphy or thyroid scan and a 24-h radioactive iodine uptake (RAIU) test are very valuable methods for the etiologic diagnosis.

A RAIU should be performed when the clinical presentation of thyrotoxicosis is not clearly diagnostic of Graves' disease and other causes of thyrotoxicosis have to be distinguished. Furthermore, a thyroid scan should be added in the presence of thyroid nodularity [2] in particular in grade 2 Shyper to guide clinicians in the choice of treatment [1].

The use of thyroid scintigraphy with the objective to identify autonomous tissue has been also recommended, despite normal TSH levels, in patients with MNG from regions of long-standing insufficient iodine supply [37, 38]. Its contribution to diagnosis has been confirmed in a study where scintiscan was the most sensitive tool to detect AFTN [39]. Moreover, a recent meta-analysis has shown that about half of the patients with AFTN discovered in a scintigraphy had a TSH value within normal references (40).

Thyrotoxicosis due to diverse forms of destructive thyroiditis exhibit RAIU near 0% similarly to iodine-induced thyroiditis where the radioiodine uptake may remain low for 1–2 months after exposure. In this case the measurement of 24-h urinary iodine excretion may help to confirm suspected excessive iodine intake [1]. On the contrary, Graves' disease patients will display a moderate or frankly elevated uptake with a homogenous scintigraphic image. Autonomous adenomas will show the typical image of the single hot nodule and TMNG multiple hot areas, although very often a characteristic speckled or heterogenous pattern will be observed [1] (Fig. 1).

Thyroid scintigraphy can be performed with ^{131}I; however an alternative to ^{131}I scintiscan is a ^{123}I or a ^{99m}Tc—(sodium pertechnetate) scintigraphy. The advantage of using these two radioisotopes is a lower total body radiation exposure than with ^{131}I. However ^{123}I is expensive and not always available, while ^{99m}Tc, that is trapped by the thyroid but not organified, can yield some false-positive (about 5%) hot nodules that are not truly autonomous [41].

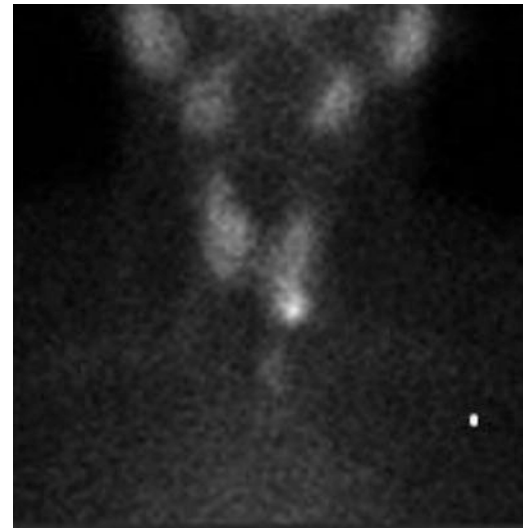

Fig. 1 Thyroid scintigraphy with 5 mCi ^{99m}Tc showing a multinodular goiter with increased uptake in lower left lobe in a patient with Shyper due to a TMNG

Another imaging test worth considering for the diagnosis of Shyper is Doppler ultrasound. Color Doppler flow of the inferior thyroid artery may be useful in the differential diagnosis of thyrotoxicosis in cases where nuclear imaging is contraindicated. Such is the situation with pregnancy and lactation, recent intake of iodine-rich food, and injection of iodine-based contrast media (coronary angiography, computed tomography, etc.) or when TRAb are not available. Diffusely increased thyroid blood flow is pathognomonic of untreated Graves' disease. Peak systolic velocity of the inferior thyroid artery was reported significantly higher (>40 cm/s) in Graves' disease patients when compared to patients with destructive thyroiditis [42]. However, in a recent study assessment of the peak systolic value at the superior rather than at the inferior thyroid artery was proposed as an easier way to differentiate between these two entities [43].

Although the likelihood of malignancy in a toxic nodule is very low [44], the presence of malignant nodules in Shyper patients is always of concern. The only indication to perform a fine needle aspiration in patients with TMNG is within the hypofunctioning thyroid nodules in a thyroid scan, particularly in those with suspicious ultrasound findings [15].

Computed tomography (CT) or magnetic resonance imaging (MRI) can also be indicated for diagnosis of patients with Shyper and large goiters. An objective measure of thyroid size evaluated by diagnostic imaging of intrathoracic (often referred to as substernal) goiter in patients with compressive symptoms can be achieved by these imaging tests. Furthermore, either CT or MRI can detect extrathyroidal extension and/or regional lymphadenopathy suggestive of thyroid malignancy and provide valuable information regarding the dimensions of the trachea and surgery strategies [45, 46].

Assessment of Clinical Significance

Due to the detrimental impact of Shyper on the cardiovascular system, several cardiac tests such as ECG, Holter ECG, and Doppler echocardiography have been recommended for symptomatic patients, the elderly, or those with cardiovascular risks or previous cardiovascular disease. Furthermore, bone mineral density should also be assessed in postmenopausal women, in elderly patients, and in patients with underlying bone risk factors [1].

Clinical Significance

The clinical consequences of overt hyperthyroidism on the general health, particularly on the cardiovascular and skeletal system, are well established, but the clinical significance of Shyper remains unclear. However, more recently a growing body of high-quality evidence has associated Shyper with an increased risk of coronary heart disease (CHD), atrial fibrillation (AF), bone fractures, and lower life expectancy. Less consistently, Shyper has been associated with a decreased quality of life, cognitive impairment, dementia, insulin resistance, and hypercoagulability (Table 2).

Quality of Life, Cognitive Impairment, and Dementia

The literature on the association of Shyper with quality of life, cognitive impairment, and dementia is large, heterogeneous, and controversial. Patients with Shyper are usually asymptomatic, but some few small studies have associated Shyper with clinical manifestations of thyrotoxicosis, particularly when applying specific clinical indexes to rate signs and symptoms of thyrotoxicosis [47, 48]. In fact, a larger prospective cohort study on hyperthyroidism in France showed that most patients with Shyper had signs or symptoms of thyrotoxicosis [49], but in another large community-based study in women, no impact of Shyper on well-being or quality of life was found [50].

In the last years, there have been an increasing number of studies exploring an association of Shyper with cognitive impairment and dementia with conflicting findings. The first data suggesting an association between Shyper and dementia or Alzheimer's disease was derived from the Rotterdam Study. In a sample of 1843 participants aged ≥55 years, subjects with reduced TSH levels at baseline had a more than threefold increased risk of dementia and Alzheimer's disease after adjustment for age and sex over 2-year follow-up [51]. More recently, a cross-sectional population-based study from Brazil with 1119 elderly ≥65 years also described an association of Shyper with any type of dementia and vascular dementia in men, but not in women [52]. In another prospective population-based study from Korea [53] with 313 participants (mean age 72.5 ± 6.9 years), a lower normal serum TSH level (but not FT4 level) was independently associated with the risk of cognitive impairment and dementia during 5-year follow-up. In an Australian prospective population-based study, comprising 3401 community-dwelling men aged 70–89 years, there was no association between TSH quartiles and incident dementia over 5.9-year follow-up. However, men who developed dementia had higher baseline FT4 levels compared with men who did not receive this diagnosis, and the association persisted significant even when the analysis was restricted to euthyroid men [54].

Conversely, in a larger retrospective cohort from Scotland including 2004 patients with Shyper, no relationship with TSH concentration was found, suggesting no causal relationships between Shyper and dementia [55]. Moreover,

Table 2 Summary of evidences on the clinical relevance of subclinical hyperthyroidism

	Shyper grade 1 (TSH 0.1–0.39 mIU/L)	Shyper grade 2 (TSH < 0.1 mIU/L)
Quality of life	Insufficient	Insufficient
Cognitive dysfunction and dementia	Moderate (elderly)	Moderate (elderly)
Metabolic consequences	Insufficient	Insufficient
Osteoporosis	Insufficient	Strong (postmenopausal women)
Fractures	Insufficient	Strong
Atrial fibrillation	Strong (≥60 year)	Strong (≥60 year)
Heart failure	Insufficient	Strong
Coronary heart disease and mortality	Insufficient	Strong
Stroke	Insufficient	Insufficient
Thromboembolism	Insufficient	Insufficient

in the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) with 5182 participants with a mean age of 75.2 years and a follow-up of 42 months, there were no differences on the self-reported functional capacity between participants with Shyper compared to those in euthyroidism [56]. In the same population, another study also found no consistent association of Shyper with altered cognitive performance on the individual cognitive tests [57]. In another prospective cohort study from Spain with 307 inhabitants aged 85 years at baseline, Shyper patients were not significantly associated with poor physical or cognitive function at baseline when compared to euthyroid subjects [58]. A negative finding was also found in a prospective cohort of the Longitudinal Aging Study Amsterdam comprising 1219 individuals aged ≥65 years. In this study, Shyper was not related to impairment in any of the tested domains of cognitive function nor to more depressive symptoms at baseline compared to euthyroid subjects [59].

Finally, in two recent reviews [60, 61] including several well-designed and well-powered studies, Shyper was significantly associated with cognitive impairment or dementia in elderly people.

In summary, at the current time, there is no definitive evidence on the association of Shyper with low quality of life, but there is moderate-quality evidence on the association of Shyper with cognitive impairment and dementia in older people. Nevertheless, there is still a need of larger, powered, and well-designed studies as to allow analysis according to TSH levels and age groups (Table 2).

Metabolic Consequences

Thyroid hormones have important effects on lipid metabolism that are clearly observed in overt hyperthyroidism [62]. With regard to the lipoprotein profile of Shyper patients, normal levels of total LDL and HDL cholesterol, triglycerides, Lp(a), apoA1, and apoB have all been reported [63]. On the other hand, in a population screening study of patients over 60 years, with persistently low TSH with normal free T4, a reduction in total cholesterol was detected [64]. Furthermore, in TMNG patients with Shyper, total serum HDL, LDL cholesterol, and triglycerides were lower when compared to a control group [65].

Similarly to what has been described in overt hyperthyroidism [66], subclinical thyrotoxicosis has also been associated with insulin resistance [67–69], although in some but not all studies [70]. The heterogenous nature of this condition can partly explain this controversy. Shyper may have a larger impact on glucose metabolism due to its chronicity and higher T3 levels when compared to exogenous administration of T4 [69].

Osteoporosis and Fractures

Thyroid hormones strongly affect the skeletal development and bone structure and strength by acting in all phases of the bone remodeling cycle, stimulating both bone formation and reabsorption [71]. Thus, either thyroid hormone excess or deficiency can have detrimental effects in the bone. In fact, overt hyperthyroidism has been consistently associated with bone mineral density (BMD) loss, osteoporosis, and fractures, but whether Shyper is associated with the same risks remains controversial [72].

In the last three decades, data derived from several small studies on the association of Shyper with loss of BMD, osteoporosis, and fractures are conflicting. However, most of these studies agree on an association between Shyper and a reduction in BMD and osteoporosis in postmenopausal women [3, 71]. Two meta-analyses found that a long-term suppressive L-thyroxine treatment is associated with a significant BMD loss in postmenopausal women, but not in premenopausal women [73, 74]. In fact, a recent review [3] found no evidence of an association between Shyper and deleterious bone consequences in men or in premenopausal women.

In the last years, Shyper has been related to an increased risk of osteoporotic fractures, but results derived from prospective studies are also conflicting. In the Cardiovascular Health Study (CHS), a prospective cohort of 3567 US community-dwelling ≥65 years, men (but not women) with Shyper had a more than fourfold increased incidence of hip fractures compared to euthyroid individuals in 13-year follow-up [75]. Interestingly, a subsequent study with an expansion of the same study population to 4936 participants found no association between Shyper and incident hip fracture in either sex. These results were strengthened by the findings in a subset of 1317 participants with dual-energy X-ray absorptiometry scans in whom Shyper was not related to loss of BMD at the lumbar spine, total hip, or femoral neck sites [76].

In another population-based prospective cohort study from Israel comprising 14,325 participants ≥65 years and a mean follow-up of 102 ± 3 months, low-normal TSH levels were associated with a higher risk of hip fractures in euthyroid women, but not men [77]. In a larger population-based cohort study from Denmark, a first and single low TSH in a patient without known thyroid disease was associated with an increased risk of hip fracture over a median follow-up of 7.5 years, which remained significant in women but not in men after adjusting for confounders. In addition, in this study the risk increased exponentially by the length of time during which TSH remained low, and the risk of fractures increased significantly with each SD unit of TSH decrease in euthyroid patients [78]. By contrast, in a large retrospective cohort study from Scotland, Shyper was associated with a higher risk of osteoporotic fracture, but there was no dose-response effect according to TSH level, suggesting no causal effect [55].

Despite controversies among prospective cohort studies, three recent meta-analyses of prospective studies have demonstrated an increased fracture risk in Shyper. In a first meta-analysis with 50,245 participants, it was reported that Shyper might be associated with an increased risk of hip and nonspine fractures, particularly for adults with a TSH ≤ 0.1 mIU/L [79]. In a second meta-analysis, individual participant data were obtained from 13 prospective cohorts comprising 70,298 participants. Compared to euthyroid participants, the HR for Shyper was 1.36 for hip fracture (95% CI, 1.13–1.64), 1.28 for any fracture (95% CI, 1.06–1.53), 1.16 for nonspine fracture (95% CI, 0.95–1.41), and 1.51 for spine fracture (95% CI, 0.93–2.45). Lower TSH (≤0.10 mIU/L) was associated with higher fracture rates [80]. Finally, the third meta-analysis included 314,146 participants from five population-based cohort studies including both endogenous and exogenous subclinical thyroid dysfunction. The relative risk (RR) for subclinical hyperthyroidism vs. euthyroid subjects was 1.25 (95% CI 1.11–1.41) in a multivariable-adjusted model, and a subgroup analysis indicated that the risk of fracture was higher in the endogenous group than the exogenous group [81].

These data show that there is high-quality evidence on the association of Shyper with an increased risk of BMD loss and osteoporosis in postmenopausal women, as with an increased risk of osteoporotic fractures in elderly, particularly for those with grade 2 Shyper (TSH ≤ 0.1 mIU/L) (Table 2).

Atrial Fibrillation

The association between Shyper and the increased risk of atrial fibrillation (AF) has been considered the most consistent evidence to recommend treatment of Shyper in elderly people with both Shyper grade 1 and Shyper grade 2 [1, 2], based on data derived from prospective studies and meta-analysis.

In a prospective cohort of the Framingham Heart Study with 2007 subjects ≥60 years, a low serum TSH (≤0.1 mIU/L) at baseline was associated with a threefold higher risk of AF in a 10-year follow-up period, while for those with slightly low TSH (0.1–0.4 mIU/L) values, no significant difference was found [82]. In the context of the CHS, which consisted of 3233 individuals aged 65 years or older, participants with Shyper had nearly twice the risk of developing AF in a 13-year follow-up period. The risks (HR) were similar for both Shyper grade 2 [1.98 (95% CI, 1.29–3.03), $p < 0.001$] and Shyper grade 1 [1.85 (95% CI, 1.14–3.00), $p = 0.007$] [83].

Compared to euthyroid subjects, in a large population-based cohort study from Denmark, comprising 586,460 individuals, the risk [incidence rate ratio—IRR (95% CI)] of AF increased with decreasing levels of TSH, from individuals with high-normal thyroid function [TSH 0.2–0.4 mIU/L, 1.12 (1.03–1.21)] to those with mild Shyper [TSH 0.1–0.2 mIU/L; 1.16 (0.99–1.36)] and more severe Shyper [TSH < 0.1 mIU/L, 1.41 (1.25–1.59)] in a median follow-up of 5.5 years [84]. Finally, in a recent individual participant data meta-analysis with 8711 participants from 5 cohorts, during a mean follow-up of 8.8 years, in age- and sex-adjusted analyses, the overall HR (95% CI) for participants with Shyper compared with euthyroidism was 1.68 (1.16–2.43; 17.1 vs. 12.5/1000 person-years). The risks were increased for both Shyper grade 1 [1.63 (1.10–2.41)] and Shyper grade 2 [2.54 (1.08–5.9) [16].

Taken together, these data suggest that the risk of AF in individuals with Shyper aged 60 years or more is higher for both grade 1 and grade 2 Shyper. In addition, these findings also suggest a dose-response relationship between low TSH levels and an increased risk of AF and justify recommendations for treating all patients >60 years with grade 1 and grade 2 Shyper [1, 2] (Table 2).

Heart Failure

Thyroid hormones have marked effects on the heart and cardiovascular system through genomic and non-genomic actions. It is well known that in overt hyperthyroidism thyroid hormone excess can lead to a hyperdynamic state, systolic and diastolic dysfunction, cardiac hypertrophy, low ventricular performance, increased pulmonary arterial pressure, and heart failure (HF) that can be reversible after euthyroidism with treatment [85]. Moreover, in some studies, but not in all, Shyper has been associated with similar abnormalities, such as with increased resting heart rate, supraventricular arrhythmias, increased left ventricular mass, impairment of systolic and diastolic functions, and hemodynamic abnormalities, which could be reversible after restoring the euthyroid state [48].

More recently, some population-based prospective studies have assessed the association between Shyper and HF. Rodondi et al. [86] studied 3044 individuals ≥65 years initially free of HF in the CHS. Compared to euthyroidism, Shyper was associated with larger left atrial size, impaired E/A ratio, and increased heart rate, although no increased risk of HF was found during the 12-year follow-up. Nanchen et al. [87] studied the incidence rate of HF hospitalization according to baseline thyroid function in 5316 patients aged 70–82 years with known cardiovascular in the context of PROSPER study. Over 3.2-year follow-up, the rate of HF was higher for Shyper compared with euthyroidism [HR = 2.93 (95% CI, 1.37–6.24, $P = 0.005$)].

Gencer et al. [88] performed a pooled analysis of individual participant data from 6 prospective cohorts which consisted of 25,390 individuals. Among 648 (2.6%) Shyper participants, in an age- and sex-adjusted analyses, risk [HR (95% CI)] of HF events was significantly increased for TSH levels ≤0.10 mIU/L [1.94 (1.01–3.72)], but not for TSH of 0.10–0.44 mIU/L [1.31 (0.88–1.95)], compared to euthyroidism. However, in a study including 758 patients hospitalized for systolic HF, Shyper was not associated with increased age-adjusted mortality risk after a median follow-up of 3 years [89], and no clinical trial has assessed yet whether treating Shyper improved HF outcome.

In conclusion, Shyper is consistently associated with an increased risk of HF in older people, particularly for those with Shyper grade 2 (Table 2).

Coronary Heart Disease and Mortality

The association between Shyper and CHD has been investigated in several prospective population-based cohort studies with variable results. Some studies have reported significant findings [90], while others have found no association between Shyper and cardiovascular risk [83]. Similarly, data from study-level meta-analyses on the topic are also conflicting. In a meta-analyses including 3385 individuals from 5 higher-quality prospective studies, Ochs et al. [91] found that Shyper was associated with only a modest increased relative risk [RR (95% CI)] for CHD [1.21 (0.88–1.68)], cardiovascular mortality [1.19 (0.81–1.76)], and total mortality [1.12 (0.89–1.42)]. By contrast, based on 7 cohorts including 290 participants with Shyper, Haentjens et al. [92] estimated that the pooled HR (95% CI) for all-cause mortality was 1.41 (1.12–1.79), being the excess mortality increased beyond the age of 60, especially in aging men.

Several factors have been implicated to justify these controversial findings, including different population characteristics (such as ethnia, age, gender), different Shyper and CHD definitions, different inclusion and exclusion criteria, and different confounder adjustments among studies. However, most recently, a well-designed, powered, and robust study based on individual participant data (IPD) analysis from large cohort studies might have reconciled these conflicting results, by having uniformed inclusion and exclusion criteria, CHD definition, and TSH cutoff levels used for Shyper definition for all participants, therefore providing pooled survival estimates less prone to bias [16].

In such IPD analysis, individual data on 52,674 (2188 with Shyper) were pooled from 10 cohorts. In age-and sex-adjusted analyses, Shyper was significantly associated with an increased risk [HR (95% CI)] of CHD events [1.21 (CI, 0.99–1.46)], CHD mortality [1.29 (1.02–1.62)], and total mortality [1.24 (1.06–1.46)]. Risks remained significant even after further adjustment for cardiovascular risk factors and did not differ significantly by age, sex, or preexisting cardiovascular disease. However, CHD mortality risks were higher in participants with Shyper grade 2 compared to those with Shyper grade 1 [16].

In summary, despite controversy among prospective studies and meta-analyses, there is now strong evidence suggesting a significant association between Shyper and fatal and nonfatal CHD, particularly for TSH levels <0.1 mIU/L. However, clinicians should take these data with caution, since there are no randomized controlled studies on the benefits of treating Shyper regarding these outcomes [93] (Table 2).

Stroke

Stroke is one of the most important causes of mortality and morbidity globally, and some of its risk factors such as hypertension and cardiac arrhythmia, particularly AF, are associated with Shyper. In fact, the link between Shyper and AF has been consistently evidenced among prospective studies [82, 83] and meta-analysis [16]; nevertheless, the association between Shyper and stroke still remains unclear. There are a few available studies with heterogeneous qual-

ity and results on the topic. In a small case-control Swedish study including 153 patients with acute ischemic stroke, unknown Shyper was significantly associated with the cardio-embolic (based on the presence of AF) compared to non-embolic group (13% vs. 3%, $p = 0.048$) [94]. In another small study with a total of 165 consecutively recruited patients admitted for ischemic stroke, patients with Shyper had a significant increased risk of functional disability 3 months after stroke compared with those in euthyroidism [odds ratio, 2.63 (95% CI, 1.02–6.82)], adjusted for age, sex, and smoking status [95]. In a population-based prospective study including 609 subjects ≥50 years from general practice in Denmark, the incidence of stroke in median of 5 years of follow-up was substantially greater among Shyper subjects compared to euthyroid [HR 3.39 (95% CI, 1.15–10.00, p = 0.027)] after adjusting for sex, age, and atrial fibrillation [96].

Conversely, in a more consistent cohort study comprising 563,700 (mean age, 48.6 ± 18.2 years) subjects without prior thyroid disease from primary care in Denmark, the incidence rate ratios [1.02 (95% CI, 0.93–1.12)] of fatal stroke were not significantly associated with Shyper during a median follow-up of 5.5 years [97]. Most significantly, in a recent systematic review and meta-analysis with 6029 participants from 4 studies, no evidence supporting an increased risk for stroke associated with Shyper compared to euthyroidism was found [HR = 1.17 (95% CI, 0.54–2.56)] [98].

In conclusion, data about the association of Shyper with an increased risk of stroke are insufficient, and new larger prospective cohort studies are needed to clarify this uncertainty (Table 2).

Venous Thromboembolism

Thyroid hormone exerts important influence on the coagulation fibrinolytic system, and overt hyperthyroidism has been related to a hypercoagulable state and an increased thromboembolic risk [99], although there are few data on Shyper.

In a systematic review including only moderate-quality case-control and cohorts studies (no high-quality study was found), Shyper was significantly associated with subclinical laboratory findings suggesting a hypercoagulable and hypofibrinolytic state with a rise in factors VIII and IX, fibrinogen, von Willebrand factor, and plasminogen activator inhibitor-1 that could induce a prothrombotic state and a higher venous thromboembolism (VTE) risk [100]. However, in a most recent prospective multicenter cohort of 561 elderly participants, in a mean follow-up of 20.8 months, the VTE incidence rate was 0.00 (95% CI, 0.00–0.58) in Shyper compared with euthyroid participants, without increased levels of thrombophilic biomarkers, suggesting that Shyper could be associated with a lower VTE risk [101]. In addition, in a larger prospective study comprising 11,962 subjects aged 25–89 years, low TSH levels were associated with only a modest and nonsignificant higher risk [HR = 1.55 (95% CI, 0.87–2.77)] of VTE during 8.2 years of follow-up, suggesting that only a minor proportion of the VTE risk in the population can be attributed to Shyper [102].

In summary, despite some evidence suggesting an association between Shyper and subclinical laboratory abnormalities on the coagulation and fibrinolytic state, there is no consistent evidence suggesting that Shyper enhances the risk of clinical outcomes associated to an hypercoagulable and hypofibrinolytic state. Further prospective cohorts might be needed to provide a more definitive information on the clinical significance of the association between Shyper and a hypercoagulability state (Table 2).

Case Finding

Screening for Shyper is not currently recommended [103]. However, as mentioned above Shyper is associated with atrial fibrillation, congestive heart failure, and osteoporosis in older persons and postmenopausal women. Therefore, aggressive case finding is advocated in these two sets of populations in particular [104]. Moreover, although the definition of Shyper is biochemical and not clinical, palpitations, weakness, heat-related signs, and disturbed sleep have been reported in patients even with mild degree of

hyperthyroidism [49]. Therefore, Shyper has to be discarded also in the presence of these signs or symptoms.

Treatment

Indications of Treatment

In the last two decades there have been continuous and exciting debates whether Shyper should be treated or not [105, 106]. Despite a growing body of robust evidence that Shyper (particularly grade 2) is associated with a higher risk of progression to overt disease; with cognitive impairment, dementia, AF, HF, and fractures in older people; with osteoporosis in postmenopausal women; and with CHD event and mortality, there are several arguments against treatment. Among them are the low rate of progression to overt hyperthyroidism, the risks associated with the treatment, and the lack of appropriately large-scale randomized trials able to detect the benefit of treating on the outcomes [93, 105]. Thus, making a decision to treat or not a patient with Shyper relies mainly in the potential risks of not treating and in on our best clinical judgment.

However, some features seem to be consensual. In a hypothyroid patient with exogenous subclinical thyrotoxicosis due to excessive dose of levothyroxine, titrating the dose to obtain the target TSH levels according to age is recommended. In patients under treatment with suppressive levothyroxine therapy for persistent or recurrent differentiated thyroid carcinoma, the use of beta-blockers should be considered, particularly for those with symptoms of adrenergic hyperactivity, age > 60 years, or with cardiovascular risk or previous cardiovascular disease. Postmenopausal women with persistent Shyper, particularly those without estrogen replacement therapy, should be monitored with bone densitometry, determination of calcium and vitamin D to assess the need for specific treatment with bone resorption inhibitors, and vitamin D and calcium supplementation. In elderly people (>60 years), with persistent Shyper and a defined thyroid disease (physiological adaptive low TSH with aging should be excluded), treatment should be considered for grade 1 or 2 Shyper. This recommendation is based on the association of Shyper with a higher risk of AF in elderly people even for those with low but not suppressed TSH levels [16].

A good suggested policy on how to manage Shyper patients in the clinical practice could be reached applying a stepwise approach in five steps [107]:

Step 1: Establish the diagnosis of persistent Shyper.

It is necessary to exclude T3 toxicosis. Non-thyroidal causes of low TSH should also be excluded. Repeat thyroid function tests over a period of 3 to 6 months to exclude transitory causes.

Step 2: Define the etiology.

The most common causes of subclinical thyrotoxicosis are exogenous. Endogenous Shyper has the same etiology of overt hyperthyroidism. Color-flow Doppler thyroid ultrasound, radionuclide thyroid scanning, TRAb determinations, and a detailed medical history will be useful to establish the etiology of most cases.

Step 3: Assessment of clinical significance.

A careful and detailed medical history may be useful in the identification of thyrotoxicosis symptoms in apparently asymptomatic patients. Patients should be evaluated regarding the potential harmful effects associated with Shyper, particularly on the cardiovascular system and skeleton. Previous cardiovascular disease and cardiovascular risk factors should be routinely investigated. According to clinical judgment, evaluate the need for ECG, ECG Holter, Doppler echocardiogram, and bone densitometry.

Step 4: Stratify patients according to the risks.

Stratify patients according to the severity of Shyper (Grade 1 or Grade 2) and the age of the patients. Grade 2 Shyper has been associated with a higher risk of progression to overt hyperthyroidism and incident coronary heart disease and mortality. Age > 60 years is associated to a significant risk of AF, HF, and fractures.

Step 5: Make a decision.

Each clinical situation should be individually analyzed considering the potential clinical conse-

quences of not treating, and the risks associated with the treatment, having in mind data from the previous steps and recommendations from recent society guidelines [1, 2]. Both ATA and ETA guidelines [1, 2] agree on the concept that the indication of treatment of Shyper highly depends on the age, degree of TSH suppression, and comorbidities present in each individual. Treatment is either "recommended" or "should be considered" accordingly (Table 3).

Treatment Modalities

Patients with Shyper are treated with antithyroid medications, radioiodine (RAI) or ^{131}I, or surgery, depending on the clinical circumstances and patient preference. Treatment modalities vary according to the etiology of Shyper, and there are no control trials comparing the efficacy among them. Furthermore symptomatic treatment includes cardioselective β-blocking agents with the aim of improving symptoms, heart rate, and supraventricular arrhythmias [108, 109].

In patients with Graves' disease, RAI therapy, antithyroid medication, and thyroidectomy are all acceptable modes of treatment. A treatment option can be chosen by the patients following comprehensive discussion with their physician. However in certain scenarios such as young Graves' disease patients with Shyper, long-term and low-dose (5–0.10 mg/day of methimazole) antithyroid drug therapy is the first choice since the remission rate is high [110]. Similarly, in patients older than 65 years with Graves' disease and grade 1 Shyper, antithyroid drugs may be used as an initial line of therapy [1], while for those elderly Graves' disease patients with grade 2 Shyper or for patients with cardiovascular disease, both antithyroid drugs or RAI can be considered as the first choice with the aim of a rapid remission of the disease [1].

Table 3 Treatment of Shyper according to age and degree of TSH suppression

Age (years)	Grade 1 Shyper (TSH 0.1–0.39 mIU/L)	Grade 2 Shyper (TSH < 0.1 mIU/L)
>65	Consider treatment	Treatment is recommended in all patients
<65	Consider treatment if symptomatic or with cardiovascular or bone fracture risk	Treatment is recommended in symptomatic patients or with cardiovascular or bone fracture risk

On the other hand, ^{131}I therapy and surgery are offered primarily to patients with TMNG or TA [111] especially in elderly patients. Although, pretreatment with antithyroid medication has been advocated to avoid exacerbation of hyperthyroidism due to RAI, its use remains controversial [112] considering that 10–15% increase in RAI activity will be needed after pretreatment with antithyroid drugs to maintain efficacy [112]. During the first week after RAI, the use of antithyroid medication may decrease complications such as atrial fibrillation; however, it may also decrease the efficacy of the RAI treatment [112]. In those elderly patients in whom neither surgery nor RAI are feasible, long-term antithyroid drugs can also be used [113].

In case of compressive symptoms, concomitant hyperparathyroidism or suspicion of thyroid malignancy, total or partial thyroidectomy is the best option. Iodine is primarily used now in conjunction with antithyroid drugs to prepare patients with Graves' disease for surgical thyroidectomy. Conversely, its use is not really needed in case of AFTN surgery since it may exacerbate thyrotoxicosis. In case of a solitary autonomous nodule, lobectomy and isthmus resection is sufficient [2]. In the presence of a patient with a large goiter with contraindication for surgery due to advanced age or comorbidities, other treatment modalities may be considered.

Low doses of recombinant human TSH before RAI have been advocated in the management of multinodular goiter to increase iodine uptake [114]. Its use however may be associated to transient exacerbation of hyperthyroidism.

With regard to possible adverse effects of all these treatments, they are the same as when administered for overt hyperthyroidism [2, 115]. However, since the proposed doses of antithyroid drugs in Shyper are low and the adverse effects with methimazole in particular are dose-related,

patients receiving this drug may not be at increased risk. With regard to the use of RAI in Graves' disease in patients with mild and active eye disease or smokers, steroid prophylaxis is recommended to avoid Graves' orbitopathy progression [116]. Another unfrequent consequence of RAI is the induction of Graves' disease in patients with TMNG. This situation has been associated to pre-existing thyroid autoimmunity in these patients despite undetectable TRAb levels [117].

In patients who are treated surgically, complications include permanent vocal cord paralysis and hypoparathyroidism, although with surgeons with high level of experience, these adverse events should be relatively low [118].

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Thyroid Storm (Thyrotoxic Crisis)

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Thyroid storm is a relatively rare life-threatening condition resulting from decompensated thyrotoxicosis with increased action of the thyroid hormones, thyroxine (T4) and triiodothyronine (T3), exceeding the patient's metabolic demands (thyrotoxic crisis). Early recognition of the clinical signs and symptoms and prompt and accurate diagnosis, taken together with an understanding of its underlying pathogenesis, will permit appropriate therapy and increase the likelihood of survival.

In thyroid storm, the usual manifestations of hyperthyroidism are severely exaggerated. In the overwhelming majority of cases of thyroid storm, the underlying cause of thyrotoxicosis is Graves' disease and, less commonly, a toxic multinodular goiter. Less commonly, thyrotoxic storm may occur as a result of a hyperfunctioning autonomous nodule, subacute thyroiditis [1], or factitious thyrotoxicosis due to intentional thyroxine or triiodothyronine overdosage [2].

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Epidemiology and Precipitating Events

Accurate assessment of the incidence of thyroid storm is difficult to determine because of the relative rarity of reported cases and the considerable variability in criteria for its diagnosis. Storm is likely to be less common today than in the past, probably due to earlier diagnosis of the hyperthyroidism with initiation of appropriate therapy such as antithyroid drugs, thereby precluding progression to storm. Based upon various reports, storm appears to occur in 1–2% of hospital inpatients, most of whom were admitted for management of severe thyrotoxicosis [3], but in other cases, the storm occurs incident to admissions for other indications, particularly surgery. For example, the setting may be postoperatively after non-thyroidal surgical procedures, while thyrotoxic storm is much less commonly seen after thyroid surgery. This is so in regard to thyroidectomies for Graves' disease because of the routine preoperative preparation of patients by treatment with antithyroid drugs. In the case of non-thyroidal surgeries, fractures, or other traumas, storm in a patient with previously undiagnosed thyrotoxicosis may be related to perioperative events, such as anesthesia, stress, and volume depletion, because these conditions are associated with an increase in free T4 and T3 concentrations. Other conditions in which thyroid

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storm has been seen and observed include pregnancy, during labor, molar pregnancy [4], and in complicated deliveries such as with placenta previa [5]. An acute discharge of hormones in the appropriate clinical setting may trigger a crisis, and cases have been reported following vigorous palpation of the thyroid, radioactive ^{131}I therapy [6], withdrawal of propylthiouracil therapy, or after administration of lithium, stable iodine, or iodinated contrast dyes. Indeed, any conditions known to be associated with increased free fraction of T4 and T3 may be associated with precipitation of storm, such as severe stress, infections, burns, cytotoxic chemotherapy for acute leukemia, aspirin overdose, ketoacidosis, organophosphate intoxication, and tyrosine kinase inhibitor therapy for malignancy [7–13] (Table 1). Amiodarone, an antiarrhythmic and antianginal drug which is 39% iodine, may cause either an iodine-induced thyrotoxicosis (type 1) or a destructive thyroiditis (type 2); the latter has been reported as a cause of thyroid storm refractory to the usual treatment [13]. There is also a case report of thyrotoxic storm precipitated by food poisoning with marine neurotoxin after ingestion of seafood [14]. There is a single case that was said to be attributed to a TSH-secreting pituitary adenoma [15]. Notwithstanding the latter multiplicity of precipitating factors, in hospitalized patients, the most common event associated with thyrotoxic storm is some form of infectious disease.

Table 1 Factors reported to precipitate thyrotoxic storm

• Withdrawal of antithyroid drug treatment
• ^{131}I treatment
• Thyroxine overdosage
• Cytotoxic chemotherapy
• Aspirin overdosage
• Iodinated contrast dyes
• Organophosphates
• Targeted chemotherapy (sorafenib)
Sepsis, infection
Gestational trophoblastic disease; molar pregnancy
Seizure disorder
Gastrointestinal bleeding
Pulmonary thromboembolism
Burn injury
Surgery, trauma, vigorous palpation of the thyroid
Metabolic disturbances
• Diabetic ketoacidosis
• Hypoglycemia
Parturition
Emotional stress
TSH-secreting pituitary adenoma

Clinical Signs and Symptoms

The diagnosis of thyroid storm on clinical grounds is based on the identification of signs and symptoms which suggest decompensation of a number of organ systems in a thyrotoxic patient. Some of these cardinal manifestations include temperature elevations out of proportion to an apparent routine infection as well as more accompanying diaphoresis than would be anticipated. Another key component of thyrotoxic storm is tachycardia out of proportion to the fever, and also gastrointestinal dysfunction, which can include nausea, vomiting, diarrhea, and, in the more severe cases, jaundice. Hyperthermia in thyroid crisis is thought to be due to a combination of defective hypothalamic thermoregulation and increased basal metabolic rate [16]. At the height of "the storm," an encephalopathic picture appears with symptoms of central nervous system dysfunction that may include increasing agitation and emotional lability, confusion, paranoia, psychosis, and coma [17]. Instances of thyroid storm have been reported that were associated with status epilepticus and stroke and with bilateral basal ganglia infarction [18]. In patients with neurological symptoms, a high index of suspicion for cerebral sinus thrombosis should be considered, because of the higher prevalence of this condition in severe hyperthyroidism [19]. The appearance of paralysis in thyroid crisis requires distinction between an uncomplicated cerebrovascular accident and the co-occurrence of thyrotoxic periodic paralysis with hypokalemia, especially in patients who are Asian men [20]. Presentation with masked or apathetic thyrotoxicosis has been seen in older patients with thyrotoxic storm [21].

Renal Manifestations and Electrolyte Disturbances

Increased blood levels of calcium can be seen due to both hemoconcentration and the release of calcium from the bone due to thyroid hormone effects on bone resorption. Sodium, potassium, and chloride levels are usually normal. Ketoacidosis and lactic acidosis may also occur due to augmented lipolysis and ketogenesis and basal metabolic demands that exceed oxygen delivery.

An accelerated glomerular filtration rate occurs in hyperthyroidism and can be associated with progression to glomerulosclerosis and excessive proteinuria. There are case reports of thyroid storm with renal failure due to rhabdomyolysis [22], urinary retention associated with dyssynergy of the detrusor muscle and bladder dysfunction [23], and an autoimmune complex-mediated nephritis concomitant with Graves' disease [24].

Cardiovascular Manifestations

Disturbances in rhythm are the most common cardiovascular manifestations in storm and include sinus tachycardia, atrial fibrillation or other supraventricular tachyarrhythmias, and rarely ventricular tachyarrhythmias, which can be observed even in patients without previous heart disease [25]. Congestive heart failure or a reversible dilated cardiomyopathy [26] also may be present even in young- or middle-aged patients without known antecedent cardiac disease. Increased preload secondary to activation of the renin-angiotensin-aldosterone axis results in a high-output state that is augmented by a decrease in afterload secondary to a direct vasodilatory relaxing effect of thyroid hormones on vascular muscle cells. This is why we tend to see systolic hypertension with widened pulse pressure. The "hyperthyroid heart" is characterized by higher than usual oxygen demands. This can lead to ischemia and myocardial infarction, even in young patients [27, 28]. Pulmonary hypertension is a relatively rare complication of severe hyperthyroidism and is presumed to be on an autoimmune basis when associated with Graves' disease. However, pulmonary hypertension also may be secondary to an augmented blood volume, cardiac output, and sympathetic tone that lead to pulmonary vasoconstriction and increased pulmonary arterial pressure. This manifestation is usually reversible after restoration of euthyroidism by treatment with antithyroid drugs. Another reason for pulmonary hypertension could be pulmonary embolism due to the thrombotic or hypercoagulable state that has been observed in severe hyperthyroidism.

Respiratory Manifestations

The principal pulmonary symptoms are dyspnea and tachypnea related to increased oxygen demand. The excessive work of the respiratory muscles may eventually lead to the diaphragmatic dysfunction [29]. Respiratory failure may result from the hyperdynamic cardiomyopathy, as well as from preexistent underlying pulmonary disease [30, 31].

Gastrointestinal Manifestations

Diarrhea and vomiting are the most common gastrointestinal symptoms and when present can aggravate existing volume depletion and postural hypotension and even progress to shock with vascular collapse. Non-specific, diffuse abdominal pain may be present, possibly due to impaired neurohormonal regulation of gastric myoelectrical activity with delayed gastric emptying [32]. The latter may progress and present as an acute abdomen [33] and intestinal obstruction [34]. The presence of progressive abnormalities in liver function tests and jaundice warrants immediate and vigorous therapy. Although the majority of presentations of an acute abdomen in thyrotoxicosis are medical in nature, surgical conditions may also occur [35].

Hematological Manifestations

Complete blood counts will often reveal a moderate leukocytosis with a mild shift to the left even in the absence of infection. The hypercoagulability noted in hyperthyroidism is believed to be on

a multifactorial basis related to increased concentrations of fibrinogen, factors VIII and IX, tissue plasminogen activator inhibitor 1, von Willebrand factor, an increase in red blood cell mass secondary to erythropoietin upregulation, and, finally, a tendency to augmented platelet plug formation [36]. Major thromboembolic complications are responsible for 18% of deaths caused by thyrotoxicosis [37–41].

Diagnosis

The clinical presentation in thyroid storm forms the basis for diagnosis, because the laboratory findings may not be much different than those observed in uncomplicated hyperthyroidism. Indeed, serum total T3 levels may be even within normal limits, as these patients may have some underlying precipitating illness that reduces T4 to T3 conversion as is seen in euthyroid individuals with non-thyroidal systemic illness [42]. In order to create a more objective basis for the diagnosis, a semiquantitative scale (Table 2) was developed to help assess the presence and severity of the most common signs and symptoms and thereby aid in establishing the diagnosis [43]. This scale has been referred to as the "Burch-Wartofsky score" and examines the presence of criteria similar to those subsequently suggested and evaluated by Akamizu et al. [44, 45]. The utility of the Burch-Wartofsky and Akamizu scoring systems was assessed by Angell et al. in a retrospective analysis comparing storm patients to otherwise compensated thyrotoxic patients [46].

Table 2 Burch-Wartofsky diagnostic scoring system

Criteria	Score
Thermoregulatory dysfunction	
Temperature 99–99.9 °F (37.2–37.7 °C)	5
Temperature 100–100.9 °F (37.8–38.2 °C)	10
Temperature 101–101.9 °F (38.3–38.8 °C)	15
Temperature 102–102.9 °F (38.9–39.3 °C)	20
Temperature 103–103.9 °F (39.4–39.9 °C)	25
Temperature ≥ 104 °F (40 °C) or higher	30
Central nervous system effects	
Absent	0
Mild agitation	10
Delirium, psychosis, lethargy	20
Seizure or coma	30
Gastrointestinal dysfunction	
Absent	0
Diarrhea, nausea, vomiting, abdominal pain	10
Unexplained jaundice 20	20
Cardiovascular dysfunction	
90–109 beats/min	5
110–119 beats/min	10
120–129 beats/min	15
130–139 beats/min	20
≥140 beats/min	25
Congestive heart failure	
Absent	0
Mild (edema)	5
Moderate (bibasilar rales)	10
Severe (pulmonary edema)	15
Atrial fibrillation	
Absent	0
Present	10
History of precipitating event	
Absent	0
Present	10

Based upon the total score, the likelihood of the diagnosis of thyrotoxic storm is as follows: unlikely <25, impending 25–44, and highly likely >45 [43]

When thyrotoxicosis is prolonged leading to the depletion of glycogen deposits, hypoglycemia may occur, particularly in older people when aggravated by malnutrition secondary to emesis or abdominal pain [47]. In contrast, a modest hyperglycemia in the absence of diabetes mellitus may be present, probably as a result of augmented glycogenolysis and catecholamine-mediated inhibition of insulin release as well as increased insulin clearance and insulin resistance. There often will be elevated levels of serum lactate dehydrogenase, aspartate aminotransferase, and bilirubin resulting from liver dysfunction. An increased level of serum alkaline phosphatase is more likely to reflect increased osteoblastic bone activity in response and augmented bone resorption rather than liver dysfunction.

Importantly, adrenal reserve may be exceeded in thyrotoxic crisis because of inability of the adrenal gland to meet metabolic demands in the face of accelerated turnover of glucocorticoids. Moreover, on a common autoimmune basis, there may be coincident occurrence of adrenal insufficiency and Graves' disease. This diagnosis should be considered when there are hypotension

and electrolyte abnormalities suggestive of Addison's disease.

Treatment of Thyroid Storm

A relatively complex approach to management is suggested in order to avoid a potential disastrous outcome [3]. Initially, antithyroid drugs of the thiourea type should be used to reduce ongoing increased thyroid production and release of T4 and T3. Secondly, a treatment should be imposed to block the effects of the remaining but excessive circulating free T4 and T3 concentrations in blood. The third leg of therapy is to treat the presence of any systemic decompensation, e.g., congestive heart failure and shock. Finally, if there is an underlying precipitating illness such as infection or ketoacidosis present, then it too must be treated, or the risk for recurrence of storm obtains.

Therapy Directed to the Thyroid Gland

The administration of thionamide antithyroid drugs, such as carbimazole, methimazole (Tapazole), and propylthiouracil, will inhibit de novo synthesis of the thyroid hormones, T4 and T3. In the comatose or uncooperative patient, these drugs are given by nasogastric tube or per rectum as enemas or suppositories [48–53]. In the USA, there currently are no available i.v. preparations of these compounds, but they have been available in some European countries such as the UK, Germany, and Poland [54, 55] and used effectively. Generally, propylthiouracil (PTU) can be started as 200 mg every 4 h (1200 mg/day), and methimazole should be administered as 30 mg every 6 h or a daily dose of 120 mg. Because PTU has the additional advantage of inhibiting conversion of T4 to T3, a property not shared by methimazole, it is thought to provide more rapid clinical improvement. Because the thiourea agents like methimazole and PTU reduce new hormone synthesis but do not block thyroidal secretion of preformed glandular stores of hormone, separate treatment must be administered to inhibit the continuing release of T4 and T3 into the blood. Either inorganic iodine or lithium carbonate may be used to inhibit proteolysis of colloid, and iodides may be given either orally as Lugol's solution or as a saturated solution of potassium iodide (3–5 drops every 6 h). As an earlier mainstay of treatment, the use of an intravenous infusion of sodium iodide (0.5–1 g every 12 h) has not been feasible recently as sterile sodium iodide has not been available for intravenous use.

Importantly, iodine should be administered no sooner than 1 h after the prior administration of a dose of thionamide. Otherwise, iodine will enhance thyroid hormone synthesis, enrich hormone stores within the gland, and thereby permit further exaggeration of thyrotoxicosis. When iodine is administered in conjunction with full doses of antithyroid drugs, dramatic rapid decreases in serum T4 are seen, with values approaching the normal range within 4 or 5 days [56]. Other agents that may be used in this manner are the radiographic contrast dyes ipodate (Oragrafin) and iopanoic acid (Telepaque), which act not only by decreasing thyroid hormone release but also will slow the peripheral conversion of T4 to T3, as well as possibly blocking binding of both T3 and T4 to their cellular receptors. However, these latter agents are not available in the USA.

Lithium carbonate may be used as an alternative agent to inhibit hormonal release [57, 58] in patients who may be allergic to iodine. Lithium is administered initially as 300 mg every 6 h, with subsequent adjustment of dosage as necessary to maintain serum lithium levels at about 1–1.2 mEq/L.

Therapy Directed at the Continuing Effects of Thyroid Hormone in the Circulation

Treatment with antithyroid drugs alone is not sufficient in instances when severe thyrotoxicosis is present, given the presence and likelihood of high levels of circulating T4 and T3 in a large vascular

pool and tissue distribution space. Effective alternative therapeutic measures that can reduce T4 and T3 levels within 36 h include plasmapheresis and therapeutic plasma exchange. Plasma or albumin solution given during therapeutic plasma exchange provides new binding sites to attract, bind, and reduce circulating levels of free thyroid hormones [59, 60]. However, this effect is transient, lasting for perhaps 24–48 h, and thus needs to be followed by more definitive therapy. In rare patients apparently unresponsive to medical therapy, early thyroidectomy has been reported to reduce the mortality rate from 20 to 40% with medical therapy alone to less than 10% [61].

Peritoneal dialysis or experimental hemoperfusion through a resin bed [62–64] or charcoal columns [65] may be attempted. Oral administration of cholestyramine resin constitutes another therapeutic adjunct, the goal of which is to remove T4 and T3, by binding thyroid hormone entering the gut via enterohepatic recirculation with the subsequent excretion of the resin-hormone complex [66].

The treatment of thyrotoxic storm with a β-adrenergic blocker was first reported by Hughes [67] as a means to ameliorate the manifestations of thyroid hormone excess. Propranolol is the most commonly used agent in the USA. The oral dosage of 60–80 mg every 4 h or intravenous doses of 0.5–1 mg followed by subsequent doses of 2–3 mg given intravenously over 10–15 min every several hours are recommended with constant cardiac rhythm monitoring [68, 69]. There may be a minor benefit derived from the inhibitory effect of propranolol on the conversion of T4 to T3 [70], but this is likely not appreciable below oral doses in excess of 160 mg/day. Usage of β-blockers not only corrects the heart rate and diminishes the oxygen demand of the cardiac muscle but also improves the agitation, convulsions, psychotic behavior, tremor, diarrhea, fever, and diaphoresis. In certain patients, there may be relative risks or contraindications to the use of these agents. For example, in patients with a history of bronchospasm or asthma, either treatment with selective β1-blockers or reserpine, guanethidine should be considered instead. A very short acting β-adrenergic blocker, esmolol, has been employed successfully in thyroid storm management. An initial loading dose of 0.25–0.5 mg/kg is followed by continuous infusion of 0.05–0.1 mg/kg per min [71, 72]. Another ultrashort-acting beta-blocker with high cardioselectivity, landiolol hydrochloride, has been employed in Japan for thyroid storm with some success [73]. An i.v. drip of 1.0–5.0 μg/kg/min was administered with close monitoring of blood pressure and heart rate.

Corticosteroids are other medications with moderately important therapeutic potency and modest ability to inhibit peripheral conversion of T4 to T3. An initial dose of 300 mg hydrocortisone followed by 100 mg every 8 h during the first 24–36 h should be adequate. Thyroid storm has been reported to recur when steroids had been discontinued after initial clinical improvement [74]. The additional rationale behind the routine usage of steroids is perhaps theoretical and unproven but relates to possible relative adrenal insufficiency secondary to increased metabolic demands and more rapid turnover of cortisol.

Some authorities have suggested that the supplemental administration of 1α (OH) vitamin D3 might accelerate the reduction of serum T4 and T3 [75]. In a recent study, the administration of L-carnitine 2 g/day in thyrotoxic storm facilitated a dose reduction of methimazole. The mechanism appears to be related to an inhibition by L-carnitine of T3 and T4 entry into cell nuclei [76, 77]. While these preliminary findings are of interest, the utility of this adjunct to therapy requires confirmation.

Therapy Directed at Systemic Decompensation

Fluid depletion caused by vomiting, diarrhea, hyperpyrexia, and diaphoresis must be vigorously replaced to avoid vascular collapse. Hypercalcemia when present is usually corrected by appropriate fluid therapy. Hypotension not readily reversed by adequate hydration may temporarily require pressor and/or glucocorticoid therapy. However, judicious replacement of fluids is necessary in elderly

patients with congestive heart failure or other cardiac compromise. Intravenous fluids containing 10% dextrose in addition to electrolytes will serve to restore depleted hepatic glycogen. Vitamin supplements may be added to the i.v. fluids to reverse vitamin deficiencies.

Acetaminophen rather than salicylates is the preferred antipyretic when fever is present, because salicylates inhibit thyroid hormone binding and could serve to increase free T4 and T3, thereby transiently worsening the thyrotoxic crisis. Hyperthermia may also respond well to external cooling with alcohol sponging, cooling blankets, and ice packs. Some authors advocate usage of the skeletal muscle relaxant dantrolene [78], but significant risk associated with its use precludes routine recommendation. When present, congestive heart failure should be treated routinely. Although less commonly used today, when digoxin is employed larger than usual doses may be required because of its increased turnover in the thyrotoxic state.

Therapy Directed at the Precipitating Illness

Therapy cannot be considered sufficient unless the possible precipitating event has been identified and addressed. Generally this is not a problem in obvious cases, when trauma, surgery, labor, and premature withdrawal of antithyroid drugs are known to have been the precipitants of thyrotoxic crisis, which may require no additional management. However, when none of the latter precipitating factors is apparent, a diligent search for some focus of infection must be carried out. Routine cultures of urine, blood, and sputum should be obtained in the febrile thyrotoxic patient, and cultures of other sites may be warranted on clinical grounds. Empiric broad-spectrum antibiotic coverage may be required initially while awaiting results of cultures. Conditions such as ketoacidosis, pulmonary thromboembolism, and stroke may underlie thyrotoxic crisis, particularly in the obtunded or psychotic patient, and require the indicated vigorous management.

Prognosis

Multi-organ system failure is the most common cause of death in thyroid crisis and often includes respiratory failure, congestive heart failure, sepsis, central nervous system dysfunction, disseminated intravascular coagulation, and arrhythmias [79–82]. Even with early diagnosis, death can occur, and reported mortality rates have ranged from 10 to 75% in hospitalized patients [83, 84]. In surviving patients, clinical improvement is dramatic and demonstrable within first 24 h. During the recovery period of the next few days, supportive therapy such as corticosteroids, antipyretics, and intravenous fluids may be tapered and gradually withdrawn on the basis of patient status, oral intake of calories and fluids, vasomotor stability, and continuing improvement. After the crisis has been resolved, attention may be turned to consideration of the definitive treatment of thyrotoxicosis. Should thyroidectomy be considered, thyrotoxicosis will need to have been adequately treated preoperatively, to obviate any likelihood of another episode of crisis either during the surgery or postoperatively. Total thyroidectomy is the procedure of choice in view of reports of recurrent severe thyrotoxicosis and thyroid crisis after less than total thyroidectomy [85].

Radioactive iodine as definitive treatment is often precluded by the recent therapeutic use of inorganic iodine in virtually all cases of storm, but it could be considered at a later date, in which case antithyroid drugs of the thiourea type may be employed to restore and maintain euthyroidism until such time as radioiodine could be administered effectively. Continuing treatment with antithyroid drugs alone in the hope of the patient's sustaining a spontaneous remission is also possible.

In summary, as should be apparent from this review, the life-threatening thyroid emergency of thyrotoxic crisis requires a high index of suspicion in the appropriate clinical setting followed by prompt and accurate diagnosis and urgent multifaceted therapy in order to reduce the risk of fatal outcome.

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Heart in Hyperthyroidism

Bernadette Biondi and George J. Kahaly

Effects of Thyroid Hormone on the Heart

Triiodothyronine (T3) is essential to control molecular pathways on the heart and vasculature. Many of the physiological effects of T3 are mediated by its genomic nuclear effects on important cardiac proteins such as sarcoplasmic reticulum calcium adenosine triphosphatase (ATPase) (SERCA2), α-myosin heavy chain (α-MHC), ß1-adrenergic receptors, sodium/potassium ATPase, voltage-gated potassium channels, malic enzyme, and atrial natriuretic hormone [1–4]. The non-genomic effects exerted by thyroid hormone (TH) on cardiac myocyte and peripheral vascular resistance involve a variety of intracellular signaling pathways, the transport of ions across the plasma membrane, glucose and amino acid transport, and mitochondrial function [5]. Non-genomic effects may explain the effects of acute T3 administration on cardiovascular (CV) hemodynamic and heart rate [6].

Here we explore the changes in cardiovascular hemodynamic in overt and subclinical hyperthyroidism (SHyper), their cardiovascular risks and the prevention and treatment of these disorders, and their CV complications.

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Hemodynamics in Hyperthyroidism

Thyroid hormone increases cardiac output by affecting stroke volume and heart rate.

Peripheral vasodilatation occurs as a result of rapid utilization of oxygen, increased metabolic end products, induction of arterial smooth muscle cell relaxation, and increase of endothelial nitric oxide availability by thyroid hormones [7–9]. Vasodilatation results in a marked decrease in systemic vascular resistance which plays a central role in the hemodynamic changes that accompany thyrotoxicosis, resulting in a selective increase in blood flow to certain organs such as skin, skeletal muscles, and heart and a drop in diastolic blood pressure with widening of the pulse pressure [2, 8]. Vasodilatation and the lack of raise in renal blood flow cause a decrease in renal perfusion pressure and an activation of the renin-angiotensin system, thus increasing sodium reabsorption and blood volume [3, 8]. The combination of expanded blood volume and improvement in diastolic relaxation of the heart contribute to increase left ventricular end-diastolic volume or preload [3, 7, 8]. Similarly, the drop in systemic vascular resistance results in a smaller left ventricular end-systolic volume or afterload [3, 7,

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8]. The net effect of an increased preload and a decreased afterload translates into a significant increase in left ventricular stroke volume [8]. In turn, the rise in heart rate and the increased stroke volume combine to cause a two- to threefold increase in cardiac output, greater than accounted for by the changes in the body metabolic rate [1–3, 8]. Therefore, a hyperdynamic cardiocirculatory state is associated with short-term hyperthyroidism. The increase in heart rate and cardiac preload and the effects of thyroid hormone on peripheral circulation play a major role in increasing LV performance in human hyperthyroidism, which is important in determining the high-output state [8] (Table 1). This suggests that the hyperthyroid heart increases its performance through the modulation of hemodynamic loads; this positive effect on energy metabolism and oxygen consumption improves the left ventricle mechanical efficiency optimizing its cardiac mechanical-energetic utilization [8]. Echocardiography data indicate that, in humans, newly diagnosed thyrotoxicosis is accompanied by an improvement in left ventricular systolic and diastolic function [7, 8, 10]. Enhancement in left ventricular relaxation, diastolic flow velocities, and isovolumic relaxation time has been reported in overt hyperthyroidism [7, 8, 10] (Fig. 1).

However, the hyperthyroid cardiovascular system is already highly "stressed" at rest, and hyperthyroid patients have an impaired cardiopulmonary function, which in part reflects their reduced cardiovascular and respiratory reserve; this may explain why they often complain of low exercise capacity and tolerance [11–13] (Fig. 2).

Table 1 Hemodynamic changes in hyperthyroidism

Systemic vascular resistance	↓
Circulation time	↓
Diastolic blood pressure	↓
Systolic blood pressure	↑
Pulse pressure	Widened
Cardiac output	↑
Cardiac index	↑
LV stroke volume	↑
LV systolic function	↑
LV diastolic function	↑
Exercise tolerance	↓

Stress-induced changes in cardiovascular and respiratory function have been demonstrated in untreated hyperthyroidism, resulting in a significantly reduced maximal work rate with a markedly decreased forced vital capacity as well as decreased oxygen uptake per heartbeat (oxygen pulse) both at the anaerobic threshold and at maximal exercise [1–3, 8, 11, 12].

In experimental studies, prolonged thyroid hormone excess induced physiological cardiomyocyte hypertrophy [14]. Long-term exposure to thyroid hormone excess may exert unfavorable effects on cardiac morphology and function because it may increase left ventricular mass, arterial stiffness, and left atrial size and may induce diastolic dysfunction, thereby impairing left ventricle performance [15–18] (Table 2).

Interactions with the Sympathoadrenal System

Sympathomimetic agents and thyroid hormones lead to similar cardiac symptoms, especially inducing tachycardia and increasing the force and velocity of cardiac contraction. Treatment of thyrotoxic patients with sympatholytic agents ameliorates rate-related cardiac changes. These observations have resulted in the hypothesis that some T3 effects are mediated by an increased activity of the sympathoadrenal system or an increased responsiveness and sensitivity of cardiac tissue to normal sympathomimetic stimuli. Since plasma and urine levels of catecholamines are normal in thyrotoxicosis, the hypothesis that the thyroid status leads to an increased sensitivity of the sympathoadrenal system has been favored. The enhanced sympathetic sensitivity of the thyrotoxic heart may be mediated by an increased number of ß-adrenergic receptors. In humans, short-term hyperthyroidism is associated with an increase in the sensitivity of heart rate and left ventricular shortening velocity to isoproterenol stimulation. In addition, an increased level of other components of the sympathetic transmission system occurs. Specifically, investigations in thyrotoxic pigs show that FT3 markedly increases the amount of stimulatory guanine nucleotide regulatory

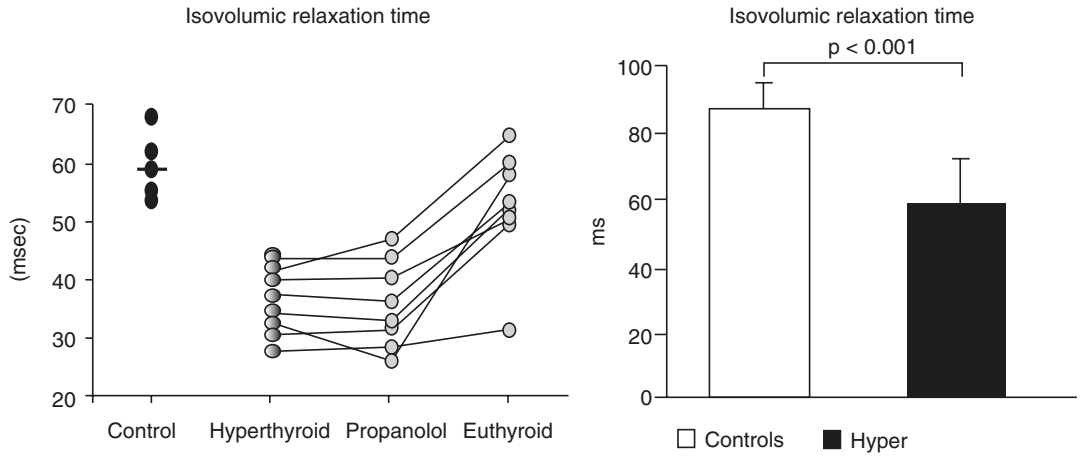

Fig. 1 Isovolumic relaxation time in euthyroid controls and in hyperthyroid patients before and after propranolol [7, 10]

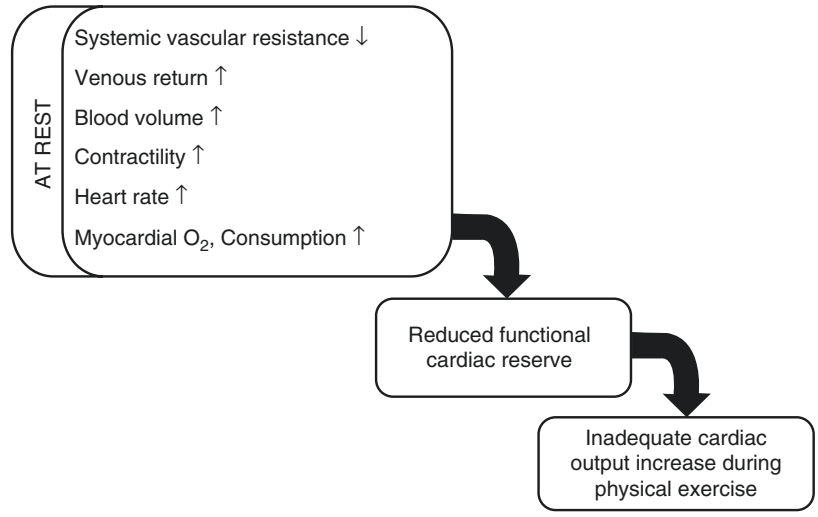

Fig. 2 Factors responsible for the reduced cardiovascular reserve in patients with hyperthyroid

protein. Studies of the various components of the adrenergic receptor complex in plasma membranes have also shown that ß-adrenergic receptors, guanine nucleotide regulatory proteins, and adenylyl cyclase types V and VI are all altered by changes in thyroid status [19–23].

Cardiac tissue contains both ß1- and ß2-adrenergic receptor subtypes. In most species studied, the ß1-receptors account for 70% of total ß-adrenergic receptors. Furthermore, ß-adrenoceptors are increased approximately twofold in the sinoatrial node compared to their level in surrounding myocytes. The proportion of ß-adrenoceptors in the sinoatrial node is comprised predominantly of ß1-receptors (75%). In contrast, ß2-receptors are the predominant species in non-myocyte vascular cells (75%). Thus, ß1-receptors are the predominant ß-adrenoceptors in cells of myocyte origin and might be

Table 2 Cardiac complications in untreated persistent subclinical hyperthyroidism

• Increased left ventricular mass
• Impaired left ventricular filling
• Increased heart rate
• Atrial arrhythmias
• Persistent atrial fibrillation
• Stroke
• Heart failure
• CHD mortality

responsive to T3 regulation. Indeed, there appears to be a differential induction of cardiac ß1- and ß2-adrenergic receptor mRNA in rat myocytes by T3. T3 causes a fourfold induction of cardiac ß1-adrenoceptor mRNA, but no significant change in ß2-receptor mRNA. The effects of T3 on ß1-adrenergic gene transcription occur within 30 min, elevations lasting for 72 h. Following the rise in ß1-mRNA, there is a threefold increase in the density of cardiac ß1-receptors, which persists for 48 h. In contrast, ß2-receptors are not significantly increased following T3 administration. These studies suggest that in cardiac tissue, the ß1-adrenoceptor gene is sensitive to T3, whereas the ß2-receptor gene is influenced minimally [24–26].

Cardiovascular Complications in Hyperthyroid Patients

Some important cardiovascular complications may develop in patients with overt and subclinical hyperthyroidism (Table 3).

Heart Failure

Any adverse event that might damage the efficiency of the cardiovascular system may precipitate congested circulation or may induce a true congestive heart failure (HF) [27]. The patient's age, the severity of hyperthyroidism, and the underlying cardiac conditions may affect the clinical manifestations and severity of HF in hyperthyroid patients (Table 2) [27, 28]. Severe hyperthyroidism in young patients without underlying heart disease may induce a "high-output HF," an inappropriate term to define the congestive circulation due to the increased heart rate and cardiac output, normal systolic function, low systemic vascular resistance, and increased blood volume [27–29]. This high-output HF may induce symptoms such as breathlessness at rest, fatigue and fluid retention with peripheral edema, pleural effusion, hepatic congestion and increased pulmonary arterial hypertension [27, 29, 30]. Rarely, untreated high-output state may lead to ventricular dilatation and persistent tachycardia, which can trigger chronic HF and fatal events [31].

Some hyperthyroid patients may develop diastolic HF [32]. Increasing age may be an independent predictor for the development of diastolic dysfunction and HF in hyperthyroid patients [32]. Elderly hyperthyroid patients may develop HF even in presence of slight thyroid hormone excess [27, 33]. Cardiac preload is increased in these patients, despite the increase in systemic vascular resistance, the reduced myocardial contractility, the impaired left ventricular filling, and consequent low cardiac output [33].

Recent data suggest that even subclinical hyperthyroidism (SHyper) may be responsible of heart failure. In a recent meta-analysis, Gencer et al. analyzed the association between SHyper and heart failure (HF) event [34]. They pooled individual participants (IPD) data from six prospective cohort studies (HF). Among the 648 participants with SHyper, the (hazard ratio) HR for HF events was significantly increased during a median follow-up of 10.4 years in age and sex-adjusted analyses [34]. The risk of HF was much higher in participants with grade 2 SHyper (TSH levels <0.1 mIU/L) (HR = 1.94; 95% CI, 1.01–3.72) than in those with grade 1 SHyper (TSH 0.1–0.39 mU/L), (HR = 1.31; 95% CI, 0.88–1.95) [34].

Table 3 Cardiac manifestations and clinical features in hyperthyroid patients

• Dyspnea on effort
• Easy fatigability
• Reduced exercise tolerance
• Systolic hypertension with widened pulse pressure
• Atrial fibrillation
• Stroke
• Heart failure
• Coronary heart disease
• Pulmonary hypertension
• Dilated cardiomyopathy
• Myxomatous valve disease

Atrial Fibrillation

Sinus tachycardia, atrial premature beats, and symptoms of adrenergic over activity are frequently observed in young patients with overt and subclinical hyperthyroidism [35]. Atrial fibrillation may be the first symptom of thyroid

hormone excess in the elderly [36]. About 7–8% of middle-aged hyperthyroid patients may develop atrial fibrillation or flutter compared to 0.5–9.0% of the general population [13]. This prevalence increases with age, being 10–20% in patients aged >60 years and even 20–40% in hyperthyroid patients with coexistent ischemic heart disease or heart valve disease [36].

Collet et al. analyzed the risk of AF in patients with subclinical hyperthyroidism [37]. They included IPD data from five prospective cohort studies and stratified the analysis by age, sex, race, and TSH categories. During a mean follow-up of 8.8 years, the overall HR for incident AF was higher in participants with SHyper than in those who were euthyroid, and the attributable risk for AF was 41.5% in patients with SHyper [37]. After age- and sex-adjusted analyses, incident AF was significantly more common in participants with grade 2 SHyper (HR = 2.54; 95% CI, 1.08–5.99) than in those with grade 1 SHyper (HR = 1.63; 95% CI, 1.10–2.41; *p* for trend 0.02), and a slightly greater risk of incident AF was observed in men and in elderly patients [37].

Stroke

Stroke is a potential complication of AF in overt hyperthyroidism where thyroid hormone excess is associated with a prothrombotic state [38]. In particular, thyrotoxic atrial fibrillation has been associated with an increased risk of cerebrovascular and pulmonary embolism [39–41]. Atrial fibrillation is responsible for embolic events in 10–15% of cases of hyperthyroidism [13]. Elderly patients with atrial fibrillation are susceptible to embolic events, especially when left atrial enlargement, risk factors for stroke, and cardiovascular disease (or other comorbidities) are also present [13].

Conflicting data have been reported on the risk of stroke in patients with subclinical hyperthyroidism. A recent systematic review assessed the results from the available prospective studies [42]. The pooled data did not support an increased risk of stroke linked to subclinical hyperthyroidism (HR of 1.17 (95% CI 0.54–2.56). [42]. However, the available literature on the risk of stroke in SHyper is insufficient, and more research is needed.

Autoimmune Cardiovascular Involvement in Patients with Autoimmune Hyperthyroidism

Interestingly, autoimmune hyperthyroidism is frequently responsible for autoimmune cardiovascular involvement; therefore, pulmonary arterial hypertension, myxomatous cardiac valve disease, and autoimmune reversible and irreversible dilated cardiomyopathy have been reported in patients with Graves' disease [13]. Pulmonary arterial hypertension is characterized by an increase in systolic pulmonary artery pressure above 30 mmHg at rest and a progressive increase in pulmonary vascular resistance, leading to right ventricular insufficiency. Pulmonary arterial hypertension has been reported in hyperthyroid patients [13]. It may be the consequence of the immune-mediated endothelial damage and the increased metabolism of vasodilating substances in hyperthyroid patients [13]. Asymptomatic pulmonary hyperthyroidism has been detected in 45% hyperthyroid patients at echocardiographic examination [43–45]. A significant reduction in pulmonary arterial pressure has been usually observed after correcting hyperthyroidism; severe pulmonary hypertension may also be completely reversible after successfully treating hyperthyroidism [13]. A specific vasoactive effect of methimazole has been postulated to explain the significant improvement in the pulmonary vasculature after medical treatment of hyperthyroidism [13]. Few cases of hyperthyroid GD with severe right ventricle volume overload, tricuspid regurgitation, and isolated right heart failure have been described in the literature [46, 47]. Some of these cases were reversible with the achievement of euthyroidism.

Autoimmune myocarditis is another cardiac complication of GD; myocardial changes are characterized by lymphocytic infiltrations, mucopolysaccharide deposits, necrosis, and fibrosis [13]. Approximately one-third of hyperthyroid patients will develop a specific reversible or irreversible dilated cardiomyopathy in GD [48–55]. The autoimmune origin of this disease is supported by endomyocardial biopsies [13].

Coronary Heart Disease Events and Cardiovascular and Total Mortality

Some recent meta-analyses suggest that patients with untreated overt and subclinical hyperthyroidism are at increased risk for cardiac mortality [37]. In an analysis of IPD data from ten prospective cohorts, grade 2 SHyper was linked to an increased risk of total mortality with an attributable risk of 14.5% [37]. The IPD analysis of the association between SHyper and coronary heart disease (CHD) in six prospective cohorts suggests that the risk of CHD events was not significantly higher in grade 2 SHyper than in grade 1 SHyper [37]. However, in age- and sex-adjusted analyses, the overall HR for CHD mortality was increased in patients with grade 2 SHyper than in euthyroid individuals (HR = 1.84; 95% CI, 1.12–3.00; p value for trend ≤0.03) [37]. Heterogeneity was present across studies for total mortality (I^2 = 49%), but not for CHD mortality and CHD events (all I^2 = 0%). Men had a slightly greater risk for total mortality and CHD mortality than women [37].

Table 4 Recommendations to improve to prognosis of patients with hyperthyroidism and cardiovascular complications

1. Prompt diagnosis of CV complications in elderly patients and in those with underlying heart disease (ECG, Holter ECG, echocardiography)
2. Restoration of a euthyroid state with antithyroid drugs as soon as possible
3. β-blocking drugs to obtain heart rate control
4. Prevention of thromboembolism in patients with AF (anticoagulation with an international normalized ratio (INR) of 2.0–3.0)
5. Pharmacological or electrical cardioversion in patients with persistent atrial fibrillation after 4 months of euthyroidism
6. Anticoagulant therapy with warfarin for at least 3 weeks before cardioversion and for at least 4 weeks after successful cardioversion to avoid the risk of embolic events
7. Antiarrhythmic drugs to avoid the recurrence of atrial fibrillation after successful cardioversion
8. Treatment with β-blockers and diuretics to improve the congestive circulatory in young patients with severe hyperthyroidism
9. Hospitalization when HF does not improve upon restoration of euthyroidism
10. Pretreatment with antithyroid drugs before definitive treatment of hyperthyroidism with RAI or surgery

Prevention and Treatment of Cardiovascular Complications in Hyperthyroid Patients (Table 4)

Data from all the available prospective cohort studies demonstrate that overt hyperthyroidism and grade 2 SHyper are associated with an increased risk of total mortality, CHD mortality, and AF with a greater risk among elderly patients and patients with underlying heart disease [34, 37]. Although the available meta-analyses do not show evidence that treatment is effective in preventing the risks associated with untreated hyperthyroidism, they clearly demonstrate that even slight thyroid hormone excess is a potentially life-threatening condition [34, 37, 56–60].

The timely recognition of overt and SHyper may improve the prognosis of cardiovascular complications in hyperthyroid patients. Subclinical hyperthyroidism should be treated with ATDs in all patients with grade 2 SHyper and in those with grade 1 SHyper older than 65 years, particularly in the presence of heart disease, diabetes, renal failure, previous stroke or transient ischemic attack, left atrial dilatation, increased risk factors for stroke, HF, CHD, valvular heart disease, and coronary or peripheral arterial disease [58, 59].

Prevention of the cardiovascular complications of thyroid hormone excess should be considered in hyperthyroid patients, especially in those with preexisting cardiac disease [27, 58, 59]. Electrocardiography and Doppler echocardiography are mandatory to assess cardiac function, pulmonary pressure, valve disease, and pleural or pericardial effusion in symptomatic patients [27, 59].

Prompt, effective treatment of cardiac manifestations in symptomatic patients with hyperthyroidism is important because cardiovascular complications account for most of the deaths in hyperthyroid patients [27]. According to the American College of Cardiology/American

Heart Association, the first-line treatment of AF and HF in patients with thyroid dysfunction should be directed primarily toward restoring a euthyroid state because cardiovascular drugs are generally unsuccessful while thyroid hormone excess persists [58]. Treatment of hyperthyroidism with ATDs should be the first-line therapy in patients with overt hyperthyroidism and AF and/or HF to obtain spontaneous conversion to sinus rhythm and to improve cardiovascular hemodynamic. β-blocking drugs may control cardiovascular symptoms before attaining euthyroidism in patients treated with antithyroid drugs [59]. Symptomatic patients should be treated with cardioselective β-blocking agents to obtain control of the heart rate [59].

In patients with hyperthyroidism and atrial fibrillation, initial therapy should aim at controlling ventricular rate to a nearly normal level by using β-blockers [57–59]. Atrial fibrillation spontaneously converts to sinus rhythm after treatment of hyperthyroidism in about two-thirds of hyperthyroid patients under 50 years of age, particularly in a new onset of AF without underlying heart disease [13, 59, 60]. Persistent AF after 4 months of euthyroidism should be treated with pharmacological or electrical cardioversion during anticoagulation [13, 59, 61, 62]. In hyperthyroid patients who do not regain normal rhythm spontaneously within 4 months of normalization of thyroid function, pharmacological or electrical cardioversion should be considered after evaluation of the age of the patient and the underlying cardiac condition [13, 61, 62]. Thromboembolism should be prevented in patients with AF. The American Heart Association suggests anticoagulation with an international normalized ratio (INR) of 2.0–3.0 for patients with hyper and AF [57].

Antiarrhythmic drugs should be used to avoid the recurrence of atrial fibrillation after successful cardioversion. Anticoagulant therapy with warfarin should be administered for at least 3 weeks before cardioversion and should be continued for at least 4 weeks after successful cardioversion, to avoid the risk of embolic events [13, 61, 62]. Hyperthyroid patients, however, have an increased sensitivity to the anticoagulant effects of warfarin, owing to the increased clearance of clotting factors and the reduced plasma protein binding of the drug [13]. Moreover, these patients are resistant to reversal of warfarin-induced hypoprothrombinemia by vitamin K. Reduced doses of warfarin should, therefore, be administered to hyperthyroid patients [13].

HF may be a complication of atrial fibrillation or sinus tachycardia and may be improved or resolved when the ventricular rate is slowed or sinus rhythm is restored. Treatment with β-blockers and diuretics may improve the congestive circulation in young patients with severe hyperthyroidism [27] .Treatment of HF in elderly patients should aim at improving cardiac hemodynamics and heart rate. Hospitalization is required to treat HF in patients with preexisting left ventricular dysfunction or when HF does not improve upon restoration of euthyroidism [27].

Antithyroid drugs (ATDs), radioactive iodine (RAI), or surgery are all effective in treating persistent hyperthyroidism, although a high relapse rate has been observed with ATDs in comparison with RAI or surgery [58, 59]. Pretreatment with MMI should be considered before definitive treatment of hyperthyroidism with RAI or surgery in patients with cardiovascular disease (AF, CHD, or HF) and in patients at an increased risk of complications due to the potential worsening of hyperthyroidism [58, 59].

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Part VI

Thyroiditis

Chronic Autoimmune Thyroiditis

Andrea Carbone, Mario Rotondi,
and Luca Chiovato

Chronic Autoimmune Thyroiditis

Chronic autoimmune thyroiditis was first described as struma lymphomatosa by the surgeon Hakaru Hashimoto in 1912. He reported enlarged thyroid glands with unique histologic features: diffuse lymphocytic infiltration, lymphoid follicles, destruction of epithelial cells, and proliferation of fibrous tissue. It was not until 1956 that the pathogenesis of Hashimoto's disease became clear due to the pioneering work of Rose and Witebsky, who reproduced the disease by immunizing rabbits with thyroid extracts, and Doniach and Roitt, who discovered thyroglobulin and thyroid cytoplasmic (microsomal) antibodies.

Chronic autoimmune thyroiditis, also known as lymphocytic thyroiditis, presents with two main clinical entities: a goitrous form (Hashimoto's thyroiditis) and an atrophic form (atrophic thyroiditis or primary myxedema). Clinical variants include juvenile thyroiditis (lymphocytic thyroiditis of childhood and adolescence) and focal or minimal thyroiditis. Silent (painless) thyroiditis and postpartum thyroiditis also recognize an autoimmune origin, but in most cases their clinical courses are transient (Table 1). Organ-specific autoimmunity is the cause of chronic autoimmune thyroiditis. In all variants the thyroid is infiltrated by lymphocytes, thyroid antibodies are present in serum, and there is a clinical or immunological overlap with other autoimmune diseases. Chronic autoimmune thyroiditis occurs frequently in certain families, particularly among the female members. The disease is also more common in patients with Down's syndrome or Turner's syndrome than in the general population.

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Basic Mechanisms in the Initiation of Thyroid Autoimmunity

Most of our current knowledge on the mechanisms involved in the development of thyroid autoimmunity derives from studies in experimental models of autoimmune disease, mainly in mice. Experimental autoimmune thyroiditis (EAT) in mice can be induced by immunization with mouse thyroglobulin (mTg) emulsified in complete Freund's adjuvant. Antigen-presenting cells (APC), such as dendritic cells (DC), present immunogenic epitopes of Tg to T cells in the context of Class II major histocompatibility molecules (MHC). Costimulatory signals are also required, which may result in either activation or downregulation of T cells. Based on the type of cytokines secreted by these DCs, a Th1, Th2, or a Th17 immune response is initiated. Th1 cells predominantly secrete IFN-γ and IL-12, whereas Th2 cells secrete IL-4 and IL-10. Th17 cells

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Table 1 A clinical classification of chronic autoimmune thyroiditis

Chronic autoimmune thyroiditis
Hashimoto's thyroiditis
Atrophic thyroiditis
Focal thyroiditis
Juvenile thyroiditis
Silent thyroiditis [a]
Postpartum thyroiditis [a]

[a]Transient

secrete IL-17. Th1 and Th17 cells have been shown to infiltrate the thyroid, resulting in chronic inflammation and eventually death of the thyrocytes in EAT (Fig. 1) [1–5]. CD4+ T cells are the major type of lymphocytic cells infiltrating the gland in thyroid autoimmune diseases. CD4+ T cells comprise a functionally heterogeneous population of T effector cells (Teff), being responsible for the development of thyroiditis, and a smaller population (10%) of T regulatory cells (Tregs), which express CD25 (the IL-2 receptor α). Tregs are critical for maintaining peripheral tolerance and are identified by their expression of Foxp3, a transcription factor, which is necessary and sufficient for Treg development. These cells typically secrete the cytokines IL-10 and transforming growth factor-β (TGF-β) to induce tolerance. Neonatal thymectomy (at age 3 days) and irradiation result in a multi-organ autoimmune disease, thus providing evidence for natural Tregs. The role of these cells is to prevent the development of organ-specific autoimmunity. Tregs are kept at a basal state of activation by low levels of circulating autoantigen; the homeostatic level is sufficient to prevent the development of autoimmunity. However, the clonal balance between Tregs and autoreactive T cells may be overcome by immunogenic stimuli, such as the administration of mTg and adjuvant [6]. As demonstrated by the group of Prabhakar [7], treatment of mTg-primed mice with granulocyte-macrophage colony-stimulating factor (GM-CSF) induces semi-matured tolerogenic DCs that are characterized by reduced levels of pro-inflammatory cytokines such as IL-1β and IL-12 and increased levels TGF-β. These tolerogenic DCs, instead of activating pathogenic Teff, induce and expand Tregs. Tregs produce IL-10 and TGF-β, two regulatory cytokines, which, by counteracting the role of pro-inflammatory cytokines, result in the suppression or prevention of EAT. Experimental evidence in mice showed that, apart from Tg, thyroid peroxidase (TPO) is also a major antigen in chronic autoimmune thyroiditis. Indeed, transgenic, TAZ1 mice expressing a human T cell receptor-specific for a cryptic TPO epitope spontaneously develop chronic autoimmune thyroiditis. This thyroid autoimmunity model is MHC II restricted but occurs independently from mature B cells and antibodies [8]. Experimental data in humans with chronic autoimmune thyroiditis do not clearly show a deficit in number of Tregs, but this T-cell subpopulation may be functionally deficient [9].

In the last few years, evidence was also accumulated supporting the concept that INF-γ-inducible chemokines, such as CXCL10, play an important role in the initial stages of thyroid autoimmunity. Chemokines are a group of low molecular weight proteins that recruit leukocyte subtypes and other cell types to sites of inflammation. When stimulated by INF-γ, thyroid follicular cells secrete CXCL10, which in turn recruits into the thyroid Th1 lymphocytes expressing CXCR3 and secreting INF-γ, thus establishing a loop which reinforces and maintains the autoimmunity process (Fig. 2) [10]. In addition, INF-γ stimulates MHC class II (HLA-DR) expression on thyroid epithelial cells, which may be important for the amplification and progression of thyroid autoimmunity [11].

Loss of Self-Tolerance to the Thyroid in Humans

According to the clonal selection theory, in the early stage of fetal-neonatal development, most autoreactive T cells are eliminated within the thymus by negative selection [12]. This mechanism is usually referred to as central tolerance. The few escaped autoreactive clones that migrate to the periphery are controlled by several mechanisms of peripheral tolerance involving ignorance (they remain non-responsive to antigenic stimulation),

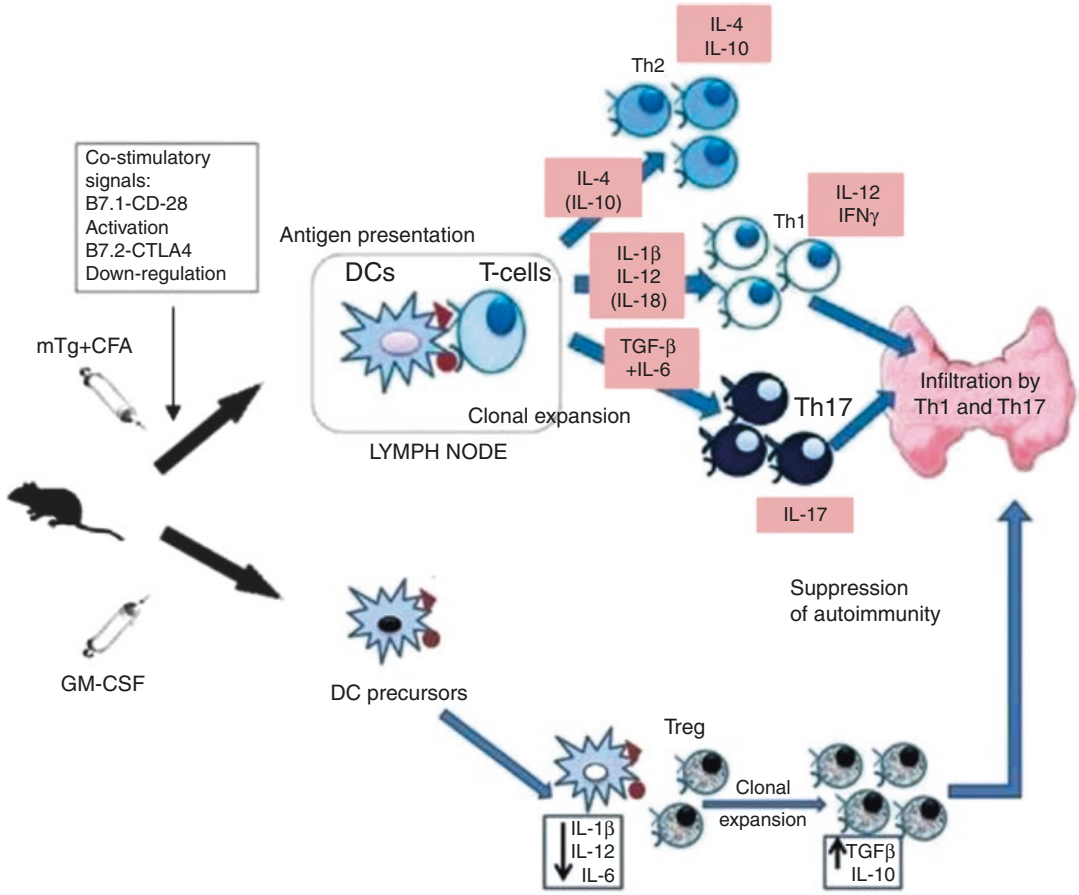

Fig. 1 Schematic representation of the immune events occurring after different immunizations of a mice. Experimental autoimmune thyroiditis (EAT) can be induced by immunization with mouse Tg emulsified in complete Freund's adjuvant (CFA) in the presence of costimulatory signals (B7–1 and B7–2). Treatment of mTg-primed mice with granulocyte-macrophage colony-stimulating factor (GM-CSF) results in suppression of EAT

anergy, activation-induced cell death, and active suppression by Tregs. Similar to other autoimmune diseases, loss of tolerance to thyroid antigens involves a complex interplay between genetic background and environmental factors [13, 14].

Role of Genetics

The role of genetics is suggested by the high frequency of autoimmune thyroid diseases affecting family members and by a significantly higher concordance of autoimmune thyroid diseases in monozygotic (up to 55% for chronic autoimmune thyroiditis) compared to dizygotic twins [15, 16]. The fact that concordance rate is not 100% in monozygotic twins indicates that environmental factors also play an important role in the etiology of autoimmune thyroid diseases [14, 17, 18]. Several susceptibility genes have been identified by whole candidate gene analysis, genome linkage studies, genome-wide association studies, and whole-genome sequencing techniques (Table 2) [14]. These genes are classified as nonspecific immune-related genes and thyroid-specific genes. The immune-related genes are divided into two groups: immunological synapse genes and regulatory T-cell genes. The immunological synapse is the interface between APCs and T cells that is formed during T-cell activation. It is a complex interface involving peptide antigen bound to an MHC-II molecule and to the T-cell receptor, costimulatory molecules, receptors on the APC and T cells, integrins, and other

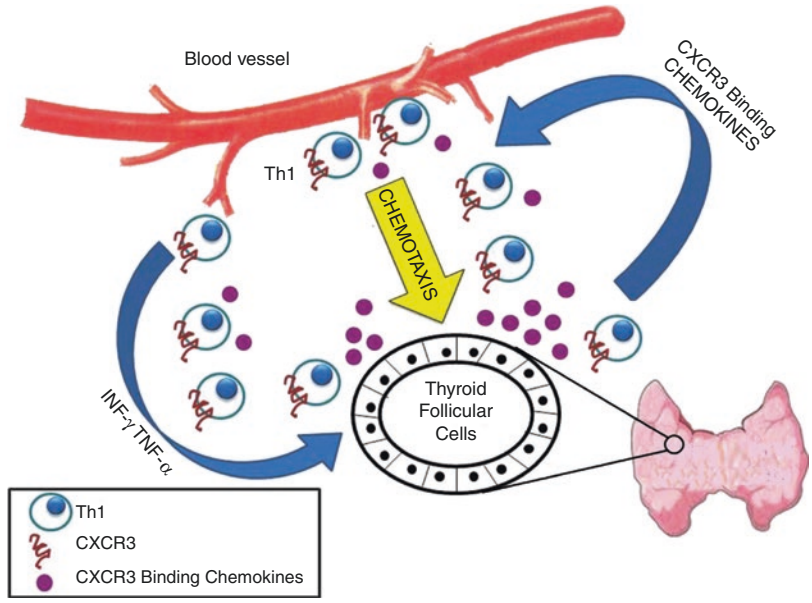

Fig. 2 Schematic representation of the proposed mechanism of lymphocyte recruitment by CXCR3 binding chemokines in thyroid autoimmunity

molecules [19]. Some alleles of the HLA-DR gene can facilitate antigen presentation. This mechanism might explain the association of DR3, DR5 with goitrous, and of DR3, HLAB8 with atrophic chronic autoimmune thyroiditis.

The CTLA-4 gene is a major negative regulator of T-cell activation. Some polymorphisms of CTLA-4 have been shown to reduce the suppression of T-cell activation by antigens [20]. The protein tyrosine phosphatase-22 (PTPN22) gene plays a role as negative regulator of T-cell activation [21]. Genes for T regulatory cells (Tregs) that can predispose to autoimmune thyroid diseases are FOXP3, a key gene in the differentiation of T cells in Tregs, and CD25. Other immune-related genetic variants involve polymorphisms of the IL6 promoter, of the INF-γ gene, and of the T-cell receptor gene [22]. Allotypes of immunoglobulin heavy chains and polymorphisms of the vitamin D receptor gene have also been associated with an increased risk of chronic autoimmune thyroiditis [23]. Among thyroid-specific genes, those for thyroglobulin (Tg) play a role in susceptibility to chronic autoimmune thyroiditis. Tg is one of the main targets of the immune response in autoimmune thyroid diseases. Amino acid variants in the Tg gene (A734S, V1027M, W1999R) can result in altered degradation of Tg in endosomes, inducing a pathogenic Tg repertoire (unique Tg peptides binding to specific HLA-DR pockets).

In spite of the impressive progress of genetics, most of the identified genes have a very minor effect. To explain the strong genetic susceptibility to autoimmune thyroid diseases, three hypotheses have been formulated: (1) an individual needs to inherit many genes of small effect; (2) gene-gene interaction may occur resulting in a combined odds ratio (OR) that is significantly higher than that expected with an additive effect alone; (3) a subset effect (also called genetic heterogeneity) may be involved producing a high OR only in a subset of patients with autoimmune thyroid diseases. As a matter of fact, really significant genes, which means that the autoimmune disease should not occur without some of the responsible polymorphisms, are still to be identified [14, 24]. A possible exception is the autoimmune regulator gene (AIRE). Its products mediate the transcription of many self-antigens in medullary epithelial cells in the thymus. Decreased AIRE expression may lead to a decrease in thymic expression of Tg and other thyroid autoantigens, resulting in the peripheral escape of autoreactive T cells [25]. Indeed, an autosomal dominant allele of AIRE has been associated with Hashimoto's thyroiditis [26]. The complex

Table 2 Immune-related genes and thyroid-specific genes associated with autoimmune thyroid diseases

Genes		Mechanism
Immunological synapse genes		
HLA-DR3, HLA-DR5 (goitrous)	(Caucasian)	Facilitated antigen presentation
HLA-DR3 and HLA-B8 (atrophic)	(Caucasian)	
HLA-DR9 and HLA-Bw46,87	(Chinese)	
HLA-DQw2 (link dis HLA DR3)	(Caucasian)	
HLA-DQ A0301 (link dis HLA-DR4)	(Caucasian)	
HLA-DQ B0201 (link dis HLA-DR3)	(Caucasian)	
CTLA4		Reduced suppression of T-cell activation by antigen
CD40		APCS B-cell activation, CD40 on thyrocytes
Protein tyrosine phosphatase-22 gene (PTPN22)		Negative regulator of T-cell activation?
Regulatory T-cell genes		
FOXP3		Reduced differentiation of T cells into natural Treg cells
CD 25		Reduced α chain IL2 R on Treg cells
Thyroid-specific genes		
Tg		Amino acid variants resulting in altered degradation of
		Tg in endosomes
		Unique Tg peptides binding to specific HLA-DR pockets
		(A734S, V1027M, W1999R)

role of epigenetic control on gene transcription is far from being exhaustively explored, while the influence of X chromosome inactivation on thyroid autoimmunity also remains to be clarified.

Role of the Environment

As reviewed by Duntas, an array of environmental factors was inculpated for their stimulatory effect on thyroid autoimmunity (Table 3) [13]. Some of these factors, such iodine excess, selenium deficiency, and, possibly, industrial pollutants, exert their effects mainly at a population level. Infective agents, immune-modulatory drugs, and stress are probably more relevant for the individual development of autoimmune thyroid diseases. Tobacco smoking deserves a specific mention because, while being a well-known risk factor for the development of Graves' orbitopathy [27], it may in fact protect against the occurrence of TPOAb and inferentially chronic autoimmune thyroiditis [28].

Hypotheses on the Pathogenesis of Thyroid Autoimmunity

Innate Immune Activation (The Danger Hypothesis)

Endogenous molecules, also referred to as danger (damage)-associated molecular patterns (DAMPs), which are produced by dying cells, may induce innate immune responses. DAMPs include genomic DNA fragments, heat shock proteins, high-mobility group B1 proteins, uric acid, collagen, and hyaluronic acid. Toll-like receptors of innate immunity are present on thyrocytes and, by recognizing DAMPs, may activate the innate immune response. Genomic DNA (but also RNA) may behave as a DAMP. When introduced into the cytosol of a viable cell, double-stranded DNA is recognized by DNA sensors (such as histone H2B) and can activate immune responses by upregulating the genes for MHC, costimulatory molecules, transporter associated with antigen processing (TAP-1), immune proteasome subunit LMP2, signal transducer of transcription 1 (STAT-1), IFN regulatory factor (IRF), protein kinase R, and type-1 IFNs. This process of autophagy also allows the processing and delivery of cytosolic antigens to be loaded on MHC-II molecules, thus enabling thyroid cells to present their antigens to CD4+ T cells [18]. The danger hypothesis has been put forward to explain the possible role of

Table 3 Environmental factors involved in thyroid autoimmunity

Dietary factors	Iodine excess
	Selenium deficiency
Pollutants	Radioiodine
	Tobacco smoking
	Polychlorinated biphenyls
	Effects of global warming (possible)
	Metals
	Solvents
Hormones	Female sex (10/1)
	Parity (OR = 4.6)
	Postpartum
	Oral contraceptives
Infections	*Yersinia enterocolitica*
	Hepatitis C virus
Therapy	Interferon α
	Interferon β
	Interleukin 2
	(Amiodarone)
	(Lithium)
	Ipilimumab (anti CTLA-4 mAb)
	Campath-1H (alemtuzumab) anti-CD-52 mAb
	Immune reconstitution syndrome • Bone marrow transplantation • Highly active antiretroviral therapy (HAART) for AIDS
Other	Stress
	Small fetal size (fetal nutrition)
	Direct trauma on thyroid
	Seasonal variation
	Allergy
	Socioeconomic environment (lower exposure to infective agents)

thyroid follicular cells as self-antigen-presenting cells to the immune system, the old Bottazzo's hypothesis [29].

Fetal Microchimerism

Fetal cell microchimerism (FCM) was also implicated in the pathogenesis of thyroid autoimmune diseases.43 FCM is due to the passage of cells from the fetus to the mother. Fetal cells commonly appear in the maternal circulation early in gestation (4–5 weeks). FCM involves T (CD4+ and CD8+) and B cells, monocytes, macrophages, NK cells, CD34+ hematopoietic stem cells, CD34+/CD38+progenitor stem cells, mesenchymal cells, and endothelial progenitor cells. Fetal cells can persist in the mother for more than 27 years after the partum. While not producing a graft-versus-host reaction, fetal cells might mature in the maternal thymus. The presence of these engrafted cells or their proteins could break tolerance or could impair the function of host autoantigen-specific immunoregulatory cells. Such a mechanism could also explain the exacerbation of autoimmune reactivity in the postpartum period and later in life. Evidence favoring a pathogenic role of FCM in thyroid autoimmunity stems from studies indicating that:

1. A significantly higher number of FCM was found within the thyroid gland of women with Hashimoto's thyroiditis and Graves' disease compared with women without thyroid autoimmunity [30].
2. Male cells can be detected in the peripheral blood of both Graves' disease patients and healthy women of reproductive age [31].
3. In a murine model of EAT, fetal immune cells (T-cell and dendritic cell lineages) were found to accumulate in maternal thyroids [32].
4. The thyroid autoimmunity susceptibility markers, HLA DQA1*0501-DQB1*0201 and DQB1*0301, are more common in mother-child pairs positive for FCM [31].
5. One case-control study indicated parity as a potential risk factor for AITD [33].

By contrast, three large epidemiological community-based studies failed to demonstrate an association between pregnancy, parity, abortion, and the presence of thyroid autoantibodies or thyroid dysfunction, indicating that FCM could be a marginal phenomenon [34–36].

The Hygiene Hypothesis

The hygiene hypothesis suggests that people born and living in countries with a high socioeconomic standard and, as a consequence, being exposed to a lower burden of infectious agents during childhood might be more prone to develop thyroid autoimmunity. An epidemiological study comparing the prevalence of thyroid antibodies in the genetically similar populations of Russian Karelia and Finland supports this hypothesis. Indeed, the

prevalence of thyroid antibodies was significantly lower in school children from Karelia compared to their counterparts in Finland. This result supports the idea that the Russian environment, which was characterized by inferior prosperity and standard of hygiene, may provide a protection against thyroid autoimmunity [37].

Antigen-Antibody Systems in Thyroid Autoimmunity

The three main thyroid autoantigens, which were identified several decades ago, are thyroglobulin (Tg), the organ-specific enzyme thyroid peroxidase (TPO), and the TSH-receptor (TSH-R) [38]. Human Tg has at least 40 epitopes, but only one or two of these bind human thyroglobulin antibodies (TgAb), which in humans are mainly IgG and belong to the IgG4 subtype, thus explaining their poor complement-fixing property. TgAb have been detected in up to 60% of patients with chronic autoimmune thyroiditis, but, similarly to TPO antibodies (TPOAb), they are also found in healthy individuals and in patients with non-autoimmune thyroid diseases such as nontoxic goiter and thyroid cancer [39]. In the latter conditions, circulating thyroid antibodies reflect the presence of focal autoimmune thyroiditis within the affected gland. Thyroid peroxidase, previously termed the microsomal antigen, evokes high affinity, IgG class autoantibodies (TPOAb), which fix complement and may be implicated in thyroid destruction [40]. However, when TPOAb are transferred passively from mothers, they do not seem to damage the fetal thyroid. This is because in the intact gland TPO is secluded from TPOAb been expressed at the apical pole of follicular thyroid cell facing the colloid [41]. Test for TPOAb is positive in nearly 90% of patients with chronic autoimmune thyroiditis [42]. Thus they have a superior diagnostic value compared with TgAb for the confirmation of chronic autoimmune thyroiditis. However, in some instances, patients have only TgAb as a marker of the disease. Antibodies directed to the thyroid-stimulating hormone (TSH) receptor with blocking activity (TSBAb) have been also detected in a minority of patients with chronic autoimmune thyroiditis, mainly of the atrophic variant [41]. They are believed to bind the C-terminal part of the extracellular domain of the receptor. However, in vitro conversion from TSBAb to TSAb after addition of antihuman IgG antibody implies that the same antibody may act as blocking or stimulating depending on the influence of other factors [43].

In the first years of this century, antibodies to the sodium-iodide symporter (NIS) and pendrin [44], an iodide transporter located at the apical pole of thyroid follicular cells, were identified in a minority of patients with chronic autoimmune thyroiditis. Their pathogenic role is probably negligible. Antibodies to thyroxine (T4) and triiodothyronine (T3) may be also found in patients with chronic autoimmune thyroiditis. These antibodies interfere with measurement of serum T4 and T3, mainly in the assays for free T4 and T3 [45].

Cellular and Humoral Effector Mechanisms in Thyroid Autoimmunity

Cell damage mechanisms in chronic autoimmune thyroiditis include antibody-dependent cell-mediated cytotoxicity (ADCC); Fas/Fas ligand-mediated apoptosis of thyroid cells (the so-called suicide or fratricide); the direct cytotoxic effect of CD8+ and CD4+ cells, which is MHC I and MHC II restricted, respectively (the so-called homicide); and the granule exocytosis pathway (perforins, granzymes) [46]. Lymphokine-activated killer cells are also involved. Although Fas ligand is expressed in thyrocytes from both normal individuals and patients with chronic autoimmune thyroiditis, Fas is present only in thyrocytes from autoimmune glands, its expression being stimulated by IL-1α [47]. Humoral mechanisms include the TSH blocking effect of TSH-R antibodies [42] and the complement-mediated cytotoxic effect of TPOAb [46]. TPOAb probably exert a secondary destructive mechanism, requiring a primary disruptive event to allow antibody access to the intra-follicular site of TPO expression.

The eventual destruction and/or apoptosis of thyroid follicular cells is responsible for the development of hypothyroidism in chronic autoimmune thyroiditis. Hypothyroidism can also occur by being mediated through the inhibiting effect of cytokines on thyroid hormone synthesis. According to this model, clinical hypothyroidism results from a chronic inhibition of thyroid function, which is induced by exposure of the thyroid to pro-inflammatory cytokines like IFN-γ and TNF-α. These factors can be present in the thyroid also independently of infiltrating lymphocytes. The novelty of this nonclassical model is that thyroid atrophy is shown to be the consequence of thyroid hypofunction rather than the cause of it [48].

Pathology

Goitrous Hashimoto's thyroiditis is characterized by lymphocytic infiltration with plasma cells and germinal centers, follicular destruction, colloid depletion, and fibrosis. Thyroid cells may show an oxyphilic cytoplasm (Hurthle or Askanazy cells). In atrophic thyroiditis, the gland is reduced in size, with lymphocytic infiltration and fibrosis replacing the thyroid parenchyma. Thyroid follicle destruction is mild and lymphocytic infiltration is minimal in focal thyroiditis. In juvenile thyroiditis, oxyphil cells, fibrosis, and germinal centers are less prominent or absent. In silent and postpartum thyroiditis, the thyroid is infiltrated with lymphocytes and thyroid follicles are collapsed, but germinal centers and Hurthle cells are absent.

Epidemiology

Chronic autoimmune thyroiditis is the most common cause of spontaneously acquired hypothyroidism in populations with sufficient iodine intake. Severe forms of autoimmune thyroiditis are detected at autopsy in 5–15% of women and in 1–5% of men. The disease is most often diagnosed between the ages of 50 and 60 years, and it is 5–7 times more frequent in women than in men. The prevalence of thyroid antibodies, which correlates with at least some degree of autoimmune thyroiditis, increases from 6 to 15% in the second to third decades of life to more than 21–27% in women 60 years old or older [49]. In communities with sufficient iodine intake, elevated serum TSH concentrations mainly result from chronic autoimmune thyroiditis. In these populations, subclinical hypothyroidism is found in 8–17% of subjects older than 55–60 years, and overt hypothyroidism is found in 1.7–3% of elderly women [50]. The rate of hypothyroidism is higher in women than in men and higher in whites than in blacks. In recent years, chronic autoimmune thyroiditis has been diagnosed more frequently than in the past due to both improved diagnostic procedures and to an increased number of affected cases. The increased iodine consumption that occurred in Western countries in the past few generations may explain this phenomenon [51].

Clinical Findings

Most patients with chronic autoimmune thyroiditis are euthyroid or have subclinical hypothyroidism and circulating thyroid antibodies. The classical presentation with goiter and overt hypothyroidism is nowadays uncommon. In many patients the diagnosis is made because blood tests, done for unrelated complaints (weight gain, anxiety, asthenia), reveal thyroid dysfunction or thyroid antibodies. On physical examination, the typical Hashimoto's gland is diffusely enlarged, but one lobe may be larger than the other, and the pyramidal lobe may be palpable. The goiter is generally moderate in size, though massive enlargements may occur. The gland is nontender, firm or rubbery in consistency, with a smooth or bosselated surface. If left untreated, the goiter either remains unchanged or enlarges gradually over many years, mainly in patients with unrecognized hypothyroidism. In other instance the goiter becomes smaller. A feeling of tightness in the neck may occur, but compression of the trachea is uncommon. Rapid growth of the goiter

and compressive symptoms should raise the suspicion of thyroid lymphoma.

Patients may present with complaints of hypothyroidism, but for each patient with overt thyroid failure, several have subclinical hypothyroidism. Patients with atrophic thyroiditis exclusively present with hypothyroidism. The incidence rate for the development of hypothyroidism in women increases with age, with 51%of cases diagnosed between 45 and 64 years of age. Thyrotoxicosis (*Hashitoxicosis*) rarely occurs, due to a combination of Hashimoto's thyroiditis with Graves' disease in the same patient or to the transient discharge of preformed thyroid hormones as a result of follicle disruption resulting from the inflammatory process. In the latter case, the disease, at least in its initial stage, should more appropriately be classified as painless thyroiditis. Hyperthyroidism in patients with a combination of Graves' and Hashimoto's disorders is produced by TSH-receptor antibodies with thyroid-stimulating (TSAb) activity and is indistinguishable from that of only Graves' disease. The only differences with Graves' disease are that the goiter is firmer, the titers of TPOAb and TgAb are higher, and the chance of spontaneous remission of hyperthyroidism is higher. Rare cases of transition from hypothyroidism to hyperthyroidism, and vice versa, are also reported, probably due to a shift from TSBAb to thyroid-stimulating antibody (TSAb) within the family of circulating TSH-receptor antibodies. Thus, the clinical features of patients depend on the balance among stimulating, blocking, and destructive aspects of humoral and cellular immunity. High iodine ingestion can aggravate chronic autoimmune thyroiditis, thus precipitating hypothyroidism.

Diagnostic Procedures

Clinical observation may be enough to diagnose the classic picture of goitrous Hashimoto's thyroiditis, but, in the majority of "subclinical" presentations of the disease, laboratory and instrumental investigations are necessary. The diagnostic workup includes tests for thyroid autoimmunity, thyroid echography, and assays for TSH and freeT4 (fT4). Serum TPOAb are detectable in up to 95% of patients with Hashimoto's thyroiditis and 90% of those with atrophic thyroiditis. TgAb are less frequently positive in patients with both types of autoimmune thyroiditis. Low or undetectable titers of both TPOAb and TgAb are encountered in a few patients with chronic autoimmune thyroiditis, the so-called serum-negative chronic autoimmune thyroiditis. Conversely, low to medium titers of TPOAb and/or TgAb are found in a minority of normal subjects and in patients with nontoxic goiter, subacute thyroiditis, or thyroid carcinoma [39]. Thus, the diagnostic specificity of thyroid antibody tests is not absolute. Antibodies to thyroid hormones may interfere with the measurement of serum T4 or T3 and result in falsely high or low thyroid hormone levels, depending on the assay used.

At ultrasound examination, a diffusely reduced echogenicity of the thyroid is detected in chronic autoimmune thyroiditis (Fig. 3). Because this hypoechogenic pattern of the gland is pathognomonic of thyroiditis, echography is nowadays the easiest tool for diagnosing chronic autoimmune thyroiditis. Thyroid radionuclide scan is not crucial to the diagnosis and shows either a diffuse, patchy uptake or a pattern that may mimic hypofunctioning or hyperfunctioning nodules (pseudo toxic adenoma). Values of thyroid radioactive iodine uptake (RAIU) may be normal, low, or high in Hashimoto's thyroiditis. In the assessment of most patients, fine needle aspiration cytology (FNAC) is not necessary, but it is advisable in those with suspicious nodules or a rapidly enlarging goiter in order to rule out malignancy. Cytological smears of Hashimoto's thyroiditis are rich in lymphocytes and oxyphil cells.

An increase in serum TSH concentration may occur long before any decline in serum fT4 levels (subclinical hypothyroidism). Decreased serum concentrations of fT4, and less frequently of free T3 (fT3), are observed in overt hypothyroidism; but low serum fT3, and lessfrequentlyfT4 levels, may be found in patients with non-thyroidal illnesses. An increased serum TSH concentration is the single best diagnostic

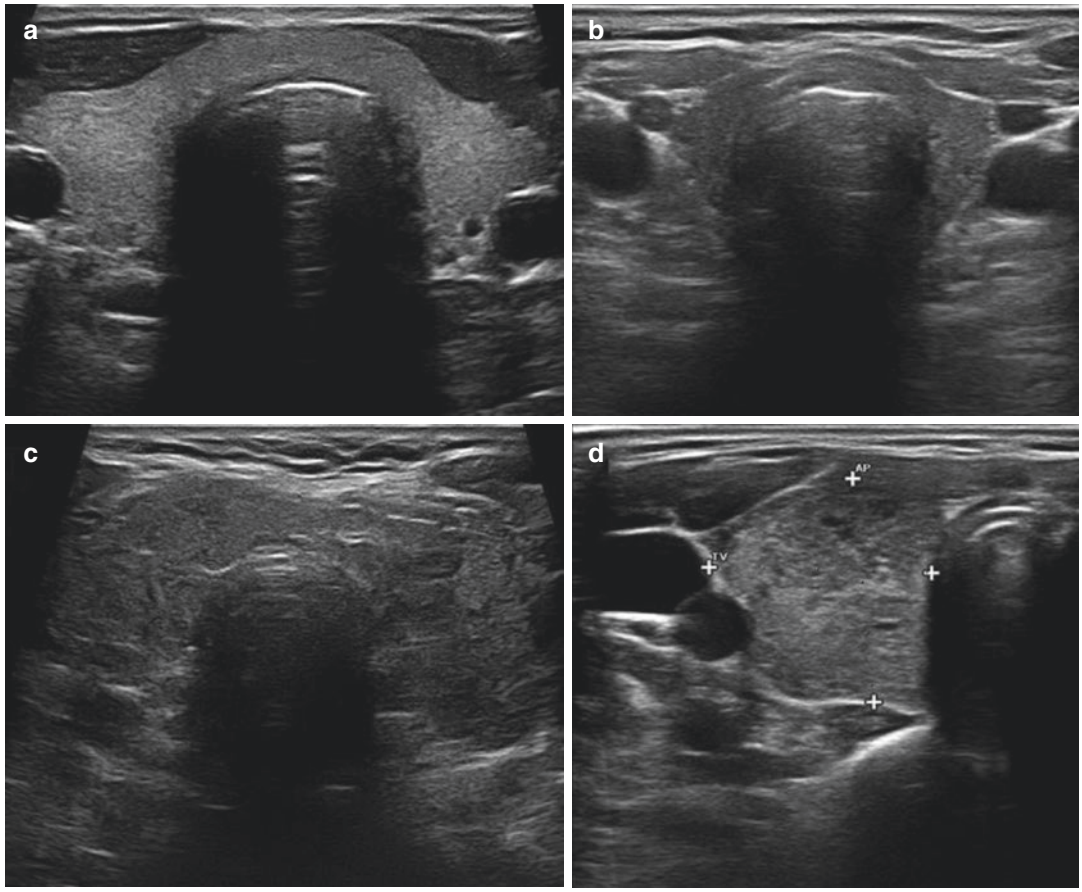

Fig. 3 Echographic aspect of normal thyroid (**a**), atrophic thyroiditis (**b**), Hashimoto's thyroiditis, (**c**) and focal thyroiditis (**d**)

test for primary hypothyroidism. Because rare cases of peripheral resistance to thyroid hormones are an exception to this rule, fT4 assays are needed as a second key test.

The diagnosis of chronic autoimmune thyroiditis is based on the detection of a typical Hashimoto's goiter in euthyroid patients or in the detection of hypothyroidism (either overt or subclinical). Positive tests for thyroid antibodies support the diagnosis, but they are not completely specific. Thyroid echography, by showing a hypoechogenic pattern of the goiter in Hashimoto's thyroiditis or a gland reduced in size in atrophic thyroiditis, provides confirmatory evidence. In patients with serum-negative chronic autoimmune thyroiditis, a typical hypoechoic ultrasound pattern supports the diagnosis. Rarely, the ultimate diagnosis may require FNAC. In iodine-deficient areas, differentiation of Hashimoto's thyroiditis from nontoxic multinodular goiter with focal thyroiditis may be difficult, and intermediate conditions are encountered. Differentiation between Hashimoto's thyroiditis with a prominent "nodule" and thyroid carcinoma requires FNAC. A rapidly enlarging nodule with regional lymphadenopathy suggests thyroid malignancy.

Natural History

Overt hypothyroidism can develop in patients who are euthyroid or have subclinical hypothyroidism when first seen, but the progression is slow and takes several years. Temporary remissions of

subclinical hypothyroidism may also occur. In the follow-up study performed in Whickham [50], the annual rate of developing overt hypothyroidism was 4.3% in women with both raised serum TSH (>6 mU/L) and positive thyroid antibodies, 2.6% if only TSH was raised, and 2.1% if only thyroid antibodies were positive. Even a serum TSH level at the upper limit of the normal range (>2–4 mU/L) was associated with an increased probability of developing hypothyroidism in the following 20 years. Graves' hyperthyroidism occasionally develops in patients with hypothyroidism caused by chronic autoimmune thyroiditis and is believed to result from a change in the nature of TSH-receptor antibodies (TRAb) from blocking to stimulating. The opposite evolution from hyperthyroidism to hypothyroidism may also occur due to changes in the biological activity of TRAb or to the progressive destruction of thyroid parenchyma produced by autoimmune thyroiditis. Hypothyroidism due to chronic autoimmune thyroiditis ultimately supervenes in 10–20% of patients with Graves' hyperthyroidism who remain in remission after antithyroid drugs (burn-out Graves's disease). Euthyroid patients with chronic autoimmune thyroiditis are more susceptible to the antithyroid effects of excess iodine, and amiodarone is a common cause of iodine-induced hypothyroidism in these patients. Remissions of hypothyroidism have been described in Japan after discontinuation of excessive dietary iodine intake [52]. Hypothyroidism may develop in up to one-third of patients treated with lithium and is more common in those with thyroid antibodies. Therapy with IFN- α, IL-2, or granulocyte-macrophage colony-stimulating factor may be associated with the development of thyroid antibodies, transient thyrotoxicosis, hypothyroidism or both, primarily in patients who have thyroid antibodies before therapy. Smoking increases the risk of hypothyroidism in patients with autoimmune thyroiditis.

Serum-Negative Chronic Autoimmune Thyroiditis

Compared to the typical disease, patients with serum-negative chronic autoimmune thyroiditis usually display lower levels of serum TSH and higher FT4 levels. As a consequence, the prevalence of overt hypothyroidism is lower in serum-negative patients. Their thyroid volume is also smaller. On the other hand, the prevalence of female gender and thyroid nodules among affected patients is similar to that observed in those with chronic autoimmune thyroiditis and circulating thyroid antibodies. Overall, it appears that serum-negative chronic autoimmune thyroiditis represents a milder form of thyroid autoimmunity [53].

IgG4 Thyroiditis

Recently, a subtype of Hashimoto's thyroiditis associated with sclerosing pancreatitis has been identified as a distinct clinical-pathological entity being termed IgG4 thyroiditis. The disease is characterized by fibrosis, lymphoplasmacytic infiltration, increased IgG4 positive plasma cells in the thyroid, and high IgG4 levels in serum. Clinically, IgG4 thyroiditis presents with a more rapid progress of goiter, subclinical hypothyroidism, high levels of circulating thyroid antibodies, and a diffuse hypoechogenic pattern of the gland at ultrasound examination. Positive immunostaining for IgG4 plasma cells and histologic evidence of invasive fibrosis, even beyond the thyroid capsule, may be required for the differential diagnosis. It is suggested, but not definitely proved, that Riedel's thyroiditis might belong to the spectrum of IgG4 thyroiditis [54].

Associated Diseases

Up to 5% of patients with thyroid-associated orbitopathy have chronic autoimmune thyroiditis with hypothyroidism [27]. A peculiar encephalopathy or encephalitis with stroke-like episodes, seizures, or altered consciousness has been reported in euthyroid patients with Hashimoto's thyroiditis, though rarely [55]. This neurologic disease is more common in females and has been described in all ages. Although corticosteroid-responsive, its course may be progressive or

relapsing. Hashimoto's encephalopathy is associated with abnormal electroencephalogram and high cerebrospinal fluid proteins without pleocytosis. Some patients have residual disability; cognitive decline and behavioral problems may occur in children. This condition may represent an association with Hashimoto's thyroiditis with a rare autoimmune encephalopathy, although the binding of TPOAb to astrocytes has been described [56].

Patients with Hashimoto's thyroiditis are prone to develop other autoimmune organ-specific, endocrine and non-endocrine (i.e., vitiligo, myasthenia gravis, multiple sclerosis, thrombocytopenic purpura, Sjögren's disease, alopecia), diseases. Non-organ-specific systemic autoimmune conditions (i.e., rheumatoid arthritis, lupus erythematosus) are also more frequent (Table 4). These associations are suggested by the presence in the patients' serum of autoantibodies to adrenal cortex (1–2%), pancreatic islet cells (l–3%), gastric parietal cells (10–30%), intrinsic factor (1%), DNA, mitochondria, phospholipids, or IgG. Celiac disease is now believed to be a common association. In a study, 15% of patients with chronic autoimmune thyroiditis had positive serology for the gastrointestinal disease, and 21% of celiac patients had circulating thyroid autoantibodies [57]. Chronic autoimmune thyroiditis is a component of type 2 autoimmune polyglandular syndrome (APS), a condition characterized by the coexistence of two or more of the following disorders: Addison's disease, autoimmune thyroiditis, and insulin-dependent diabetes mellitus [58]. The association with other autoimmune conditions, apart from Addison's disease, is categorized as type 3 polyglandular syndrome (Table 5).

Focal thyroiditis is seen in many patients with papillary thyroid carcinoma, and it may represent a secondary immune response to cancer. Although still controversial, recent studies have shown that patients with chronic autoimmune thyroiditis may have an increased risk of developing papillary thyroid cancer, and molecular findings demonstrated that RET/PTC oncogene is more commonly expressed in nonneoplastic follicular cells in Hashimoto's glands [59]. From a clinical point of view, the coexistence of chronic autoimmune thyroiditis (even focal) with differentiated thyroid cancer is of relevance because circulating TgAb interfere with the measurement of serum Tg, thus rendering unreliable the use of this marker in the follow-up of the neoplastic disease [60].

In patients with chronic autoimmune thyroiditis, the prevalence of primary B-cell lymphoma of the thyroid is 80 times greater than expected, and most patients with this malignancy have preexistent Hashimoto's thyroiditis [61]. Sequence determination of immunoglobulin heavy chain gene rearrangements suggests that primary thyroid lymphoma evolves from lymphocytes infiltrating the gland in Hashimoto's thyroiditis. Despite this increased incidence, lymphomas of the thyroid remain rare tumors.

Table 4 Hashimoto's thyroiditis and its association with autoimmune diseases

	Hashimoto's thyroiditis		
Associated autoimmune disease	Women $N = 427$	Men $N = 68$	Total $N = 495$
Type 1 diabetes	5 (1.17%)	0 (0%)	5 (1.01%)
Rheumatoid arthritis	20 (4.68%)	1 (1.47%)	21 (4.24%)
Pernicious anemia	19 (4.45%)	0 (0%)	20 (4.04%)
Systemic lupus erythematosus	3 (0.70%)	0 (0%)	3 (0.61%)
Addison's disease	5 (1.17%)	2 (2.94%)	7 (1.41%)
Celiac disease	5 (1.17%)	0 (0%)	5 (1.01%)
Vitiligo	12 (2.81%)	1 (1.47%)	13 (2.63%)
Multiple sclerosis	3 (0.70%)	1 (1.47%)	4 (0.81%)
Myasthenia gravis	1 (0.23%)	0 (0%)	1 (0.20%)
Inflammatory bowel disease	3 (0.70%)	1 (1.47%)	4 (0.81%)

Table 5 Autoimmune polyendocrine syndrome 3 (APS 3)

Chronic autoimmune thyroiditis			
Type 1 diabetes mellitus	Chronic atrophic Gastritis	Vitiligo	SLE
Hirata's disease	Pernicious anemia	Alopecia	Rheumatoid arthritis
Hyper-gonadotropic hypogonadism	Celiac disease	Idiopathic urticaria	Systemic sclerosis
Lymphocytic hypophysitis	Protracted diarrhea of infancy	Bullous skin diseases	Seronegative Arthritis
Idiopathic diabetes insipidus	Inflammatory bowel diseases	Myasthenia gravis	Scleroderma
Chronic hypoparathyroidism	Autoimmune hepatitis	Stiff-man syndrome	Vasculitis
Sjögren's syndrome	Primary biliary cirrhosis	Multiple sclerosis	
Breast lymphocytic lobulitis	Sclerosing cholangitis	Guillain-Barrè syndrome	
Autoimmune pancreatitis		Werlhof's disease	
		Autoimmune anemia	
		Antiphospholipid syndrome	
		Idiopathic myocarditis	
Endocrine or exocrine diseases 3A	*Gastrointestinal or liver diseases 3B*	*Skin, neuromuscular, hematological, cardiac diseases 3C*	*Rheumatic diseases or vasculitis 3D*

Treatment

Immunosuppressive agents such as corticosteroids are not recommended in a benign disease that can be safely and economically treated with L-thyroxine (L-T4). Corticosteroids cause some regression of Hashimoto's goiter and decrease thyroid antibody titers, but the activity of the disease returns after treatment is withdrawn. In rare cases, painful subacute exacerbations of Hashimoto's thyroiditis may benefit of a short course of corticosteroid treatment.

Patients with overt hypothyroidism require substitution therapy with L-T4 at a dose that normalizes serum TSH levels. The average daily replacement dose of L-T4 in adults is 1.6 μg/kg body weight. Full replacement doses are 75–100 μg per day in most women and 100–150 μg per day in most men. Elderly hypothyroid patients require a dose 20–30% lower. In hypothyroid patients with coexistent cardiac disease, L-T4 therapy should be initiated with 12.5–25 μg per day, followed by careful increments of 12.5–25 μg per day every 4–8 weeks. L-T4 substitution may precipitate angina or myocardial infarction in the elderly with coronary artery disease, but it ameliorates reversible coronary dysfunction inherent with hypothyroidism and produces beneficial effects on hypothyroid hyperlipidemia. Coronary by-pass or angioplasty can be safely performed before starting L-T4 administration. Long-term L-T4 substitution was not found to reduce bone mineral density, provided that serum TSH concentrations are kept in the normal range. Successful L-T4 therapy is often accompanied by a decrease in thyroid antibody titers in patients with elevated serum TSH levels before treatment. Indications for L-T4 substitution therapy in subclinical hypothyroidism are not univocal, but an improvement in some hypothyroid features was observed in two placebo-controlled trials of L-T4 therapy [62]. Restoration of normal TSH levels with L-T4 also produces a slight reduction in the elevated serum cholesterol levels sometimes observed in patients with subclinical hypothyroidism. Replacement therapy is usually advised when serum TSH concentration is higher than 10 mU/L and in those patients with borderline high serum TSH (5–10 mU/L) and positive thyroid antibody who are at high risk for progression to overt hypothyroidism. The presence of goiter and symptoms consistent with thyroid hormone deficiency favor treatment. Substitution treatment should be lifelong because hypothyroidism

tends to recur when therapy is discontinued. A spontaneous recovery of thyroid function that persists when L-T4 treatment is withdrawn occasionally occurs when hypothyroidism has been precipitated by dietary or pharmacological overload, by administration of lithium or cytokines, or when TSBAb present in serum before starting L-T4 treatment disappear in the subsequent follow-up. The permanence of such remissions is uncertain, but most of these patients might not remain euthyroid during the follow-up. Selenium treatment was shown to reduce circulating thyroid antibodies and possibly TSH in some, but not all studies. The issue is still controversial, and there is no evidence enough to recommend this type of treatment [63].

The proper treatment of euthyroid Hashimoto's goiter is controversial. The use of L-T4 may be considered for two main reasons. The first is that a reduction in goiter size was observed in euthyroid patients with Hashimoto's thyroiditis randomly allocated to L-thyroxine therapy as compared to those who were observed with no active treatment [64]. The second reason pertains to the possible evolution to hypothyroidism and to the fact that L-T4 therapy may limit a further growth of goiter. The decision to treat with L-T4 is optional. Untreated euthyroid patients with autoimmune thyroiditis require periodic thyroid function tests: once a year in patients with goitrous Hashimoto's thyroiditis and every 6 months in those with a gland of reduced size.

Surgery is indicated, though this happens rarely, in cases of extremely large Hashimoto's goiters with obstructive symptoms or when associated thyroid malignancy is suspected. L-T4 therapy is mandatory after surgery, as hypothyroidism invariably results.

Juvenile Thyroiditis

Among children and adolescents living in areas of iodine sufficiency, juvenile (lymphocytic) thyroiditis is the cause of euthyroid goiter in one-half to two-thirds of patients. Atrophic thyroiditis may also occur and is associated with hypothyroidism. Thyroid antibodies are less frequently positive than in adults. In a 20-year follow-up study of goitrous juvenile thyroiditis, spontaneous resolutions occurred in about 25% of cases, but hypothyroidism developed in 33% of patients. A more recent Italian study [65] investigated children with Hashimoto's thyroiditis being euthyroid or showing mild subclinical hypothyroidism (TSH <100% of the upper limit) when first seen. After a 3-year observation period, 72% of originally euthyroid children maintained normal thyroid function, while 13% and 15% developed subclinical or overt hypothyroidism, respectively. Among children with initial subclinical hypothyroidism, 41% normalized their function, while 19% remained stable and 40% progressed to overt hypothyroidism. The presence of celiac disease and elevated TSH and TPOAb increased the risk of developing hypothyroidism by 4.0-, 3.4-, and 3.5-fold, respectively. The increase in TSH levels during follow-up was strongly predictive of the development of hypothyroidism. Active L-thyroxine treatment in euthyroid children with juvenile thyroiditis is pointless, while elevated serum TSH may indicate the need for substitution therapy.

Silent Thyroiditis

Silent (painless) thyroiditis is characterized by transient thyrotoxicosis with low RAIU, and a small, painless, nontender goiter. Silent thyroiditis occurs either sporadically or in the postpartum period (postpartum thyroiditis). The overall prevalence of silent thyroiditis as a cause of thyrotoxicosis ranges from 4 to 15%, but the latter figure appears high. A seasonal and geographic variation in the prevalence has been reported, with those areas previously iodine-deficient, but recently exposed to sufficient iodine, having a greater prevalence [66]. The female/male ratio is approximately 2/1. Thyrotoxicosis results from damage of the follicular cells by the inflammatory process, with leakage of preformed thyroid hormones in the bloodstream. The inflammatory process impairs the capacity of the thyroid to make thyroid hormones. As a consequence, patients undergo a euthyroid phase and then,

when thyroid hormone stores are depleted, become hypothyroid. Silent thyroiditis has been reported in patients with other autoimmune disorders, with a personal or family history of thyroid autoimmunity, and in those experiencing a rebound of immunity after treatment of Cushing's syndrome. Excess iodide intake, amiodarone, IFN-α and IL-2, simple palpation, or parathyroid surgery have been reported as initiating events of silent thyroiditis.

Silent thyroiditis presents with a relatively abrupt onset of symptoms of mild thyrotoxicosis (tachycardia, heat intolerance, sweating, nervousness, and weight loss). The thyroid may be normal in size (50% of cases), or a small, modestly firm, nontender goiter may be palpable. After 2–9 weeks, thyrotoxicosis subsides, and patients progress to euthyroid and hypothyroid phases before recovering normal thyroid function. About 40% have a hypothyroid phase, which usually lasts between 4 and 10 weeks. Euthyroidism is ultimately restored in most cases, but persistent hypothyroidism may also develop (5%). Recurrences may develop in up to 11% of cases. Impaired thyroid reserve or goiter may occur after a bout of silent thyroiditis.

Free T4 and free T3 are elevated in the thyrotoxic phase, but the increase of T4 relative to T3 is disproportionate owing to the release of preformed thyroid hormones into the circulation. Serum Tg and urinary iodide concentrations are also increased. RAIU is suppressed (often 1–2% at 24 h). The erythrocyte sedimentation rate is normal or only slightly elevated. The white blood cell count is usually normal. TPOAb and TgAb are present in sera of 60% and 25% of patients, respectively. Serum TSH levels are low in the thyrotoxic phase, but they may increase to hypothyroid levels before recovery. Silent thyroiditis should be considered in all cases of thyrotoxicosis with low RAIU and a nontender gland. Other causes of thyrotoxicosis with low RAIU should be excluded, such as thyrotoxicosis factitia (serum Tg is low), iodine-induced hyperthyroidism (urinary iodine excretion >1000 μg/24 h), or ectopic thyroid hormone production (struma ovarii). Differentiation from Graves' hyperthyroidism is important (Table 6). FNAC reveals lymphocytic thyroiditis, but it is usually not necessary to make the diagnosis. Thyroid ultrasound shows decreased echogenicity.

Table 6 Differences between silent thyroiditis and Graves' disease

	Silent thyroiditis	Graves' disease
Onset	Abrupt	Gradual
Severity of thyrotoxicosis	Mild-moderate	Moderate-marked
Duration of symptoms	<3 months	>3 months
Thyroid bruit	Absent	May be present
Ophthalmopathy, dermopathy	Absent	May be present
T3 (ng/mL)/T4 (μg/dL) ratio	<20/1	Mostly >20/1
RAIU	Low	High
TSH-R antibodies	Usually negative	Usually positive
Thyrotoxicosis	Transient	Persistent

Antithyroid drugs or radioiodine are inappropriate for the treatment of silent thyroiditis, because thyrotoxicosis is due to the release of preformed thyroid hormones. Thyrotoxic symptoms are managed with β-adrenergic blocking agents, such as propranolol (30–40 mg orally 3–4 times daily). Prednisone, started at 40–60 mg per day orally and then tapered over 4 weeks, may cause a decline of T4 and T3 serum concentrations within 7–10 days. L-T4 replacement therapy is usually not needed because symptoms of hypothyroidism are often mild and transient. When hypothyroidism is symptomatic, a suboptimal replacement dose of L-T4 should be given, and then it should be tapered after 6 months and the patient should be checked to determine whether recovery has occurred.

Autoimmune Thyroiditis During Pregnancy and Postpartum

Chronic autoimmune thyroiditis, mainly asymptomatic, is relatively common in women of childbearing age. During pregnancy all autoimmune reactions are downregulated by a number of physiologic factors. Following delivery there is a

reversal of these alterations with a rebound of autoimmune phenomena. Thus, TPOAb, TgAb, and TSH-receptor antibodies decrease or may even disappear during the third trimester of pregnancy, but a rebound increase is observed after delivery [67]. Patients with asymptomatic chronic autoimmune thyroiditis may undergo a deterioration of their thyroid function during pregnancy with a slight increase in serum TSH (up to 5–10 mU/L) at the end of pregnancy. It is therefore recommended that in these women serum TSH and fT4 levels are frequently checked during gestation and L-thyroxine replacement therapy initiated when serum TSH levels increase over trimester-specific reference values. Hypothyroid patients with autoimmune thyroiditis on replacement therapy frequently require an increase in the dose of L-T4 during pregnancy, which mainly occurs in the first trimester of gestation and is highlighted by an elevation in serum TSH level. The dose of L-T4 should be adjusted to keep serum TSH within trimester-specific normal limits [68]. TPOAb and TgAb cross the placenta, but they do not directly damage the fetal thyroid. Because of this, the incidence of congenital hypothyroidism is not increased in neonates of mothers with autoimmune thyroiditis. Rare neonates born to mothers with atrophic thyroiditis and serum TSBAb may develop transient neonatal hypothyroidism.

The rebound of immunity that follows delivery may be accompanied by destructive thyroiditis resulting in transient thyrotoxicosis evolving to hypothyroidism or by hypothyroidism occurring de novo, followed by gradual recovery (postpartum thyroiditis, PPT). The incidence of PPT ranges from 1.1 to 16.7% in different studies. Risk factors for the development of PPT include positive TPOAb in the first trimester of pregnancy, type 1 diabetes mellitus, a history of chronic autoimmune thyroiditis or Graves' disease, or a previous episode of PPT during a preceding pregnancy. Circulating TPOAb are found in the majority of women with PPT. FNAC show diffuse lymphocytic infiltration.

The classical clinical course is observed in about 26% of patients. The first phase, within 2–3 months after delivery, is characterized by mild symptoms of thyrotoxicosis and lasts from 1 to 6 weeks. Mild hypothyroidism of 2–6 weeks' duration may then occur between 3 and 8 months after delivery. Lack of energy, poor memory, dry skin, and cold intolerance predominate. Postpartum depression is more common in women with positive thyroid antibodies irrespective of their thyroid status. A small, diffuse, firm, nontender, usually painless goiter is palpable. Transient thyrotoxicosis alone or transient hypothyroidism alone occurs in 38% and 36% of patients, respectively. In the long run, persistent hypothyroidism develops in 20–30% of patients.

Postpartum thyroiditis is suspected in women who have fatigue, palpitation, emotional lability, or goiter during the first year after delivery. Thyroid hormone and TSH changes are similar to those found in silent thyroiditis. High titers of TPOAb are found in most patients. Serum Tg is elevated. Diffuse or multifocal reduction of thyroid echogenicity is found at ultrasound. The differential diagnosis with Graves' hyperthyroidism, which may actually appear or relapse in the postpartum period, is based on the same criteria suggested for silent thyroiditis (Tabella). RAIU evaluation using ^{123}I may be ordered, but breast feeding should be interrupted for at least 3 days. Long-term follow-up of thyroid function is mandatory in patients with PPT.

Administration of B-adrenergic blocking drugs ameliorates symptoms of thyrotoxicosis. In patients with marked hypothyroid symptoms, L-T4 treatment at medium-low dose (50–75 μg/day) is required, and it should be maintained for the first year after parturition. Thereafter an attempt to withdraw therapy should be done to check whether hypothyroidism is transient or permanent.

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Acute Infectious Thyroiditis (Acute Suppurative Thyroiditis)

Hiroo Masuoka and Akira Miyauchi

Introduction

Acute infectious thyroiditis, also known as acute suppurative thyroiditis (AST), is a rare inflammatory thyroid disease that is caused by bacterial or fungal infection. The thyroid gland is an endocrine organ having no external connections and is remarkably resistant to bacterial infection due to its high iodine content. This generally prevents the gland from becoming infected unless there is some underlying abnormality. Anatomical abnormalities found in patients with acute infectious thyroiditis include pyriform sinus fistula, thyroid nodules, and cancers. One of the present authors was among those who, in 1979, first reported the frequent phenomenon of a congenital internal fistula originating from the pyriform recess and running to the thyroid lobe in AST patients and named this entity pyriform sinus fistula [1]. Patients with thyroid nodules or cancers who develop infectious thyroiditis have also been shown to be susceptible to other forms of infection.

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Clinical Manifestations

Most patients with AST caused by infection through a pyriform sinus fistula complain of an abrupt onset of painful swelling in the thyroid region accompanied by fever. The pain increases on swelling and is usually accentuated by swallowing. Some adult patients present with a vague firm mass in the thyroid region and slight pain without the typical features of acute inflammation, suggestive of an unusual malignant tumor. With the progression of inflammation, the overlying skin becomes edematous and erythematous, and an abscess develops that may rupture to the skin spontaneously (Fig. 1). Infants with a large cystic fistula may develop acute respiratory distress due to tracheal compression following feeding or crying, and this risk increases markedly with inflammation. The left side is predominantly involved [2], with the left-to-right ratio being 166:8 in our updated data. In only two patients with AST through a pyriform sinus fistula were bilateral sides involved.

In most cases, the clinical features are typical of AST at the initial episode. In the many cases with recurrence of the disease, the inflammation tends to be local, probably due to adhesions in the perithyroidal space, and shows the features of a localized abscess in the region of the thyroid lobe. Recurrence of the episodes is quite common. In a previous series of 43 patients, 29 had had a number of previous episodes (up to 12

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episodes; mean, 2.8), while 14 patients were being seen for their first episode [2]. In our updated series of 176 patients with a pyriform sinus fistula, the intervals between episodes varied from 1 month to 37 years, and the patients were asymptomatic before the onset and during the remission of the inflammation.

Age at first episode varies widely, as the disease can be seen in infants and is common in youth but can appear as late as middle age. Among our 176 patients, the age at first episode ranged from 11 months to 58 years with a mean of 10 years and was less than 20 years in 79.1% of the patients (Fig. 2).

The onset of AST often follows upper respiratory tract infection and occurs frequently in the fall and winter. In a few cases, it follows blunt trauma to the thyroid region [3].

Patients with AST caused by infection of a thyroid nodule also have painful swelling in the thyroid region and fever. These patients may have noticed a preexisting nodule and may have had fine-needle aspiration on the nodule [4].

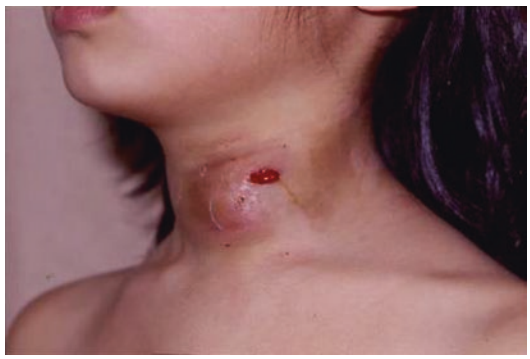

Fig. 1 A 10-year-old girl with acute suppurative thyroiditis showing swelling in the region of the left thyroid lobe. The overlying skin is erythematous. The abscess ruptured spontaneously to the skin

Laboratory Findings

Laboratory investigations typically show leukocytosis and a positive C-reactive protein reflecting acute inflammation. Serum levels of thyroid hormones, thyrotropin, and thyroglobulin are normal in most patients, while some patients with severe destruction of thyroid follicles show transient thyrotoxicosis with elevations of serum thyroxine and thyroglobulin [5].

Pathogenesis and Management

1. Infection through a pyriform sinus fistula
   (a) Pathogenesis

   A congenital fistula, pyriform sinus fistula, is the most common route of infection in AST. The fistula originates from the apex of the pyriform recess of the hypopharynx, penetrates the cricopharyngeal muscle, runs anteroinferiorly, and ends in or adjacent to the thyroid lobe.

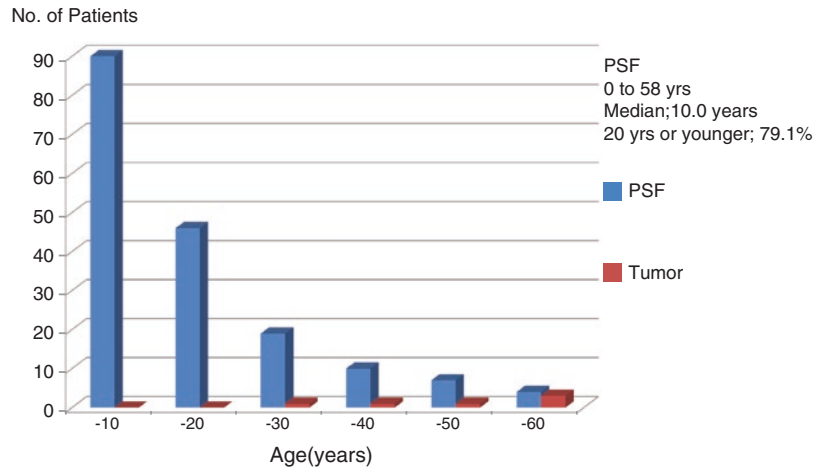

Fig. 2 Age at onset of acute suppurative thyroiditis in patients with a pyriform sinus fistula and patients with an infected thyroid tumor

Bacteria from the fistula cause inflammation in the thyroid lobe in cases with a fistula entering the lobe, or they may spread along the perithyroidal space around the thyroid and invade the thyroid gland secondarily.

Recurrence of inflammation is very common in patients with a pyriform sinus fistula. Successful surgical removal of the fistula terminates the recurrence of the inflammation. However, this surgical procedure is not easy and is associated with the risk of incomplete resection of the fistula and injury to the recurrent laryngeal nerve. In order to overcome these limitations, a method of chemically cauterizing the pharyngeal opening of the fistula causing the fistula to close secondarily was developed by Kim et al. [6]. The success rate in closure of the fistula was 85% in our series. Spontaneous closure of the fistula following inflammation may be possible in some patients, especially in those with a very fine fistula.

(b) Embryology

A pyriform sinus fistula arises from the hypopharynx and ends in or adjacent to the thyroid lobe. We examined resected specimens of the thyroid glands and the pyriform sinus fistulae from 15 patients immunohistochemically with rabbit antisera to human calcitonin and thyroglobulin. The fistulae were lined by squamous, columnar, or ciliated epithelium and sometimes formed branches in the thyroid lobe. Near the branches there were solid cell nests, which are regarded as remnants related to the ultimobranchial body. Mucous glands, follicular structures, and thymic tissue were found in the fistula. The follicular structures stained positive for thyroglobulin. Immunostaining for calcitonin revealed aggregates of many C cells in the thyroid near the fistula. A few calcitonin-positive cells were also found in the fistulae. These findings, along with the anatomical relation of the fistulae to major structures of the neck, strongly suggest that the fistulae are remnants related to the ultimobranchial body and that fistulae trace the migration route of the ultimobranchial body to the thyroid gland to become C cells [7].

(c) Imaging studies

Thyroid scintigraphy with radioactive iodine or sodium pertechnetate ^{99m}Tc demonstrates a decreased uptake in the affected lobe. Plain roentgenograms of the neck show the trachea shifted laterally by soft tissue swelling, which occasionally contain a gas shadow. Ultrasonography (US) and CT scan can reveal the inflammatory status more accurately than any other modalities. In the acute inflammatory stage, US shows a hypoechoic lesion spreading in and around the affected thyroid lobe, destruction of the lobe, and abscess formation in the neck. CT scans demonstrate similar features with clearer anatomical involvement and edema in the ipsilateral hypopharynx (Fig. 3). These findings allow easy diagnosis of

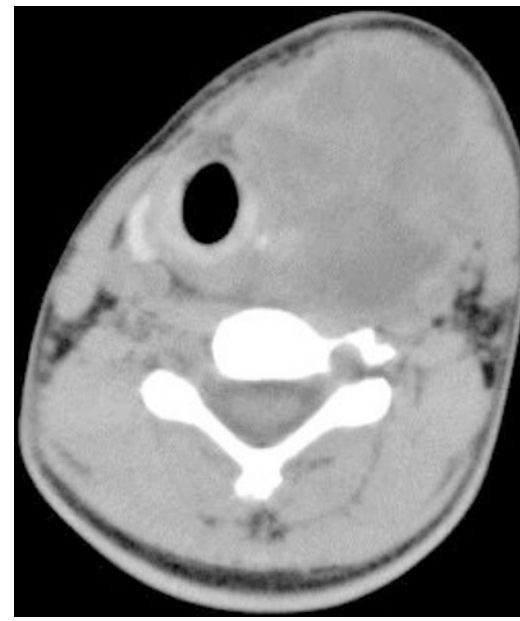

Fig. 3 A CT scan of a patient with acute suppurative thyroiditis caused by bacterial infection via a pyriform sinus fistula, showing massive swelling in and around the left thyroid lobe, which was destroyed by the inflammation

AST. However, in the early inflammatory stage, US may show an unclear hypoechoic area in the affected lobe, and CT scans show a nonspecific low-density area (Fig.4). These findings often lead to erroneous diagnoses of subacute thyroiditis. In the convalescent stage of the inflammation, US and CT scans often show atrophy and an unclear hypoechoic or low-density area in and around the affected lobe. In detecting pyriform sinus fistulae, barium swallow studies are more sensitive than US or CT scans (Fig. 5). A careful search of the hypopharynx discloses an internal fistula originating from the apex of the pyriform recess on the affected side. In some cases, there is no detectable fistula during the acute inflammatory stage, but a fistula is detected upon follow-up during convalescence [8]. A CT scan under the trumpet maneuver can also reveal a pyriform sinus fistula. For this maneuver, patients are instructed to hold and blow into the outer part of an empty syringe as if they are blowing up a balloon or playing a trumpet so that air can be used as a contrast agent. The CT scan taken under the trumpet maneuver clarifies the anatomical path and the relationship of the fistula to the laryngeal organs and the thyroid, which cannot be shown by barium swallow studies [9].

(d) Differentiation from subacute thyroiditis
The most important consideration in the initial evaluation is differentiating AST from subacute thyroiditis [10]. Subacute thyroiditis is a relatively common entity associated with anterior neck pain and an increase in inflammatory markers such as white blood count (WBC) and C-reactive protein (CRP). During the active inflammatory stage, subacute thyroiditis often shows thyrotoxicosis. As AST at its early onset may mimic subacute thyroiditis, some patients with AST have been referred to our hospital after being erroneously diagnosed with subacute thyroiditis and prescribed prednisolone, which aggravated the inflammation [5]. The differential diagnosis at the early inflammatory stage can be difficult, especially if it is the first episode and the inflammation is mainly located within the thyroid lobe. The most straightforward methods to differentiate subacute thyroiditis and AST

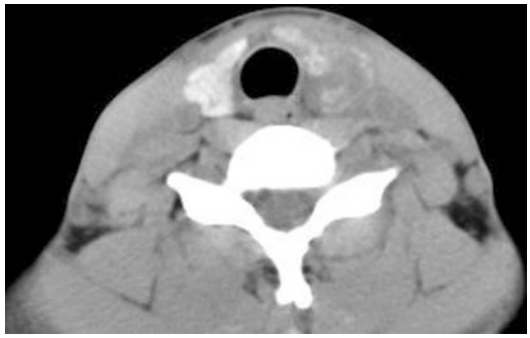

Fig. 4 A CT scan at an early stage of acute inflammation showing mild swelling of the left lobe with a low-density area, mimicking subacute thyroiditis

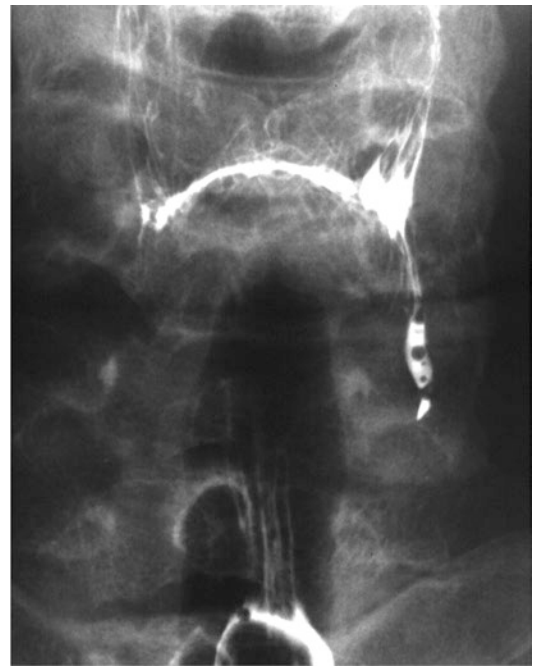

Fig. 5 A barium swallow study revealing a left pyriform sinus fistula. Note that this fistula is much thicker than the average fistula

are careful US examination and fine-needle aspiration biopsy (FNA) targeting any mass and/or fluid collection. US can demonstrate the collection of small amounts of fluid around the affected thyroid lobe, a small heterogeneous low-density area in the thyroid gland, and can determine whether hypoechoic lesions are unifocal, which is a characteristic finding in patients with early-stage AST (Fig. 6). On the other hand, in patients with subacute thyroiditis, ultrasound examinations usually show multifocal hypoechoic areas and no fluid collection around the thyroid lobe. FNA usually shows evidence of multinucleated giant-cell granulomas and mononuclear cell infiltration in subacute thyroiditis, while it shows pus formation with many leukocytes in AST patients. The age of the patient is also important. We should remember that subacute thyroiditis is extremely rare in patients under 20 years of age [11], while the vast majority of AST cases due to infection through a pyriform sinus fistula occur in this age group.

(e) Management

- Initial treatment

If an abscess formation is detected by imaging studies such as CT scan and US in patients with AST, adequate drainage should be performed, which can be done through a small incision in the skin. Intravenous empiric broad-spectrum antibiotics should be given. If a specific organism is identified by culture, antibiotic selection can be more focused. The acute inflammation and abscess formation will readily subside if these initial treatments are performed. Only patients with mild symptoms and a small abscess formation are likely to be candidates for conservative treatment with antibiotics, with or without fine-needle aspiration. After the inflammation has subsided and while waiting for definitive treatment, administration of antibiotics is usually not necessary [12].

- Definitive treatment
  – Fistulectomy

Because recurrence of the inflammation is very common, a definitive treatment should be performed in the convalescent stage, usually several months after the initial treatment. Until the invention of chemocauterization treatment described below, we used to perform fistulectomy. In our series of patients with AST through a pyriform sinus fistula, we confirmed that patients in whom the fistula was completely removed had no recurrence after surgery [2]. However, a fistulectomy for a pyriform sinus fistula is not an easy operation, because the pyriform sinus fistula is usually covered with fibrous or granulation tissue, and the recurrent laryngeal nerve usually runs close to the fistula. Thus there are risks of incomplete resection of the fistula and injury to the recurrent laryngeal nerve on this operation.

The surgical procedure is described as follows: a collar skin incision is made on the upper neck slightly cranial to the cricoid cartilage. The superior thyroid vessels are ligated and cut, taking care not to injure the external branch of the superior laryngeal nerve. Next, the upper pole of the thyroid lobe is retracted anterolaterally to expose the surfaces of the cricothyroid and the cricopharyngeal muscles, and the fistula is identified at the lower border of the thyroid cartilage (Fig. 7). The inflamma-

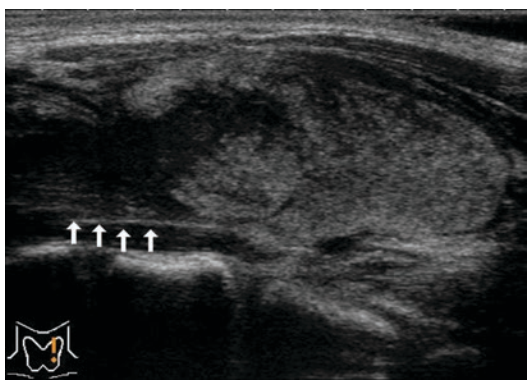

Fig. 6 Ultrasonogram showing swelling of the left thyroid lobe with a hypoechoic area extending dorso-cranially from the posterior surface of the lobe

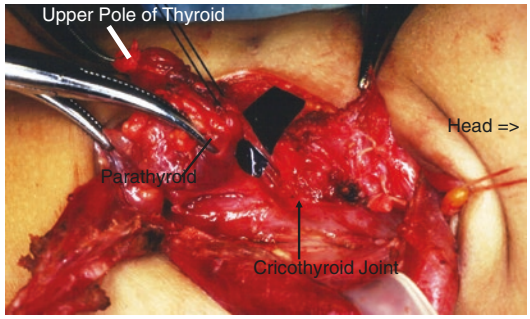

Fig. 7 An intraoperative image showing a left pyriform sinus fistula, which penetrated the cricothyroid muscle just medial to the cricothyroid joint and entered the left thyroid lobe. The superior thyroid vessels were ligated and divided, and the upper pole of the left lobe was retracted anteriorly and caudally. The head is to the right

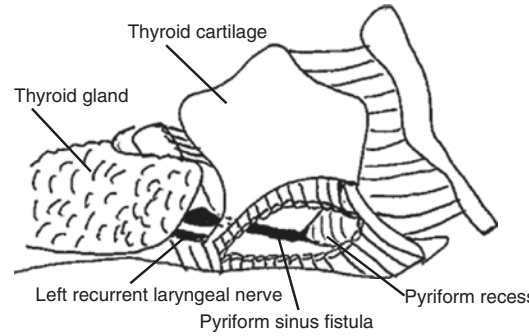

Fig. 8 The scheme of the Nonomura procedure. The inferior pharyngeal constrictor muscle was divided along the lateral edge of the thyroid cartilage

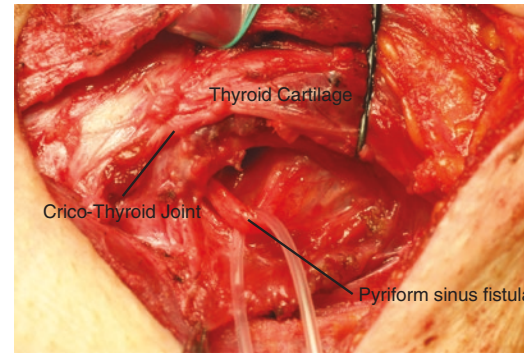

Fig. 9 A pyriform sinus fistula was found by the Nonomura procedure. The inferior pharyngeal constrictor muscle was divided along the lateral edge of the thyroid cartilage, which was retracted with a fine hook. Note that the pyriform recess was not incised. The head is to the right

tory changes are usually less severe at this point. In this procedure, we remove the fistula (and part of the thyroid lobe in the cases where it is attached to the lobe) and refrain from attempting to remove all the fibrous tissue around the thyroid to avoid injury to the recurrent laryngeal nerve. In some cases with severe adhesion between the upper pole of the thyroid and cricopharyngeal muscle, another surgical approach (we call it the Nonomura method) can be adopted [13]. After incising the inferior pharyngeal constrictor muscle vertically over the posterior edge of the thyroid cartilage ala, the thyroid cartilage is retracted anteriorly to reveal the pyriform recess and the pyriform sinus fistula. Special care should be taken not to incise the pyriform recess. If it is opened, a pharyngeal fistula might occur as a difficult complication. This approach enables us to identify the fistula, in cases where we could not find the fistula outside the larynx because of severe adhesion and fibrosis from previous inflammations (Figs. 8 and 9).

– Chemocauterization

Kim et al. invented a chemocauterization technique as an alternative to surgical fistulectomy [6]. In this procedure, the pharyngeal orifice of a pyriform sinus fistula is chemically cauterized under direct laryngoscope, and the orifice closes secondarily, eliminating the infectious spread from the pharynx to the thyroid. The actual procedure of the chemocauterization is as follows: under general anesthesia, the patient's neck is flexed dorsally, and the head is placed in a suspended position. Under suspension laryngoscopy, the opening of the pyriform sinus fistula is identified at the bottom of the pyriform recess. A small cotton ball measuring about 4 mm in diameter soaked in 30% trichloroacetic acid is placed in the opening for 1 min. The color of the pharyngeal mucosa changes white following this procedure. The procedure is repeated three times (Fig. 10). The pharynx is washed with saline after the procedure. The patients are given intravenous infusion of antibiotics for 3 days, and oral feeding is started on the fourth postoperative day. The cauterized

wound is allowed to heal secondarily (Fig. 11). The procedure is usually scheduled during the remission of the inflammation. However, this can be done during a mild inflammatory period, although incision and drainage for the abscess might be necessary. We performed a barium swallow study and CT scan taken under the trumpet maneuver 3 and 12 months after the treatment in order to confirm the closure of the fistula. In our experience, 85% of the patients have achieved closure of the fistula, and none of them have experienced recurrent inflammation.

The merits of this nonsurgical obliteration technique of the pyriform sinus fistula include the absence of a surgical scar, lack of serious complications such as vocal cord paralysis, and less pain [6, 14]. Moreover, this procedure can be performed during an inflammatory period, although simultaneous incision and drainage is necessary, while fistulectomy can be performed only during remission. However, this method has limitations, as the endoscopic procedure is difficult or impossible to perform in patients whose mouths do not open widely or whose necks do not flex dorsally enough to insert a laryngoscope. This procedure also carries a risk of tooth injuries. Some patients might require multiple treatments to achieve obliteration of the fistula. Retention cysts may possibly develop during long-term follow-up, since the thyroidal part of the fistula remains.

2. Infection of a thyroid nodule or cancer
   (a) Pathogenesis

   Infection of a thyroid nodule or cancer is one of the possible causes of AST (Fig. 12). The infection may be caused by repeated aspiration of the nodule [4]. Other mechanisms include a direct spread of infective organisms and hematogenous or lymphatic spread from distant infected

Fig. 10 The chemocauterization procedure. Under a suspension laryngoscope, the orifice of the pyriform sinus fistula was identified and cauterized chemically. The monitor shows a change in the color of the pharyngeal mucosa

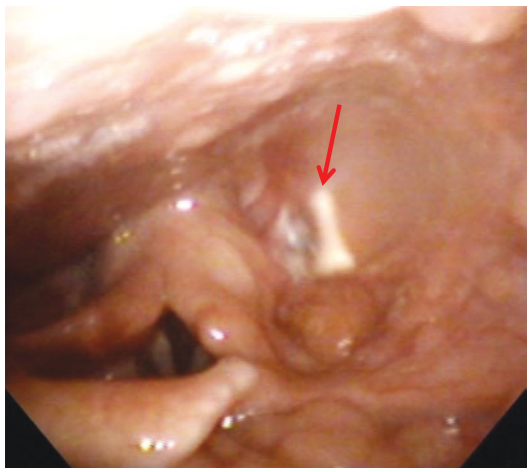

Fig. 11 Laryngoscope showing the white color change in the pharyngeal mucosa around the orifice of the pyriform sinus fistula

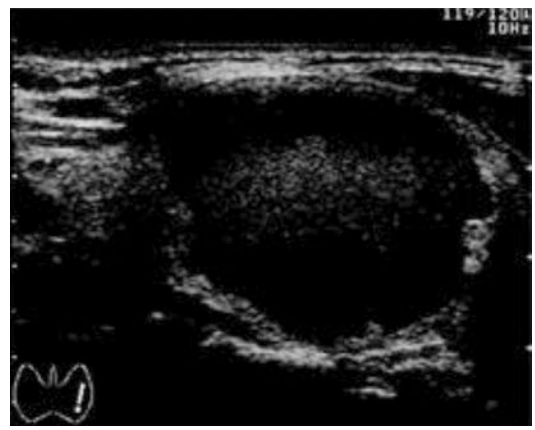

Fig. 12 Ultrasonogram of the infected thyroid nodule. The nodule has a thick fibrous capsule surrounding unhomogeneous hypoechoic content

foci [10]. Infection of a thyroid nodule or cancer often occurs in elderly patients who are susceptible to infection due to diabetes mellitus, use of glucocorticoid or immunosuppressive agents or HIV infection, and so on.

(b) Management

The treatment for AST through an infected thyroid nodule is usually antibiotics and surgical drainage. In case of mild inflammation, symptoms may subside only by the administration of antibiotics and aspiration of pus. However, in many cases, these therapies fail to ameliorate the inflammation, probably because of degenerated tissues and fibrosis in the nodules. Such patients may require thyroidectomy for recovery [4].

3. Infection through the esophagus due to a foreign body or esophageal cancer

(a) Pathogenesis

Penetration of a foreign body such as a fish bone through the cervical esophagus, infection of the cervical esophagus due to a foreign body, or perforation of esophageal cancer may also cause AST (Fig. 13). Such patients complain of pain and tenderness in the neck and fever. US examination and/or CT scan may detect swelling of the esophagus and an obscure border between the thyroid gland and the esophagus due to the inflammation and foreign body.

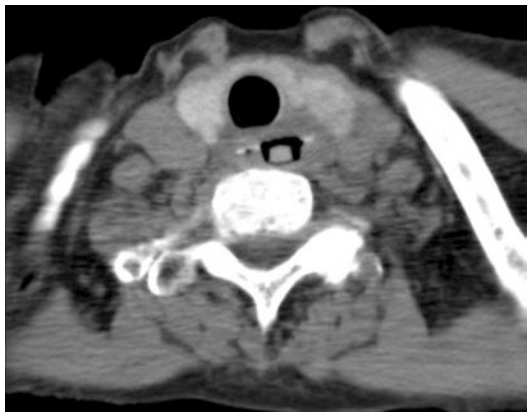

Fig. 13 A foreign body in the cervical esophagus causing inflammation around the esophagus involving the left thyroid lobe

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Postpartum Thyroiditis

Juan C. Galofré and L. D. Premawardhana

Introduction

Postpartum thyroiditis (PPT) is defined as the occurrence of a sporadic destructive thyroiditis in the first year postpartum, in women with no overt thyroid disease before pregnancy [1]. Clearly, this definition does not take into account those women who have had thyroid dysfunction predating their pregnancy, as we now know that they too can develop PPT (see below). Several types of thyroid dysfunction may occur as a consequence of PPT. The classic presentation is of a triphasic illness with a transient thyrotoxic phase followed by a hypothyroid phase and then recovery. However, incomplete forms are not unusual, and many women present solely with either a hyperthyroid or a hypothyroid phase.

The fact that a vast majority of women who develop PPT have antithyroid peroxidase antibody (TPOAb) in their blood prior to pregnancy is a marker of the autoimmune nature of this condition.

This chapter reviews the epidemiology, clinical manifestations, diagnosis and management of this often overlooked condition.

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Epidemiology

Prevalence

The reported prevalence of PPT from systematic reviews of prospective studies of women during the first year after delivery is around 4–8% [2–4]. However there is a wide range from 1 to 20% in different patient groups (Table 1). The variability in the reported prevalence depends on several factors such as patient selection, geographic and ethnic differences, variable diagnostic criteria, frequency and timing of blood samples and concomitant illnesses.

The Role of Autoimmunity

As expected, reflecting its autoimmune origin, the incidence of PPT increases dramatically in seropositive women (those with elevated antibody titres). Although the presence of TPOAb is usual, anti-thyroglobulin antibodies (TgAb) may occur alone in less than 5% of cases. However, PPT has been reported in a small

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Table 1 The incidence of postpartum thyroiditis (PPT) in different groups of women

Condition	Incidence (%)
General population	5–8
Seropositive women (positive TPOAb)	50
Women with previous PPT	40–70
Type 1 diabetes mellitus	20–25
Previous Graves' disease	50–70

(TPOAb)-antithyroid peroxidase autoantibodies

percentage of women without evidence of autoimmune thyroid disease. Experimental evidence shows that seropositive females have a six times higher risk of developing PPT; in other words, around 40–60% of women with positive TPOAb and normal thyroid function during pregnancy will develop PPT compared with 5–8% of women without TPOAb [5]. In this context the prevalence of PPT in patients with Graves' disease (GD), which is an autoimmune condition, is comparable to that in the population with positive TPOAb [6], and the differential diagnosis between these two conditions could be challenging (see below). It has been described that PPT can develop in patients who had Hashimoto's thyroiditis prior to pregnancy and who were being treated with levothyroxine [7]. Interestingly, in line with this finding, it has also been described that PPT is more common in those women with a prior history of hypothyroidism, independently of the autoimmune status [2].

The history of previous PPT is a clear risk factor for relapse. A past history of PPT indicates a 70% chance of developing PPT in a subsequent pregnancy. An increased incidence has also been reported in type 1 diabetes (DM1) patients: around 20–25% of DM1 women will develop an episode of PPT after delivery [3]. Similarly it has also been described in patients with other autoimmune diseases such as rheumatoid arthritis, lupus erythematous and scleroderma. On the contrary, seronegative women (those with negative autoantibodies) who do not develop PPT after the first delivery rarely acquire the condition after successive pregnancies.

The Role of the Environment

In addition to genetic influences, the incidence of PPT might be influenced by environmental factors such as iodine intake and smoking [1]. PPT is more prevalent among smokers. Iodine consumption may have a role in the appearance of PPT, but to date there have been no reports as to whether iodine supplements during pregnancy increase its risk.

Screening

Universal Screening: Remains Controversial

The need for universal screening for PPT is controversial. Although the benefits of preventing PPT are clear, no firm single strategy is currently recommended. The significant morbidity that PPT produces, the fact that nearly three-fourths of women who have PPT will have an episode in a future pregnancy, the high prevalence of long-term thyroid dysfunction following PPT and the availability of effective treatment should make screening for PPT useful. But there have been no randomized controlled trials examining the utility of screening [8].

The Pros and Cons

In order to analyse the need for screening for PPT, several aspects of screening merit consideration. First, whether screening for PPT is needed; second, if required, the aim of screening should be defined; third, it would be crucial to define the best tool and time for screening; and lastly the logistics of screening should be clarified.

The aim of screening for PPT is twofold. On the one hand, an adequate screening test should detect women at risk of developing PPT, giving healthcare workers a window of opportunity to potentially *prevent* the onset of disease. On the other hand, a good screening programme also allows healthcare personnel to provide *early treatment* as soon as the disease becomes clinically manifest, which undoubtedly is also good

clinical practice. To date, there are no clear measures to predict or prevent the appearance of PPT in predisposed women, but early treatment is fairly straightforward and prevents potential complications.

Case Finding vs. Screening

Although the ATA guideline for the management of thyroid disease during pregnancy and postpartum [9] is presently (2016) under revision, the 2012 Endocrine Society Guidelines on the same topic stated that there is insufficient data to recommend screening of all women for PPT [1]. In a broader sense the recommendations favour a policy of selective case finding in women who are at risk of developing PPT. Those individuals belong to the following six groups: (1) DM1 patients, (2) those with a previous episode of PPT, (3) all TPOAb-positive women, (4) women with a past history of miscarriage, (5) women with postpartum depression and (6) women with a family history of autoimmune diseases. In all these circumstances, it is advisable to follow a screening programme of thyroid function 3 months after delivery.

However these suggestions are sometimes contradictory, cause confusion and are almost impossible to follow, because they show a lack of consistency. For instance, in order to follow a case finding strategy in all TPOAb-positive women, it would be necessary to have prior knowledge of their antibody status. Thus, in theory the recommendation entails the previous screening for the presence of TPOAb in all potential mothers, and this is what the Guidelines recommend against.

It has been demonstrated that a case-finding approach focused only on high-risk patients will miss around a third of PPT cases. Taking all this information together, it is not surprising that many experts currently recommend a screening programme for PPT [9, 10, 11]. Similarly the current authors also think that there are important reasons to do so [12]. The scenario is similar to screening for thyroid disease during pregnancy. Although many scientific societies argue against universal screening, the Spanish Endocrine Society and others have a clear position supporting it [11]. For instance, the American College of Obstetricians and Gynecologists recommends TSH testing (and thyroid hormone testing if indicated), if clinical features suggest postpartum thyroid disease.

Some authors may argue that a screening programme is not cost-effective. Nevertheless, there is positive evidence that screening for PPT is cost beneficial [13].

The Suitable Screening Test

The presence of TPOAb is a risk factor for PPT, and the presence of TPOAb before or during pregnancy identifies women at risk. Furthermore, there is evidence that the TPOAb titre at 16 weeks of gestation is related to the severity of PPT [2]. Specifically about 90% of patients with PPT have positive TPOAb. However, only around 40–50% of patients with positive TPOAb will develop PPT. By contrast, less than 1% of TPOAb-negative patients have PPT [5]. Thus, TPOAb measured in the first trimester is the most sensitive screening tool to detect individuals at risk that we currently have.

It is well established that the serum TPOAb levels decrease during pregnancy and recover in the postpartum period peaking around the third to fourth month postpartum as the release from pregnancy induced immune modulation leads to a rebound of the autoimmune process. Certainly, seronegative euthyroid women would not need further follow-up. For this reason, despite the fact that current guidelines do not recommended a universal screening strategy, some experts advocate the routine measurement of TPOAb in all childbearing age women.

The Postpartum Follow-Up

Seropositive women (or those who meet any known risk factor criteria) need careful follow-up. As mentioned, the presence of TPOAb has a positive predictive value (about 50%) for development of thyroiditis in the postpartum period. Normally the onset of PPT occurs 2–3 months in the postpartum period (Fig. 1). In addition to clinical assessment, it is recommended that biochemical screening of thyroid function is undertaken in all TPOAb-positive women 2–3 months after delivery. According to the

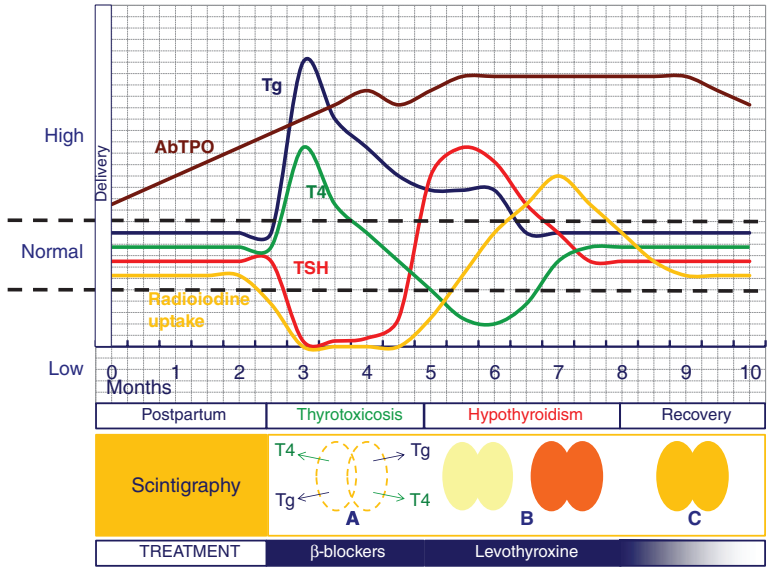

Fig. 1 The clinical course of postpartum thyroiditis. Typically, postpartum thyroiditis (PPT) starts about 3 months after delivery with a thyrotoxic period (upper panel). This is the consequence of a massive and continuous release of intra-thyroidally stored thyroxine (T4) and thyroglobulin (Tg) as a result of destructive thyroiditis. This period usually lasts several weeks and leads to a sudden increase of serum T4 and Tg level. As a consequence of the negative feedback mechanism that operates, the elevated T4 inhibits pituitary thyrotropin (TSH) secretion. After several weeks circulating T4 levels are cleared from the blood consistent with the long half-life of T4. In this period, decreasing T4 levels begin to stimulate the release of TSH from the pituitary. However this TSH increase is not immediately translated into an increase in circulating T4 levels because the thyroid gland is still recovering and is unable of synthetize T4. This situation leads to the second phase (hypothyroidism) that usually lasts several months. Eventually the gland is fully restored, and the situation turns normal in a few months thereafter. In a parallel way, the circulating anti-peroxidase antibodies (TPOAb) levels that normally have been declining during pregnancy start to rise following delivery and plateaus after several months. All three phases could be identified by nuclear medicine techniques (middle panel). During the T4 and Tg release period (A), the gland is unable to trap radionuclides, and the scintigraphy image is blank. This corresponds to the thyrotoxic phase. Subsequently, the gland gradually recovers during the second period (B) that corresponds to the hypothyroid phase. At the end of this period the scintigraphy may show an intense uptake that could lead to confusion in the interpretation of the result (yellow line) because the blood hormones levels are almost normal. Finally (C) the gland completely recovers, and all circulating hormones (T4 and TSH) and Tg levels are back to the normal ranges. The scintigraphy images display a normal uptake. Therapy (lower panel) should be tailored to suit each phase of the illness. The thyrotoxic phase is treated with β-blockers (thionamides are not useful), while the hypothyroid phase responds to levothyroxine replacement therapy

2011 ATA guidelines, all TPOAb-positive women (or those who have DM1) should have a serum TSH performed at 3 and 6 months postpartum. The determination of TSH in the postpartum period will identify women that have developed the disease. If the TSH level is abnormal, it should be repeated along with a free T4 level and T3 (if TSH is low), within 1–2 weeks. In addition, increased thyroid hypoechogenicity in the ultrasound (if done) is also predictive of PPT [14].

In the long-term follow-up, it is worth considering that women with a history of PPT have a markedly increased risk of developing permanent hypothyroidism in the 5 to 10 years after PPT, and therefore an annual TSH assessment is recommended in these women.

Clinical Manifestations

Destructive forms of thyroiditis may demonstrate a triphasic clinical course that reflects the peripheral action of thyroid hormones on different body tissues at different times in the course of the illness. Approximately one third of women with PPT have the classic thyrotoxicosis-hypothyroidism-recovery phases, whereas the rest have incomplete forms (Fig. 1). In the classic form, the thyrotoxic period typically begins 1–4 months after delivery and lasts around 2–10 weeks. The hypothyroid phase that follows lasts from about 2 weeks to 6 months, followed by recovery. The most frequent incomplete form that occurs in approximately 40–50% of cases is an isolated transient hypothyroid episode. The remaining 20–40% cases have transient hyperthyroidism only (Table 2) [14].

Signs and Symptoms

The clinical manifestations of the hyperthyroid phase (both in the classic and isolated forms) are usually mild (palpitations, irritability, fatigue, weight loss, nervousness, lack of energy, heat intolerance, anxiety and tremor). Due to its minor symptoms, the disease may go frequently unnoticed, and it is necessary to have a high level of awareness to detect this phase. On the other hand, hypothyroidism normally produces more significant symptoms. Women usually complain of cold intolerance, lack of energy, sluggishness, poor memory, dry skin, tiredness, constipation and probably depression. On physical examination it is frequent to find a mildly enlarged, diffuse, nontender goitre. Lactating women may notice that postpartum hypothyroidism may decrease milk volume.

The Sequelae

In around 20–50% of women, hypothyroidism is permanent. According to a single prospective study, this association increases with time, and the risk continues beyond 10 years after the PPT episode [4]. Unfortunately there are no clear predictors that indicate which patients are at risk of progressing to permanent hypothyroidism. Some data suggest that this evolution is more common when higher initial TSH levels or TPOAb titres are present, as well as in older mothers or when the baby is female.

Diagnosis and Differential Diagnosis

Thyrotoxic Phase of Postpartum Thyroiditis

The thyrotoxic phase of PPT occurs early and needs to be differentiated from postpartum GD. This is important as the management of postpartum GD differs from PPT. From an epidemiological point of view, PPT is 20 times more common than postpartum GD. It is estimated that in Denmark, Japan and the USA, 40–45% of women who develop GD do so in the postpartum period [15]. There are several features, which

Table 2 Clinical presentation of postpartum thyroiditis

Presentation	Mean onset (month postpartum)	Duration (months)	Prevalence (%)
Transient			
Classic (hyperthyroidism and hypothyroidism)	1–4	2–8	25–35
Isolated hypothyroidism	3–6	1–6	40–50
Isolated thyrotoxicosis	1–3	2–4	20–40
Asymptomatic (hypoechogenicity by US)	1–6		40–80
Permanent			
Hypothyroidism	>6	Permanent	25–50
Abnormal US	>6	Permanent	50–75

US ultrasound

may be useful in differentiating between thyrotoxic PPT and postpartum GD (Table 3).

The Onset of Symptoms and Clinical Features

Most studies of PPT report the onset of thyrotoxic symptoms at a median time of 12–13 weeks postpartum [16, 17]. This contrasts with the late onset of postpartum GD, which usually occurs between 6 and 12 months. Although the timing of the onset of symptoms may help in differentiating between PPT and postpartum GD, there may be an overlap, as 22% of postpartum GD occurred earlier in one study [16].

PPT symptoms are mild (with occasional reports of severe disease), short lasting and self-limiting. There is no single symptom or symptom complex that can herald the onset of thyroid dysfunction, as most postpartum women are known to develop significant but non-specific symptoms whether they have PPT or not. Symptoms may be more severe in TPOAb-positive women who develop PPT, compared to TPOAb-positive women who do not develop PPT and TPOAb-negative postpartum women [18]. The existence of extrathyroidal manifestations of GD such as orbitopathy, and a smooth symmetrical goitre with a bruit heard over it, would favour the diagnosis of postpartum GD.

Table 3 A comparison of thyrotoxic PPT and postpartum Graves' disease

Condition/feature	Postpartum GD	Thyrotoxic PPT
Prevalence in thyrotoxic subjects (%)	0.2	4
Presentation		
Months after delivery	4–12	2–4
Onset of symptoms	Late	Early
Severity of symptoms	Often severe	Usually mild
Clinical signs		
Goitre (%)	90	0–40
	Smooth, symmetrical goitre	Small smooth goitre
Neck thrill and bruit	May be present	Absent
Ophthalmopathy (%)	10–50	Absent
Pretibial myxoedema and/or acropachy (%)	5–10	Absent
Laboratory		
T4/T3 ratio	T3 predominant	T4 predominant
TPOAb (%)	75	80
TRAb	Positive	Negative
Ultrasound TBF	High	Low
Scintigraphy uptake[a]	High	Low

In clinical practice, the most useful distinguishing features are clinical signs, the presence of TRAb and increased TBF in Graves' disease

PPT postpartum thyroiditis, *GD* Graves' disease, *TPOAb* antiperoxidase autoantibodies, *TRAb* thyrotropin receptor antibody, *TBF* thyroid blood flow

[a]Scintigraphy with radioactive iodine is contraindicated during breastfeeding

Free T3 and T4 Concentrations

Thyrotoxic PPT results in T4 predominance in the blood, compared to postpartum GD where T3 predominates [19]. This T4 predominance reflects the release of stored intra-thyroidal hormones following the "destructive thyroiditis" of PPT (T4 is stored in excess of T3 within the gland). An elevated Free T4/T3 ratio in the blood has been used in the past to differentiate, but clinically this ratio is not accurate or very useful.

Thyrotropin Receptor Antibody

The sensitivity and specificity of thyrotropin receptor antibody (TRAb) in diagnosing GD is very high and is estimated to be 97–99% using second- and third-generation assays [20]. Most studies using TRAb report successful differentiation of postpartum GD (TRAb positive) from thyrotoxic PPT (TRAb negative) [16]. The previously seen overlap between the two conditions with second-generation assays has been eliminated with highly sensitive third-generation assays. TRAb testing is very useful during the overlap period of 3–6 months postpartum where other tests may be confusing [16].

Ultrasound Scans and Thyroid Blood Flow

Measuring thyroid blood flow (TBF) using ultrasound scanning techniques may also be used to

differentiate between thyrotoxic PPT and GD [21]. TBF is high in GD reflecting a vascular gland, compared to PPT, where TBF is low. However, this investigation is not readily available in all centres rapidly enough for diagnostic purposes. Also, TBF may be unhelpful in differentiating PPT from postpartum GD, during the overlap period between 3 and 6 months postpartum.

Take Home Message

In practice, the most useful factors in differentiating between thyrotoxic PPT and postpartum GD are the timing of the onset and severity of clinical features, TRAb positive status and increased TBF of GD.

Although rarely necessary, scintigraphy with radionuclides can help in the differential diagnosis as uptake is near 0% in PPT, whereas it is very high in GD. However, it is necessary to stress that radioiodine uptake scans are contraindicated during lactation [16].

Hypothyroid Phase of Postpartum Thyroiditis

As mentioned before the hypothyroid phase of PPT may occur alone or as part of a triphasic PPT. It occurs late in the first postpartum year—a median time to onset of about 20 weeks or later—and this phase is also self-limiting in the majority of cases. Women who develop PPT are generally thyroid antibody positive (TPOAb usually, but TgAb in some), and differentiation from Hashimoto's thyroiditis is therefore not easy. The therapeutic test of thyroxine withdrawal at the end of the first postpartum year may however be useful. Those with Hashimoto's thyroiditis will continue to require thyroxine upon withdrawal (as evidenced by a return of symptoms and elevated TSH levels), but the majority of those with hypothyroid PPT will not: they will remain symptom free with TSH levels in the reference range. However, it is prudent to remember that a significant minority may require long-term thyroxine therapy when withdrawal is attempted at the end of the first postpartum year, said to be between 4 and 54% in various studies [14].

Postpartum in Hypothyroid Women on Thyroxine Replacement Therapy

Two previous studies have examined the incidence and pattern of postpartum thyroid dysfunction in women previously diagnosed to have hypothyroidism and taking thyroxine replacement therapy [22]. Both reported fluctuations in thyroid function suggestive of PPT in around 68% of subjects studied (n = 97 and 31). It is to be noted that this is a significantly higher incidence of PPT, compared to those without previous thyroid disease at the onset of pregnancy. In the more recent study, a third of women each developed hyperthyroidism or hypothyroidism alone or had a triphasic pattern [22].

Association with Depression

The prevalence of depression is about 10% in the postpartum period. Data are unclear whether there is a link between depression and PPT or TPOAb status. The rationale behind a possible association between depression and PPT is that hypothyroidism is associated with depression outside the postpartum period. The association of TPOAb and depression is less evident. However, the link between the two is not entirely consistent. This association described in several reports suggests that an association may exist in a subset of women that, to date, has not been clarified. In any case, as hypothyroidism is a reversible cause of depression, women with postpartum depression should be screened for thyroid dysfunction.

Management of Postpartum Thyroiditis

The management of PPT is largely empirical and directed towards symptom control. There is no firm evidence base from clinical trials. There is very little evidence for intervention that alters the course or duration of the disease following the onset of symptoms either (see selenium below).

The only intervention that may be required during the thyrotoxic phase of PPT is a beta-

blocker. The minority of women with troublesome adrenergic symptoms of thyrotoxicosis (such as palpitations) may benefit from a small dose of propranolol or bisoprolol (or another beta-blocker) for a few weeks. This can be safely withdrawn when the thyrotoxic phase subsides spontaneously. Thionamides are not useful and should not be given, as the thyrotoxic phase is the result of a destructive thyroiditis, as mentioned before, and not caused by increased synthesis of thyroid hormones.

However, those with the hypothyroid phase often require thyroxine replacement because of significant symptoms. Treatment should also be considered for women who are contemplating a further pregnancy soon after the index pregnancy or those who are breastfeeding. Treatment can be safely started with a full dose of thyroxine, e.g. 100–125 μg/day, as these are young and otherwise healthy women. It is customary to stop thyroxine at the end of the first postpartum year, as the majority of those women with hypothyroid PPT will not require thyroxine thereafter. Clearly this should not be done if the woman is breastfeeding, is planning another pregnancy or is actually pregnant.

Prevention of Postpartum Thyroiditis

Factors Affecting the Incidence of Postpartum Thyroiditis

Selenium

There has been a study from Italy of the effects of selenium (Se) supplementation in pregnancy, evaluating the incidence and type of thyroid dysfunction in the postpartum period. One hundred and fifty-one TPOAb-positive women and a cohort of TPOAb-negative women were recruited at 10 weeks of pregnancy. They had Se levels at the lower end of the reference range for the population. Se was given to 77 of the 151 TPOAb-positive pregnant women. TPOAb fell by a significantly greater amount in Se-treated women compared to those without treatment (62.4% vs. 43.9%; $p < 0.01$) and TPOAb-negative control women. The mean levels and rebound postpartum peak TPOAb titres were also significantly lower in the Se-treated group. PPT developed in 28.6% of Se-treated women, and 11.7% had permanent hypothyroidism at the end of the study, compared to 48.6% and 20.3%, respectively, in the untreated group. This is the only study to have demonstrated the benefits of Se supplementation in pregnant women with low normal selenium levels, in relation to thyroid autoimmunity during and after pregnancy and the incidence of PPT and permanent hypothyroidism [23]. Further studies are needed to corroborate this evidence.

Radioiodine for Graves' Disease and Lower Incidence of Postpartum Thyroiditis

There are reports of a lower incidence of PPT in subjects who had been treated with radioiodine (RAI) for GD compared to those who had a subtotal thyroidectomy or antithyroid drugs (ATD) before pregnancy [24]. A retrospective review of 118 women who had RAI before pregnancy found a reduced incidence of PPT (2.1%), compared to the group who had a subtotal thyroidectomy (23.6%) or ATD (55.1%). The authors commented on the protective effect of RAI against the occurrence of PPT compared to the other two modalities, despite the reduction in thyroid volume in those who had had a subtotal thyroidectomy. However, more evidence is required before firm conclusions can be drawn.

Type 1 Diabetes Mellitus and Gestational Diabetes and Increased Risk of Postpartum Thyroiditis

Following Gerstein's and Alvarez-Marfany's initial studies, there have been several reports of an increased incidence of PPT in subjects with DM1—a reported incidence of approximately 25%. However, a recent study from Iran [25] suggests that this increased risk of developing PPT also extends to lesser forms of glucose intolerance in pregnancy, such as gestational diabetes mellitus. They found an incidence of 19.2% in 341 women with gestational diabetes compared to an incidence of 10.2% in 313 women without GDM. These data need to be confirmed in further studies.

Thyroxine and Iodine Administration and Postpartum Thyroiditis

Previous studies of thyroxine and iodine administration in TPOAb-positive women did not confer any benefits in terms of the incidence of hypothyroidism postpartum, although there was a reduction in hypothyroid symptoms of PPT in the thyroxine-treated group. Also, the degree of thyroid dysfunction was more severe in the iodine-treated group [14].

Another study evaluated the usefulness of iodine during pregnancy only, compared to iodine administration during both pregnancy and the postpartum period. There was no reduction in the incidence or severity of PPT in women given iodine in either group.

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Amiodarone-Induced Thyroid Dysfunction

Simone De Leo and Lewis E. Braverman

Introduction

Amiodarone is a benzofuranic drug, whose chemical structure resembles that of thyroid hormones, and contains 37.5% iodine by weight [1]. Therefore, a 200 mg tablet contains 75 mg of iodine. After deiodination, 6 mg of free iodine (3 mg per 100 mg of amiodarone) is released into the circulation [2]: an amount that is 40 times higher than the recommended daily iodine intake for adults. The iodine load markedly increases the urinary iodide excretion [3].

Amiodarone is metabolized in the liver to desethylamiodarone (DEA), through N-dealkylation by the cytochrome P450 enzyme group. DEA is the main active metabolite; other metabolites can be formed after deiodination and glucuroconjugation [4]. Amiodarone is markedly lipophilic, with a large distribution volume, including adipose tissue, liver, lung, and thyroid. Due to its high fat solubility, the drug has a slow turnover, which explains the long elimination half-life of around 40 days for amiodarone and 57 days for DEA [4]. Some studies reported an even longer half-life for amiodarone, up to 100 days, after withdrawal of long-term treatment [5]. This explains why the adverse effects of amiodarone can occur long after drug withdrawal. Amiodarone is mainly eliminated through biliary excretion; other pathways are minor and include saliva, tears, semen, and sweat [4].

Amiodarone is a class III antiarrhythmic agent, since it blocks myocardial potassium channels, and it shares some of the properties of class I, II, and IV antiarrhythmic agents [6]. It is approved by the US Food and Drug Administration for the treatment of patients with life-threatening recurrent ventricular fibrillation or hemodynamically unstable ventricular tachycardia, who are refractory or intolerant to other antiarrhythmic drugs used for these conditions [7]. Amiodarone is also commonly used for the treatment of atrial fibrillation and other supraventricular tachyarrhythmias and for cardiac arrest (mainly due to ventricular fibrillation or pulseless ventricular tachycardia) resistant to other resuscitative measures [8]. Amiodarone was previously reported to promote successful defibrillation of shock-resistant cardiac arrest and to increase survival rate from home to hospital admission [9, 10]; however, a recent prospective randomized controlled

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trial reported that in patients who received amiodarone, survival rate to hospital discharge is similar to that of patients who received lidocaine or placebo [11].

Amiodarone is associated with many side effects, since it can cause thyroid, pulmonary, gastrointestinal, neurologic, ocular, and dermatologic toxicities. Adverse effects are common, with a prevalence of up to 15% in the first year of amiodarone treatment and 50% during long-term use [8]. This high prevalence can partly explain why discontinuing amiodarone treatment is common. A recent study reported that 52% of younger patients (≤65 years) treated for non-life-threatening atrial fibrillation discontinued amiodarone during the first year of treatment [12].

Thyroid dysfunction is particularly dangerous in these patients, because of the underlying cardiovascular dysfunction. It has been clearly reported that both hypothyroidism and hyperthyroidism have a detrimental effect on cardiovascular function, causing manifestations ranging from bradycardia/tachycardia to onset or recurrence of arrhythmias and even heart failure [13, 14]. One retrospective study reported that one-third of patients who developed thyrotoxicosis during amiodarone treatment developed major adverse cardiovascular events, in particular new onset or recurrence of ventricular arrhythmias that required hospitalization, and this risk was significantly higher compared with patients who remained euthyroid (hazard ratio 2.68, CI 1.53–4.68, $p < 0.01$) [15]. Another retrospective study showed that thyrotoxicosis induced by amiodarone had a significant increased risk of mortality compared with thyrotoxicosis due to other causes, such as Graves' disease and toxic nodular goiter. Patients with amiodarone-induced thyrotoxicosis (AIT) were at higher risk if severe left ventricular dysfunction was present [16].

Effect on Thyroid Function Tests

Amiodarone exerts multiple effects on thyroid function tests, due to the high iodine content of the drug and an effect of the drug. The thyroid adapts to iodine excess by inhibiting iodide organification in the thyroid, a mechanism called the acute Wolff-Chaikoff effect. T_3 and T_4 production decreases and serum TSH increases. Subsequently, the thyroid escapes from the acute Wolff-Chaikoff effect by decreasing the sodium/iodide symporter (NIS) and resumes its normal function of iodide organification: serum TSH returns to baseline values [see the iodine-induced thyroid dysfunction chapter].

Amiodarone also exerts direct drug effects. Amiodarone and DEA are concentrated into thyroidal and non-thyroidal cells [17], where they induce a peripheral "hypothyroid-like" effect, due to inhibition of T_3 binding to its nuclear receptor and cytotoxic effects on thyroid cells [18, 19]. The latter can be explained, at least partly, by the iodine contained in this drug. These toxic effects can induce a release of autoantigens by thyroid cells and can trigger thyroid autoimmune reactions, even though the development of thyroid autoimmunity secondary to amiodarone treatment is controversial [20].

The main direct drug effect of amiodarone is a decrease in the activity of deiodinase enzymes (Fig. 1) [20–22]. Thyroid function changes occur acutely within 2 weeks [23]:

- Serum T_4 concentration increases and serum T_3 concentration decreases, due to:
  - Inhibition of type I 5′-deiodinase (D1) activity, which converts T_4 to T_3 in peripheral tissues, mainly in the liver [24]
  - Inhibition of thyroid hormone entry in peripheral tissues
- Serum rT_3 concentrations increase, due to:
  - Inhibition of type I 5′-deiodinase (D1) activity, which converts rT_3 to T_2 in peripheral tissues, mainly in the liver [24]
- Serum TSH concentration transiently increases, due to inhibition of type II 5′-deiodinase (D2) activity, which converts T_4 to T_3 in the pituitary. Less T_3 is generated and TSH synthesis and release are increased [25].

Chronically, a steady state is reached. T_4 and rT_3 remain at the upper limit or slightly elevated, while T_3 is slightly reduced. Conversely, serum TSH concentration returns to normal levels after 3 months [2].

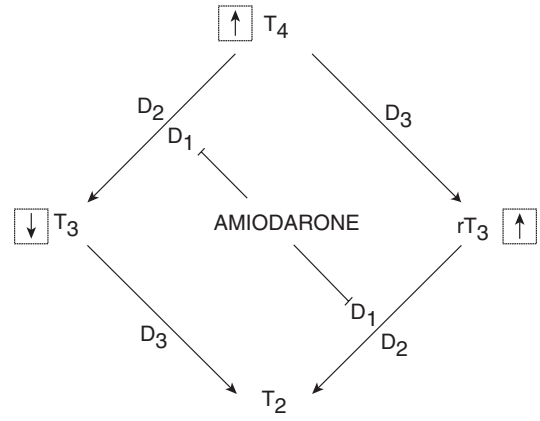

Fig. 1 Effect of amiodarone on the activity of deiodinase enzymes. T_4 thyroxine, T_3 triiodothyronine, rT_3 reverse triiodothyronine, T_2 diiodothyronine, *D1* type I 5′-deiodinase, *D2* type II 5′-deiodinase, *D3* type III 5′-deiodinase

Epidemiology and Risk Factors for Amiodarone-Induced Thyroid Dysfunction

The incidence of amiodarone-induced thyroid dysfunction is estimated to be around 15–20%; however, it varies widely among studies, because of various selection criteria (inclusion of subclinical dysfunction and use of thyroid hormone reference ranges adjusted for amiodarone use) and different geographical areas [4].

Indeed, it is known that different geographical iodine intake is responsible for the different epidemiology of amiodarone-induced thyroid dysfunction: in areas where iodine intake is low, amiodarone-induced thyrotoxicosis (AIT) is more frequent than amiodarone-induced hypothyroidism (AIH); conversely, in areas where iodine intake is sufficient, AIH is more frequent than AIT [26]. It has been estimated that AIT occurred in 8% and in 2–6% of patients treated with amiodarone, in iodine-deficient and iodine-sufficient areas, respectively, while AIH occurred in 6% and 14–30%, respectively [27–31].

AIH can develop in patients with or without underlying thyroid dysfunction [32]. Some risk factors have been identified, including thyroid autoimmunity [32], female gender, older age (>60–65 years old), and higher serum TSH concentration at baseline (>1.4 mU/l) [27, 33]. One study reported a significant increased risk of AIH in patients with β-thalassemia, who are chronically exposed to blood transfusions and, therefore, thyroidal iron accumulation [34] (Table 1).

On the other hand, AIT is more typical in males [35] and younger patients (<60–65 years old) [27, 29, 36] (Table 1). The incidence of AIT has been increasing over the last decades. According to one study conducted in Italy, in iodine-deficient area, type 2 AIT incidence is dramatically increasing, and this form is now more frequent than type 1 AIT [37]. The causes of this increase are not clear. A possible explanation might be the avoidance of amiodarone administration in patients with preexisting thyroid dysfunction, since patients are screened for thyroid disease more frequently than in the past and improved dietary iodine intake in Italy.

Patients with underlying thyroid dysfunction have a well-known increased risk of developing amiodarone-induced thyroid dysfunction (both AIH and AIT), and some studies reported an increased risk (up to 30%) in adults with congenital heart disease (CHD) [38, 39].

Onset Time of Amiodarone-Induced Thyroid Dysfunction and Monitoring

Before starting amiodarone therapy, physicians should assess thyroid function (including serum TSH concentration, serum free T_4 or free T_4 index, and free or total T_3 concentrations) and

Table 1 Characteristics of patients at risk for developing AIH and AIT

	AIH	AIT
Ambient iodine intake	Adequate	Low
Underlying thyroid dysfunction	Present or not	Present or not
Gender	Female	Male
Age	Older	Younger
TSH at baseline	Higher	Lower
Onset time	First months of therapy	Unpredictable

AIH amiodarone-induced hypothyroidism, *AIT* amiodarone-induced thyrotoxicosis

thyroid autoimmunity (including thyroid peroxidase (TPO) and thyroglobulin antibodies). During amiodarone therapy, physicians should check thyroid function every 3 months, but no later than 6 months. If thyroid dysfunction is suspected, adjunctive diagnostic tools may be necessary (see paragraph about diagnosis). Monitoring should be continued for at least 2 years after amiodarone withdrawal [40], because of the slow drug turnover, in particular in patients without underlying thyroid disease, who more frequently develop type 2 AIT. AIT after amiodarone withdrawal was reported to develop in 7% and 20% of patients residing in iodine-sufficient and iodine-deficient areas, respectively [41, 42]. In particular, in an iodine-deficient area, more than 20% of patients developed type 2 AIT after amiodarone withdrawal, while this percentage was significantly lower (5%) in patients who developed type 1 AIT (with underlying thyroid disease) [42].

The onset time of thyroid dysfunction after starting amiodarone treatment is unpredictable. AIT can occur at any time, and the appearance is generally sudden and explosive [4]. Type 1 AIT tends to occur earlier than type 2 AIT: according to a recent retrospective study, median onset time of thyrotoxicosis was 3.5 months in type 1 AIT and 30 months in type 2 AIT [42]. Conversely, AIH seems to occur more frequently in the first months of therapy [36, 43]. One prospective study reported that AIH occurred in the first 6 months of therapy in 76% of patients who developed AIH [31]. Although it was previously reported that the risk of amiodarone-induced thyroid dysfunction increased with exposure to higher cumulative doses [44], more recent studies reported that the daily or cumulative dose of amiodarone are not significant risk factors [27–29, 36].

Amiodarone-Induced Hypothyroidism

Pathogenesis

The basis of hypothyroidism during amiodarone treatment is the failure to escape from the acute Wolff-Chaikoff effect [see the iodine-induced thyroid dysfunction chapter]: this explains the significant increased risk of AIH in patients with underlying thyroid disease, in particular thyroid autoimmunity, which was detected in around 50% of patients with AIH [20]. In patients with an apparent normal thyroid gland, pathogenesis is less clear and is postulated to be the presence of a subtle abnormality of hormone synthesis [20]. This hypothesis is corroborated by the restoration of euthyroidism in patients with AIH (and without underlying thyroid abnormalities) treated with potassium perchlorate and subsequent reoccurrence of hypothyroidism when potassium perchlorate was withdrawn [45]. Potassium perchlorate reduces intrathyroidal iodide content by inhibiting thyroid iodide transport (NIS). As a result, thyroid hormone synthesis is restored, since the inhibitory effect due to excessive intrathyroidal iodide is no longer present [46]. Some experimental studies reported an iodine-independent inhibition of iodide transport exerted by amiodarone [47], but the relevance of this mechanism in AIH development is still debated [45].

Diagnosis

The clinical manifestations of AIH are similar to those of hypothyroidism due to other causes. Hypothyroidism develops in the first months of therapy (usually in the first 6 months), and the diagnosis is based on the findings of increased serum TSH concentrations and low or normal serum thyroid hormones. Thyroid antibodies are frequently present (usually preexisting).

It should be noted that slight increases in serum TSH concentrations are common shortly after amiodarone therapy initiation. These alterations are transient and not clinically significant. Therefore, AIH should be diagnosed and treated when hypothyroidism is overt or persists.

Treatment

AIH does not require amiodarone discontinuation, since it is easily treated with levothyroxine.

Levothyroxine replacement should aim at normalizing TSH, which should be maintained at the upper half of normal TSH range. In general, this goal is achieved using larger-than-usual doses of levothyroxine [48], because of the intrapituitary and peripheral inhibitory effect of amiodarone on T_4 conversion to T_3 [25].

TSH should be revaluated 6–12 months after amiodarone withdrawal to determine if levothyroxine therapy is still necessary, since euthyroidism is restored in most patients [32]. One study reported that half of patients became euthyroid within 6 months after discontinuing amiodarone [49]. Patients with thyroid autoimmunity are at increased risk of permanent hypothyroidism after amiodarone withdrawal and require permanent levothyroxine therapy.

Amiodarone-Induced Thyrotoxicosis

Types of Amiodarone-Induced Thyrotoxicosis

There are two types of amiodarone-induced thyrotoxicosis (AIT): type 1 AIT, a form of iodine-induced thyrotoxicosis, and type 2 AIT, a drug-induced destructive thyroiditis. These two forms should be distinguished, since they have different pathogenesis and consequently different treatment modalities. In some cases, it is not possible to differentiate between the two types: these so-called mixed types share the pathogenic mechanisms of both AIT types (Table 2).

Table 2 Characteristics of patients with type 1 AIT and type 2 AIT

	Type 1 AIT	Type 2 AIT
Pathogenesis	Iodine-induced thyrotoxicosis	Drug-induced destructive thyroiditis
Incidence	Rarer	More frequent
Ambient iodine intake	Low	Variable
Onset time of AIT	Unpredictable but generally during first months of therapy	Unpredictable but generally after 2–3 years of therapy
Preexisting thyroid abnormalities	Generally yes	Generally no
Physical examination	Usually goiter or nodules	Sometimes small firm goiter
Thyroid autoimmunity	Generally present	Generally absent
Thyroid radioiodine uptake	Variable (low, normal, high)	Low or absent
^{99m}Tc-sestaMIBI uptake	Diffuse retention	Absent uptake
Thyroid ultrasound scan	Generally enlarged and nodular gland	Generally normal gland
Color-flow Doppler sonography	Increased vascularity (pattern I-III)	Absent vascularity (pattern 0)
Spontaneous remission	Unlikely	Likely
Subsequent hypothyroidism	Unlikely	Likely
Thyrotoxicosis recurrence during amiodarone-free period	Likely	Unlikely
Thyrotoxicosis recurrence after amiodarone restarted	Very likely	Likely

AIT amiodarone-induced thyrotoxicosis

Pathogenesis

The pathogenesis of type 1 AIT is the same as that of the iodine-induced thyrotoxicosis and is based on the *Jod-Basedow* phenomenon. The iodine load acts as a substrate for the thyroid, which has areas of underlying autonomy, and is responsible for the uncontrolled overproduction of thyroid hormones. Generally, these patients have underlying thyroid abnormalities and reside in areas of iodine deficiency [4]. Conversely, in areas with adequate or high iodine intake, the thyroid gland is more able to adapt to iodine excess, and this type of AIT is less common [29].

Type 2 AIT is caused by a destructive thyroiditis, induced by a direct cytotoxic effect of amiodarone and DEA on thyroid follicular cells [18, 19]. Consequently, preformed thyroid hormones are released into the circulation. Thyroid hormone synthesis does not increased, and, generally, these patients do not have an underlying thyroid abnormality. Destruction of the thyroid gland frequently results in permanent hypothyroidism. In a prospective study, 17% of patients with type 2 AIT developed permanent hypothyroidism, a percentage significantly higher than that for subacute thyroiditis (5%, $p < 0.03$), even in the absence of subsequent iodine exposure [50].

Diagnosis

The clinical manifestations of AIT are similar to those of other causes of thyrotoxicosis [51]. Signs and symptoms are due to hypermetabolism and hyperactivity of physiological processes, induced by excess thyroid hormones, and include unexplained weight loss, sweating, tremor, weakness, heat intolerance, hyperdefecation, and cardiologic manifestations such as palpitations, tachycardia, and worsening of the preexisting cardiac disease [52, 53]. Diagnosis of AIT is based on the finding of a suppressed serum TSH concentration and elevated levels of serum thyroid hormones, in a patient receiving amiodarone or who recently (from few months to 1–3 years) has stopped the drug. Serum free T_3 concentration can be elevated or normal but generally less elevated than T_4 because of the inhibition of deiodinases, which convert T_4 to T_3.

Differential Diagnosis Between Type 1 and Type 2 AIT

Differential diagnosis between type 1 and type 2 AIT is challenging because no gold-standard diagnostic test is available. Physical examination and biochemical evaluation, including TSH, free T_3 (or total T_3), and free T_4 (or free T_4 index), should be performed in all patients. Additional diagnostic procedures, which may help in differentiating the two types of AIT, include nuclear medicine imaging tests (such as radioactive iodine with ^{131}I or ^{123}I, technetium-99 pertechnetate ($^{99}TcO_4^-$) scintigraphy, and 99m technetium (^{99m}Tc) sestaMIBI) and ultrasound imaging, including thyroid ultrasound and color-flow Doppler sonography (CFDS) [54].

Surveys aimed at ascertaining the preferred modalities for diagnosing and treating the two types of AIT showed a lack of consensus among endocrinologists [55–58]. CFDS is considered the preferred diagnostic procedure by European and American endocrinologists (decreased blood flow in type 2 AIT and increased in type 1 AIT), even though the large majority of respondents would add an additional test to CFDS, such as the thyroid radioiodine uptake [55–57]. However, the thyroid radioiodine uptake has been demonstrated to have a poor diagnostic value and, probably, is currently far less used, especially in the United States. The high iodine intake would lower the thyroid radioiodine uptake, and it is indeed puzzling that the radioiodine uptake would be normal or elevated in patients treated with amiodarone. Conversely, ^{99m}Tc-sestaMIBI scan has been recently suggested as a useful tool and was reported to be superior to CFDS in differentiating type 1 from type 2 AIT [59]. However, this is an expensive procedure, and these preliminary results need to be confirmed by larger prospective studies [60, 61].

Physical Exam

Some patients can have a goiter or nodules, which suggest type 1 AIT, even though this is not a specific finding. In contrast, type 2 AIT generally presents with a normal thyroid gland or a small firm goiter [4, 54].

Biochemical Evaluation

In order to differentiate between the two types of AIT, free T_4 and free T_3 levels are not specific, even though these are higher in type 2 AIT than type 1 AIT [42, 62]. Similarly, the T_4/T_3 ratio is not useful and was reported to be on average more than 4 in AIT [42].

Thyroid autoimmunity evaluation, including TPO and thyroglobulin antibodies, is helpful, since antibodies are generally positive in type 1 AIT and negative in type 2 AIT. However, TPO and thyroglobulin antibodies can be positive in patients with type 2 AIT [63], up to 8% of patients according to a retrospective study [37]. Evaluation of TSH-receptor antibodies (TRAb) or TSH-stimulating immunoglobulins (TSI) is useful to diagnose patients with underlying Graves' disease.

Interleukin-6 (IL-6) evaluation has been previously recommended, since IL-6 levels were reported to be significantly higher in type 2 than type 1 AIT [64]. IL-6 is synthetized by thyrocytes and represents a useful parameter of thyroid destructive process; indeed, IL-6 concentrations are markedly increased during the thyrotoxic phase of subacute painful thyroiditis. However, subsequent studies failed to replicate this finding [62, 65]. In addition, IL-6 evaluation is an expensive test, so that its usefulness in clinical practice is limited [57, 58, 66]. Other inflammatory markers, such as serum C-reactive protein, are equally ineffective [67].

Thyroid Radioiodine Uptake

Thyroid radioiodine uptake is higher in type 1 than type 2 AIT, both at 3rd and 24th hour [42]. Patients with type 2 AIT have an invariably low or absent uptake. Conversely, patients with type 1 AIT have a variable uptake, ranging from low to high, in areas with low iodine intake, such as Europe, while in areas with higher iodine intake, such as the United States, the uptake is generally low [68]. Therefore, the test might be superfluous in areas where iodine intake is adequate [65].

Thyroid $^{99}TcO_4^-$ Scintigraphy

Thyroid $^{99}TcO_4^-$ uptake should be present in type 1 AIT and absent in type 2 AIT [69]. However, the method has low sensitivity and specificity [54], and some studies reported failure in differentiating the two AIT types, since thyroid scintigraphy had, in almost all cases, low or absent uptake [59, 70].

99m Technetium (^{99m}Tc) SestaMIBI Scan

^{99m}Tc-sestaMIBI scan is a new method that is proving highly effective. ^{99m}Tc-sestaMIBI uptake is increased in hypermetabolic cells, which contain a high number of mitochondria, such as in hyperfunctioning thyroid tissue. Conversely, ^{99m}Tc-sestaMIBI uptake is reduced or absent in thyroid tissue with glandular destruction and fibrosis [71]. In patients with AIT, diffuse MIBI uptake was observed in patients with type 1 AIT and absent uptake in patients with type 2 AIT. A mixed form may occur, and, in this case, faint persistent uptake or an uptake with a rapid washout (within 10 min) was observed [59]. This method was reported to be superior to both thyroid radioiodine uptake and $^{99}TcO_4^-$ scintigraphy [59]. One study proposed the use of quantitative thyroid-to-background ratio displayed on a time-activity curve to improve the interobserver reliability of ^{99m}Tc-sestaMIBI scans for differentiating the two types [72]. Another advantage of this procedure is that the thyroid uptake appears not to be altered by iodine overload and antithyroid drug use [73].

Thyroid Ultrasound

Thyroid ultrasound is a noninvasive and cost-effective tool but with a low diagnostic value in AIT. Type 1 AIT is generally characterized by an enlarged thyroid gland and nodularity because of the underlying thyroid disease, while type 2 AIT presents with a normal thyroid gland. However, some studies reported similar thyroid gland size in the two AIT types [70]. Thyroid echogenicity was also not discriminatory [69, 74].

Color-Flow Doppler Sonography (CFDS)

CFDS is one of the most helpful diagnostic tools to differentiate type 1 from type 2 AIT. Four different CFDS patterns have been described. Pattern 0 refers to absent vascularity and patterns I–III to progressively increased vascularity, ranging from the presence of parenchymal blood flow with patchy uneven distribution to a markedly increased blood flow with diffuse homogenous distribution [66]. Patients with type 1 AIT show increased vascularity (patterns I–III), while patients with type 2 AIT show absent vascularity (pattern 0) [62, 68].

CFDS can also be helpful in determining the presence of nodules. This differentiation proved useful in diagnosing the underlying thyroid disease in patients with type 1 AIT. A diffuse hypervascular parenchymal pattern without detectable nodules is typical of Graves' disease, while perinodular or intranodular vascularization is detected in toxic multinodular goiter or toxic adenoma [69]. CFDS has been reported to be a useful tool in directing therapy [62], even though one study reported a high heterogeneity in treatment responses within the same CFDS patterns, especially in the presence of increased vascularity (patterns I–III) [70].

Management and Treatment

Decision Regarding Continuation/Discontinuation of Amiodarone Therapy

Once AIT is diagnosed and the type is ascertained, the first question to be addressed pertains to the decision whether to continue amiodarone administration. The decision to discontinue amiodarone is often difficult to determine. Amiodarone effects are long lasting (and benefits of drug withdrawal are not immediate), and there might be a worsening of thyrotoxic symptoms because there is no longer an inhibition of T_4 to T_3 conversion. Moreover, amiodarone discontinuation is not always feasible because it might put the patient at increased risk for worsening of the cardiovascular problem. On the other hand, thyrotoxicosis itself has a detrimental effect on cardiovascular function and significantly increases the risk of major adverse cardiovascular events [13–15].

Generally, patients with type 2 AIT can safely continue amiodarone [75, 76]. Restoration of euthyroidism and median time to first normalization of thyroid function are not significantly different in patients who continue compared to those who discontinue amiodarone treatment [76, 77]. However, patients who continue amiodarone have an increased recurrence rate of thyrotoxicosis, thus delaying the stable restoration of euthyroidism [77]. Therefore, if cardiac conditions are stable, it is preferable to discontinue amiodarone treatment.

In patients with type 1 AIT, the decision to continue amiodarone is controversial because of lack of data. Although available data seem to be reassuring if amiodarone is continued [78], it seems prudent to discontinue amiodarone treatment in these patients [13], whenever possible. This practice is commonly shared by the large majority of American and European endocrinologists [55–57]. However, in any given patient, the decision to withdraw amiodarone needs to be discussed with endocrinologists and cardiologists regarding the potential risks and benefits.

Management and Treatment of Type 1 AIT

Patients with type 1 AIT should be treated with thionamide drugs, which are actively transported into the thyroid where they inhibit thyroid hormone biosynthesis [51]. Due to thyroid exposure to excess iodine, higher doses of thionamide drugs are usually required. The starting dose of methimazole should be 40–60 mg once daily (or equivalent doses of carbimazole, in countries where methimazole is not available, or propylthiouracil (600 mg daily), when methimazole is contraindicated) [60, 79]. Once euthyroidism is restored, methimazole is tapered to a lower maintenance dose.

In patients with a poor or absent treatment response after 4–8 weeks of thionamide treatment, potassium perchlorate should be added, since it inhibits thyroid iodide transport and, therefore, reduces intrathyroidal iodide content.

Potassium perchlorate should be administered at doses of 1000 mg daily (250 mg four times daily) or lower [60–79]. The suggested dose should not be exceeded in order to reduce the risk of side effects, such as aplastic anemia [80]. The drug is not available in some countries, including the United States.

The combination treatment with thionamide and potassium perchlorate should be continued for 2–6 weeks. If euthyroidism is restored, methimazole is tapered and potassium perchlorate discontinued. If the patient remains refractory to therapy, definitive treatment should be considered (see paragraph about definitive therapy), or a "stepwise" approach may be tried. In the latter case, if after a month of therapy with methimazole and potassium perchlorate, free T_4 or free T_4 index is not decreased by more than 50%, glucocorticoids are added (prednisone 40 mg daily) and tapered over 1 month after free T_4 or free T_4 index concentrations normalize [81] (Fig. 2).

Management and Treatment of Type 2 AIT

Unlike type 1 AIT, patients with type 2 AIT may have a self-limiting disease, which might not require therapy if mild and the cardiovascular condition is stable. Patients with overt thyrotoxicosis or those who require restoration of euthyroidism because of the cardiac condition should be treated with glucocorticoids [6].

Glucocorticoids are the treatment of choice for type 2 AIT. Iopanoic acid proved effective but cured patients less rapidly compared with glucocorticoids [82]; moreover, the drug is not available in some countries, such as the United States. Thionamide drugs have no role in the treatment of patients with type 2 AIT [83] but can be used in when the diagnosis is unclear or mixed forms are present [84] (see paragraph about management and treatment of mixed forms of AIT).

Generally, the glucocorticoid drug most commonly used is prednisone. The starting dose is usually 40 mg once daily, for 2–4 weeks, followed by a gradual tapering over 2–3 months, depending on the patient's clinical and biochemical response [79]. Improvement is generally rapid, and euthyroidism is restored in 4–8 weeks, whether amiodarone is continued or not [77, 85]. In a prospective study, patients who discontinue amiodarone had a median cure time of 30 days to restore euthyroidism. However, 15% were still thyrotoxic after 3 months. Higher baseline thyroid hormone concentrations (serum free $T_4 > 50$ pg/mL) and larger thyroid volume (thyroid volume normalized for body surface area > 12 mL/m^2) were the main determinants of a delayed response to glucocorticoids [85].

As mentioned above, patients with type 2 AIT, who continue amiodarone, are at increased risk of reoccurrence of thyrotoxicosis [77]; however, other studies reported a low reoccurrence rate (6–8%), and these patients had mild episodes, which quickly responded to therapy [75, 86] (Fig. 3).

Management and Treatment of Mixed Forms of AIT

Mixed forms of AIT should be treated with a combination of thionamide and glucocorticoids [60]. The starting doses should be methimazole 40 mg daily and prednisone 40 mg daily. After 2 weeks, if serum thyroid hormone concentrations normalize, methimazole can be stopped and glucocorticoids tapered over 2–3 months, since the patient is likely to have type 2 AIT. If thyrotoxicosis persists, the most likely diagnosis is type 1 AIT. Therefore, methimazole should be continued, possibly adding potassium perchlorate, and glucocorticoids tapered and discontinued [87]. If the patient is still irresponsive to therapy, definitive treatment should be considered (see below) (Fig. 4).

Definitive Therapy

Some patients may be treated by thyroidectomy. Regardless of AIT type, thyroidectomy is recommended in patients refractory to medical therapy or unable to tolerate a protract thyrotoxic state, because of worsening cardiac disease. AIT patients with left ventricular (LV) dysfunction have a mortality rate as high as 30–50% [16, 53]. These patients need a rapid restoration of euthyroidism, and total thyroidectomy was reported to reduce the mortality risk and improve cardiac

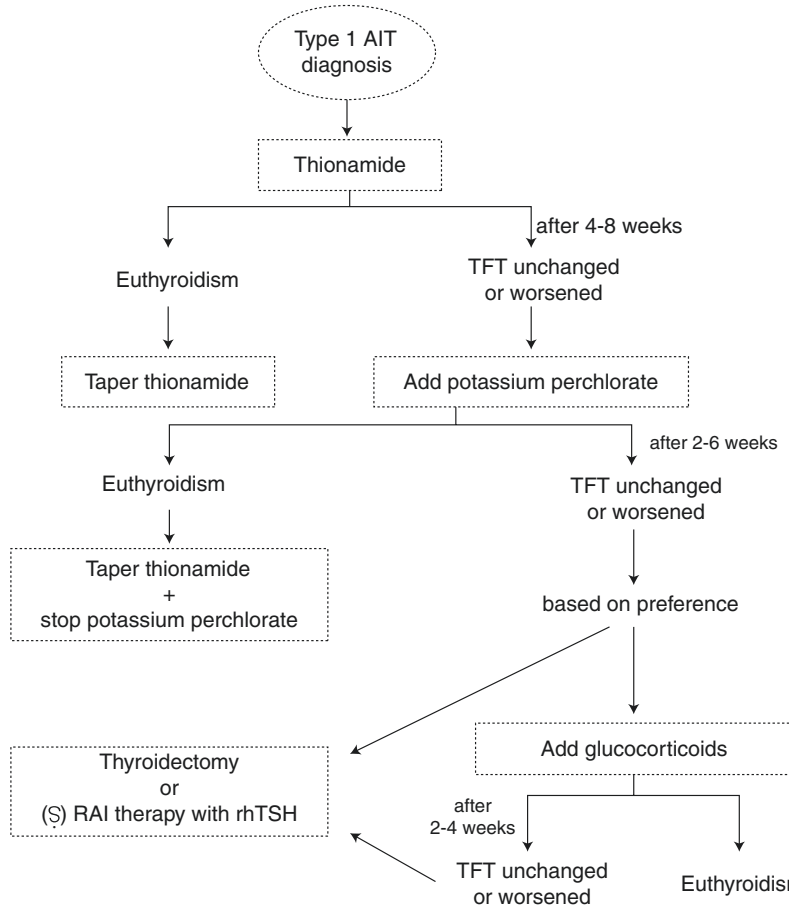

Fig. 2 Suggested algorithm for the management of patients with type 1 AIT. *RAI* radioactive iodine, *TFT* thyroid function test

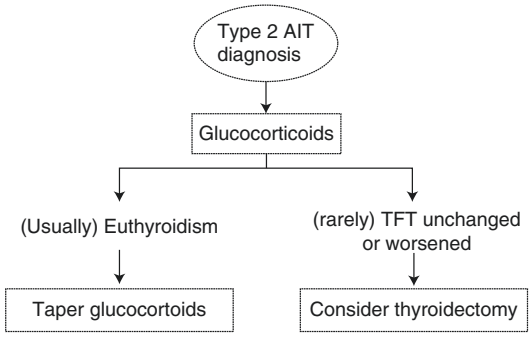

Fig. 3 Suggested algorithm for the management of patients with type 2 AIT. *TFT* thyroid function test

function in patients with severe LV dysfunction (LV ejection fraction <50%) [88, 89].

Patients should be euthyroid prior to surgery in order to reduce surgical risk. A short course of iopanoic acid, for 1–3 weeks, at a dose of 1000 mg/day orally, is suggested to quickly restore euthyroidism. Iopanoic acid is iodine rich, inhibits type I 5′-deiodinase (D1) activity, and decreases serum free T_3 concentration in few days [90, 91]. However, as mentioned above, the drug is not available in some countries, including the United States.

Total thyroidectomy, under general anesthesia, is the procedure of choice and is considered safe [88, 92–94], even though one study reported a high complication and mortality rate (29% and 9%, respectively), probably due to poor cardiac state of the enrolled patients [95]. Some authors suggested minimally invasive video-assisted thyroidectomy under regional anesthesia to reduce complications [96], but this data need to be confirmed in larger studies.

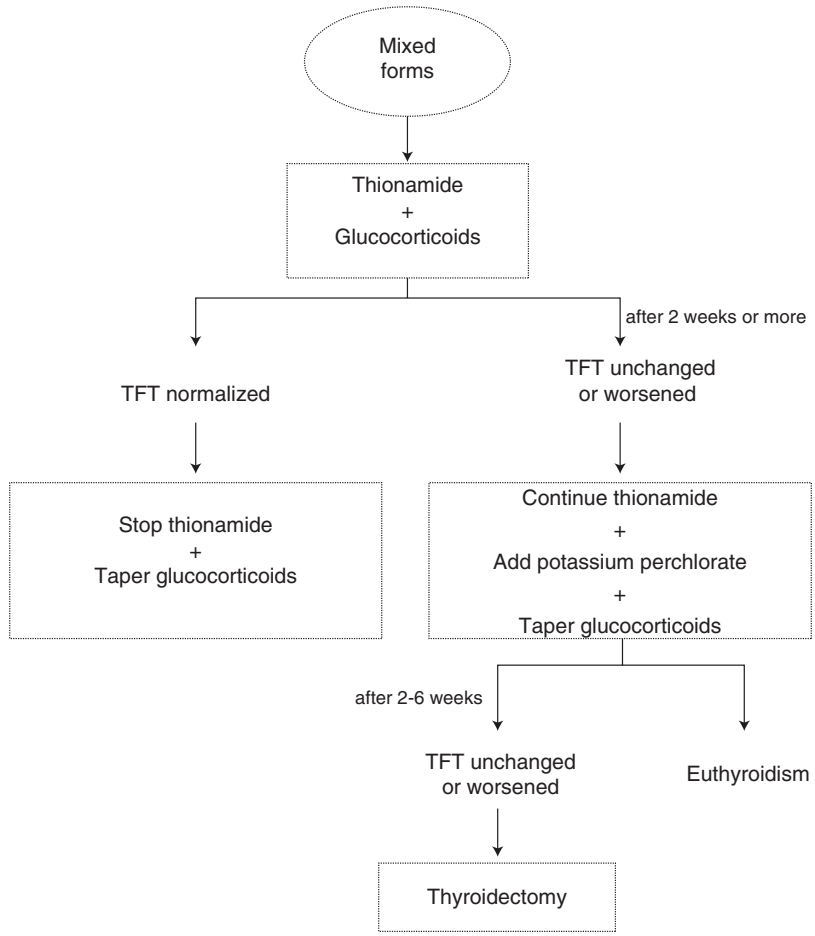

Fig. 4 Suggested algorithm for the management of patients with mixed forms of AIT. *TFT* thyroid function test

Radioactive iodine therapy is rarely feasible because patients with AIT have usually a low radioiodine uptake. However, some studies reported a role for radioactive iodine therapy in patients with type 2 AIT with a low radioiodine uptake [97–99]. However, relapse or persistence of thyrotoxicosis is not uncommon in these patients, and, according to one study, 15% were still thyrotoxic after 2-year follow-up [99]. Therefore, restoration of euthyroidism is delayed, and the underlying cardiac disease can worsen due to protracted thyrotoxicosis. In patients with type 1 AIT and a low radioiodine uptake, recombinant human TSH (rhTSH) increases uptake, and radioactive iodine may be effective in treating the thyrotoxicosis [100]. However, rhTSH should be used cautiously in these patients, since it stimulates the hyperfunctioning thyroid tissue and can induce worsening of thyrotoxicosis, which poses a further risk to the heart [101].

Management of Patients After Restoration of Euthyroidism and if Amiodarone Needs to Be Restarted

After restoration of euthyroidism and discontinuation of amiodarone, patients who had type 1 AIT should receive definitive therapy because of the underlying thyroid disease. Approximately one-third of European and American endocrinologists recommend definitive therapy in these patients, and almost 50% of thyrotoxicosis recurs [55–57]. Patients who had type 2 AIT, and do not need to restart amiodarone, rarely have a recurrence of thyrotoxicosis. Therefore, American and European endocrinologists generally prefer to monitor these patients [55–57]. Patients who had

type 2 AIT are at risk for subsequent hypothyroidism [50], and this risk is increased in patients with thyroid autoimmunity [63]. Close monitoring is recommended, and levothyroxine replacement should be instituted when permanent hypothyroidism develops.

When a euthyroid patient, with a history of AIT, needs to restart amiodarone, a decision on whether to begin prophylactic definitive therapy is still controversial because of a lack of studies [55, 57, 102]. A recent retrospective study reported a recurrence of AIT in 30% of patients, and this percentage seems to be underestimated, since a subgroup of patients received preventive thionamide treatment. Thyrotoxicosis recurred in 19% of patients with a history of type 2 AIT. In patients with a history of type 1 AIT thyrotoxicosis recurred in 37% of cases; however, when considering only the subgroup of patients without preventive thionamide treatment, thyrotoxicosis recurred in 73% of cases [103].

In conclusion, euthyroid patients with a history of AIT (in particular type 1 AIT) who need to restart amiodarone should receive prophylactic definitive therapy (thyroidectomy or radioactive iodine at high doses). Patients who urgently need amiodarone therapy and are awaiting thyroidectomy may benefit from a low dose of thionamides for a short period [103]. After thyroidectomy, levothyroxine can easily be given during amiodarone treatment, keeping in mind that a larger dose of levothyroxine may be required since amiodarone inhibits conversion of T_4 to T_3.

Patients Treated with Warfarin

A complex interaction between warfarin, amiodarone, and thyroid hormones has been reported. Warfarin is an anticoagulant drug, which inhibits vitamin K-dependent clotting factors II, VII, IX, X, and proteins C and S. Thyroid function abnormalities play a role in the coagulation-fibrinolytic system regulation: hyperthyroidism increases the risk of thrombosis, while hypothyroidism increases the risk of bleeding [104, 105]. Nevertheless, in patients treated with warfarin, thyrotoxicosis increases the risk of bleeding, since warfarin effects are potentiated by thyrotoxicosis. Indeed, thyrotoxic patients have an exaggerated degradation of functional clotting factors (II, VII) in response to warfarin [106]. In addition, amiodarone itself inhibits hepatic warfarin metabolism and potentiates warfarin's anticoagulant effect [107]. Therefore, in AIT patients, warfarin therapy should be started with a very low dose and international normalized ratio (INR) monitored closely in order to adjust the warfarin dose [108].

Dronedarone

Dronedarone is a non-iodinated benzofuran derivative related to amiodarone. A methane-sulfonyl group is added to dronedarone, which reduces lipophilicity and the half-life of the drug (to 24 h), and iodine moieties are not present, so thyroid complications typical of amiodarone are absent [109].

Dronedarone has been approved for the use in patients with paroxysmal or persistent atrial fibrillation but is contraindicated in patients with permanent atrial fibrillation or with concomitant heart failure [110]. When compared to amiodarone, dronedarone proved less effective in reducing atrial fibrillation recurrences post cardioversion but had a better safety profile, specifically less thyroid and neurologic events [111]. In the DIONYSOS trial, 1% of patients treated with dronedarone had thyroid toxicity (mainly hypothyroidism) compared with 6% of patients who developed thyroid dysfunction during amiodarone treatment [111]. Therefore, dronedarone may be beneficial in patients at increased risk or with a history of amiodarone-induced thyroid dysfunction [1]; however, poor efficacy and the contraindications of the drug limit its use.

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Iodine-Induced Thyroid Dysfunction

Simone De Leo and Lewis E. Braverman

Introduction

Iodine is an essential trace element required for the synthesis of the thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3). It is present in varying amounts in the Earth's soil, which in turn is responsible for the different iodine content of crops and foods. In general, the highest environmental iodine is available in coastal areas.

There is a U-shaped relationship between iodine intake and thyroid disorders, since both low and high iodine intakes are responsible for increased thyroid dysfunction. Several studies reported a significantly higher prevalence of hypothyroidism, hyperthyroidism, and thyroid autoimmunity in the population with excessive iodine intake compared to those with an adequate iodine intake [1–5]. The prevalence of thyroid nodules and the association with iodine excess is not clear, and further studies are needed.

The Institute of Medicine [6], the WHO (together with the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) and the United Nations Children's Fund (UNICEF)) [7], and the European Food Safety Authority (EFSA) [8] recommend a daily iodine intake of 150 μg for adults. Iodine intake should be increased during pregnancy and lactation (Table 1). Moreover, the Scientific Committee on Food of the European Commission and the Institute of Medicine have established a tolerable upper iodine intake in adults of 600 and 1100 μg daily, respectively (Table 2) [9]. The Endocrine Society recommended that iodine intake not exceed 500 μg of iodine daily in pregnant and breastfeeding women [10], and the WHO considered iodine intake excessive when the median UIC is higher than 300 μg/L in children (>6 years) and adults and higher than 500 μg/L in pregnant women [7].

Urinary iodine (UI) correlates with iodine intake, since more than 90% of ingested iodine is excreted in the urine within 24–48 h [11, 12]. Many urinary iodine measurements have been proposed for evaluation of iodine status in populations [13]; however, urinary iodine excretion is not reliable in individuals, because it varies widely within the day and day-to-day depending on a circadian rhythm and alimentary iodine intake [14, 15], limiting its use in clinical practice.

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Table 1 Recommended daily iodine intake (μg/day)

	Recommended dietary intake according to WHO, ICCIDD, and UNICEF	RDA according to Food and Nutrition Board (US Institute of Medicine)	Adequate intake according to EFSA
Infants (0–12 months)	90	RDA not determined AI 0–6 months—110 AI 7–12 months—130	0–6 months—ND 7–11 months—70
Children (1–18 years old)	1–6 years old—90 6–12 years old—120 12–18 years old—150	1–8 years old—90 9–13 years old—120 14–18 years old—150	1–10 years old—90 11–14 years old—120 15–17 years old—130
Adults (>18 years old)	150	150	150
Pregnancy	250	220	200
Lactation	250	290	200

RDA recommended dietary allowance, *AI* adequate intake, *WHO* World Health Organization, *ICCIDD* International Council for Control of Iodine deficiency disorders, *UNICEF* United Nations Children's Fund, *EFSA* European Food Safety Authority

Table 2 Tolerable upper intake limits for iodine (μg/day)

	Scientific Committee on Food (European Commission)	Food and Nutrition Board (US Institute of Medicine)
Infants (0–12 months)	ND	ND
Children (1–18 years old)	1–3 years old—200 4–6 years old—250 7–10 years old—300 11–14 years old—450 15–17 years old—500	1–3 years old—200 4–8 years old—300 9–13 years old—600 14–18 years old—900
Adults (>18 years old)	600	1100
Pregnancy	600	≤18 years old—900 >18 years old—1100
Lactation	600	≤18 years old—900 >18 years old—1100

Some studies reported that 10–12 repeated spot urine samples or 7–10 24 h urine collections are needed to estimate individual urinary iodine excretion, with a precision range ±20% [16, 17]. In population studies, the 24 h urinary iodine excretion is considered the reference standard [13, 18]. However, because of the difficulty in collecting 24 h urines, WHO recommends using median urinary iodine concentration (UIC), obtained by single spot urine samples [7]. It is worth noting that some studies demonstrated that the two evaluations are not interchangeable [19, 20], and because of variability in hydration status, some suggest estimating 24 h iodine excretion using a creatinine correction [21, 22], although WHO considers the creatinine correction not reliable [7].

Thyroglobulin has been proposed as an alternative method to assess iodine status in the general population. Serum thyroglobulin significantly decreased in an iodine-deficient population after the introduction of an iodization program or after iodine supplementation [23, 24], and it was more reliable than thyroid volume evaluation, which requires decades to reflect a new iodine status. In addition, an increased thyroid volume has been reported to reflect an extremely high dietary iodine intake (>500 μg/day), but not a moderately high dietary intake (300–500 μg/day) [25]. A more practical way to assess thyroglobulin concentrations is using dried blood spots (DBS). This method is recommended by the WHO, UNICEF, and ICCIDD for the evaluation of iodine status in school-age children (≥6 years old) [7]. In 2006, an International Reference Range for DBS-thyroglobulin of 4–40 μg/L in iodine-sufficient

children 5–14 years old was established [26]. DBS-thyroglobulin shows a U-shaped distribution related to iodine intake, because children with excess intake had significantly higher DBS-thyroglobulin concentration [27].

Adaptation to Excess Iodine

The thyroid gland is able to adapt to iodine excess, due to the acute Wolff-Chaikoff effect. This phenomenon was first described in 1948, when Wolff and Chaikoff reported that, in rats injected with excess iodine, the thyroid transiently inhibited the incorporation of inorganic iodide into the thyroid hormones [28]. To date, the acute Wolff-Chaikoff effect is not completely understood. One hypothesis is based on the thyroidal generation of iodinated lipids, such as iodolactones and iodoaldehydes [29]. α-Iodohexadecanal (α-IHDA), the major iodolipid formed after iodide administration, has been shown to reduce intrathyroid cyclic adenosine monophosphate (cAMP) levels and to inhibit thyroid peroxidase activity, necessary for thyroid hormone synthesis [30, 31] (Fig. 1).

Some studies aimed at ascertaining possible mechanisms involved in the decrease of thyroid hormone release during the acute Wolff-Chaikoff effect. In particular, monocarboxylate transporter 8 (MCT8), which transports thyroid hormones in and out of the cells, has been reported to be downregulated by iodine overload [32]. Therefore, iodide excess may decrease T_4 secretion during the acute Wolff-Chaikoff effect, by not only decreasing thyroid iodide organification but also impairing the transporters responsible for thyroid hormone release.

In normal individuals, the acute Wolff-Chaikoff effect is only transient; subsequently the thyroid gland resumes its function of iodine organification: the so-called "escape" from the acute Wolff-Chaikoff effect. Braverman and Ingbar, in 1963, postulated that adaptation to long-term iodide exposure is due to reduction of iodine transport into the thyroid gland and, therefore, reducing the thyroidal iodide concentration to a level inadequate to sustain the acute Wolff-Chaikoff effect [33]. In 1996, Dai et al. cloned the sodium/iodide symporter (NIS) [34]. NIS is a 13-transmembrane glycoprotein, located in the basolateral membrane of thyroid follicular cells, and responsible for the active transport of iodide into the thyroid [35]. NIS plays an important role in the "escape" phenomenon, because it has been demonstrated that NIS mRNA expression and protein levels are strikingly reduced after 1 and 6 days of iodide administration to rats [36]. Moreover, TPO mRNA expression was significantly decreased after 6 days of iodide administration [36]. High doses of iodide inhibit NIS function by increasing reactive oxygen species

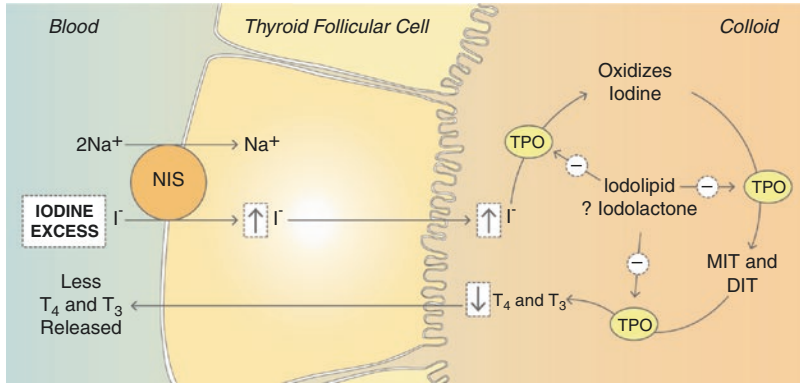

Fig. 1 The acute Wolff-Chaikoff effect. *DIT* diiodotyrosine, *I* iodide, *MIT* monoiodotyrosine, *Na* sodium, *Tg* thyroglobulin, *TPO* thyroid peroxidase, T_3 triiodothyronine, T_4 thyroxine (Adapted from Pramyothin P, Leung AM, Pearce EN, Malabanan AO, Braverman LE. Clinical problem-solving. A hidden solution. N Engl J Med. 2011;365:2123-7)

(ROS) production [37, 38]. Iodide-induced ROS were reported to activate the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway, which downregulates NIS function [39, 40].

Other thyroid mechanisms, such as dehalogenases and pendrin might take part in the escape from the acute Wolff-Chaikoff effect. Dehalogenases are deiodinases that recycle iodide by iodotyrosines (MIT and DIT) and by inactive iodothyronines. The decrease of thyroidal iodide concentration may be partly due to reduction of iodide recycling by inhibition of thyroidal dehalogenases induced by iodide excess [41]. Pendrin is a glycoprotein, located at the apical membrane of thyrocytes, and responsible for iodide efflux from the thyroid cell into the colloid. Iodine excess has been shown to increase pendrin expression and half-life, so that reduction of thyroidal iodine concentration might in part be due to the higher iodide efflux through pendrin [42, 43] (Fig. 2).

Sources of Excess Iodine

Iodinated Contrast Agents

One important cause of transient excess of iodine intake is the use of iodinated contrast agents, which has grown more rapidly than that of other physician-ordered services in the past decade [44]. CT scans have doubled from 2000 to 2012 [45].

Iodinated contrast agents or media (ICM) are used because iodine has a high atomic number so that it enhances visibility of organs or vessels in medical imaging by attenuating X-rays. In general, ICM can be high-osmolar or low/iso-osmolar [46]. Low-osmolar ICM are preferred because they have less side effects and they are generally better tolerated than high-osmolar ICM. However, low-osmolar ICM have a higher iodine concentration than the high-osmolar [46, 47] (Table 3). The organically bound iodine contained in an ICM is only minimally free iodide. In an ICM containing 300 mg of iodine/mL, the upper limit of free iodide is generally below 50 μg/mL after production. Nevertheless, 100 mL of ICM, containing 50 μg/mL, provides 5000 μg free iodide, which is 20 times the recommended daily intake [48]. In addition, ICM molecules are deiodinated in the body, and free iodide is increased especially in patients with kidney dysfunction, although available studies have not shown an increased risk of thyroid dysfunction after ICM in patients with impaired renal function [46, 48].

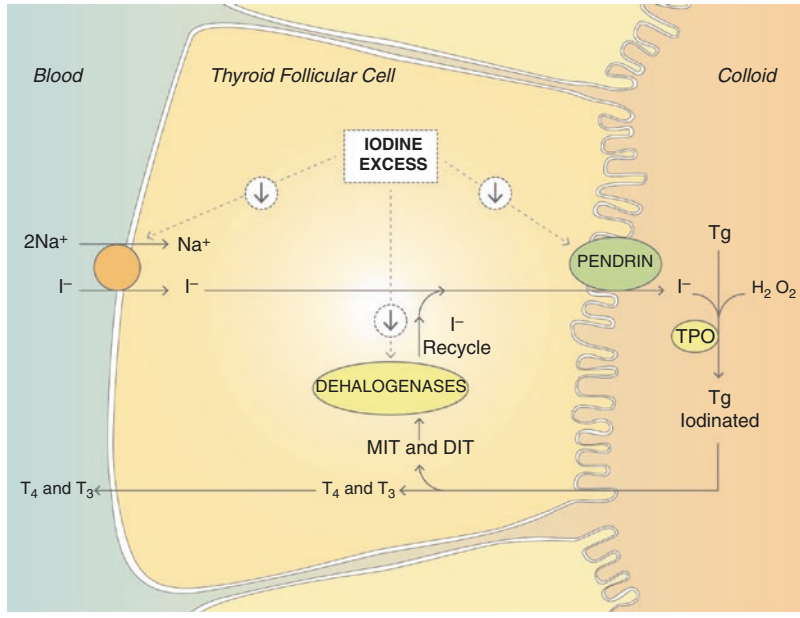

Fig. 2 The escape from the acute Wolff-Chaikoff effect. *DIT* diiodotyrosine, *I* iodide, *MIT* monoiodotyrosine, *Na* sodium, *Tg* thyroglobulin, *TPO* thyroid peroxidase, T_3 triiodothyronine, T_4 thyroxine

Table 3 Radiographic contrast media [47]

	Name (trade name)	Iodine content mg/mL	Osmolality (mOsm/kg H_2O)
Ionic contrast media	Diatrizoate (Hypaque 50)	300	1515—High
	Metrizoate (Isopaque 370)	370	2100—High
	Ioxaglate (Hexabrix)	320	600—Low
	Iothalamate (Conray)	282	1400—High
Nonionic contrast media	Iopamidol (Isovue 370)	370	796—Low
	Iohexol (Omnipaque 350)	350	844—Low
	Ioxilan (Oxilan 350)	350	721—Low
	Iodixanol (Visipaque 320)	320	290—Low
	Ioversol (Optiray 350)	350	792—Low
	Iopromide (Ultravist 370)	370	774—Low

In iodine-sufficient areas, several studies demonstrated an association between ICM exposure and development of thyroid dysfunction. Children without thyroid abnormalities had an increased risk of iodine-induced hypothyroidism after ICM exposure [49]. Similarly, larger studies reported that adults without known thyroid dysfunction, to whom ICM was administered for cardiac catheterization and/or computed tomography, had an increased risk of both hypothyroidism and hyperthyroidism [50–52]. Moreover, thyroid disorders appear to be more frequently detected if ICM exposure is repeated [51], although other studies failed to demonstrate this result [52]. Slight and transient modifications of thyroid function were reported after ICM exposure: in iodine-deficient areas, TSH decreased after coronary angiography [53, 54] and computed tomography [55]. In contrast, in an iodine-sufficient area, a significant increase in serum TSH, up to 24 weeks after hysterosalpingography, was reported in euthyroid patients, while free T3 and free T4 concentrations remained stable [56].

Metabolism of ICM

After ICM administration, UIC peaks after 1 week and returns to baseline levels on average in 5–6 weeks in euthyroid patients without kidney dysfunction, although some patients may require more than 2 months [57, 58]. Thyroidectomized patients require 4 weeks to normalize UIC after ICM [59–61].

Iodine introduced with contrast media competes with radioactive iodine (RAI) for uptake by thyroid tissue, so that ICM can reduce the efficacy of diagnostic or therapeutic RAI [48]. Therefore, the American Thyroid Association guidelines regarding management of patients with thyroid nodules and papillary thyroid cancer suggest performing RAI therapy and diagnostic scans 4–8 weeks after administration of ICM. Moreover, a random urinary iodine (and creatinine) evaluation should be measured to ensure that iodine concentration is not high [62]. However, it is worth noting that biliary contrast media may circulate longer in the body, and an interval of 3–4 months between ICM administration and RAI may be appropriate [48].

Thyroid Function Monitoring After ICM Administration

Overall, even though an increased risk of thyroid dysfunction and abnormal thyroid function tests after ICM exposure was reported in euthyroid patients, these are transient and the incidence is low [51]. Routine monitoring of thyroid function prior to ICM administration is not recommended [48, 55, 63]. Conversely, patients with risk factors (see below), in particular latent Graves' disease and nodular goiter with thyroid autonomy that increase the risk of iodine-induced thyrotoxicosis, require special attention. Guidelines from the Contrast Media Safety Committee of the European Society of Urogenital Radiology

contraindicate ICM in patients with overt hyperthyroidism and recommend close monitoring of thyroid function after ICM administration in patients at risk [48]. However, because many patients who developed iodine-induced thyroid dysfunction were not reported to have underlying risk factors, monitoring of thyroid function may also be appropriate in patients unable to tolerate thyroid dysfunction, such as patients with unstable cardiovascular disease [64].

Special Circumstances

ICM Exposure In Utero

ICM administration in pregnant women can induce hypothyroidism in the fetus since iodine readily crosses the placenta [65]. Fetal iodine uptake and thyroid hormone synthesis is possible after 20 weeks of gestation, while up to 36 weeks of gestation, the fetal thyroid may not be able to escape from the acute Wolff-Chaikoff effect. The fetal thyroid is, therefore, at risk for iodine overload [46]. However, the available studies, which evaluated the adverse effects of ICM administration in pregnant women, failed to demonstrate a detrimental effect on neonatal thyroid function [66–69]. The ICMs currently administered are water soluble and rapidly cleared from the body. Moreover, they have a high molecular weight and cross the placenta less readily than smaller water-soluble molecules. Therefore, fetal exposure to ICM is transient [70]. Although the available results are reassuring, there is still little direct evidence. Guidelines of the Contrast Media Safety Committee of the European Society of Urogenital Radiology recommend screening all infants exposed to ICM during the first week, using a blood test [70]. The American College of Radiology suggests that the routine evaluation of all newborns for congenital hypothyroidism (measuring TSH levels at birth) is sufficient [47].

ICM Exposure in Infants

According to the Institute of Medicine, the recommended dietary adequate iodine intake for infants aged 0–6 months is 110 μg/day and in infants aged 6–12 months 130 μg/day [6] (Table 1). The tolerable upper intake level for iodine has not been determined in infants up to 12 months because of the lack of data of adverse effects in this age group, while in children 1–3 years of age, the upper limit is 200 μg/day [6] (Table 2). Infants may be exposed directly to ICM or breastfed by a mother who was exposed to ICM. ICM can be secreted into the milk, but minimally compared to the iodine administered because ICM has low affinity for binding to milk proteins and the duration of iodine exposure is short. Therefore, breastfeeding is considered safe after the mother has received ICM [47, 70].

In contrast, direct ICM exposure in infants was reported to increase the risk for iodine-induced hypothyroidism and of abnormal thyroid function [71, 72]. These risks are particularly higher in premature infants, compared to term infants. According to a meta-analysis, 8% of term infants and 18% of premature infants developed hypothyroidism after exposure to ICM [71]. Thyroid glands of premature infants may be immature and unable to escape from the acute Wolff-Chaikoff effect. Therefore, the risk of exposure should be minimized in this at-risk population, and thyroid function monitoring is recommended.

Diet and Iodine Supplementation

Excessive Dietary Iodine Intake

The diet is particularly important for achieving iodine sufficiency in a population. Some foods contain elevated concentration of iodine, in particular seaweed. Other foods contain variable amount of iodine and contribute to the total dietary iodine intake [73, 74].

The main sources of dietary iodine are [74–76]:

- Dairy products, because of the use of iodophor disinfectants in pre- and post-milking teat dips and udder washes, and because of iodine supplementation of cattle feed.
- Grain or breads, since crops might grow in iodine-rich soils or iodine might be added as iodate bread conditioners (which may be added to prolong shelf life of the products), table salt, seaweed, or other seafood.

In infants, it has been estimated that 90% of iodine intake is obtained from dairy products and baby foods (including infant formulas), unlike all other ages in whom iodine intake is predominantly from dairy products and grains [76].

Some populations ingest excessive amounts of seaweed, such as Japanese and those from other Asian countries, and their iodine intake may be excessive [77]. There are several different types of seaweed, which contain various amounts of iodine ranging from a small amount to more than 8000 μg/g in *laminaria* [78]. Moreover, the iodine content of seaweed varies with food preparation and cooking methods. For example, about 99% of iodine contained in Kombu may be lost after 15 min of boiling in water, since iodine in edible seaweed is generally water soluble [77].

Daily consumption of seaweed in adults, even though for a short term (7–10 weeks), increases UIC and serum TSH levels [79, 80]. Therefore, populations at risk, such as lactating women, should avoid ingestion of seaweed. Korean lactating women often use seaweed during the early postpartum period [81], and subclinical hypothyroidism has been described in their preterm infants because of the excessive iodine in their breast milk [82]. A large epidemiological study in postmenopausal women reported an association between seaweed consumption and differentiated thyroid cancer [83], although other studies failed to find this association [84].

Iodine Supplementation

Iodine supplementation worldwide is fundamental to correct iodine deficiency [85]. Different methods are available, including salt iodization, fortification of bread with iodine, iodization of drinking and irrigation water, use of iodophors in the dairy industry, administration to cattle of iodine-fortified fodder, or administration to particular populations of iodized oil orally or intramuscularly [73, 86].

However, excessive iodine supplementation, for example, by salt iodization, may be responsible for iodine excess, as reported in some countries [87–89]. In general, iodine supplementation confers benefits that outweigh the risks of iodine excess, but it should be monitored to avoid excess while ensuring adequate iodine intake.

Salt iodization is mandatory in approximately 120 countries, while in others it is voluntary, such as the United States [90]. In some countries, the excessive iodization of salt was responsible for iodine-induced thyroid dysfunction, especially goiter and thyrotoxicosis, more common if other source of iodine were already available in the diet [91, 92].

After universal salt iodization, even though an excessive iodine level is not reached, the sudden exposure to increased iodine intake might be responsible for higher rates of thyroid dysfunction. In Denmark, the DanThyr program was developed to monitor iodine intake and thyroid diseases after the introduction of mandatory iodine fortification of bread salt and household salt [93]. Median UIC significantly increased from 61 μg/L pre-fortification to 101 μg/L 4–5 years after mandatory iodization [94]. In this population, after iodine fortification, increased serum TSH concentrations were reported, as was the prevalence of mild hypothyroidism, with a decrease of overt hypothyroidism. The prevalence of hyperthyroidism, after an earlier increase, subsequently declined [95]. Changes in thyroid gland structure were also described; there was an increased prevalence of multinodularity, while one-third of single nodules disappeared [96]. Finally, a marked increase in thyroid cancer incidence was reported, which was almost exclusively explained by an increase in papillary thyroid carcinoma [97]. This finding was also confirmed in studies conducted in other countries after universal salt iodization [98]. However, the increased incidence of thyroid cancer is partly explained by overdiagnosis, due to improvements in diagnostic and screening activities in the last decades [99].

Some studies reported an excessive iodine intake due to drinking water. Endemic goiter was reported in these populations, both in adults [100] and in children [4, 101, 102]. In lactating women, subclinical hypothyroidism was demonstrated to be significantly more prevalent in women with iodine excess compared with adequate iodine intake. Thyroid autoimmunity was also more fre-

quent, although not statistically significant [103]. Similarly, Saharawi lactating women with excessive iodine intake due to increased iodine content in the drinking water had high rates of thyroid dysfunction, up to 33%, including hypothyroidism, hyperthyroidism, and thyroid autoimmunity [104]. These thyroid abnormalities persisted after 3 years of follow-up [105]. Breast milk iodine concentration was high and correlated well with thyroid dysfunction in these women [104–106]. Other regions of the world in which high iodine in groundwater has been reported to be responsible for iodine excess include some areas of China [107–109], Somali [110], and probably in Somali refugees in Kenya [111]. A study conducted in China defined a safe upper limit of iodine contained in water to be 100 μg/L [112].

Nutritional Supplements

Multivitamins might be a source of excess iodine because of administration errors or elevated iodine content of the supplements. Multivitamins contain iodine typically as potassium iodide (which is the preferred form) and kelp. In the United States, it was reported that iodine content of multivitamins containing kelp was frequently discordant with the values on their labels and ranged from 33 to 610 μg per capsule [113].

Administration of excessive doses of iodine supplements has been shown to induce thyroid dysfunction. In a double-blind prospective trial, euthyroid adults were randomized to an intervention group receiving iodine supplements and to a placebo control group. Participants who received excessive amounts of iodine developed subclinical hypothyroidism after 4 weeks, and the percentage varied between 5% in those who received 400 μg/daily and 15–45% in those who received 500–2000 μg/daily. Therefore, the authors suggested that the total daily iodine intake should not exceed 800 μg/daily [114]. Similarly, in a randomized controlled trial, patients receiving elevated doses of iodine (>50 mg daily) for 8 weeks significantly increased serum TSH concentrations, 25% of participants developed hypothyroidism and 7% hyperthyroidism [115].

Moreover, congenital hypothyroidism and neonatal goiter were reported in infants whose mothers had taken iodine supplements or herbal iodine-containing medicine during pregnancy [116, 117]. In one report, congenital hypothyroidism was reported in newborns following ingestion of Iodoral daily, an iodine supplement containing 12.5 mg of iodine [118].

Because of the demonstrated adverse effects of excessive doses of iodine, the ATA Public Health Committee has advised against the administration of iodine and kelp supplements containing more than 500 μg iodine daily for children and adults and during pregnancy and lactation [119].

Iodine-Rich Medications and Other Sources

One of the most important causes of iodine-induced thyroid dysfunction is amiodarone (see chapter “Pathology of the Thyroid: A Review”). Other sources of iodine include iodine-containing antiseptics, such as those containing povidone-iodine. Povidone-iodine is a water-soluble complex and, in the form of a 10% topical solution, contains 10 mg of iodine per milliliter. Povidone-iodine can be absorbed by the skin [120], and iodine excess was demonstrated by surgical staff after scrubbing with iodine-containing solutions [121]. Iodine is also employed as a preoperative antiseptic in many surgical settings [122–125], for vaginal disinfection [126] or disinfection before catheterization [127]. Iodine absorption is higher in cases of skin damage or thinner skin, such as in infants [128], and it can alter thyroid function, increasing serum TSH concentrations in exposed patients. Preterm infants are at a particular higher risk of developing hypothyroidism, because of the immaturity of the thyroid gland and inability to escape from the acute Wolff-Chaikoff effect [129–131]. Some alternatives to povidone-iodine are the alcohol-based cleansers, such as chlorhexidine, which was demonstrated to be superior in preventing surgical-site infection compared with cleansing with povidone-iodine [132]. The adverse effects with the two antiseptics seem to occur in equal proportion [132],

although there are still concerns regarding the use of alcohol-based disinfectants in premature infants because of the increased risk of systemic absorption and skin irritation or burns.

Finally, potential sources of excess iodine exposure are mouthwashes [133], expectorants, food preservatives, and water when it is enriched with iodine to avoid microbial contamination [134]. In American Peace Corp workers in Niger, it was reported that exposure to iodine-enriched water was responsible for iodine excess and thyroid dysfunction. After removal of the iodine exposure source, serum iodine levels significantly decreased and thyroid abnormalities resolved [134].

Iodine-Induced Hypothyroidism

Pathogenesis and Risk Factors

Failure to escape from the acute Wolff-Chaikoff effect results in iodine-induced hypothyroidism. Hypothyroidism might also be a result of thyroid autoimmunity induced by iodine excess [135] or because of direct toxic effect of iodine on thyrocytes [136].

Although iodine-induced hypothyroidism may develop in subjects without apparent risk factors, others may develop hypothyroidism because of underlying thyroid abnormalities or other risk factors [29, 73, 137, 138] (Table 4).

Diagnosis

Patients with hypothyroidism induced by iodine excess commonly report symptoms similar to those of primary hypothyroidism. Physical examination may reveal a goiter, and patients with Hashimoto thyroiditis are at increased risk of developing iodine-induced hypothyroidism.

The diagnosis is based on the finding of increased serum TSH concentrations. The serum thyroid hormones, free and total thyroxine (T_4) or the free T_4 index and serum total and free triiodothyronine (T_3), may be low or normal. Overt hypothyroidism is characterized by a high serum TSH concentration and low serum thyroid hormone concentrations, while subclinical hypothyroidism is defined as a high TSH and normal serum thyroid hormone concentrations.

The physician should obtain a history of excess iodine ingestion or administration, including all the possible sources of excess iodine.

Subsequent evaluation is useful to ascertain the possible presence of underlying thyroid disease. Serum TPO and thyroglobulin antibodies are present in patients with underlying thyroid

Table 4 Risk factors for iodine-induced hypothyroidism

Underlying thyroid dysfunction	Hashimoto thyroiditis
	Euthyroid Graves' disease previously treated with RAI, surgery, or ATD
	History of postpartum thyroiditis
	History of subacute (painful) thyroiditis
	History of type 2 amiodarone-induced thyrotoxicosis
	Post-hemithyroidectomy for benign nodules
	Thyroid dysfunction induced by treatment with IFN-α
Nonthyroidal illness	Thalassemia major with repeated blood transfusions
	Chronic renal diseases (in particular during dialysis treatment)
	Anorexia nervosa
Synergism with other goitrogens	Lithium, sulfonamides, sulfonylureas
Healthy population at risk	Transplacental transfer of iodine to fetus
	Neonates and infants (in particular preterm)
	Elderly (>65 years old)

RAI radioactive iodine, *ATD* antithyroid drug, *IFN-α* interferon-α

autoimmunity. Thyroid ultrasound may identify an enlarged gland with increased blood flow. A careful history of previous thyroid dysfunction and concomitant treatment with goitrogen drugs and amiodarone should be obtained.

The thyroid radioactive iodine uptake is not useful since it is almost always low due to the excess iodine, but an elevated uptake has been reported [139, 140].

Management and Treatment

Iodine-induced hypothyroidism is typically transient. Euthyroidism is usually restored in a few weeks (2–8 weeks) after iodine withdrawal. However, some compounds may require more time if the iodine-containing substances responsible for iodine excess are not rapidly eliminated.

Levothyroxine replacement is not generally necessary in patients with iodine-induced hypothyroidism. However, if hypothyroidism persists, is severe, or the iodine-containing compounds cannot be withdrawn, treatment of the hypothyroidism with levothyroxine replacement therapy is recommended.

Patients who developed transient iodine-induced hypothyroidism require subsequent follow-up with periodic monitoring of serum TSH and thyroid hormone concentrations because of the underlying thyroid disease predisposing to hypothyroidism [29]. These patients may be at increased risk of developing permanent hypothyroidism.

Iodine-Induced Hyperthyroidism

Pathogenesis and Risk Factors

Iodine-induced hyperthyroidism usually develops in patients who have underlying autonomy of the thyroid, which can be in small foci or nodules or can be diffuse [141]. The iodine load acts as a substrate for the thyroid to produce excess amount of thyroid hormones. This response is called the *Jod-Basedow* phenomenon, in recognition of the German physician Von Basedow who first described iodine-induced hyperthyroidism.

Patients at increased risk of developing iodine-induced hyperthyroidism are those with (1) nontoxic nodular or diffuse goiter, especially in elderly patients, (2) latent Graves' disease, and (3) long-standing iodine deficiency. However, iodine-induced hyperthyroidism can also develop in patients with an apparent normal thyroid gland [73, 142].

Diagnosis

Clinical presentation is similar to that of other causes of thyrotoxicosis, with the signs and symptoms due to excess circulating thyroid hormones. The physical examination may reveal a goiter, often multinodular.

The diagnosis is based on the finding of suppressed levels of serum TSH, while serum thyroid hormone concentrations can be high (overt thyrotoxicosis) or normal (subclinical thyrotoxicosis). Iodine-induced hyperthyroidism should be distinguished from other causes of thyrotoxicosis.

A history of iodine ingestion or administration should be ascertained, taking into account that iodine exposure might have occurred weeks or even months before development of thyrotoxicosis.

Underlying thyroid disease should be excluded, such as nodular goiter and Graves' disease. Radioactive iodine uptake might be useful in some circumstances, such as confirming the diagnosis of Graves' disease in cases of doubt or for differentiating functioning from nonfunctioning nodules that might require fine-needle aspiration biopsy. However, the radioactive iodine uptake in a patient with iodine-induced hyperthyroidism is not reliable, since the uptake is typically low because of dilution of the radioiodine tracer and a decrease in the transport of iodine from blood to thyroid. However, the uptake is generally not as low as detected in patients with subacute thyroiditis or ectopic hyperthyroidism, where the uptake is typically lower than 1% [143]. Clinicians who need to perform a thyroid radioactive iodine uptake should await at least 4 weeks after iodine exposure, so that iodine is cleared from the body [48],

although some organic iodine products may take far longer to be cleared.

If the diagnosis of iodine-induced hyperthyroidism is likely, but a source of iodine excess cannot be determined, a 24 h urinary iodine measurement can confirm the diagnosis, especially if results are greater than 1000 μg/day [144].

Management and Treatment

Iodine-induced hyperthyroidism is usually transient lasting 1 to 18 months after the source of iodine is discontinued. In mild hyperthyroidism, patients can be treated with β-adrenergic blocking agents alone, after having excluded contraindications to these drugs, such as heart failure and asthma. β-blockers may decrease the peripheral effects of excess thyroid hormones and, in high doses, slightly decrease T_4 to T_3 conversion [145].

All β-blockers are efficacious. An initial dose of atenolol 25–50 mg daily is usually adequate. Subsequently, β-blockers should be titrated and discontinued once euthyroidism is restored.

In case of severe or persistent hyperthyroidism, patients might require treatment with antithyroid drugs. Antithyroid drugs prevent the synthesis of new thyroid hormones, and propylthiouracil in large doses, but not methimazole, slightly decreases T_4 to T_3 conversion. However, methimazole is the preferred drug, except during the first trimester of pregnancy or in patients with adverse reactions to methimazole [146].

Doses are generally higher than those generally used, since patients with iodine-induced hyperthyroidism may have a relative resistance to antithyroid drugs. Therefore, the American Thyroid Association (ATA) and American Association of Clinical Endocrinologists (AACE) guidelines for hyperthyroidism and thyrotoxicosis recommend methimazole at a dose of 20–40 mg daily for treatment of iodine-induced hyperthyroidism [145].

After initiation of therapy, thyroid function tests should be checked after 4–6 weeks and subsequently every 2–3 months. Antithyroid drugs can be tapered and discontinued once euthyroidism is achieved. After resolution of the acute episode, clinicians should treat the underlying thyroid disease, if needed.

In patients with underlying Graves' disease, treatment with antithyroid drugs should be continued for 12–18 months to reduce the risk of relapse of hyperthyroidism. If hyperthyroidism returns, without exposure to iodine excess, definitive treatment with radioactive iodine or thyroidectomy is recommended [146]. In patients with nodular goiter, definitive treatment may not be necessary. However, because of the high risk of relapse of hyperthyroidism, if patients with nodular goiter are exposed again to iodine, definitive treatment with radioactive iodine or thyroidectomy can be considered. Radioactive treatment should be performed at least 4–8 weeks after withdrawal of iodine, if the radioactive iodine is high normal or elevated [48].

Prevention and Prophylaxis

When the administration of iodine cannot be avoided, it would be helpful to predict which patients will develop overt thyroid dysfunction. Some studies attempted to assess this risk by performing a thyroid scintigraphy using ^{99m}Tc pertechnetate. Technetium thyroid uptake is able to measure the amount of functional thyroid autonomous tissue: the higher the uptake, the more the risk of hyperthyroidism is increased, in patients with a suppressed serum TSH. Iodine administration, such as ICM, is considered safe when the technetium thyroid uptake is less than 1% [147]. However, sensitive TSH assays currently available and the cost of scintigraphy resulted in the test being performed only occasionally.

Some regimens were attempted to decrease the risk of thyrotoxicosis after ICM administration in high-risk patients. Prophylactic drugs that have been proposed are methimazole, which blocks thyroid hormone synthesis, and perchlorate, which competitively inhibits the sodium-iodine symporter. These drugs should be used together from the day before ICM administration and continued for 14 days [148]. However, this prophylaxis method, even though it has a protec-

tive effect against iodine excess, does not completely prevent thyrotoxicosis. Moreover, perchlorate is not available in all countries, such as the United States, and antithyroid drugs have some risk that would limit their routine use [64]. In conclusion, prophylactic treatment should not be routinely used, while limited use may be considered for selected high-risk patients.

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Part VII

Thyroid Neoplasia

Pathology of the Thyroid: A Review

Virginia A. LiVolsi, Kathleen T. Montone, and Zubair W. Baloch

Introduction

The thyroid may be involved by a variety of disease processes that may be neoplastic and non-neoplastic as well as focal or diffuse. This chapter will provide a brief overview and update of thyroid pathology.

Processes that *Diffusely* Involve the Thyroid

The most common clinically and histopathologically encountered thyroid lesions are autoimmune in origin including Graves' disease or diffuse toxic goiter and chronic lymphocytic thyroiditis particularly Hashimoto thyroiditis. These processes usually produce thyroid enlargement without discrete nodules and involve the entire thyroid uniformly. Both entities are morphologically characterized by lymphocytic or lymphoplasmacytic infiltration of the gland with reactive changes in the follicular epithelium. Care must be rendered not to confuse these reactive follicular epithelial changes with those seen in papillary thyroid carcinoma [1].

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Recently, certain diffuse fibrosing lesions of the thyroid including fibrosing Hashimoto's thyroiditis and Riedel's thyroiditis have been shown to be part of the spectrum of IgG4-related diseases [2]. In this condition there is extensive fibrosis and a predominant plasma cell infiltration of the gland. Special staining techniques have shown that the majority of the plasma cells in these conditions stain for IgG4 [3, 4]. Many of the patients affected by this thyroid disease can have systemic manifestations of hyper-IgG4 production including involvement of the retroperitoneum, the pancreas, the kidney, the salivary glands, and the mediastinum [5].

Nodular Thyroid Lesions

Thyroid nodules are very common and vary in their incidence in different parts of the world. In endemic goiter regions, the frequency of thyroid nodules may be as high as 25%, whereas in non-iodine-deficient areas, 4–7% of the population has palpable thyroid nodules [6]. However, with the increasing use of ultrasound as screening tool, thyroid nodules can be encountered in up to 60% of the population [7–10].

Nodular thyroid disease comprises the majority of lesions which are examined by pathologists. Thyroid nodules are initially examined either by fine-needle aspiration (FNA) biopsy or by large-core needle biopsies [11–13]. FNA

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samples are often obtained under radiologic guidance, and the pathology can be classified according to various schemes. In the United States, the most common classification for thyroid FNA cytology is the "The Bethesda System for Reporting Thyroid Cytology (TBSRTC)" [14]; see Table 1. This system not only attempts to classify the nature of the biopsied nodule but also assigns a risk level for malignancy. FNA is most useful for identifying benign nodules (i.e., hyperplastic nodules in multinodular goiter), papillary thyroid carcinoma, and medullary thyroid carcinoma [15]. However, FNA has limitations for diagnosing follicular or oncocytic (Hürthle cell) lesions since due to the nature of the specimen, it cannot assess both capsular and angioinvasion, the two criteria to determine malignancy in follicular thyroid lesions. However, molecular analysis of thyroid FNA cytology is becoming a useful ancillary tool for predicting malignancy in thyroid nodules. Numerous recent studies have elucidated the value of molecular testing of FNA specimens to further characterize the nature of the nodule. Excellent negative and positive predictive values are reported for these analyses [16–18].

Core needle biopsies have become more recently utilized for analyzing thyroid nodules. Excellent results can be obtained for diffuse thyroid diseases, benign nodular conditions, and papillary thyroid carcinomas [19]. However these types of biopsies suffer from the same issues as to FNA in that follicular nodules that have a monotonous and cellular pattern require evaluation of the lesional capsule or edge in order to define benign from malignant lesions [20, 21]. However, similar to FNA, molecular analysis may be useful to determine malignancy risk in many, but not all, of these biopsy samples.

Table 1 The Bethesda system for reporting thyroid cytopathology (TBSRTC): diagnostic categories and implied risk of malignancy

Diagnostic category	ROM(%)[a]	ROM(%)[b]
Nondiagnostic or unsatisfactory	1–4	11–26
Benign	0–3	4–9
Atypia of undetermined significance or follicular lesion of undetermined significance	~5–15	19–38
Follicular neoplasm or suspicious for a follicular neoplasm	15–30	26–40
Suspicious for malignancy	60–75	50–79
Malignant	97–99	98–99

ROM risk of malignancy
[a]2007-calculated based on available literature
[b]Calculated based on current literature

Hyperplastic/Adenomatoid Nodule

The most common nodule produced by the thyroid gland is the hyperplastic (also known as adenomatous, adenomatoid, or follicular nodule). These lesions most commonly occur in background of multinodular goiter. Such lesions which may be solitary, but most often are multiple, are composed of follicles which are varied in size and shape and amount of luminal colloid. Some hyperplastic nodules show papillary growth with edematous "pseudo-papillae" lacking fibrovascular core. These papillae are lined by small-sized, dark polarized nuclei and are often seen focally in an otherwise recognizable adenomatous follicular nodule [1, 15, 22].

Hyperplastic lesions are usually well circumscribed and unencapsulated (although rarely, thin partial capsulation may be evident). It is not uncommon to see scattered lymphocytes, hemorrhage, hemosiderin, and varying degrees of fibrosis in hyperplastic nodules. In hyperplastic nodules that have undergone biopsy, an additional assortment of inflammatory changes may be seen including granulomatous reaction, calcification, and squamous metaplasia of the follicular epithelium. Rarely, along biopsy needle tracts nuclear changes reminiscent of those seen in papillary carcinoma nuclei may be found [23]. In addition, FNA may result in lesion infarction which is more commonly seen in lesions biopsied with large gauge needles (18G or less).

A very rare probably familial entity is so-called multiple papilloid nodules. This condition is characterized by a multinodular gland in which the nodules are circumscribed and cystic and show papillary ingrowth into the cyst by very thin papillae; these papillae are not edematous and are different from the papillary hyperplastic nodule

(see below). The genetics of this condition is not yet known; a few affected patients have developed papillary thyroid carcinoma [24].

The FNA specimens form nodular goiter can range in cellularity from specimen barely meeting the criteria for cell adequacy to those specimens containing large numbers of cells which can be mistaken for a neoplasm. The cytology specimen from a goitrous nodule stained with Romanowsky stain/Diff-Quik® stain shows abundant watery colloid, which usually appears bluish-pink magenta in color and shows a "chicken wire" artifact due to air-drying. The follicular cells appear small and round to oval in shape with dark nuclei and are arranged in monolayer sheets, groups with follicle formation, or as single cells. In some cases of nodular goiter especially the ones with cystic changes, the follicular cell group may assume spindle shape and appear similar to cells growing in "tissue culture." Macrophages, usually filled with hemosiderin granules are also noted; however, their number depends upon the presence or absence of degenerative changes or a cystic component [15].

The FNA specimens of hyperplastic/adenomatoid nodules are usually more cellular and can be mistaken for a follicular neoplasm (Figs. 1 and 2). *The papillary hyperplastic nodules* besides being cellular can show papillary formations with transgressing vessels; however, diagnostic nuclear features of papillary thyroid carcinoma are not seen [22, 25].

Follicular Adenoma

The most common type of benign neoplasm of the thyroid is the follicular adenoma. Such lesions are characterized by a capsule (often thin, but complete) around the lesion; the cytology of the lesion is monotonous, often cellular and composed of microfollicles, macrofollicles, or trabeculae (Fig. 3). Examination of the capsule shows no evidence of invasion. In addition vascular (angio)invasion is not seen (Figs. 4 and 5). Follicular adenomas can vary in size from subcentimeter to several centimeters and are often solitary; however multiple adenomas may arise in glands with multinodular goiter or various types of thyroiditis [26–28].

In adenomas that have undergone preoperative biopsy, changes related to the biopsy procedure may be identified. These vary with the time between the biopsy and the surgical excision of the nodule. Such changes include hemorrhage, hemosiderin, reactive nuclear changes (which

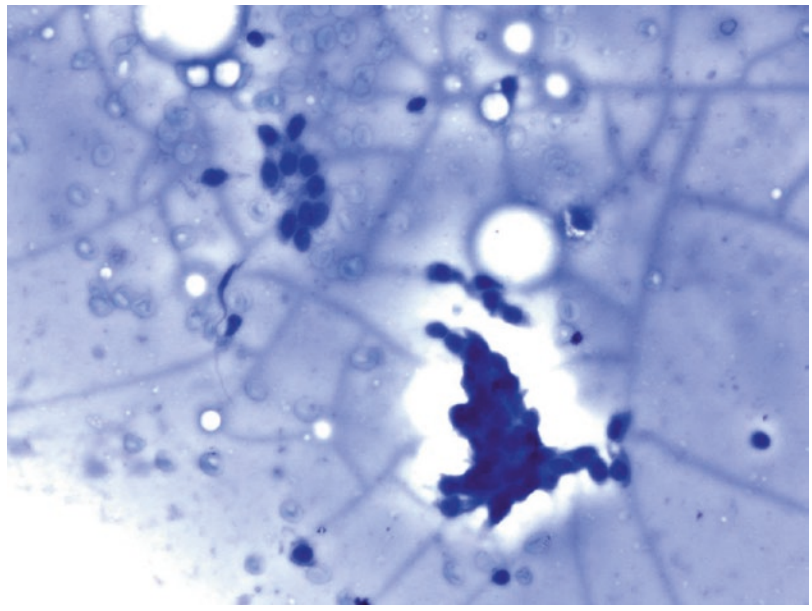

Fig. 1 Fine-needle aspiration specimen from a hyperplastic nodule arising in nodular goiter, showing watery colloid in the background with small groups of benign follicular cells (air-dried Diff-Quik stain)

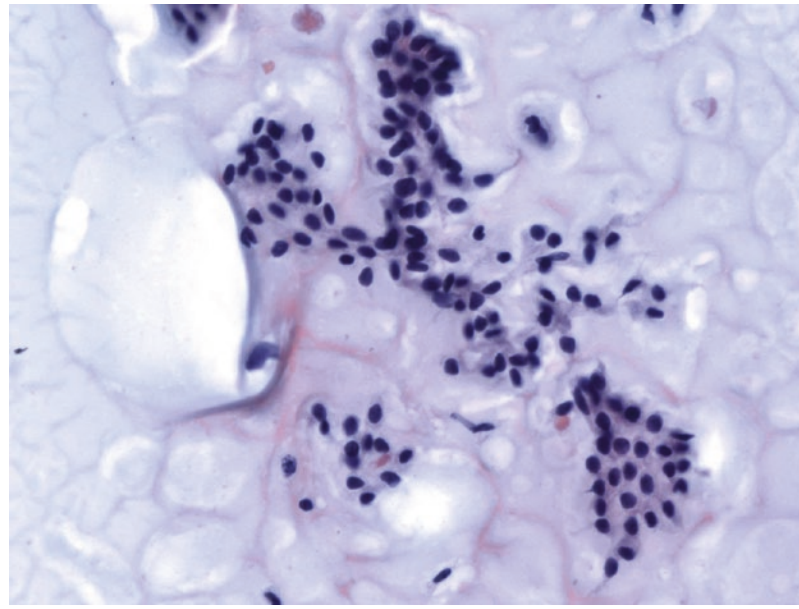

Fig. 2 The corresponding alcohol-fixed smear shows the same features; notice the follicular cells have small dark nuclei and lack nuclear features of papillary thyroid carcinoma (Papanicolaou stain)

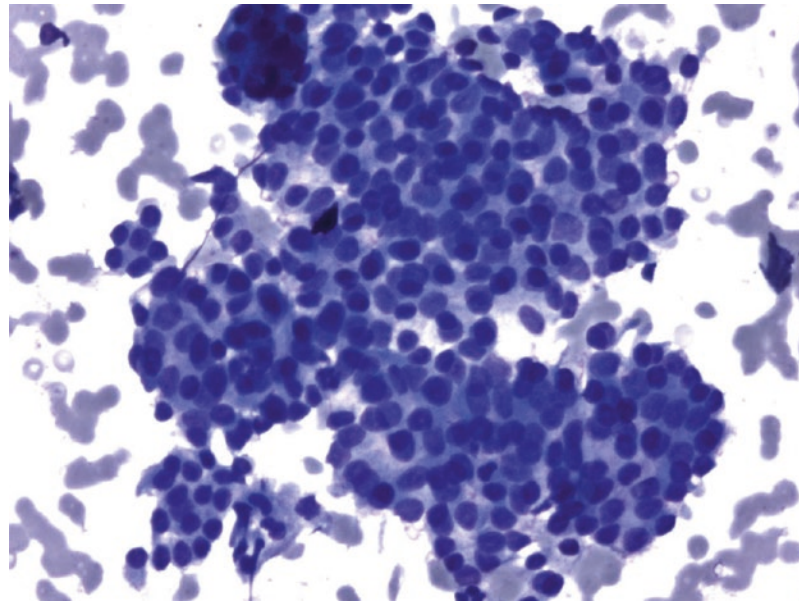

Fig. 3 Follicular neoplasm. Fine-needle aspiration specimen of a solid hypoechoic nodule showing a monotonous population of follicular cells arranged in cohesive follicular groups with nuclear overlapping and crowding. No nuclear features of PTC are seen

may mimic the nuclear changes of papillary thyroid carcinoma), granulomatous/histiocytic and/or lymphocytic reaction, linear fibrosis, calcification, and epithelial (usually squamous) metaplasia. In some examples a biopsy needle tract is identified and may extend into and through the lesion's capsule mimicking invasion. Microscopic examination of these areas will show the presence of inflammatory tissue and hemosiderin admixed with lesional follicular cells. The geographic linear configuration of the biopsy tract at low power magnification is a helpful clue that one is dealing with a post biopsy artifact and not true invasive growth. On occasion, partial or complete infarction of an adenoma may occur after biopsy [23]. This is most commonly seen

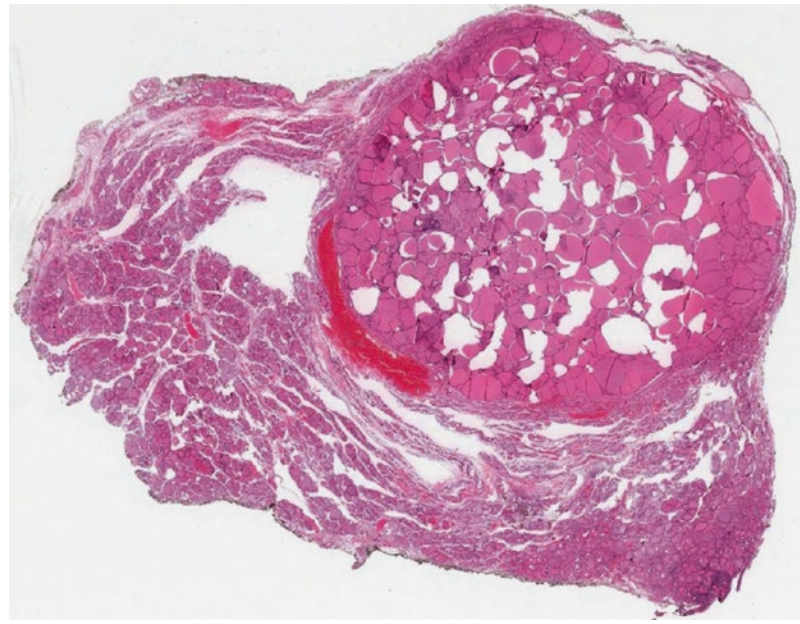

Fig. 4 Follicular adenoma with thin capsule and macrofollicular growth pattern

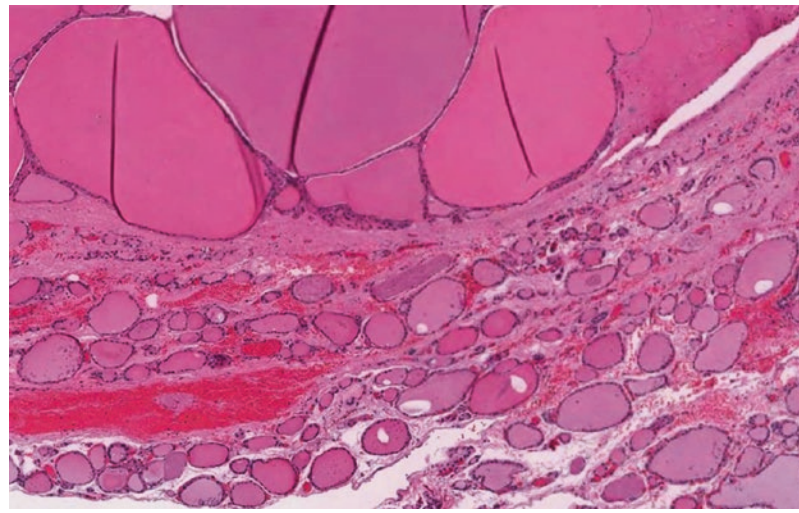

Fig. 5 Same lesion as Fig. 1. Note smooth capsular edge without invasion

with oncocytic (usually Hürthle cell) lesions biopsied with larger than 18-guage needles.

Special Subtypes of Adenoma

Oncocytic or Hürthle Cell Adenoma

This is essentially a follicular adenoma in which at least 75% (usually 100%) of the cells comprising the lesion are eosinophilic in their cytoplasm which may be granular. The nuclei of these cells are large and rounded and show prominent centrally located nucleoli. Some of these tumors show the cytology of unequivocal Hürthle cells, whereas others show just eosinophilic cytoplasm. These lesions are surrounded by a complete capsule, and as long as there is no invasion of the capsule of blood vessels, they behave in a benign fashion [29–31].

So-called papillary hyperplastic nodules ("papillary adenoma," follicular adenoma with

exuberant papillary hyperplasia) (Figs. 6 and 7) are clonal proliferations and therefore neoplasms. They are characterized by encapsulation, are often centrally cystic, and show papillary proliferations often with edematous papillae containing small benign follicles. The nuclei are round dark and polarized often to the base of the proliferating cells. The great majority of such lesions occur in young women around the age of menarche; a small minority about 10–15% are associated with hyperthyroidism, whereas another 25 or 30% show abnormal thyroid function especially low TSH [22, 25].

Fine-needle aspiration biopsy cannot distinguish between follicular adenoma and carcinoma (same applies to oncocytic lesions); both demonstrate similar cytomorphology and most are diagnosed as follicular neoplasm (Figs. 8 and 9). Several authors have shown that, at most, 20–30% of cases diagnosed as "follicular neoplasm" are diagnosed as malignant on histological examination, and the rest are either follicular adenomas or cellular adenomatoid nodules, i.e., benign [32, 33]. Interestingly half of the malignant cases are diagnosed as follicular variant of papillary thyroid carcinoma [33]. However, these rates will change as reclassification of noninvasive follicular variant of papillary thyroid carcinoma as neoplasm rather than carcinoma [34, 35]. FNA specimens obtained from an oncocytic follicular neoplasm usually demonstrate a monotonous population of oncocytic cells arranged in cohesive groups/tissue fragments and as single cells. Random nuclear atypia in the form of nuclear enlargement, multinucleation, cellular pleomorphism, and prominent "cherry red" nucleoli is commonly observed in oncocytic follicular lesions. Intranuclear grooves are common in nonpapillary oncocytic follicular lesions; however, the nuclei maintain a round shape with prominent nucleoli, and other major diagnostic features of papillary carcinoma are not seen. It has been shown that the presence of intra-cytoplasmic lumens and transgressing vessels is common in FNA specimens of neoplastic oncocytic follicular lesions [36].

Hyalinizing Trabecular Tumor (HTT)

This controversial lesion, originally described by Dr. Carney and his colleagues from the Mayo Clinic as "hyalinizing trabecular adenoma," is a circumscribed but nonencapsulated tumor comprised of trabeculae of follicular cells containing nuclei with exag-

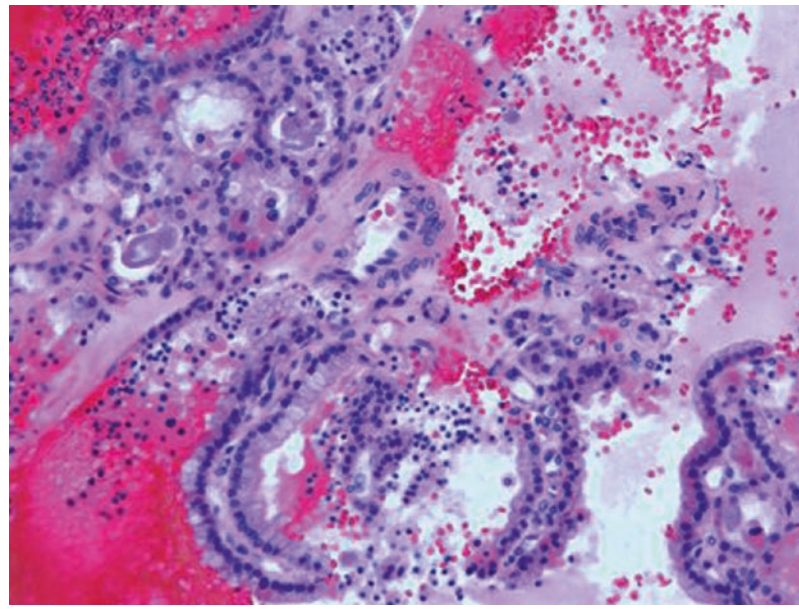

Fig. 6 Papillary hyperplastic nodule. Note polarized even nuclei

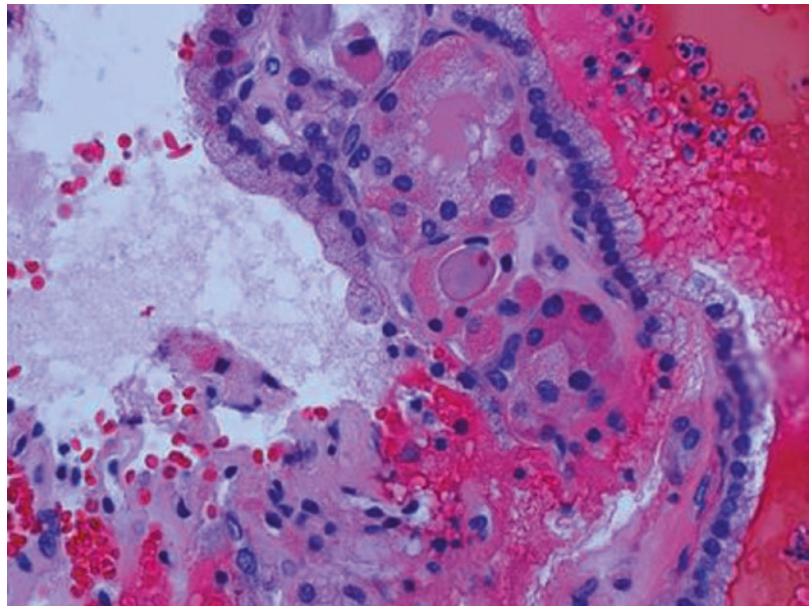

Fig. 7 Same case as Fig. 3. Upper left shows cystic area. Nuclear polarization is well illustrated

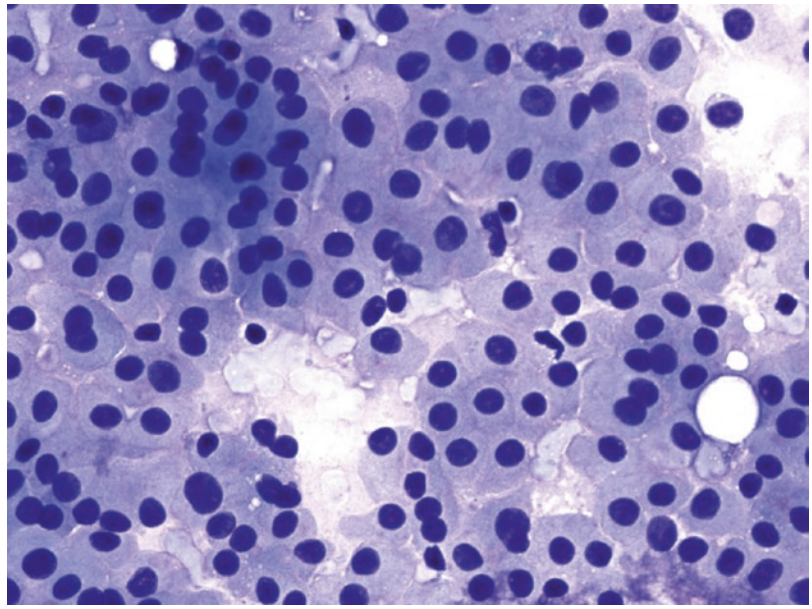

Fig. 8 Follicular neoplasm with oncocytic features. Monotonous population of cells with eosinophilic cytoplasm and round nuclei (Diff-Quik air-dried smear)

gerated features of papillary carcinoma (elongation, intranuclear inclusions, intranuclear grooves) (Figs. 10 and 11). The stroma intervening between and among the trabeculae is fibrous and hyalinizing [37]. Within the tumor cells in about 60% of cases, there are so-called yellow bodies presumably a degenerative change. In addition, the cytoplasm of the tumor cells may stain with antibodies to MIB–1 (but the conditions of this stain are unusual for a positive reaction to take place) [38]. These lesions may be multifocal; they may occur in the background of chronic lymphocytic thyroiditis; and at least 30% of them will show a papillary thyroid carcinoma in the background thyroid.

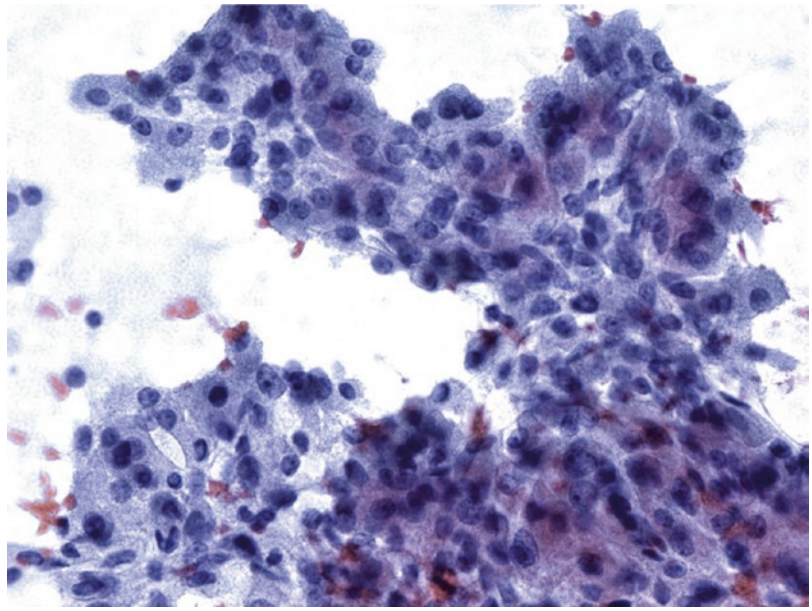

Fig. 9 Same case as Fig. 8. The corresponding alcohol-fixed smear shows the same features and prominent nucleoli (Papanicolaou stain)

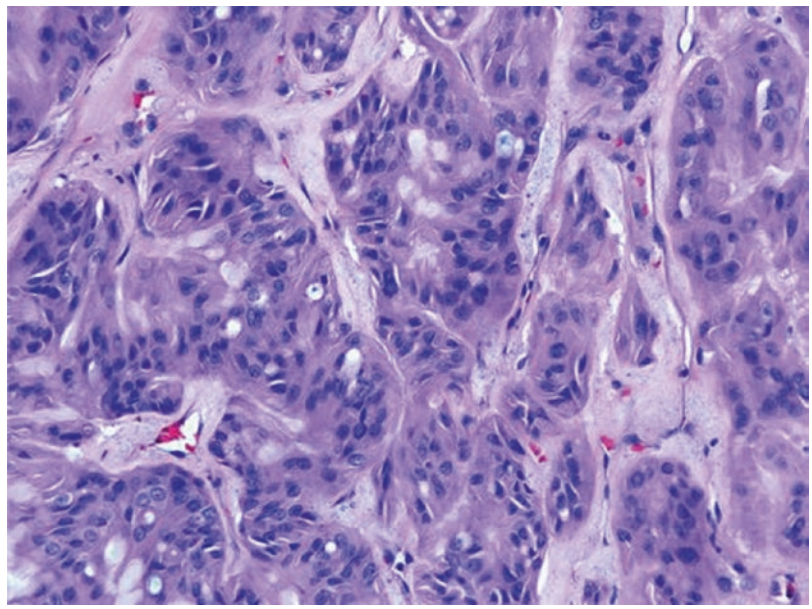

Fig. 10 Hyalinizing trabecular tumor. Note trabecular growth pattern and large irregular nuclei

The controversy with this lesion is whether or not they represent an unusual variant of papillary carcinoma although there have been only rare reports of metastases from these lesions. (In the opinion of the current authors, the examples of HTT with evidence of metastases are not actually HTT but are actually papillary thyroid carcinoma and are not classic hyalinizing trabecular tumors.) Molecular analysis has shown that classic HTT does show rearrangements in ret proto-oncogene but no mutations in Braf 600Ve [39]. In deference to the controversy, the World Health Organization classification in 2004 [40] termed these lesions "hyalinizing trabecular tumor" rather than

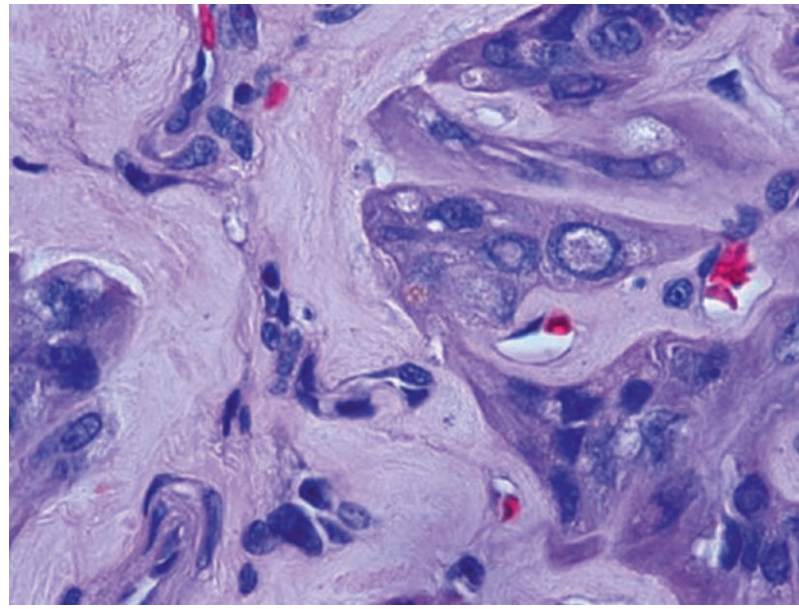

Fig. 11 Higher power of Fig. 5. Note large nuclei with intranuclear inclusions

"hyalinizing trabecular adenoma" or "hyalinizing trabecular carcinoma."

The fine-needle aspirates of HTT show cohesive lesional cells with easily identifiable well-formed intranuclear inclusions and grooves embedded or closely associated with an acellular matrix. These are often diagnosed as suspicious for or compatible with papillary thyroid carcinoma due to the presence of diagnostic nuclear features [41, 42].

Malignant Lesions of the Thyroid: Carcinomas

The vast majority of malignancies of the thyroid gland are carcinomas. Most of these are of follicular cell derivation, and as seen in Table 2, they comprise a variety of histologic patterns with variable prognostic risks. These will be discussed below.

Table 2 Carcinomas of the thyroid of follicular derivation

Considered "low risk"
Encapsulated, non-invasive, papillary carcinoma, including follicular variant
Classic papillary carcinoma
Warthin variant papillary carcinoma
Spindle cell variant papillary carcinoma
Papillary micro-carcinoma
Macrofollicular variant papillary carcinoma
Minimally invasive (capsule only) follicular carcinoma
Considered "intermediate risk"
Diffuse sclerosis variant papillary carcinoma
Solid variant papillary carcinoma
Hürthle cell carcinoma, minimally invasive
Tall cell variant papillary carcinoma
Columnar cell variant papillary carcinoma
Invasive follicular variant papillary carcinoma
Considered "high risk"
Angioinvasive follicular carcinoma
Angioinvasive Hürthle cell carcinoma
Hobnail variant papillary carcinoma (especially associated with micropapillary carcinoma)
Poorly differentiated carcinoma
Anaplastic (undifferentiated) carcinoma

Papillary Thyroid Carcinoma

Papillary thyroid carcinoma (PTC) is the most common malignancy of the thyroid and of the entire human endocrine system. Common and uncommon histological variants comprise about 80–85% of thyroid cancers [43–45].

Classical papillary carcinoma can present at any size including minute microcarcinomas (<0.1

cm) and ranging up to several centimeters. In the modern era, most clinically evident carcinomas are in the 1.5–3 cm range [43, 45].

Grossly, PTC of usual or classic type can appear as a circumscribed (rarely encapsulated) nodule or more commonly as a firm infiltrative tumor mass. Some PTCs show calcification and even ossification. In a few lesions, cyst formation may be present and in fact PTC may be entirely cystic [46, 47].

Microscopically, PTC is comprised of papillae and follicles with a wide variation in which pattern predominates. In those with a majority papillary pattern, the papillae are composed of fibrovascular cores lined by 1–2 layers of tumor cells (Figs. 12, 13, 14, 15, and 16). The cells themselves show amphophilic cytoplasm (rarely this is clear) and the characteristic nuclei (Fig. 17) which allow for both cytologic and histologic diagnosis of this entity. The nuclear features (listed in Table 3) include enlargement, oval shape, intranuclear grooves and intranuclear cytoplasmic inclusions as well as thick nuclear membranes and eccentrically located nucleoli. (It is important to note that some of these nuclear features may be seen in non-papillary thyroid tumors (benign and malignant) and in reactive or reparative sites; however, the constellation of nuclear features is characteristic of papillary carcinoma [47, 48].)

The majority of PTCs show an infiltrative pattern of growth and invade the surrounding thyroid tissue. Lymphatic invasion is commonly noted. An important diagnostic feature, the psammoma body which represents a lamellated calcification produced by gradual infarction of a papilla, is commonly seen within the tumor stroma and within lymphatics (Fig. 18). The presence of psammoma bodies in lymphatics in areas of thyroid removed from the main tumor mass may be associated with regional node metastases. The presence of only psammoma bodies in a neck lymph node is diagnostic of metastatic papillary carcinoma even if no viable tumor cells are present [49–51]. In addition, sometimes the primary tumor will consist solely of a fibrous scar with occasional psammoma bodies within it; such lesions represent totally involuted papillary carcinoma.

The concept of multifocality in PTC has created some controversy. The main question is whether the tumor in these cases reflects true multiclonal origin in several sites in the thyroid or multiple foci of intraglandular intralymphatic spread [52–55]. Evidence suggests that both may

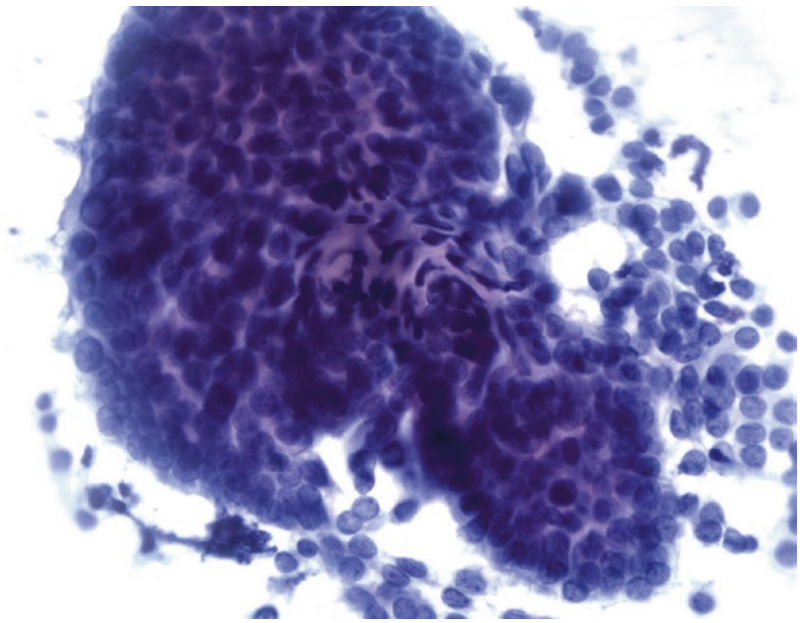

Fig. 12 Papillary thyroid carcinoma. A case of papillary carcinoma diagnosed on fine-needle aspiration shows papillary formation (Papanicolaou stain)

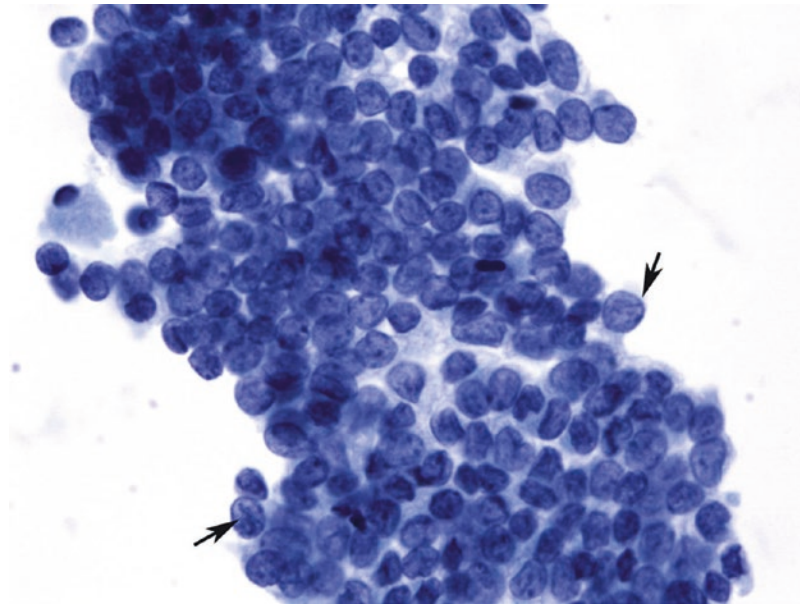

Fig. 13 The lesional cells demonstrate elongated nuclei with chromatin clearing and delicate intranuclear grooves (arrows, Papanicolaou stain)

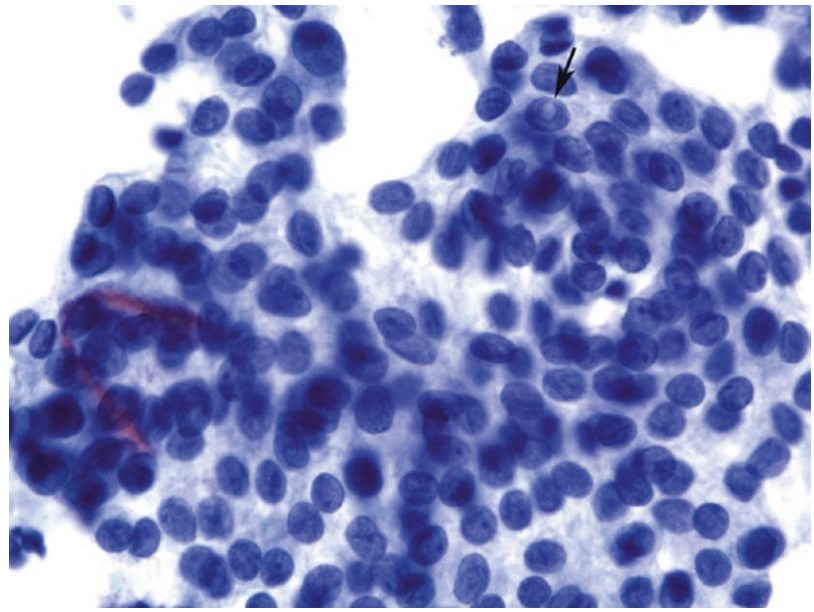

Fig. 14 Papillary thyroid carcinoma. Intranuclear pseudoinclusions are shown (arrows, Papanicolaou stain)

occur although in most classic PTC the second option is more common [55–57].

Another common finding in PTC is the presence of lymph node metastases which occurs in over 50–60% of patients at the time of diagnosis. In some cases, the nodal metastases are tiny and subcapsular and truly "micrometastases (<0.2cm)"; in others the nodal disease is large and may be the presenting symptom of the cancer [50, 58, 59]. In large nodal metastases, large areas of cyst formation may occur, and the clinical misdiagnosis of "branchial cleft cyst" may be given [60].

The cytomorphologic diagnosis of PTC is based on major and minor diagnostic criteria. The major diagnostic feature is the characteristic nuclear morphology regardless of the features of cytoplasm, growth pattern, special stains, and

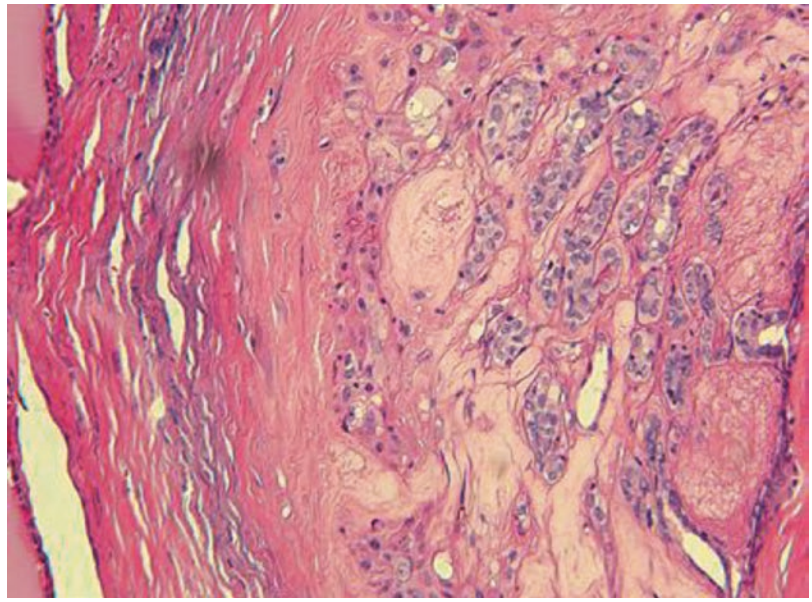

Fig. 15 Typical sclero-elastotic stroma seen in some examples of papillary thyroid carcinoma

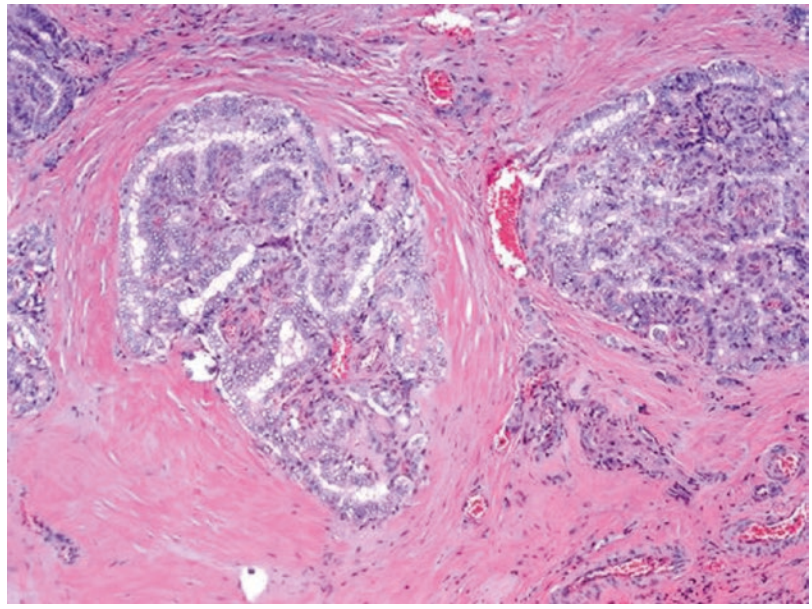

Fig. 16 Papillary carcinoma with papillary growth pattern and fibrosis

immunohistochemical markers. This holds true for a majority of cases of PTC; however, some variants of PTC may be difficult to diagnose due to the lack of some of the nuclear features. The FNA specimen of PTC is usually cellular and shows tumor cells arranged in papillary groups, three-dimensional clusters, or as single cells in a background of watery or thick "ropy" colloid (aka chewing gum colloid), nuclear or calcific debris, macrophages, and stromal fragments. The cell groups may show a typical concentric arrangement of lesional cells described as "cellular swirls" (Figs. 12, 13, and 14).

The individual tumor cells are enlarged; the nuclei show elongation, membrane thickening, chromatin clearing, grooves, and inclusions [61].

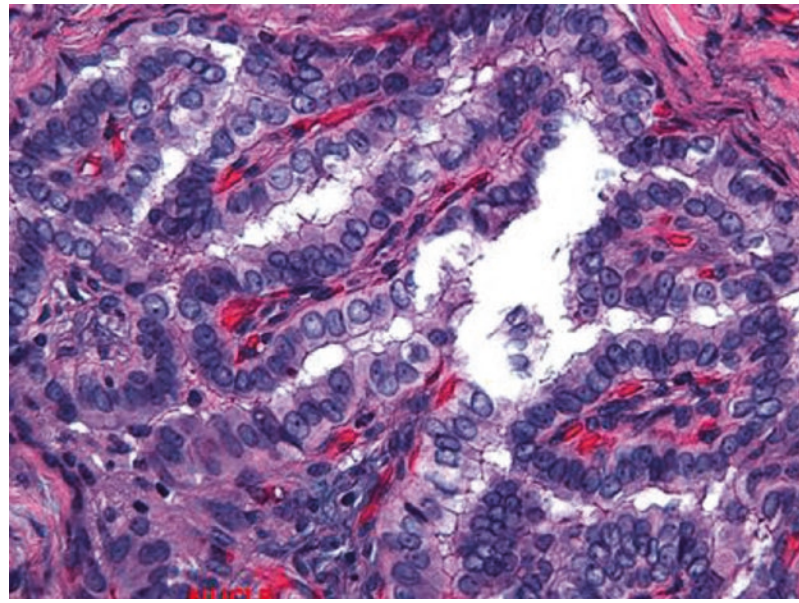

Fig. 17 The classic nuclei of papillary carcinoma lining papillae with fibrovascular cores

Table 3 Nuclear features of papillary carcinoma of thyroid

Enlargement
Elongation (oval shape)[a]
Thick nuclear membranes
Small nucleoli near or attached to thick nuclear membrane
Intranuclear inclusions
Intranuclear grooves extending across or almost across nucleus
Nuclear overlapping

[a]The follicular variant of papillary carcinoma and the solid variant (solid follicular variant) often show rounded nuclei

The nucleoli are usually small and eccentric. Multinucleated histiocytes are common in FNA specimens of papillary thyroid carcinoma. These can be variable in size, shape, and number of nuclei. Squamous metaplasia can be seen in FNA specimens of papillary thyroid carcinoma; however, it is more common in cases with cystification [62].

Papillary Carcinoma Variants

Numerous papers have described variants and subvariants of papillary thyroid carcinoma. Some of these point out the diagnostic difficulties in recognizing these lesions; some however give indication of prognostic differences and point the clinical relevance of the recognition of these subtypes [63]. The following section will discuss those variants which have clinically relevant importance, their frequency, histological features, and prognostic meaning.

Tall Cell Variant Papillary Carcinoma

The tall cell variant has been defined differently over the years since it was originally described. In the modern era, the tall cell is described as a thin cell with eosinophilic cytoplasm in which the length of the cell is three times its width [40, 48]. The nuclei are those of papillary carcinoma, and in many examples, they show multiple intranuclear inclusions (this finding in an FNA sample may allow the cytopathologist to suggest the diagnosis of tall cell papillary carcinoma) (Figs. 19 and 20) [64]. A second controversial issue in the definition of this subtype of carcinoma has been the amount of tall cell cytology needed for a diagnosis of "tall cell variant papillary carcinoma." Percentages of tall cell morphology have ranged from 10 to 70% [40, 48, 65]. A study from Memorial Sloan Kettering Cancer Center of almost 500 cases indicated that the diagnosis

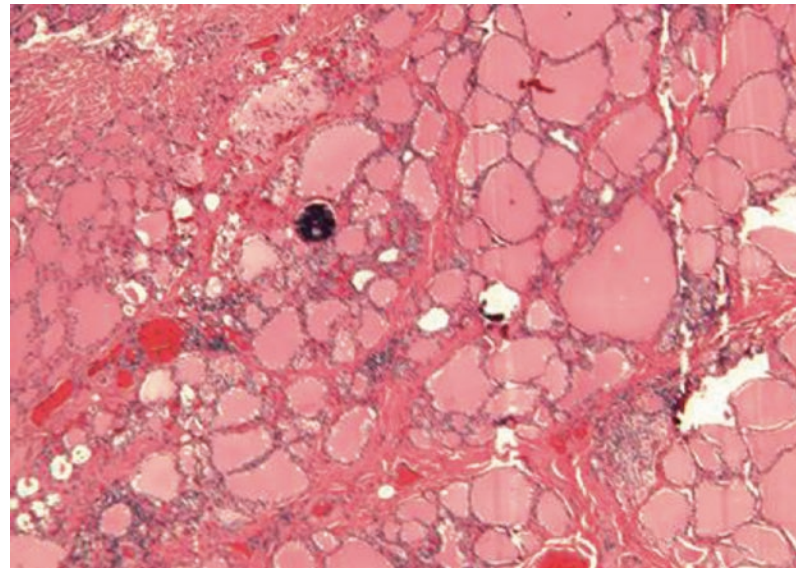

Fig. 18 Psammoma body to left of center. It is in a lymphatic space

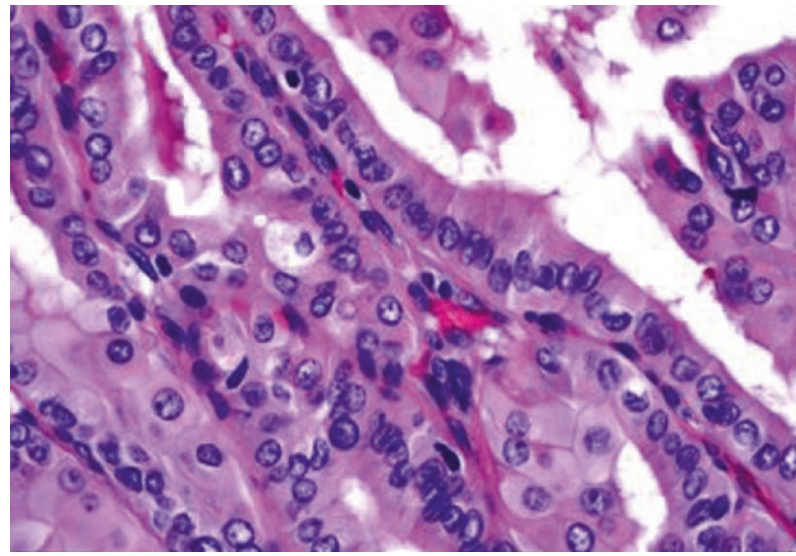

Fig. 19 Tall cell PTC. Note voluminous eosinophilic cytoplasm

"tall variant papillary carcinoma" is appropriate if 30% or more of the tumor has tall cell features, whereas a diagnosis of "papillary carcinoma with tall cell features" is appropriate for those tumors that show 10–30% tall cell morphology [65]. However, this paper admonishes pathologists to mention any amount of tall cell features since in recurrences and/or metastatic foci, the percentage of tall cell change may increase [65].

It is critical to point out that the identification of tall cell morphology can be problematic for pathologists. Hence, in our experience, about 40% of tall cell papillary carcinomas or tall cell features in these tumors are not recognized [66]. Similarly, overdiagnosis of oncocytes in benign nodules or even in Graves' disease can cause diagnostic errors.

Tall cell papillary carcinoma tends to occur in older individuals and is often a large tumor which is extraglandular in extent. Lymphatic as well as vascular invasion (up to 20% of cases) is noted in this variant. The vascular (venous) involvement

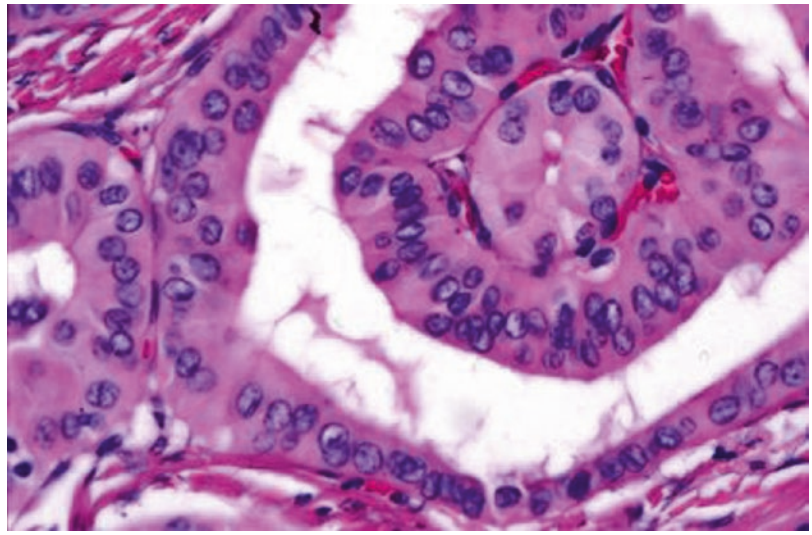

Fig. 20 Tall cell variant PTC. Note nuclei with loss of polarization

is often found in the extrathyroidal component of the tumor; this is also the one subtype of papillary carcinoma in which perineural invasion may be found [67–69].

Lymph node metastases are often present and extranodal involvement is identified in many cases. Extracervical metastases to the lungs, pleura, bone, and brain may also develop [69, 70].

A small number of patients with tall cell papillary carcinoma will progressively develop changes of less differentiated tumor (often with spindle cell areas associated with hemorrhage), and these are found in recurrent or metastatic foci. Some of these patients will eventually develop anaplastic transformation usually with a spindle cell squamous component [71].

Molecular studies have shown that over 70% of tall cell papillary carcinoma harbor Braf V 600E mutations [72, 73], the highest percentage of any subtype of papillary cancer (the one exception is in patients of Asian heritage [74]).

The cytologic samples from tall cell variant of PTC contain elongated cells with sharp cytoplasmic borders, granular eosinophilic cytoplasm, and variably sized nuclei with nuclear features of papillary carcinoma. The diagnostic nuclear features of PTC are readily found in aspirates of this variant. The intranuclear inclusions can be multiple within the same nucleus giving rise to a "soap-bubble-like" appearance [64].

Columnar Cell Variant Papillary Carcinoma

This variant is rare, and some reported examples show an intermingling with tall cell papillary carcinoma [63, 75, 76]. When originally described, it featured a predominance in male patients with extra thyroidal tumors and a rapidly fatal course [77]. However, additional reports indicate that this tumor if contained within the thyroid may have a less worrisome prognosis than originally thought [78].

Morphologically the tumor is composed of papillae and trabeculae lined by cells with stratified nuclei. Portions of the tumor may show cytoplasmic clearing which in conjunction with the nuclear stratification resembles the morphology of normal early secretory endometrium [48].

A curious and still unexplained phenomenon is the finding in over 50% of cases studied of nuclear localization of CDX2 protein, a growth factor predominantly found in the gastrointestinal tract. Virtually no other thyroid cancer shows this finding [79, 80].

Cytologic preparations of this tumor demonstrate cohesive cell fragments with a prominent papillary architecture. The tumor cells appear columnar in shape with pale cytoplasm which tapers at one end. Nuclear palisading and stratification are prominent at the periphery of papillary

fragments. Intranuclear grooves and intranuclear inclusions are rare as are psammoma bodies and multinucleated tumor giant cells. Due to the scarcity or lack of diagnostic nuclear features, the aspirates of CCV-PTC can be mistaken for medullary carcinoma or metastasis (especially from the colon) to the thyroid gland [80].

Hobnail Cell Variant Papillary Carcinoma

This recently described subtype of papillary carcinoma is characterized by papillary growth, and the cells lining the papillae are large oncocytic and have appropriate nuclei. However, the luminal surface of the cells shows apocrine-like secretion giving the "hobnail" appearance [81–83]. The lesions described have been large, often extrathyroidal, with aggressive clinical behavior (50% mortality at 5 years). Over half of the described cases are Braf V 600E mutated. Some show associated tall cell features and a few have micropapillary pattern [84].

Diffuse Sclerosis Variant

This variant is rare comprising about 10% of cancers in the pediatric thyroid tumor epidemic that occurred following the Chernobyl nuclear accident [85]. This variant of papillary cancer disproportionately affects young individuals (often females who are teenagers or in their early 20s); the gland is frequently diffusely enlarged and very hard. A dominant nodule may be present, but often the tumor is found diffusely infiltrating gland stroma and lymphatics without a main tumor mass [86, 87]. The tumor has a small number of papillae, prominent squamous or epidermoid metaplasia, and numerous psammoma bodies (which are responsible for the firm to hard gland). The background thyroid demonstrates well-developed lymphocytic thyroiditis (which is histologically and immunologically identical to autoimmune thyroiditis) [87, 88]. One case of diffuse sclerosis variant of papillary carcinoma has been reported in a gland involved by Graves' disease [89].

Because of the extensive lymphatic invasion in the gland and in the surrounding extrathyroidal tissues, virtually 100% of patients with this tumor subtype have regional node metastasis at diagnosis, and 25% of cases show lymphangitic spread to the lungs. However, although it is difficult to get data on many patients with long-term follow-up, it appears (perhaps because the patients are so young) that the patients live many years often with the presence of pulmonary metastases which can respond to radioactive iodine therapy and remain stable [90, 91].

The FNA specimens of diffuse sclerosis variant of PTC show tumor cells with nuclear features of papillary carcinoma arranged in nests and numerous psammoma bodies. Some cases may also demonstrate a brisk lymphocytic infiltrate around the tumor cell groups and in the background. Squamous metaplasia is commonly seen in aspirates of this tumor [92, 93].

Follicular Variant Papillary Carcinoma

Of all the variants of papillary thyroid carcinoma, none has elicited more controversy than the follicular variant. Its definition varies from tumors which are totally follicular in pattern to those that have 20 or even 50% papillary growth [94]. Recent published studies have separated those lesions which have a nonencapsulated invasive growth pattern and those that show complete or partial encapsulation and/or circumscription [95].

The encapsulated lesions which show capsular and/or vascular invasion are considered true carcinomas, but despite nuclear features of papillary carcinoma, they rarely spread to regional nodes and metastasize via the hematogenous route particularly bone. Some pathologists in the past have considered these lesions a type of "follicular carcinoma" ignoring the nuclear morphology [94].

The results of extensive molecular analysis through the Cancer Genome Atlas Project have shown that at the molecular level, the encapsulated follicular variant lesions are more closely related to true follicular tumors and not to papillary carcinoma [96]. (Hence whereas the infiltrative lesions can show Braf V600 E mutations and

ret/PTC translocations as do classic papillary carcinomas, the encapsulated lesions demonstrate mutations in RAS and translocations in PAX 8/PPAR gamma.)

The most intense debate has centered on the totally encapsulated or circumscribed and *noninvasive* lesions which fall into the "follicular adenoma" group of tumors except for the presence of papillary nuclear features which may be multifocally present within the lesion or diffusely distributed in the tumor. Many studies by endocrine pathologist "experts" have shown large interobserver diagnostic variability [97–99]. Since many such noninvasive lesions show no aggressive behavior, a recent international study of such lesions recognized their indolent clinical course and suggested removing the "carcinoma" diagnosis from such cases. The proposed diagnostic term is NIFT-P (noninvasive follicular tumors with papillary-like nuclei) that is supported by the studied series of cases showing no metastasis or recurrence with a median follow-up of 14 years (even in the absence of total thyroidectomy and radioiodine) [100]. The new diagnostic designation confirms the clinical behavior while recognizing the papillary carcinoma nuclear features (Figs. 21 and 22).

The cytologic interpretation of follicular variant of PTC can be challenging due to a paucity of diagnostic nuclear features. The cytologic samples from FVPTC usually show enlarged follicular cells arranged in monolayer sheets and follicular groups in a background of thin and thick colloid. The individual tumor cells show nuclear elongation, chromatin clearing, and thick nuclear membranes. The intranuclear grooves in FVPTC are delicate and do not traverse the entire length of nucleus; however, nuclear grooves and inclusions are very scarce. Thus, a majority of cytologic samples of FVPTC are either diagnosed follicular neoplasm or as suspicious for papillary carcinoma [34].

Other Variants with Similar Prognosis to Classic Papillary Carcinoma

A number of histological variants of papillary carcinoma share clinical behavior with classic papillary cancer of similar stage. These include Warthin-like variant a usually circumscribed lesion often arising in a thyroiditic gland and showing oncocytic cytology and extensive lym-

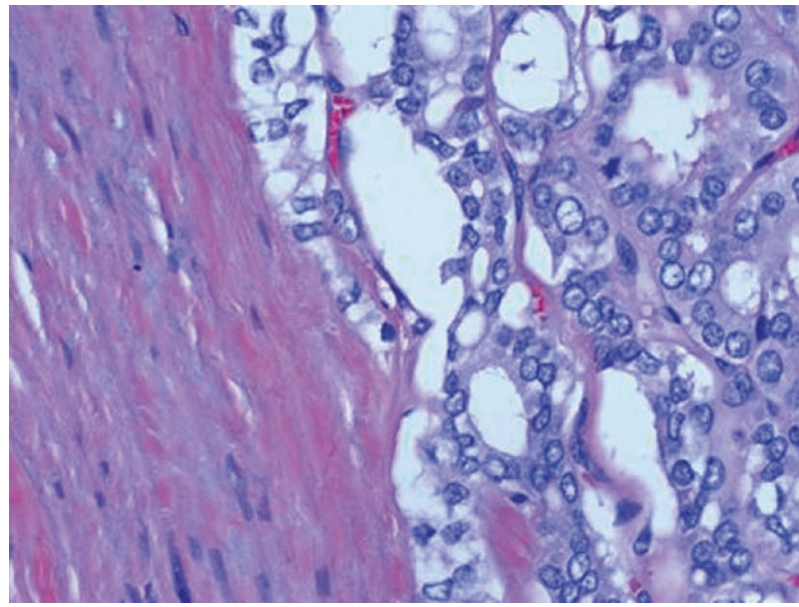

Fig. 21 Encapsulated follicular variant of papillary carcinoma. The capsule of the lesion is not invaded in this portion of the tumor, although invasion was noted in other areas. Not classic nuclei of papillary carcinoma

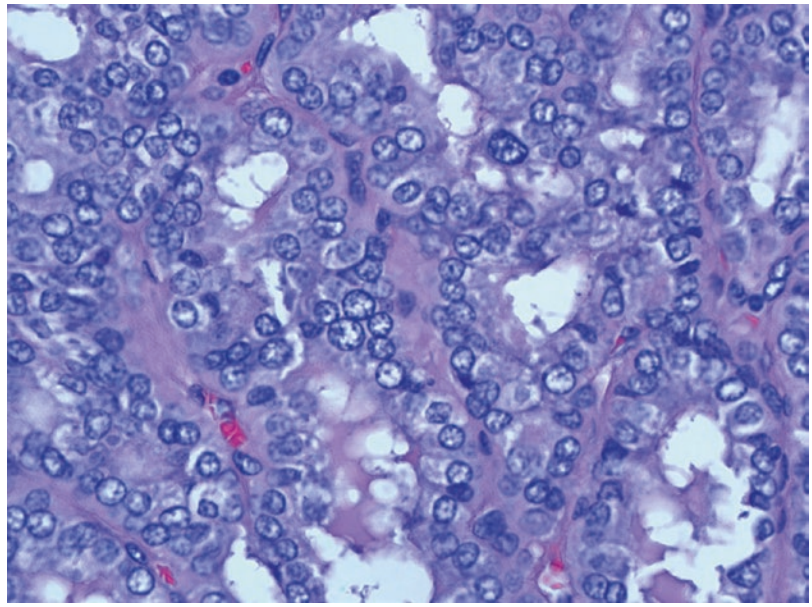

Fig. 22 Center of an encapsulated follicular neoplasm. Nuclei are those of papillary carcinoma. If no invasion is present in the capsule, this would be diagnosed as "noninvasive follicular thyroid neoplasm with papillary like nuclei or NIFT-P"

phoplasmacytic infiltrate in the papillary cores of the tumor [101, 102]; Papillary carcinoma with fasciitis like stroma or spindle cell metaplasia in which part of the tumor is composed of a bland spindle cell proliferation which shows TTF1 positivity by immunostaining; rare mitotic activity and which blends with the epithelial component showing classic papillary cancer morphology [103–105]; and papillary carcinoma with lipomatous stroma in the cores of the papillae [106]. Finally the macrofollicular variant of papillary carcinoma needs to be noted. Such lesions resemble adenomatous or hyperplastic nodules but demonstrate multifocal nuclear features of papillary carcinoma. These are extremely low-grade lesions, and although a few reported cases have shown regional node spread, none has been documented to show distant metastases [107, 108].

Other Variants with Worse Prognosis than Classic Papillary Carcinoma

There are a few additional histologic variants of papillary carcinoma that have a more aggressive clinical course; these are very rare lesions and do not occur with the frequency that tall cell, columnar, or even hobnail types do [109]. The trabecular variant was originally considered as a more aggressive lesion; however, too few cases are reported with long-term follow-up for a definitive assessment [110]. In addition, pathologists must be aware that most trabecular patterned thyroid carcinomas are either of C cell derivation or are poorly differentiated carcinomas (see below).

The micropapillary variant has been described in fewer than eight patients. However, similar to histologically identical tumors occurring in the ovary, breast, and urinary bladder, these lesions avidly invade thyroid and lymphatics and spread throughout the body rapidly with an associated high mortality [111].

Variants in Which Prognostic Influence Is Not Known

There are some papillary carcinoma types in which because of rarity or confusion over diagnostic criteria, data on outlook is not known.

The solid variant of papillary carcinoma is a common subtype in children, and its features have been elucidated and summarized by studies of the cases that were identified following the Chernobyl nuclear accident [63, 85, 112, 113]. Morphologically this tumor shows a nested pat-

tern of growth with minute follicles, a prominent vascular capillary network, and weak expression of thyroglobulin by immunostaining. Vascular and extrathyroidal invasion have been noted in studies of pediatric cases. This tumor is associated with radiation exposure and is distinctive in its molecular signature showing translocation in ret/PTC 3 [114, 115]. In adults, this tumor almost always occurs in a thyroiditic gland, and in our experience, the patients often have systemic autoimmune disorders.

Rare tumors that are distinct from tall cell variant or the Warthin-like variant of papillary carcinoma are oncocytic variant. These tumors are papillary and the lesional cells show oncocytic cytoplasm, but the other features of tall cell morphology are not present. Because of their infrequent occurrence, their behavior is not known [116]. Clear cell variant may show coexistence with oncocytic tumor cells. These unusual lesions are not well characterized as to clinical behavior and prognosis; the pathologist must be certain that they do not represent metastasis to the thyroid from clear cell renal carcinomas [117–119].

Immunohistochemistry of Papillary Carcinoma

It is beyond the purview of this chapter to extensively review the immunohistochemistry of thyroid papillary carcinoma. Almost all stain strongly and diffusely for thyroglobulin (cytoplasmic) and thyroid transcription factor-1 (TTF-1) (nuclear staining). About 70% show cell membrane staining for HBME-1; CK19 and galectin-3 staining is less reliable in our experience and may give false-positive results. Thyroid epithelium and its tumors stain for beta catenin (cytoplasmic) and PAX8 (nuclear) [74, 120–123].

Molecular Markers of Significance in Papillary Carcinoma

Characteristically papillary carcinomas of classic and tall cell type show a high frequency of Braf mutations (V600E); about 30–40% will show translocation or rearrangement in ret proto-oncogene; interestingly the two molecular changes are not seen in the same tumor—they apparently are mutually exclusive [72, 124].

Although some authors insist that the presence of Braf mutation indicates the tumor is a high-risk lesion, not all agree. It appears that if additional mutations such as TERT or p53 are present, it is these that predict a clinically aggressive course [125–128].

The encapsulated forms of follicular variant of papillary carcinoma show a different molecular signature. Similar to follicular adenomas and carcinomas, the follicular variant lesions show mutations in the RAS family of genes or show translocation in PAX8/PPAR gamma [96].

Follicular Carcinoma

True follicular carcinomas of the thyroid are rare lesions in countries with sufficient or excess iodide in the diet. In the United States, these tumors comprise 5–10% of all thyroid cancers [129].

Such tumor present as solitary masses and can occur in any part of the gland (including in substernal/mediastinal extensions of the thyroid). They are rarely microcarcinomas (1 cm or less) and range in size from 2 to several centimeters. Grossly, they most often appear as encapsulated tumors and can resemble adenomas. On occasion, careful macroscopic examination will disclose foci of capsular invasion. The rare widely invasive subtype may show some nodules with partial encapsulation, but it usually displays multiple nodules within the gland or in extraglandular soft tissue. Gross vascular invasion may be noted intraoperatively.

Based on these appearances, follicular carcinomas have been divided into two or three groups: Minimally invasive versus widely invasive or minimally invasive, grossly encapsulated, angioinvasive, and widely invasive types.

The encapsulated follicular carcinoma shows a thick capsule (1mm or thicker) that surrounds the lesional cells. The cells can be arranged in follicles (most commonly microfollicles although macrofollicular variants are known to occur) admixed

with solid or even trabecular areas. The sine qua non of the diagnosis of follicular carcinoma is the presence of invasion. Controversy surrounds what constitutes sufficient invasion to diagnose follicular carcinoma: some authors indicate that there must be trans-capsular invasion and invasion into the capsule is insufficient for a malignant diagnosis [48]. Others believe any capsular invasion is sufficient although such cases rarely give rise to metastatic disease [26, 130]. It is imperative that the pathologist be aware of the changes that can occur after biopsy of thyroid nodules including "pseudoinvasion of the capsule"; this is a geographic (linear) extension of tissue (including tumor cells, histiocytes, lymphoid cells, and endothelial cells usually with associated hemosiderin) through the capsule [131].

Studies have shown that lesions which show only capsular invasion rarely behave aggressively in the patients who have had at least 10 years of follow-up. However, tumors with vascular (venous) invasion are the group that shows hematogenous metastasis (most to the bone, lungs, brain, or liver). There is some dispute as to whether tumors that have four or fewer foci of venous invasion harbor high risk. It has been shown that follicular carcinomas with over four foci of venous involvement can metastasize and often this occurs within the first 5 years of diagnosis [95] (Fig. 23).

It is critical for pathologists to recognize that vascular invasion to be meaningful must be found in the capsule or in the neighboring thyroid (or even in extrathyroidal tissues); what may appear to represent vascular invasion within the substance of the tumor is not important since such a finding alone has not been shown to be associated with metastasis.

Hürthle Cell Carcinoma

Although many pathologists including the present authors believe that Hürthle cell tumors are different from non-Hürthle follicular counterparts, some authors group Hürthle cell carcinomas with follicular ones [129]. From the gross appearance and the histological criteria required to separate benign from malignant tumors, similarity exists [132]. However, there is a correlation between tumor size and risk of malignancy in Hürthle cell lesions; some Hürthle cell tumor can metastasize to regional nodes; the tumors are often larger than usual follicular cancers and have more venous invasion. In addition, a peculiar finding in Hürthle cell carcinomas is the finding in extrathyroidal extension or in post-thyroidectomy neck recurrences to present as nodules mimicking lymph nodes but in reality representing intravenous tumor thrombi [133].

Finally, some pathologists do not separate tumors composed of Hürthle cells (eosinophilic tumor cells with granular cytoplasm) (Fig. 24), which at the ultrastructural level contain numerous enlarged, abnormal mitochondria from oncocytic cells (delicate abundant eosinophilic minimally granular cytoplasm with modest numbers of mitochondria) [134–136]. It is unclear if oncocytic follicular carcinoma shares identical clinical risk with true Hürthle cell carcinomas.

It is important to remember that although 3–4 decades ago much of the literature indicated that all Hürthle cell neoplasms were potentially clinically malignant, numerous studies from various laboratories have shown that application of criteria for invasion in these tumors separates benign lesions from carcinomas. The size of the neoplasm does matter, and in our experience, tumors 4 cm or greater have an 80% risk of demonstrating invasion; the work of Chen et al. showed that tumors of 3.5 cm or larger harbored a 67% risk of malignancy [137]. However, we have also reported on the fact that small Hürthle cell cancers can be aggressive lesions as well [138].

The number of invaded vessels in the tumor capsule in Hürthle cell carcinoma correlates with molecular signatures for these lesions (4 or fewer vessels versus more than 4) has been reported [139].

Poorly Differentiated Carcinoma

Until recently this group of tumors was poorly understood. Lesions from this group had been classified under the term "widely invasive

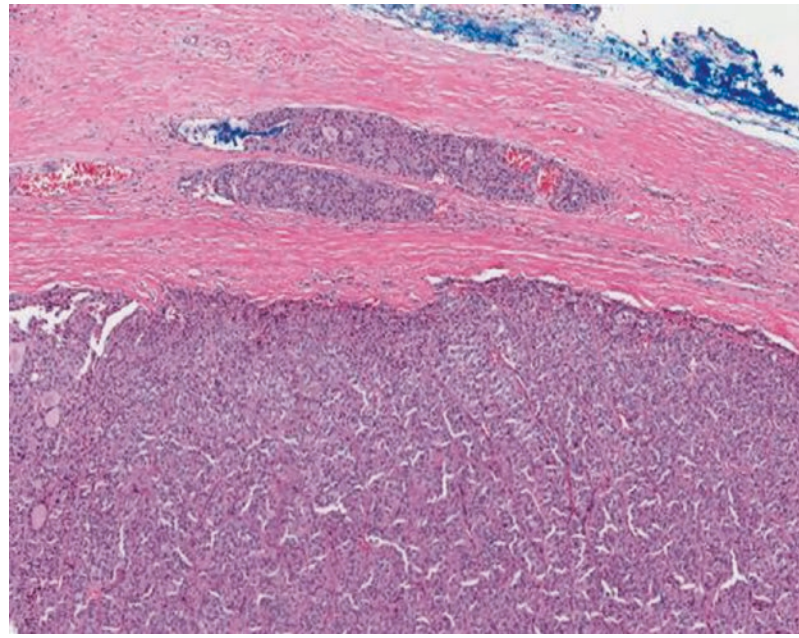

Fig. 23 Encapsulated very cellular follicular lesion with vascular invasion as noted. This is angioinvasive follicular carcinoma

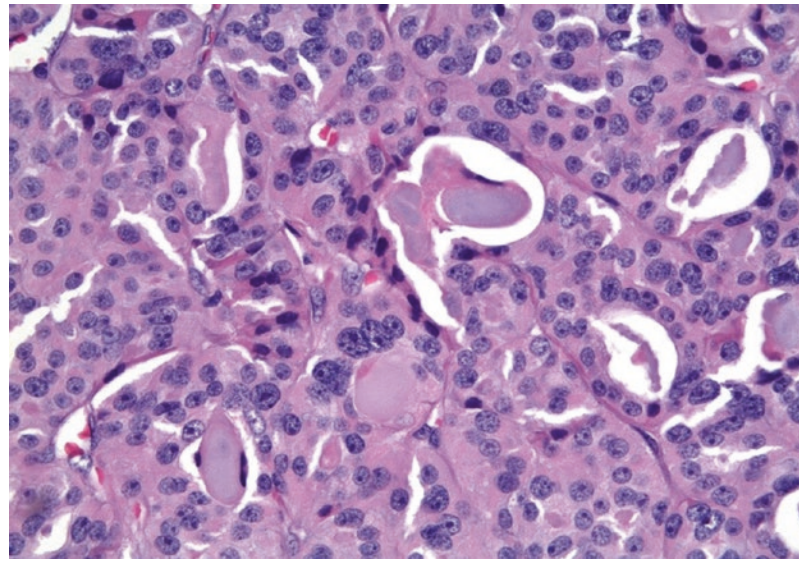

Fig. 24 Hürthle cell tumor. Note pleomorphism and multinucleation. This tumor showed vascular invasion and eventual lung metastases

follicular carcinoma." Several studies predominantly from Europe described the pathological features of these lesions, and a proposal known as the "Turin classification" offers a system for the recognition and diagnosis of these lesions [140–143].

The tumors in this category are often large extrathyroidal lesions with grossly noted hemorrhage and necrosis. Microscopically they show one, or more commonly a combination of growth patterns including solid, trabecular, and insular (insular is now considered a pattern of growth, and most do not use this term as a diagnosis) [48, 142, 143]. In addition to the growth pattern, other findings that place a tumor in this category are the presence of mitotic figures (at

least 3/10 high-power fields), abnormal mitoses, and coagulative tumor necrosis (distinction of true necrosis from infarction following biopsy is a crucial one for the pathologist to make) [144–146].

These tumors often show invasion into the thyroid and periglandular soft tissues; prominent vascular and lymphatic invasion is also present. The tumor cells are small to medium in size and do not show nuclei of papillary carcinoma despite a possible temporal association with a lower-grade lesion. In fact, many of these tumors are associated with low-grade lesions either in the past or concurrently at the time of diagnosis. The low-grade tumors may be any of the follicular-derived carcinomas: classical papillary carcinoma, follicular variant of papillary carcinoma, and follicular carcinoma/Hürthle cell carcinoma [142]. These examples strongly suggest that the poorly differentiated tumor is derived from the well-differentiated one (Fig. 25) [142]. A number of studies of poorly differentiated Hürthle cell carcinoma have been published and each has pointed out the finding of a small cell component in the tumors as they become poorly differentiated. It is important to recognize that these lesions may lose not only the ability to stain for thyroglobulin but also TTF-1 in metastatic sites; these results could be misinterpreted as the tumor not being of thyroid derivation and an incorrect diagnosis given [147].

The prognosis of poorly differentiated carcinoma is poor with about 50–60% mortality at 5 years after diagnosis from widely metastatic disease [148].

Aspirates of poorly differentiated thyroid carcinoma are usually cellular and demonstrate a monotonous population of cells arranged in large solid groups with cell crowding and overlapping, mitoses, and single-cell necrosis (apoptosis). On high-power examination, nuclear pleomorphism is readily evident. Endothelial wrapping of the tissue fragment can be seen in some cases. Since this growth pattern can also be encountered in MTC and secondary tumors of the thyroid such as metastatic neuroendocrine carcinoma, it is prudent to confirm the diagnosis of PDTC by performing immunostains for TTF-1, thyroglobulin, and calcitonin [149].

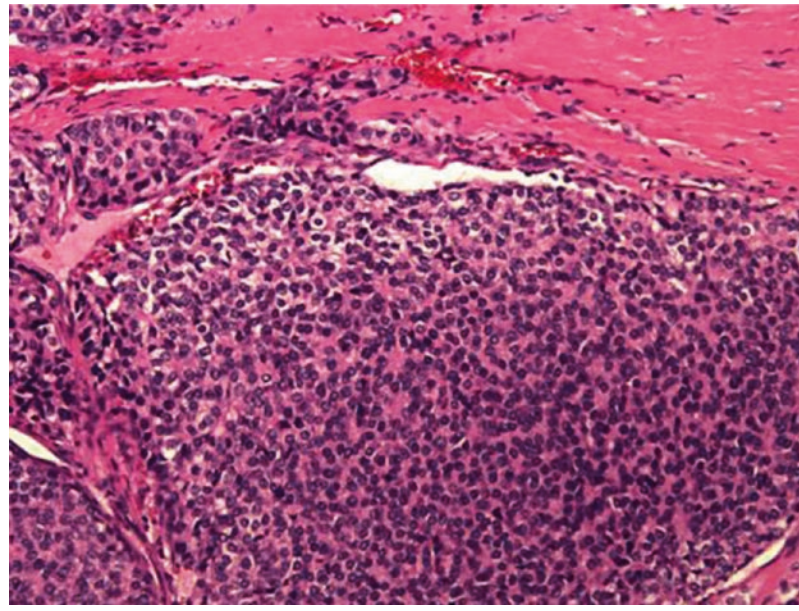

Fig. 25 Poorly differentiated carcinoma with solid growth pattern. Tumor necrosis and vascular invasion were present

Grading of "Differentiated" Thyroid Carcinoma, So-Called High-Grade Carcinoma

This concept attributed in separate studies to Drs. Akslen [150] and Tallini [148] is defined as a group of tumors (usually papillary) in which the lesion is recognized as a papillary carcinoma (papillae, appropriate nuclei), but there are high-grade features (mitoses, abnormal mitoses, and necrosis). Retention of recognizable well-differentiated tumor throughout the lesion distinguished this group of tumors from the poorly differentiated carcinoma group. There is little to no data on follow-up of this group of cases although one study suggests they are biologically aggressive when compared to usual papillary carcinoma (Fig. 26).

Anaplastic Carcinoma

One of the most virulent of all human tumors, anaplastic or undifferentiated carcinoma (similar in other systems to sarcomatoid carcinoma) is associated with a 90% 1-year mortality rate. Occurring in older patients, and showing massive extrathyroidal extension at presentation, most are unresectable, and diagnosis is accomplished by FNA, core, or wedge biopsy. These tumors which are believed to be derived from lower-grade differentiated carcinomas (papillary, follicular, or Hürthle cell) grow very rapidly with numerous mitoses, vascular invasion, and necrosis. The tumors which may metastasize to distant sites often kill by local invasion of the trachea [151]. Molecular studies have shown that these lesions often maintain the underlying changes seen in the lower-grade tumors with which they are associated but acquire additional mutations linked with aggressive biology (TERT, p53) [124, 152–156].

Hence there is a spectrum of thyroid cancers of follicular derivation ranging from tumors with an excellent prognosis to somewhat more aggressive clinical subvariants, to higher-grade tumors, poorly differentiated lesions, and finally anaplastic carcinoma (Fig. 27) (see Table 2).

The fine-needle aspiration biopsy specimens from anaplastic carcinoma usually do not pose any diagnostic difficulties; they can be readily classified as malignant due to extreme cellular pleomorphism and obvious malignant features [157, 158].

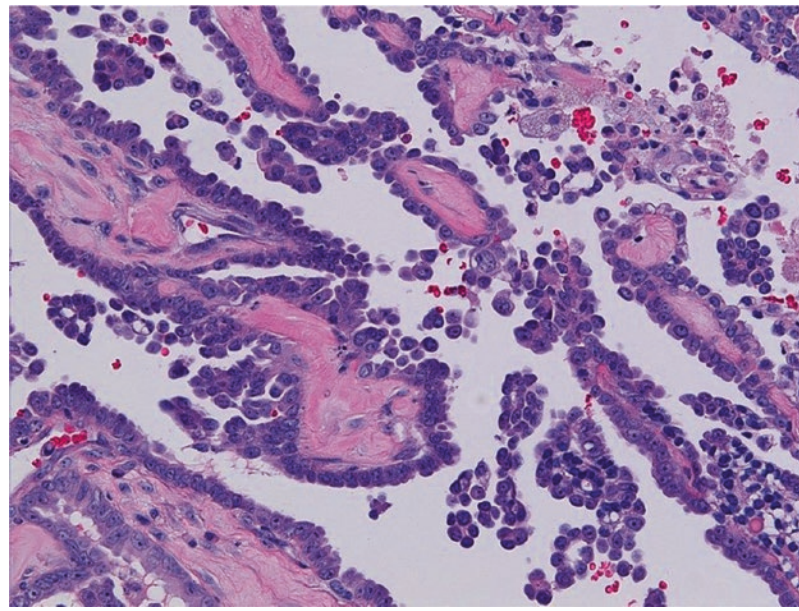

Fig. 26 High-grade papillary carcinoma. Not nuclear pleomorphism despite maintenance of papillary growth pattern. In upper right is focus of necrosis

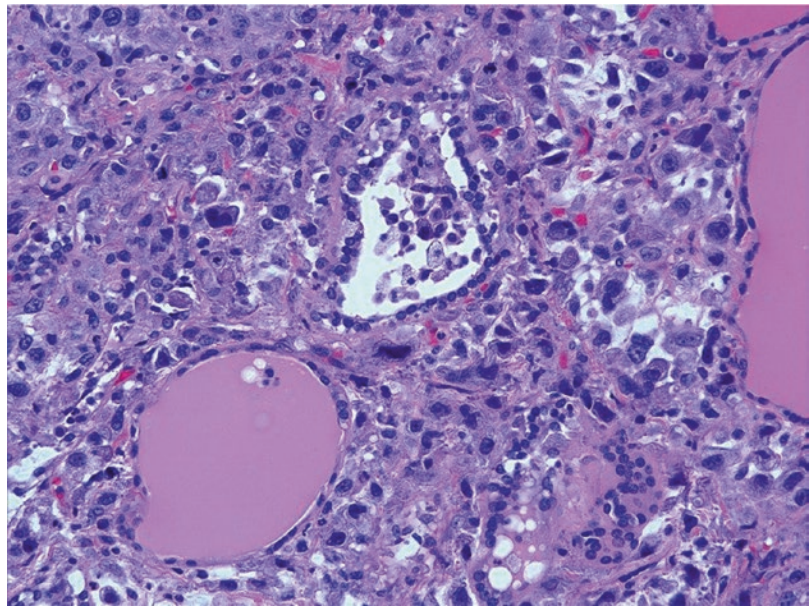

Fig. 27 Anaplastic carcinoma with tumor giant cells. Tumor is invading preexisting thyroid follicles

Medullary Carcinoma

Medullary carcinoma (sometimes called C cell carcinoma) is a distinct tumor derived from nonfollicular cells in the thyroid—the parafollicular or C cells (Fig. 20). These cells secrete the hormone calcitonin, a fact that can be used both diagnostically and to follow patients after therapy. These tumors occur as sporadic lesions (75%) or as familial tumors and some with multiple endocrine tumor syndromes (25%). In the familial cases, germline mutations in ret proto-oncogene are noted. Worldwide studies have identified specific clinical scenarios associated with certain mutations. Mutations at codon 918 in the intracellular domain of ret are associated with multiple endocrine neoplasia type 2B (MEN2B) and aggressive medullary carcinomas. Such patients need to have surgical therapy at very young age (below age 2) so that tumors do not develop or are so small as to not have spread to regional nodes. The correlation of molecular signature and clinical tumor behavior has allowed the development of guidelines for determining at what age(s) therapeutic intervention (usually surgical thyroidectomy) needs to take place [159, 160].

Grossly medullary carcinoma is often circumscribed but rarely encapsulated and may be yellow-tan in color (as many neuroendocrine tumors from various organs tend to be). Microscopically the tumor can show a variety of patterns including nested and epithelioid, spindled, papillary, follicular, giant cell, oncocytic, and clear cells. Unusual examples of melanin production by medullary carcinoma have been reported. About 75% of the tumors produce stromal amyloid which is a product of procalcitonin (Fig. 28) [40].

The tumors produce calcitonin but also many other neuroendocrine markers such as chromogranin A, synaptophysin, and CD56 [40]. Other hormones of nonthyroid origin can be produced and may lead to associated metabolic syndromes, e.g., ACTH or CRF leading to Cushing syndrome [161].

In familial tumors, the C cells in the rest of the thyroid are usually hyperplastic and increased in numbers around follicles (Fig. 29). This can be a diffuse or show a micronodular pattern of proliferation. C cell hyperplasia may be associated with micromedullary carcinomas (1.0 cm or smaller) and are often multifocal [162–165]. Some evidence exists that C cell hyperplasia of syndromic type is neoplastic and represents preinvasive neoplasia. Terms such as "medullary carcinoma in situ" or "thyroid intraepithelial neoplasia of C cells" (THINC) have not been accepted by the recent WHO endocrine book (personal communication).

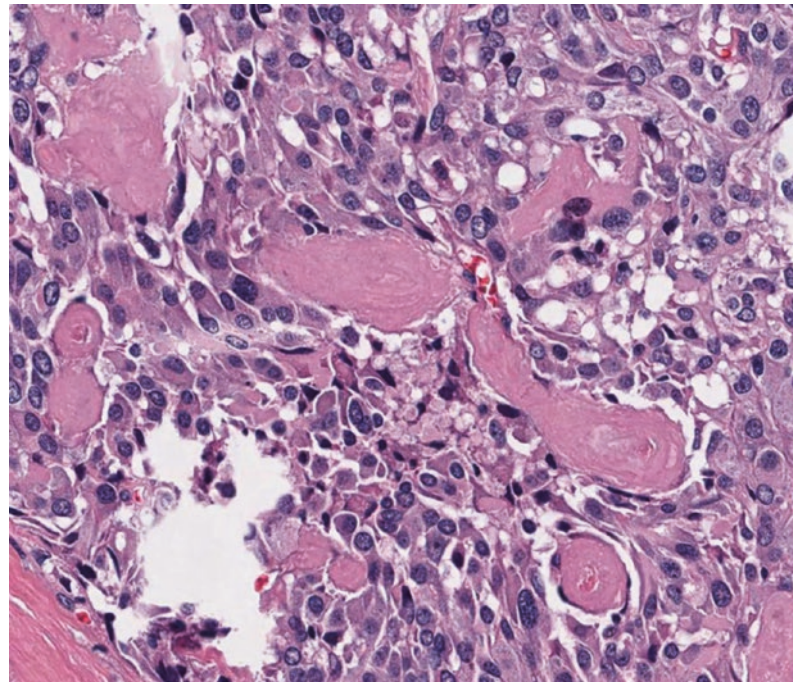

Fig. 28 Medullary carcinoma, sporadic type. Note deeply eosinophilic areas representing amyloid

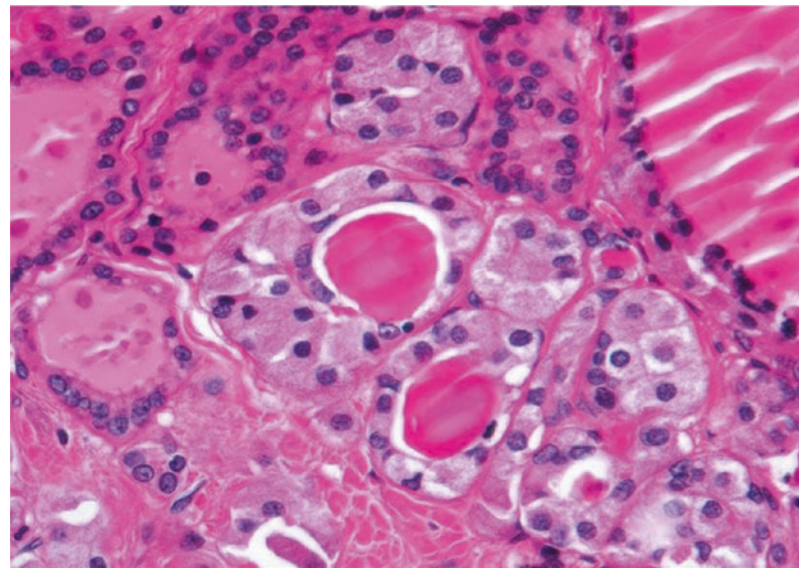

Fig. 29 C cell hyperplasia is shown. This focus was one of many in a thyroid removed prophylactically in a patient with MEN 2A

The definition of micromedullary carcinoma and distinction from C cell hyperplasia include size, total replacement of follicles, infiltrative growth at edge of the nodule, and the presence of amyloid in the stroma [165–167].

Familial medullary carcinoma occurs as part of at least three well-defined syndromes (see Table 4). In all of these, the medullary carcinoma is the most significant oncologic lesion and is the major cause of morbidity.

In sporadic cases, clinically evident tumors may show small metastases (especially early on in level 6 lymph nodes); the course for these patients may be indolent or rapidly fatal. Some pathologic features have been shown to correlate with rapid growth and metastasis; these include tumor necrosis, high mitotic count, paucity, or absence of amyloid and large tumor size. Alternatively, amyloid-rich tumors often with calcification in the amyloid, those with uniform

Table 4 Multiple endocrine neoplasia syndromes with medullary thyroid carcinoma as primary lesion

1. MEN2A (aka Sipple syndrome) components:
(a) Medullary thyroid carcinoma and C cell hyperplasia
(b) Adrenal pheochromocytoma and adrenal medullary hyperplasia
(c) Parathyroid adenoma/hyperplasia
2. MEN2B or 3 (aka Gorlin syndrome) components:
(a) Medullary thyroid carcinoma and C cell hyperplasia
(b) Adrenal pheochromocytoma and adrenal medullary hyperplasia
(c) GI and mucosal neuromas
(d) Marfanoid habitus
(e) Ocular lens abnormalities
3. Familial medullary carcinoma[a]

[a]Some authors believe that the families with only medullary carcinoma and (C cell hyperplasia) may have represented mild MEN2A syndromes

cytology either epithelioid, spindled, or both, circumscribed lesions, and those of small size, tend to do well with long-term survival. Patients in the former group may have a rapidly fatal course even if the tumor stage is low, and those in the second group may live a long time with recurrent and/or metastatic carcinoma [168–173].

FNA specimens of MTC display a spectrum of morphologic patterns similar to surgical pathology specimens. The majority of MTC FNA specimens are cellular consisting of round to oval cells arranged mainly as single cells or loosely cohesive groups. The tumor cells show ample granular cytoplasm with eccentric nuclei imparting a plasmacytoid appearance to the cells. The nuclear chromatin is similar to that seen in neuroendocrine tumors; intranuclear inclusions and multinucleated cells may be seen. Marked nuclear pleomorphism is uncommon; however, when present the cases are indistinguishable from aspirates of anaplastic thyroid carcinoma. The neoplastic cells can assume a "spindle shape" and appear mesenchymal in origin. Amyloid may be observed as acellular material and can be distinguished from the thick colloid of papillary carcinoma by performing a Congo red stain. The diagnosis of MTC can be confirmed either by performing immunostains for calcitonin, correlating with serum calcitonin levels, or measuring calcitonin levels in the FNA aspirate specimen [174–178].

The cytomorphologic diagnosis of MTC can be challenging due to morphologic variability. The differential diagnosis of MTC includes hyalinizing trabecular neoplasm, oncocytic follicular neoplasm (aka Hürthle cell neoplasm), papillary thyroid carcinoma, follicular neoplasm with solid and trabecular growth pattern, poorly differentiated carcinoma/insular carcinoma, anaplastic carcinoma, plasmacytoma, and metastatic tumors to the thyroid especially melanoma [174].

Noncarcinoma Malignancies of the Thyroid

Hematopoietic and Related Lesions

The thyroid gland may be a site of involvement in disseminated hematologic/lymphoid malignancies such as leukemias and lymphomas of any type. Rarely plasma cell myeloma may involve the gland.

However, lymphomas may arise primarily in the thyroid usually in glands affected by thyroiditis. These lymphomas are predominantly of B cell lineage and may be tumors of MALT-associated lymphoid tissue [179–181]. Histologically, immunologically, and by genetic studies, these tumors are either large diffuse B cell lymphomas or small cell MALTomas. However, all lymphoma subtypes including those of T cell lineage and Hodgkin disease have been described as primary thyroid tumors. The relationship between the underling autoimmune thyroiditis and the lymphoid neoplasm is very strong. Indeed, if a patient with known lymphocytic thyroiditis develops a mass lesion in the thyroid, the risk that nodule is a lymphoma 80 times greater than that it represents an epithelial tumor [180–182].

Some thyroiditic glands will develop a lymphoid lesion that appears exclusively composed of plasma cells. In a patient without systemic plasma cell dyscrasia, such as multiple myeloma, such a lesion likely represents a large B cell lymphoma with marked plasma cell differentiation [183, 184].

Mycosis fungoides (i.e., T cell lymphoma originating in the skin) [185] and histiocytic lesions such as Rosai-Dorfman disease [186] and Langerhans histiocytosis [187] may manifest in the thyroid gland and on occasion may be the presenting symptom of the disease. Rare cases of Langerhans histiocytosis in the thyroid admixed with or coexisting with papillary thyroid carcinoma have been reported [188].

The diagnosis of lymphoma can be made by FNA; if it is suspected, the entire FNA rinse can be sent for special studies including flow cytometry and molecular analysis in order to characterize the subtype of lymphoma [189].

Soft Tissue Tumors

Both benign and malignant tumors of soft tissue are known to occur in the thyroid and are presumed to derive from stromal elements or vessels within the gland. Tumors of neural origin [190–193] (including schwannomas and granular cell tumors), smooth muscle, and venous or lymphatic lineage have been reported to have solitary fibrous tumors [194]. Sarcomas usually of neural or smooth muscle origin are documented [195]. One must be cognizant of the fact that extrathyroidal origin of sarcomas in the thyroid is more common than primary tumor, i.e., metastatic sarcoma. In our experience, gastrointestinal stromal tumor, uterine leiomyosarcoma, and malignant phyllodes tumor of the breast seem to be the mesenchymal lesions that more commonly spread to the thyroid [196].

Unusual Tumors of Thyroid

Tumors in the thyroid can be considered unusual because of their rarity of occurrence, because they are histologically unique, or because they are rare but occur in the setting of genetic/familial disease and should be considered "marker lesions."

Cribriform Morular Carcinoma

This tumor, still classified among the papillary carcinoma group, is histologically unusual and important to recognize because it is strongly associated with familial adenomatous polyposis (FAP aka Gardner syndrome) of the gastrointestinal tract and adenocarcinomas of the colon and ampulla of Vater [197, 198].

From a histologic point of view, the tumor is often circumscribed and even partially encapsulated and is composed of broad-based "papillae" with morules in the stroma resembling "squamous morules" seen in endometrial hyperplasia/metaplasia [199]. The tumor rarely makes follicles but can assume a sieve-like appearance. On first glance, the pathologist who is unfamiliar with the appearance of these tumors may think of a metastatic tumor to the thyroid. This is further emphasized by the relative lack of immunostaining for thyroglobulin (TTF-1 is strongly positive). In patients with the familial form, multifocal tumors may be found in the gland. In sporadic cases, the lesion is unifocal [197].

The familial cases often occur in young (teenage) females (very rarely in males), and the thyroid lesion may represent the initial event in identifying families at risk for gastrointestinal cancers. The thyroid tumor is indolent in most cases [197].

An important pathologic contribution to the identification of familial cases is the staining of the tumor with antibody to beta catenin [198, 200]. In cases associated with FAP, there is translocation of beta catenin to the nucleus so that immunostains will demonstrate both cytoplasmic and nuclear staining. This does not occur in sporadic cases [198].

Tumors Associated with Cowden (PTEN Mutation) Syndrome

Although not as histologically specific as the cribriform morular carcinoma, identifying patients with germline APC gene mutations,

there are other thyroid tumors that give clues to an underlying genetic disorder. Thyroids in patients with PTEN mutations (Cowden's syndrome) are often multinodular, and almost all the nodules show oncocytic or even Hürthle cell cytology. If this histology is found in a young patient (under age 45), the pathologist should raise the possibility of Cowden-related syndrome. About 15% of the thyroids will also harbor a carcinoma, either papillary, follicular variant of papillary cancer, or follicular/Hürthle cell carcinoma [201].

The importance of recognizing these thyroid lesions as possibly part of this syndrome is that the risk for breast and uterine cancers is very high in these patients [202].

Attempts to utilize immunostaining for PTEN to assist the pathologist in defining these cases better have met with little success. In theory, the stain for PTEN should be negative as the germline mutation results in loss of the protein. However, the results can be confusing with both false-positive and false-negative results reported [203].

In summary, the pathologist plays an important role in identifying possible germline genetic changes and risk to family members of the patient whose thyroid is being studied. These major family disorders include medullary carcinoma, cribriform morular carcinoma, and FAP and lesions suggestive of Cowden syndrome.

Sclerosing Mucoepidermoid Carcinoma with Eosinophilia (SMECE)

This unusual tumor arises in the background of severe lymphocytic thyroiditis and often shows hyperplasia of solid cell nests (rests of ultimobranchial body (UBB) derived from the fourth-fifth branchial pouch). These tumors occur in the lateral aspects of the thyroid lobes (where the UBB are located). Although still debated, many authors feel these tumors may develop from the UBB. The tumors do not stain for thyroglobulin or calcitonin, but show cytokeratins, and some have TTF-1 staining. Originally considered to be low-grade lesions, some authors have reported metastases [204, 205].

Microscopically the tumors are comprised of nests of squamoid cells intermixed with glandular spaces lined in part by mucous containing cells (mucocytes). This histology is reminiscent of mucoepidermoid carcinoma of the salivary glands. The tumor nests are arranged in a fibrous stroma, and the latter is infiltrated by lymphocytes, plasma cells, and many eosinophils [205, 206].

Mucoepidermoid Carcinoma

Different from the SMECE, the mucoepidermoid carcinoma often shows admixture with classic or follicular variant papillary carcinoma [206]. Portions of the tumor show glands with mucous cells and squamoid metaplasia. The neoplasms can be found in any area of the gland including the isthmus. These tumors have a similar prognosis to papillary carcinoma of similar stage. Occasional cases have been associated with anaplastic transformation [204, 207, 208].

The cytology FNAB specimens of both sclerosing mucoepidermoid carcinoma with eosinophilia and mucoepidermoid carcinoma show epidermoid and glandular elements with stromal fragments. Eosinophils can be seen in the aspirates of sclerosing mucoepidermoid carcinoma with eosinophilia. The squamous elements can be mistaken for a primary or secondary squamous carcinoma and anaplastic carcinoma of the thyroid [204, 209].

Mammary Analog Secretory Carcinoma (MASC)

These distinctly rare tumors (two reported cases) resemble MASC of salivary glands. They are partly papillary and have a secretory look. Immunohistochemistry shows lack of thyroglobulin, TTF-1, and calcitonin. As with the salivary gland counterpart, molecular analysis shows the characteristic ETV6-NTRK3 t translocation [210–212].

Branchial-Related Tumors

A variety of tumors related to branchial-thymic tissues can occur in or around the thyroid. These include thymoma (benign, invasive, or malignant) and the ectopic hamartomatous thymoma (a benign lesion usually of childhood) which arises in extrathyroidal soft tissue.

Spindle Epithelial Tumor with Thymus-Like Differentiation (SETTLE)

This tumor, which occurs in young individuals including children, may resemble synovial sarcoma (which can occur in the neck) and involve the thyroid or medullary carcinoma. SETTLE lesions show spindle cells with associated epithelial nests [213–217]. They may contain cytokeratin but are negative for calcitonin (which is helpful for excluding medullary carcinoma), thyroglobulin, and TTF-1. The characteristic translocation found in synovial sarcoma, t(X;18)(p11;q11) SSX18, has been shown to be absent by fluorescent in situ hybridization studies (FISH) [215].

Carcinoma Showing Thymus-Like Differentiation of the Thyroid (CASTLE)

This lesion is the most malignant of the thyroid branchial-related tumors with about a 50% mortality. It resembles lymphoepithelial carcinoma of the nasopharynx and consists of large epithelial cells with significant nuclear pleomorphism growing in a nested pattern surrounded by a dense lymphocytic infiltrate. Reports of these cases prior to their description in the branchial-related group indicated that these tumors were diagnosed as anaplastic carcinoma (which had a much better survival rate). Despite its resemblance to nasopharyngeal carcinoma, the tumor shows no evidence of a relationship to Epstein-Barr virus infection [217–222].

Paraganglioma

Whether true paraganglioma occurs within the thyroid or whether the reported cases represent tumors arising in perithyroidal paraganglia remains controversial. Both benign and aggressive paragangliomas have been described [223–226]. The major differential diagnoses include medullary carcinoma (calcitonin positive) and hyalinizing trabecular tumor (thyroglobulin positive).

Parathyroid Tumors Intrathyroidal

Parathyroid tissue including entire parathyroid glands may be found entirely within the thyroid. More frequently the parathyroid is located close to the thyroid tissue edge ("capsule") and may appear grossly to be intrathyroidal. Microscopically the true location is noted [227–232].

Parathyroid tissue actually embedded within the thyroid gland may give rise to adenomas and mimic clinically and radiologically thyroid neoplasms [228–231]. Many of these nodules are biopsied, and FNA diagnoses of "follicular neoplasm" or even "follicular lesion of undetermined significance" may be rendered. The parathyroid adenoma cells are more uniform and have rounder smaller nuclei. These features may trigger the possible diagnosis of a parathyroid lesion, and the pathologist may suggest sending some of the FNA material for parathyroid hormone assay (which would verify the correct diagnosis) [233, 234].

Any disease that affects the parathyroid tissue can involve intrathyroidal parathyroids; hence multigland hyperplasia may lead to hyperplasia of the intrathyroidal tissue. Rare cases of parathyroid carcinoma arising within the thyroid are known [235, 236].

Metastatic Tumors

Metastasis to the thyroid gland usually presents as multiple nodules in patients with known malignancies. The most common tumors that spread to the thyroid in this setting are lung and breast carcinomas [237].

However, it has been noted that the tumor which metastasizes to the thyroid and presents as

a solitary nodule mimicking a tumor of thyroid origin is clear cell renal carcinoma [237]. Such lesions may be the only site of metastasis in the affected patient. In some individuals, the thyroid metastasis is the initial evidence of a kidney cancer. The interval between the diagnosis of the renal lesion and the thyroid tumor may be many years including decades [238–240].

Other relatively common metastatic lesions (often numerically disproportionate to their frequency in the cancer population) include malignant melanoma and colonic adenocarcinoma [241].

Finally it is important to recognize that because patients with malignancies are surviving longer with modern therapies, the number of FNA specimens that represent metastases to the thyroid is increasing. Two series decades apart from the Mayo Clinic elegantly showed this clinical change [241].

Similarly to histopathology, FNA of metastatic tumors to the thyroid gland can also be difficult to differentiate from primary thyroid neoplasms, clear cell carcinoma of kidney vs. follicular carcinoma or adenoma with clear cell change, metastatic neuroendocrine carcinoma vs. medullary thyroid carcinoma, and poorly differentiated lung carcinoma vs. anaplastic or poorly differentiated thyroid carcinoma. However, immunohistochemistry and a detailed history are always helpful for differentiation between primary and secondary tumors of thyroid [242].

This chapter has summarized and illustrated pathologic lesions of the thyroid stressing neoplasms. Practical topics, including differential diagnoses, approaches to defining these lesions and differentiating them from mimics, and summaries of the role of the pathologist in approaches to familial and genetically defined conditions have been included.

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Thyroid Nodule: Current Evaluation and Management

Alan A. Parsa and Hossein Gharib

Introduction

The prevalence of palpable thyroid nodules has not changed significantly since the 1960s and remains around 3–7% [1–5]. The increase in nodular thyroid detection is, therefore, predominantly incidental and correlates with the increased use of sensitive imaging. Each imaging modality has a different sensitivity to detect thyroid nodules but also requires an experienced radiologist. For example, thyroid nodules discovered incidentally with ultrasound (US) are around 30–70% [6, 7]; with computerized tomography (CT), 16–18% [8–10]; with magnetic resonance imaging (MRI), 6–16% [10, 11]; and with fluorodeoxyglucose positron emission tomography (FDG-PET), 1–4% [12–16].

The overall incidence of thyroid nodules in the United States (USA) is around 100 cases per 100,000 persons per year [17], with an annual incidence of thyroid cancer around 14.3 per 100,000 individuals per year, nearly tripled from 1975 [18]. It is projected that by 2019, papillary thyroid cancer (PTC) will become the third most common cancer in women in the USA, with an estimated incidence of 37 per 100,000 population [19]. Worldwide, during the past 30 years, thyroid cancer rates have increased by 48% in males and 67% in females [20]. This substantial increase in rates under lines the importance of new strategies for evaluation, surveillance, and treatment of thyroid nodules.

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Diagnosis

History and Physical Exam

Clinical evaluation begins with a detailed history and physical exam. Thyroid nodules typically present asymptomatically as a mass discovered on neck palpation by a physician, by a patient, or by imaging for an unrelated condition. Once discovered, familial conditions associated with a risk of thyroid malignancy such as multiple endocrine neoplasia 2 (MEN2) [21], familial nonmedullary thyroid cancer (NMTC) [22, 23], Cowden syndrome (PTEN hamartoma tumor syndrome) [24], familial adenomatous polyposis (FAP) [25, 26], and Gardner syndrome [27] should be considered (Table 1).

History of head and neck irradiation, female gender, iodine deficiency, age of puberty, and family or personal history of thyroid disease are associated with an increased risk for carcinoma and should be evaluated and documented [28]. A

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Table 1 Risk of developing thyroid cancer and type based on familial syndromes [21, 22, 25, 27]

Condition	Risk of thyroid malignancy	Predominant thyroid cancer type
MEN2	90% ([23])	Medullary
FNMTC	3–15% ([24, 25])	FCD
Cowden syndrome	38%	Follicular
FAP	0.4–12%	Papillary
Gardner syndrome	2% (predominantly women)	Papillary

MEN2 multiple endocrine neoplasia type 2, *FNMTC* familial nonmedullary thyroid carcinoma, *FCD* follicular cell-derived, *FAP* familial adenomatous polyposis

focused thyroid exam should include and document: growth rate of the thyroid and/or nodule(s) by ultrasound or palpation, tenderness, presence of cervical lymphadenopathy, voice changes, and compressive symptoms. Further evaluation may be required based on physical features or family history.

About 1.5% of children will have identifiable thyroid nodules that possess a 26% risk of malignancy [29, 30], compared to 3–7% of adults with a 5–10% risk of cancer [4, 31–33]. Childhood thyroid malignancies tend to be more aggressive and present with lymph node metastasis in 90% of cases compared to 35% in adults [34]. Children with newly discovered nodules deserve full evaluation, and a more extensive surgical approach, because of high locoregional metastasis, realizing that long-term outcome still remains excellent [35]. A recent report suggests that, while with advancing age the prevalence of thyroid nodule increases, the actual risk of malignancy decreases [36].

Ultrasonography

Brightness-mode (B-mode) ultrasound is the most sensitive test available to detect and define thyroid parenchyma. Commercially available US machines are equipped with 7.5 MHz (for deep tissue evaluation) to 15.0 MHz (for superficial tissue evaluation) linear or curvilinear array transducers. US allows for a clear and continuous real-time visualization of the thyroid gland [32, 37].

An US exam should identify and record the number of nodules, size, position, shape, margins, content, echogenicity, and the vascular patterns of the gland and its nodules. This helps stratify risk of thyroid malignancy and requirements for further workup. If multiple nodules exist, the report should include all nodules with suspicious features of malignancy and not just the dominant (largest) nodule. Population screening for thyroid nodules by US is not recommended due to the high likelihood of detecting nodules of doubtful clinical significance [38]. Thyroid US is indicated when palpation is suspicious for, or confirms a nodule, for those at risk for thyroid malignancy (i.e., history of childhood head/neck irradiation, MEN2, familial thyroid cancer), for those with suspicious neck adenopathy, as well as those with incidental nodular findings by other imaging modalities.

Ultrasound-Guided Fine Needle Aspiration

Once a thyroid nodule is confirmed, US-guided fine needle aspiration (US-FNA) follows. US-FNA is a safe, accurate, and cost-effective method of evaluating sonographically indeterminate thyroid nodules [39, 40]. Real-time imaging allows direct visualization of the needle as it is guided to the desired location within a nodule while avoiding vital structures (i.e., carotid artery, trachea, jugular vein). US-FNA has an accuracy of 80% compared to 61% reported by palpation-guided FNA (P-FNA) [41].

Two common techniques applied in US-FNA are the parallel approach (US-guided FNA), where the needle is inserted along the same plane of the transducer, or the perpendicular or out of plane approach (US-assisted FNA), where the needle is inserted at the center point of the transducer entering the skin perpendicularly to the transducer. Details of the different techniques have been described elsewhere [42–45].

Inadequate/nondiagnostic specimen rates decrease significantly from 9–17% with P-FNA to 4–7% using US-FNA [41, 46]. This improvement in rate may be attributed to real-time visual-

ization of needle placement, allowing the aspirating physician the ability to avoid areas of fibrosis, calcification, and cystic degeneration [42] while targeting the wall and solid portions of lesions [47, 48] to maximize sampling adequacy. For example, to prevent false-negative results in cystic carcinoma, the needle should be directed to the base of the cyst where tumor cells tend to reside [49] which without US would be an area difficult to target. Rates of inadequacy vary depending on the experience of the physician performing FNA and the cytopathologist reading the slides. In cytologically adequate samples, with an experienced cytopathologist, cancer prevalence is similar, around 5–7% in both palpable and impalpable lesions [50, 51].

Thyroid cysts collapse after drainage (Fig. 1) but tend to recur in 10–80% of cases [52]. A hemorrhagic recurrence should raise suspicion of a cystic carcinoma [53]. To assist in minimizing delay of therapy, fluid drained from a cyst should always be sent to the lab for cytological analysis. In predominantly cystic complex nodules (>50% cystic), cystic areas should be drained initially to remove necrotic debris and cells prior to sampling the potentially malignant solid components. This improves cytology reports by minimizing artifacts.

The most significant complication is mild hematoma at the biopsy site, especially in patients on aspirin or warfarin [42], although usually not an issue. Other rare but possible complications include transient localized pain, infection at puncture site, and slight ecchymosis. Seeding of tumor cells along the needle track has been described in head and neck cancers but appears exceedingly rare with either FNA or core needle biopsy [54].

Indications for US-FNA in adults are listed in Table 2. To avoid the inappropriate use of US-FNA, malignancy risk assessment based on US features or other imaging techniques is necessary. In children, all nodules greater than 10 mm should undergo FNA regardless of US features, due to the higher risk of malignancy [55].

The reported incidence of false-positive results with FNA is about 1% but may be as high as 7%, according to some reports [56]. False-negative results are also around 1% [57]. Most sampling errors occur from specimen inadequacy, inappropriate sampling site, degenerative changes, or cytopathologist inexperience [58].

Ultrasound Prediction of Malignancy

Numerous reports have demonstrated that US features are useful in assessing malignancy risks, thereby necessitating FNA [31, 59, 60]. Guidelines assist in stratifying malignancy risks to make US reports more descriptive and consistent. For example, the 2015 ATA guidelines categorize sonographic features into five patterns: benign, very low suspicion, low suspicion, intermediate suspicion, and high suspicion [61]. FNA is strongly recommended for nodules in the high and intermediate suspicion categories, having malignancy rates of 70–90% and 10–20%,

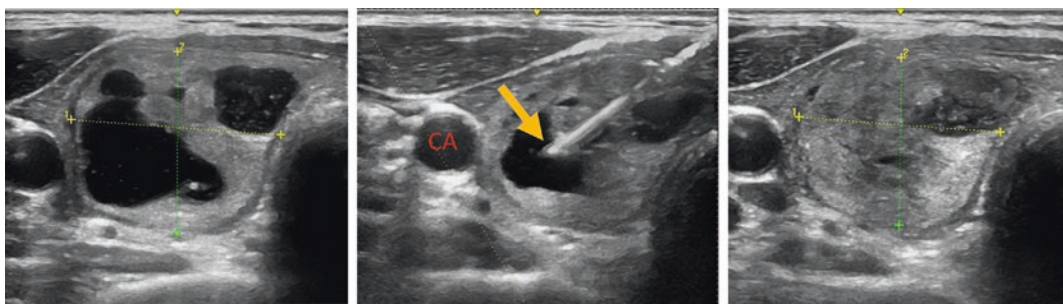

Fig. 1 Drainage and collapse of cystic component of a complex nodule before (upper left image), during (middle), and post (upper right) drainage using parallel approach. Arrow is pointing at the needle tip within the cystic portion of nodule. *CA* carotid artery

Table 2 Indications to perform US-FNA adapted from AACE/AME 2016 clinical practice guidelines for the diagnosis and management of thyroid nodules

Indications for US-FNA
• Nodule 5–10 mm if: Subcapsular or paratracheal lesion Suspicious lymph nodes or extrathyroid spread Positive personal or family history of thyroid cancer
• Nodule >10 mm with high US risks
• Nodule >20 mm with intermediate US risks
• Spongiform or predominantly cystic nodules >20 mm with growth
• Presence of abnormal cervical nodes
• Nondiagnostic palpation-guided FNA
• Incidentalomas noted on FDG-PET scan
• Complex (solid/cystic) nodule

respectively [61]. The 2016 AACE/AME guidelines suggest a simplified three-class system (% malignancy risk): low risk (1%), intermediate risk (5–15%), and high risk (50–90%) with FNA recommended in high-risk nodules if > 10 mm and intermediate-risk lesions if > 20 mm (Gharib et al., EP 2016 in print May) [62]. Radiology literature has adopted a ten sonographic feature system known as the TIRADS (thyroid imaging reporting and data systems) classification, with increasing risks based on increasing number of suspicious features [63]. The TIRADS system, while thorough, seems complex and impractical for most clinical practices.

US imaging allows for a detailed assessment of thyroid nodular features. Individually, each feature possesses a significant degree of diagnostic uncertainty, but, together, posttest probability of malignancy increases dramatically [64].

Solitary versus *multiple nodules*. There are no significant differences in malignancy rates between patients with a single thyroid nodule and those with a multinodular goiter (MNG) [31, 50]. To avoid missing malignancy in MNG, identifying an indeterminate or suspicious nodule for US-FNA is more important than identifying the dominant (largest) nodule [50, 65].

Size. With a high prevalence of nodules <10 mm (micronodule), difficulties in establishing malignancy risks and fear of missing a malignancy may lead to overtreatment. Recent ATA guidelines suggest active surveillance for nodules <10 mm [61], while AACE guidelines favor FNA for high-risk 5–10 mm lesions (e.g., young patients, invasive nodules, etc...) [66]. Regardless of size, all nodules should be thoroughly evaluated for features suggestive of malignancy [67] allowing individualized decision-making based on risk factors (i.e., history of neck irradiation or familial history of thyroid malignancy).

The rationale for *not* performing FNA on nodules <10 mm is based on observations that the majority of such nodules change little with time, even if malignant [68–70]. Also, the excellent oncological outcome associated with active surveillance appears to outweigh complications associated with surgical resection (i.e., vocal cord paralysis, hypoparathyroidism) [71]. Ito and colleagues [72] monitored 340 patients with papillary thyroid microcarcinoma (PTMC) for 10 years. Tumor excision was carried out when signs of progression was noted (i.e., tumor enlargement, novel nodal metastasis) and demonstrated no recurrence of tumors post resection. Active surveillance did not seem to adversely influence long-term outcome in PTMC.

This form of "active surveillance" is slowly becoming accepted as a clinical approach for low-risk microcarcinomas [61, 73, 74] while identifying those requiring a more aggressive treatment (i.e., biopsy or surgical resection) [75]. The risk of psychological stress associated with the perception of living with cancer may prevent some patients from accepting an active surveillance protocol [76]. This emphasizes the importance of patient education, support, and patient selection for long-term follow-up.

Solid, echogenicity: The echogenicity of a thyroid nodule is assessed by comparing it to surrounding normal thyroid parenchyma [77]. Thus, a hypoechogenic nodule is one which is less echogenic than the surrounding normal thyroid tissue. Approximately 50–90% of PTC and its variants are hypoechoic [78–80], while a benign nodule, typically, is isoechoic or slightly hyperechoic compared to surrounding normal tissue [77] (Fig. 2). Many studies identify hypoechogenicity as a marker of malignancy with a sensitivity and specificity of 27–87% and

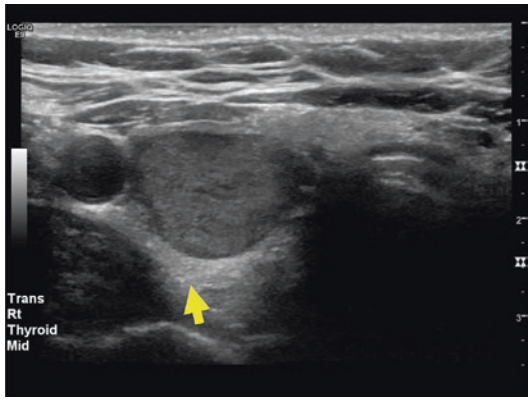

Fig. 2 Hypoechoic nodule. Arrow shows normal extranodal thyroid tissue

43–94%, respectively, and positive and negative predictive value of 11–68% and 74–94%, respectively [50, 81, 82]. Thus, while a hypoechogenic nodule should raise suspicion, it should not be considered diagnostic, and other features should be evaluated and taken into consideration.

Color Doppler: US can examine the vascular flow of a nodule but holds limited value in determining malignancy risks. Most malignant nodules have intranodular vascularity, also found in benign nodules [83, 84]. Most benign nodules possess a predominant peripheral flow pattern; however 20% of malignant nodules show a peripheral ring [79]. Studies suggest that flow is a poor predictor of malignancy [83, 84] but should be documented in US reports (Fig. 3).

Extracapsular growth. Capsular abutment, contour bulging, loss of echogenic thyroid capsule, invasion into perithyroid muscles, and infiltration of the recurrent laryngeal nerve are features of extracapsular growth and increased risk of malignancy, warranting further cytological evaluation [85, 86].

Complex or cystic lesions: Simple cystic lesions are almost never malignant (0.32 cancers per 1000) [31]. Nodules containing both cystic and solid components (complex cysts), with a predominantly cystic component (> 50% cystic), while typically benign, may contain PTC within the solid component, and US-FNA may be warranted [77, 87]. A predominantly solid, complex nodule (> 50% solid) has a risk of malignancy similar to a pure solid nodule and should be managed as such.

Nodule shape: Taller (anteroposterior)-than-wide (transverse) shape (Fig. 4) as well as irregular borders are possible malignant features, while wider than tall is a suggestive benign finding [88, 89].

Suspicious cervical adenopathy: Normal and reactive lymph nodes are typically oval with an echogenic hilum. Suspicious cervical lymph nodes are rounded, possess cystic changes, microcalcifications, chaotic hypervascularity, and lack hilum [90]. If any or all of these features are present, the lymph node as well as ipsilateral thyroid nodule(s), irrespective of size or features, should undergo US-FNA [91].

Elastography: Two commonly used techniques in thyroid elastography are real-time elastography (RTE) and shear wave elastography (SWE), described in detail elsewhere [92–94]. Elastography uses the concept that stiffer or less elastic tissue correlates with malignancy, while softer, more elastic tissue is benign [93]. Repeatability and reliability are operator-dependent, and excessive pressure or angle misalignment may lead to misinterpretation [94]. Sensitivities with elastography range between 62 and 93% [95–97], with positive predictive values ranging from 36 to 100% [98, 99]. Elastography is currently not widely available, and though it may be useful, neither the ATA nor AACE recommends it as a useful routine test in nodular evaluation [61, 66].

Other Imaging Techniques

High costs combined with low specificity limit the application of MRI, CT, or FDG-PET for routine evaluation of nodular thyroid disease. Advantages include assessment of airway compromise, substernal extension, and positional relationship of the goiter to surrounding structures (Fig. 5). In general, use of CT contrast material in iodine-deficient geographic areas may trigger hyperthyroidism and/or decrease the gland's sensitivity to radioiodine treatment [100]. Incidental findings of nodules by CT, MRI, or focal thyroid uptake by FDG-PET scan usually warrant further US evaluation [101, 102].

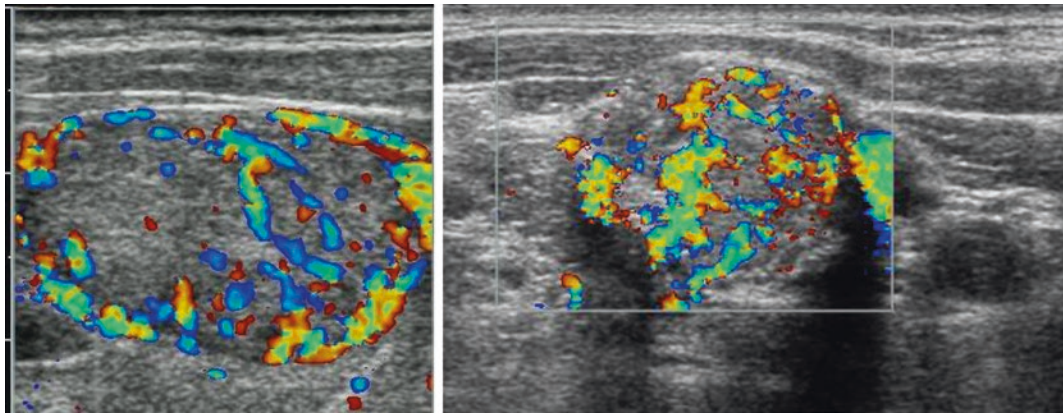

Fig. 3 Benign nodule with intranodular vascularity (left) and malignant PTC nodule with internal vascularity (right)

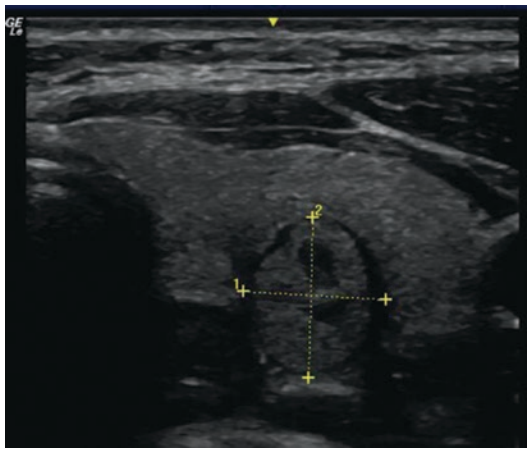

Fig. 4 Taller (1.3 cm)-than-wide (1.0 cm) nodule is suspicious for malignancy

Fine Needle Aspiration (FNA) Biopsy

The most accurate, cost-effective, and widely available method to determine thyroid malignancy is FNA [103]. It is estimated that well over 600,000 fine needle aspirations (FNA) are performed yearly in the USA, with approximately 70% labeled as "benign," preventing over 420,000 unnecessary surgeries annually. Thyroid FNA has a reported sensitivity and specificity of approximately 67–98% and 56–100%, with a positive and negative predictive value of 75–94% and 77–84%, respectively [104, 105]. This is higher than any other modality currently available making it the ideal method to "rule in" or "rule out" thyroid malignancy [55].

Palpation-Guided Fine Needle Aspiration

P-FNA is no longer performed in clinical practice due to the wide availability and increased accuracy of US-FNA. Descriptions of P-FNA and its limitations have been published by us, and others, elsewhere [106–109].

Cytological Diagnosis

In 2007, the Bethesda System for Reporting Thyroid Cytopathology (BSRTC) was introduced and has gained significant popularity [110, 111]. It is an extension of the conventional thyroid cytological classification [109], based on sample adequacy. An adequate sample is defined as: no less than six groups of well-preserved thyroid epithelial cells consisting of at least ten cells in each group. BSRTC categorizes adequate samples into five groups based on risks of malignancy: unsatisfactory/nondiagnostic benign, indeterminate (further divided into atypia of undetermined significance (AUS)/follicular lesion of undetermined significance (FLUS) or follicular neoplasm (FN)/suspicious for follicular neoplasm (SFN)), suspicious for malignancy, or malignant [110, 111]. Inadequate, nondiagnostic, or unsatisfactory samples are those containing fewer cells or groups that are defined as adequate and should be correlated with US reports, as re-biopsy is not always indicated. For instance, pure cystic nodules, a predominantly benign finding, are

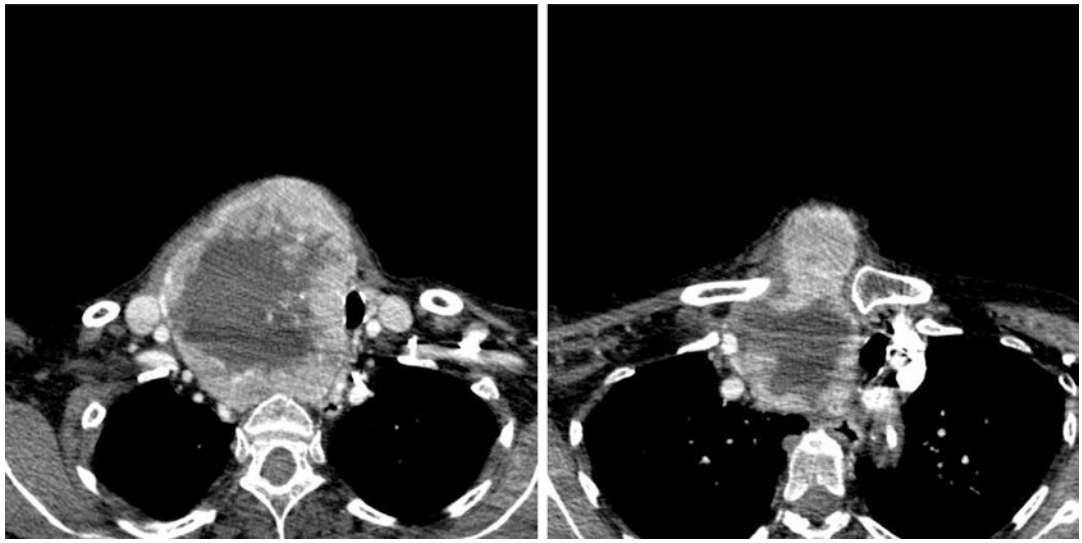

Fig. 5 CT scan showing substernal extension of a huge multinodular goiter

fairly acellular and reported cytologically as nondiagnostic and typically do not require re-biopsy. Inadequacy rates are lower in US-FNA (6.4%) than in palpation-FNA (13%) [112]. The inadequate rates may vary between labs due to different standards set by each for defining adequate specimens.

The "malignant" category carries a high malignancy risk of 97–99% [111]. Papillary thyroid carcinoma (PTC), the most common thyroid cancer, accounts for 70–80% of the "malignant" category [19]. Cytologically, PTC is characterized by pale chromatin, linear chromatin ridges (grooves), intranuclear cytoplasmic inclusions, and nuclear crowding often overlapping. Medullary thyroid carcinoma (MTC), anaplastic carcinoma, lymphomas, poorly differentiated carcinoma, and metastatic cancers are cytologically distinguishable and are also categorized as "malignant" [32]. Since histological features and cancer type impact treatment, prognosis, and recurrence, they should reported by the cytopathologist when possible.

"Suspicious for malignancy," by the BSRTC, suggests that malignancy is strongly suspected on cytology but cannot be established with high certainty [110, 111]. This category carries a 50–75% risk of malignancy; PTC dominates this category.

"Indeterminate" cytology is further subdivided into AUS/FLUS and FN/SFN. If a benign follicular pattern with either cellular atypia (AUS) or follicular cells with architectural and/or nuclear atypia (FLUS) is identified, a 5–15% malignancy risk is expected [110, 111]. Depending on associated US features, re-biopsy is typically recommended rather than excision [113]. Cytological criteria for FN/SFN include high cellularity and altered follicular cell architecture (microfollicles) with scant or absent colloid. These lesions carry a 15–30% risk of malignancy, and based on US features, a more aggressive step may be required (i.e., hemithyroidectomy or total thyroidectomy) due to the inability to differentiate between benign and malignant follicular neoplasms by FNA [114]. For ease to the provider, the most recent AACE/AME recommendations suggest re-categorizing indeterminate lesions to low-risk indeterminate lesion (AUS/FLUS) and high-risk indeterminate lesion (pure follicular patterned lesions; FN/SFN) to stress the difference in malignancy rates and to help direct therapy [66]. Table 3 summarizes the Bethesda categories with risks of associated malignancy.

"Benign" cytology has a < 3% risk of malignancy based on the BSRTC [110]. Opinions vary as to if and when benign nodules should be

Table 3 Bethesda system for reporting thyroid cytopathology

Diagnostic category	Type	Malignancy risk (%)	Management	Frequency reported (%)
Nondiagnostic/ unsatisfactory	Pure cyst poor sample quality	1–4	Repeat US-FNA	<10
Benign	Lymphocytic thyroiditis Adenomatoid nodule Colloid nodule Granulomatous thyroiditis	0–3	Clinical follow-up	54–74
Indeterminate	Low risk (AUS/FLUS)	5–15	Repeat FNA	10–20
	High risk (FN/SFN)	15–30	Lobectomy	
Suspicious for malignancy	Suspicious for			
	PTC			2.5–5.0
	MTC	60–75	Total thyroidectomy or lobectomy	
	Metastatic carcinoma			
	Lymphoma			
Malignant	PTC			
	MTC			
	Poorly differentiated carcinoma	95–99	Total thyroidectomy	4.0–5.4
	Anaplastic			
	Lymphoma			
	Metastatic carcinoma			

AUS atypia of undetermined significance, *FLUS* follicular lesion of undetermined significance, *FN* follicular neoplasm, *SFN* suspicious for follicular neoplasm, *PTC* papillary thyroid carcinoma, *MTC* medullary thyroid carcinoma [32, 63, 111–113]

re-aspirated. Some reports suggest that routine re-biopsy of benign lesions will significantly decrease false-negative rates [115, 116], whereas recent guidelines recommend re-biopsy of cytologically benign nodules only if those nodules are enlarging and >4 cm in size, possess malignant features, or are recurrent cysts (Table 4) [117].

Thyroglobulin in FNA of Cervical Lymph Nodes

Cervical lymph node (LN) involvement in thyroid cancer is indicative of metastatic disease. It is an integral component of cancer staging and determining treatment options. To improve detection of metastatic disease, Pacini and colleagues in 1992 reported thyroglobulin (Tg) measurements in needle aspirates from nonthyroidal neck masses can increase sensitivity from 85 to 100% in the detection of LN metastasis [118]. This is consistent with current sensitivity reports of around 95–100% [119, 120]. This simple procedure does not require additional punctures (washout is performed after smear preparation). The lack of standardized Tg levels leads to possible increases in false-negative reports in a subset of samples (i.e., "hook effect," dedifferentiated neoplastic tissue, or, in some studies, the interference with Tg antibody) [121].

Immunohistochemical Markers

Assessment of malignancy risk of indeterminate nodules remains a clinical challenge. Several immunohistologic markers have been introduced to assist in differentiating between benign and malignant smears [122]. For example, specific transcript variants of Clusterin, involved in neoplastic transformation, have shown promise in detecting PTC [123]. HBME-1, a monoclonal antibody, is highly expressed in PTC and may serve as a potential biomarker for PTC with a sensitivity and specificity of 72% and 72%, respectively [124]. Currently, no single marker is sensitive or specific enough to be considered for routine use. Some centers use immunochemical panels to assist in the diagnosis of indeterminate

Table 4 When to repeat FNA

Enlarging nodule
Clinically suspicious, cytologically negative nodule
Initial FNA nondiagnostic
Large nodule (>4 cm)
Recurrent cyst

nodules [125] though the lack of standardization makes this method impractical for routine use at this time.

MicroRNA

MicroRNAs (miRNAs) are short (no longer than 24 nucleotides), noncoding, typically negative regulators of gene expression with a high specificity to a given cell and disease [122, 126, 127]. Several different miRNAs have been identified to be associated with thyroid cancer [128, 129], tumor aggressiveness [130, 131], and potential therapeutic use [132, 133]. Currently, there are no specific tumor markers available to regularly and reliably distinguish between a benign and a malignant thyroid lesion. As studies continue, miRNAs may prove to be valuable in the diagnosis and treatment of thyroid cancer.

Molecular Markers

BRAFV600E (BRAF), an amino acid substitution at position 600 in BRAF, leads to increased kinase activity [134] and is found in approximately 45% of all PTCs [135]. Reportedly, BRAF predicts tumor aggressiveness with associated increased mortality [136, 137]. An independent association between BRAF mutation and tumor recurrence in all forms of PTC has also been reported [138]. BRAF mutations appear to activate various molecular mechanisms accelerating the tumors natural course [137]. With a 100% specificity for PTC, thyroidectomy is recommended in FNA aspirates detecting BRAF mutations. Since BRAF analysis is not widely available, current recommendations have not incorporated its routine use.

The three isoforms of RAS (NRAS, HRAS, KRAS) along with PAX8/PPARγ and RET/PTC rearrangements are detected at a lower frequency than BRAF [122]. Some evidence suggests that RAS, PAX8/PPARγ, or RET/PTC rearrangement-positive nodules may be histologically benign but carry a high potential of becoming malignant [139] or are associated with distant metastasis [140].

Gene expression classifiers (GEC) have a reported 95% negative predictive value (NPV) [141] but a low positive predictive value (PPV) of 15–38% [142], suggesting that GEC may be useful to "rule out" malignancy [143]. The low PPV of 38%, though, makes it a poor predictor of cancer if GEC is positive [143]. A recent study evaluating a vast number of genomic markers found that they should not be used as a sole means of detecting thyroid cancer due to a poor 50% sensitivity and 80% specificity [144]. Next-generation sequencing (NGS), testing for a 7-oncogenic panel, has recently reported a sensitivity and specificity of 90% and 93%, respectively, with a PPV and NPV of 83% and 96% for FN/FSN [145] and a sensitivity and specificity of 91% and 92%, respectively, with a PPV and NPV of 77% and 97% for nodules with AUS/FLUS [146]. When positive, it is highly suggestive of malignancy, a "rule in" test, and patients should thus be submitted for thyroidectomy.

Recommendations regarding the use of molecular markers in clinical practice differ between societies. For example, AACE guidelines are neither in favor of nor against use of molecular markers for cytologically indeterminate nodules [61, 66], whereas the ATA guidelines favor molecular testing for nodules with low-risk (AUS/FLUS) cytology [61].

We realize that this is a very dynamic area of clinical research, with the usefulness of molecular markers in thyroid practice continually evolving. At this time, it seems reasonable that nodules with AUS/FLUS cytology and negative GEC be closely followed, whereas those with FN cytology and positive NGS panel be surgically excised.

Laboratory Evaluation

Serum TSH is an accurate and sensitive measurement of thyroid function. Low TSH levels are suggestive of hyperthyroidism and should be followed with a free thyroxine (FT4) and total triiodothyronine (T3) measurement. An elevated TSH is suggestive of hypothyroidism and warrants

measurement of FT4 and thyroperoxidase antibodies (TPOAb) which, when positive, is highly suggestive of autoimmune disease. TPOAb should be measured whenever autoimmune thyroid disease (Hashimoto) is suspected, especially with a nodular goiter [61].

Recent data suggest that higher serum TSH levels, even within the normal range, may be associated with increased risk of thyroid malignancy and can be considered an independent marker to assist in malignancy prediction when considering FNA [147–149]. This association has been also suggested in the pediatric population [150].

The link between Hashimoto's thyroiditis (HT) and PTC continues to be debated [151–153]. Several studies have shown the expression of RET/PTC oncogenes in 68–95% of HT individuals [154, 155]. While the RET/PTC rearrangement is specific for PTC and is highly expressed in Hashimoto's, HT continues to be considered a relatively benign condition. A clear association between the two seems doubtful [156, 157], and we do not consider HT as a risk factor in nodule evaluation and management.

Serum thyroglobulin (Tg) concentration correlates with iodine intake and the size of the thyroid gland rather than the nature or function of a nodule [158]. It does not offer useful information in nodule diagnosis and should not be measured.

Serum calcitonin (Ct) is a marker of MTC and correlates with tumor burden [159]. Though MTC accounts for only 3–5% of all thyroid cancers, the prevalence of small MTC may range from 0.4 to 1.4% in those with nodular thyroid disease [160, 161], without clear evidence of clinical relevance of these medullary microcarcinoma foci. Moreover, calcitonin elevations can be seen in a variety of nonthyroidal diseases such as pulmonary endocrine tumors, renal failure, hypergastrinemia, alcohol use, and smoking, which should be taken in consideration when Ct is measured [162, 163].

The recent US guidelines do not recommend routine Ct measurement in individuals with thyroid nodular disease [61, 66]. This is in contrast to the European Panel of Experts (EPE) which recommends routine serum Ct measurements in all patients with thyroid nodules [164]. We favor selective Ct determination in those at risk for MTC, including a positive family history of MTC, MEN2, pheochromocytoma, or when FNA suggests MTC.

Radioisotope Scanning

Technetium-99 m pertechnetate (^{99m}Tc) and iodine-123 (^{123}I) scintigraphy are the two most commonly used techniques to evaluate autonomous thyroid function. ^{99m}Tc, a monovalent anion trapped by the thyroid gland by an active transport mechanism, is an inexpensive and readily available isotope administered intravenously. ^{123}I, a relatively expensive cyclotron produced radioisotope of iodine given orally, permits the evaluation of the entire metabolic iodine pathway including trapping, organification, coupling, hormone storage, and secretion [165]. ^{123}I is typically preferred over ^{99m}Tc due to better imaging quality in the mediastinum and for showing cellular function rather than just trapping ability. Increased uptake of ^{123}I or ^{99m}Tc is indicative of nodular hyperfunction, classified as "hot," while the lack of uptake is considered "cold" or hypofunctioning or indeterminate.

"Hot" nodules are considered benign, while "cold" or indeterminate nodules have a reported malignancy risk of 3–15% [166]. Of note, since hot nodules in children may be malignant, evaluation with FNA is recommended. Occasionally, nodules appear "hot" on ^{99m}Tc while "cold" on ^{123}I scanning due to the trapping of ^{99m}Tc within the nodule while unable to organify ^{123}I, exposing the true nonfunctioning ("cold") nature of the nodule. This discordance occurs in less than 5% of thyroid carcinomas [167].

Scintigraphy, with a resolution limit of around 1 cm, has a limited role in routine thyroid practice. With the majority of nodules being cold (80–85%), and only a small minority being malignant, the predictive value of cold or indeterminate nodules remains low [168]. Scintigraphy can provide useful information in the following settings: a single nodule with suppressed TSH, a large MNG with or without suppressed TSH to identify cold or indeterminate nodule requiring FNA, MNG with substernal extension, and in

congenital hypothyroidism and when searching for ectopic thyroid tissue, such as struma ovarii or sublingual thyroid [32].

Management and Therapeutic Approaches

Clinical management and therapeutic approaches of thyroid nodules are based predominantly on FNA cytology and US characteristics.

FNA-Malignant Nodule

With malignant FNA cytology, surgical resection is almost always indicated [61, 169, 170]. While the extent of surgery remains a matter of debate, recent trends favor a more limited approach for small, node-negative thyroid tumors. For example, lobectomy is recommended for nodules 1–4 cm with no extrathyroid extension or lymphovascular invasion [61].

The role of routine prophylactic central neck dissection for nonapparent lymphadenopathy (cN0) also continues to be debated [171, 172]. PTC is the most common thyroid malignancy, and approximately 30–90% of patients present with clinical or occult cervical LN involvement [173–175]. Recent guidelines recommend a detailed preoperative US evaluation to identify lymph node involvement to assist the surgeon's clinical decision-making on the extent of surgical resection [61, 169]. In cases when a patient is unable to undergo surgical resection, active surveillance is recommended.

FNA-Benign Nodule

Benign FNA cytology warrants follow-up, and nodule(s) should be monitored with an US and TSH measurement in 1–2 years [66, 91]. Since a 1–3% false-negative risk exists with benign cytology, a repeat FNA should be considered if: follow-up US is suspicious for malignancy [176, 177], if > 50% nodule volume increase and/or > 20% increase in at least two nodular dimensions over a 12 month period [66, 178].

Recent data confirm that approximately 75% of benign nodules remain stable, while 10–15% spontaneously enlarge over a 5-year period with minimal risks of subsequent cancer development [87, 179, 180]. Shrinkage of nodules with levothyroxine (LT4) suppressive therapy, though theoretically possible, is negligible in the vast majority of these patients [181, 182]. Additionally, LT4 suppressive therapy may lead to complications, including bone, heart, and quality of life issues [183–185]. Chronic low TSH in menopausal women decreases bone mineral density [186, 187] and increases fracture risks. There is a threefold increase in atrial fibrillation [188], with increased morbidity and mortality from cardiovascular diseases [189, 190]. For these reasons, neither ATA nor AACE guidelines recommend routine use of LT4 therapy in FNA-benign nodules [61, 66].

Volume debulking surgery should be reserved for large, locally symptomatic goiters. Most large asymptomatic benign nodules or goiters may be followed with periodic thyroid palpation and imaging.

FNA-Indeterminate Nodule

Lobectomy is the appropriate treatment for a solitary, cytologically indeterminate nodule [61, 66]. Total thyroidectomy may be preferred in those with sonographically suspicious features, large (>4 cm) lesions, bilateral nodules, or those with familial thyroid carcinoma history or personal history of neck irradiation. A repeat FNA may provide a definitive diagnosis in some cases [191, 192] but could also be problematic for the clinician who has to choose between two conflicting reports for a given patient. GEC or gene mutations (BRAF, RAS, RET/PTC, PAX8/PPARγ) may adjust malignancy risks [193–195] but can be cost-restrictive and currently are not recommended for routine clinical use.

FNA-Suspicious Nodule

With an estimated cancer risk of 60–75% [110, 111], total thyroidectomy is indicated. GEC (gene expression classifier) testing is not recommended in this category as it often fails to change the treatment course.

FNA-Nondiagnostic Nodule

An unsatisfactory specimen may result from a cystic nodule that yields few or no follicular cells; reaspiration yields satisfactory smears in about 50% of these cases [196]. Pure cysts, with a very low risk of malignancy, do not require FNA. Complex or solid nodules with nondiagnostic initial FNA should be re-biopsied. Large nodules (>4 cm), recurrent cysts, or solid nodules, with repeated nondiagnostic FNA, should be treated surgically due to a not-insignificant risk of malignancy [110].

TSH-Suppressed Solitary Nodule or MNG

A patient presenting with a solitary thyroid nodule, or a MNG, *and* a suppressed TSH, should undergo scintigraphy with technetium or radioiodine to evaluate for nodule autonomy. Autonomous nodules (hot) are rarely malignant in the adult patient and do not require FNA, but careful evaluation for suspicious features of malignancy should not be overlooked [197, 198]. By contrast, as many as 30% of children are reported to have incidental PTC in autonomous nodules, and FNA is needed when nodule is suspicious by US [199].

Multinodular Goiter (MNG)

MNGs are common, frequently benign, and often asymptomatic [200]. When a goiter causes local compressive symptoms such as dysphagia, choking, or airway obstruction, surgical intervention becomes necessary [180, 201] (Fig. 5). Approximately 10–15% of patients with goiters require surgical intervention, with up to 12% requiring reoperations due to nodular recurrence when initial thyroidectomy was partial or subtotal [202, 203]. The use of T4 suppressive therapy postoperatively does not have a significant effect in reducing recurrence rates [203, 204], except in iodine-deficient areas [205]. Currently, experienced surgical groups prefer total rather than subtotal thyroidectomy as the procedure of choice due to the very low risk of recurrence and minimal risk of permanent hypoparathyroidism or recurrent laryngeal nerve injury [202, 206, 207].

MNGs may become toxic (Plummer's disease) and, while not malignant, require treatment for hyperthyroidism [208].

Surgery

Surgical options for thyroid nodules include lobectomy, with or without isthmectomy, near-total, or total thyroidectomy [61]. The type of surgery is dependent on FNA cytology (suspicious vs. malignant), extent of disease (unilateral vs. bilateral disease), and presence of local symptoms. If presenting with dysphagia, choking, dyspnea, hoarseness, neck pressure, or pain caused by an enlarged thyroid gland, total thyroidectomy is preferred. Surgical complications include hemorrhage, vocal cord paralysis, and hypoparathyroidism [206], which are much less frequent with high-volume surgical practices [209].

Medical Rx

Radioiodine Therapy for Benign Nodular Goiter

The goals of radioiodine (^{131}I, RAI) therapy are to ablate thyroid autonomy, restore normal thyroid function, and reduce thyroid volume [210]. ^{131}I is a β- and γ-radiation emitter, which rapidly concentrates in the thyroid gland after oral ingestion [211, 212]. It is used for both hyperfunctioning and symptomatic nontoxic goiters. RAI therapy is effective and leads to an 80–90% euthyroid

state within 8 weeks posttreatment [213]; those with toxic MNG experience a 40% reduction in thyroid volume after 1 year and up to 60% by 5 years [214]. Radioiodine doses vary from 25 to 50 mCi, with approximately 80% of RAI-treated individuals at risk for hypothyroidism within 25 years [215].

Although some authors suggest the use of methimazole prior to ^{131}I ablation in toxic goiters [216, 217], randomized trials show lower efficacy with lower cure rates with this approach [218, 219]. Transient hypersecretion of T4 may occur in 1–5% of those treated with RAI for nontoxic MNG [220, 221]. RAI is otherwise well-tolerated, and if TSH has not normalized within 6 months, retreatment with RAI may be considered.

When malignancy is not a concern, RAI therapy is preferred in patients with nodular goiters at risk for surgical intervention, those with previous surgical resection, and for small nontoxic goiters (volume < 100 mL) [32]. RAI is not recommended in those requiring immediate resolution of hyperthyroidism or with symptomatic airway compromise. We do not favor RAI use in children or adolescents due to long-term risks of malignancy [222]. The only absolute contraindication of RAI therapy is pregnancy [223]; women of childbearing potential should have a pregnancy test prior to administration of radioiodine.

Recombinant Human Thyroid-Stimulating Hormone

In areas of mild iodine deficiency, ^{131}I is easily taken up by the thyroid gland to successfully treat nontoxic goiters. In iodine-sufficient regions, ^{131}I uptake in nontoxic goiters may be low or low-normal, and significantly higher ^{131}I doses are needed for ablation and thyroid volume reduction [224]. To minimize excessive dosing while maximizing efficacy, recombinant human TSH (rhTSH) has been used with increasing favor. Low doses (0.01 or 0.03 mg) of rhTSH given 24 h prior to ^{131}I administration result in a twofold increase of RAI uptake to the thyroid gland compared to nontreated subjects [225] while requiring a mean 36% reduction in ^{131}I dose [226]. rhTSH works by intensifying radiation absorption to the thyroid gland as a consequence of increased TSH [227, 228]. Thyroid volume reportedly decreases by around 40% [229]. Complications with rhTSH may include mild transient hyperthyroidism (40%), painful thyroiditis (30%), permanent hypothyroidism (65%), or transient goiter enlargement (24%) [230, 231]. Prior to any ^{131}I treatment, all patients should undergo US evaluation and, if necessary, US-FNA to rule out malignancy requiring thyroidectomy.

Nonsurgical Minimally Invasive Procedures

Percutaneous Ethanol Injection

Percutaneous ethanol injection (PEI) involves injecting 95–99% ethanol solution under US guidance into a cystic thyroid mass. It induces small vessel thrombosis, coagulative necrosis, interstitial edema, and granulomatous inflammation, followed by fibrotic changes leading to significant volume reduction [232, 233]. Occasionally multiple treatments are required to permanently shrink the cystic mass.

Thyroid cysts. Cystic nodules tend to recur 10–80% of the time post-percutaneous drainage and are dependent on the number aspirations, solid component, and cystic volume [52] (Fig. 1). The recurrence rate of cysts post PEI is much less, around 13–38% [234], with success rates of over 80% [52, 235, 236]. PEI has been reported to reduce symptoms in 75% of patients, whereas simple fluid aspiration reduced symptoms in only 24% of treated patients [52]. Mean volume reduction is around 65% for cysts [237]. A retrospective study noted that PEI-treated cysts and cystic nodules develop US features of malignancy in 75% of patients without real increased malignancy compared to nontreated cysts [238]. Risks associated with the procedure include pain, ethanol leakage into surrounding tissues, and dysphonia, which are typically mild and transient. We consider PEI as treatment of choice for symptomatic or recurrent benign thyroid cysts [233].

Autonomously functioning thyroid nodules. Use of PEI has shown variable results in autonomously functioning nodules. Larger nodules (>60 mL) tend to have higher failure rates [239], while smaller or pre-toxic nodules show more success [240, 241]. A study of 125 patients noted a mean shrinkage of 66% and normalization of thyroid function in over 90% of subjects [242]. Currently, ^{131}I and surgery remain the better alternative therapy for hyperfunctioning nodules.

Cold solid nodules. Use of PEI in FNA-benign, nonfunctioning solid nodules leads to a 38% volume reduction [237]. The procedure appears to be more effective than T4 suppressive therapy in decreasing nodule volume and relieving local pressure symptoms. Complications include transient pain, and some nodules may require multiple treatments. Lack of long-term follow-up, and the fact that majority of benign nodules remain stable in size, mitigates against use of PEI for benign, solid nodules [233].

PTC malignant lymph nodes. PEI has been used in patients with PTC metastatic lymph nodes, who are unresponsive to ^{131}I and/or non-surgical candidates. PEI is well-tolerated and effective in limited nodal disease (1–5 involved lymph nodes) with initial therapy [243] and long-term outcome [244]. It is considered to be both safe and an effective alternative to nodal dissection but sadly is not offered by many centers in the USA [244].

In summary, PEI is effective and should be the treatment of choice for recurrent, large, or symptomatic benign thyroid cysts. It should seldom be used to treat autonomously functioning thyroid nodules; RAI remains the procedure of choice in most of these cases. PEI is not recommended for cold thyroid nodules but should be considered for malignant cervical nodes in some circumstances.

Other Approaches

Laser thermal ablation (LTA). LTA involves using laser light transmitted through silica optical fibers guided through a 21G spinal needle into the desired location [233]. LTA is a safe and effective method of ablating cold, autonomous, and cystic nodules [245–247]. Though complication risks are minimal, they must not be ignored and include tracheal wall damage from operator lack of experience and vocal cord paralysis with recurrent laryngeal nerve damage. To minimize these risks, treatment should be performed by well-trained operators [248]. Other risks include transient cervical burning during the procedure with possible persistence for a few days post procedure or low-grade fever, controlled with acetaminophen [233].

Radio-frequency ablation (RFA). RFA uses electromagnetic energy to induce thermal injury to its target. Large 14G or 17G needles are used to deliver 30–50 Watts of radio-frequency power to large lesions under conscious sedation [233]. RFA has been shown to reduce benign nodules by 50–90% with resolution of symptoms within 6 months of treatment [249]. While RFA is very effective, it requires special equipment and experienced personnel [233]. RFA has also been described as effective in the management of locally recurrent thyroid cancer patients who are poor candidates for or refuse surgery. RFA has shown significant volume reduction, with low recurrence rates post treatment [250].

Summary

We have witnessed a significant improvement in the management of patients with thyroid nodules over the last few decades. Not long ago, thyroid masses were detected by palpation, either by the patient or by an examining physician, evaluated by radioisotope scan, and mostly treated by surgical excision. The majority of excised nodules were benign, meaning that most surgeries were unnecessary. Those not surgically treated were placed on chronic thyroxine (T4) therapy with TSH suppression.

Today, we use reliable techniques to identify benign nodules and medically manage most patients with nodular thyroid disease. We employ sensitive US machines, perform accurate FNA guided by US, apply complex cytological classifications, and use nonsurgical approaches to treat and follow thyroid nodules.

Long-term T4 suppressive therapy is no longer used and must be avoided, because of lack of benefit and potential harmful complications. Identifying various molecular markers helps guide treatment to minimize or eliminate unnecessary surgery.

It should also be noted that the application of sensitive imaging to thyroid practice, while quite beneficial, has had unintended consequences. Discovery of small, incidental thyroid masses often creates a dilemma for the physician taking care of a patient who has no prior history of thyroid disease and now is faced with a new thyroid mass. Frequently, small incidentalomas are evaluated by FNA, and when cytology is abnormal, surgery follows. Many of these micronodules are clinically insignificant and may never become relevant in the patient's lifetime. This scenario likely explains the significant rise in the incidence of PTC in recent decades and should be considered a setback in the management of nodular thyroid disease.

This chapter describes advances, advantages, and future directions in thyroid nodular disease. The Bethesda system for reporting thyroid cytopathology has improved our ability to further separate low- from high-risk indeterminate cytology. At this time, the role of biomarkers to better classify indeterminate cytology is not well established, though we remain hopeful that improving technology will lead to better clinical tests. Minimally invasive techniques offer a high degree of therapeutic promise, not only for benign nodules but also for limited, recurrent locoregional malignancy.

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Nontoxic Multinodular Goiter

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Introduction

Multinodular goiter (MNG) is defined as the enlargement of the thyroid gland, in the absence of inflammation, autoimmune thyroid disease, and malignancy. Furthermore, the term MNG refers to a thyroid gland that presents many nodules, in the absence of thyroid dysfunction. The natural history of MNG is characterized by thyroid growth, followed by nodule formation, and the progression, in many cases, to nodule autonomy and overt hyperthyroidism (i.e., toxic MNG; TMNG) due to hyperfunctioning nodules that secrete thyroid hormones independent of TSH stimulation. Iodine deficiency is the most important etiologic factor predisposing to the development of MNG.

The clinical presentation of a patient with MNG is diverse. Some affected patients can be asymptomatic; on the other side of the spectrum, individuals with large goiters can present upper airway compression and respiratory insufficiency. MNG is more prevalent in females than in males, in a proportion of 6:1 to 15:1. The diagnostic evaluation includes thyroid function tests; imaging studies such as thyroid ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI); cytological studies of nodular samples obtained through US-guided fine-needle aspiration (US-FNAB); and assessment of radioactive iodine uptake (RAIU). The management of a patient with MNG depends on the clinical presentation and preference, which includes surgery or radioiodine therapy in most cases.

Epidemiology

Endemic nodular goiter is defined as thyroid enlargement that affects 5% or more of children from 6 to 12 years old [1]. The most importance etiological factor for MNG is iodine deficiency; it has been observed that the incidence of MNG is negatively correlated with the iodine intake of a population living in a given area. Besides iodine, other risk factors for the development of MNG include smoking, intake of natural goitrogens, age, sex, and heredity [1]. However, epidemiologic studies assessing the risk factors for the development of goiter, as well as its prevalence, have several limitations, such as different methods employed for the determination of thyroid volume, heterogeneous criteria defined for the selection of affected individuals (including thyroid function), and interference of environmental factors [2].

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Low environmental supply of iodine accounts for thyroid abnormalities such as increased nodular size and goiter, in which prevalence can be as low as 15% in case of mild iodine deficiency and as high as 23% in case of moderate iodine deficiency [3]. It is estimated that the prevalence of thyroid nodular disease is approximately 4% in iodine-sufficient countries. This prevalence can be four- to fivefold higher in iodine-deficient areas [4].

Based on ultrasonographic studies conducted in iodine-deficient areas, the reported prevalence of nodular disease in adults is about 30–40% in women and 20–30% in men [2]. In the Wickham study (County Durham, UK), a survey of 2749 individuals living in an iodine-sufficient area detected small goiters (palpable but not visible) in 8.6% of that sample and obvious goiters (palpable and visible) in 6.9%. Goiters were four times more common in females [5].

In another survey conducted in Framingham (Massachusetts, USA), an iodine-sufficient area, the prevalence of MNG was 1% (diagnosed by palpation). By US, 3% of individuals older than age 60 and 36% of women aged 49–58 had thyroid nodules [6]. As the disease progresses, thyroid volume and nodularity increase. Subclinical and overt hyperthyroidism develops in many cases, as a consequence of the overproduction of thyroid hormones due to the increase in functioning thyroid parenchyma and to thyroid autonomy [6].

Etiology

The adaptive response of the thyroid follicles to endogenous and exogenous factors that impair thyroid hormone synthesis leads to the development of goiter. Iodine insufficiency and persistent (even discrete) thyrotropin (TSH) elevation lead to thyrocyte proliferation and diffuse enlargement of the gland during childhood and adolescence [7]. However, this traditional concept has to be challenged to contemplate the many aspects of goiter [8].

While the important roles of iodine deficiency and of the growth-promoting effect of TSH are, without any doubt, recognized as important factors in the MNG etiology, this hypothesis has been revised. The notion that iodine deficiency is the sole factor responsible for the development of goiter appears to be an oversimplification [8]. Endemic goiter has been described in countries with no iodine deficiency and even in some regions with iodine excess. Curiously, it has not been seen in some areas with severe iodine deficiency [8]. MNG can develop as a consequence of an inherent predisposition of the thyroid gland to develop nodules during aging, amplified by the presence of additional factors further promoting thyrocyte proliferation and nodule formation [9]. Superimposed iodine deficiency, even at moderate degrees, enhances the clinical presentation of MNG in younger individuals, with the additional influence of augmented TSH secretion [8].

Environmental factors associated with the development of goiter include cigarette smoking, infections, use of certain drugs, and exposition to goitrogens [10]. Several substances have been shown to have goitrogenic effects: cruciferous vegetables (such as cabbage, kale, cauliflower, broccoli, turnips, and rapeseed) contain glucosinolates, which metabolites compete with iodine for thyroidal uptake. Similarly, cassava, lima beans, linseed, sorghum, and sweet potato contain cyanogenic glucosides, which may be metabolized to thiocyanates and compete with iodine for thyroidal uptake. Cigarettes also contain thiocyanates. Other goitrogens include perchlorate, disulfides from coal processes, and flavonoids present in soy [11, 12].

Smoking is an unquestionable environmental factor that contributes to the development of MNG, especially in areas of mild iodine deficiency. It has been confirmed that the thiocyanate present in tobacco competes with solute carrier family 5 sodium-iodide symporter, member 5 (SLC5A5), also known as sodium-iodide symporter (NIS) for the active uptake of iodine in the basal membrane of the thyrocyte [13, 14].

The effects of goitrogenic substances are attributed to their inhibitory action on iodine uptake and thyroperoxidase (TPO) activity, to their effect leading to the displacement of thyroxine (T4) from the serum thyroid-binding protein transthyretin, and to their influence on reducing

T4 half-life [14]. Those changes lead to a slight state of hypothyroidism with an increase in TSH, which stimulates thyroid growth.

The deficiency of selenium, iron, and vitamin A predisposes to the development of goiter by (1) increasing peroxides that damage the thyroid gland and by impairing deiodinase activity (selenium deficiency), by (2) reducing TPO activity (iron deficiency), or by (3) decreasing vitamin A-mediated suppression of the pituitary *TSHβ* gene [12]. On the other hand, iodine excess, observed in many countries such as Brazil, Chile, Algeria, Ivory Coast, Zimbabwe, and Uganda, also has a goitrogenic effects due to its action on decreasing the synthesis and secretion of thyroid hormones [15].

The effects of estrogens on the pathogenesis of goiter seem to be determined by several complex pathways, depending on whether endogenous or exogenous estrogens are involved [16]. Estradiol increases FRTL-5 cell growth in a time- and concentration-dependent manner in either the absence or presence of TSH [17]. Because iodine is known to inhibit thyroid cell growth, the effect of estradiol on the expression of NIS is considered as a potential target of estrogen action. Estradiol blocks TSH-induced NIS expression, demonstrating that the Fischer rat thyroid cell line 5 (FRTL-5) contains functional estrogen receptors that enhance cell growth and inhibit NIS expression [17]. Thus, there is a direct effect of female sex hormones on thyrocytes and consequent thyroid growth, which explains the higher prevalence of MNG among women [2]. In addition, it has been suggested that 17β-estradiol amplifies the growth factor-induced signaling in the normal thyroid and in thyroid tumors [18].

Besides TSH, other growth factors are also involved in the pathogenesis of nodular goiter. Insulin-like growth factor-1 (IGF-1) levels are positively correlated with thyroid volume in both genders and with the presence of nodules in men [19, 20]. Increased levels of IGF-1, as seen in acromegalic patients [21] and in transgenic mice overexpressing IGF-1/IGF-1 receptor [22], are also associated with increased thyroid volume.

Genetic and environmental aspects may play an important role in the genesis of diffuse and nodular goiter, and some of these factors may act synergistically [23]. In a study that evaluated twins, it was demonstrated that genetic factors accounted for 67% and environmental factors for 33% of the individual differences in the susceptibility to the development of thyroid nodularity [24], suggesting that genetic factors are of etiological importance for thyroid nodularity in clinically healthy and euthyroid individuals [24]. The occurrence of familial cases of nodular goiter, at an early age in many cases, strengthens the hypothesis that genetic factors are involved in the pathogenesis of goiter [25]. Because of their important role in thyroid physiology and hormone synthesis, genes involved in various aspects of thyroid physiology and hormone synthesis such as thyroglobulin (*TG*), *TPO*, *NIS*, dual oxidase 2 (*DUOX2*), and TSH receptor (*TSHR*) are major candidate genes for familial euthyroid goiters [16]. Besides the aforementioned genes, other candidate loci as determinants of nodular goiter are the multinodular goiter 1 *(MNG*) locus on chromosome 14 and loci on chromosomes Xp22 (*MNG2*), 2q, 3p, 3q (*MNG3)*, 7q, and 8p [26]. In genome-wide association studies, four genetic loci were associated with thyroid volume: two independent loci located upstream of and within capping actin protein of muscle Z-line beta subunit *(CAPZB*), one within fibroblast growth factor 7 *(FGF7*), and one in chromosome 16q23 [27]. A recent study combining genome-wide linkage analysis with whole-exome sequencing identified gene variants (in multigenerational goiter families with an autosomal dominant inheritance pattern) that may be involved in familial goiter (*RGS12, GRPEL1, CLIC6,* and *WFS1*) [28] (Table 1).

Pathology

Histologic analysis reveals a broad range of morphologies, from hypercellular regions to cystic areas filled with colloid. Fibrosis is frequently extensive, with areas of hemorrhage or lymphocytic infiltration. Using molecular techniques, most nodules within a MNG are polyclonal in origin, suggesting a hyperplastic response to

Table 1 Examples of situations predisposing to goitrogenesis [16]

Origin	Substance	Examples
Iodine intake	Iodine deficiency	Individuals living in Australia and certain European countries
	Iodine excess	Individuals living in Brazil and Japan
Chemicals	Thiocyanate	Cigarettes
	Perchlorate	Fertilizers, solid propellants, fireworks, road flares, matches, and airbag inflation systems
	Disulfides	Coal processes
Food substances	Flavonoids	Soy
	Glucosinolates	Cruciferous vegetables
	Cyanogenic glucosides	Cassava, lima beans, linseed, sorghum, and sweet potato
Drugs	Lithium, aminoglutethimide	Mood stabilizing and anti-steroid drugs
Pollutants	Nitrates	Nitrogen-rich fertilizers
Micronutrient deficiencies	Deficiency of selenium, iron, and vitamin A	Patients on total parenteral nutrition, iron deficiency anemia, cystic fibrosis
Hormonal alterations	IGF-1 excess, TGF decrease	Acromegaly, systemic lupus erythematosus
Genetic factors	Mutations and polymorphisms predisposing to goiter	Recessive or dominant mutations, polymorphisms on genes such as 16q23

locally produced growth factors and cytokines. TSH, which is usually not elevated, may play a permissive or contributory role. Monoclonal lesions also occur within a MNG, reflecting mutations in genes that confer a selective growth advantage to the progenitor cells [29].

Thyroid glands in the early phase of development of MNG show areas of hyperplasia with considerable variation in follicle size. Macroscopic examination shows nodular consistency and varied appearance, with the normal homogeneous parenchymal structure deformed by the presence of nodules [30]. The nodules may vary considerably in size (from a few millimeters to several centimeters), in borders (from sharp encapsulation as seen in adenomas, to poorly defined margination), and in architecture (from solid follicular adenomas to gelatinous, colloid-rich nodules or degenerative cystic structures) [30].

Often, there is extensive fibrosis, and calcium may be deposited in internodular septae. Areas of normal thyroid tissue are scattered between the nodules, and there are often focal areas of lymphocytic infiltration. Radioautography shows a variety of appearance, with uptake localized sometimes in the adenomas and sometimes in the paranodular tissue. Occasionally, most of the radioactivity is confined to a few nodules that seem to dominate the metabolic activity of the gland [30].

Clinical Manifestations

Patients with relatively small goiters and with normal thyroid function are usually asymptomatic. However, the goiter can gradually increase in size, leading to the development of multiple nodules, local compressive symptoms (such as difficulty in swallowing, cough, respiratory distress, and feeling of a lump in the throat), and/or cosmetic complaints. Almost 70% of patients with sporadic nontoxic goiter complain of neck discomfort; the remainder has cosmetic concerns or fear of possible malignancy [16]. Thyrotoxicosis may develop, particularly in older patients, and can present from asymptomatic subclinical hyperthyroidism to overt hyperthyroidism [31].

There are no specific parameters to predict, in a given patient, the natural history of MNG. Usually, the physician follows the patient, and further approaches are undertaken if clinical symptoms and signs arise. In some cases, treatment can be chosen before the goiter grows any further, avoiding adverse clinical outcomes for the patient [10].

Younger patients tend to have smaller, diffuse goiters, with few or no nodules and without intrathoracic extension. Many times, the goiter grows gradually for a period of a few to many years, and then growth becomes stable with little tendency for further enlargement [16]. Patients with large goiters may develop compressive symptoms, and the clinical manifestations are influenced not only by the size of the goiter but also by its possible substernal extension. In that case, compression of the trachea, esophagus, and great vessels is more likely to occur in the confined space of the thoracic inlet. The close relationship of the thyroid gland with adjacent structures is the key factor that determines the most significant clinical manifestations. Large goiters can compress the trachea, esophagus, and neck vessels and be associated with symptoms of inspiratory stridor and dysphagia [10, 16].

Rarely, compression of carotid arteries may cause carotid bruit, a systolic sound heard over the carotid artery area during auscultation [10]. Compression of the recurrent laryngeal nerves may lead to unilateral or bilateral vocal cord paralysis, with consequent transient or permanent dyspnea and hoarseness [32]. Compression of the cervical sympathetic chain may cause paralysis of the phrenic nerve (which can be asymptomatic or cause dyspnea) [33]. Horner's syndrome (ptosis, mitosis, and decreased sweating of the face on the same side) [34], thrombosis of the jugular vein, and superior vena cava syndrome have also been described [35].

Hemorrhage into a nodular cyst can provoke sudden increase in the size of the gland, associated with sharp pain and tenderness. In that case, US reveals a cystic nodule at the hemorrhage site, confirming this event. Within some days, the symptoms disappear; within a few weeks, the gland may revert to its previous dimensions [30]. Malignant nodules tend to be firm, irregular, and fixed to adjacent tissues. Regional lymphadenopathy also raises suspicion for malignancy [16].

In cases of goiter migration to the retrosternal and upper mediastinal regions, compression of the jugular and subclavian veins in the area around the superior vena cava may occur. Pemberton's maneuver (extension of the arms above the head) leads to dislocation of the goiter into the upper thoracic inlet, causing respiratory difficulty, distension of the neck veins, facial congestion, and stridor due to increased pressure on the trachea (Pemberton's sign) [36] (Table 2).

The management of substernal goiters has challenged surgeons for decades. Their treatment is important, as they can represent up to 7% of mediastinal tumors [37]. Huins et al. commented on the diverse definitions used for retrosternal goiters [38] and concluded that the classification based on the anatomical location (1, above the aortic arch or above T4; 2, the aortic arch to the pericardium; and 3, below the right atrium) provides a common standard for preoperative planning [38].

The CSI (CT scan cross-sectional imaging) classification system is useful for risk stratification and defines substernal goiters based on (1) the craniocaudal extension, as grade 1 (above the aortic arch), grade 2 (at the level of the aortic arch), and grade 3 (below the aortic arch); (2) the anteroposterior extension, as type A (prevascular), type B (retrovascular-paratracheal), and type C (retrotracheal); and (3) the latero-lateral extension, as monolateral or bilateral [39]. The prevalence of intrathoracic goiters, with substernal or mediastinal extension, ranges between 2.6 and 30.4% [16].

Table 2 Possible clinical manifestation in patients with multinodular goiter and their causes [16]

Effect	Clinical manifestation
Tracheal compression	Dyspnea Respiratory stridor Cough Choking sensation
Esophageal compression	Dysphagia
Recurrent laryngeal nerve compression	Hoarseness
Cervical sympathetic chain compression	Horner's syndrome (ptosis, miosis, decreased facial sweating) Phrenic nerve paralysis
Vascular compression	Pemberton's sign
Thyroid hyperfunction	Signs and symptoms of hyperthyroidism

Diagnostic Evaluation

History and Physical Examination

Careful anamnesis and physical examination are crucial for the diagnostic evaluation of a patient with MNG. The incidence of malignancy in MNG appears to be the same as in uninodular goiter and is one of the main concerns when evaluating MNG [40]. On the contrary, a recent systematic review and meta-analysis confirmed the slightly higher risk of malignancy in solitary nodules, compared to MNG. This cancer incidence appears to be valid mainly in iodine-deficient populations [41]. Features suggesting benign disease include family history of Hashimoto's thyroiditis, benign thyroid nodule, or goiter; symptoms of hypothyroidism or hyperthyroidism; and a sudden increase of nodule size with pain or tenderness, which suggests a cystic hemorrhage or localized subacute thyroiditis [10].

Investigating the family history, comorbidities and potential exposure to goitrogens may help to determine the etiological factors. Thyroid nodules are generally benign colloid nodules, and only 5–10% of nodules coming to medical attention are carcinomas. With the widespread practice of medical checkups in healthy individuals, and the increasing use of imaging technology, this problem is likely to become more common [8]. High-resolution US studies suggest that the prevalence of nodular thyroid disease in healthy adults is greater than 60% [42]. Many studies have shown that nodule size is not predictive of malignancy and that the incidence of cancer in incidentally identified or nonpalpable thyroid nodules is the same as in patients with palpable nodules [40, 42]. On the other side, some authors suggest that the incidence of carcinoma in thyroid nodules equal to or larger than 4 cm is high, with an elevated false-negative rate for preoperative benign cytology [43]. Given the excellent prognosis of micropapillary carcinoma measuring less than 1 cm in diameter, most authors recommend investigation of only those nodules larger than 1 cm and of nonpalpable nodules with clinical or sonographic suspicious findings [40, 42, 44].

Laboratory Investigation

In any patient with goiter, serum TSH is by far the most used test in the initial evaluation, which allows the determination of thyroid function. Patients with normal serum TSH are considered euthyroid and do not need further laboratory investigation [40].

A low or undetectable serum TSH is consistent with thyroid hyperfunction due to autonomously functioning nodular areas and warrants thyroid scintigraphy [16, 40]. Serum thyroid hormones must be measured in order to diagnose subclinical or overt hyperthyroidism. If clinical findings strongly raise suspicion of hyperthyroidism, total triiodothyronine (T3), total T4, and free T4 could be measured concomitantly with TSH [16]. Patients with thyroid hyperfunction, especially elderly ones, should have additional cardiac investigation, as the risk of atrial fibrillation may be increased as much as threefold when serum TSH levels are less than 0.1 mU/L [45].

A recent panel of the American Thyroid Association (ATA) could not recommend either for or against routine measurement of serum calcitonin in patients with thyroid nodules [40]. If measured, calcitonin levels must be interpreted with caution, since it has low positive predictive value and can lead to unnecessary surgery [46]. Thyroglobulin measurements are not recommended in the evaluation of malignancy [40]. In patients with MNG and overt hyperthyroidism, the determination of the titers of TSH receptor antibodies (TRAb) may be considered to support or exclude the diagnosis of Graves' disease co-existing with multiple nonfunctioning thyroid nodules (the so-called Lenhart-Marine Syndrome). Also, elevated anti-TPO antibodies (TPOAb) are associated with an increased risk of post-radioiodine hypothyroidism [47] and of Graves' hyperthyroidism [48, 49].

Imaging

The presence of MNG may be detected incidentally during a chest X-ray done for other reasons as a mass occupying the upper mediastinum, fre-

quently with tracheal deviation. Thyroid US is mandatory in the evaluation of a patient with MNG [40]. It is an inexpensive, easy to perform test and can be used to guide fine-needle aspiration biopsy (US-FNAB). It provides an estimate of thyroid volume and also identifies and characterizes benign-appearing and more suspicious thyroid nodules.

The US report should convey nodule size (in three dimensions), as well as areas adjacent to the carotid artery and the jugular vein, in search of suspicious cervical lymphadenopathy [40]. US may assess not only the characteristics of the thyroid nodules but is also used to follow those nodules [42, 10]. The usefulness of US is very limited in patients with intrathoracic goiters, as the US beam cannot penetrate the bone. Cross-sectional imaging with computerized tomography (CT) or magnetic resonance imaging (MRI) are invaluable tools that fully characterize thyroid volume, degree of substernal extension, and compression of the trachea [16]. CT and MRI can also determine the cross-sectional area of the trachea, a useful measure of tracheal compression [16]. None of these methods have any advantages over sonography when it comes to detailed visualization of the intrathyroidal structure [10].

The major strength of CT and MRI is the ability to diagnose and assess the extent of substernal goiters much more precisely than any other method. A comparative study between CT and MRI showed an accuracy of 85.7% for CT and 100% for MRI regarding the correlation between anatomo-topographic and intraoperative findings, without a significant statistical difference between these two diagnostic procedures [50]. Another advantage of CT and MRI is the possibility to estimate planimetric volumes, especially useful in cases of irregularly enlarged goiters [16] (Fig. 1) [51].

Calculation of thyroid volume based on US recordings of cross-sectional areas is a reproducible method in patients without substernal goiter extension [52]. Wide differences are observed in the size of nodular goiters measured by scintigraphy and US [53]. All of the methods described above have little value in the differentiation between malignant and benign thyroid lesions (Table 3), but new techniques are promising in this respect. Increased glucose metabolism, measured by [^{18}F]2-deoxy-2-fluoro-d-glucose positron emission tomography (FDG-PET), can, with high precision, differentiate malignant from benign thyroid nodules [54]. Nearly 50% of thyroid nodules detected incidentally by FDG-PET harbor thyroid cancer [54].

Fine-Needle Aspiration Biopsy (FNAB)

Patients with toxic MNG or atoxic MNG have the same risk of malignancy as the risk observed in those with single nodules [40, 55]. Patients with nodular goiter and low TSH levels appear to have a lower risk of papillary thyroid cancer [56]. The recommendations for US-FNAB in patients with MNG are the same as those for patients with single nodules: the dominant nodule, as well as any sonographically suspicious nodules, should be biopsied [40, 57]. It is preferable that FNAB be performed under US guidance (US-FNAB), rather than without US. This procedure is cost-effective and increases accuracy [58]. Nodules that are suspicious for malignancy are hypoechoic, have irregular margins, do not have a sonolucent halo, and have intranodular vascularity and microcalcifications [40, 59].

US-FNAB reports should follow the classification proposed at the National Cancer Institute Thyroid Fine-Needle Aspiration State of the Science Conference, held in Bethesda: (1) nondiagnostic or unsatisfactory, (2) benign, (3) atypia of undetermined significance or follicular lesion of undetermined significance, (4) follicular neoplasm or suspicious for follicular neoplasm, (5) suspicious for malignancy, and (6) malignant [40, 60]. If the cytological findings are indeterminate (Bethesda 3), a second cytologist opinion can be helpful. If the cytological analysis is the same, it is recommended to repeat fine-needle aspiration in 6–12 months, or to perform mutational analysis or molecular profiling, to better estimate the risk of cancer [40] (Table 4).

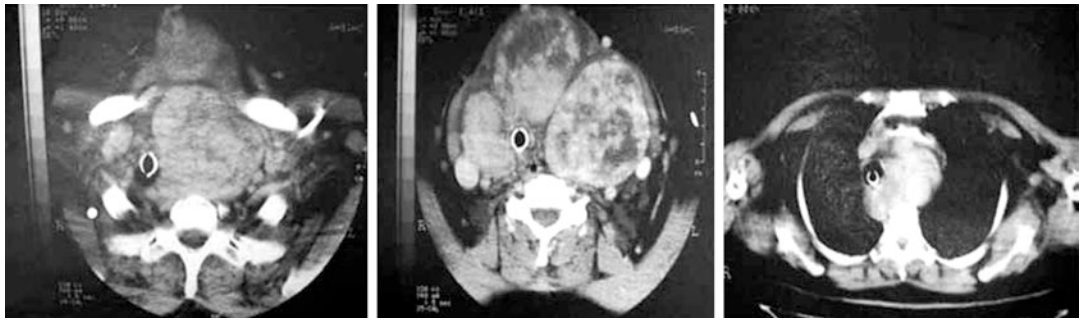

Fig. 1 CT scan showing the large substernal goiter compressing and deviating the trachea (Reproduced from [51])

Pulmonary Function Tests

Although large MNG can lead to tracheal compression and pulmonary function impairment, it is common that this aspect is not evaluated routinely in the clinical practice [61]. Pulmonary evaluation is fundamental in cases of large and/or intrathoracic goiters compressing the trachea, in order to determine the presence and degree of pulmonary functional impairment and to demonstrate the efficacy of therapeutic approaches such as surgery and radioiodine therapy. The airflow rate, particularly in the inspiratory phase, is obviously critically compromised if the lumen of the trachea is reduced beyond a certain point [62]. Even asymptomatic patients may have abnormal results and therefore may benefit from this evaluation [61, 62]. Improvement in pulmonary function after surgery or radioiodine therapy can be objectively quantified with pulmonary function tests [61] (Fig. 2).

Management

The management of patients with MNG is guided by the clinical presentation and by the patients' preference, which is comprised of expectant clinical observation, surgery, or radioiodine therapy. Occasionally, long-term, low-dose treatment with methimazole is warranted to some patients with subclinical hyperthyroidism or toxic multinodular goiter and with limited longevity, such as elderly or otherwise ill patients, provided they can be monitored regularly and they prefer this option [40]. Iodine supplementation and suppressive therapy with levothyroxine are not recommended therapeutic options. In spite of these observations, there is not a unique treatment for MNG. This can be confirmed by surveys involving European [63], American [64], and Latin-American [65] endocrinologists, showing that there are different views in the treatment of patients with MNG.

Clinical Observation

Clinical observation is a reasonable approach in the management of patients with nontoxic and asymptomatic benign MNG that do not cause any cosmetic issues to the patient. The natural history of goiters during menopause shows that thyroid growth is extremely slow, with no significant change in mean nodule volume over 5 years [66]. In a survey among European clinicians, one third of them would refrain from treating a patient with moderate discomfort due to a multinodular nontoxic goiter of 50–80 μg, in which malignancy had been ruled out [63]. A conservative approach was preferred, based on periodic assessment of the thyroid hormonal status and on the monitoring of goiter and thyroid nodule sizes by US and CT scans [63].

For thyroid nodules, ATA suggests that monitoring should be conducted according to risk stratification: nodules with high suspicion, low to intermediate suspicion, and low suspicion sonographic pattern should be evaluated every 12, 12–24, and ≥24 months, respectively [40]. If a

Table 3 Advantages and disadvantages of the main imaging tools [16]

Tool	Advantages	Disadvantages
Ultrasound	Widely available	Operator-dependent
	Has high resolution	No information on thyroid function
	No exposure to ionizing radiation	Not useful for substernal goiters
	Dynamic picture	Poor prediction of malignancy
	Ability to visualize blood flow (Doppler)	
	Moderate precision to estimate thyroid volume	
	Low patient discomfort	
	Bedside investigation	
Scintigraphy	Information on thyroid function	Requires nuclear medicine units
	Differentiates between destructive and hyperthyroid conditions	Ionizing radiation
	Measures iodine uptake	Poor resolution
	Predicts feasibility of radioiodine therapy	Poor differentiation between solid and cystic cold nodules
	Detects ectopic tissue	Inaccurate volume estimation
		^{99m}Tc may falsely show nodular uptake
		Affected by iodine contamination
		Poor prediction of malignancy
CT scan	Good availability	Ionizing radiation
	Has high resolution	No information on thyroid function
	Visualization of adjacent structures	Poor prediction of malignancy
	Useful for substernal goiter	
	Planimetric volume estimation	
	Accurate volume estimation	
MRI	No exposure to ionizing radiation	Moderate availability, expensive
	Has high resolution	Long procedure time
	Visualization of adjacent structures	Contraindicated for patients with implanted metallic objects
	Useful for substernal goiter	No information on thyroid function
	Planimetric volume estimation	Poor prediction of malignancy
	Highly accurate volume estimation	Claustrophobia

nodule has been submitted to a repeated US-FNAB with a second benign cytology result, US surveillance for malignancy is no longer indicated [40]. In patients harboring intrathoracic or substernal goiters, US-FNAB is anatomically difficult and malignancy, while rare, cannot be always ruled out [67]. As surgery is almost always recommended for this particular goiter presentation, thyroid cancer can be confirmed or ruled out [67].

US-FNAB should be repeated only if characteristics that raise suspicion for malignancy are newly identified, if a thyroid nodule volume increases by ≥50% in size or by ≥20% in at least two of the three diameters, or if other worrisome clinical features develop, such as persistent hoarseness, dysphagia, or adenopathy [40]. Ultrasonography altered the clinical management for 63% of the patients referred to a thyroid nodule clinic after abnormal results on thyroid physical examination [68]. Routine follow-up FNAB has been shown not to be cost-effective in patients whose initial FNAB was benign [69].

Iodine Supplementation

Iodine supplementation does not have sufficient therapeutic effect on MNG, in spite of the knowledge that iodine deficiency is the most important

Table 4 The Bethesda System for Reporting Thyroid Cytopathology: Recommended Diagnostic Categories [60]

I. Nondiagnostic or unsatisfactory	Cyst fluid only
	Virtually acellular specimen
	Others (obscuring blood, clotting artifact, etc.)
II. Benign	Consistent with a benign follicular nodule (includes adenomatoid nodule, colloid nodule, etc.)
	Consistent with lymphocytic (Hashimoto) thyroiditis in the proper clinical context
	Consistent with granulomatous (subacute) thyroiditis
	Others
III. Atypia of undetermined significance or follicular lesion of undetermined significance	
IV. Follicular neoplasm or suspicious for a follicular neoplasm	Specify if Hürthle cell (oncocytic) type
V. Suspicious for malignancy	Suspicious for papillary carcinoma
	Suspicious for medullary carcinoma
	Suspicious for metastatic carcinoma
	Suspicious for lymphoma
	Others
VI. Malignant	Papillary thyroid carcinoma
	Poorly differentiated carcinoma
	Medullary thyroid carcinoma
	Undifferentiated (anaplastic) carcinoma
	Squamous cell carcinoma
	Carcinoma with mixed features (specify)
	Metastatic carcinoma
	Non-Hodgkin lymphoma
	Others

etiological factor for goiter development worldwide. Even in iodine insufficient parts of the world, iodine therapy does not effectively decrease thyroid volume, once MNG is established. In a recent study, a 1-year combination of iodine and levothyroxine leading to incomplete TSH suppression reduced thyroid nodule volume compared to either component alone or placebo. This finding may be explained by the fact that patients with thyroid nodules included in this trial were clearly iodine deficient (mean urinary iodine excretion 49.7–59.5 μg/L) [70]. Due to the risk of inducing hyperthyroidism (Jod-Basedow effect), iodine supplementation is not used a therapeutic option in patients with MNG [71].

Suppressive Therapy with Levothyroxine

Thyrotropin is well known as the most important thyroid growth factor, and its suppression by pharmacological doses of levothyroxine (LT4) supposedly could inhibit thyroid growth or even reduce its volume. Several studies have evaluated LT4 in patients with diffuse goiter, but few studies have involved euthyroid patients with MNG in a randomized placebo-controlled design [72, 73]. In these studies, conducted mainly in iodine-deficient regions, thyroid shrinkage ranged from 7 to 32% after 1 year [16]. However, in a controlled randomized clinical trial, treatment discontinuation was associated with thyroid volume increase in the responders, with catch-up thyroid growth to baseline values after 9 months of follow-up [72]. Compared to radioactive iodine (^{131}I) therapy, suppressive therapy is greatly inferior: patients treated with ^{131}I achieve 35% volume reduction in the first year, whereas goiter shrinkage in patients given suppressive treatment is approximately 7% [74].

Due to its low efficacy, the need for continuous treatment, and its adverse effects, suppressive therapy with LT4 is not recommended as a therapeutic option for MNG, in spite of being inexpensive and easily applicable [75]. Suppressive therapy can lead to increased bone mineral turnover and to decreased bone mineral density, which can augment the risk of fractures [76]. It also has cardiac effects, leading to increased left ventricular mass and increased risk of atrial fibrillation [77]. Low serum TSH in individuals aged 60 or older is associated with increased mortality from all causes and, in particular, with increased mortality due to circulatory and cardiovascular diseases [78].

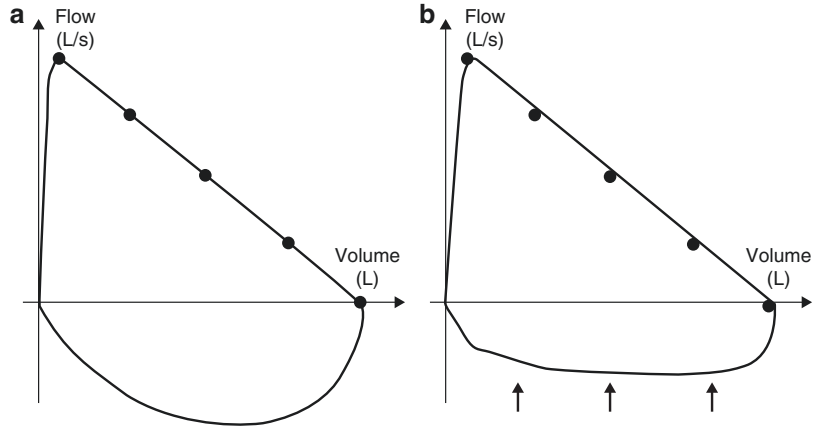

Fig. 2 Spirometry studies in patients with goiter (Reproduced from [16]). Flow-volume loops in a normal patient (**a**) and in a patient with substernal goiter (**b**). In (**b**), the expiratory part of the flow-volume loop is normal because the force of the expiration overcomes the obstruction. The inspiratory part of the loop is flattened due to a partial obstruction of the trachea

Surgery

Surgical management is considered by many groups as the main therapeutic option for patients with MNG, especially those with compressive symptoms [79, 80]. After thyroidectomy, improvements in breathing and swallowing are rapidly observed [16].

Preoperative evaluation is very important, allowing the determination of the most suitable type of anesthesia and intubation. In addition, planning the extent of surgery and postoperative care is necessary to achieve optimal results [79]. The surgical team must be aware of possible complications arising from massive goiter surgery, such as bleeding, airway distress, recurrent laryngeal nerve injury, and transient hypoparathyroidism [79].

The advantage of surgery, in addition to the prompt relief of symptoms, is that it provides a definite histologic diagnosis [10, 16]. Total or near-total thyroidectomy has become the treatment of choice because it eliminates the risk of recurrence, can detect the presence of cancer, cures co-existing hyperthyroidism (if present), and is not associated with an increase in surgical risk (when performed by experienced surgeons) [80–82]. Bilateral subtotal thyroidectomy is also an option, but the higher surgical risks that are associated with reoperation must be considered—reoperation results in a three to tenfold risk of hypoparathyroidism or permanent vocal cord paralysis [83, 84].

Specific complications related to thyroid surgery are injury to the recurrent laryngeal nerve and to the parathyroid glands. Permanent lesions of these structures occur in less than 1% of the patients in specialized units. Patients with MNG and substernal extension requiring surgery should be referred to high-volume surgeons. Intrathoracic goiters can usually be managed by cervical incision, but 10–30% of the cases require sternotomy or thoracotomy [85]. More recently, minimally invasive thyroidectomy has been evaluated as a technique that is associated with less surgical risks [86], but the large thyroid volume excludes this technique as a therapeutic option in MNG.

Hyperthyroid patients must be rendered euthyroid prior to the procedure with methimazole, without iodine supplementation. Beta-blockers can also be employed if necessary [87]. After total or near-total thyroidectomy, thyroid hormone replacement can be promptly initiated at 1.5–1.7 μg/kg/day or in lower doses in the elderly. If subtotal thyroidectomy is chosen, LT4 replacement should be initiated only if hypothyroidism develops, and not as a prophylactic agent against thyroid regrowth, since evidence for this is lacking [1, 16]. A randomized prospective non-placebo controlled study showed that LT4 did not

prevent recurrence in a 9-year post-partial thyroidectomy follow-up [88]. Surgery is relatively contraindicated in patients with significant comorbidities and when a high-volume thyroid surgeon is not available. Surgery should also be avoided during pregnancy, but if it is necessary, it should be performed in the later portion of the second trimester [89].

Radioactive Iodine

For more than seven decades, radioactive iodine (^{131}I) therapy has been used to treat thyroid diseases, mainly Graves' disease [87]. ^{131}I is not only effective for curing hyperthyroid states but also leads to shrinkage of the thyroid gland [90]. Owing to this effect on the gland volume, ^{131}I has been used for a long time in the treatment of compressive nontoxic nodular goiters. In 1988, Hegedus et al., using US, demonstrated that ^{131}I treatment of nontoxic MNG leads to significant goiter volume reduction after 1 year of ^{131}I administration [91].

Treatment with ^{131}I is an option for patients with contraindications to surgery, for those who reject surgical procedures, and for patients who have had previous surgery or radiation to the neck (making further surgical procedures more difficult) [16]. Pretreatment with methimazole before ^{131}I therapy is indicated to patients with subclinical hyperthyroidism that are at an increased risk for complications due to worsening of hyperthyroidism, including elderly and those with cardiovascular disease or severe hyperthyroidism [87].

A number of studies employing US or CT/MRI (for accurate measurements of thyroid volume) have shown that ^{131}I therapy reduces the volume of MNG by 35–50% within 1 year [47, 74, 92–97], with further reduction observed after 3–5 years [92, 96, 98] and with improvement in obstructive symptoms in most patients [91, 92].

The therapeutic efficacy of radioiodine depends, to some extent, on the goiter radioactive iodine uptake (RAIU). Low isotope accumulation in inactive and partially suppressed areas around the nodule is a limitation for radioiodine treatment in patients with MNG [16]. This low and heterogeneous RAIU in multinodular goiters requires higher activities and sometimes repeated administrations of ^{131}I [16]. The improvement in compressive symptoms after therapeutic activities of ^{131}I is accompanied by significant tracheal widening, as measured by CT or MRI [10, 16, 96]. Treatment is usually accomplished by the administration of a single oral dose of radioiodine. An effective administered activity is calculated to deliver 100–150 μCi per gram of thyroid tissue, corrected for 24-h RAIU [16]. The calculated activity is directly proportional to the thyroid volume and inversely proportional to the radioiodine uptake, aiming at an absorbed thyroid dose of 100 Gy [10, 16]. Activities may range from 15 mCi for small goiters with normal/high RAIU to 100 to 150 mCi for large glands with low RAIU and heterogeneous scintigraphic tracer distribution [10]. Higher ^{131}I activities cause considerable irradiation of extrathyroidal organs and tissues [98, 99]. In most cases, patients require hospitalization and isolation [16]. A survey about safety practices among members of major societies of physicians and allied specialists who treat patients with thyroid disorders showed a diversity of responses related to ^{131}I administration, suggesting the importance of a multispecialty collaboration in defining more uniform recommendations for patients receiving ^{131}I treatment [100].

Due to the usually low and heterogeneous RAIU seen in MNG, many strategies to enhance uptake have been evaluated, such as the use of recombinant human TSH [101].

Radioiodine and Recombinant Human TSH (rhTSH)

In healthy subjects without thyroid disease, 0.1 mg intramuscular injections of recombinant human TSH (rhTSH) significantly increase mean serum TSH after 2 h (2.4–40.7 mU/L), with a peak after 4 h (50.9 mU/L) [102]. TSH levels remain significantly elevated for 1 day and decrease significantly below baseline (0.8 mU/L) 7 days after rhTSH administration. Serum T3

increases significantly after 4 h (115–190 ng/dL), peaks after 24 h (217 ng/dL), and remains significantly elevated for 3 days (151 ng/dL). Conversely, serum T4 significantly increases after 8 h (7.3–9.8 ng/dL), peaks at 24 h (11.2 ng/dL), and remains significantly elevated for 4 days (9.4 ng/dL) [102]. In another study, the administration of 0.9 mg rhTSH increased the 24-h thyroid RAIU from 23.0% at baseline to 41.0%, showing that there are important interindividual variations regarding the RAIU increase after rhTSH administration [103]. In healthy individuals, rhTSH induces thyroid swelling in a dose-dependent manner (0.1, 0.3, and 0.9 mg of rhTSH). Fast et al. suggest that these adverse effects are probably without clinical significance following doses of rhTSH that are equal to or lower than 0.1 mg [104].

The use of rhTSH has changed the management of patients with differentiated thyroid cancer (DTC) and is routinely used for diagnostic and therapeutic purposes in many centers [105, 106]. After rhTSH stimulation, RAIU is increased in thyroid tumor cells [106]. After thyroidectomy, many patients with DTC receive adjunct ^{131}I for thyroid remnant ablation [105], and it has been demonstrated that the two existing regimens (rhTSH stimulation vs. thyroid hormone withdrawal) are equally effective for TSH stimulation and thyroid remnant ablation [107, 108]. Remnant thyroid ablation with rhTSH avoids the deterioration of quality of life that is caused by thyroid hormone withdrawal [109].

The administration of a single low dose of rhTSH in patients with MNG significantly enhances and homogenizes thyroid radioiodine uptake [110] (Fig. 3). Remarkable RAIU increases after rhTSH, from very low baseline RAIU, have been described, with large interindividual variations. This indicates that individual factors, most of which are yet unidentified, are involved. Much of the variation is explained by differences in the baseline thyroid RAIU, since the effect is highly negatively correlated with this variable [16]. Baseline serum TSH may be a confounding factor, since the increase in RAIU correlates negatively with serum TSH. Since patients with MNG frequently present low serum TSH, radioiodine is only taken up by some "hot" areas encircled by suppressed thyroid tissue that is inactive on scintigraphy. After RAIU stimulation with rhTSH, these inactive thyroid areas concentrate, reactivate, and amplify the effect of ^{131}I in the gland, with further thyroid reduction [111].

During the last decade, different rhTSH doses have been utilized: 0.2 mg or more in some studies [112–121], while 0.1 mg or less in others [99, 122–127]. Few safety concerns have been observed with the latter doses. rhTSH has been shown to distribute the therapeutic radioiodine more homogeneously in the nodular goiter, allowing a decrease in the dose of ^{131}I to be administered. Consequently, a major reduction of the radiation burden is achieved, with retained efficacy [124] (Fig. 4).

In a multicentric randomized controlled study, modified-release rhTSH (MRrhTSH) was used to treat patients with MNG [128]. MRrhTSH is an analog of rhTSH that has the same potency to increase thyroid RAIU and that determines a lower peak plasma TSH concentration. Potentially, MRrhTSH could reduce the side effects of rhTSH due to its altered pharmacokinetics, with a slightly delayed serum TSH peak after injection, compared to aqueous rhTSH [128]. In this study, the objective was to compare the efficacy and safety of 0.01 and 0.03 mg MRrhTSH as an adjuvant to ^{131}I therapy, vs. ^{131}I alone. Thyroid volume decreased significantly in all groups after 6 months: by 23% in patients prestimulated with either placebo or 0.01 mg MRrhTSH and by 33% in patients prestimulated with 0.03 mg. The smallest cross-sectional area of the trachea increased more in the latter group, without significant difference from the two other groups [128]. The long-term (36 months) results of the same trial demonstrated that patients who received 0.03 mg of MRrhTSH with baseline RAIU <20% achieved a greater reduction in goiter size [129].

Side Effects of rhTSH Use in MNG

It is important to recognize that some side effects of rhTSH in MNG can occur [130, 131]. Nielsen et al. demonstrated that patients with MNG can

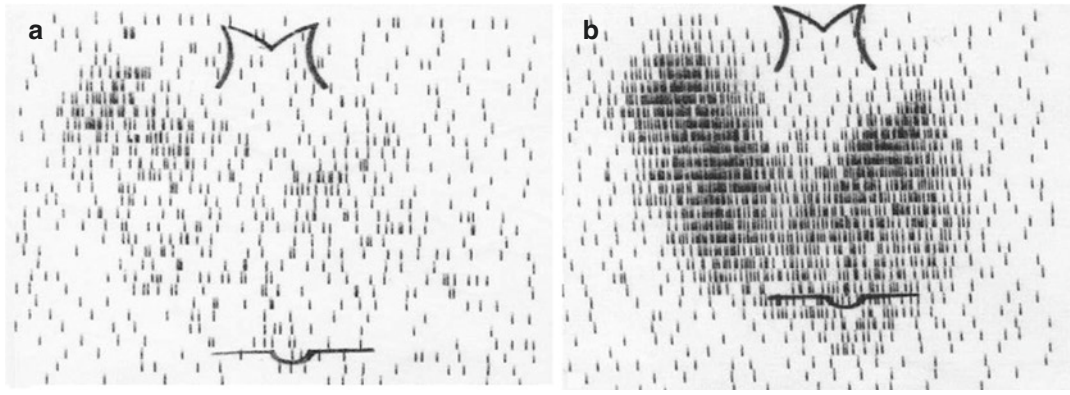

Fig. 3 Radioiodine uptake in a patient with goiter. (Reproduced from [16]). Scintigraphy before (**a**) and 24 h after the administration of rhTSH 0.1 mg in a single dose (**b**). Besides making the uptake of ^{131}I more homogeneous, rhTSH increased the 24-h uptake from 4.5 to 39.3%. From "Graf, H. Multinodular Goiter: Pathogenesis and Management"

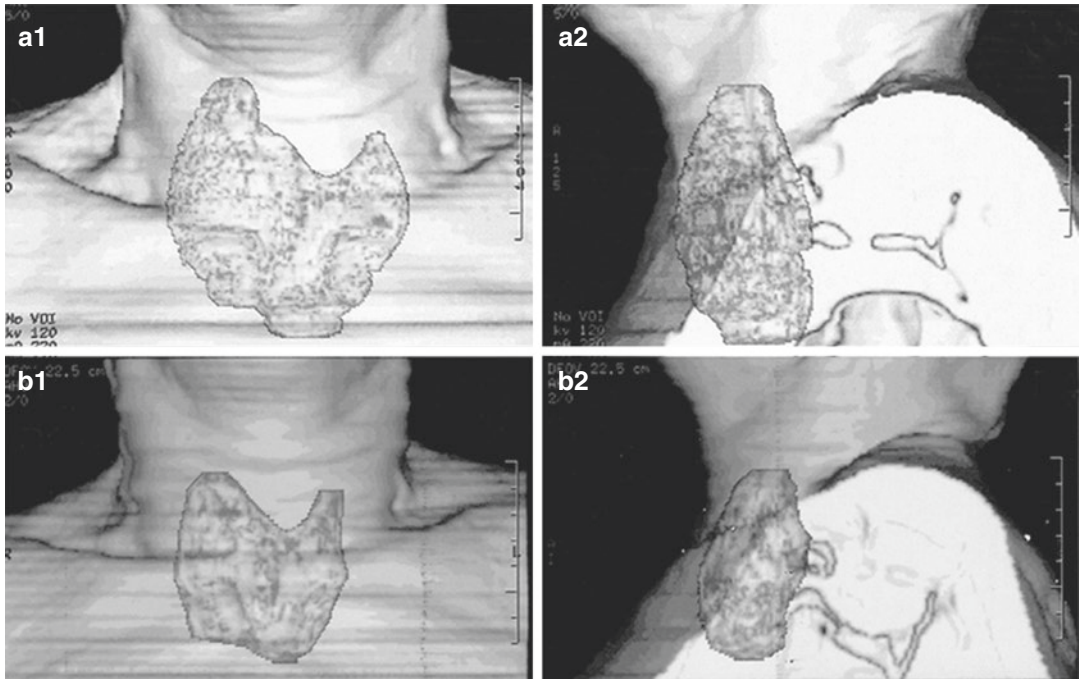

Fig. 4 Changes in thyroid volume after treatment with rhTSH plus ^{131}I (Reproduced from [16]). Computerized tomography with multiplanar reconstruction of a patient with multinodular substernal goiter treated with 30 mCi radioiodine after 0.1 mg rhTSH. A1 and A2, baseline (thyroid volume 147 mL); B1 and B2, 1 year after treatment (thyroid volume 42 mL)

report a sensation of thyroid swelling after administration of 0.3 mg rhTSH, but no acute compressive effects have been observed [119]. In this study, patients with a rather small MNG (median volume of 40.0 mL) presented a 24% transient goiter enlargement [119]. Safety measures such as the use of beta-blockers should be considered in patients with MNG and subclinical hyperthyroid-

ism submitted to ^{131}I. One study showed that hyperthyroid patients had higher increases in thyroid hormone levels after 0.1 mg rhTSH plus ^{131}I, with a higher frequency of side effects [132]. Currently, the rhTSH adjunct therapy is not indicated for patients with TMNG [87]. Glucocorticoid therapy is almost never necessary in patients receiving ^{131}I for the treatment of MNG [105], but prophylactic glucocorticoid therapy should be considered in patients with critical tracheal narrowing, to prevent thyroid swelling and further respiratory compromise [87]. Painful transient thyroiditis may occur within the first month after treatment [122], and the development of Graves' disease (with high levels of TSH receptor antibodies) is reported in euthyroid MNG patients with preexisting TPOAb [48, 49, 120].

The development of hypothyroidism is common and depends on the size of the treated goiter and on the administered rhTSH dose and ^{131}I activity. However, this should not be seen as an adverse effect and is a rather common event [16, 112–114, 119, 120, 125, 131, 133]. Based on the present knowledge, the optimal rhTSH dose for enhancing ^{131}I therapy is most likely in the range of 0.03–0.1 mg. In this dose range, a significant improvement of thyroid RAIU is obtained while minimizing the risk of goiter swelling and temporary thyrotoxicosis [16, 129].

A recent meta-analysis demonstrated that the administration of rhTSH before radioiodine therapy resulted in a greater reduction in thyroid volume than radioiodine therapy alone and in an increased incidence of hypothyroidism in patients receiving high-dose rhTSH. The authors concluded that low-dose rhTSH before radioiodine therapy was more efficacious than radioiodine therapy alone, when used for treating nontoxic benign thyroid nodules [134].

As an alternative to rhTSH, recent studies showed that, in patients with MNG, methimazole-induced hypothyroidism increases endogenous TSH levels, augmenting RAIU and allowing the administration of more effective activities of ^{131}I [135–138]. Albino et al. treated nine female patients with MNG with methimazole for 2.8 ± 0.8 months (10–20 mg, with monthly adjusted doses based on thyroid hormone levels), leading to increases in mean serum TSH to to 11.7 ± 5.4 mU/L, and to increases in mean 24-h RAIU, from 21.3 to 78.3%. One year after a fixed activity of 1.11 GBq (30 mCi) of ^{131}I was given, median thyroid volume decreased from 97 to 56 mL (mean reduction of 46.2%). Eight patients (89%) had initially subclinical hyperthyroidism, which was reversed in all patients after 1 year. Five patients (56%) developed overt hypothyroidism, and no clinical adverse events were observed [135]. Therefore, pretreatment with methimazole appears to be as effective as rhTSH in the treatment of MNG with subclinical hyperthyroidism. It does seem that a larger, longer, and more closely monitored prospective, randomized study comparing pretreatment with methimazole versus pretreatment with rhTSH would be of some value [138]. It is important to mention that the adjunct therapy of MNG with rhTSH and ^{131}I is not approved by the FDA or EMEA. Moreover, the cost-effectiveness of the combined rhTSH therapy has not been demonstrated [131].

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Part VIII

Thyroid Cancer

Epidemiology of Thyroid Cancer

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Incidence

Thyroid cancer is the most common endocrine malignancy, accounting for 2.1% of all new malignancies (excluding skin cancer and in situ carcinomas) diagnosed annually worldwide, while in the USA, it is even more common accounting for 3.2% of all new cancers [1]. The annual incidence of thyroid cancer has kept rising for the last decades; it has nearly tripled from 4.9 per 100,000 in 1975 to 14.3 per 100,000 in 2009 [2]. In 2018, approximately 53,990 new cases of thyroid cancer are expected to be diagnosed in the USA [3]. As shown in a study with cancer incidence projections in the UK up to 2035, thyroid cancer is expected to be the most rapidly growing cancer [4]. Similarly, if the current trends persist, thyroid cancer may become the fourth most common cancer in the USA by 2030 [5].

The annual incidence varies by geographic area, age, and gender. Despite the fact that the increasing incidence rate is a common finding in all geographic regions, there are distinct area-specific patterns [6]. In Denmark, the age-standardized incidence increased in both sexes from 1943 to 2008: in men from 0.41 to 1.57 per 100,000 and from 0.90 to 4.11 per 100,000 in women, corresponding to a significant average annual percentage change of 1.7 and 1.8%, respectively [7]. In Luxembourg, the overall age-standardized incidence rate over the two 5-year periods 1990–1994 and 1995–1999 increased from 7.4 to 10.1 per 100,000 in females and from 2.3 to 3.6 per 100,000 in males [8]. The highly variable and highly geographic-dependent incidence rates of thyroid cancer have been described in multiple studies for Europe [9, 10], Australia [11], Asia [12–14], North America [15, 16], and South America [17].

Thyroid cancer is very rare among children under the age of 15. The annual US incidence is 2.2/million girls and 0.7/million boys [18]. More recent data from the SEER registry reported a total of 1753 pediatric patients with thyroid cancer from 1973 to 2004, with an annual incidence of 0.54 cases per 100,000 people [19]. The annual incidence of thyroid cancer increases with age, peaking by the fifth through eighth decade of life [20]. Corroborating data from the USA, results from the UK showed that the number of thyroid cancer diagnoses in individuals under 20 years of age was low [21]. For women diagnosed with thyroid cancer between 2006 and 2010, the incidence rate peaked for 40–44 years of age and

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then remained stable, while for men, the incidence rate increased steadily with age [21].

Females have approximately a threefold to fourfold higher incidence of thyroid cancer than men, a ratio consistently observed across countries that has remained fairly unchanged over time [22]. This overall increased incidence of thyroid cancer in females is observed across different geographic populations, with a female-to-male ratio ranging from 1.44 in Australia to 7.40 in Spain [23], as well as across ethnicities, ranging within the USA from 2.92 in Caucasians to 3.69 in Hispanics [3]. Multiple reasons have been proposed in an effort to explain this distinct difference in incidence, which range from purely behavioral [women's greater tendency to seek medical advice and more active participation during medical visits [24, 25]] to biological differences [increased exposure to higher TSH levels for a longer period of time, greater susceptibility to cancer development, sex steroid hormones [26]]. Interestingly, the increasing incidence of thyroid cancer is similar between racial and ethnic groups in the USA [27].

Recently, there has been an ongoing discussion about the issue of overdiagnosis of thyroid cancer. Davies et al. in their very comprehensive analysis reported a marked increase in thyroid cancer incidence and a stable rate of thyroid cancer mortality over time and attribute these trends entirely to "increased diagnostic scrutiny" [2]. The widespread use and technical improvement of imaging modalities as well as fine-needle aspiration biopsies certainly account for some of that increase, especially in cases like South Korea where these modalities are used as a screening tool and create the potential for observational bias with discovery of small, subclinical papillary thyroid cancers (PTC) [14]. However, substantial increases were also observed for advanced stage and larger (>4 cm) size PTCs [16]. In California, the incidence of PTC increased regardless of size, stage, and socioeconomic status [28]. Moreover, the molecular landscape of PTC has been changing with different mutations being identified. Thus, we believe that the increased incidence is likely the result of two coexisting processes: increased detection (apparent increase) and increased number of cases (true increase) due to unrecognized thyroid-specific carcinogens [29].

Prevalence

Given the increasing incidence and the good prognosis of thyroid cancer, the prevalence of the disease is also expected to be rising. In 2015, there were an estimated 765,547 people living with thyroid cancer in the USA [3]. Thyroid cancer prevalence rates, mimicking the incidence rates, vary widely by geographic area and patient population. Moreover, the prevalence rates are highly dependent on the method used to calculate them. Autopsy series have been used to calculate the thyroid cancer prevalence, and rates ranging from 0.03 to 36% have been reported in the literature [30–37]. In one of the largest series, Mortensen et al. reported on 1000 consecutive routine biopsies and found a 2.8% prevalence rate of thyroid carcinoma [30]. In a study performed in Helsinki, Finland, a prevalence of 36% of differentiated thyroid cancer was reported after examination of 101 consecutive necropsies [31]. Kanamori et al. recently published an excellent meta-analysis of 35 studies including 12,834 autopsies conducted from 1949 to 2007 [38]. The prevalence of incidental differentiated thyroid cancer was calculated to be 4.1% when partial thyroid autopsy was performed, while it was calculated as 11.2% when the entire thyroid was examined irrespective of the evidence or the absence of macroscopic evidence of disease. Interestingly, the time period that the autopsy series was performed was not a major predicting factor of the differentiated thyroid cancer prevalence. Thus, despite the fact that the incidence of thyroid cancer has been increasing since the 1980s, there has not been a concomitant increase in prevalence rates suggesting that the reservoir remains relatively stable, and we are diagnosing a lot of previously undiagnosed cancers. The issue of overdiagnosis of thyroid cancer is discussed separately in this chapter.

In a separate systematic review of 24 autopsy series from 1970 to 2011, PTC prevalence rate

was reported as 7.6%, while the rate of medullary thyroid cancer (MTC) was 0.14% [39]. This study demonstrated that a small percentage of the population has occult MTC without mortality and morbidity. The tumor size was virtually all sub-centimeter, and no aggressive characteristics (extrathyroidal extension, distant metastasis, lymph node involvement) were observed. This observation of the uncommon subclinical MTC can be especially useful for future prevalence studies.

As shown above, thyroid cancer prevalence rates are significantly higher than incidence rates, reflecting that a substantial number of patients survive several decades or longer since the diagnosis.

Mortality

In 2018, it is estimated that approximately 2,060 deaths from thyroid cancer will occur, accounting for 0.3% of all cancer deaths in the USA [3]. Ferlay et al. reported that the estimated number of deaths from thyroid cancer in Europe in 2012 was 6300, with thyroid cancer having one of the lowest mortality age-standardized rates (0.6 per 100,000) [40]. Mortality has remained seemingly unchanged in the USA since 1970, with 0.5 cases per 100,000 being reported [3]. However, from 1992 to 2012, there has been an average annual percent increase in mortality of 0.82% [41]. The 5-year survival rate is excellent reaching 98.1% in 2012 and improving from 92.3% in 1975 [3]. Age-specific mortality rates rise gradually from around age 40 to 44 and more sharply from around age 60 to 64, with the highest rates being reported in the 85–89 age group in females and the 90+ age group in males [42]. The same trend is also encountered around the world, with the most impressive example being that of South Korea where, despite the fact that the incidence of thyroid cancer has risen 15-fold from 1993 to 2011, the mortality has remained stable [14]. Earlier diagnosis, improved treatment modalities, and decreased incidence of anaplastic carcinoma certainly account for the stabilization in mortality rates, even though the incidence has been rising exponentially. Sex, age, and distant metastases are some of the variables that can influence mortality rates, with response to radioactive iodine being the strongest predictor of a good outcome in a study that examined the natural history of DTC [43]. However, an increasing number of investigators advocate in favor of active surveillance for the low-risk micropapillary thyroid cancers [44]. Our opinion is congruent with the guidelines of the American Thyroid Association that, in general, nodules below 1 cm should not be biopsied but each case should be individualized as there are reports of distant metastases from micropapillary thyroid cancers that can lead to increased mortality rates [45, 46].

Distribution by Histological Type

Thyroid cancer can be further subcategorized in one of the three following major histologic subtypes: differentiated thyroid cancer (DTC), medullary thyroid cancer (MTC), and anaplastic thyroid cancer (ATC).

DTC comprises more than 90% of all thyroid cancer cases. DTC derives from epithelial thyroid cells and is classified histologically as papillary, follicular, Hürthle cell and poorly differentiated [47]. PTC accounts for approximately 80% of thyroid cancers in the USA, while approximately 10% of the cases are follicular thyroid cancer (FTC) [48]. These two types of cancer, despite being grouped together, have distinct molecular backgrounds. The most common mutation in PTC is the BRAF V600E (present in 45% of the cases), while the most prevalent mutations in FTC are RAS mutations (present in 40–50% of the cases) [49, 50]. Both histologic types are more common in women than in men, and their peak incidence is during the fourth and fifth decade of life. Dietary iodine intake is a major factor influencing the relative proportion of DTC in a given geographic area [51]. PTC are more predominant in iodine-sufficient areas, such as the USA or Iceland where the percentages were 85% PTC and 15% FTC [52]. On the contrary, in iodine-deficient areas, the percentage of FTC increases to 30–40% [51].

MTC is a neuroendocrine tumor that arises from the neural crest-derived parafollicular C cells [53]. MTC comprises approximately 3–5% of all thyroid cancers in the USA but accounts for 15% of all thyroid cancer-induced deaths [54]. Approximately 25% occur as a hereditary disease, with most cases associated with multiple endocrine neoplasia syndrome (MEN) types 2A and 2B, while 75% of the cases occur in a sporadic form. The sporadic form presents mostly in the fifth and sixth decades of life and is more common in females with a ratio of 1.5:1 [55]. Patients with MEN2 develop clinically apparent MTC, often early in life [56]. In MEN2A-associated MTC, the peak incidence is in the third decade of life, while it is usually earlier in MEN2B (often within the first year of life).

ATC is also derived from epithelial thyroid cells. It accounts for less than 2% of thyroid cancers, although it has been reported as high as 9.8% of thyroid cancers globally [57]. It is one of the most aggressive solid malignancies in humans, with a mean survival of less than 6 months from the time of diagnosis and 1-year survival of 20% [58, 59]. At diagnosis, more than one-third of patients have extrathyroidal extension and/or regional nodal metastases, whereas distant metastases are present in more than 40% [59]. The incidence of ATC is decreasing worldwide, and that is one reason why thyroid cancer mortality has been relatively stable despite the increasing incidence in general, as ATC accounts for 14–50% of the mortality [60]. Most patients at diagnosis are 65 years or older, and 60–70% of tumors occur in women [59, 60].

Finally, thyroid lymphomas should always be in the differential with a rapidly enlarging thyroid tumor. Thyroid lymphomas are almost always non-Hodgkin, as Hodgkin lymphomas are very rare [61]. The annual incidence is approximately 2.1 cases per million people, while they account for less than 2% of all thyroid cancers [62, 63]. The mean age of diagnosis is similar to ATC (between 65 and 75 years of age), while there is a clear female predominance with a female-to-male ratio ranging from 4:1 to 8:1 [62, 64, 65].

Risk Factors for Thyroid Cancer

The increasing incidence of thyroid cancer has triggered multiple efforts in order to identify potential risk factors for the development of thyroid cancer. However, there are only a few well-established risk factors. Among them, radiation exposure of the thyroid during childhood is the most clearly defined environmental factor associated with benign and malignant thyroid tumors [66]. Potential sources of radiation exposure include nuclear power plant accidents (e.g., Chernobyl) or atomic weapons (Nagasaki, Hiroshima) and/or therapeutic uses of radiation (which was much more pronounced in the past). Pathophysiologically, a translocation of the RET gene has been found in patients that developed thyroid cancer after Chernobyl and after therapeutic radiation [67]. Female gender is also a risk factor for the development of thyroid cancer, although male gender is a risk factor for the development of more aggressive tumors [26]. Family history is certainly a well-established risk factor. Thyroid cancer may develop in the context of familial cancer syndromes (familial adenomatous polyposis, Gardner's syndrome, Cowden's disease, Carney's complex type 1, Werner's syndrome, and papillary renal neoplasia) [54]. It can also develop in the context of familial non-medullary thyroid cancer (FNMTC), which is defined as the presence of non-MTC thyroid cancer in two first degree relatives of the patient. The genetic inheritance of FNMTC remains unclear; but it is believed to be autosomal dominant with incomplete penetrance and variable expressivity with environmental factors also contributing [68]. Concerning MTC, MEN2A and MEN2B syndromes have been discussed above. The presence of Hashimoto's thyroiditis is a very well-defined risk factor for the development of thyroid lymphoma, with the risk being at least 60 times higher than in patients without thyroiditis [69].

In addition to the well-established risk factors, there are some postulated risk factors for which a definite causative relationship is yet to be proven. Iodine intake may influence the incidence and prevalence of thyroid malignancy, and more specifically it may increase the risk for PTC [70, 71].

Moreover, excess consumption of carbohydrate and protein has been associated with increased risk for DTC [72]. Surprisingly, smoking has been found to have an inverse correlation with DTC in several studies [73, 74], but there are studies demonstrating the opposite [75], and the effect of the toxic compounds found in tobacco is not clearly understood. High incidence of thyroid cancer has been described in volcanic areas, with trace elements found in those areas being implicated in thyroid tumorigenesis [76]. Viruses could also be implicated in thyroid tumorigenesis with data already existing on herpes viruses and Epstein-Barr virus [77, 78], but further studies are needed to confirm these preliminary results. Finally, the presence of Graves' disease has been proposed as a risk factor for the development of thyroid cancer and indeed represents an intriguing hypothesis since the thyroid-stimulating immunoglobulins could have a TSH-like stimulatory effect. Some studies have suggested that Graves' disease is associated with larger, multifocal, and potentially more aggressive thyroid cancer [79], but others have reported very low cancer rates (0.06%) in patients with Graves' disease [80]. The data for the above risk factors remain inconclusive, and prospective studies are needed in order to evaluate the presence or absence of a causative relationship with thyroid cancer.

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Molecular Genetics and Diagnostics of Thyroid Cancer

Susan J. Hsiao and Yuri E. Nikiforov

Introduction

In the age of personalized medicine, molecular markers are being increasingly utilized to provide diagnostic, prognostic, and therapeutic information. Thyroid cancer, in particular, is ideally suited to incorporating molecular markers into clinical management. Several factors contribute toward this: thyroid nodules are easily accessible for fine-needle aspiration (FNA) biopsy (which generates sufficient material for both diagnostic evaluation and ancillary testing on nearly all patients), a substantial proportion (20–30%) of thyroid nodules are diagnostically indeterminate by cytopathologic analysis, and thyroid cancer is well characterized with a relatively smaller number of genomic alterations (many of which are highly specific for malignancy).

Ultrasound and cytologic examination of thyroid nodules is standard in the diagnostic evaluation of thyroid nodules and reliably classifies the majority (70–80%) of thyroid nodules as benign or malignant [1, 2]. Those thyroid nodules classified as benign have a low risk (approximately 0–3%) of malignancy, while those nodules classified as malignant have a high risk of malignancy (97–99%) [3]. The remaining thyroid nodules are classified cytologically using the Bethesda reporting system as fitting into one of three indeterminate categories: atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), follicular or oncocytic (Hürthle cell) neoplasm/suspicious for a follicular or oncocytic (Hürthle cell) neoplasm (FN/SFN), and suspicious for malignant cells (SUSP) [4, 5]. The risk of malignancy for an indeterminate cytology thyroid nodule ranges from 5 to 75% (5–15% risk for AUS/FLUS nodules, 15–30% risk for FN/SFN nodules, and 60–75% risk for SUSP nodules) [3]. Based on the Bethesda classification, recommended management is repeat FNA biopsy for AUS/FLUS, diagnostic lobectomy for FN/SFN, and thyroidectomy or lobectomy for SUSP nodules [3].

The majority of surgically resected nodules are benign and the remaining 10–40% of nodules are malignant [4, 6, 7]. Thus, for many patients, surgery is unnecessary. Furthermore, in the patients with malignant thyroid nodules greater than 1 cm in size who have undergone diagnostic lobectomy, a completion lobectomy is typically performed to remove the remaining thyroid lobe. These patients could have benefitted from an upfront thyroidectomy rather than two separate

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surgeries. In addition, well-differentiated thyroid cancer is overall an indolent disease with a small proportion (5–10%) expected to have an aggressive course. Many patients can be spared more aggressive therapy if cancer is low-risk, and conversely, high-risk cancers need appropriate treatment. So, molecular markers may assist tumor prognostication.

To further refine the risk conferred by Bethesda classification, to reduce the need for diagnostic lobectomies and two-step surgeries, and to aid in tumor prognostication, several ancillary approaches have been pursued. These include the use of microRNAs, gene mutations/rearrangements, and gene expression panels [8–11]. Several of these ancillary studies are being used in clinical management and will be discussed below.

Molecular Alterations in Thyroid Cancer

The genomic alterations underlying thyroid cancer pathogenesis have been well characterized (Table 1). Studies from multiple laboratories have identified the driver mutations for the majority of thyroid tumors, and recent large scale sequencing projects have identified genomic alterations in many of the remaining thyroid tumors as well as provided an overview of the landscape of alterations. These findings have been important in shaping and evolving the classification of thyroid tumors to reflect histologic, molecular, and behavioral features.

Recently, papillary thyroid carcinoma was extensively studied through The Cancer Genome Atlas (TCGA) initiative [12]. Using data on single nucleotide variants, small indels, translocations, mRNA expression, miR expression, protein expression, DNA methylation, and copy number alterations from 496 papillary thyroid carcinomas, driver mutations were identified in 96.5% of cases [12]. Papillary thyroid carcinomas were found to have a low frequency of somatic variants, and most tumor genomes were "quiet," with few copy number gains or losses [12]. Most of the alterations seen in the TCGA study as well as in previous studies involved genes that function in the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI3K) pathways.

Table 1 Average frequency of main mutations and gene fusions in different types of thyroid cancer

Papillary thyroid carcinoma	
RAF	40–45%
RET/PTC	10–20%
RAS	10–20%
TERT	10%
NTRK	<5%
Follicular carcinoma	
RAS	40–50%
PAX8-PPARG	30–35%
TERT	10–20%
PIK3CA	<10%
PTEN	<10%
Poorly differentiated carcinoma	
TERT	40%
RAS	25–30%
CTNNB1	10–20%
TP53	20–30%
BRAF	10–15%
EIF1AX	10%
Anaplastic carcinoma	
TP53	70–80%
CTNNB1	60–70%
TERT	70%
RAS	40–50%
BRAF	20–30%
EIF1AX	10%
Medullary carcinoma	
RET	40–50%
RAS	20%
STK11	10–20%

BRAF, a serine threonine kinase, is a key player in the MAPK pathway. Activating mutations in *BRAF* are estimated to occur in approximately 40–45% of papillary thyroid cancers [13, 14]. Most *BRAF* mutations are the activating V600E mutation, although other mutations such as K601E mutation or small in-frame insertions or deletions have also been reported [15–18]. An association of the *BRAF* V600E mutation with conventional and tall cell variant of papillary thyroid carcinoma has been reported, while the *BRAF* K601E mutation has been reported to be associated with follicular variant of papillary

thyroid cancer [19–23]. Alternate activation of *BRAF* and MAPK signaling in papillary thyroid carcinoma occurs through the generation of BRAF fusion proteins. Reported fusions such as *AKAP9-BRAF*, *SND1-BRAF*, or *MKRN1-BRAF* preserve the C-terminal kinase domain of BRAF while removing and replacing the N-terminal regulatory domain of BRAF with a fusion partner [12, 24].

Oncogenic mutations in *NRAS*, *HRAS*, or *KRAS* are also seen in papillary thyroid carcinoma. These mutations most frequently occur at codon 61 in *NRAS* and *HRAS*, although mutations at codons 12 and 13 are also seen. *RAS* gene mutations are primarily seen in the follicular variant of papillary thyroid cancer [19, 25, 26]. The observation that *RAS* mutations primarily occur in noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) and invasive follicular variant of papillary thyroid carcinoma has led to the suggestion that NIFTP may represent a precursor to invasive follicular variant of papillary thyroid carcinoma [25].

Driver fusion genes are also important in papillary thyroid carcinoma pathogenesis. The most common rearrangements are RET/PTC1 (fusion of RET with CCDC6) and RET/PTC3 (fusion of 47\RET with NCOA4). These fusions were previously observed at approximately 20–30% frequency two decades ago and are now seen in approximately 10% of cases [27–29]. In 5% of papillary thyroid carcinomas, rearrangements involving *NTRK1* and *NTRK3* are seen [30–34], although a recent report suggests that the frequency of *NTRK* rearrangements in pediatric papillary thyroid carcinoma may be much higher [35]. Other fusions, such as those involving *THADA* and *ALK* genes, are observed in approximately 1% of papillary thyroid carcinomas [12, 36].

The TCGA study of papillary thyroid carcinoma identified a novel significantly mutated gene, *EIF1AX* [12]. This gene encodes an essential eukaryotic translational initiation factor. Recurrent mutations in *EIF1AX* were observed, primarily in tumors lacking known MAPK pathway driver mutations, suggesting a possible novel driver role for *EIF1AX* in papillary thyroid carcinoma [12]. However, a subsequent study found that although *EIF1AX* mutations were seen in approximately 2% of papillary thyroid carcinomas, mutations were also seen in two follicular adenomas and one hyperplastic nodule, possibly limiting the utility of *EIF1AX* as a highly specific marker of papillary thyroid carcinoma [37].

For follicular adenomas and follicular carcinomas, the *RAS* genes have been implicated as major driver genes [38–40]. Approximately 40–50% of follicular carcinomas and 20–40% of follicular adenomas have been reported to harbor *RAS* gene mutations [38–41]. Also seen at a significant frequency (30–40%) in follicular carcinoma is *PAX8/PPARG* rearrangement [42–44]. This rearrangement may also be seen, at lower frequencies, in follicular adenomas as well as in the follicular variant of papillary thyroid carcinoma [42–46]. Another alteration that has been reported in both follicular adenoma and follicular carcinoma is mutation of *PTEN*, a tumor suppressor gene that functions as a negative regulator of the PI3K/AKT pathway [34, 47–50].

Poorly differentiated and anaplastic thyroid carcinomas, as compared to well-differentiated follicular tumors, often harbor multiple driver mutations (Fig. 1). In addition to mutations in *BRAF* or *RAS*, these tumors typically acquire additional mutations in genes like *TP53*, *PIK3CA*,

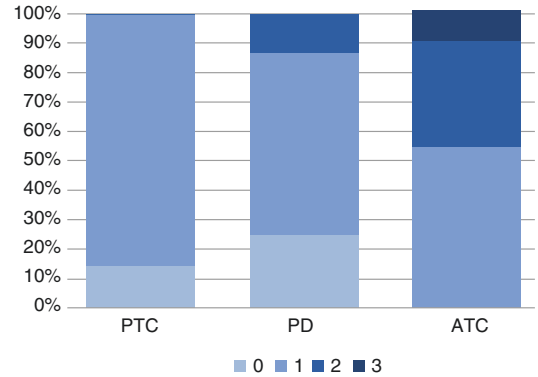

Fig. 1 Driver mutation/fusion frequency in thyroid cancer. Number of driver mutations (in *BRAF*, *NRAS*, *KRAS*, *HRAS*, *EIF1AX*, or *TP53* genes) or driver fusions across papillary thyroid carcinomas (PTC), poorly differentiated carcinomas (PD), and anaplastic thyroid carcinomas (ATC)

and *AKT1*. *TP53* is an important tumor suppressor and, in many tumor types, including thyroid cancer, is associated with aggressive behavior and tumor progression. Approximately 20–30% of poorly differentiated carcinomas and 70–80% of anaplastic thyroid carcinomas are reported to harbor *TP53* mutations [51–55]. Other genetic alterations that have been described in poorly differentiated and anaplastic thyroid carcinomas involve activating mutations in the *PIK3CA* and *AKT1* genes, both of which function in the PI3K/AKT pathway [49, 56, 57].

Recurrent mutations in the telomerase (*TERT*) promoter have been described in the last few years and have been described in a multitude of tumors including melanoma, glioblastoma, bladder, and thyroid cancers. These mutations, located 124 bp (C228T) and 146 bp (C250T) upstream of the initiating ATG, are thought to increase *TERT* promoter activity [58, 59]. *TERT* promoter mutations have been reported in follicular cell thyroid cancers, but have not been detected in benign thyroid lesions [60–63]. Although seen in well-differentiated papillary thyroid and follicular carcinomas, the frequency of *TERT* promoter mutations is significantly higher in aggressive tumors such as widely invasive oncocytic carcinoma, poorly differentiated carcinoma, and anaplastic thyroid carcinoma [60–63]. The presence of *TERT* promoter mutations is associated with increased risk for persistent disease, distant metastases, and disease-specific mortality for well-differentiated thyroid cancer [63].

Recently, two studies further characterized poorly differentiated and anaplastic thyroid carcinomas using either a 341-gene cancer panel or whole exome sequencing [64, 65]. Both studies confirmed previous findings of *BRAF* or *RAS* mutations, which often co-occurred with variants in *TP53*, *TERT*, or PI3K/AKT/mTOR pathway components. Interestingly, *EIF1AX* mutations were seen in 11% of poorly differentiated carcinomas and 9% of anaplastic carcinomas in one study and in 14% of anaplastic carcinomas in the other study [64, 65]. In both studies, a strong tendency toward co-occurrence of *EIF1AX* and *RAS* mutations was seen, in contrast to papillary thyroid carcinoma, where *EIF1AX* mutations were mostly mutually exclusive with other driver mutations [12, 64, 65]. These findings raise the possibility of a cooperative effect of *EIF1AX* and *RAS* mutations in poorly differentiated and anaplastic carcinomas.

Medullary thyroid carcinomas are also primarily driven by MAPK and PI3K/AKT pathway mutations. Mutation of *RET*, a receptor tyrosine kinase expressed in thyroid C cells, is seen in both familial and sporadic forms of medullary thyroid cancer. The activating tyrosine kinase domain M918T mutation in *RET* is the most common *RET* mutation seen in sporadic medullary thyroid carcinomas and accounts for greater than 75% of *RET* mutations [66, 67]. The M918T mutation is also commonly seen in tumors arising in MEN2B syndrome [68–70]. In MEN2A syndrome and familial medullary thyroid carcinoma, *RET* mutations typically do not occur in the tyrosine kinase domain and instead occur at one of five conserved cysteine residues in the extracellular domain [71, 72]. Mutation of the cysteine residues allows the mutant RET protein to undergo ligand-independent dimerization and activation. In addition to *RET* mutation, mutation of the *RAS* genes has been described in sporadic medullary thyroid carcinomas [73–77]. These mutations are mutually exclusive and account for up to 90% of sporadic medullary thyroid carcinomas [73].

Recent work has shown the presence of *ALK* gene fusions in medullary thyroid carcinoma [75]. An *EML4-ALK* fusion, as well as a novel *GFPT1-ALK* fusion, was reported [75]. ALK fusions have not been previously observed in medullary thyroid carcinoma, but have been observed in approximately 1–2% of papillary thyroid carcinomas, 4–9% of poorly differentiated carcinomas, and 4% of anaplastic thyroid carcinomas [36, 65].

Finally, other genetic alterations may be seen in benign lesions and may be of utility in differentiating between benign and malignant lesions. Somatic activating mutations in *TSHR* have been reported to occur in approximately 50–80% of hyperfunctioning nodules. [78, 79] Activating mutations of *GNAS* occur in approximately 3–6%

of hyperfunctioning nodules [80–82]. Mutations in either gene are seen primarily in benign hyperfunctioning nodules and have only rarely been seen in follicular carcinomas [34].

Gene Mutation/Rearrangement Testing

One approach is single-gene mutational testing of thyroid nodules. Several groups have reported experiences with the use of *BRAF* V600E mutational analysis preoperatively. *BRAF* V600E mutation is seen in approximately 45% of papillary thyroid cancers and is not seen in benign thyroid nodules [13, 14]. *BRAF* V600E mutation is detectable by a variety of molecular technologies, such as real-time PCR, sequencing (Sanger and next generation), or single-base (primer) extension assays, which contribute to ease of adoption and incorporation into routine diagnostics and clinical management. Testing for *BRAF* V600E mutation has been reported to result in increased sensitivity in papillary thyroid cancer detection [83, 84]. In a recent meta-analysis of *BRAF* V600E mutation testing in thyroid FNA specimens, the addition of *BRAF* V600E testing to FNA cytology increased the sensitivity from 81.4 to 87.4% [85]. However, although the specificity of *BRAF* V600E mutation testing is very high (86.1–99.7%), the sensitivity is low (19.5–59.4%) [85]. Use of ultrasensitive techniques to detect *BRAF* V600E mutation may lead to false-positive results [86]. Preoperative *BRAF* V600E mutation testing may also have utility in predicting disease persistence and recurrence [87]. However, although *BRAF* V600E testing offers some utility in increasing sensitivity and predicting disease recurrence, as a stand-alone test, it offers insufficient sensitivity and specificity for thyroid cancer.

To address this, a seven-gene panel of genetic mutations and gene rearrangements was developed. This panel includes the genes and rearrangements most frequently implicated in thyroid cancer (*BRAF*, *NRAS*, *HRAS*, *KRAS*, *RET/PTC1*, *RET/PTC3*, and *PAX8/PPARG*), which together account for driver genes of approximately 70% of thyroid cancers. Each of these genes and rearrangements shows a high specificity and positive predictive value (PPV) for cancer, although the positive predictive value for *NRAS*, *HRAS*, or *KRAS* mutations is lower at 74–87% [11, 88, 89]. This seven-gene panel, or a similar eight-gene panel that also includes *TRK* rearrangements, was initially described and validated at two institutions in three prospective studies [11, 88, 89]. These studies all showed this gene panel to have high specificity (97–100%) and high PPV (86–100%) for cancer in indeterminate thyroid nodules [11, 88, 89].

Subsequent validation of similar seven-gene mutational tests, either in one retrospective study at a single institution or in two prospective studies at multiple institutions of the commercially available offering of a seven-gene panel, the ThyGenX test (formerly the miRInform test) offered by Interpace Diagnostics, has shown similar results [90–92]. In FN/SFN thyroid nodules, these studies showed a specificity of 86–92% and PPV of 71–80% [90–92].

To test variants that encompass a greater percentage of thyroid cancers and to further increase the sensitivity of mutational testing, next-generation sequencing (NGS) testing—either pan-cancer or thyroid specific panels—can be utilized [93, 94]. NGS technology is suited for high-throughput, massively parallel sequencing needs and can interrogate multiple genes simultaneously. A large, thyroid cancer-specific next-generation sequencing-based assay was recently developed and characterized (ThyroSeq v2). The genes on the ThyroSeq v2 panel include the seven genes in the other mutational panels but additionally include mutational hotspots in *AKT1*, *PTEN*, *TP53*, *TSHR*, *GNAS*, *CTNNB1*, *RET*, *PIK3CA*, *EIF1AX*, and *TERT*, as well as rearrangements of *RET*, *BRAF*, *NTRK1*, *NTRK3*, *PPARG*, and *THADA* [94]. Mutations or rearrangements involving the majority of these additional genes are primarily seen in thyroid carcinomas. A subset of these genes, such as *PTEN* and *EIF1AX*, are mutated in both benign and malignant lesions [12, 34, 37, 47–50], and activating mutations of *TSHR* and *GNAS* are mostly seen in hyperfunctioning nodules [78–82]. In the validation study

of ThyroSeq v2, a combined retrospective and prospective study at a single institution of 143 FN/SFN thyroid nodules, the test performed well with good specificity (93%) and PPV (83%) and additionally, showed good sensitivity (90%) and NPV (96%) [94].

Expression Classifier Testing

mRNA Gene Expression Classifier

Another methodology widely used in testing indeterminate thyroid nodules is gene expression profiling. The mRNA expression profiles of thyroid nodules were used to train a molecular classifier [95]. By examining the pattern of expression of 142 genes, which are involved in diverse processes such as energy metabolism or cell differentiation/development, thyroid nodules are classified into benign or suspicious categories [8, 95]. This test is currently offered commercially as the Afirma gene expression classifier (Veracyte).

The Afirma test was initially validated in a multi-institutional study of 265 indeterminate thyroid nodules and was found to have a high sensitivity (90%) and NPV (94%). The validation study was somewhat limited by small sample size, and some subsequent studies performed in institutions with higher disease prevalence, reported lower NPVs for the Afirma test [96–99]. Recently, a meta-analysis of seven studies of the Afirma test was performed [100]. In these studies, true negative and false negative rates were somewhat difficult to ascertain as many patients with benign results by the Afirma gene expression classifier did not undergo surgery. The prevalence of malignancy in the pooled cohort was 37.1% [100]. The meta-analysis found a pooled sensitivity of 95.7% and pooled specificity of 30.5% [100].

miRNA Expression Classifier

The differential expression of miRNAs has also been used in the classification of indeterminate thyroid nodules. miRNAs are small, noncoding RNAs. miRNAs regulate gene expression by binding to the 3′ untranslated region of target mRNAs and result in mRNA degradation or translation inhibition. Many miRNAs have been characterized in thyroid carcinoma, and the expression of a subset has been associated with not only the presence of carcinoma but additionally with prognostic features such as advanced disease or extrathyroidal extension [101, 102].

A panel of 10 miRNAs (miR-29-b-1-5p, miR-31-5p, miR-138-1-3p, miR-139-5p, miR-146b-5p, miR-155, miR-204-5p, miR-222-3p, miR-375, and miR-551-3p) is used to classify nodules as “positive” or “negative.” This testing is currently available commercially as the ThyraMIR test (Interpace Diagnostics) and is offered as reflex testing on thyroid nodules that are negative by the ThyGenX panel [92]. In the initial validation study of this miRNA classifier, the reported sensitivity was 57%, specificity 92%, NPV 82%, and PPV 77% [92].

Test Performance Comparisons and Potential Improvements

Evaluation of diagnostic tests typically involves comparisons of specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV). Tests with clinical utility in ruling out malignancy should have high sensitivity and NPV, and tests with clinical utility in ruling in malignancy should have high specificity and PPV. Sensitivity and specificity reflect test performance characteristics, but NPV and PPV may vary significantly depending on the prevalence of disease. In the context of thyroid nodules, this may reflect differences in patient population demographics or institutional differences in the malignancy rates in each indeterminate cytology category.

Although there were institutional differences because of variability in disease prevalence, in general, available follow-up studies have supported the findings in the initial validation studies. Seven-gene mutation/rearrangement studies have high specificity and PPV and show utility in

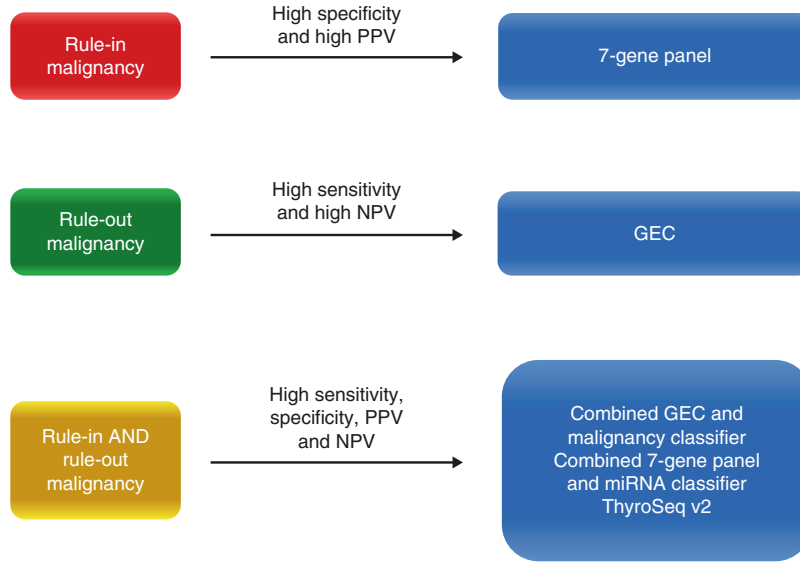

Fig. 2 Utility of currently available diagnostic tests and their performance characteristics

"ruling in" malignancy, while the gene expression classifier has high sensitivity and NPV and shows utility in "ruling out" malignancy (Fig. 2). The ideal diagnostic test, however, would have high PPV, and high NPV would be able to both rule in and rule out malignancy. One possible approach would be to add-on or combine testing. Afirma, for example, in addition to the gene expression classifier, also offers two malignancy classifiers for nodules suspicious by GEC or cytopathology, Afirma MTC and Afirma BRAF. These are mRNA gene expression classifiers specific for genes differentially expressed in either medullary thyroid cancer or *BRAF* V600E mutation-positive thyroid cancer. A positive result for the Afirma MTC or Afirma BRAF test may add additional specificity to the Afirma GEC, although data regarding this has not yet been published. Interpace Diagnostics combines the miRNA-based classifier (ThyraMIR) in thyroid nodules that are negative by the seven-gene mutational panel (ThyGenX). In their validation studies, they report that by combining tests, they are able to achieve a sensitivity of 89%, specificity of 85%, NPV of 94%, and PPV of 74%. Further studies of this test are needed to explore this test.

Of the currently available tests, ThyroSeq v2 with a sensitivity of 90%, specificity of 93%, NPV of 96%, and PPV of 83% in FN/SFN nodules currently shows much promise as a potential test to both rule in and rule out malignancy. Potential increases in specificity and sensitivity may be both from further expanding the panel and from increased understanding of thyroid pathogenesis and "cooperating" genes that drive malignancy. For example, whereas *BRAF* mutation and *RET/PTC* rearrangement are seen virtually exclusively in thyroid cancer, *RAS* mutations are also seen in benign or indolent neoplasms such as follicular adenomas or NIFTP, and thus the PPV of *RAS* mutations for malignancy ranges from 74 to 87% [11, 88, 89]. Recent studies, however, suggest that coexisting *RAS* and *TP53* or *RAS* and *EIF1AX* mutation may, with further study, prove to be associated with increased risk.

Clinical Utility of Molecular Testing of Indeterminate Thyroid Nodules

Based on the performance characteristics of seven-gene mutation/rearrangement panels (high specificity and high PPV) and gene expression classifiers (high sensitivity and high NPV), clinical algorithms have been suggested to guide perioperative decision-making [103]. With seven-gene mutation/rearrangement panels, the suggested management for a positive result for AUS/FLUS, FN/SFN, or SUSP nodules is oncologic thyroidec-

tomy. Negative results for AUS/FLUS nodules may be managed by observation or diagnostic thyroid lobectomy, whereas negative results for FN/SFN or SUSP nodules should be managed by at least a diagnostic thyroid lobectomy. For gene expression classifier testing results, suspicious results for AUS/FLUS or FN/SFN nodules should be managed by at least a diagnostic thyroid lobectomy, and benign results may be managed by observation or diagnostic thyroid lobectomy. Testing of SUSP nodules by gene expression classifier is generally not recommended as both benign and suspicious results should still be managed with at least a diagnostic thyroid lobectomy.

Initial results on application of molecular testing results into the management of indeterminate thyroid nodules have been reported for both seven-gene mutation/rearrangement panels and gene expression classifiers [104, 105]. For the seven-gene mutation/rearrangement panel, in a series of 471 patients with AUS/FLUS or FN/SFN nodules at a single institution, patients who did not undergo seven-gene mutation/rearrangement panel testing were found to be 2.5-fold more likely to require a two-step (initial lobectomy followed by completion thyroidectomy) surgery [105]. For gene expression classifier testing, a study of 273 patients at a single institution reported a change in clinical management in 8.4% of patients who underwent testing [104]. Further studies are needed to more fully assess the impact of molecular testing on clinical management.

Prognostic Applications of Molecular Markers

Molecular profiling of mutations and gene rearrangements not only provides helpful diagnostic information that can help rule in malignancy but can also simultaneously identify molecular alterations with prognostic and therapeutic applications. Molecular profiling may inform surgical management as some patient may benefit from a more extensive initial surgery, may affect post-surgical surveillance for disease recurrence, and may provide therapeutic targets for metastatic or recurrent disease.

The *BRAF* V600E mutation has been extensively characterized as a possible prognostic marker. Multiple studies have found an association in papillary thyroid cancer between the *BRAF* V600E mutation and factors such as extrathyroidal invasion, metastatic disease, and disease recurrence. However, other studies did not show a strong association [106–108]. In a meta-analysis of 14 studies, the *BRAF* V600E mutation was found to be associated with tumor recurrence and persistent disease (25% in *BRAF* mutation-positive tumors vs. 13% in mutation-negative tumors). Furthermore, in a large, multicenter study, the *BRAF* V600E mutation was shown to be significantly associated with mortality (5% in mutation-positive patients vs. 1% in mutation-negative patients) [109]. For both tumor recurrence and mortality, the increases were small but statistically significant, suggesting that *BRAF* V600E mutation alone is a relatively sensitive, but not specific marker of tumor recurrence and tumor-related mortality.

TP53 has been described as a prognostic marker in several tumors and, in thyroid cancer, is a well characterized genetic event governing thyroid tumor dedifferentiation. *TP53* mutations occur in well-differentiated tumors but occur at highest frequency in poorly differentiated and anaplastic thyroid cancers [51, 52]. Further studies are needed, but *TP53* mutations in well-differentiated tumors may herald the potential for dedifferentiation or a more aggressive clinical course.

The recurrent mutations of the *TERT* promoter are seen more frequently in aggressive thyroid tumors such as widely invasive oncocytic carcinoma and anaplastic thyroid carcinoma [60–63]. *TERT* promoter mutations have been reported to be an independent risk factor for poor prognostic factors such as persistent disease, distant metastases, and disease-specific mortality for well-differential thyroid cancer [63]. In addition, *TERT* promoter mutations were found to frequently co-occur with *BRAF* V600E mutation, which suggested a possible interplay between MAPK pathway and telomerase activation in aggressive tumors [60, 62]. Indeed, in a recent study of 551 patients with differentiated thyroid cancer, the coexistence of *BRAF* or *RAS* mutations with

TERT promoter mutations was found to be associated with increased recurrence and mortality [110].

As we continue to elucidate the genomic landscape of thyroid cancer, it is likely that more markers of aggressive tumor behavior will be found. It is becoming clear that rather than the presence of a single biomarker, a profile of genomic alterations may be more useful in predicting tumor behavior. Coexisting mutations in driver genes such as *BRAF* or *RAS* with mutations in *PIK3CA*, *AKT1*, or *TP53* occur in poorly differentiated and anaplastic tumors [49, 56, 111]. Multiple mutations have also been seen in a small number of well-differentiated tumors, which were aggressive and presented with distant metastases [112]. Detection of multiple mutations can be achieved in FNA samples of even very small tumors, allowing for both diagnosis and prognostication prior to surgery [113].

Summary

Improved diagnostic accuracy of thyroid nodules and clinical management decision support is achievable by incorporating molecular mutation/rearrangement or gene expression information. Currently available tests excel in ruling in or ruling out malignancy, and further improvements are expected with expanded panels that include more thyroid cancer markers. Gene mutation/rearrangement panels additionally offer prognostic information that can guide the extent of the initial surgical management as well as postsurgical management. With further improvements in technology and decreased costs of testing, routine molecular profiling of thyroid tumors can help achieve a personalized treatment and management plan for every patient.

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Thyroid Cancer in Children and Adolescents

Young Ah Lee and Andrew J. Bauer

Introduction

The incidence of pediatric thyroid cancer has gradually increased over the last decades [1, 2]. Pediatric thyroid cancer most commonly presents as a visible or palpable thyroid nodule with or without cervical lymphadenopathy. Although the majority of pediatric thyroid nodules are likely to be benign, thyroid nodules diagnosed in the pediatric age carry a greater risk of malignancy compared to those in adults (22%–26% vs. 7%–15%) [3, 4]. In pediatrics, differentiated thyroid carcinoma (DTC) is the most common form of thyroid malignancy, with 90% or more being papillary thyroid carcinoma (PTC). Follicular thyroid cancer (FTC) and medullary thyroid cancer (MTC) are less common, and poorly differentiated tumors, including anaplastic thyroid carcinoma, are exceedingly rare in young patients. Recently, the American Thyroid Association (ATA) released the first consensus guidelines specific to pediatric patients with thyroid nodules and DTC [5]. This chapter reviews the practical evaluation of pediatric thyroid nodules as well as the management of pediatric thyroid carcinoma.

Presentation at Diagnosis

Pediatric thyroid cancer is most often detected by a visible nodule and/or a palpable neck mass [6]. Recently, with the prevalent use of radiologic imaging, the number of pediatric patients referred for the evaluation of incidentally detected thyroid nodules is increasing. The incidence of pediatric thyroid nodules ranges from 0.05 to 2% [4, 7, 8], but up to 18% of pediatric patients may have an incidental thyroid lesion discovered on non-thyroid-related neck ultrasound (US) studies [9]. Although cystic lesions are more commonly identified compared to nodules (57% vs. 1.5%) [8], there does not appear to be a correlation between how the nodule is discovered (incidental or purposeful exam of the thyroid) and the risk of malignancy.

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Diagnostic Evaluation of Thyroid Nodule

History Taking and Physical Examination

There are several risk factors associated with an increased risk for developing thyroid cancer, including a previous history of radiation exposure [10, 11], coexisting autoimmune thyroid disease [12–14], family history of thyroid malignancy [15–18], and several genetic syndromes [19–24] (Table 1). Although most patients are asymptomatic, a query of hoarseness and a sensation of a lump or obstruction with swallowing should be evaluated.

A complete thyroid exam includes inspection and palpation of the thyroid gland as well as the lateral neck cervical lymph nodes (see https://www.youtube.com/watch?v=Z9norsLPKfU). The size, symmetry, texture, and firmness of the thyroid gland, thyroid nodule(s), and lateral neck lymph nodes should be described. The presence of a thyroid nodule with cervical lymphadenopathy is a significant predictor for malignancy, especially if lymphadenopathy is noted in levels III, IV, and V of the lateral neck [25, 26].

Physical findings related to genetic syndromes (Table 1) should be evaluated. PTEN hamartoma syndrome (PTHS) is associated with macrocephaly, trichilemmomas, lipomas, and genital freckling [20–22], Carney complex [24] and familial adenomatous polyposis [19] are associated with lentigines, and MEN2B is associated with alacrima, marfanoid facies, and mucosal neuromas [27].

Table 1 Risk factors for the development of thyroid nodules and cancer

Family or personal history	Genetic syndromes[a]
Exposure to radiation	PTEN hamartoma syndrome
Autoimmune thyroid disease	APC-associated polyposis
Familial multinodular goiter	*DICER1* syndrome
Familial non-medullary thyroid cancer	Carney complex

[a]Although DTC has also been reported to occur in patients with Beckwith-Wiedemann syndrome, the familial paraganglioma syndromes, McCune-Albright syndrome, and Peutz-Jeghers syndrome, it remains unclear if these tumors are a direct result of the underlying genetic defect

Laboratory Evaluation

A normal or elevated thyrotropin (TSH) level may confer an increased likelihood of thyroid malignancy [28, 29]; however, not all studies support this observation [30]. A suppressed TSH may be associated with the presence of an autonomously functioning thyroid nodule (AFTN), a nodule with a lower risk of malignancy in both adult (1–10%) and pediatric age patients [31]. In adult patients, there are mixed reports on the utility of preoperative serum thyroglobulin (Tg) as a predictive factor of thyroid malignancy [32], as well as disease burden [33]. The utility of preoperative Tg in children and adolescents with thyroid nodules has not been established, and one needs to consider confounding variables that may be associated with an elevated Tg, including iodine deficiency and excess [34] and thyroiditis. In contrast, calcitonin needs to be measured if there is a family history of multiple endocrine neoplasia type 2 (MEN2) or clinical features suggestive of MEN2B or if the cytology is suspicion for MTC [35].

Radiologic Imaging

A thyroid and neck ultrasound (US) is the best imaging modality to assess thyroid tissue morphology and lymphadenopathy. The US report should describe the size, location, composition (solid, cystic, or spongiform), echogenicity (hypoechoic, isoechoic, hyperechoic, or mixed echogenicity), margins (regular, infiltrative, or microlobulated), presence of calcification, shape (taller than wide or not on transverse imaging), and vascularity of the thyroid nodule(s). US evaluation of the lateral neck should be performed for patients with suspicious nodules with documentation of shape, echogenicity, vascular pattern (central, peripheral, or both), and presence of microcalcifications of any suspicious cervical lymph nodes [36]. Thyroid nodules with solid composition, hypoechoic or

mixed echogenicity, microlobulated or infiltrative margin, taller than wide shape of transverse imaging, and the presence of microcalcification and lymph nodes with a rounded shape, increased echogenicity, microcalcifications, and peripheral blood flow are associated with an increased risk of malignancy [37–39].

For adults, the particular US pattern of the thyroid nodule correlates with the risk of malignancy and may be used to stratify which nodules should undergo fine needle aspiration (FNA) [35]. Adult criteria may be applicable to children, with the exceptions that (1) US features and clinical context should be used rather than size alone to identify nodules that warrant FNA [5], (2) solid nodules may have an increased risk of malignancy in pediatric patients compared to adults, and (3) a widely invasive PTC, called diffuse sclerosing variant PTC (dsvPTC), is not associated with nodular disease [40, 41], but is associated with microcalcifications throughout the gland ("snowstorm" appearance on US) and macroscopic metastasis to lateral neck lymph nodes [42–44]. Thyroid nodules with >50% cystic component are associated with a lower risk of malignancy [3]; however, while several US features are associated with an increased risk of malignancy, including an irregular margin, presence of calcifications, and presence of abnormal lymph node appearance, no US features have a diagnostic accuracy as high as FNA [38, 39, 45, 46].

US-guided FNA should be considered for nodules >1 cm and for those 0.5 to 1 cm if US features show suspicious findings [47]. The presence of cervical lymphadenopathy increases the likelihood of malignancy for the primary thyroid lesion [25, 26] and complete assessment by US with confirmation by FNA is critical to optimize the surgical plan [36]. For patients undergoing FNA to confirm metastatic lymph node disease, measurement of Tg in the FNA washout of the lymph node may help to confirm equivocal cytological evidence of regional metastasis [48, 49]. Axial imaging with neck computed tomography (CT) or magnetic resonance imaging (MRI) may increase the sensitivity of identifying lymph node metastasis not readily visible by preoperative US, including level VI (central neck), subclavicular, and upper mediastinum [50]. Although US elastography may be a helpful tool to distinguish between benign and malignant nodules and lymph nodes, there is no consensus on universally incorporating this technique into clinical practice [35, 51, 52].

Thyroid scintigraphy may be considered for patients with a suppressed TSH (Fig. 1); however, there does not appear to be any benefit to obtaining preoperative studies with other radioisotope imaging modalities, including [^{18}F]-fluorodeoxyglucose positron emission tomography (^{18}F-FDG-PET/CT). However, there is an approximate 20% risk of malignancy for a thyroid nodule that is incidentally identified during ^{18}F-FDG-PET/CT performed during evaluation and management of non-thyroid disease [53].

Fine Needle Aspiration

Similar to adults, The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) is used to classify the FNA results in pediatrics with equal sensitivity, specificity, and overall accuracy [3, 38]. The results of the FNA are then used to stratify an appropriate management plan [5].

1. Nondiagnostic or unsatisfactory cytology has a 1–4% DTC risk in adults [54], with limited data in children. Repeat FNA is an option, but it should be delayed for at least 3 months (6 weeks at minimum) to avoid potential post-FNA reactive cellular atypia [55]. Cytological confirmation of sample adequacy at the bedside can decrease the rate of nondiagnostic results.
2. Benign cytology has a 0–10% DTC risk according to recent pediatric studies [25, 56]. This is higher than a 0–3% risk in adults [54]. Thus, despite benign cytology, follow-up is mandatory. If nodule grows and develops compressive symptoms, surgery may be indicated. If children have large thyroid nodule ≥4 cm with benign cytology, lobectomy should be considered, because FNA of nodule ≥4 cm may have decreased sensitivity with higher false-negative rates [57, 58].

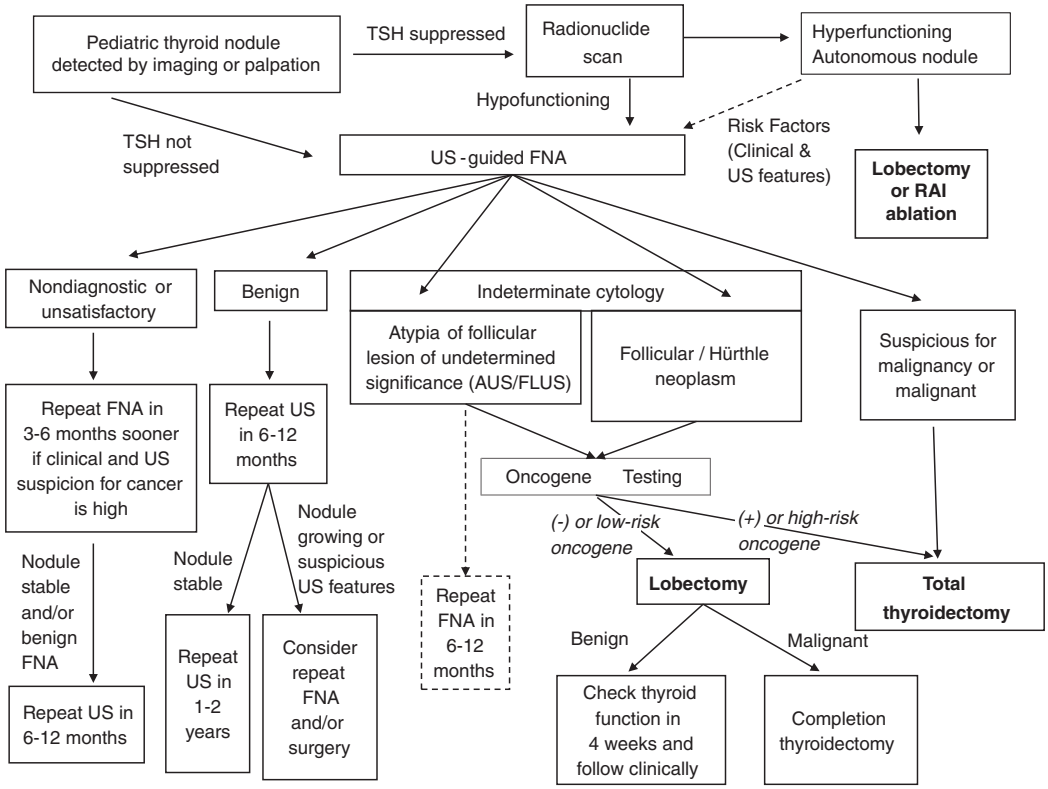


Fig. 1 Initial evaluation, treatment, and follow-up of a thyroid nodule in a child or adolescent

3. The indeterminate categories include "atypia or follicular lesion of undetermined significance (AUS/FLUS)" and "follicular/Hürthle neoplasm" or "suspicious for follicular/Hürthle neoplasm (FN or SFN)." The limited pediatric data suggest the indeterminate FNA categories account for 35% of results and that there is a higher risk of malignancy within these categories with up to 28% of AUS/FLUS and 58% of FN ultimately found to be malignant [59, 60]. In adults, the risk of malignancy is approximately 5–15% in the AUS/FLUS category and 15–30% in the FN or SFN group [61]. Based on the increased risk of DTC in children, surgery is favored over repeat FNA for most nodules with indeterminate cytology [5]. Lobectomy is recommended for children with low-risk US features and total thyroidectomy (TT) for those with US features suspicious for PTC or with bilateral thyroid nodules.

In an effort to decrease reliance on diagnostic surgery, there is increasing use of supplemental molecular profile testing in pediatrics [35] following the more widespread use of oncogene panels and gene expression classifier testing in adults [62, 63]. In children, the presence of a thyroid oncogene mutation or fusion (*BRAF, RET/PTC, NTRK fusion, and others*) in an indeterminate FNA specimen is associated with an increased risk of malignancy [25, 59, 64].

Based on current, limited data, the following approach is supported:

1. Oncogene panels are the only tests that have clinical utility to predict an increased risk for malignancy in patients under 19 years of age [59, 65–67]. Gene expression classifiers have not been validated in patients <21 years of age and are highly influenced by the prevalence of disease within the test population.

Table 2 Thyroid oncogene, risk of invasive disease, and anticipated surgical approach

Point mutation or oncogene fusion	Increased risk of DTC with invasive disease	Surgical approach
BRAFV600E RET-PTC fusion NTRK fusion AGK-BRAF ALK fusion TERT + (additional mutation)	Yes Yes Yes Yes (very limited data) No data in pediatrics	Total thyroidectomy with central neck dissection; lateral neck lymph node dissection based on clinical findings and FNA confirmation of metastasis
RAS PAX8-PPARG	No → Increasing risk of FTC and fvPTC if >20% of cells with mutation No	Lobectomy → Consider completion thyroidectomy if invasive histology
TSHR THADA GNAS	No No No	Surveillance or definitive treatment if associated with autonomous function (TSHR or GNAS)
AKT1, CTNNB1, EIF1AX, and others	Unknown	No specific recommendation

2. Oncogene panels should only be ordered on samples with indeterminate cytology (TBSRTC categories III and IV). Oncogene panel testing may also be considered for nodules with benign cytology but suspicious ultrasound features (hypoechoic solid nodule, infiltrating or microlobulated border, increased intranodular blood flow, and/or microcalcifications).
3. Only a few mutations or rearrangements appear to be associated with an increased risk for PTC with invasive disease (Table 2) [65–70]. The presence of other mutations or fusions (*RAS, PAX8-PPARg*, and others) is associated with an increased risk of malignancy; however, until further data is available, lobectomy may still be the surgery of choice as the associated variants often have a more indolent, less invasive phenotype.
4. A low mutation level detected by next-generation sequencing (<10 of alleles, corresponding to <20% of cells with a mutation or fusion) in *RAS* may be associated with benign nodules [63]. In these cases, diagnostic lobectomy should also be considered as the initial surgical approach.
    - The recommendation for lobectomy is based on the absence of predisposing risk factors, including the absence of familial tumor predisposition syndrome or a history of previous radiation exposure, the presence of a unilateral nodule, the absence of pathologic lymphadenopathy, and the absence of autoimmune thyroid disease.
5. Suspicious for malignancy (SUSP) and (6) malignant cytology correlate with a 75–100% risk of PTC [25, 56]. TT with prophylactic central neck lymph node dissection (CND) is recommended [5].

DTC Variants

Papillary Thyroid Cancer

At the time of diagnosis, pediatric patients with PTC have high rates of bilateral and multifocal disease [6, 71], regional lymph node metastasis (40–90%), and, for patients with lateral neck metastasis, an increased risk of lung metastasis (10–25%) [72–75]. Pediatric patients with PTC also have high recurrence rates [6, 73, 76, 77], and many children with lung metastasis show persistent, albeit stable, disease following ^{131}I therapy [5, 78]. Despite these concerning characteristics, pediatric patients with PTC have a more favorable prognosis and a significantly lower disease-specific mortality rate compared to adults [73, 79–81]. Irrespective of the tumor size, extra-

thyroidal extension, and lymph node or distant metastasis, the overall survival rate for pediatric patients with differentiated thyroid cancer (PTC and follicular thyroid cancer, FTC) is estimated to be greater than 95% [6, 82, 83], with appropriate management.

There is ongoing debate whether differences in clinicopathological presentation and long-term outcome between children and adults result from differences in genetic alterations [84]. The low prevalence of the *BRAF*V600E mutation, the most common driver mutation in adult PTC and associated with a poorer prognosis [85, 86], was initially proposed as a potential explanation for why pediatric patients have lower disease-specific mortality compared to adults [87, 88]. With the development of more sensitive technologies [89], more recent studies report that between 30 and 40% of pediatric PTC harbor a *BRAF*V600E mutation; however, in contrast to adults, the presence of a *BRAF* mutation does not correlate with increased invasive behavior or poor prognosis [65–69]. In children and adolescents, PTC with a fusion oncogene, specifically *RET-PTC1, RET-PTC3, ETV6-NTRK3, and TPR-NTRK1,* present with more extensive disease and aggressive pathology, including diffuse sclerosing variant (dsvPTC), solid variant (sPTC), and diffuse or widely invasive follicular variant PTC [65, 67, 70].

Further investigation is needed to confirm if these molecular markers are associated with specific variants of PTC as well as to determine if they can be used to predict clinical behavior. Ultimately, these driver mutations, as well as other alterations in the oncogenome, may be used to stratify surgery and select which patients have a higher likelihood of benefiting from radioiodine and whether a more or less intense surveillance plan should be followed [90].

As the presence of *RET-PTC* and *NTRK* gene fusions appears to predict invasive variants of PTC, the presence of *RAS*, *TSHR*, *GNAS* mutations and *PAX8-PPARγ* fusion may be associated with more indolent forms of PTC, to include encapsulated follicular variant PTC (enc-fvPTC) or FTC (*PAX8-PPARγ*) [84]. These lesions are typically associated with indeterminate cytology as well as increased intra- and interobserver variability in histological diagnosis [91, 92]. The recent change in nomenclature of encapsulated fvPTC to "noninvasive follicular thyroid neoplasm with papillary-like nuclear features" (NIFTP) reflects a recognition that some of these lesions have no risk for invasive behavior [93]. While only a subgroup of tumors will meet the strict diagnostic criteria for NIFTP, other tumors that continue to be considered carcinomas may also display very indolent behavior. The presence of one of these genetic alterations, combined with US features and histologic findings, including the presence of a tumor capsule with the absence or minimal, partial invasion of the tumor into the capsule, and the absence or minimal lymphatic and vascular invasion (<4 vessels), may support for a less aggressive approach, with remission achieved by lobectomy alone.

Follicular Thyroid Cancer

Pediatric FTC is uncommon malignancy and represents 10% or less of pediatric DTC patients. Although pediatric FTC remains unstudied, *RAS* point mutation and the *PAX8-PPARγ* rearrangement have been implicated as genetic alterations in adult FTC [94, 95]. FTC may develop as part of *PTEN* hamartoma tumor syndrome; thus, there should be a high index of suspicion in children with FTC, particularly in those with macrocephaly, lipoma, penile freckling, or a suggestive family history [20, 22, 23]. FTC may also be related to other genetic syndromes (Table 1).

The diagnosis of FTC is based on the pathologic identification of capsular and/or vascular invasion. Tumors with microscopic capsular invasion alone and/or very limited vascular invasion are subdivided into minimally invasive FTC, whereas grossly invasive tumors that show widespread invasion into blood vessels and/or adjacent thyroid tissue are classified into widely invasive FTC [96, 97]. Pediatric FTC may be less aggressive than PTC and is typically unifocal rarely metastasizing to regional lymph nodes [73, 98–100]. However, FTC is prone to hematogenous metastasis, usually the lung and bone, even

in the absence of lymph node metastasis [101, 102]. Minimally invasive FTC has excellent prognosis, whereas widely invasive FTC is associated with significant morbidity and mortality in adults [96, 97, 101]. Limited data suggest that pediatric patients with FTC may be at risk for recurrence but have low disease-specific mortality compared to adult patients [100].

Patients with clear evidence of vascular invasion (more than three involved blood vessels), known distant metastasis, and/or tumor size >4 cm should be treated with TT and staged postoperatively with ^{131}I therapy [97, 101–103]. Minimally invasive FTC <4 cm in size and with no or minimal vascular invasion (three or fewer involved vessels) should be treated on a case-by-case basis, but lobectomy alone rather than TT with ^{131}I therapy may be sufficient [5]. Due to limited data regarding pediatric FTC, further studies are needed to risk-stratify children who would benefit from extensive surgery and ^{131}I therapy. The surveillance and follow-up of pediatric FTC is similar to PTC and includes serial serum Tg and TgAb measurements as well as TSH suppression. Routine neck US monitoring is less useful due to the low incidence of regional lymph node metastasis [5].

Surgery and Radioiodine Treatment

The goals for the 2015 ATA pediatric consensus guidelines are to maintain the low disease-specific mortality currently experienced by children with DTC while reducing potential complications from therapy [5]. The first treatment is surgery, ideally performed by a high-volume thyroid surgeon [104, 105] after complete preoperative assessment and FNA confirmation of cervical disease. The second treatment is TSH suppression with levothyroxine, to decrease the likelihood of TSH-induced tumor regrowth. TSH is a well-established growth factor of thyroid cancer cells, and TSH suppression has been an important cornerstone of therapy [106]. Third, therapeutic ^{131}I therapy is considered based on the risk of persistent or recurrent disease as defined by the ATA pediatric risk levels (low, intermediate, and high) [5]. With increased awareness of the potential short- and long-term risks of ^{131}I therapy, there are renewed efforts to identify which patients may (ATA pediatric intermediate and high risk) or may not (ATA pediatric low risk) benefit from ^{131}I therapy [5, 107, 108].

Surgery

For the majority of patients with PTC, a total thyroidectomy (TT) with prophylactic central compartment lymph node dissection is recommended [5] secondary to the increased incidence of bilateral/multifocal disease in pediatric PTC [6, 71], the increased risk for recurrence and subsequent second surgery when less than a near-TT or TT is performed [75, 79, 81, 109], and the high incidence of lymph node metastasis. For patients with unifocal papillary microcarcinoma (<1 cm), lobectomy may be adequate with surveillance and counseling for the potential need for additional surgery if persistent or recurrent disease is found [110, 111].

Thyroid surgery should ideally be performed by a high-volume thyroid surgeon [5], defined as a surgeon who performs 30 or more cervical endocrine procedures annually, to minimize the risk of operative complications [104, 105]. While the exact number of surgeries performed annually may not reflect the quality of the surgeon, it increases the likelihood that the surgeon understands the disease process in children and adolescents in an effort to balance complete resection while minimizing the risk of incomplete surgical resection, permanent hypoparathyroidism, and recurrent laryngeal nerve damage. A prophylactic central lymph node dissection (CND), either ipsilateral or bilateral, is recommended for children and adolescents with malignant cytology, and a therapeutic CND is recommended if there is evidence of gross extrathyroidal extension (ETE) and/or regional lymph node metastasis on preoperative staging [5]. A compartment-based lateral neck dissection should only be pursued on patients with cytological evidence of metastases to the lateral neck on preoperative FNA [5].

Routine prophylactic lateral neck dissection is not recommended.

The development of postsurgical complications such as hypoparathyrodism and recurrent laryngeal nerve damage, spinal accessory nerve injury, and Horner syndrome should be monitored, although the risk is reduced in a high-volume surgical practice. The use of intraoperative parathyroid hormone levels may help identify patients at risk of hypoparathyroidism in an effort to ensure early administration of calcium and calcitriol. The perioperative calcium and phosphorus must be monitored to ensure stable values prior to discharge from the inpatient setting [112, 113]. Early identification of hypoparathyroidism with subsequent initiation of calcitriol and calcium decreases the risk of symptomatic hypocalcemia as well as shortens the duration of postoperative hospitalization [114].

ATA Pediatric Risk Levels and Radioiodine Administration

In contrast to adults with thyroid cancer, there is no staging system for children and adolescents with PTC secondary to the extremely low disease-specific mortality [115]. However, the American Joint Committee on Cancer (AJCC) Tumor, Nodes, Metastases (TNM) classification system [116] is used to describe the extent of disease and stratify an approach to further evaluation and management (Table 3) [5]. These three groups are excerpted from 2015 ATA pediatric guidelines as follows:

1. *ATA Pediatric Low Risk*: Disease grossly confined to the thyroid with N0 (no lymph node metastasis) or NX (no lymph nodes assessed) disease or patients with incidental N1a metastasis in which "incidental" is defined as the presence of microscopic metastasis to a small number of central neck lymph nodes (typically, less than 3, although there is no consensus on the exact number). These patients appear to be at lowest risk for distant metastasis but may still be at risk for residual cervical disease, especially if the initial surgery did not include a CND.
2. *ATA Pediatric Intermediate Risk*: Extensive N1a (level VI) or minimal N1b disease (levels II, III, IV, or V). These patients appear to be at low risk for distant metastasis but are at an increased risk for incomplete lymph node resection and persistent cervical disease. The impact of the pathologic identification of microscopic extrathyroidal extension (ETE; T3 disease) on management and outcomes has not been well studied in children with PTC, but patients with minimal ETE are likely either ATA pediatric low or intermediate risk, depending on other clinical factors.

Table 3 The American Joint Committee on Cancer TNM classification system for differentiated thyroid carcinoma

Primary tumor (T)		
TX		Size not assessed, limited to the thyroid
T1	**T1a** **T1b**	≤1 cm, limited to the thyroid >1 cm but ≤2 cm, limited to the thyroid
T2		>2 cm but ≤4 cm, limited to the thyroid
T3		>4 cm, limited to the thyroid, or any tumor with minimal extrathyroid extension
T4	**T4a**	Tumor extends beyond the thyroid capsule to invade subcutaneous soft tissues, larynx, trachea, esophagus, or recurrent laryngeal nerve
	T4b	Tumor invades prevertebral fascia or encases carotid artery or mediastinal vessels
Lymph nodes (N)		
NX		Regional lymph nodes not assessed
N0		No regional lymph node metastasis
N1	**N1a**	Metastasis to level VI (pretracheal, paratracheal, and prelaryngeal/Delphian lymph nodes)
	N1b	Metastasis to unilateral, bilateral, or contralateral cervical levels I, II, III, IV, or V or retropharyngeal or superior mediastinal lymph nodes (level VII)
Distant metastasis (M)		
MX		Distant metastasis not assessed
M0		No distant metastasis
M1		Distant metastasis

Used with the permission of the American Joint Committee on Cancer (AJCC), Chicago, Illinois. The original source for this material is the *AJCC Cancer Staging Manual*, 7th edition (2010), published by Springer Science and Business Media LLC, www.springer.com [116]

3. *ATA Pediatric High Risk*: Regionally extensive disease (extensive N1b) or locally invasive disease (T4 tumors), with or without distant metastasis. Patients in this group are at the highest risk for incomplete resection, persistent disease, and distant metastasis.

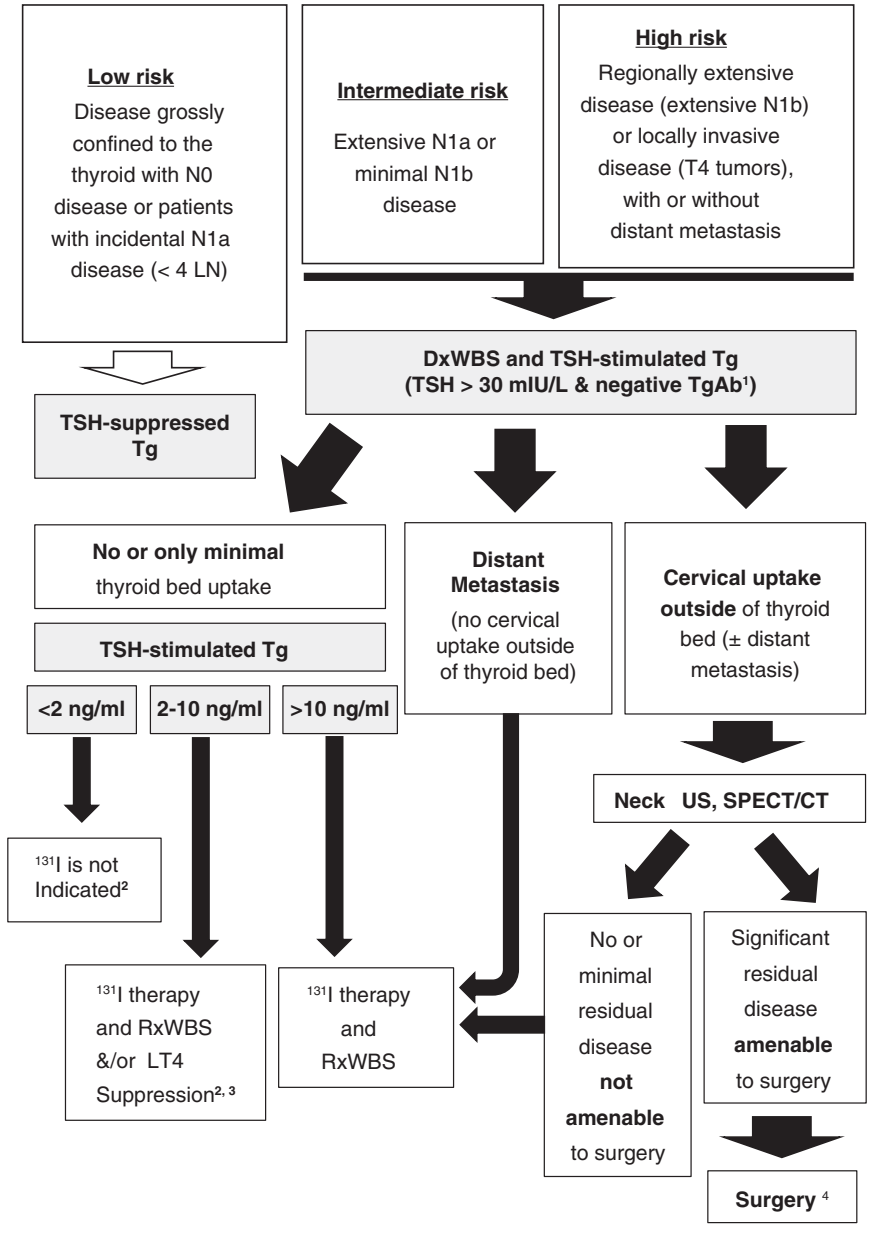
Postoperative evaluation for evidence of persistent disease is typically performed within 12 weeks of surgery to identify which patients may or may not benefit from further therapy, to include additional surgery or ^{131}I therapy. For ATA low-risk patients, one may consider following the TSH-suppressed Tg level with repeat neck US instead of pursuing a stimulated Tg level with diagnostic whole body scan (DxWBS) in the immediate postoperative time frame. The stimulated Tg and DxWBS can be performed at a later time if there is an elevated Tg and no evidence of disease based on US and/or anatomic imaging (CT or MRI) [5, 107, 108].

For intermediate- and high-risk patients, a TSH-stimulated Tg and a ^{123}I- DxWBS are recommended to search for residual or metastatic disease in the neck, mediastinum, lungs, or elsewhere (Fig. 2) [72, 117]. Two to three weeks before the DxWBS, liothyronine (LT3) or levothyroxine (LT4) is withheld with a goal of achieving a TSH of at least or greater than 30 mIU/L. The ability to achieve adequate TSH elevation using recombinant human TSH (rhTSH) has been studied in children using the typical adult dose (0.9 mg given 24 h apart), although pediatric data on the efficacy of rhTSH on treatment outcomes are limited [118, 119]. For selected patients who cannot tolerate endogenous hypothyroidism or have a pituitary TSH deficiency, rhTSH needs to be considered. The patient is also placed on a low-iodine diet over this time frame to optimize absorption of radioiodine (see www.ThyCa.org). For children who received iodinated contrast agents, it is recommended to wait 2–3 months or to confirm low 24-h urine iodine levels prior to administration of RAI [120].

Because the diagnostic activity of ^{131}I may theoretically "stun" the iodine-avid tissue and reduce therapeutic ^{131}I uptake, the lowest possible activity of ^{131}I (2.7–4.0 mCi) or, preferably, ^{123}I should be used for the DxWBS [121, 122]. The use of ^{123}I is favored due to advantages including superior imaging quality, decreased radiation exposure, and prevention of stunning in children [121]. For patients with cervical uptake, neck US and/or single-photon emission computed tomography with integrated conventional CT (SPECT/CT) may provide more accurate anatomic localization to differentiate metastatic regional lymph node from remnant thyroid tissue [123].

For patients with negative or only minimal thyroid bed uptake on DxWBS after initial surgery, ^{131}I therapy can be considered based on a TSH-stimulated serum Tg level and a case-by-case basis (Fig. 2). A TSH-stimulated Tg <2 ng/mL has a predictive value of 94.9% for the absence of disease in adult patients, which is similarly considered to be disease-free in children [124]. If TSH-stimulated Tg is 2–10 ng/mL, ^{131}I therapy should be considered for patients with thyroid bed uptake, invasive histology (dsvPTC, sPTC, and widely invasive follicular variant PTC), or tumors with extensive regional metastasis (extensive N1a or any N1b disease). If the TSH-stimulated Tg is >10 ng/mL, ^{131}I therapy is indicated. Repeat surgery prior to administration of ^{131}I should be pursued if there is evidence of "bulky," macroscopic, persistent disease noted during this initial postoperative time frame (Fig. 2). FNA should always be considered prior to surgery to confirm the presence of persistent or recurrent disease. For treatment of nodal or other locoregional disease that is not amenable to surgery, as well as known or presumed iodine-avid distant metastasis, ^{131}I therapy is indicated (Fig. 2) [5].

There is no consensus on a standardized dose of ^{131}I in children. Therapeutic ^{131}I is commonly dosed empirically or determined based on bone marrow dose limited dosimetry. Empiric dosing is given as a fraction (child's weight in kg divided by 70 kg) of a typical adult dose used to treat similar disease extent [125, 126] or based on weight (1.0–1.5 mCi/kg) [127]. Bone marrow dose limited dosimetry is targeted to limit lung retention to <80 mCi at 48 h and blood/bone marrow exposure to <200 cGy [128]. Dosimetry should be considered in younger children (<than 10 years), those with diffuse pulmonary metastases, and those who received radiation therapy for other malig-



[1] In positive TgAb patients (except in patients with T4 disease or clinical M1 disease), consideration can be given to deferred evaluation to allow time for TgAb clearance.

[2] Consider ^{131}I in patients with thyroid bed uptake and T4 tumors or known residual microscopic cervical disease.

[3] While there are no prospective studies in pediatric patients, the use of ^{131}I remnant ablation may not decrease the risk for persistent or recurrent disease.

[4] Re-evaluate to determine if ^{131}I therapy would be beneficial (evidence of persistent, non-operable disease)

Fig. 2 Evaluation and management for the risk of persistent post-surgical disease using the American thyroid association (ATA) pediatric risk stratification system

nancies. A posttreatment WBS (RxWBS) should be obtained 4–7 days after all ^{131}I treatments and is associated with a greater sensitivity for detecting persistent disease when compared to the DxWBS [129]. The addition of SPECT/CT may help to localize residual cervical disease [5, 130].

The short-term side effects of radioiodine therapy include salivary gland dysfunction (sialadenitis, xerostomia, dental caries, and stomatitis), ocular dryness, and nasolacrimal duct obstruction. Bone marrow suppression and gonadal toxicity may also occur, with an increased risk at higher ^{131}I cumulative doses and shorter intervals of administration. Males should avoid attempts at conception for at least 4 months and females for 6–12 months after ^{131}I therapy [131, 132]. Postpubertal males should be counseled, and sperm banking should be considered, for those receiving cumulative activities ≥400 mCi [5, 133]. Acute bone marrow suppression may develop, but all hematologic parameters usually normalize within 3 months' post therapy [134]. Children with pulmonary metastasis may have diffuse micronodular disease and are at an increased risk of developing treatment-induced pulmonary fibrosis [78, 110]. Thus, pulmonary function testing and non-contrast chest CT should be monitored in all children with diffuse pulmonary metastasis, especially if multiple ^{131}I therapies are considered [5]. Furthermore, long-term follow-up studies over the last decades have reported an increase in mortality attributed to second primary malignancies (SPM) that appear to be possibly related to ^{131}I therapy [6, 79, 135, 136]. Many of the SPMs are in iodine-avid glands (i.e., salivary glands) or in non-avid tissues passively exposed to ^{131}I during physiologic clearance (bone marrow, colon, bladder, and others) [79, 137]. Thus, the challenge is to identify the children for whom the benefits of ^{131}I therapy outweigh the risks. Families should be provided full information of the benefits and risks of ^{131}I therapy [5].

Surveillance, Restaging, and Follow-Up of PTC in Children

Serial serum thyroglobulin (Tg) levels and neck US are the most useful tests for monitoring patients with DTC. First-generation Tg assays have a functional sensitivity of ~1.0 ng/mL, and newer second-generation immuno-chemiluminescent assays (ICMA) have improved sensitivity down to 0.1 ng/mL. Tg and TgAb levels should be simultaneously measured on all samples as up to 20–25% of patients with DTC have detectable TgAb that can interfere with Tg result with the direction of the false Tg value dependent on which assay is used [138]. For patients with TgAb, Tg will be underestimated using ICMA. For these patients, the trend in TgAb should be followed as a marker of disease status [138]. Many laboratories will reflexively run the Tg by radioimmunoassay (RIA) or liquid chromatography/tandem mass spectrometry (LC/MS-MS) if TgAb is detected. One needs to remember that there is significant variance in the reliability in detecting Tg and TgAb between different assays and that in the presence of TgAb the Tg may only be detected in 35% of samples run on RIA, with a high proportion of false-positive results, and that LC-MS/MS may have a false-negative detection rate of approximately 40% [139]. One must be aware of the shortfalls in measuring Tg in the presence of TgAb, and in an effort to obtain reliable results, it is critical to use the same assay and laboratory for serial surveillance laboratory monitoring to reduce inter-assay variance and improve assessment of the Tg and/or TgAb trend [140].

In the absence of TgAb, a basal (non-TSH-stimulated) Tg level below 0.2 ng/mL is consistent with remission from disease [141]. For patients that did not receive postsurgical ^{131}I therapy, the TSH-suppressed Tg should decrease to <0.5 ng/mL 6 to 12 months after total thyroidectomy [141]. If the Tg remains mildly elevated, between 2 and 10 ng/mL, continued monitoring may be pursued depending on the trend in Tg over time as well as evidence of persistent or recurrent disease based on radiologic imaging. Increasing or frankly elevated levels of TSH-stimulated Tg (>10 ng/mL) warrant further evaluation to localize disease and to decide whether additional surgery and/or ^{131}I therapy would be beneficial [5].

Neck US is the most effective and efficient radiologic tool to monitor for persistent or recurrent anatomic disease. The first US

should be performed approximately 6 months after the initial surgery and then at 6- to 12-month intervals for ATA pediatric intermediate- and high-risk patients and at annual intervals for ATA pediatric low-risk patients. Serial chest CT at 6- to 12-month intervals should be used to monitor patients with known pulmonary metastasis. A TSH-stimulated Tg and a DxWBS may be obtained in ATA pediatric intermediate- and high-risk patients previously treated with ^{131}I with known iodine-avid metastatic disease if the Tg level has plateaued or is increasing and anatomic disease is not detected on neck US or cross-sectional imaging (chest CT or MRI) [5]. The use of ^{18}F-FDG-PET/CT should be limited to patients suspected to have persistent anatomic that is non-RAI avid based on previous RAI treatment and WBS imaging [142].

No evidence of disease (NED) is defined as the absence of structural abnormalities on radiologic imaging and undetectable Tg and TgAb levels. Persistent disease is defined by TSH-suppressed >1 ng/mL or any evidence of anatomic disease on neck US, cross-sectional (CT or MRI), or functional imaging (RAI whole body scan or ^{18}F-FDG-PET/CT). Recurrent disease is characterized by the detection of new biochemical or anatomic abnormalities in patients that were previously considered to have NED [108].

All pediatric patients with DTC should receive thyroid hormone replacement following surgery with or without ^{131}I therapy dosed to achieve a TSH of <0.5 mIU/L. The degree of TSH suppression should be based on the extent of disease [143] adjusted to avoid signs and symptoms of hyperthyroidism. Based on ATA pediatric risk level and current disease status, ATA pediatric guidelines recommend that the goal of TSH suppression is to achieve a TSH level 0.5–1.0 mIU/L for low-risk patients, 0.1–0.5 mIU/L for the intermediate-risk patients, and <0.1 mIU/L for the high-risk patients [5]. In children with NED, TSH can be normalized to the low-normal range after an appropriate period of surveillance. Lifelong surveillance is indicated in all pediatric patients because recurrence has been reported in approximately 30% of children with DTC as long as 20–40 years after initial surgery [144].

Children with Persistent/Recurrent Cervical Disease

Cervical lymph nodes are the most common location for residual and recurrent PTC [73, 81, 144]. The decision to treat or to observe identifiable cervical disease should be individualized according to the size, anatomic location, prior treatment history, the iodine avidity of cervical disease, and the presence of distant metastasis. If macroscopic cervical disease (≥1 cm in size) is identified by imaging and confirmed via FNA, surgery is preferable to ^{131}I therapy [145, 146]. Children with iodine-avid small-volume cervical disease (<1 cm) can be observed while continuing TSH suppression or can be considered for therapeutic ^{131}I therapy depending on the individual risk-to-benefit ratio as well as the absence or presence of distant metastasis. [147]. US-guided percutaneous ethanol (UPEA) or radiofrequency ablation may be considered as nonsurgical treatment options in patients with a limited number of neck metastases from PTC depending on the location [147–149]. The therapeutic success rates of ethanol injection and RFA in adult studies have been reported to be 70.8–98% and 75–91.6%, respectively [148].

Children with Pulmonary Metastasis

The majority of children with pulmonary metastasis have micronodular disease demonstrating excellent iodine avidity. Thus, in contrast to adults, children and adolescents with pulmonary metastasis have low disease-specific mortality [78, 150]. However, while many pediatric patients achieve remission from pulmonary disease [73, 78, 150], at least 1/3 of children will have stable, persistent disease that will not respond to repeat doses of ^{131}I [78]. Re-treatment of ^{131}I iodine-avid pulmonary metastases should be considered in children who have demonstrated previous improvement but continue to have persistent dis-

ease based on a plateau in Tg and/or cross-sectional imaging or evidence of disease progression. The timing of additional ^{131}I should be at least 12 or more months from the previous treatment with several studies demonstrating a continuous decline in serum Tg levels for 18 to 24 months, or longer, following the previous RAI therapy [150, 151].

Children with Detectable Tg but Negative Neck US or Cross-Sectional Chest Imaging

In children and adolescents with a detectable or increasing Tg or TgAb, but negative neck US and cross-sectional imaging of the chest, a DxWBS should be performed to look for persistent RAI-avid disease. If disease is found, additional treatment with surgical resection and/or ^{131}I is indicated, based on extent, location, and previous treatment. If no disease is found, one can consider a single empiric dose of ^{131}I with a posttreatment WBS (RxWBS) to assess for RAI-avid disease. If the RxWBS is negative and the Tg or TgAb continues to increase, then ^{18}F-FDG-PET/CT should be considered to determine the location of disease; however, additional RAI treatment is not warranted [152–154].

New Approaches for Children with Progressive Disease

Most children with asymptomatic and nonprogressive ^{131}I refractory disease can be safely followed up while continuing TSH suppression. However, a very small proportion of children will have progressive disease refractory to ^{131}I therapy. In adults, ^{18}F-FDG-avid lesions are associated with poor prognosis [155]. The prognostic value of ^{18}F-FDG-PET/CT scan in children remains unclear.

For adults with ^{131}I refractory and progressive thyroid cancer, there are an increasing number of systemic therapies that target protein tyrosine kinase-dependent pathways (tyrosine kinase inhibitors, TKIs) [156]. For adults, where the mortality rate is higher than in children, the benefit of extending progression-free survival is significant [157, 158], and two TKIs, sorafenib and lenvatinib, have received FDA approval for use in adult patients with ^{131}I refractory, progressive thyroid cancer [159].

A phase 2 trial was recently conducted to investigate the efficacy and safety of sorafenib in pediatric patients and young adults with refractory solid tumors or leukemias [160], but, unfortunately no pediatric patients with thyroid cancer were recruited for participation. Thus, to date, the pediatric data are limited to case reports and anecdotal clinical experience [161, 162]. National clinical trials are needed to determine efficacy and toxicities in children with advanced thyroid cancer. In the rare and exceptional situation in which a child warrants systemic therapy, the use of systemic therapies should be carefully considered in consultation with experts in this area [5].

Additional agents are also being developed and studied in an effort to improve survival for adult patients with dedifferentiated disease. One example is selumetinib, a MEK1/MEK2 inhibitor, that has been shown to increase expression of the sodium-iodine symporter with an associated increase in RAI uptake [163]. Data on the incidence and association of oncogene mutations and rearrangements in relation to the clinical behavior of DTC variants in children and adolescents with RAI-refractory disease is needed in an effort to determine which systemic therapy may be most beneficial for this uncommon situation in pediatrics.

Medullary Thyroid Cancer

MTC is a rare neuroendocrine malignancy that derives from the neural crest originated parafollicular C-cells of the thyroid gland [164]. Thus, in contrast to follicular-cell-derived thyroid tumors, MTC cells are not responsive to TSH, do not express the sodium-iodine symporter, and do not produce Tg; rather they secrete calcitonin and carcinoembryonic antigen (CEA), both of which serve as tumor markers of MTC. In children, MTC is a monogenic disorder caused by a dominantly inherited or de novo gain-of-function

Table 4 Risk levels and management based on common *RET* mutations detected on genetic screening [164]

MTC risk level	*RET* mutation	Age for prophylactic thyroidectomy
Highest (MEN2B)	M918 T	TT in the first year or the first months of life. Risk of metastasis increases after 5 years of age
High (MEN2A)	A883F and C634F/G/R/S/W/Y	TT at or before 5 years of age based on serum calcitonin levels
Moderate (MEN2A)	G533C, C609F/G/R/S/Y C611F/G/S/Y/W, C618F/R/S, C620F/R/S, C630R/Y, D631Y, K666E, E768D, L790F, V804L, V804M, S891A, R912P	TT to be performed when the serum calcitonin levels become elevated or in childhood if the parents do not wish to embark on a lengthy period evaluation, which might last for years or decades

mutation in the RET proto-oncogene. With rare exception, MTC in children and adolescents is associated with multiple endocrine neoplasia type 2, either MEN2A or 2B, depending on the specific mutation [165, 166]. The development of MTC in children with MEN2A typically occurs after 5 years of age, especially for patients with ATA moderate-risk (M) mutations (Table 4) [164–167]. In contrast, in children with hereditary MEN2B, macroscopic MTC may be detectable within the first year of life, and nodal metastases may occur prior to 5 years of age [168]. Sporadic MTC associated with somatic mutations of *RET* and *RAS* are very uncommon in the pediatric population [164].

There is a strong genotype and phenotype correlation in the RET proto-oncogene allowing for prediction of the rapidity with which an individual may develop MTC as well as the risk and timing of the other MEN2-related diseases, including pheochromocytoma and hyperparathyroidism [164–167]. The ATA divides the most common *RET* mutations into three risk categories, highest risk, high risk, and moderate risk, and bases the recommended age for initial screening and the timing of prophylactic thyroidectomy to coincide with the goal of achieving surgical remission from disease (Table 4) [164]. If a germline *RET* mutation is detected on genetic screening, TT is recommended as follows: within the first year of life for carriers of the highest-risk mutation (codon 918), at or before age 5 years for those with a high-risk mutation (codons 634 and 883), and for all other moderate-risk mutations when the serum calcitonin level shows an increasing upward trend, when a nodule is found on surveillance thyroid US, or if the parents do not wish to continue to embark on a long period of laboratory and radiological surveillance (Table 4) [164].

For patients undergoing thyroidectomy, a central lymph node dissection is recommended in children whose basal calcitonin is >40 pg/mL or with any evidence of lymph node metastasis [164, 169]. After initial surgery, levothyroxine medication is given to normalize, not suppress, the TSH. In contrast to the follicular cell in nonmedullary DTC, parafollicular C-cells do not accumulate iodine. Thus, postoperative ^{131}I therapy is not indicated following thyroidectomy for MTC [170]. Calcitonin and CEA levels should be monitored as tumor markers every 6–12 months, with decreasing frequency once remission is confirmed. Neck US should be followed up for patients with persistently detectable tumor markers or initial lymph node metastasis. If the tumor markers remain significantly elevated or show rapid doubling time, additional imaging such as chest CT, contrast-enhanced liver MRI/CT, bone scan, MRI of the axial skeleton, or ^{18}F-FDG-PET/CT should be performed. In pediatric patients with MEN2B, remission may not be an achievable goal secondary to an increased incidence of de novo mutations associated with delay in diagnosis [27, 171].

Patients with hereditary MTC should receive continued and lifelong follow-up including genetic counseling, psychosocial support, and prospective screening of pheochromocytoma and primary hyperparathyroidism. Annual screening for pheochromocytoma with a urine or serum fractionated metanephrine panel is initiated at age 11 years for ATA highest-risk patients (918) and at 16 years for ATA high- and moderate-risk patients with the addition of annual screening for hyperparathyroidism for ATA high- and moderate-risk patients at the same time.

Metastatic MTC is generally incurable, but may show an indolent clinical course with stable disease over decades. A more aggressive progression and poorer prognosis can be predicted by the inability of the MTC cells to produce calcitonin, a rapidly rising CEA out of proportion to calcitonin, and a fast tumor marker doubling time [164, 172]. For MTC patients with symptomatic or progressive metastatic disease, the treatment of molecular targeted therapies that inhibit *RET* and other receptor tyrosine kinases involved in angiogenesis is indicated. Vandetanib and cabozantinib have been FDA-approved for the treatment of adults with progressive, metastatic MTC [156, 159], and limited data suggests that vandetanib is effective and well-tolerated in children with advanced MTC in the setting of MEN2B [173].

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Thyroid Cancer Surgery

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Well-Differentiated Thyroid Cancer

Well-differentiated thyroid cancer (WDTC) is the most common type of thyroid cancer and includes three histologic variants: papillary thyroid carcinoma (PTC), follicular thyroid carcinoma (FTC), and Hurthle cell carcinoma (HCC). Localized WDTC has an excellent prognosis, and surgical resection remains the foundation of treatment for most patients. Survival of metastatic WDTC is often measured in years, and systemic treatments (radioactive iodine, chemotherapy, kinase inhibitors) are often reserved for patients demonstrating significant progression or symptoms. The surgeon should be familiar with the pattern and propensity of the different subtypes to locoregional spread that influence the surgical procedure. PTC initially spreads to central and lateral neck nodes, whereas FTC will often spread hematogenously to the lung and bone [1]. HCC is now recognized as distinct from PTC and FTC and often spreads hematogenously but rarely may involve lymph nodes as well [2].

Dedifferentiated Thyroid Cancer

Poorly differentiated and anaplastic thyroid cancers (PDTC and ATC) have a high mortality rate as compared to WDTC. Disease-specific survival rates after resection of PDTC are approximately 60% at 5 years, while patients presenting with ATC have one of the shortest median survival of all human cancers at approximately 3 months [3, 4]. PDTC is defined histologically by tightly packed groups of cells that lack papillary or follicular architecture, have frequent mitoses, and demonstrate necrosis [5]. When PDTC is localized, surgery can effectively control disease. Primary and recurrent tumors can invade local structures in the neck and can require extended resections (trachea) and lymphadenectomy. Recurrences can occur quickly, and treatment options include radioactive iodine, tyrosine kinase inhibitors, targeted therapy, and surgery.

ATC classically presents as a rapidly expanding neck mass with metastatic disease. Biopsy demon-

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strates spindle or giant cells with frequent mitosis and at times can appear similar to sarcoma [6]. It is rare to come across a patient with ATC that is resectable and is without distant metastases. More commonly, ATC presents with locally advanced disease involving neck structures including the carotid and trachea. ATC is extremely rare with an incidence of 1–2/1,000,000 per year in the USA [7]. Several centers have developed significant experience with the disease, and every effort should be made to enroll patients in clinic trials. Multidisciplinary care is key and the surgeon can play an important role in palliation of symptoms particularly as it relates to timing of tracheostomy and feeding tubes in the setting of chemotherapy, radiation, and progressive tumor.

Medullary Thyroid Cancer

Medullary thyroid cancer (MTC) originates from calcitonin secreting cells (c-cells) of the thyroid. MTC can be sporadic in 75% of cases, but the rest of the cases are familial and can be grouped into multiple endocrine neoplasia types IIA and IIB. These MEN syndrome subtypes are defined by their mutation in the RET oncogene. Families aware of their genetic status are recommended to have prophylactic thyroidectomy based on their RET mutation status [8]. Localized MTC has a survival rate of >80% at 5 years. Calcitonin and CEA measurements can help detect persistent/recurrent or metastatic disease [9]. Calcitonin doubling time is a well-established prognostic factor [10]. Surgery for localized MTC includes a total thyroidectomy, a central neck dissection, and typically an ipsilateral lateral neck. Symptomatic or progressive metastatic disease can be treated with tyrosine kinase inhibitors.

Papillary Microcarcinomas (PMC)

The frequent use of neck ultrasound has led to a dramatic increase in the diagnosis of thyroid cancer in the USA and many westernized countries [11]. This is attributed to increased incidence of papillary thyroid cancer as the incidence of other types of thyroid cancer has been found to be stable. On a closer look, it was realized that the increase was mainly due to increased detection of small papillary thyroid cancers that were <2 cm, 49% of which were PMC (PTC < 1 cm). While the thyroid cancer incidence has increased 2.9-fold, the mortality has remained stable over the years [12]. Active surveillance program for PMC patients has demonstrated feasibility of this approach in Japanese patients [13]. Several centers in the USA have introduced clinical protocols to offer observation with careful monitoring of tumors as a management option for PMC; the decision to operate is based on evidence of tumor growth on serial ultrasounds. As this approach gains popularity, it is critical that surgeons play a role in decision-making [14]. Eligibility for active surveillance approach should be stringently defined; factors such as tumor proximity to adjacent structures on imaging studies should be considered. Further, the relative risks of surgery versus observation should be explained, and informed consent should be documented. Since PTCs tend to spread to local lymph nodes before distant sites, observation with careful serial imaging assessment should be implemented. It should be noted that there are patients with PTCs <1 cm that display aggressiveness and have distant metastases. For this reason, surveillance program should be carefully structured with multidisciplinary group of physicians having expertise in thyroid cancer.

Preoperative Assessment: Exam, Imaging, and Laryngoscopy

Prior to proceeding to surgery, a thorough preoperative assessment should be performed including history and physical exam concentrating on the head and neck, appropriate imaging, and evaluation of the larynx. Disease extent should be carefully evaluated.

Preoperative laryngeal exam is increasingly recognized as essential procedure prior to thyroid surgery especially for patients with thyroid cancer and has been the subject of recent American Academy of Otolaryngology published guidelines [15]. Preoperative laryngoscopy is important as it can identify vocal cord dysfunction in the absence of voice complaints/changes and also can provide

a baseline for comparing postoperative laryngeal outcome. It can offer essential information about functionality of the RLN, so a surgeon is better equipped to address a RLN that is found to be invaded by the tumor intraoperatively. Examination of the vocal cords can be performed by mirror exam, laryngoscopy, or more recently with ultrasound [16, 17]. Laryngoscopy provides excellent visualization of the glottis and is well tolerated in the clinic with the use of topical anesthetics.

Imaging studies include high-resolution ultrasound and a CT scan with contrast; sometimes MRI can be employed as well. If a CT scan is performed, RAI is given in a delayed fashion. Ultrasound is excellent for evaluating lymph node involvement, particularly small nodes that may have abnormal morphology but not enlarged by CT scan criteria. Additionally, US can identify small contralateral thyroid nodules if relevant to the extent of thyroidectomy. However, US has limited ability to assess for tracheal invasion and cannot assess structures posterior to the trachea due to acoustic shadowing or in the extreme neck base. US and CT have complementary strengths and combined preoperative US and CT provides a complete preoperative macroscopic nodal metastasis map to design rational nodal surgery in primary as well as recurrent setting [18]. MRI of the neck can also be used to evaluate thyroid tumors. However, its use is primarily for patients with very large tumors for assessment of soft tissue invasion and is often complimentary to other imaging tests. FDG-PET scans are not routinely used for preoperative evaluation of WDTC patients. However, for patients with aggressive thyroid cancer (PDTC/ATC) in long-term follow-up, there is a reciprocal relationship between PET positivity and iodine uptake, and so FDG PET is an excellent test to determine the extent of disease and is an excellent baseline-imaging test prior to deciding on a therapeutic plan [19].

Extent of Thyroidectomy

The extent of surgery (lobectomy versus total thyroidectomy) has been a long-standing controversy in the field of thyroid cancer, particularly for smaller primaries. Proponents of total thyroidectomy for all patients point to the relatively high incidence of unknown contralateral thyroid cancers, the need for long-term imaging surveillance of the contralateral gland, the increased difficulty of reoperation in the central compartment, the ability to give RAI, and controversial large database studies that suggest a survival benefit of total thyroidectomy over lobectomy [20]. Additionally, total thyroidectomy facilitates detection of recurrence with the thyroglobulin (Tg) levels; Tg elevation and rate of increases can be used to time and plan a therapeutic approach. Advocates of lobectomy cite increased rate of complications with total thyroidectomy (hypoparathyroidism, RLN injury) and excellent disease-specific outcomes for WDTC [21]. The most recent American Thyroid Association (ATA) guideline for thyroid cancer has attempted to increase the threshold for RAI use as an adjuvant after resection, suggesting that it should be employed only in cases with significant risk of recurrence, typically when there is a large tumor (>4 cm), invasive histological features, or bulky lymph node metastases [22]. As such these guidelines suggest that lobectomy is reasonable for non-metastatic intrathyroid tumors given the state of the current evidence. The goals of these recommendations are to prevent medical and surgical overtreatment for small thyroid cancers that are unlikely to become life threatening. Risk of RLN injury and permanent hypoparathyroidism is infrequent in experienced hands but can be considerably debilitating for affected patients [23]. The goal of treatment for small, intrathyroidal cancers (<4 cm) is to avoid making the treatment worse than the disease.

Thyroidectomy for Thyroid Cancer

Once the patient has been assessed preoperatively (as described above), the surgical procedure has been planned, and informed consent obtained, the patient is brought to the operating room. After induction, the patient is positioned on the operating room table with neck extension. This can be facilitated by placing a shoulder roll at the level of the scapulae and placing the patient in a "beach chair" position (Fig. 1). Prior neck surgery or

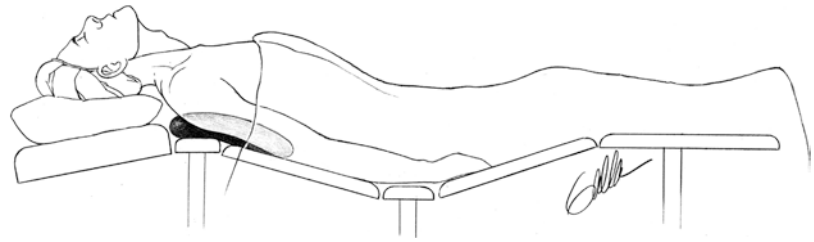

Fig. 1 "Beach chair" position for thyroidectomy

injury should be noted and range of motion assessed in order to prevent overextension and injury. Sequential compression devices are placed on the calves, and a warming blanket covers the patients to maintain temperature. Venous thrombosis rates are low in thyroid surgery patients, and preoperative prophylaxis is only given to high-risk patients [24]. Many surgeons selectively use preoperative antibiotics usually in reoperative cases, in patients with a history of neck radiation or with medical conditions placing them at high risk of infection (diabetes). The patient is prepped and draped from the chin to suprasternal notch with appropriate lateral neck exposure.

Incision and Initial Dissection of the Strap Muscles

Incisions for total thyroidectomy are generally 4–5 cm in length and typically 1–2 cm below the cricoid in the midline. However, the patient body habitus and the intended operation are variables that may require a different incision length. Optimally these incisions can be hidden well in one of creases of the neck. Symmetry can be assessed by finding the midline of the neck by examining the relationship of the suprasternal notch to the chin. Equidistant measurements from the lateral ends of the incision to the suprasternal notch can also help determine a symmetrical incision. After incision is made, the platysma is identified and divided. The platysma can be attenuated in the midline and attention should be paid to avoid injury to the anterior jugular veins. Subplatysmal flaps are created with cautery directly under the muscle. Countertraction on the skin helps to identify this plane when the incision edges are retracted superiorly with skin hooks. After adequate flap creation, the midline strap muscle fascia is identified and dissected. The relevant strap muscles of the neck during thyroidectomy include the most ventral muscle, the sternohyoid, and the muscle that directly envelops the thyroid, the sternothyroid. The plane in between these two muscles can be dissected to reveal the carotid sheath and jugular vein. Retractors can facilitate exposure of the thyroid lobe and the sternothyroid muscle carefully evaluated. The thyroid is palpated through the muscle; any inflammatory changes or extrathyroidal extension is looked for. In case of extrathyroidal extension, the sternothyroid muscle can be divided to ensure an appropriate margin (Fig. 2). Some surgeons prefer to divide the sternothyroid routinely to facilitate exposure of the superior pole vessels and

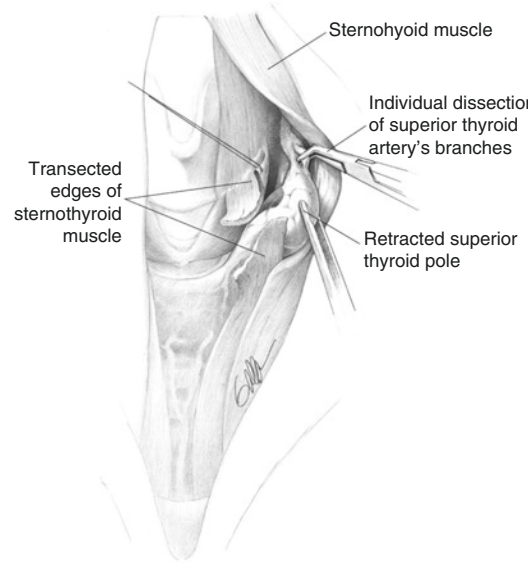

Fig. 2 Front view depicting left thyroid superior pole dissection with adequate exposure of the superior pole region to facilitate dissection of the branches of superior thyroid artery

identification of the superior laryngeal nerve; division of the muscle does not result in any functional deficit [25]. Prior to muscle division, the carotid sheath should be identified and the muscle dissected away in order to prevent injury. If the muscle is not divided, it is reflected off the thyroid.

Thyroid Dissection

The dissection of the thyroid lobe is dictated by the blood supply, the desire to protect the parathyroid glands and laryngeal nerves (both recurrent and superior), and the tumor characteristics.

The thyroid is retracted medially with a peanut or finger with gauze, and the middle thyroid vein is identified and divided (Fig. 3). In general blood vessels of the thyroid can be isolated with ties, a vessel sealing device, or clips. Bleeding in and around the tracheoesophageal (TE) groove should be avoided, as thyroid surgeons must rely on anatomic landmarks and characteristic colors of parathyroid glands, normal thyroid tissue, lymph nodes, and nerves that can be altered with bleeding. Division of middle thyroid vein facilitates the exposure of the TE groove. At this point the trachea can be identified in the midline by division of the inferior pole vessels. The rings of the trachea and its characteristic white color are useful landmarks to assess the location of the TE groove. Additionally, the isthmus can be divided at this point to facilitate mobilization of the thyroid lobe. A clamp can be passed behind the thyroid along the trachea and then the thyroid divided with a vessel sealing device as long as the area is free of tumor. With the inferior pole vessels divided attention is turned to identifying the inferior parathyroid gland and maintenance of its blood supply. This structure is swept inferiorly as the dissection is carried out cranially in the TE groove. RLN has a characteristic vascularized epineurium. If nerve monitoring is used, the nerve can be stimulated to confirm its function. Dissection along the nerve should be minimal at this inferior aspect so as not to inadvertently interrupt blood supply to the inferior parathyroid gland. The RLN courses behind the subclavian artery on the right and behind the aorta on the left, which affects the angle of entry into the glottis (Fig. 4). On the right the nerve passes at a more oblique angle to the trachea given its more lateral origin and the right RLN courses parallel to the trachea through the TE groove. The RLN should be safely identified and preserved during the TE groove dissection, and careful manipulation is well tolerated. As dissection progresses cranially in the TE groove, the inferior thyroid artery, a branch of the thyrocervical trunk, is identified and preserved. The RLN can be often found deep to the branching vessels of the artery, but this relationship can be variable. Dissection and ligation of the branching vessels distally at the capsule of the thyroid should be performed. As the dissection continues, the thyroid is gradually retracted away from the TE groove and toward the midline. A variety of techniques are

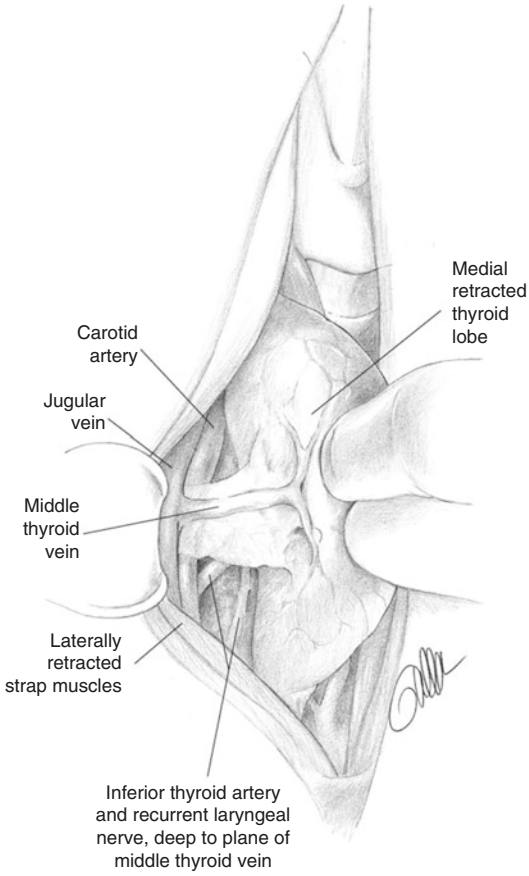

Fig. 3 Medial retraction of the thyroid gland with a surgeon's hand to identify and divide the middle thyroid

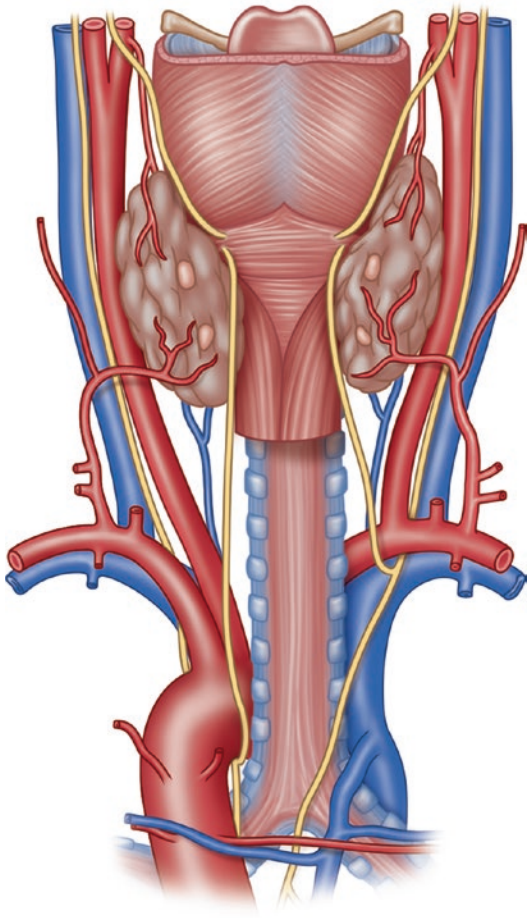

Fig. 4 Normal pathway of the left (L1) and right RLN (R1) through respective paratracheal regions

employed to retract from babcock clamps to small hemostats or finger retraction with gauze. If clamp retraction is carried out, retraction has to be gentle in order to avoid tearing of the thyroid capsule, and the clamp should be placed on benign thyroid tissue to avoid interruption of the tumor capsule.

The next step is to identify the superior parathyroid gland. This gland is typically located deep to the RLN. The parathyroid glands can be distinguished from lymph nodes and thyroid tissue in this location by their distinct capsule and mobility in the fibro fatty tissue of the TE groove. The glands are typically flatter and are more tan/brown in color than lymph nodes. The dissection should be meticulous, and every effort is made to preserve blood supply. One aim of the caudal to cranial dissection approach is to identify the superior parathyroid gland prior to superior pole vessel ligation. As the blood supply to the parathyroid glands is appreciated, the superior pole vessels can be taken more deliberately, avoiding devascularization of the parathyroid.

The ligament of berry is an area of dense fibrous tissue extending from the thyroid capsule to the trachea. It is often in close proximity to the RLN or the RLN travels through its fibers. Due to potential of nerve injury in this location, vessels and tissue should be divided with bipolar cautery or divided using ties. If bleeding occurs around the RLN, cautery and excessive manipulation should be avoided. Often, simple digital pressure and time can control the bleeding. Hemostatic agents are another good alternative to further manipulation.

The superior pole vessels are divided as the last maneuver. The vessels can be separated from surrounding tissue with blunt dissection, which is recommended in order to avoid injury external branch of superior laryngeal nerve. This nerve travels in the fascia along the cricothyroid muscle as a branch of the vagus nerve. Injury to this nerve results in the inability to project the voice. As such, the superior pole vessels should be divided with a vessel sealer or ties directly at their point of entry into the thyroid. This also avoids interrupting any vessel that may provide blood supply to the superior parathyroid. With the entire thyroid lobe mobilized and retracted medially, the rest of the attachments on the trachea are divided with cautery, avoiding injury to the trachea and cricothyroid muscle. Attention is then turned to the contralateral side if a total thyroidectomy is being performed and the steps repeated. At the completion of the case and if no central neck dissection is performed, the TE groove and the pretracheal lymphoid tissue are visually inspected and palpated to determine lymph node involvement. If none is detected, the parathyroid glands are visualized one last time to assess viability, and the RLNs are inspected along the course and tested if RLN monitoring is being used. It is some surgeons' practice to mark the parathyroid glands with suture or clips in case of reoperation.

If a parathyroid gland is thought to be devascularized after careful inspection, it can be explanted and reimplanted in the sternocleidomastoid (SCM) after pathological confirmation. While awaiting frozen section, the parathyroid is maintained in cold saline. A bloodless muscle pocket within the SCM is created with gentle clamp spreading. After histologic confirmation, the parathyroid is divided with a scalpel into 2–3 mm pieces and inserted into 1–3 muscle pockets followed by blue permanent suture closure of the SCM fascia. The central compartment is inspected carefully for hemostasis, and any remnant bleeding is dealt with appropriately. The strap muscle fascia is closed in the midline followed by platysma reapproximation and dermal closure. Drains are not mandatory but are used by some surgeons. The skin closure can be performed in a variety of different ways, but the ultimate goal is to limit the inflammatory reaction that can result in more pronounced scarring. Placement of monofilament suture with a topical wound glue or dressing typically suffices.

Central Neck Dissection

Central neck dissection (CND) refers to the removal of lymph nodes in the central compartment (level VI). Level VI lymph nodes extend from the hyoid bone superiorly to the carotid sheaths to the innominate artery. Caudal to the artery but accessible through a cervical incision are level VII lymph nodes. Therapeutic lymph node dissection refers to the removal of lymph nodes that are clinically evident as oppose to prophylactic dissection, which is to remove lymph nodes that are not obviously involved with tumor. For those surgeons that perform prophylactic lymph node dissection for WDTC, they cite reduced recurrence rates, improved staging, and avoidance of reoperative morbidity as considerations [26, 27]. However, there are clear disadvantages such as greater risks of complications most notably increased rates of hypoparathyroidism and RLN injury [28, 29].

CND can be performed unilaterally or bilaterally depending on the clinical presentation and type of thyroid cancer. For prophylactic CND in WDTC, randomized data to guide decision-making is lacking and clinical trials are unlikely to be forthcoming given the power required demonstrate a benefit [30]. The retrospective data that describes outcomes after CND is challenging to interpret given the various definitions and stations of lymph nodes within level IV [29]. Central neck lymph nodes can be divided into right and left paratracheal, prelaryngeal, and pretracheal. As the literature is difficult to interpret, there are a variety of practice patterns in regard to prophylactic CND. Some authors have suggested prophylactic CND when larger primary tumors are present (T3/T4), while others perform the procedure routinely. Yet others argue that it should not be performed as increased detection of micrometastatic disease is unlikely to become clinically relevant which may result in overstaging and treatment with radioactive iodine [31]. It should be noted that prophylactic lateral neck dissection was performed routinely for WDTC but is no longer employed despite up to 50% of patients having micrometastatic lymph nodes. To further the argument against routine prophylactic CND, several high volume centers have suggested that reoperative central neck dissection can be performed with minimal morbidity [32].

CND Technique

There are four lymph node compartments that are addressed during CND. Dissection of prelaryngeal nodes (typically performed at the time of thyroidectomy) is typically followed by pretracheal and paratracheal dissections. Although the order of dissection can be variable from surgeon to surgeon, in general the CND is performed after removal of the thyroid specimen. The medial borders of the carotid sheaths are dissected down to the level of innominate artery bilaterally. The lymphoid tissue between the carotid arteries is dissected, but only after inferiorly tracing and protecting both the RLNs. Parathyroid gland blood supply should also be identified and preserved. The innominate should be noted on preoperative imaging studies in order to avoid injury

(particularly if coursing high in the mediastinum). The lymphoid tissue is removed en bloc and divided with a vessel sealing device or cautery. Finally, the central compartment is evaluated for any additional lymph nodes in the surgical bed, thorough visual inspection and palpation. Drains are not typically used.

Lateral Neck Dissection

For patients with clinically involved lymph nodes of the lateral neck (levels II, III, IV, V), lymph node dissection is carried out to remove gross macroscopic WDTC which is not effectively treated with RAI, and surgery represents the best opportunity for locoregional control. For patients with MTC, neck dissection is recommended for gross disease and in cases of high calcitonin levels. Dissection of levels II through V is referred to as a modified radical neck dissection (MRND) or selective neck dissection (SND – II, III, IV) as opposed to the radical neck dissection that includes removal of the SCM, the internal jugular vein, and the accessary nerve. Removing only involved nodes (berry picking) should be avoided and has been associated with persistent disease and higher recurrence rates.

Lateral Neck Dissection Technique

A cervical crease incision extending from the thyroidectomy incision laterally but keeping it low is adequate for an SND. In most cases a hockey stick incision that extends parallel with the SCM can be avoided. The subplatysmal flaps are elevated to the clavicle, the mandible, and the trapezius posteriorly as needed for the extent of dissection. Removal of lymphoid tissue begins at level II with identification of the fascial vein and the posterior belly of the digastric muscle. The SCM fascia is entered and the accessory nerve identified. Injury to this nerve results in shoulder weakness which can result in permanent disability. Levels IIA and IIB lymph nodes are dissected with the specimen being passed posterior to the accessory nerve. The SCM is dissected away from the specimen with identification and preservation of the cervical roots. The lymph nodes are then removed in continuation down to level IV; dissection ends in proximity to the transverse cervical artery and branches. The phrenic nerve lies on the floor of the dissection bed and is avoided. To dissect level V, the accessory nerve is dissected as it enters the trapezius muscle. Lymphoid tissue is removed from superiorly/laterally, and the dissection proceeds toward the SCM/clavicle avoiding the brachial plexus.

Lateral Neck Dissection Complications

Chyle leak (fistula) on the left and injury to various nerves are the primary concerns during lateral neck dissection. A chyle leak occurs when the thoracic duct is not completely tied off. During dissection of the left lower neck, surgeons will often use ties instead of cautery or a vessel-sealing device in an attempt to avoid this complication. If a leak is observed during the case, administration of thick cream via NGT can help localize the leak. If it occurs postoperatively, the patient can either undergo conservative treatment with octreotide, medium chain triglyceride diet, and a pressure dressing or can be taken for re-exploration. Nerves at risk during a lateral neck dissection include the hypoglossal nerve, the marginal mandibular nerve, the spinal accessory nerve, the phrenic nerve, the vagus nerve, and the cervical sympathetic chain in the carotid sheath. Injury to these nerves can be temporary or permanent.

Recurrent Laryngeal Nerve Monitoring and Injury

Intraoperative neuromonitoring (IONM) is an adjunct to visualization of the nerve. IONM is used variably by thyroid surgeons. IONM shows a trend toward lower RLN injury rates, but there is lack of statistical proof in part due to inadequate statistical powering of most studies. IONM facilitates mapping the RLN course, assessment of nerve func-

tionality, and identification of an injured nerve segment [33–35]. Adherence to the international nerve monitoring study group guidelines is key to accurate and uniform neural monitoring [33]. The ATA 2015 Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer state that intraoperative neural stimulation (with or without monitoring) may be used to facilitate nerve identification and confirmation of neural function [37]. Although proving a benefit to RLN monitoring based on retrospective data is difficult, experience dictates that in certain instances it can assist the surgeon with intraoperative decision-making. RLN anatomy can be highly variable in location, size, and branching patterns. While most patients have a typical trajectory, the presence of a mass can result in the nerve resting in an abnormal location being pushed either ventrally/laterally on the left or medially/ventrally on the right. In addition, abnormal embryology can lead to a nonrecurrent laryngeal nerve which is rare on the right (1%) and exceedingly infrequent on the left (0.04%) [36]. An anatomical and electrophysiologic algorithm based on IONM can help identify NRLN prior to proceeding with the dissection in the related area [37] (Fig. 5). RLN may be invaded or abutted by tumor creating challenging intraoperative circumstances. AAOHNS guidelines suggest significant utility of IONM in cases of (1) bilateral thyroid surgery, (2) revision thyroid surgery, and (3) surgery in the setting of an existing RLN paralysis [15]. IONM can serve to aid in dissection in difficult cases but can also help prognosticate a nerve injury. If a nerve injury is suspected on one side and confirmed with the RLN monitor, the contralateral side surgery can be delayed as needed to allow for recovery and preventing the need for tracheostomy.

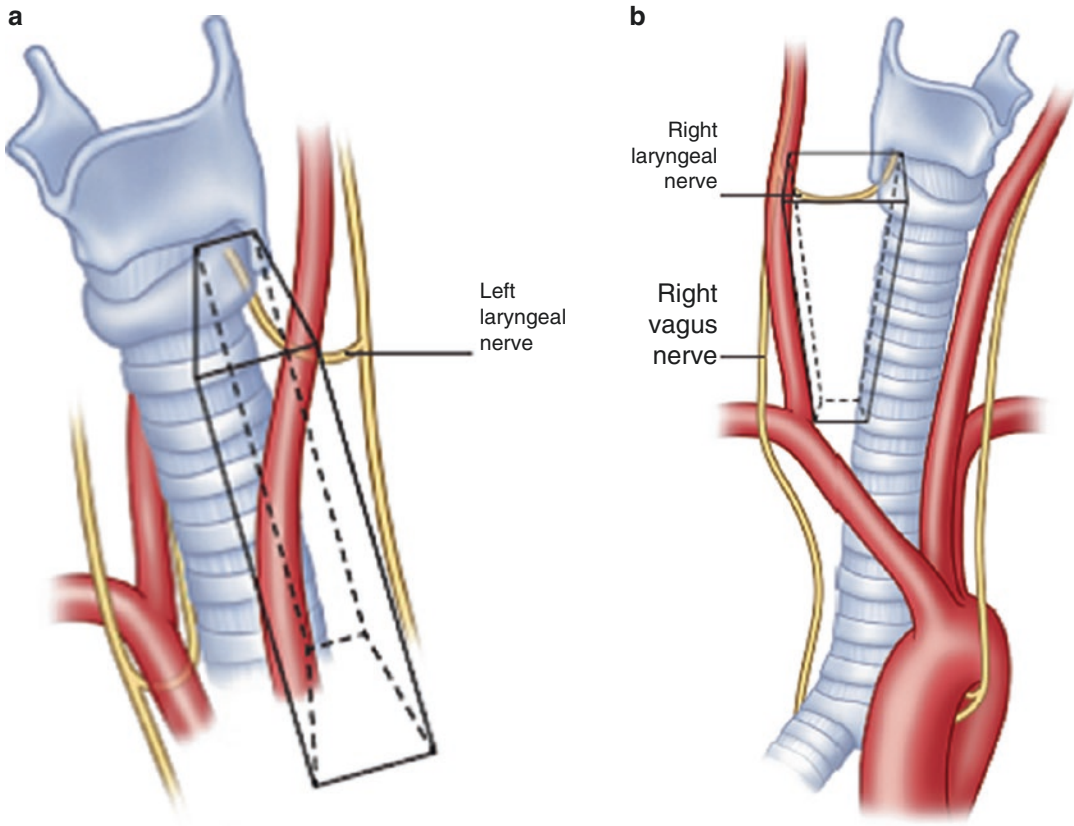

Fig. 5 (**a**) Left nonrecurrent laryngeal nerve (NRLN). (**b**) Right nonrecurrent laryngeal nerve (NRLN)

RLN injuries can be the result of thermal injury, traction, or transection. Monopolar cautery should be avoided near the RLN and ligament of berry dissection and bipolar cautery should be used judicially. Suture ties and clips can be used to prevent thermal injury, but these can also result in nerve injury if not used carefully. Traction injury can occur as the gland is retracted medially and the RLN is pulled near the ligament of berry near its muscular insertion site as it travels to the glottis. A tumor in the proximity to the RLN can also entrap/abut the nerve, and distraction of the gland during dissection can result in injury. In general, if a tumor abuts or pushes against a nerve, the nerve does not need to be sacrificed, if all gross disease can be removed. However, if the tumor completely surrounds the nerve and/or gross disease is left behind, then the nerve should be resected. The surgeon should assess the TE groove carefully for these variables and proceed with an approach that limits RLN injury risk. If a transection injury occurs and is recognized immediately, primary anastomosis may improve long-term glottic function, although a complete recovery is variable [38]. If the RLN cannot be repaired, then an anastomosis with the ansa hypoglossi nerve can provide a resting tone that may also improve function over time, although normal function should not be expected.

Complete recovery of normal RLN is possible when the RLN remains intact but loses signal during surgery. A majority of patients will recover in 6 months. Swallowing and voice assessment and therapy can help clarify the severity of neurologic deficit and provide the patient with strategies as they adjust post-surgery. Once recovery is thought to be unlikely (post 6 months), intervention aimed at improving glottic tone and function can be attempted. These include surgical manipulation of the larynx and injection laryngoplasty.

External Branch of Superior Laryngeal Nerve (EBSLN) Monitoring and Injury

EBSLN injury can be of great significance to patients, particularly to voice professionals. Lack of distinct laryngoscopy findings and variable and subtle voice changes associated with EBSLN injury are underlying reasons for difficulty in identification of EBSLN injury, and hence it is alleged to be the most commonly underestimated complication of thyroid surgery. The laryngeal head of the sternothyroid muscle is an important landmark for the EBSLN as it descends along the inferior constrictor to the cricothyroid muscle. A standardized approach for IONM can be a useful adjunct for intraoperative EBSLN identification, as up to 20% of EBSLN are subfacial and visually unidentifiable [39, 40]. The EBSLN is at a high risk of injury during dissection of the superior thyroid pole. Knowledge of EBSLN anatomic variations, a precise surgical technique with meticulous superior pole dissection, and ligation of the superior pole vessels on the gland are important in avoiding the injury [41, 42].

Surgery for Recurrent Thyroid Cancer

Recurrent thyroid cancer can be in the form of structural and/or biochemical recurrence. A surgical approach requires presence of a structural recurrence; it is vital to clearly identify a structural disease on imaging studies, typically on ultrasound, axial CT, and MRI scan with contrast [41]. Factors that are considered for surgical intervention include primary tumor histology, the presence of extranodal extension, disease progression, presence of distant metastases, and comorbidities such as vocal cord paralysis from previous surgery. Surgery for recurrent thyroid cancer is typically associated with higher rate of complications, as it presents technical challenges due to altered anatomy, scarring, as well as altered natural history. Consequently surgery for recurrent thyroid cancer entails certain expertise. Use of IONM in reoperative and complex thyroid surgeries can help reduce associated rate of vocal cord paralysis [43, 44]. Meticulously performed revision thyroid surgeries can have good oncological and surgical outcomes [45, 46].

A recent study of 181 revision surgeries performed in a tertiary center reported occurrence of temporary and permanent hypocalcemia in 9% and 4.2% of the patients, respectively, and rate of

cervical node recurrence in 5% of patients (3.4 years of median follow-up). No permanent or temporary vocal cord palsy was reported; biochemical complete remission was achieved in 58% of all revision cases [46].

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Staging and Prognosis of Thyroid Cancer

Joanna Klubo-Gwiezdzinska

The incidence of thyroid cancer is rising faster than any other type of cancer and has increased by 5.1% per year from 2003 to 2012 in the USA and tripled over the past 20 years worldwide [1, 2]. The increased detection of thyroid "incidentalomas" due to utilization of sensitive imaging techniques contributes significantly to the observed trend. Therefore, the identification of high-risk thyroid cancer patients among a large number of patients with indolent thyroid cancer is particularly important [1]. Despite the fact that differentiated thyroid cancer is associated with overall excellent prognosis with 5, 10, and 15 years relative survival rate of 98%, 97%, and 95%, respectively, there is a subgroup of high-risk patients characterized by 5 years survival rate of 45% and even larger subgroup of patients at risk of morbidities associated with thyroid cancer recurrence [3, 4]. Therefore, the optimal management plan requires accurate risk stratification both at diagnosis and throughout the disease course. Appropriate risk stratification guides the distinction between the candidates for conservative approach for patients with excellent prognosis and for aggressive therapy for individuals with increased risk of dying from thyroid cancer. Moreover, a unified staging system enables appropriate professional communication between clinicians.

There are several staging systems utilizing various prognostic factors such as age, tumor size, presence of lymph node and distant metastases, gender, extrathyroid extension, multifocality, completeness of the surgical resection, presence of vascular and capsular invasion, histology subtype and grade, and DNA ploidy summarized in Table 1.

Of note, existence of numerous prognostic scoring systems suggests that the optimal and universally accepted one is still to be developed. One of the pitfalls of practical applications of prognostic scoring systems is the fact that they often require lengthy calculations that make individualized risk prediction quite difficult in a busy clinical practice. The stratification methods utilized by different prognostic systems are summarized in Table 2. Some of the staging systems were developed and validated to evaluate the predictors of overall survival (OS) and disease-specific survival (DSS), while the other focus on the risk of thyroid cancer recurrence and disease-free survival (Table 2).

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Table 1 Summary of variables utilized in different prognostic systems

Staging systems	T size	LN met	Distant met	Age	Sex	Extrathyroid extension	Surgical margins	Vascular/capsular invasion	Response to therapy	Grade	Histology	DNA ploidy	Multifocality
AJCC-TNM seventh edition/ATA [7]	+	+	+	+		+							
ATA 2015 [7]	+	+	+	+		+		+	+		+		
MACIS [52]	+		+	+		+	+	+					
AGES [17]	+		+	+		+				+			
AMES [8]	+		+	+				+					
DAMES [18]	+		+	+		+		+		+		+	
MSK (GAMES) [19]	+		+	+		+				+	+		
EORTC [9]	+		+		+			+			+		
Murcia [86]	+			+		+					+		
Ohio State [20]	+		+			+							+
NTCTCS [21]	+	+	+	+		+		+			+		+
UAB-MDACC [22]	+		+	+									
Manitoba Nomogram [23]	+	+	+	+	+	+	+				+		
Clinical Class [25]		+	+			+							
Munster [10]	+		+			+		+					
Noguchi [26]		+		+	+								
Yildrim [27]	+		+	+				+					
SAG [28]				+	+			+		+			+
Cancer Institute Hospital in Tokyo [29]	+	+	+	+		+							

AJCC/TNM American Joint Committee on Cancer/tumor nodes metastases, *ATA* American Thyroid Association, *MACIS* metastasis, age, completeness of resection, invasion, and tumor size, *AGES* age, grade, extension, size, *AMES* age, metastasis, extrathyroidal extension, and size, *DAMES* DNA ploidy, age, metastasis, extrathyroidal extension, and size, *MSK* Memorial Sloan Kettering (*GAMES* grade, age, metastases, extension, size), *EORTC* European Organization for Research and Treatment of Cancer, *NTCTCS* National Thyroid Cancer Treatment Cooperative Study, *UAB-MDACC* University of Alabama (Birmingham) and M.D. Anderson Cancer Center, *SAG* sex, age, grade, *T* tumor, *LN met* lymph node metastases

Table 2 The methods utilized to estimate prognosis in thyroid cancer patients

Prognostic system	Calculation risk groups	OS	DSS	Risk of recurrence
AJCC-TNM seventh edition [7]	**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 extrathyroid extension			
	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; **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			
	Age<45 years old **Stage I** Any T Any N M0; **Stage II** Any T Any N M1			
	Age≥45 years old			
	Stage I T1a N0M0; T1b N0M0; **Stage II** T2N0M0			
	Stage III T1a N1a M0; T1b N1a M0; T2 N1a M0; T3N0M0; T3 N1a M0			
	Stage Iva T1a N1b M0; T1b N1b M0; T2 N1b M0; T3 N1b M0; T4a N0M0; T4a N1a M0; T4a N1b M0			
	Stage Ivb T4b Any N M0; **Stage Ivc** Any T Any N M1			
ATA [7]	*TNM* Histology, response to treatment, *ER* excellent response, *SIR* structural incomplete response, *BIR* biochemical incomplete response, *IR* indeterminate response			+
	Three groups			
	Low risk—T1, T2, N0 or ≤ 5 pathologic N1 micrometastases (<0.2 cm in largest dimension), M0; no aggressive cytology, no metastatic foci outside the thyroid bed; intrathyroidal FTC with <4 foci of capsular invasion, micro-PTC including the ones with BRAF mutation, ER to treatment			
	Intermediate risk—T2 with BRAF mutation, T3, N1— >5 pathologic N1 with all involved lymph nodes < 3 cm; M0; RAI avid metastatic foci in the; aggressive histology PTC with vascular invasion; IR or BIR—response to treatment			
	High risk T4, N1—with any metastatic lymph node ≥3 cm; M1; FTC >4 foci of vascular invasion; SIR—response to treatment			
MACIS [52]	Total score = 3.1 (if aged ≤39 years), or 0.08 × age (if aged ≥40 years), + 0.3 × tumor size in cm + 1 (if not completely resected) + 1 (if locally invasive) + 3 (if distant metastases)		+	
	Four risk groups:			
	Group 1 <6.0; **Group 2**—6.0–6.99; **Group 3**—7.0–7.99; **Group 4** >8.0			
AGES [17]	Total score = 0.05 × age in years (if aged ≥40) or + 0 (if aged <40) + 1 if tumor grade 2 or + 3 if tumor grade 3 or 4 + 1 if extrathyroidal invasion + 3 if distant spread + 0.2 × tumor size (maximum diameter in cm)		+	
	Four risk groups			
	Group 1—score <4.00; **Group 2—**score 4.01–4.99; **Group 3—**score 5.00–5.99; **Group 4—**score ≥6			

(continued)

Table 2 (continued)

Prognostic system	Calculation risk groups	OS	DSS	Risk of recurrence
AMES [8]	Two groups	+		
	Low risk—all younger patients without distant metastases (men <41 years, women <51 years) (b) all older patients with intrathyroidal papillary cancer or minor tumor capsular involvement follicular carcinoma and tumor size <5 cm, and no distant metastases			
	High risk—all patients with distant metastases; all older patients with major capsular involvement papillary cancer or major capsular involvement follicular carcinoma and tumor size ≥5 cm			
DAMES [18]	**Three risk groups**		+	+
	Low risk—patients in AMES low-risk group with euploid* tumors			
	Intermediate risk—patients in AMES high-risk group with euploid* tumors			
	High risk—patients in AMES high-risk group with 14 neuploidy* tumors			
MSK (GAMES) [19]	**Three risk groups**		+	
	Low risk—age <45, no distant metastases, tumor size <4 cm, and PTC on histology			
	Intermediate risk—age <45, with distant metastases, tumor size ≥4 cm or FTC on histology; age ≥45, with no distant metastases, tumor <4 cm, and PTC on histology			
	High risk—age ≥45, with distant metastases, tumor size ≥4 cm, or FTC on histology			
EORTC [9]	Total score = patient's age + 12 if male + 10 if poorly differentiated FTC + 10 if invaded the thyroid capsule + 15 if one distant metastasis + 30 if 2 or more distant metastases	+		
	Five risk groups			
	Group 1—score <50; **Group 2—**score 50–65; **Group 3—**score 66–83, **Group 4—**score 84–108, **Group 5—**score ≥108			
Murcia [86]	Prognostic index = (3 × age score) + (2 × size score) + (6 × spread score) + (2 × histologic variant score)			+
	Age score—1 if aged <50, 2 if aged ≥50			
	Size score—1 if tumor size from 1 to 4 cm, 2 if tumor size ≥4 cm			
	Spread score—1 if intrathyroidal, 2 if extrathyroidal			
	Histologic variant score—1 if well-differentiated, follicular variant or diffuse sclerosis variant PTC, 2 if solid or tall cell variant PTC, 3 if poorly differentiated PTC			
	Three risk groups			
	Low risk—index <18; **medium risk**—index 18–22; **high risk**—index ≥22			
Ohio State [20]	**Four stages**		+	+
	Stage 1—tumor <1.5 cm in diameter			
	Stage 2—tumor 1.5–4.4 cm, cervical lymph node metastases, or more than three intrathyroidal foci of tumor			
	Stage 3—tumor ≥4.5 cm or presence of extrathyroidal invasion			
	Stage 4—any tumor distant metastases			

Table 2 (continued)

Prognostic system	Calculation risk groups					OS	DSS	Risk of recurrence
NTCTCS [21]	**Four tumor stages I, II, III, IV**						+	+
	Histology	PTC		FTC				
	Age	<45 years	≥45 years	<45 years	≥45 years			
	Tumor size (cm)<1 cm	I	I	I	II			
	1–4 cm	I	II	I	III			
	≥4 cm	II	III	II	III			
	Microscopic multifocal	I	II	I	III			
	Macroscopic multifocal	I	II	II	III			
	Microscopic extrathyroidal	I	II	I	III			
	Macroscopic extrathyroidal	II	III	II	III			
	Poor differentiation	n/a	n/a	III	III			
	Cervical lymph node mets	I	III	I	II			
	Distant mets	III	IV	III	IV			
UAB-MDACC [22]	**Three risk groups**						+	
	Low risk—patients <50 years of age without distant metastases							
	Intermediate risk—patients ≥50 years of age without distant metastases							
	High risk—patients of any age with distant metastases within the risk groups; there is a further subdivision based on tumor size (≤3 cm and >3 cm)							
Manitoba Nomogram [23, 24]	Probability of DSS correlates with total score in the **nomogram** incorporating:						+	+
	Age 0–100 points + male gender 15 points + FTC 2 points or MTC 25 points + distant metastases 35 points + tumor stage (T1 0 points, T2 10 points, T3 15 points, T4 20 points) + presence of residual tumor post-surgery 15 points							
	Probability of recurrence correlates with a total score of the **nomogram** incorporating:							
	Age 0–70 points + male gender 20 points + PTC 10 points or MTC 45 points or PD/Hurthle cell 50 points + distant metastases 100 points + LN metastases 40 points + tumor stage T2 10 points, T3 30 points T4 40 points							
Clinical Class [25]	**Four classes**						+	+
	Class I—disease limited to the thyroid gland							
	Class II—locoregional lymph node involvement							
	Class III—extrathyroidal tumor invasion							
	Class IV—distant metastases							
Munster [10]	T1, tumor size <1 cm; T2, size 1–4 cm; T3, size ≥4, limited to thyroid; T4, any size beyond capsule; M0, no distant metastases; M1, distant metastases					+		
	Two risk groups							
	Low risk—T1–3 and M0							
	High risk—T4 or M1							
Noguchi [26]	**Three risk groups**						+	+
	Men							
	Excellent group—age <45 years old or <60 without gross LN metastasis							
	Intermediate group—age ≥60 years old without gross LN mets and age 45–55 years old with gross LN metastasis							
	Poor group—age >55 years old with gross LN metastasis							
	Women							
	Excellent group—all patients <50 years old and age 50–55 without metastases							
	Intermediate group—age 50–55 years old with gross LN metastasis, age >65 years old with primary tumor <3 cm							
	Poor group—remaining patients							

(continued)

Table 2 (continued)

Prognostic system	Calculation risk groups	OS	DSS	Risk of recurrence
Yildrim [27]	Score = exp[(0.2 × tumor size in cm) + (1 if age more than 45 years) + (0.7 if angioinvasion in primary tumor) + (1 if distant metastasis at presentation)]		+	+
	Probability of cancer-specific mortality = (score)/(1 + score)			
	0 risk groups			
	Very low risk—pretreatment probability <55%			
	Low risk—pretreatment probability 56% to 85%			
	High risk—pretreatment probability 86% to 95%			
	Very high risk—probability >96%			
	Posttreatment score = exp[(0.2 tumor size) + (0.8 if age>45 years) + (0.5 if angioinvasion) + (0.6 if distant metastasis) − (0.9 if total/near total thyroidectomy) − (0.7 if use of adjuvant radioiodine)]			
SAG [28]	Total score = 1 if male + 1 if aged >70 years + 1 if any one of the three microscopic features such as vascular invasion, marked nuclear atypia, and tumor necrosis are present		+	
	Three risk groups			
	SAG I—score is 1			
	SAG II—score is 2			
	SAG III—score is 3			
Cancer Institute Hospital in Tokyo [29]	**Two risk groups**		+	
	High risk—patients of any age with distant metastasis or patients >50 years with ≥3 cm nodal metastasis and/or extrathyroidal invasion			
	Low risk—those who did not meet the high-risk criteria			

AJCC/TNM American Joint Committee on Cancer/tumor nodes metastases, *ATA* American Thyroid Association, *MACIS* metastasis, age, completeness of resection, invasion, and tumor size, *AGES* age, grade, extension, size, *AMES* age, metastasis, extrathyroidal extension, and size, *DAMES* DNA ploidy**,** age, metastasis, extrathyroidal extension, and size, *MSK* Memorial Sloan Kettering (*GAMES* grade, age, metastases, extension, size), *EORTC* European Organization for Research and Treatment of Cancer, *NTCTCS* National Thyroid Cancer Treatment Cooperative Study, *UAB-MDACC* University of Alabama (Birmingham) and M.D. Anderson Cancer Center, *SAG* sex, age, grade, *PTC* papillary thyroid cancer, *FTC* follicular thyroid cancer, *LN* lymph nodes

Prognostic Systems Assessing the Likelihood of Overall Survival (OS)

Approximately 85% of patients with differentiated thyroid cancer have a normal life expectancy and are nearly twofold likely of dying from causes other than thyroid cancer [5]. However, patients who are at least 45 years of age and have extensive local tumor invasion, lateral lymph node, or distant metastases are characterized by significantly reduced life expectancy [6]. The risk factors affecting OS in thyroid cancer patients are summarized in four staging systems—AJCC-TNM seventh edition [7], AMES [8], EORTC [9], and Munster [10]. The most commonly used AJCC-TNM evaluates the association between the overall survival and patient's age, tumor size and extrathyroid extension, and presence of central and lateral neck lymph node metastases and distant metastases (Table 2). It enables stratification of the patients into: stage I with 5 years OS of 100%, 10 years OS of 98.5%; stage II with 5 years OS of 100%, 10 years OS of 98%; stage III with 5 years OS of 98%, 10 years OS of 98%; stage IVa with 5 years OS of 85%, 10 years OS of 76%; stage IVb with 5 years OS of 76%, 10 years OS of 62%; stage IVc with 5 years OS of 70% and 10 years OS of 50–63%.

The variables implemented in AJCC-TNM risk stratification were validated in the analysis of SEER database which documented that among 9904 patients with papillary thyroid cancer (PTC), lymph node metastases, age>45 years, distant metastases, and large tumor size significantly predicted decreased OS [11]. Some

authors suggest that cervical lymph node metastases are associated with decreased OS only in patients with follicular thyroid cancer (FTC) and older than 45-year-old patients with PTC [12], while others did find small but significantly increased risk of death for patients with metastatic lymph nodes, who are younger than 45 years, and that incrementally more metastatic lymph nodes (up to six involved) confer additional mortality risk in this age group [13].

AMES system stratifies patients to either low risk or high risk of death based on age, tumor size, extrathyroid extension, and distant metastases, without including lymph node metastases as additional variable. Low-risk patients are characterized by the 20 years death rate of 1.8% while high-risk patients by 46% mortality rate.

EORTC system classifies patients to five groups based on score calculated from patient's age, gender, poorly differentiated histology, capsular invasion, as well as presence and number of distant metastases (Table 2). In a recent validation of EORTC system, 5 years OS was 98% for group 1, 94% for group 2, 79% for group 3, 47% for group 4, and 33% for group 5 [14]. What is unique for EORTC system is the inclusion of poorly differentiated histology as an important prognostic factor. Poorly differentiated carcinomas have significantly worse outcome as compared to well-differentiated PTC and FTC, with a 10-year survival of approximately 50% [7, 15].

Finally, very simplistic Munster system stratifies the patients into the low-risk group with 5 years OS of 98% and high-risk group with 5 years OS of 83% based on the tumor size and extent as well as presence of distant metastases (Table 2).

Importantly, prognostic systems predicting OS perform slightly differently in different populations; thus, it is difficult to assess which one is the most accurate. Nevertheless, a recent analysis of 2257 patients with DTC documented that AJCC-TNM seventh version was more accurate in prognostication of OS than Munster and AMES systems and is currently the most widely used by the clinicians to assess the risk of poor overall survival [16].

Prognostic Systems Assessing the Likelihood of Disease-Specific Survival (DSS)

The majority of staging systems identify risk factors associated with disease-specific mortality—AJCC-TNM seventh edition [7], MACIS [6], AGES [17], DAMES [18], MSK (GAMES) [19], Ohio State [20], NTCTCS [21], UAB-MDACC [22], Manitoba Nomogram [23, 24], Clinical Class [25], Noguchi [26], Yildrim [27], SAG [28], and Cancer Institute Hospital in Tokyo [29] (Table 2). The risk estimates for DSS derived from original studied cohorts are summarized in Table 3.

DSS and Age

Most of prognostic systems, except from Ohio State and Clinical Class, include age as one of the key variables in predicting thyroid cancer-related mortality. The age is either treated as continuous variable (e.g., Manitoba Nomogram) or categorical variable with different age thresholds utilized: 40 years old for MACIS and AGES; 45 years old for AJCC-TNM, MSK (GAMES), NTCTCS, and Yildrim; 50 years old for UAB-MDACC and Cancer Institute Hospital in Tokyo; 50–55 years old for Ngoushi; and 70 years old for SAG system.

This discrepancy between different prognostic systems might be due to the different populations sampled to create and/or validate the risk stratification. One of the exemplifications is a study of a cohort of 3664 thyroid cancer patients analyzed to determine the significance of age at diagnosis at a variety of age cutoffs (5-year intervals between 30 and 70 years of age). The study revealed that there was no specific cutoff, suggesting that using age as a continuous variable may be the most appropriate tool for predicting the outcome [30]. In fact, a predictive nomogram using age as a continuous variable with other predictive variables had a high concordance index of 96% in a study cohort and 73% in the external validation cohort [30]. Similarly, a SEER database analysis including 53,581 patients revealed

Table 3 Disease-specific mortality (DSS) and proportion of variance explained based on different staging systems

Staging system	DSS	PVE
AJCC-TNM [7]	Stage 1—99–100% 5 years	4–33% [56]
	Stage II—99–100% 5 years	
	Stage III—93% 5 years	
	Stage IV—51% 5 years	
MACIS [52]	Group 1—99% 20 years	3.1–48% [21, 27, 56, 59, 87–90]
	Group 2—89% 20 years	
	Group 3—56% 20 years	
	Group 4—24% 20 years	
AGES [17]	Group 1—1–2% 20 years	23.1–46% [88, 90]
	Group 2–4—35–65% 20 years	
DAMES [18]	Low risk—100% 10 years	n/a
	Intermediate risk—92% 10 years	
	High risk—0% 10 years	
MSK (GAMES) [19]	Low risk—100% 5 years	4.8–19.2% [27, 59, 87, 88]
	Intermediate risk—96% 5 years	
	High risk—72% 5 years	
Ohio State [20]	Stage 1—100% 5 years	1.6–22.9% [21, 56, 59, 87, 88]
	Stage 2—94% 5 years	
	Stage 3—86% 5 years	
	Stage 4—35% 5 years	
NTCTCS [21]	Stage I—100% 5 years	3.5–18.4% [21, 56, 58, 91]
	Stage II—100% 5 years	
	Stage III—93.8% 5 years	
	Stage IV—78.5% 5 years	
UAB-MDACC [22]	Low risk—100% 5 years	2.7–18.7% [56, 58, 87, 91]
	Intermediate risk—90% 5 years	
	High risk—40% 5 years	
Manitoba Nomogram [23, 24]	n/a	n/a
Clinical Class [25]	Class I—10% 10 years	1.3–21.2% [21, 56, 58, 87, 88, 91]
	Class II—100% 10 years	
	Class III—87% 10 years	
	Class IV—35% 10 years	
Noguchi [26]	Excellent—98.4% men, 99.3% women 10 years	2.8–14.3% [56, 58, 91]
	Intermediate—90.1% men, 96.4% women 10 years	
	Poor—74.4% men, 88.8% women 10 years	
Yildrim [27]	Very low—100% 10 years	1.4–23.4% [27, 56]
	Low—88% 10 years	
	High—30% 10 years	
	Very high—5% 10 years	
SAG [28]	n/a	n/a
Cancer Institute Hospital in Tokyo [29]	Low—99% 10 years	0.7–12.6% [58, 91]
	High—69% 10 years	

AJCC/TNM American Joint Committee on Cancer/tumor nodes metastases, *MACIS* metastasis, age, completeness of resection, invasion, and tumor size, *AGES* age, grade, extension, size, *DAMES* DNA ploidy, age, metastasis, extrathyroidal extension, and size, *MSK* Memorial Sloan Kettering (*GAMES* grade, age, metastases, extension, size), *NTCTCS* National Thyroid Cancer Treatment Cooperative Study, *UAB-MDACC* University of Alabama (Birmingham) and M.D. Anderson Cancer Center, *SAG* sex, age, grade

an incremental continuum of increased disease-specific mortality with age [31].

A multicenter study including 9484 participants tested 45 years of age cutoff and 55 years cutoff in AJCC-TNM seventh edition predictive model. Interestingly, using age 45 years as a cut-off, 10-year DSS rates for stage I–IV were 99.7%, 97.3%, 96.6%, and 76.3%, respectively, while using age 55 years as a cutoff, 10-year DSS rates for stage I–IV were 99.5%, 94.7%, 94.1%, and 67.6%, respectively. The increased age threshold resulted in downstaging of 12% of patients and improved the statistical validity of the model [32]. Similarly, Hendrickson-Rebizant et al. in a study including 2125 consecutive thyroid cancer patients found that the age threshold of 55 years was found to be the best for TNM stage grouping [33]. The 55-year-old cutoff was also the optimal risk stratification tool in another study of 2115 consecutive DTC patients [34].

McLeod et al. utilized a sample of 4721 patients with thyroid cancer and tested different age cutoffs in a NTCTCS model. Among papillary thyroid cancer the best model utilizing the age threshold of 50 years old did not significantly outperform the model with 45-year-old age cutoff. However, for FTC utilizing the age cutoff of 50 years led to a significant improvement in the risk stratification and resulted in downstaging of a significant proportion of patients [35].

To summarize, the increased age is associated with increased likelihood of death from thyroid cancer, but the arbitrary cutoffs utilized in different staging systems might not be the most accurate predictors of the outcome.

DSS and Primary Tumor Extension

Another key variable utilized in the DSS risk stratification by almost all predictive models is the extent of the primary tumor (Table 2). The extrathyroid extension is uniformly and consistently found to be a significant and independent risk factor worsening the prognosis [7]. On the other hand, a lack of tumor invasion and specifically the encapsulated follicular variant of PTC (FVPTC) have such an indolent clinical course that might not be even called "cancer" but "noninvasive follicular thyroid neoplasm with papillary-like nuclear features" (NIFTP) [36].

Several prognostic systems utilize capsular/vascular invasion as an important prognostic variable (Table 1). Tumors without vascular invasion are characterized by the mortality rate of less than 5%, while angioinvasive follicular carcinomas, depending on the number of invaded blood vessels, have a mortality ranging from 5 to 30% [37]. In fact, current requirements for pathology reporting necessitate inclusion of the number of invaded vessels as a part of tumor description as invasion of four or more blood vessels is associated with poorer outcomes, particularly in follicular carcinomas [7].

DSS and Histology Subtype

Several prognostic systems underscore the importance of histology subtype as an important variable independently affecting the outcome (Table 1). The variants with more unfavorable outcomes are the tall cell, columnar cell, and hobnail variants of PTC as well as a poorly differentiated thyroid cancer [7].

The tall cell variant is characterized by predominance (>50%) of tall columnar tumor cells whose height is at least three times their width. Several studies documented that tall cell variant of PTC is associated with higher rate of extrathyroid extension and lymph node metastases and decreased DSS [38–40].

The columnar cell and hobnail variants of PTC are characterized by a higher risk of distant metastases, tumor-related mortality, specifically in patients with an advanced disease stage at diagnosis [7, 41, 42].

Consistently, recent data analysis from The Cancer Genome Atlas, including 6282 papillary thyroid cancer patients, revealed that a tall cell variant PTC is characterized by the worst prognosis (disease-specific mortality 9.1%), while the best prognosis is associated with the follicular variant of PTC (disease-specific mortality 0.6%). Classic variant PTC is characterized by the intermediate risk of cancer-specific death (2.5%). Patients with classic PTC were 3 times more likely to die from thyroid cancer than patients with follicular variant PTC (HR 3.44; 95% CI, 1.07–11.11), while patients with tall cell variant PTC were 15 times more likely to die from thyroid cancer (HR 14.96; 95% CI, 3.93–56.89) [43].

The poorly differentiated thyroid carcinoma has a much worse prognosis, with the 5-year DSS of 72% and 10-year DSS of 46% [44].

DSS and Gender

Only few staging systems utilize gender as an independent predictor of DSS—Manitoba Nomogram, Noguchi, and SAG (Tables 1 and 2).

The reason behind is the fact that the influence of gender on DSS is controversial and studies testing its prognostic value have inconsistent results.

Jonklaas et al. based on the analysis of NTCTCS Registry (n = 3572) found that DSS of women was similar to men after adjusting for disease stage and age at presentation. Interestingly, the subgroup analysis revealed that women with stage I and II disease had better outcomes than men when comparing individuals diagnosed before age 55 years old [45]. Yang et al. analyzed a large cohort of patients from SEER database (n = 29,225) and found that male patients showed higher cumulative incidence of death from thyroid cancer than their female counterparts, while Nilubol et al. utilizing the larger cohort from the same SEER database (n = 61,523) did not find gender to be a significant and independent prognostic factor for DSS [5, 46]. At this point it's unclear if thyroid cancer is characterized by more aggressive behavior in men than in women or if we are witnessing a gender-related ascertainment bias as men tend to reach medical attention at an older age and with more advanced disease [47].

DSS and Lymph Node Metastases

Several staging systems include lymph node metastases as an independent prognostic variable (Table 1). However, in contrast to well-established association of clinically positive lymph node metastases with increased thyroid cancer recurrence rate, the role of lymph node metastases as prognostic variable affecting disease-specific survival is controversial. Importantly, a thorough inspection of lymph nodes reveal that up to 90% of patients have micrometastatic disease, which does not translate to a mortality in this group of patients as disease-specific mortality has never approached such a high value [7, 48]. Therefore, a very practical approach has been proposed by Schneider et al. [49]. The authors analyzed the association between DSS and lymph nodes ratio, calculated as proportion of positive lymph node among all lymph nodes examined, excluding patients with less than three nodes examined. Based on analysis of 10,955 patients included in the SEER database, they found that patients with a lymph node ratio ≥0.42 experienced a 77% higher disease-specific mortality rate compared to all patients with metastatic lymph nodes [49].

On the other hand, another SEER database analysis of 11,453 thyroid cancer patients revealed that only patients aged 45 and more years old with lateral and/or mediastinal lymph node metastases have an increased risk of death from PTC [50]. Analysis of 20,357 patients with follicular variant of papillary thyroid cancer revealed that clinically significant lymph node metastases occur only in 10% of patients and do not affect the DSS [51].

To summarize, there is an ongoing controversy regarding the association of lymph node involvement in DTC and survival and whether prophylactic central lymph node dissection should be performed in thyroid cancer patients. This controversy could be resolved by the well-designed randomized prospective controlled trial determining the prognostic role of prophylactic central lymph node dissection in the management of thyroid cancer patients.

DSS and Distant Metastases

Presence of distant metastases is a variable utilized in all prognostic systems (Table 1) and has been found to be an independent and significant variable affecting DSS uniformly in all staging systems [7, 18, 20–22, 24–28, 52].

DSS and Multifocality

Multifocal tumor growth has been implemented as a prognostic variable in three predictive models—Ohio State, NTCTCS, and SAG (Table 1). One of the reasons for the lack of uniformed inclusion of tumor multifocality in different predictive models is the fact that the association of multifocality with poor prognosis remains controversial. Qu et al. documented that cancer-specific survival rates decreased significantly with increasing number of tumor foci (p = 0.041) [53].

However, several studies suggested that the tumor multifocality do not have a prognostic value [54, 55]. Moreover, the incidence of tumor multifocality in PTC is relatively high (up to 40% of PTC cases)—the rate which does not correspond nor correlate with a disease-specific mortality rate.

Summarizing the impact of the prognostic systems estimating the risk of cancer-specific death, it's worthwhile to underscore that none of the staging systems has been shown to be clearly superior over the other. The statistical tool used to compare different staging systems—proportion of variance explained (PVE)—describes how well a staging system can predict the outcome when applied to a broad range of patient cohorts. None of the staging system has exceeded PVE of 5–48%, leaving a significant uncertainty of the accurate prognostication of DSS (Table 3) [56]. Nevertheless, several studies have demonstrated that the AJCC/TNM and the MACIS system consistently provide the highest PVE [7, 16, 27, 47, 56–63]. Interestingly, the nomograms tend to outperform traditional scoring systems but require external validation in large populations [5, 23, 24, 63].

Prognostic Systems Assessing the Likelihood of Thyroid Cancer Recurrence

Several staging systems focus on the evaluation of prognostic factors affecting thyroid cancer recurrence rate and disease-free survival—ATA, DAMES, Murcia, Ohio State, NTCTCS, Manitoba Nomogram, Clinical Class, Noguchi, and Yildrim (Tables 2 and 4). Recent studies have emphasized the need for dynamic risk stratification, in which the various clinical data obtained over time modify the prognosis. Two staging systems implement this dynamic model and evaluate the baseline and posttreatment patient status—ATA and Yildrim risk stratification. The ATA system has been found to outperform the remaining systems (Table 4).

The initial staging systems can be informative in guiding therapeutic and early diagnostic follow-up strategy, but ongoing dynamic risk stratification enables more tailored and individualized management of the patient [7]. Multiple studies have shown that many patients initially classified

Table 4 Prognostication of persistent/recurrent disease and proportion of variance explained based on different staging systems

Staging system	Persistent/recurrent disease	PVE
ATA [7]	Low risk—1–14%%	62–84% [64, 65, 92]
	Intermediate risk—8–48%	
	High risk—69–86%	
DAMES [18]	Low risk—8%	n/a
	Intermediate risk—55%	
	High risk—100%	
Murcia [64]	Low risk—12%	11.4% [56]
	Medium risk 30%	
	High risk—100%	
Ohio State [20]	Stage I—8%	5–18% [62]
	Stage II—31%	
	Stage III—36%	
	Stage IV—62%	
NTCTCS [21]	Stage I—5.7%	18–20% [62]
	Stage II—6.9%	
	Stage III—22.2%	
	Stage IV—75.4%	
Manitoba Nomogram [23, 24]	n/a	n/a
Clinical Class [25]	Class I—9.3%	12–20% [62]
	Class II—23.6%	
	Class III—89.5%	
	Class IV—90%	
Noguchi [26]	Excellent—men 18%, women 15%	16–18% [62]
	Intermediate—men 35%, women 20%	
	Poor—men 65%, women 45%	
Yildrim [27]	Very low risk—0%	2–23.4% [27]
	Low risk—25%	
	Intermediate risk—84%	
	High risk—100%	

ATA American Thyroid Association, *DAMES* DNA ploidy, age, metastasis, extrathyroidal extension, and size, *NTCTCS* National Thyroid Cancer Treatment Cooperative Study, *UAB-MDACC* University of Alabama (Birmingham) and M.D. Anderson Cancer Center, *SAG* sex, age, grade

as intermediate or high risk of recurrence can be reclassified as having a subsequent low risk of recurrence based on an excellent response to initial therapy [64–74]. Response to therapy is evaluated based on all clinical, biochemical, imaging (structural and functional), and cytopathologic findings obtained during the follow-up. Tuttle et al. proposed the following definitions of the response to therapy [7, 62]:

- Excellent response is defined as a TSH-stimulated thyroglobulin (Tg) of less than 1 ng/mL in the absence of structural or functional evidence of disease (for patients who underwent thyroidectomy and RAI therapy).
- Biochemical incomplete response is defined as abnormal thyroglobulin values in the absence of localizable disease—non-stimulated Tg values >1 ng/mL or TSH-stimulated Tg values >10 ng/mL.
- Structural incomplete response is defined as persistent or newly identified locoregional or distant metastases.
- Indeterminate response or acceptable response is defined as biochemical or structural findings which cannot be classified as either benign or malignant.

An excellent response to initial therapy is achieved in 86–91% of ATA low-risk patients, 57–63% of ATA intermediate-risk patients, and 14–16% of ATA high-risk patients [7]. Patients who obtain an excellent response to initial therapy are characterized by very low 5–10 years recurrence rate, ranging between 1 and 4% [64–74]. The potential impact of the reclassification to an excellent response group is particularly important for 57–63% of ATA intermediate-risk patients, whose risk of recurrence drops dramatically from initially predicted 8–49% to 1–4% predicted by response to therapy reclassification. Furthermore, the reclassification holds truth also for the few high-risk patients that achieve an excellent response to initial therapy, as majority of the studies show that subsequent recurrence rates are in the 1–4% range with maximum recurrence rate documented in one study of 14% [64, 66, 70, 71, 74, 75]. Thus, high-risk patients that achieve an excellent response to therapy may require more intense follow-up than ATA low- and intermediate-risk patients demonstrating an excellent response to therapy [7].

The patients characterized by the biochemical incomplete response have persistently abnormal suppressed and/or stimulated Tg values or rising anti-thyroglobulin antibodies without structural evidence of disease. A biochemical incomplete response to therapy has a prevalence of 11–19% in ATA low-risk group, 21–22% in ATA intermediate-risk group, and 16–18% in ATA high-risk patients [7]. Eventually, majority of the patients can be classified as "no evidence of disease" over the course of 5–10 years of follow-up, while 19–27% continue to have persistently abnormal Tg values without structural correlate, and the minority—8–17%—of patients progresses to a structurally identifiable disease [7, 64, 65]. Patients who progress to a clinically significant disease tend to be characterized by the thyroglobulin (Tg) doubling time of less than 1–3 years or by the rise in unstimulated Tg level of more than 0.3 ng/mL per year [76, 77].

A structural incomplete response to initial therapy evident by either structural or functional imaging is observed only in 2–6% of ATA low-risk patients, 19–28% of ATA intermediate-risk patients, and as many as 67–75% of ATA high-risk patients [7]. Unfortunately, only minority of patients with distant metastases would respond to treatment and eventually obtain remission, while persistent locoregional disease tends to respond to surgical treatment in 29–51% of cases [7, 78].

The reminder of the patients is classified as obtaining the indeterminate response to therapy. Some authors suggest a synonym of "acceptable response" as this group of patients consists of individuals for whom biochemical, structural, or functional findings cannot confidently document either excellent response or persistent disease. This group includes patients with sub-centimeter avascular thyroid bed nodules, atypical cervical lymph nodes that have not been biopsied, faint uptake in the thyroid bed with undetectable Tg, non-stimulated Tg values that are detectable but <1 ng/mL, TSH-stimulated Tg values between 1 and 10 ng/mL, and patients with stable or declin-

ing Tg antibodies [7]. An indeterminate response to initial therapy is observed in 12–29% of ATA low-risk patients, 8–23% of ATA intermediate-risk patients, and 0–4% of ATA high-risk patients [7]. Importantly, only 13–20% of patients with an indeterminate response to therapy will eventually develop a structurally evident disease over 10 years of follow-up [7]. In the remaining 80–90% of patients, the non-specific findings either remain stable or resolve without an intervention.

To summarize, it's important to underscore that initial risk estimates are used to guide initial therapeutic approach, while evaluation of the response to treatment helps in the establishment of the individual, patient-tailored approach to subsequent management and follow-up.

Although dynamic risk stratification definitively improves the accuracy of the predictive model, there is still room for gaining more precision, as PVE does not even approach 100%. There are studies showing potential utility of incorporating molecular diagnostics in predictive models, as BRAFV600E, TERT promoter, and TP 53 mutations have been found to be associated with increased mortality and recurrence rate [79–85].

Further studies should also focus on assessing the utility of predictive models in guiding the intensity of the follow-up strategy.

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Radioiodine Therapy in Differentiated Thyroid Carcinoma

Jasna Mihailovic and Stanley J. Goldsmith

Introduction

The adequacy and timeliness of initial treatment is the most important determinant of the outcome for patients with differentiated thyroid carcinoma (DTC). Currently, the most widely accepted initial treatment protocol includes total thyroidectomy followed by ablation of remnant tissue with ^{131}I. Radioactive iodine [RAI] as sodium iodide was administered for the first time to a patient with metastatic DTC in 1943 by Dr. Sam Seidlin in the Bronx, NY, Montefiore Hospital in the USA. Based on Seidlin's experience and the subsequent observations of others that ^{131}I uptake in metastases appeared to be enhanced in the absence of significant residual thyroid tissue, RAI ablation of remnant thyroid became established worldwide as a routine part of the management algorithm for DTC [1, 2].

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Governmental and regulatory requirements, however, have a significant impact on the therapeutic use of RAI. Consequently, the treatment of DTC varies not only from country to country but even within countries and among medical centers as well.

There are two roles for the use of RAI in DTC. The first is ablative and adjuvant therapy, to eliminate normal thyroid remnants in the postoperative period as well as to eliminate microscopic or occult DTC which may not be demonstrable. The second role is curative or palliative therapy, to destroy persistent or recurrent tumor tissue (metastatic lymph nodes and distant metastases).

Radioactive Iodine Ablation and Adjuvant Therapy

Since, in general, the long-term prognosis of DTC is favorable, it is difficult to determine the efficacy of RAI as adjuvant therapy without initial evidence of residual tumor. Nevertheless, RAI therapeutic efficacy has been discussed for decades. Mazzaferri evaluated patients with primary tumors ≥1.5 cm and concluded that RAI is effective in reducing the recurrence of DTC in patients of all ages and reduces the risk of death from thyroid carcinoma in patients over age 40 at the time of diagnosis. However, there appears to be no incremental benefit in patients with isolated tumors, tumors >1.5 cm, without lymph node metastasis or invasion of thyroid capsule

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[3]. In a 30-year follow-up study, Mazzaferri later reported a significantly decreased recurrence rate in patients receiving RAI (for ablation or adjuvant treatment) versus thyroxine alone-treated patients (16% vs. 38%, respectively). In addition, he reported reduction of cancer-related deaths in ^{131}I-treated patients versus patients not receiving RAI (for ablation or adjuvant treatment) (3% vs. 8%, respectively) [4].

Since those reports, RAI efficacy as adjuvant therapy has been evaluated in numerous studies [3–15]. In the low-risk category, most authors detect no beneficial role of RAI on survival [5–7, 10] or recurrence [9, 10]. However, meta-analyses and systematic literature reviews reported inconsistent data. The majority of studies did not confirm a decreased recurrence rate [8, 11, 12]. Hay et al. report no RAI effect in either low- or high-risk patients [9], while others observe significant effects of RAI (lower recurrence rate, longer overall and disease-specific survival) [5, 10, 13–15] (Table 1).

In a recent review, Goldsmith considers that low-risk patients still represent a dilemma after initial total thyroidectomy: whether to undergo RAI or not. In order to determine a statistically significant and valid effect of RAI on recurrence and mortality, randomized controlled trials are necessary. In the meantime, decision-making for RAI ablation should be based on an individual patient criteria, considering the benefits and risks, pros and cons, as well as the physician-patient relationship [16].

At present, two ongoing prospective randomized trials in Europe compare RAI ablation in treated and non-treated DTC patients. One is the British non-inferiority study—"the IoN study" (*Is Ablative Radio-iodine Necessary for Low Risk Differentiated Thyroid Cancer Patients*)—comparing low-risk and selected intermediate-risk patients treated with total thyroidectomy and RAI ablation versus patients treated with total thyroidectomy alone [17]. Another study is the French non-inferiority study "ESTIMABL2" (*Differentiated Thyroid Cancer: Is There a Need for Radioiodine Ablation in Low Risk Patients*) comparing low-risk patients treated with surgery (total thyroidectomy with or without neck dissection) followed by RAI ablation vs. patients treated with surgery alone. Completion of these two randomized clinical trials may clarify the effect of RAI ablation in low-risk DTC patients.

The treatment of microcarcinoma in the low-risk category (patients with tumor size ≤1 cm) is controversial also. Some investigators report that RAI ablation does not improve disease-specific or disease-free survival [18–23] nor significantly decreases recurrence rates [24] in papillary microcarcinoma, uni- or multifocal disease, without high-risk features. In contrast, others advocate administration of RAI ablative therapy to this patient population [25–27] with reports of significantly lower probability of recurrence in treated patients compared to those who were not treated with RAI ablation: 18% vs. 3%, respectively ($p = 0.005$) [25].

Currently, several guidelines provide recommendations that make RAI ablation and adjuvant treatment of DTC patients uniform among different centers and practitioners. This review addresses only the most cited and commonly used guidelines: European Thyroid Association (ETA), American Thyroid Association (ATA), European Association of Nuclear Medicine (EANM), and the Society of Nuclear Medicine and Molecular Imaging (SNMMI) [28–31]. In general, these guidelines support the statement that RAI ablation is not indicated in low-risk patients (pTNM stage I patients and pT1/pT2 without extrathyroidal extension or regional or distant metastases). However, there are variations among the guidelines on this subject. The ATA Guidelines proposed a three-tiered clinic-pathologic risk stratification system that classifies DTC patients as having low, intermediate, or high risk of recurrence. Low-risk patients are defined with no evidence of extrathyroidal extension, vascular invasion, no clinically metastatic lymph node or ≤5 pathologic lymph node micrometastases (<0.2 cm in largest dimension), no aggressive histology (e.g., tall cell, hobnail variant, columnar cell carcinoma), and no RAI-avid disease outside the thyroid bed on the first post-treatment whole-body RAI scan (if ^{131}I is given). Intrathyroidal, papillary microcarcinoma, unifocal or multifocal, including *BRAF*^{V600E} mutated

Table 1 Efficacy of RAI Ablation

Authors	Study type	Patients/category risk	Treatment effect of RAI ablation				
			DSS	DFS	REC	OS	DSM
Mazzaferri [3]	Original study	Low-risk PTC and FTC patients with tumor size>1.5 cm			(+)		(+) in patients>40 years
Mazzaferri Kloos [4]	Original study	Low-risk PTC and FTC patients>40 years and tumor size>1.5 cm			(+)		(+)
Jonklaas et al. [5, 6]	Prospective multicentric study of the NTCTCS registry	Low-risk PTC/FTC patients staged I/II (AJCC/TNM ed.5)	(−)	(−)		(−)	
Schvartz et al. [7]	Retrospective multicenter study	ATA Low-risk PTC/FTC patients		(−)		(−)	
Sacs et al. [10]	Systematic analysis of peer-reviewed literature (January 1966–April 2008)[a]	Low-risk DTC patients<45 years staged I and patients>45 years staged I/II (AJCC 6.ed); and Low-risk DTC patients with MACIS<6	(−)		(−)	(−)	
Sawka et al. [11]	Meta analysis of 1543 English-language publications	Low-risk PTC/FTC patients	Inconsistent results among centers regarding the benefit in decreasing DSM and REC				
Sawka et al. [12]	Systematic review of the literature	Low-risk PTC/FTC patients	(−)		(−)[a]		(−)
Lamartina et al. [8]	Systematic review of the literature	ATA Low- and Intermediate-risk DTC patients			(−)[b]		
Hay et al. [9]	Review of the Mayo Clinic database (1940–1999)	ATA Low-risk PTC patients with N1 and MACIS<6			(−)		(−)
Jonklaas et al. [5]	Prospective multicenter study	High-risk PTC/FTC patients staged III/IV (AJCC/TNM ed.5)	(+)	(+)		(+)	
Podnos et al. [13]	Analysis of the SEER database	High-risk PTC patients>45 years and tumor size>2cm with N1 and M1				(+)	
Podnos et al. [14]	Analysis of the SEER database	High-risk FTC patients >45 years with tumor size >2cm with N1 and M1				(+)	
Taylor et al. [15]	Prospective multicentric study of the NTCTCS registry	High-risk DTC patients: PTC: FTC:	 (+) (+)	 (−) (+)			
Hay et al. [9]	Review of the Mayo Clinic database (1940–1999)	High-risk PTC patients with MACIS>6			(−)		(−)
Sacs et al. [10]	Systematic review of peer-reviewed literature (January 1966–April 2008)	High-risk DTC patients staged III/IV and patients<45 years staged II (AJCC 6.ed)	(+)		(+)	(+)	

(−)[a] majority of studies show no significantly benefit of RAI ablation

(−)[b] conflicting data; 11 studies detected reduced recurrence rates while 13 studies did not

(+) significant benefit (improvement); (−) No benefit; *REC* recurrence; *DSS* disease-specific survival; *DFS* disease-free survival; *OS* overall survival; *DSM* disease-specific mortality; *DTC* differentiated thyroid cancer; *PTC* papillary thyroid cancer; *FTC* follicular thyroid cancer; *N1* presence of lymph node metastases; *M1* presence of distant metastases; *NTCTCS* National Thyroid Cancer Treatment Cooperative Study; *SEER* surveillance, epidemiology, and end results

(if known) is also included in this risk category. Intermediate-risk patients demonstrate either microscopic extrathyroidal extension, vascular invasion, clinically apparent lymph node metastases or >5 pathologic lymph nodes with all involved lymph nodes <3 cm in largest dimension, RAI-avid disease in the neck outside the thyroid bed, or aggressive tumor histology. Multifocal papillary microcarcinoma with extrathyroidal extension and *BRAF*^{V600E} mutated (if known) is also included. ATA high-risk patients present with gross extrathyroidal extension, incomplete tumor resection, distant metastases, inappropriate postoperative serum thyroglobulin (Tgb) values, and pathologic lymph nodes with any metastatic lymph node ≥3 cm in largest dimension. Follicular thyroid cancer with extensive vascular invasion (>4 foci of vascular invasion) is included in this category. The ATA Guidelines state that RAI is not indicated in low-risk patients with tumor size ≤1 cm (so-called microcarcinoma, uni- or multifocal) and tumors >1–4 cm without local or distant metastases [29]. In addition, RAI adjuvant therapy should be considered after total thyroidectomy in ATA intermediate-risk level DTC patients.

The ETA Guidelines do not recommend RAI ablation in patients with unifocal microcarcinoma without extrathyroidal extension and regional or distal metastasis [28]. The SNMMI Guidelines that support the decision not to ablate include the absence of unfavorable histology, lymphatic/vascular invasion, capsular invasion or penetration, and perithyroidal soft tissue involvement as additional factors to consider ^{131}I in deciding whether or not to pursue RAI ablation [30]. The EANM Guidelines consider RAI ablation as routine in DTC except for patients presenting with unifocal microcarcinoma, without invasion of thyroid capsule, metastases, prior radiation exposure, or unfavorable histology (columnar cell, diffuse sclerosing, and tall cell subtype). In addition, low-risk patients who underwent less radical initial surgery need not undergo completion thyroidectomy; simple monitoring may be sufficient [31]. In all guidelines, for high-risk DTC patients RAI ablation is recommended as a routine initial component of postoperative adjuvant therapy.

In addition to a variety of opinions about the utility of RAI ablation in low-risk patients, there are additional differences concerning the role of pretreatment whole-body imaging (WBS) and the amount of ^{131}I activity to administer as the ablative dose.

^{131}I Ablation or Adjuvant Therapy Dose

There is general agreement among professional society guidelines concerning a range of RAI activities for patients without evidence of residual thyroid tumor or metastatic disease. Until recently, this range was from 1.85 to 5.55 GBq (50–150 mCi). There is, or has been, variation in the activity to be administered to each patient with some centers using 3.7 or 5.55 GBq (100 or 150 mCi) ^{131}I for all patients in this category, whereas other centers selected an activity within this range based upon an estimate of the risk of tumor recurrence. More recently, it has been demonstrated that a high degree of successful ablation (approximately 80%) can be achieved with 1.1 GBq (30 mCi) [29–31].

Some authors advocate the administration of high activities, suggesting that administration of 3.7 GBq ^{131}I results in more effective ablation than treatment with lower activities (1.1 or 1.85 GBq ^{131}I) [32–34]. However, in recent years, in order to prevent potential side effects of RAI, there is an increasing initiative to use lower RAI activities [35, 36]. Several factors such as the duration and cost of hospital isolation and the level of radiation exposure to the patients, staff, and environment motivate this approach. There are reports that low-dose radioiodine achieves similar success rates for ablation (with either low- or high-dose ^{131}I) [35–43], regardless of whether patients were prepared with thyroid hormone withdrawal or with rhTSH [35, 41, 43]. A comparison of ablation success rate using the low- and high-dose radioiodine is presented in Table 2.

Table 2 Comparison of RAI ablation success rate using low-activity vs. high-activity of ^{131}I

Authors	Patients (N)	Preparation for RAI ablation	Criteria for successful ablation	Success of RAI ablation % (patients)			
				Low dose (GBq)		High dose (GBq)	
				1.1	1.85	3.7	>3.7
Creutzig et al. [42]	1) 20 with pT2/T3N0M0 (1.1 GBq vs. 3.7 GBq) 2) 20 with pT2/T3NxM1 (1.1 GBq vs. 11.1 GBq)	Ablation was done 3 weeks after TT	RIU in thyroid bed≤0.5%	50 (5/10)	NS	60 (6/10)	NS
Johansen et al. [43]	63	THW and rhTSH	Negative WBS after the first ablation[b]	58 (21/36)	NS	52 (14/27)	NS
Bal et al. [38]	149	THW	1. Negative WBS 2. 48-h RAIU< 0.2% 3. Tgb<10 ng/mL	63 (17/27)	77.8 (42/54)	73.7 (28/38)	73.7 (28/38)
Bal et al. [39][a]	509	THW	1. Negative WBS 2. 48-h RAIU≤ 0.2% 3. Tgb≤10 ng/mL	83.6 (61/73)	81.8 (63/77)	NS	NS
Mallick et al. [35][b]	421	THW and rhTSH[c]	1. Negative WBS 2. Tgb<2 ng/mL 3. Negative WBS and Tgb<2 ng/mL	85 (182/214)	NS	88.9 (184/207)	NS
Pilli et al. [40]	72	rhTSH	Negative WBS Undetectable rhTSH-stimulated Tgb (<1 ng/mL)[a]	NS	88.9 (32/36) 78.9 (15/19)	88.9 (32/36) 66.7 (10/15)	NS
Zaman et al. [32]	40	THW	1. Negative WBS 2. Tgb<2ng/mL		40 (8/20)	60 (12/20)	NS
Prpic et al. [33]	138	THW	1. Negative WBS 2. Tgb<1ng/mL 3. Negative neck US	1.1–1.85 GBq 77.5 (62/80)		89.7 (52/58)	NS
Maenpaa et al. [36]	160	THW	1. Tgb<1ng/mL and negative WBS* 2. Negative WBS	52 (42/81)+	NS	56 (43/77)	NS
Sclumberger et al. [41]	684	THW versus rhTSH	1. Negative neck US 2. Tgb<1ng/mL 3. Negative WBS 4. RIU in thyroid bed<0.5%	92 (156/170)+ 90 (160/177)+	NS	94 (156/166)+ 93 (159/171)+	NS

(continued)

Table 2 (continued)

Authors	Patients (N)	Preparation for RAI ablation	Criteria for successful ablation	Success of RAI ablation % (patients)			
				Low dose (GBq)		High dose (GBq)	
				1.1	1.85	3.7	>3.7
McCowen et al. [37]	64	TT+T3 treatment for 6 weeks+THW	1. Negative WBS 2. PB^{131}I CR<0.005%/L in seven days 3. WBR< 3% in seven days	0.925–1.1 GBq 58 (21/36) NS		2.96–5.5 GBq 64 (18/28)	
Fallahi et al. [34]	341	TT+T3 treatment for 2 weeks+THW	1. Neck US/WBS 2. Tgb<2 ng/ml 3. Anti-TgbAb<100 IU/ml	39.2 (67/171)	NS	64.1 (109/170)	NS

*WBS was not performed in 4 patients treated with 1.1 GBq ^{131}I and in 5 patients who received 3.7 Gbq ^{131}I, due to detectable Tgb while patients were on LT4 therapy

[+]Success of RAI ablation according to local Tgb determination (obtained in each center involved in the study)

[‡]Success of RAI ablation according to central Tgb determination [in a central laboratory (Institut Gustave Roussy) using the immunometric thyroglobulin Access Immunoassay kit in patients without detectable TgbAb]

[a]In patients without TgbAb and after excluding patients with undetectable rhTSH-stimulated Tgb before ablation.

[b]40% of the patients ablated with 1.1 GBq and 44% ablated with 3.7 GBq had elevated Tgb at the time of complete scintigraphic ablation

[c]Ablation success rates were 87.1% in the rhTSH group versus 86.7% in the group undergoing THW

TT total thyroidectomy; *THW* thyroid hormone withdrawal; *rhTSH* preparation using recombinant human TSH; *NS* non studied; *PB ^{131}ICR* protein bound iodine (^{131}I) conversion rate; *WBR* whole body retention

The ATA, the EANM, and the SNMMI Guidelines provide similar recommendations regarding the activities of RAI ablation. Radioiodine-131 WBS with 111-185 MBq is usually performed 6–12 months after the RAI ablation to determine success. Successful ablation is achieved if there is negative thyroid bed uptake or thyroid bed uptake <0.1%, absence of focal uptake of ^{131}I WBS, and undetectable level of stimulated Tgb in the absence of interfering Tgb antibodies [31](Fig. 1). In general, at the present time, if ablation is not successful, patients are not re-ablated to remove minimal thyroid remnant tissue detected following the initial RAI ablation [16].

The activities in a range of 1.0 GBq–3.7 GBq ^{131}I are recommended for ablation of thyroid remnants, while higher activities of RAI (3.7 GBq–7.4 GBq) are suggested in patients with metastases (regional and/or distant) and aggressive histology [29–31] (Figs. 2 and 3).

Remnant or lesion-based dosimetry estimates the radiation absorbed dose in order to destroy desired lesion or tissue remnant—to ablate thyroid remnant or to treat metastatic disease. Maxon et al. studied the relationship between the thyroid radiation absorbed dose and successful ablation of remnant thyroid tissue and noted a regular relationship between them. They observed that thyroid remnants were ablated in 81% of cases when a calculated dose of 300 Gy was delivered. They did not obtain better results with larger doses in excess of 300 Gy. In order to ablate the tissue focus, the number of GBq selected should deliver at least 300 Gy (30,000 rad) to the thyroid residues if there is no evidence of metastatic tissue in the neck [44]. However, since the pretreatment WBS may not identify all tumor sites, it is not always possible to determine the radiation absorbed dose to tumor sites before treatment. As a practical matter, the GBq or mCi activity used

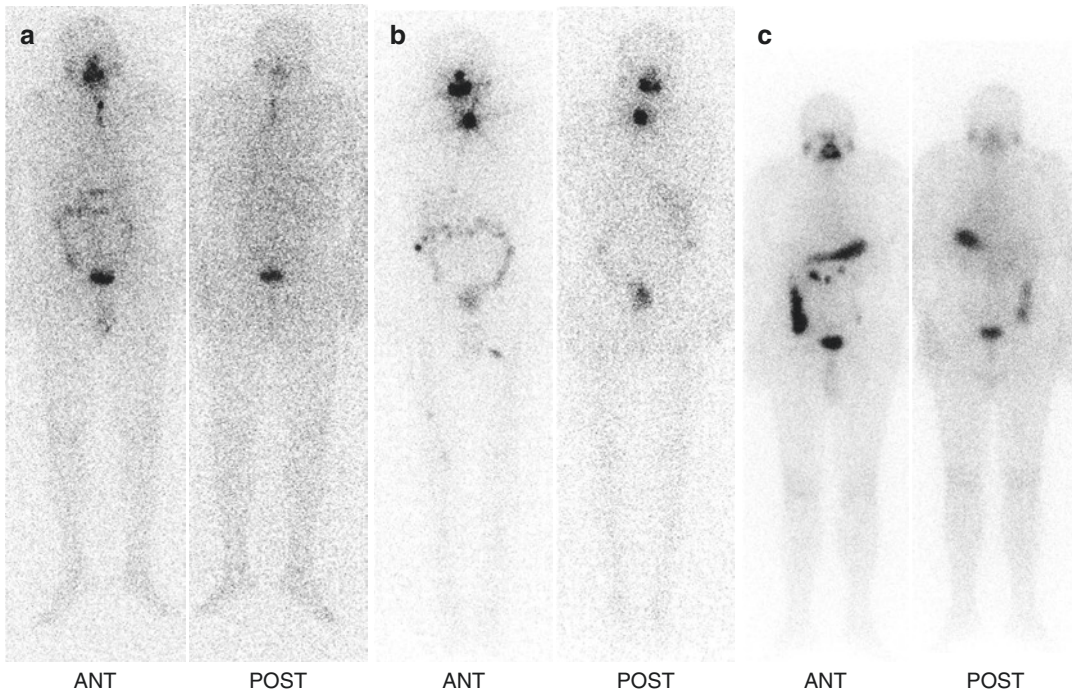


Fig. 1 Successful RAI Remnant Ablation; **(a)** Pre ^{131}I Ablation: Dx ^{123}I WBS (81.4 MBq) detects functioning residual thyroid tissue in the thyroid bed. Normal distribution of radiotracer is seen in the stomach (extraction by gastric mucosa), some bowel activity (transit from gastric secretion) and bladder (renal excretion); **(b)** Rx ^{131}I WBS performed one week after ablation radioiodine therapy (5.77 GBq). Functioning residual thyroid tissue is seen in the thyroid bed. Note "Star Artefact". No evidence of additional abnormal foci compared to the Dx ^{123}I WBS; **(c)** One year following RAI ablation: Dx ^{131}I WBS (185 MBq) detects no evidence of residual functioning thyroid tissue and no evidence of cervical or distant metastases (successful remnant ablation). TgAb not detectable; Tgb < 2 μg/L

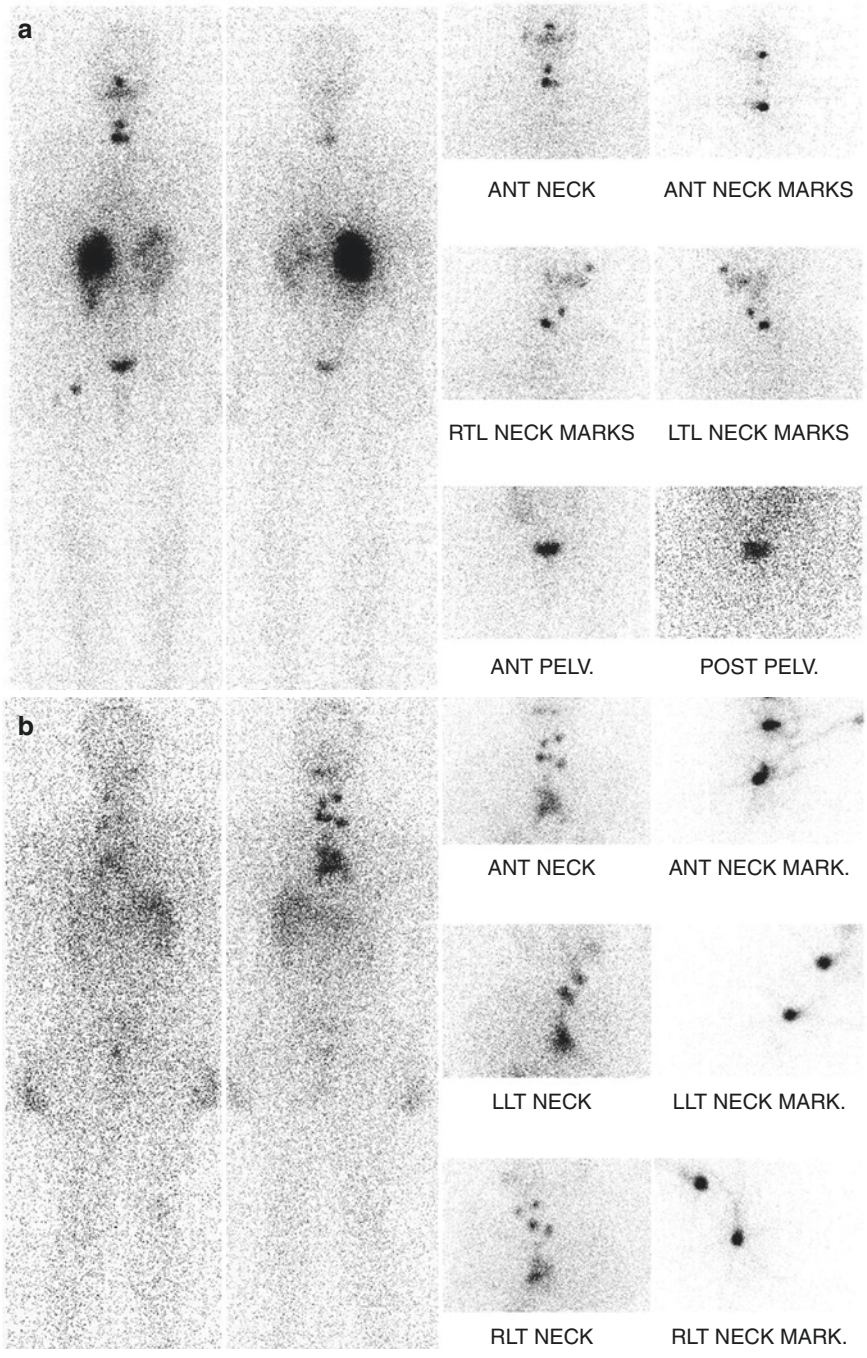


Fig. 2 Identification of local nodal involvement (detected on Rx ^{131}I WBS only, not on Dx ^{131}I WBS); **(a)** Dx ^{131}I WBS (185 MBq) identifies functioning residual thyroid tissue in the thyroid bed without evidence of cervical and distant metastases. Large mass in the right upper abdominal quadrant is dilated renal collecting system & pelvis. Right inguinal focus is an artefact (radioisotope contamination); **(b)** Rx ^{131}I WBS scan performed one week after RAI therapy (13.06 GBq) reveals functioning residual thyroid tissue in the thyroid bed, multiple foci in the lower neck (cervical metastatic disease) not seen on the pre-therapy scan. Additionally, thymic tissue is identified in the anterior mediastinum.

This figure demonstrates that decision about dose selection cannot simply be made based on pretherapy scan. There are several factors that should be considered prior to dose selection such as: extent of surgery, tumor size, tumor capsule invasion, nodal involvement and evidence of metastases

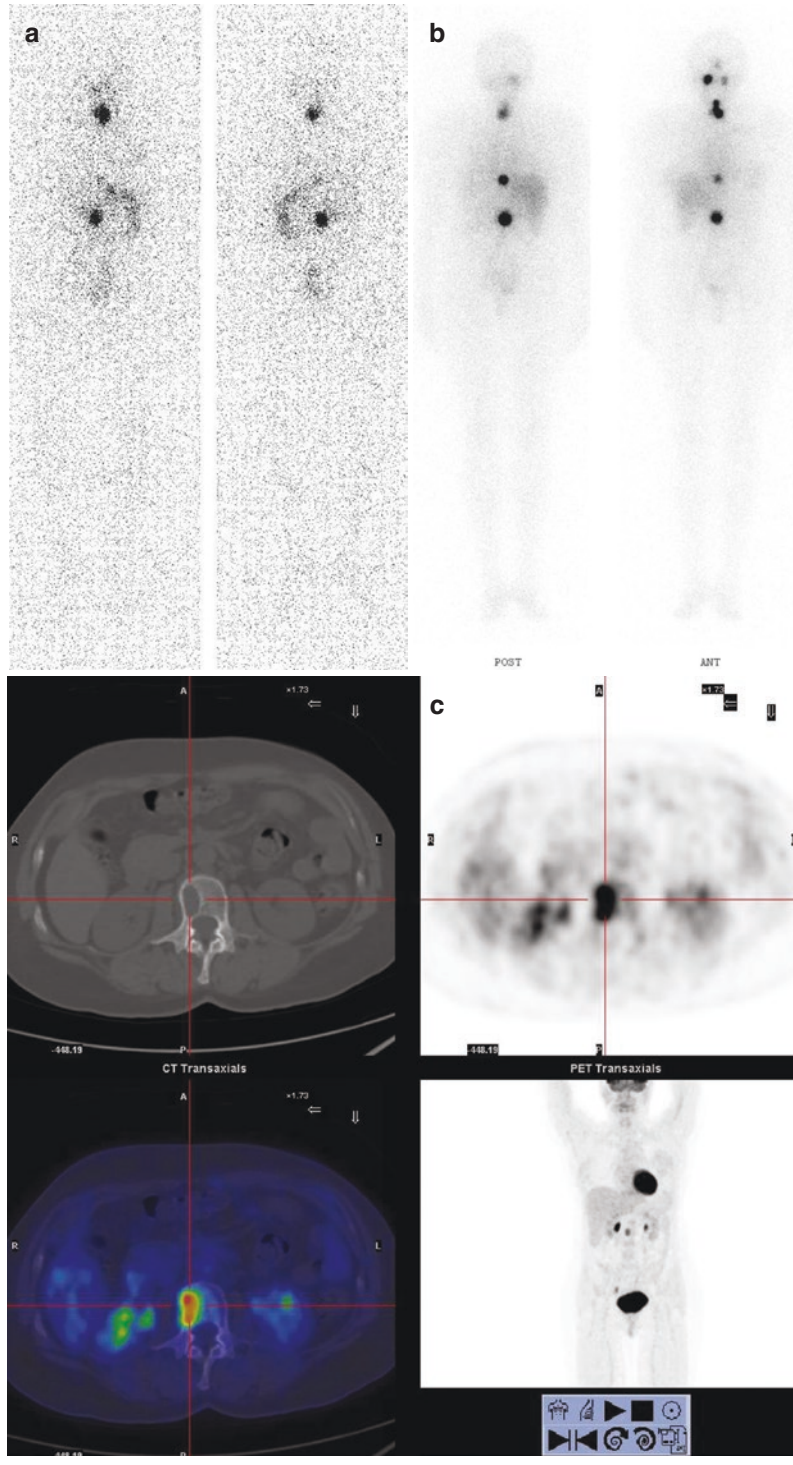
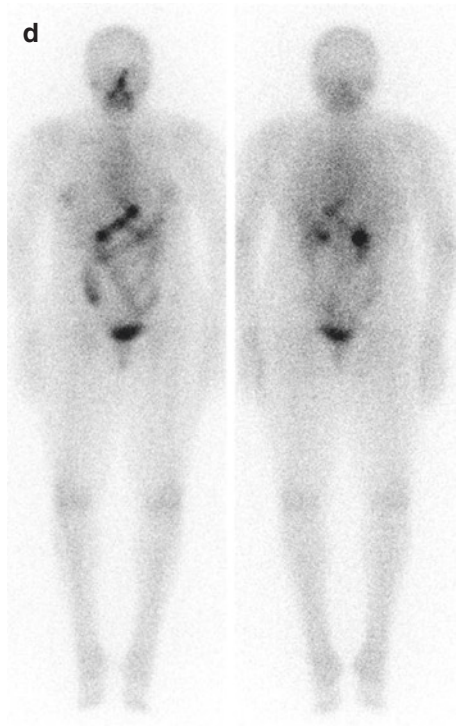
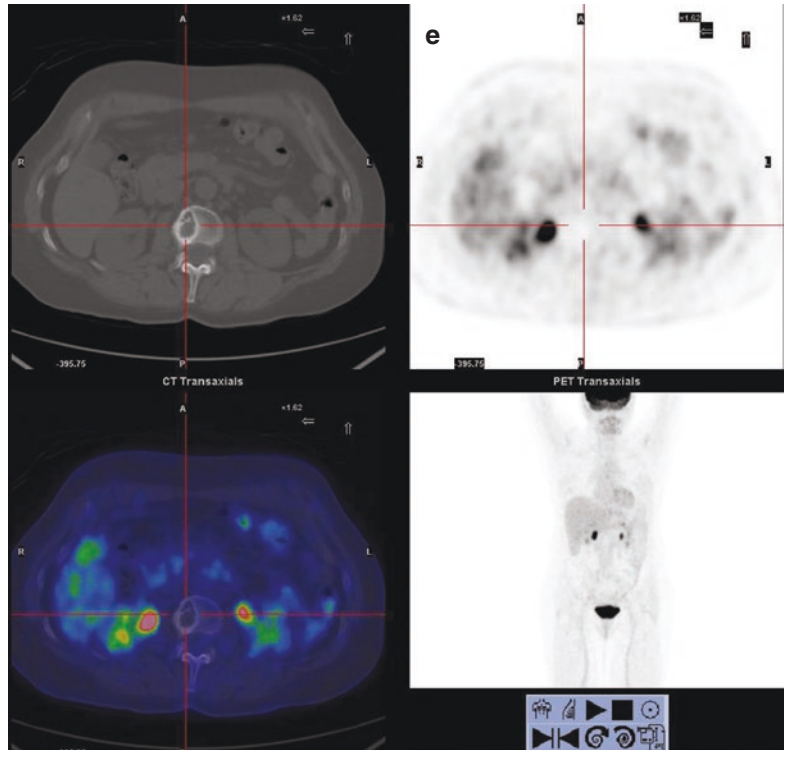


Fig. 3 Identification of Distant Metastatic Disease; **(a)** Dx ^{131}I WBS (185 MBq) detects functioning residual thyroid tissue in the thyroid bed and a midline focus at the level of the mid-abdomen (likely in the lumbar spine); **(b)** Rx ^{131}I WBS performed seven days after RAI therapy (13.39 GBq) detects thyroid remnants and a midline focus, likely in the lumbar spine is again demonstrated; **(c)** ^{18}F-FDG-PET/CT performed 4 months after RAI therapy identifies a hypermetabolic focus, SUV 4.1, measuring 0.6 × 1.6 cm in a thoracic vertebral body; **(d)** Dx ^{131}I WBS (185 MBq) performed one year after RAI therapy. No evidence of residual functioning thyroid tissue and no evidence of vertebral metastasis; **(e)** ^{18}F-FDG-PET/CT performed one year after RAI therapy. Stable non-hypermetabolic lytic lesion within the vertebral body

Fig. 3 (continued)





in the ablation/adjuvant setting is based upon the surgical pathology findings and assessment of other risk factors rather than tissue dosimetry.

Preparation for RAI Ablation and Treatment

Preablation Whole-Body Scan

^{131}I WBS after primary surgery prior to RAI ablative or therapeutic doses is also still a matter of debate. The role of pretreatment WBS is to assess the amount of remnant thyroid tissue and to detect previously unknown metastases that might result in adjusting the RAI activity. In recent years, pretherapy WBS has been abandoned in many centers. The guidelines suggest that preablation WBS may be useful if the extent of thyroid remnants or residual disease cannot be assessed from the surgical report or neck ultrasonography and if the scan findings would alter the decision to treat or RAI activity selection [28–31].

There is concern by some that pretreatment WBS will produce "stunning." This was first described by Rawson et al. Stunning is defined as a condition in which the first diagnostic dose of ^{131}I reduces subsequent trapping of RAI so that the uptake of the subsequent therapeutic dose of ^{131}I is impaired [45]. Others disagree or at least conclude that stunning does not occur if low diagnostic activity in range of 74–185 MBq is used [46]. An alternative to avoid stunning is the use of pure γ emitters (^{123}I or ^{99m}Tc) with a shorter half-life. However, the disadvantages of ^{123}I use include lower imaging sensitivity and higher cost of application [31].

Patient Preparation

To increase the effectiveness of RAI ablation, patients should be prescribed a low-iodine diet before treatment. According to a recently published systematic review, daily iodine intake ≤50 μg of iodine for 1–2 weeks before treatment is associated with increased RAI uptake and reduced urinary iodine excretion [47]. Patients are advised to avoid food and medications containing iodine (i.e., amiodarone, expectorants, topical antiseptics). Before discontinuing any medications, patients should contact their prescribing physician. In addition, diagnostic procedures using iodine-rich X-ray contrast media should be avoided for several weeks.

There are differences of opinions about the influence of a low-iodine diet on the efficacy of remnant ablation. Higher success rate for RAI ablation was reported in patients prepared with low-iodine diet compared to patients without low-iodine diet preparation [48]. Other authors, however, did not observe significant differences in effectiveness of remnant ablation between the two groups [49].

Prior to RAI ablation, thyrotropin stimulation has been established as a routine procedure. It has been suggested that TSH ≥30 mU/L is required for optimal uptake of RAI by remnant thyroid tissue [50]. This can usually be achieved 4–6 weeks after a total or near-total thyroidectomy if thyroid hormone replacement is withheld. Patients receiving hormone replacement [levothyroxine (LT4)] must discontinue the treatment 4–6 weeks prior to ablation. Patient preparation is also possible by substituting T3 for LT4 and then stopping medication for at least 2 weeks, thus shortening the discomfort. A modern and convenient alternative involves maintaining thyroid hormone replacement and administering recombinant human thyrotropin (rhTSH, Thyrogen®), two 0.9 mg IM injections 24 h apart just prior to RAI administration. The use of rhTSH was approved in the USA by the Food and Drug Administration in 1998 and in Europe by the European Agency for the Evaluation of Medical Products in 2001. This technique is well tolerated; the main advantage is to increase TSH levels without inducing hypothyroidism. In addition, rhTSH is necessary for patients unable to produce TSH, particularly hypopituitary patients or those with a significant tumor burden producing thyroid hormone. The use of rhTSH is of particular importance in patients in whom hypothyroid-related complications might worsen associated comorbidity (unstable coronary artery disease, psychiatric disease, congestive heart failure, respiratory or cen-

tral nervous system compromise) [51]. Since during the thyroid hormone withdrawal, patients suffer hypothyroid symptoms and are not able to resume work and physical activities, preparation with rhTSH for RAI ablation provides better quality of life [52, 53]. In a meta-analysis of pooled data from 1535 patients, similar remnant ablation success was observed in both groups comparing patients prepared with rhTSH and those who underwent thyroid hormone withdrawal [54]. However, high cost renders rhTSH unavailable for many countries.

Regulations

In many countries, RAI therapy is performed in a special radiation-protected area of the hospital. This area usually includes single-bed shielded rooms with separate bathrooms (toilets) built and approved for this purpose by the radiation protection agency or local radiation protection personnel. After the administration of RAI, patients remain hospitalized under the supervision of personnel qualified to work in a radiation zone. The duration of hospitalization depends on the administered RAI activity and local and state regulations. Patients should receive written and oral information prior to treatment. Patients are discharged when the total body radioactivity falls below the permitted level [55, 56]. In some countries, such as the USA, if permitted by local regulatory agencies, RAI therapy can be administered on an outpatient basis. In several studies monitoring exposure of family and others involved in outpatient care of patients treated with activities of ^{131}I between 2.8 and 5.6 GBq, no exposures above regulatory levels were detected. This method has been shown to be cost-effective [57].

Precautions and Side Effects

Pregnancy should be excluded before administration of RAI therapy. Breast-feeding should be discontinued for at least 6–8 weeks before the treatment, to allow regression of the stimulated glandular tissue. After RAI treatment, contraception is recommended to avoid pregnancy for at least 6 months [31].

Radioactive iodine is administered as a sodium iodide either as a liquid or capsule, the latter being more convenient and safer for handling. Adequate hydration is suggested during hospitalization. To simulate salivary glands and minimize side effects, some practitioners prescribe diluted lemon juice for 2–4 days after the RAI treatment as well as sour sucking candies or chewing gum. Jentzen et al. evaluated the dosimetry of salivary glands by using ^{124}I PET/CT and detected that lemon juice shortly after RAI administration increased the radiation absorbed dose to the salivary glands [58].

Patients scheduled for RAI ablation should have baseline complete blood count and assessment of renal function. Special procedures are needed for patients with kidney malfunction. Renal failure reduces RAI clearance and results in retention of RAI in the patient's body. Depression of bone marrow may appear if red marrow is exposed to excessive radiation doses during treatment of thyroid cancer. Radiation doses to red marrow depend upon the activity administered and the rate of RAI elimination from the body [59].

RAI therapy in patients requiring renal dialysis involves several important issues: the RAI activity needs to be modified, the pre- and post-therapeutic dialysis sessions need to be adjusted in regard to the RAI administration, and radiation safety issues need to be considered during and after the dialysis. Although some practitioners use empirically adjusted ^{131}I activity (25–50% of that prescribed for patients with normal kidney function) [60–62], the best option is to use dosimetry when available. Dosimetry should be performed to adjust RAI activity, so that red marrow and total body radiation absorbed dose does not exceed that in patients with normal renal function [59, 63]. All dosimetric methods for RAI treatment depend on assessment of the kinetics of a tracer dose of ^{131}I prior to treatment [64]. Since the dosimetry is a complex subject, it will not be reviewed in details in this chapter.

A pre-radioiodine treatment dialysis should be performed immediately before the RAI adminis-

tration so that the patient's plasma pool of stable iodine will be as low as possible. The timing of the first posttreatment dialysis session, however, varies among the different centers. Some suggest that optimal timing is 24 h following the RAI treatment in order to reduce the whole-body radiation dose [61, 65]. Others however, in order to make sure that the thyroid radioiodine uptake will reach its maximum, recommend that the first post-RAI treatment dialysis be scheduled at 48 h [62, 66]. Only the first post-therapy dialysis session needs to take place during the ^{131}I therapy hospitalization.

RAI ablation is generally well tolerated and rarely brings complications. Short-term complications, if they occur, are often transitory and mild. The most common adverse effect is transient sialoadenitis during the first 72 h after the administration. Symptoms include salivary gland pain, swelling, dry mouth, and metallic taste, which may last for several weeks. Ample hydration and sour candy are given during the first 24 h to promote saliva secretion [67–70]. Although sialoadenitis is a transient complication in most of the cases, it may persist for months or appear as a long-term side effect. Chronic sialoadenitis associated with xerostomia depends on the administered RAI activity, increasing the risk with larger RAI doses [71]. In addition, some reported that chronic salivary gland toxicity was more common in patients receiving multiple RAI courses [72].

Painful thyroiditis is more likely to occur in patients with large postoperative thyroid remnants and may be associated with neck swelling and recurrent laryngeal nerve paralysis. Nausea and sometimes vomiting may occur 2–8 h after the treatment but usually resolve after 1–3 days. Bone marrow toxicity develops in 5% of patients; it includes thrombocytopenia and leucopenia and is usually transient. It is dose dependent and most often appears after administration of large cumulative activities and in patients with disseminated bone metastases [73].

Long-term complications are rare. Radiation pneumonitis can be avoided if the lungs localize less than 80 mCi at 48 h. This can be determined based on quantification following pre-therapeutic WBS with 5 mCi or less of ^{131}I or ^{123}I. Aside from the acute morbidity involved with radiation pneumonitis, there is concern that it could lead to pulmonary fibrosis. Pulmonary fibrosis, in fact, presents as a serious long-term complication that may occur in patients with diffuse lung metastases receiving multiple RAI courses in a short time, those who receive high doses, or both [79].

Gonadal tissue is irradiated by the RAI present in the blood, urine, and feces. However, radiation exposure of gonads is reduced with adequate hydration, proper and frequent emptying of the bladder, and avoiding constipation [74]. Administration of a single RAI treatment usually has low gonadal irradiation. At higher activities, greater than 5.55 GBq (150 mCi), RAI treatment may result in transient hypospermia associated with increased serum follicle-stimulating hormone (FSH) [75, 76]. Large RAI cumulative activities (18.2 GBq or 800 mCi), however, increase risk of permanent FSH elevation [77–79]. In young males who may receive high cumulative RAI activity >400 mCi (14.8 GBq), sperm banking is suggested before RAI treatment. Some advocate conception for 3 months, in order to prevent eventual temporary chromosomal damage [76].

In women treated with RAI, transient amenorrhea/oligomenorrhea has been observed [80]. Results obtained on small number of patients indicate that long-term rates of infertility, miscarriage, and fetal abnormalities are not increased in women after RAI treatment [81–83]. Some authors suggest that pregnancy should be postponed for 1 year after the RAI treatment because of an increased miscarriage rate [84], although this was not confirmed in their recent study [85]. In addition, ovarian damage therapy may influence slightly earlier onset of menopause in women who received RAI therapy than in general population [80].

The risk of secondary neoplasm of bone, breast, colorectal, kidney, salivary cancer and leukemia in long-term DTC survivors treated with RAI treatment is very low [86, 87]. Increased risk of leukemia is observed in patients younger than 45 years treated with RAI [88]. Although an early increased risk for thyroid carcinoma

patients to latter develop breast carcinoma is detected, the potential role of RAI treatment is still unresolved [89]. However, the risk (if any) is dose related and increases with larger administered activities. Patients treated with cumulative ^{131}I activity above 22.2 GBq (600 mCi) have an increased risk of second malignancy [87]. In order to reduce radiation exposure of the bowel, laxatives are recommended and hydration to reduce exposure of the bladder and gonads [31].

Follow-Up and Outcome of Disease

Post-therapy whole-body scans are performed 3–7 days after the RAI ablation. In addition to the postoperative thyroid remnants, ^{131}I WBS may detect previously occult metastases. Large thyroid remnants may produce a "star" artifact at ^{131}I scintigraphy that can mask detection of cervical, less likely mediastinal or pulmonary involvement. In about 20% of cases, the post-therapy WBS upstages patients. Additionally, SPECT/CT increases the specificity of the post-therapy scan [55].

Ablation success is evaluated 6–12 months after the treatment. According to the ATA Guidelines, the criteria for successful ablation include no clinical or imaging evidence of tumor and low Tgb serum levels (on LT4 treatment, Tgb <0.2 ng/mL or stimulated Tgb, <1 ng/mL) [29].

RAI Treatment in Children and Adolescents

Juvenile differentiated thyroid carcinoma is a rare neoplasm, accounting for 0.5–3% of all malignant tumors in childhood. DTC incidence of 7% and 10% is reported in prepubertal and adolescent period, respectively [90]. Juvenile DTC often has an aggressive initial presentation, with involvement of cervical lymph nodes and frequently distal metastases. Compared to the adult population, however, the overall mortality rate in children is not increased. Usually, juvenile DTC has a good clinical outcome. Statistical analysis of the overall 5-year survival rate and comparison between 1975 and 2010 revealed improvement, 97.5% vs. 99.6%, respectively, with only 0.1% of thyroid cancer deaths in patients <20 years old [91]. Disease-specific survival of 98% was reported 30 years following the initial surgery [92, 93]. However, the recurrence rate is high, with the probability of recurrence, 16.7% at 5 years, 22.3% at 10 years, and 33.3% at 15 and 23 years after the primary treatment [92].

Several prognostic factors were reported to significantly influence the development of recurrence. The extent of surgery proved to be the most important prognostic factor for disease-free survival in children. Less radical primary surgery increases the relapse rate. The risk of recurrence was 10 times higher (range 2.3–39.1) among children who undergo less extensive surgery [94]. In another study, a significantly higher recurrence rate was obtained after hemithyroidectomy than after total thyroidectomy (38% vs. 7.5%, respectively) [95]. In addition, patients postoperatively treated with RAI show lower risk for recurrence than patients who did not receive RAI treatment [92, 94, 96]. Although some report that age is not a significant risk factor for recurrent disease [97, 98], there are opposing studies claiming that age at diagnosis is the significant patient-related factor in univariate analysis [92, 94]. In general, younger children have a higher risk of relapse [94, 99, 100], particularly among patients ≤10 years old [92, 94, 101]. Tumor multifocality is reported as additional predicting factor—patients with multifocal tumors show higher recurrence rate than those with unifocal tumors [92, 102].

Differentiated thyroid cancer in children and adolescent is usually treated the same way as in adults. Until last year, there were no guidelines specifically addressing treatment of childhood DTC. In 2015, the American Thyroid Association Guidelines Task Force on pediatric thyroid cancer released management guidelines for children with thyroid nodules and differentiated thyroid cancer. The aim was to make recommendations for the optimal care for children and adolescent with DTC. These guidelines, however, are not based on randomized double-blinded controlled

clinical trials. The guidelines are almost exclusively based on retrospective studies with limited follow-up. The guidelines recommend a selective use of RAI in children with DTC because of concern about the increased risk of second malignancies. According to the guidelines, the AJCC TNM classification system should be used in pediatric patients with papillary thyroid cancer for postoperative stratification into the three risk levels: low, intermediate, and high. The aim of this stratification is to select patients who may or may not benefit from additional treatment (surgery and/or RAI therapy) based on clinical presentation, tumor size, and metastases. ATA low-risk patients have disease limited to the thyroid gland and evidence of microscopic tumor foci in the lymph nodes level VI. Ablation is not recommended for this category. Extensive extrathyroidal invasion and metastases are increased risk factors. Intermediate-risk level patients have extensive N1a or minimal N1b disease, while patients with extensive N1b or locally invasive disease (pT4 tumors) with or without distant metastases are stratified into high-risk level category. Decision about RAI treatment in patients from the intermediate- and high-risk categories should be based on TSH-stimulated Tgb and ^{123}I WBS. RAI therapy should be considered in patients with thyroid bed uptake and T4 stage or known residual microscopic disease [103].

There are several rationales which are important for selection of the amount of RAI used for ablation in childhood: longer life expectancy, higher sensitivity to possible complications after treatment, and smaller body and organ size resulting in greater bone marrow and extrathyroidal tissue radiation dose and higher cross-radiation [31].

Some practitioners calculate the RAI activity adjusted for the body weight (1.85–7.4 MBq/kg) or the patient's age (1/3 of the adult activity is administered to a 5-year-old; 1/2 of the adult activity in a 10-year-old; and 5/6 of the adult activity in a 15-year-old) or the surface area [104]. The German guidelines for RAI therapy in pediatric DTC patients suggest adjusted iodine activity by the 24-h thyroid bed uptake of an iodine test activity and by body weight: <5% uptake should be appropriate to 50 MBq/kg; 5–10% uptake should be appropriate to 25 MBq/kg; and 10–20% uptake should be appropriate to 15 MBq/kg [105]. RAI ablation based on patient characteristics (weight, surface area, thyroid bed radioiodine uptake) seems to be an adequate strategy versus fixed dosing or flexible dosing based on age.

If RAI ablation is not successful, several approaches may be considered. In patients with small persistent residues without influence on the outcome, additional RAI treatment is not recommended—careful monitoring only may be sufficient. In patients with large persistent tumors, second surgery should be the first treatment option [30]. In patients with extensive lung metastases, there is concern about high radiation absorbed dose to the lungs; dosimetry should be performed. If retained radioiodine activity in the lung would exceed 3 GBq, the dose is reduced to avoid the risk of RAI-induced lung fibrosis [106, 107].

RAI treatment in juvenile DTC appears otherwise to be safe without complications. In a 30-year follow-up study, there were no effects on subsequent fertility and pregnancy outcome and no secondary malignancies [96, 100]. Nevertheless, there remains concern about second malignancies in children after the administration of RAI therapy. A study combining patients of all ages indicated that RAI therapy is associated with an increased risk for second malignancies and an increased mortality for DTC patients [87]. After a review of data collected on 30,000 subjects, a significant increase in second malignancies in patients treated with RAI was reported, particularly in patients younger than 45 years [86]. In a report of children treated with external radiation therapy, radium implants, or ^{131}I, a variety of secondary malignancies was detected, such as leukemia and salivary gland, breast, colon, and bladder tumors including an isolated case of acute myelogenous leukemia after 3.1 GBq, lung cancer after 5.55 GBq ^{131}I, and adenocarcinoma after 7.4 GBq ^{131}I [93]. Since the number of reported sporadic cases of secondary malignancies is very low, it is impossible to determine if they are related to RAI treat-

ment or not. Nevertheless, some authors suggest a limit of 7.4 GBq or 11.1 GBq cumulative activity, beyond which the risk for second malignancy may increase [87, 108]. However, a safe cumulative exposure to RAI has not exactly determined; additional studies are needed.

Treatment of Metastatic DTC

In patients with functioning residual, recurrent, or metastatic thyroid carcinoma, with detectable Tgb levels and RAI uptake on WBS, additional RAI treatment is recommended. "Wait and see" strategy is, however, considered in patients with small residual or persistent tumor tissue and stable disease. Patients with large persistent tumors should be evaluated for surgical tumor excision [31].

The most frequently so-called fixed method is recommended with the use of standard fixed activities in the range 3.7–7.4 GBq (100–200 mCi). Beierwaltes chooses an adjusted activity based on the involvement site: tumor in the thyroid bed is treated with up to 5.55 GBq (150 mCi), lymph node metastases with 6.5 GBq (175 mCi), and metastases outside of the neck with 7.4 GBq (200 mCi) [109].

Metastatic disease in DTC may be detected at presentation; however, 5–20% of patients develop local or regional recurrences during the follow-up period. In general, papillary thyroid carcinoma extends via lymph node to the lungs, while follicular thyroid cancer spreads hematogeneously to the lungs and bones [56].

In patients with known or suspected of metastatic disease beyond cervical lymph node involvement, the largest safe dose is considered. The term "maximum tolerated dose" describes the largest safe dose in terms of bone marrow toxicity. In order to determine the radiation absorbed dose to a specific site, it is necessary to measure the uptake and clearance of ^{131}I in each lesion and the mass of the lesion or volume of distribution. The WBS images are obtained at different time points, up to 96 h after tracer administration, but later images might be necessary in some cases. Selected ROIs (regions of interests) on gamma camera (planar, SPECT, or ^{124}I PET) are required to determine the activity in the lesion. Additionally, the attenuation and scatter correction is suggested, obtained through transmission or scatter images [110].

The final calculations of the activity required to achieve the certain absorbed dose are usually based on modifications of the generic MIRD equation for the absorbed dose [111]:

$$\bar{D} = \frac{\tilde{A} x S x m_r}{m_t}$$

where $\bar{D}$ is the remnant or lesion absorbed dose, $\tilde{A}$ is the cumulative activity, m_r is the reference tissue mass and m_t is the remnant or lesion mass, and S is the MIRD-defined value for thyroid self-irradiation.

Bone marrow (blood) dosimetry was originally described by Benua et al. suggesting the use of maximum tolerated dose with the blood as the critical organ in order to avoid myelotoxic radiation effects on the bone marrow. This method is based on the clinical observation that there are no permanent adverse bone marrow effects if patients receive <2 Gy to the blood. This approach includes the measurement of radiation counts of serial blood samples and uptake probe measurements of the patient's whole-body activity during the 4- or more-day examination after administration of ^{131}I tracer. This method permits administration of doses >5.55 GBq (150 mCi) and even 12.95–18.5 GBq (350–500 mCi) or greater. Moreover, blood dosimetry identifies patients who cannot receive even 5.55 GBq safely [106]. Additionally, the whole-body retention at 48 h after the administration of ^{131}I should not exceed 2.96 GBq in patients with iodine-avid diffuse pulmonary metastases or 4.44 GBq if these lesions are absent [112].

In order to determine the activity of ^{131}I that will not exceed 2 Gy of blood absorbed dose, the EANM Dosimetry Committee has recently published guidelines for standard procedure in dosimetry. The detailed measurements and calculations are described in detail in the guidelines [107].

Regional metastatic disease is located in cervical lymph nodes and accounts for 60–75% of all neck recurrences. Metastatic lymph nodes are usually located in the lower part of the jugulocarotid chains or in the central compartment. Involved regional lymph nodes in the central compartment of the neck and mediastinum may be associated with lung metastases. The incidence of recurrent disease as regional metastases depends on the extent of initial lymph node surgery. Locoregional recurrent disease is detected by elevated Tgb levels and ultrasonography. Fine-needle aspiration biopsy of suspicious lymph nodes should be performed under ultrasound or CT guidance, for cytological analysis and Tgb measurements on the aspirate. About 20% of DTC patients on thyroxine treatment having isolated metastatic lymph nodes do not show raising Tgb serum levels. However, Tgb levels following TSH stimulation remain undetectable in about 5% of patients with small metastases or previously treated with RAI. Pathologic foci are detected on ^{131}I WBS in 60–80% of DTC patients with clinical lymph node metastases particularly if higher activities of ^{131}I are used [113].

Regional metastases detected on WBS may be treated by RAI therapy alone or as adjuvant therapy following surgical removal. In patients with macroscopic bulky malignant nodal disease, surgery should be the first-line treatment. In patients with small metastatic lymph nodes, RAI therapy should be administered due to the ability of ^{131}I to eradicate tumor foci <1 cm in diameter [29, 113].

Other options include external beam therapy or localized treatment—including thermal ablation, ethanol ablation or chemo-embolization, and thyroid hormone suppressive treatment for patients with stable disease or slowly progressive disease [114–116]. In patients with reduced NIS (natrium iodine symporter) expression and loss of capacity for iodine uptake, further RAI treatment is not recommended. Recently, there has been progress in pharmacological manipulation of the iodide transport mechanism. A class of drugs called tyrosine kinase inhibitors has shown promise in reversing the loss of the ability to trap iodine [117, 118]. This is a complex subject that is still evolving and is discussed in greater detail in another chapter.

Metastatic disease at distant sites appears in about 10–21% of DTC patients [113, 119]. The most frequent location of distant metastases is the lungs (49–57%), while other sites are less frequently involved: skeletal metastases in 24–25%, lung and bone metastases in 15%–16%, and brain or other soft tissues in 10% [120, 121].

It is important to consider several criteria for treatment of DTC patients with pulmonary metastases, such as the size of metastases (micro- or macronodular metastatic disease), avidity for RAI, and the patient response to previous RAI treatment. It is recommended to treat patients with RAI-avid pulmonary micrometastases (<2 mm, often not detected on anatomic imaging) with repeated activities of ^{131}I every 6–12 months. The RAI activity for treating patients with lung micrometastases may be selected empirically (100–200 mCi or 100–150 mCi for patients older than 70 years) or be estimated by dosimetry to limit whole-body retention to 80 mCi at 48 h and 200 cGy to the bone marrow [29]. Some authors report that DTC patients with iodine-avid lung micrometastases achieve the best rates of complete remission after RAI treatment [122–125].

Macronodular RAI-avid pulmonary metastases are preferably also treated with RAI. The decision about the number of RAI courses is made based on the patient's response to treatment, the patient's age, and general condition [121, 122]. Complete remission, however, in these patients is rare and the outcome is poor. Radioiodine therapy may be performed using empirical fixed activities or blood and whole body (blood or bone marrow) dosimetry.

While metastatic lymph node, lung, and soft tissue metastases show high rates of remission, bone and brain metastases are seldomly cured. Bone metastases are rarely curable with RAI therapy, but there are reports about beneficial effects on some patients. Other therapeutic options also may include external beam therapy or systematic treatment [113]. Surgery is the treatment of choice for bone and brain metastases [121].

Distant metastatic disease shortens the life duration of DTC patients, with reported disease-specific survival of 63% after 5 years and 49% after 10 years and 38% after 15 years [126, 127].

In addition, patients with multiple sites of distant metastases have a poorer prognosis, with none alive after 10 years [128]. The iodine avidity of metastases is an important prognostic factor. It has been reported that there is a significant reduction of disease-specific survival in patients with non-iodine-avid tumors. A 10-year disease-specific survival was significantly reduced in patients with non-iodine-avid lung metastases compared to patients with iodine-avid metastatic disease (55% vs. 18%, respectively) [129].

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Dosimetry for Iodine-131 Therapy for Metastatic Differentiated Thyroid Cancer

Douglas Van Nostrand

Definitions of Terms Used in Dosimetry and ^{131}I Therapy

To understand dosimetry in the selection of prescribed activity of ^{131}I for treatment of patients with differentiated thyroid cancer, the definitions of terms are important to reduce misunderstandings.

Remnant Ablation, Adjuvant Treatment, and Treatment of Distant Metastases

In differentiated thyroid cancer, ^{131}I therapy may be for *remnant ablation*, *adjuvant treatment*, or *treatment of distant metastases*. The objective of *remnant ablatio*n is to destroy normal thyroid tissue remaining after initial thyroid surgery with the objectives of maximizing the utility of serum thyroglobulin levels as a tumor marker for following the patient with DTC and maximizing any future radioiodine scan and/or ^{131}I therapy. The objective of *adjuvant treatment* is to treat suspected but unproven residual DTC to reduce recurrence, increase progression-free survival, and/or increase cure. The objective of *treatment of metastases* is to treat known residual local or distant thyroid cancer to increase survival and/or palliation.

Dosimetry

The term *dosimetry* is used in many different ways. In radiation oncology, it is used to refer to the determination of a treatment plan to deliver a specific amount of radiation to the patient's tumor using external radiotherapy. Within radiation safety programs for radiation safety workers or individuals in the public, dosimetry is used to refer to the monitoring of the exposure of radiation workers and/or individuals in the public to internal or external radiation sources. In nuclear medicine and specifically for the treatment with ^{131}I for differentiated thyroid cancer, it is used in two ways: (1) for the calculation of a maximum tolerated activity (MTA) of ^{131}I that can be administered to a given patient and that would not exceed some specified radiation-absorbed dose to the blood (e.g., bone marrow) and (2) for radiation dose that would be delivered to a metastatic lesion. This usage of the term is identical to its traditional usage in external radiotherapy.

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Dose

The term *dose* is used in two ways: the amount of *radioactivity administered* to a patient or the amount of *radiation exposure*. They are related but are distinctly different. Using the former definition, the amount of radioactivity administered to the patient is measured using international units of becquerel or, in the United States, millicuries. One becquerel is defined as the activity of a quantity of radioactive material in which one nucleus decays per second [1]. The second use of *dose* is very important in the understanding of the objectives of dosimetry, and it is measured using international units of gray (Gy) or, in the United States, rad. Because it is important to distinguish whether one is speaking about the *amount of radioactivity* or the *amount of radiation exposure*, the terms *prescribed activity* and *dosage* are frequently used in formal writing and scientific discussions in place of the term *dose*, for the amount of radioactivity, with *dose* reserved for the *amount of radiation exposure.* These terms will be used in this chapter, helping to reduce the confusion that results when the term *dose* is used in relation to two very different concepts. Radiation exposure, or more correctly phrased *absorbed dose*, is the concentration of energy deposited in the tissue as a result of an exposure to ionizing radiation. In this case, it means the energy absorbed by human tissue.

Of note, even though the exact same amount of *prescribed activity (dosages)* of ^{131}I may be administered to two patients for a therapy for DTC, the *dose* (absorbed dose) to each patient's tumor and/or normal organs may be significantly different and a result of many factors (see Table 1). The units of *prescribed activity* (*dosage*) and dose and the international to the United States conversions are noted in Table 2.

Dose may also be subdivided into *radiation-absorbed dose*, *equivalent dose*, or *effective dose*, terms beyond the scope of this chapter; information of increased detail is available from Vetter et al. [2].

Table 1 Factors that may result in different absorbed doses to normal organs and/or tumors for the same administered prescribed activity in two different patients

– Absorption of ^{131}I
– Size of patient
– Uptake of ^{131}I by normal thyroid tissue
– Residence time of ^{131}I in normal thyroid tissue
– Uptake of ^{131}I in thyroid cancer
– Residence time of ^{131}I in thyroid cancer
– Renal function
– Rate of clearance of ^{131}I from the whole body
– Patient's thyroid hormone levels
– Aggressiveness to reduce side effects by increasing clearance of ^{131}I from normal tissue prior to, during, and after ^{131}I treatment
– Others

Table 2 Units of ^{131}I prescribed activity (dosage) and radiation-absorbed dose

Prescribed activity (*dosage*)
Standard international units
• Becquerels (Bq): 1 disintegration per second
• Megabecquerels (MBq): 10^6 Bq
• Gigabecquerels (GBq): 10^9 Bq)
Non-international units
• Millicuries (mCi): $3.7 \cdot 10^{10}$ disintegrations per second
• Curies (ci) = 10^3 mCi
Conversions of Bq vs. mCi"
• 1 Bq = 2.7×10^{-11} ci = 2.7×10^{-5} μCi
• 1 MBq = 0.027 mCi
• 1 μCi = 37,000 Bq = 37 kBq
• Ci: 3.7×10^{10} Bq = 37 GBq
Radiation-absorbed dose (*dose*)
Standard international units
• Grays (Gy): the absorption of one joule of energy in the form of ionizing radiation, per kilogram of matter
• 1 gray = 1000 milligray (mGy)
Non-international units
• 1 rad = 1000 millirad (mrad)
Conversion of rad vs. gray
• 1 Gy = 100 rad
• 1 cGy = 1 rad

*Equivalent dose and effective dose are not discussed here

Dosimetry

Fundamentals of External Radiotherapy

The two fundamentals of external radiotherapy are to (1) determine and minimize the absorbed dose to the normal tissues and (2) determine and deliver an absorbed dose to the tumor that is effective in controlling the tumor with an objective such as cure, adjunctive therapy, or palliation. Although these fundamentals also apply to internal radiotherapy when radioisotopes (e.g., ^{131}I) are administered into a patient, calculating the absorbed dose to normal tissues and tumor(s) prior to actually administering the radioisotope internally is more problematic. As a result, facilities and nuclear medicine physicians employ two approaches to select the prescribed activity of ^{131}I to be administered.

Empiric Vs. Dosimetrically Determined Prescribed Activity of ^{131}I for Treatment of DTC

The two approaches for determining the prescribed activity (dosage) of ^{131}I for the treatment of metastatic DTC are the *empiric* and the *dosimetric*. The empiric approach is based on the experience of one individual or a group of individuals such as at a specific facility, and frequently these empiric approaches have been "handed down" over the years with or without various modifications. Several empiric approaches are shown in Table 3 [3–8]. Of note, the empiric approaches listed in Table 3 do not necessarily imply that the respective authors continue to use these approaches. Rather, Table 3 data demonstrate the wide spectrum of empirically prescribed activities that have been and/or are continuing to be used for the determination of the prescribed activity of ^{131}I for the treatment of DTC. The dosimetric approach is based on either or both objectives: calculating the maximum prescribed activity that will not exceed more than a predetermined maximal tolerable absorbed dose to a specific normal organ, the latter frequently called the *critical organ*, and/or calculating the minimum prescribed activity to deliver an effective therapeutic absorbed dose to the patient's tumor(s). There are also several approaches to performing the *whole body* and *lesional d*osimetries, which are discussed in Sects. "Whole Body Dosimetry" and "Lesional Dosimetry," but the objectives and concepts are essentially the same.

Table 3 Examples of empiric approaches for ^{131}I prescribed activity for treatment of distant metastases

Beierwaltes [3]	For the lung, 6.58 GBq (175 mCi); for the bone, 7.3 GBq (200 mCi)
Schlumberger et al. [4]	3.6 GBq (100 mCi) every 2–6 months until post-therapy scan is negative
Petrich et al. [5]	If metastases identified on post-therapy scan after ablation with 3.7 GBq (100 mCi), then immediately retreat with 7.4 GBq (200 mCi) Next follow-up treatment at 4–6 months with mean activity of 1.11 GBq (300 mCi)
Brown et al. [6]	5.55 GBq (150 mCi) every 3 to 4 months until scan is negative or there is evidence of progression
Menzel et al. [7]	11.1 GBq (300 mCi)
Hindié et al. [8]	If + lung uptake and neg chest-X-ray: 3.7 GBq (100 mCi) every 6 months. If uptake is present in lungs after cumulative dosage of 18.5 GBq (500 mCi), reduce to 1 year and then every 2 years

*This table does not necessarily mean that these authors do or do not continue to use the empiric prescribed activities noted herein; rather the data are included to demonstrate the spectrum of empiric prescribed activities that have been, and/or are continuing, to be used

The Fundamentals of ^{131}I Dosimetry

To perform radioiodine dosimetry, we need to know (1) the types of radiation that had been emitted in each disintegration such as a beta particle (e.g., electron) and/or a photon (e.g., gamma ray), (2) the number of disintegrations that occurred in each organ over time, and (3) the fraction of the total energy of each type of radiation that had been released in a given organ that is absorbed in another organ or itself. The data for the first item is obtained from the physics litera-

ture [9], and the data for the second item is from measurements of the uptake and clearance of the radioactivity within a specific patient, which is discussed in Sect. "Whole Body Dosimetry." For the third item, one needs to know not only the absorption and penetration characteristics of the various radiations emitted but also the size, shape, volume, and geometry of the various organs within that specific patient. Ideally, one would want to perform direct measurements of the absorbed dose at selected points or organs within each specific patient. However, as an alternative, we use theoretical estimates from models and measurements performed in standardized humanoid phantoms. More detailed discussions of dosimetric calculations are beyond the scope of this chapter but are available [10, 11]. The following is an overview of the acquisition of the patient-specific data to calculate the maximum tolerated activity to the whole body and lesion dosimetry, display of data, and additional restrictions.

Whole Body Dosimetry

Patient Preparation and Data Acquisition

In order to gather the data to perform the patient's ^{131}I dosimetry, the patient is started on a low-iodine diet, and the patient's thyroid-stimulating hormone (TSH) levels are increased either by withdrawing the patient's thyroid hormone (THW) or by injecting the recombinant human thyroid-stimulating hormone (rhTSH). After the TSH levels are increased, the patient is administered orally a low diagnostic prescribed activity of ^{131}I, typically in the range of 37–74 MBq (1–2 mCi) or, in some facilities, as high as 148 MBq (4 mCi). At periodic times, such as 2, 24, 48, 72, and 96 hours after that administration, blood samples and counts of the radioactivity in the whole body of a specific patient are obtained (see Fig. 1). The whole body counts may be obtained with either an uptake probe or a gamma camera. If a gamma camera is used, the length of

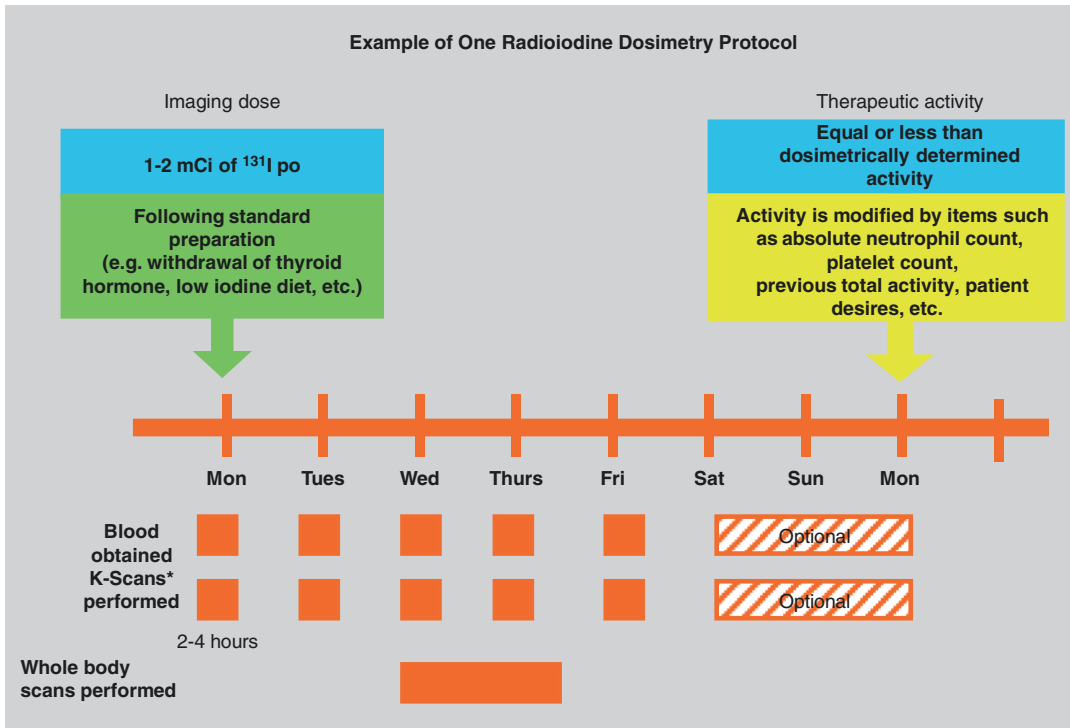


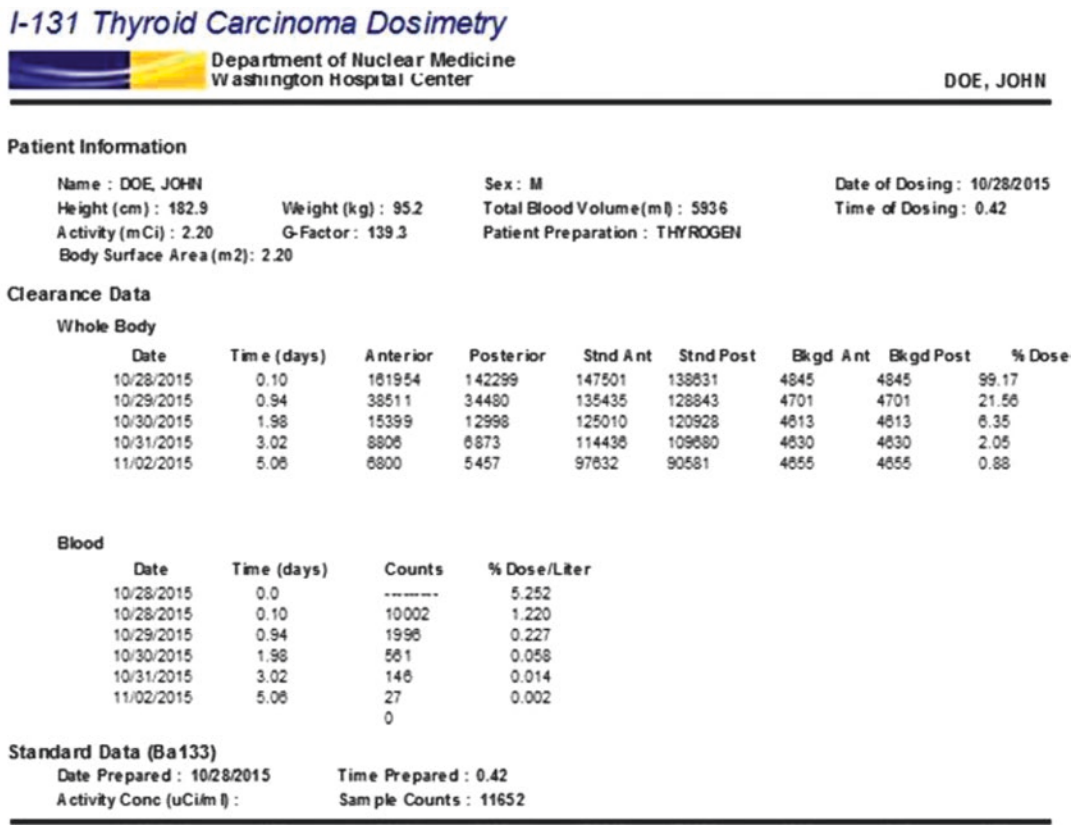
Fig. 1 It is an example of one dosimetric protocol, and there are variations thereof, including preparation with recombinant human thyroid-stimulating hormone, (rhTSH), prescribed activity of ^{131}I, urine collections, etc. (Reproduced with permission from Keystone Press, Inc.)

acquisition of the whole body count is significantly less than the length of acquisition of a diagnostic scan (e.g., 10 min vs. 30 min to 1 h). In our facility, these scans for whole body counts are called *kinetic scans* or *K-scans*. At approximately 48 h, a diagnostic whole body scan is obtained and again longer acquisition time is used. Typically, additional pinhole images of the thyroid bed, selected regional ("spot") scans, and/or SPECT-CT scans (*s*ingle-*p*hoton *e*mission *c*omputer *t*omography scans) are obtained. All of the blood samples that are obtained are counted together at the end of the procedure, and the whole body and blood counts are all corrected for decay of the radioisotope. Figure 2 provides a copy of the printed data. The 48-h images are used for identifying sites of possible metastases.

The dosimetry protocols at various facilities are for the most part conceptually the same, but over the years, these facilities have implemented various modifications with the objectives of simplification and/or improvement. These modifications include (1) elimination of the urine collection; (2) replacement of the use of an uptake probe with a gamma camera for whole body counting; (3) use of geometric means for whole body counting, timing, and number of data points; (4) analytical curve fitting techniques; (5) reduction of the number of blood samples obtained; (6) transition from the classical model (e.g., Marinelli et al. [12]) to the MIRD (Medical Internal Radiation Dose) schema; (7) biphasic model rather than a mono-exponential model; and (8) "mock" ^{131}I standard for the prior standard (e.g., Ba-133; 10.6-year half-life) [10].

Data Display

With these data, the time activity curves (TACs) are obtained, and examples of TACs representing the level of activity in the whole body and blood



I-131 Thyroid Carcinoma Dosimetry

Department of Nuclear Medicine
Washington Hospital Center

DOE, JOHN

Patient Information

Name : DOE, JOHN
Sex : M
Date of Dosing : 10/28/2015
Height (cm) : 182.9
Weight (kg) : 95.2
Total Blood Volume(ml) : 5836
Time of Dosing : 0.42
Activity (mCi) : 2.20
G-Factor : 139.3
Patient Preparation : THYROGEN
Body Surface Area (m2): 2.20

Clearance Data

Whole Body

Date	Time (days)	Anterior	Posterior	Stnd Ant	Stnd Post	Bkgd Ant	Bkgd Post	% Dose
10/28/2015	0.10	161954	142299	147501	138631	4845	4845	99.17
10/29/2015	0.94	38511	34480	135435	128843	4701	4701	21.56
10/30/2015	1.98	15399	12998	125010	120928	4613	4613	6.35
10/31/2015	3.02	8806	6873	114436	109680	4630	4630	2.05
11/02/2015	5.06	6800	5457	97632	90581	4655	4655	0.88

Blood

Date	Time (days)	Counts	% Dose/Liter
10/28/2015	0.0	-------	5.252
10/28/2015	0.10	10002	1.220
10/29/2015	0.94	1996	0.227
10/30/2015	1.98	561	0.058
10/31/2015	3.02	146	0.014
11/02/2015	5.06	27	0.002
		0	

Standard Data (Ba133)

Date Prepared : 10/28/2015
Time Prepared : 0.42
Activity Conc (uCi/ml) :
Sample Counts : 11652

Fig. 2 It demonstrates the display of the data obtained as part of dosimetry (Reproduced with permission from Keystone Press, Inc.)

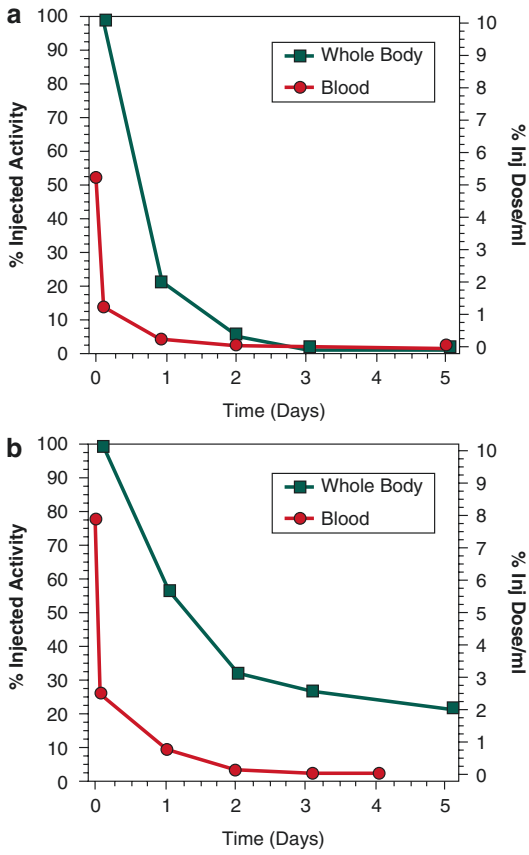


Fig. 3 (**a**) and (**b**) demonstrate time activity curves (TAGs) with activity on the *Y* axis and time on the *X* axis. The whole body counts are shown in blue, and the blood counts are shown in red. These TACs represent the clearance (e.g., washout) of radioactivity from the whole body and blood (e.g., bone marrow), respectively. The areas under the blue and red curves are proportional to the absorbed dose to the whole body and blood. The patient shown in **a** has relatively faster clearance than the patient in **b**, and the faster clearance typically results in lower absorbed dose to the whole body and blood (e.g., bone marrow) per MBq of ^{131}I administered. In turn, this typically allows the administration of a potentially larger therapeutic prescribed activity relative to the patient in **b** (Reproduced with permission from Keystone Press, Inc.)

over time are shown in Fig. 3a, b. Figure 3a demonstrates a patient with very rapid clearance; Fig. 3b demonstrates a patient with much slower clearance. The patient in Fig. 3a with the rapid clearance will typically receive a less absorbed dose (e.g., cGy or rad) to the whole body, blood (e.g., bone marrow), and other normal organs per GBq (mCi) of ^{131}I administered; therefore, this patient's maximum tolerated prescribed activity will typically be higher than the patient in Fig. 3b.

Critical Organ

The *critical organ* is " … that part of the body that is most susceptible to radiation damage resulting from the specific exposure conditions under consideration, taking into account the dose the various parts of the body receive under the exposure conditions [13]." For ^{131}I therapy, the *critical organ* is the bone marrow, and the maximum tolerated absorbed dose is 200 cGy (rad). The latter was established based on studies on animals (e.g., mice, rats, pigs), accidental exposure to radiation workers, and atomic bomb survivors. Although the bone marrow is the critical organ, this does not mean that other organs are not susceptible to significant absorbed doses. The salivary gland is one such organ, but a maximum tolerated absorbed dose to the salivary gland has not been established. Although studies are ongoing, determining a threshold for maximum absorbed dose for the salivary gland is problematic as the calculation of an estimate of absorbed dose to the salivary gland per GBq (mCi) of ^{131}I administered is very complicated. The radiopharmacokinetics of iodine within the salivary gland changes minute by minute and significantly over time depending on stimuli [14]. In addition, the sodium iodine symporter is not uniformly distributed throughout the salivary gland but appears to be predominantly located in the striated ducts [15]. Again, further study is ongoing.

Data Calculations

A detailed discussion of the calculations and formulas to determine the maximum tolerated activity (maximum prescribed activity that will not exceed 200 cGy [rad]) to the blood (e.g., bone marrow) from the above data exceeds the scope of this chapter, but examples of some of the formulas that have been used and are still used are noted in Fig. 4.

In addition to the calculation of the maximum tolerated activity to the blood (e.g., bone marrow), Rall et al. [16], Benua et al. [17], and Leeper [18, 19] implemented additional restrictions. Based on their experiences, patients

without pulmonary metastases had an increase of bone marrow side effects when the patient's percent of 48-hour whole body retention exceeded 4.44 GBq (120 mCi), and when the patient had pulmonary metastases, pneumonitis and pulmonary fibrosis increased when the percent 48-hour body retention exceeded 2.96 GBq (80 mCi). Accordingly, these two additional restrictions were recommended, and with the implementation of these restrictions over the last approximately 60 years, the frequency of bone marrow or pulmonary complications has been very low [20].

Lesional Dosimetry

Presently, tumor (e.g., lesional) dosimetry is infrequently performed because of the difficulties inherent in performing dosimetry with either ^{131}I or ^{123}I. However, with the increasing availability of ^{124}I, research is increasingly being performed in the area of lesional dosimetry (discussed in Section "Outcomes"). As for whole body dosimetry, lesional dosimetry requires the same information but is in the latter case about the tumor. This is more problematic because unlike organs that have humanoid phantoms, lesions vary in location, have significantly varying sizes and shapes, and have significant inhomogeneous uptake and clearance within the lesion itself. Additional software has been developed to improve the calculation of the absorbed dose to the lesions. See the detailed discussions that are available [11].

Advantages and Disadvantages of Empiric and Dosimetrically Determined Prescribed Activity for ^{131}I Therapy for DTC

The many advantages and disadvantages of the empiric and dosimetric approaches are noted in Table 4.

Simplified Dosimetry

As noted in Sect. "Lesional Dosimetry," one of the major disadvantages with dosimetry is the increased complexity and inconvenience to the patient and staff. However, multiple researchers over the years have been evaluating alternative dosimetric approaches that simplify the process of dosimetry and may be performed within any nuclear medicine facility with a gamma camera [21–27]. Several of the earlier reports to simplify whole body dosimetry were by Thomas et al. [20, 21], who evaluated one blood sample and performed measurements on the patient's thigh with a collimated probe 20 cm from the thigh. Sisson et al. [22] proposed the use of thyroid hormone levels and a single measurement of the relative whole body retention of ^{131}I at 48 h. Van Nostrand et al. [23] and Hänscheid et al. [24] further evaluated the percent whole body retention of ^{131}I at 48 h, and Atkins et al. [25] validated the former method of the percent whole body retention of ^{131}I at 48 h. Subsequently, Jentzen et al. [26] proposed multiple potential parameters, which, if validated, would be very useful. A discussion of these various alternative simplified dosimetries are beyond the scope of this manuscript, and further reading is available [28] (Table 5).

With the validated method of Dr. Atkins, any nuclear medicine facility with a gamma camera can perform simplified dosimetry as an alternative to full dosimetry, thus allowing physicians to more appropriately identify which patients may tolerate more prescribed activity of ^{131}I or, in fact, may need to have their planned empiric prescribed activity reduced.

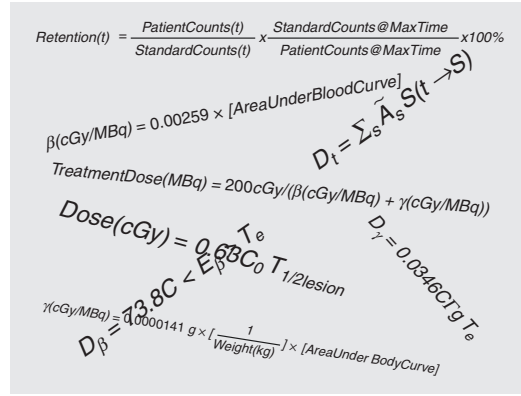


Fig. 4 It demonstrates multiple formulas that have been or continue to be used for dosimetric calculations. However, and as noted, in-depth discussions of the dosimetric calculations are beyond the scope of this chapter (Reproduced with permission from Keystone Press, Inc.)

Table 4 Potential problems and limitations of lesion-based dosimetry

• A single-exponential model may not accurately reflect the kinetics of the radioiodine in the lesion
• Assumption of instantaneous uptake and maximum at time zero
• Estimation of the lesion mass
• Assumption of uniform distribution of ^{131}I in the lesion
• Statistical errors in the measurements
• Therapeutic response relative to dose rate
• Prescribed activity reduction for lesions <5 mm in diameter

Literature

Outcomes

A detailed review is available regarding the outcomes of the use of ^{131}I in the treatment of metastatic differentiated thyroid cancer [29]. However, a major problem with selecting prescribed activity of ^{131}I for treatment of metastatic DTC is that despite the use of ^{131}I for 60 years, no prospective study is available comparing empiric approaches to dosimetric approaches. Likewise, no prospec-

Table 5 Advantages and disadvantages of empiric vs. dosimetric approach for determining prescribed activity for the ^{131}I treatment of distant metastases of differentiated thyroid cancer

Advantages	Disadvantages
Empiric approach	
Convenient	No attempt is made to determine either the absorb dose to the lesion(s) or the critical organ (e.g., the bone marrow)
60+ year history of use	Patients may exceed 200 cGy (rad) to the bone marrow
Acceptable rate and severity of complications of the lower empiric prescribed activity approaches (but not necessarily true for higher prescribed activities approaches)	Patient may receive a lower prescribed activity than the patient could have tolerated, and hence, the patient has a higher likelihood of a less effective absorbed dose to the lesion than if a dosimetric-guided prescribed activity was administered
Permits treating without having to use ^{131}I diagnostically or dosimetrically, thereby avoiding any theoretical or real risk of stunning from ^{131}I	Multiple prior lower therapeutic prescribed activities may reduce efficacy of subsequent ^{131}I therapies
	Empiric approaches that fractionated prescribed activity to reduce side effects may reduce efficacy of subsequent ^{131}I therapies
	No prospective studies comparing outcomes from the many different empiric prescribed activities, which can be quite variable
	No prospective studies comparing outcomes from the many different empiric approaches to the several dosimetric approaches
Dosimetric approach	
60+ year history of use	Increased cost
Identification of as many as 1 in 10 to 1 in 5 patients whose maximal treatment dose is less than the empiric dose	Increased inconvenience to staff and patients
Determination of maximal treatment dose specific for the patient	Does not estimate the radiation dose to the metastasis and thus one may be giving the maximal treatment dose, but it is not having any therapeutic effect
Reasonable complication rate relative to the severity of the disease (with implementation of the restrictions)	Present dosimetric approaches use ^{131}I diagnostically, which has the potential for stunning and thus reduced therapeutic effect
Less therapeutic effect because of previous therapy treatments and less “fractionation”*	
	Only modest third-party payment is available through the radiation therapy dosimetry codes
	Inadequate reimbursement
	No prospective studies comparing outcomes from the several dosimetric approaches
	No prospective studies comparing outcomes from the many different empiric approaches to the several dosimetric approaches

tive study is available comparing the many different empiric approaches for distant metastases. The studies are retrospective and predominantly report results on either empiric or dosimetrically guided prescribed activities of ^{131}I alone. However, Klubo et al. [30] evaluated outcomes in a retrospective study which compared empiric vs. dosimetrically guided ^{131}I prescribed activities used in patients with lymph node metastases and distant metastases. Eighty-seven patients were followed for a mean of 51 ± 35 months. Forty-four and 43 patients were treated with empiric and dosimetrically guided prescribed activity of ^{131}I, respectively. By multivariate analysis, the patients receiving a dosimetrically guided prescribed activity of ^{131}I were 70% less likely to progress (odds ratio, 0.29; 95% confidence interval, 0.087–1.02; $p < 0.052$) and more likely to obtain complete response compared to the patients receiving an empirically selected prescribed activity (odds ratio, 8.2; 95% confidence interval, 1.2–53.5; $p < 0.029$). In patients with locoregionally advanced disease, complete remission was significantly higher in the patients receiving dosimetrically guided prescribed activity vs. empiric prescribed activity (35.7 vs. 3.3%; $p < 0.009$). The rates of partial response, stable disease, and progression-free survival, as well as the frequency of side effects, were not significantly different between the two groups.

In regard to good prospective studies in the near future comparing empiric vs. dosimetrically guided prescribed activities of ^{131}I for the treatment of metastatic DTC, I believe such will be unlikely. The difficulties lie in (1) the increasing use and variability use of additional non-^{131}I treatment modalities such as surgery, external beam radiotherapy, CyberKnife®, radiofrequency ablation, cryotherapy, embolization, and radiolabeled embolization and (2) new developments such as radioiodine redifferentiating agents. Instead, I believe additional data will be available from ^{124}I dosimetry that will help with lesional dosimetry, which in time will be compared, not with empiric prescribed activities but with comparison of specific lesional calculated absorbed dose with specific patient outcomes such as RECIST criteria [31] and progression-free survival. With dosimetric results correlated to these various biological metrics, I believe we will be better able to determine whether a particular prescribed activity of ^{131}I will be effective or not for a specific lesion. In fact, that has already started such as by Maxon et al. [32, 33] using ^{131}I and now Sgouros et al. [34], Freudenberg [35], Khorjekar et al. [36], and Jentzen et al. [37] using ^{124}I.

In several earlier studies, Maxon et al. [31, 32] reported that the rate of successfully treated metastases to the lymph nodes significantly increased in those lesions that received over 8000 cGy (rad), as determined by his ^{131}I dosimetric approach, and the success of treatment was low when the calculated absorbed dose to the lymph nodes was less than 3500 cGy (rad). Sgouros et al. [33] evaluated 15 patients with metastatic DTC using ^{124}I PET dosimetry and 3D-ID software demonstrating not only the feasibility of using a 3D PET-based approach to 3D-absorbed dose estimations but also the substantial variability of absorbed dose between lesions and within individual lesions. Freudenberg et al. [34] reported on 28 patients using ^{124}I PET to determine the absorbed dose for prescribed activities of ^{131}I for the treatment of metastatic lesions, and in nearly one third of their patients, the patients' management was altered to include alternative multimodality treatments because of anticipated insufficient treatment with ^{131}I. In a preliminary study, Khorjekar et al. [35] evaluated 15 lesions using ^{124}I PET and 3D-RD software and demonstrated that three pulmonary lesions receiving less than 8 Gy (8000 rad) progressed, while 11 lesions receiving ≥8 Gy (8000 rad) remained stable (5), had partial improvement (2), or achieved complete remission (4). Jentzen et al. [36] evaluated 34 patients with 227 lesions and concluded that a minimum absorbed dose of ~10 Gy (10,000 rad) was associated with a high response rate for treatment of lymph node metastases. Although significantly more research is warranted and is underway, regardless of what particular dosimetric procedures are used or thresholds established, these studies indicate that the approach for determining the prescribed activity for the treatment of locoregional and distant metastases with ^{131}I is in the determi-

nation of absorbed dose to a specific lesion and not an empirically chosen prescribed activity. With the dosimetrically determined information, the treating team and patient will have to reflect and decide whether the administration of the necessary prescribed activity to achieve their treatment objective(s) warrants the risk of the potential side effects for that prescribed activity of ^{131}I.

Side Effects

In regard to side effects, a detailed review is available [19]. Again, no good prospective study is available comparing the various side effects secondary to empiric vs. dosimetrically guided prescribed activities of ^{131}I for the treatment of DTC. However, multiple authors, including Leeper [19], Tuttle et al. [38], Kulkarni et al. [39], and Esposito et al. [40], have demonstrated that the likelihood that various empiric prescribed activities, if administered, would have exceeded 200 cGy (rad) to the blood (e.g., bone marrow) in as many as 20% of patients. This in turn would increase the frequency and severity of complications such as bone marrow suppression, pulmonary pneumonitis, and/or fibrosis.

Recommendations

When selecting a prescribed activity for ^{131}I for treatment of metastatic differentiated thyroid cancer, one may choose either an empiric or dosimetric approach. In regard to selecting an empiric approach, many alternatives have been noted in Table 3. A frequent argument for choosing one of the many empiric approaches is that "Until there is a prospective study demonstrating that the outcomes are superior for dosimetrically-guided prescribed activity of ^{131}I for treatment of metastatic DTC relative to empiric approaches, one should choose an empiric approach." Although I certainly agree with this argument for selecting ^{131}I for remnant ablations and selected adjuvant treatments as defined earlier in this chapter, I do not agree with this argument for patient with extensive locoregional disease or distant metastases. I propose that until there is a prospective study demonstrating that the outcomes are superior for *empirically chosen prescribed activity of ^{131}I* for treatment of metastatic DTC relative to empiric approaches, one should choose *a dosimetric approach*. The dosimetric approaches are based on at least one, if not two, of the fundamental principles of radiation therapy, which, again, are either determining a maximum tolerated activity that one should not exceed and/or determining an effective prescribed activity to deliver, if possible, the desired number of cGy (rad) to the tumor for the desired therapeutic objective. The empiric approaches use neither of these fundamental principles to determine prescribed activity; as discussed in Sect. "Empiric vs. Dosimetrically Determined Prescribed Activity of ^{131}I for Treatment of DTC," the empiric approaches use the "experiences" or opinions of one or more individuals that may have been "handed down" over the years. In fact, if one uses the argument that one should not use dosimetrically guided prescribed activities of ^{131}I for the treatment of metastatic DTC until a prospective study has shown that such dosimetrically determined activities are superior to empirically chosen prescribed activities, should one avoid the use any empiric prescribed activity until one of the many different empiric activities has been shown in a prospective study to be superior to the other empiric approaches? An additional argument presented to choose an empiric activity is that one's facility cannot do or does not want to perform dosimetry. Again, it is time consuming for patients and staff as well as expensive, with insufficient, if any, reimbursement. However, that facility indeed has two other choices: send the patient to a facility that does perform full dosimetry or perform one of the simplified alternative dosimetric approaches that has been validated and that can be performed in any nuclear medicine facility with a gamma camera.

Although a significant number of publications have reported that "less is as effective as more" when selecting the prescribed activity of ^{131}I for remnant ablation, when one is approaching the treatment of a patient who has distant metastases and/or extensive locoregional disease with

increased morbidity and mortality, I do not believe that one should approach this with a concept that "less will be as effective as more." Rather, I submit that one should pursue the following concepts to maximize the potential of an ^{131}I treatment to achieve one's therapeutic objective:

- Administer the maximum tolerable activity possible guided by dosimetry in order to present the maximum amount of ^{131}I for the tumor to take up while not exceeding a tolerable absorbed dose to the blood (e.g., bone marrow).
- Do everything reasonable to maximize the percent uptake by the tumor of the ^{131}I presented to it (e.g., patient preparation such as low-iodine diet with documentation of low 24-h urine excretion, adequate TSH stimulation, and, possibly in the future, administration of drugs that stimulate uptake).
- Do everything reasonable to maximize the cGy (rad) delivered to the tumor per MBq (mCi) taken up by the tumor (e.g., lithium, post-therapy "cold" iodine loading).
- Do everything reasonable to minimize side effects (e.g., continuous sialagogues, hydration, frequent urination, etc.).
- Modify the dosimetrically guided prescribed activity based on patient factors such as low blood counts, patterns of pulmonary findings on radiographs, a high likelihood of poor compliance in following recommendations to minimize side effects, etc.).
- Pursue all of these concepts, but modify the finally determined prescribed activity based on the objectives and desires of the patient.
- Pursue all of these concepts with the patient's informed consent.

Based on the principle that one must exceed a threshold of delivering enough cGy (rad) in order to achieve the desire therapeutic effect, one should maximize the likelihood that one is delivering those necessary cGy (rad)—rather than believing "less will be as effective as more" in the treatment of metastatic DTC and/or extensive locoregional disease.

Summary

In summary, whole body and lesional dosimetry are founded on the principles of external radiation therapy, which are to (1) determine and minimize the absorbed dose to the normal tissues and (2) determine and deliver an absorbed dose to the tumor that is effective in controlling the tumor with the predetermined physician and patient objective such as cure, adjunctive therapy, or palliation. ^{131}I whole body dosimetry has helped guide the selection of prescribed activity of ^{131}I for the treatment of functioning metastatic differentiated thyroid cancer for over 60 years. Specifically, whole body dosimetry can help identify many patients who may be administered not only higher prescribed activities than many empiric approaches but can also help determine how much higher prescribed activity may be. In turn, this allows a potentially higher absorbed dose to the tumor while maintaining an acceptable absorbed dose to the critical organ (e.g., the blood/bone marrow). In addition, whole body dosimetry can help identify approximately 5–20% of patients who may have exceeded 200 cGy (rad) to the blood (e.g., bone marrow) when a "one-size-fits-all" empiric prescribed activity would have been administered to the patient. Although dosimetry is time consuming and complex, the patient can be referred to a site that performs full dosimetry, or one's facility may adopt an alternative simplified dosimetric approach, which has been published, validated, and can be performed in any nuclear medicine facility with a gamma camera. I anticipate the future validation of additional simplified dosimetric approaches, thereby allowing even more options for nuclear medicine facilities to perform alternate simplified dosimetry for their patients. Although lesional dosimetry with ^{131}I and ^{123}I remains difficult to perform, research is in progress with ^{124}I, which holds significant promise because of the superiority of PET for quantitation.

For the selection of patients for full dosimetry or validated, simplified dosimetry to help guide the selection of ^{131}I prescribed activity, I recommend patients with differentiated thyroid cancer

who have functioning distant metastatic and/or extensive locoregional disease. This is especially important in pediatric and adolescent patients. Choosing dosimetry in other patients should be managed on an individual basis.

Finally, with the research underway evaluating (1) whole body and lesional dosimetry with ^{124}I, PET-CT, and more sophisticated software such as 3D-RD, (2) methods to increase the uptake of ^{131}I in the tumor (e.g., redifferentiating agents), (3) maximizing the number of cGy (rad) delivered to the tumor for the number of MBq (mCi) administered (e.g., lithium, large post-therapy "cold" iodine loads), and (4) methods to reduce absorbed dose to other organs (e.g., salivary gland), I anticipate that whole body and lesional dosimetry for guiding the selection of the prescribed activity of ^{131}I for the treatment of functioning metastatic-differentiated thyroid cancer and locoregional disease will become widely available in those institutions' management of these patients.

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Targeted Molecular Therapy

Arabella Hunt and Kate L. Newbold

Introduction

Thyroid cancer represents the most common type of endocrine malignancy. It can be divided into three broad categories, differentiated (including papillary, follicular and poorly differentiated variants), medullary and anaplastic. With the exception of anaplastic disease, thyroid cancer can generally be regarded as having a good prognosis with the vast majority of patients cured by surgical resection and in the case of differentiated disease, radioactive iodine (RAI) treatments and thyroid-stimulating hormone (TSH) suppression. However, a proportion of patients will present with extensive metastatic disease or progress despite standard treatment. Traditional cytotoxic and radiotherapy treatments have been poorly efficacious for this patient cohort with disappointing outcomes. However, in recent years, increasing attention has been paid to 'targeted molecular therapies' in particular small molecule multikinase inhibitors (MKIs).

This chapter will focus on the rationale behind the use of these agents in thyroid cancer, with particular attention paid to the four approved drugs in this setting. Future directions in this field will also be discussed.

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Molecular Alterations in Thyroid Cancer and Small Molecule Multikinase Inhibitors

In recent years increasing focus has been paid to the molecular alterations that enable the development of cancer. Within the context of thyroid cancer, two pathways, in particular the MAPK pathway and PI3k/Akt pathway, appear to be of particular importance [1]. These pathways are both activated via the intracellular transducer RAS and are responsible for expression of genes needed for growth, proliferation, cell migration and inhibition of apoptosis.

Genetic Alterations Found in Thyroid Cancer

Genetic alterations to these pathways take on many forms. Individual gene mutations, amplification and copy number gains and gene translocations have all been implicated in the development of thyroid cancer.

In differentiated disease, key mutations include BRAFV600E, found in approximately 45% of papillary thyroid cancer [2] and up to 80–100% of the tall cell variant, conferring a poor prognosis; RAS mutations (either H, K or N) found in 30–45% of follicular variants and 20–40% of poorly differentiated tumours; and PTEN deletions found in 30% of follicular tumours [1].

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Common copy number gains include EGFR, VEGFR1, PIK3CA or B [3]. More recently the importance of IQGAP-1 amplification has also become apparent [4]. The most well-characterised gene translocations involve RET-PTC, of which over ten different combinations are known [1]. These translocations cause ligand-independent dimerisation resulting in the persistent activation of RET. Translocations of PAX8-PPARG are also common in papillary thyroid cancers, in particular the follicular subtype [5].

Medullary thyroid cancer (MTC) can occur spontaneously or as an inherited condition forming part of the type 2 multiple endocrine neoplasia syndromes, MEN2A and 2B, or the familial MTC syndrome [6]. The inherited form is caused by a germline RET mutation in almost 100% of cases, whilst 50% of sporadic cases also demonstrate somatic RET mutations [7]. Mutations at codon M918 T are associated with a particularly poor prognosis. Those sporadic cases lacking RET mutations tend to have mutations to RAS (H, K or N) instead [7].

Small molecular multikinase inhibitors (MKIs) have been designed to target the MAPK and PI3k/Akt pathways through inhibition of cell surface receptors such as EGFR or VEGFR or intracellular kinases such as RAF or MEK (Fig. 1). Disruption of these pathways leads to a reduction in downstream phosphorylation events and a slowing of tumour growth.

The use of MKIs has been investigated in many tumour types, in particular, melanoma, renal cell and hepatocellular carcinomas where their use is now well established. Similarly there is now a growing body of evidence around their use in differentiated and medullary thyroid cancer, and in recent years four of these drugs have gained approval in the thyroid cancer setting.

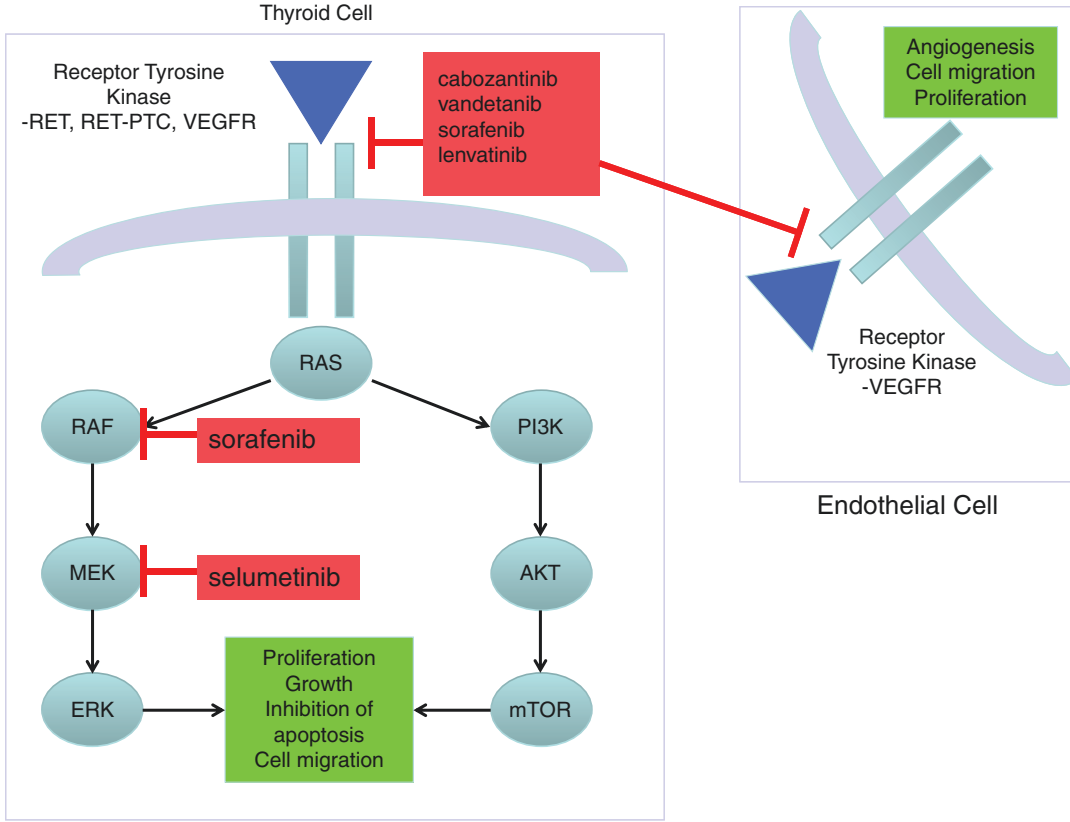

Fig. 1 Key signalling cascades, MAPK and PI3K-AKT pathways, important in the development of thyroid carcinoma and the sites of drug inhibition

Approved Drugs

Vandetanib

Vandetanib was the first small molecule kinase inhibitor licensed for use in advanced MTC. It is an oral tyrosine kinase inhibitor with activity against RET, VEGF and EGFR [8]. Approval was gained from the Food and Drug Administration (FDA) and European Medicines Agency (EMA) based on the results of the ZETA trial [9]. This phase III international placebo-controlled study compared vandetanib (300 mg/day) to placebo in 331 patients randomised in a 2:1 fashion. All patients had advanced MTC, either sporadic (90%) or hereditary (10%), with a serum calcitonin level of ≥500 pg/mL. Patients continued on their allocated drug until objective disease progression at which point, following unblinding, those on the placebo arm were offered the chance to begin open-label vandetanib. The primary endpoint was progression-free survival (PFS), with secondary endpoints including objective response rates (ORR) and overall survival (OS). Median follow-up was 24 months, with 139 patients continuing to received blinded treatment at this point, 111 (48%) of the vandetanib and 28 (28%) of the placebo groups. Ninety-three percent of eligible placebo patients entered into open-label use of vandetanib at the time of unblinding. Vandetanib produced a statistically significant improvement in PFS compared to placebo, with a predicted median of 30.5 months vs. 19.3 months (HR 0.46, 95% CI 0.31–0.69, $p < 0.001$). ORR was 45% vs. 13%. An OS benefit was not seen although this is likely to be strongly influenced by the high rates of crossover in the placebo group and the immaturity of the data.

With respect to safety and tolerability, the drug was felt to be well tolerated with a median duration of treatment of 90.1 weeks for the vandetanib group vs. 39.9 weeks for placebo. Thirty-one patients discontinued treatment due to adverse events, 12% in the vandetanib arm and 3% in placebo. The most common side effects for vandetanib were diarrhoea, rash, nausea and hypertension occurring in >30% of patients. Grade 3/4 toxicities occurring in >10% of patients included diarrhoea, hypertension and fatigue. QTc prolongation occurred in 8% of patients although there were no reports of torsades de pointes. Five deaths were reported in the vandetanib arm as a result of adverse events. These included aspiration pneumonia, respiratory failure, respiratory arrest, staphylococcal sepsis and arrhythmia followed by acute cardiac failure. The study team did not comment on whether these deaths were likely to be directly related to vandetanib. On the whole the drug was well tolerated with most side effects managed with dose reductions (35% in the vandetanib arm) and supportive measures.

The role of vandetanib in the treatment of differentiated thyroid cancer (DTC) is currently under investigation. A phase II trial by Leboulleux et al. [10] showed evidence of efficacy in this patient group with a PFS advantage over placebo (11.1 months vs. 5.9 months). A phase III trial (VERIFY) of vandetanib vs. placebo in the context of differentiated disease has completed recruitment; the results are currently awaited.

Cabozantinib

Cabozantinib was approved for use in metastatic/locally advanced MTC in the USA in 2012 and in Europe in 2014. A small molecule kinase inhibitor, it has potent activity against VEGFR2 and MET as well as RET, KIT, AXL and FLT3 [11]. Approval was gained following the results of the phase III EXAM trial [12]. This international double-blind placebo-controlled trial recruited patients with histologically confirmed, unresectable, metastatic or locally advanced MTC. In contrast to the ZETA trial, participants needed radiological evidence of disease progression within the last 14 months to be eligible. Patients could have sporadic or inherited disease and were allowed to have had prior exposure to tyrosine kinase inhibitors. They were randomised in a 2:1 fashion (cabozantinib 140 mg vs. placebo) and stratified according to age, mutation status and prior MKI use. Dose reductions down to a minimum dose of 60 mg/day were allowed, but crossover at time of progression was not. 330 patients

were enrolled, 21% had prior MKI exposure and 48% were known to be RET mutation positive.

The study's primary endpoint of PFS reached statistical significance at an estimated 11.2 months vs. 4.0 months in the cabozantinib and placebo groups, respectively (hazard ratio 0.28, 95% CI 0.19–0.40; $p < 0.001$). This prolongation of PFS was seen across all prespecified subgroups including all RET mutation subgroups. With respect to secondary endpoints, there was an ORR of 28% in the cabozantinib group compared to 0% in the placebo group ($p < 0.001$) and a median estimated duration of response of 14.6 months (95% CI 11.1–17.5 months). OS did not reach statistical significance.

The drug was reasonably well tolerated with the majority of adverse events managed with either dose reductions (79%) or dose interruptions (65%); adverse events were noted as the primary reason to stop the drug in 16% of cases. Grade 3/4 toxicity was reported in 69% of cabozantinib patients and 33% of placebo patients (reflecting a generally unwell population). In the cabozantinib arm, the most frequent grade 3/4 toxicities were diarrhoea (15.9%), palmar-plantar erythrodysesthesia (12.6%) and fatigue (9.3%). As might be expected, side effects common to VEGF inhibition such as hypertension, perforation and haemorrhage were also more common in the cabozantinib group. Grade 5 adverse events were noted in 7.9% of the cabozantinib group; this was similar to the placebo group (7.3%). Grade 5 events, including three episodes of fistula formation, felt likely to be drug related, two episodes of respiratory failure of which one was felt likely to be caused by cabozantinib, two episodes of multi-organ failure (not felt to be related), two cases of haemorrhage (one felt to be related), one case of unrelated sepsis, one case of hepatic failure (not related), one pneumonia (unrelated), one general health deterioration not felt to be related, one cardiopulmonary failure, one sudden death and one death not otherwise specified all felt to be related.

Unlike vandetanib, cabozantinib was not associated with QTc prolongation.

Although the PFS for cabozantinib appears shorter than that of vandetanib in this setting, the EXAM and ZETA populations are not comparable. The PFS for the placebo group was considerably shorter in EXAM compared to ZETA suggesting a population with more advanced or aggressive disease. As a result it is not possible to draw conclusions regarding which drug is better. Instead decisions regarding which drug to commence first should be based on individual patient comorbidities (and funding considerations) with particular attention being paid to the different toxicity profiles.

Sorafenib

Sorafenib gained approval by the EMA in 2013 for use in progressing radioiodine refractory DTC. It had gained previous approval for use in renal cell and hepatocellular carcinoma. An oral tyrosine kinase inhibitor, it has activity against Raf-1, BRAF, VEGFR2 and 3, PDGFR β, Flt-3 and C-Kit [13]. Approval in DTC was achieved based on the results of the phase III DECISION trial [14]. This multinational, randomised, double-blind placebo-controlled trial recruited patients with progressing, radioiodine refractory, differentiated thyroid cancer without prior MKI use. Patients were randomised to receive 400 mg twice daily of sorafenib vs. placebo; dose reductions and crossover at the time of progression were allowed. 419 patients were enrolled, across 18 countries. The study's primary endpoint of PFS reached statistical significance with a median of 10.8 months for the sorafenib arm vs. 5.8 months for placebo (HR 0.59, 95% CI 0.45–0.86; $p < 0.0001$). The ORR for sorafenib was 12.2%. Overall survival did not significantly differ, although median overall survival had not been reached by the time of data cut-off. 71.4% of patients crossed over to sorafenib at progression.

Adverse events were experienced by 98.6% of sorafenib patients although it should be noted that 87.6% of the placebo arm also showed side effects reflecting the poor health of the study population. The most common adverse events

were hand-foot skin reaction, diarrhoea, alopecia, rash, fatigue, weight loss, hypertension and hypocalcaemia. Dose interruptions, reductions and withdrawals occurred in 66.2, 64.3 and 18.8% of patients, respectively. There was one death (caused by a myocardial infarction), which was felt to be directly related to sorafenib use. A higher rate of skin cancers (mostly squamous cell carcinomas) was also noted.

Lenvatinib

Lenvatinib is an oral tyrosine kinase inhibitor with activity against VEGFR1–3, FGFR1–4, PDGFR α, RET and KIT [15]. It is currently licensed for use in advanced radioiodine refractory DTC following approval from the FDA and EMA in 2015. Approval was granted based on the results of the SELECT trial [16]. This was a phase III, randomised, double-blind, placebo-controlled, multicentre trial that recruited 392 patients from 21 countries. Patients required a diagnosis of metastatic or locally advanced DTC with evidence of radioiodine resistance and radiological progressive disease within the last 13 months. One line of prior MKI use was allowed. Patients were randomised in a 2:1 fashion to receive lenvatinib at 24 mg per day taken continuously vs. placebo. At the time of progression, crossover to the lenvatinib arm was allowed; 95.6% of eligible patients opted for this. The study's primary endpoint of PFS reached statistical significance with a median of 18.3 months for lenvatinib vs. 3.6 months for placebo (HR 0.21, 99%; CI 0.14–0.31; $p < 0.001$). Response rates were seen in 64% of lenvatinib users vs. 1.5% of placebo. The secondary endpoint of OS did not reach significance (HR 0.73, 95% CI 0.50–1.07; $p = 0.10$); however the effect of crossover must be remembered, and when this was considered, the HR improved to 0.62 (95% CI 0.4–1.00, $p = 0.05$).

As might be expected, patients experienced many of the side effects common to this drug class. 75.9% within the lenvatinib group and 9.9% of the placebo group experienced ≥ grade 3 toxicity. The most common side effects were hypertension, diarrhoea, fatigue, weight loss, decreased appetite and rash. Greater than or equal to grade 3 toxicity rates of ≥10% were seen for hypertension (42.4%) and proteinuria (10%). 118 deaths were reported at data cut-off, 27.2% of the lenvatinib group and 35.9% of the placebo group. The majority of deaths were due to disease progression, with 6 (2.3%) deaths in the lenvatinib group felt to be treatment related. These included three deaths not otherwise specified, one pulmonary embolism, one deterioration of general health and one haemorrhagic stroke. 67.8% of the lenvatinib group required a dose reduction; adverse events causing a termination of treatment were experienced by 14.2% of this group, most commonly due to hypertension and fatigue. A subsequent further analysis [17] showed that most adverse events occurred early and were responsive to dose reductions.

Although not currently licensed for these indications, lenvatinib has also been evaluated in phase II trials of patients with medullary and anaplastic thyroid cancer. Schlumberger et al. [18] reported 59 patients with progressive MTC. They found a response rate of 30% with a median PFS of 9.0 months. Rates of ≥grade 3 toxicity were 63%. Tahara et al. [19] have also shown activity of lenvatinib in anaplastic thyroid cancer; 17 patients were recruited, with a median PFS 7.4 months and a median overall survival of 10.6 months. There were no treatment-related deaths. Although only a small number were enrolled, this seems promising considering the usual poor prognosis of this group.

Summary Table of 1 licensed MKIs.

Toxicity Management

Given that MKIs are administered continuously, prompt management of side effects is essential to ensure treatment can continue with the minimal negative and maximum positive impact to the patient. Review prior to commencement of drug by other specialities such as cardiology can ensure medical optimisation prior to MKI use. Whilst on treatment, patients should initially be seen on a regular basis in order for an early review of toxic-

Table 1 Summary of the key design features and results of these four studies

	Sorafenib	Lenvatinib	Vandetanib	Cabozantinib
Indication	DTC	DTC	MTC	MTC
Main molecular targets	Raf-1, BRAF, VEGFR2 and 3, PDGFR β, Flt-3, C-Kit	VEGFR1–3, FGFR1–4, PDGFR α, RET, KIT	RET, VEGFR, EGFR, KIT	VEGFR2, MET, RET, KIT, AXL, FLT3
Trial evidence	DECISION Phase III	SELECT Phase III	ZETA Phase III	EXAM Phase III
Number of patients involved	417	392	331	330
Progressive disease required for eligibility	Yes	Yes	No	Yes
Prior MKI use allowed	No	Yes (25%)	Yes (39%)	Yes (21%)
Median progression-free survival vs. placebo (months)	10.8 vs. 5.8	18.3 vs. 3.6	30.5 vs. 19.3	11.2 vs. 4.0
Objective response rate	12.2%	64.8%	45%	28%
Starting dose	800 mg/day (2 divided doses)	24 mg/day	300 mg/day	140 mg/day
Median daily dosing	651 mg/day	17.2 mg/day	Not recorded	Not recorded
Important adverse events	Hand-foot skin reaction Diarrhoea Alopecia Rash Fatigue Weight loss Hypertension Hypocalcaemia Skin cancers	Hypertension Proteinuria Diarrhoea Fatigue Weight loss Decreased appetite Rash	Diarrhoea Rash Nausea Hypertension Fatigue Prolongation of QTc	Diarrhoea Hand-foot skin reaction Fatigue Hypertension

ity and to implement dose reductions or concomitant, supportive medication if necessary. Once established on a manageable regimen, clinic reviews can become less frequent.

There are several review articles available which give advice on toxicity management [20, 21]. Grande et al. [22] have produced a paper on management of vandetanib side effects that is particularly detailed. Although focussing on vandetanib, many of the management recommendations would be pertinent to the other MKIs.

Future Directions

At the time of writing, there are several ongoing phase III trials looking at MKI efficacy in thyroid cancer.

The EXAMINER trial (NCT01896479) is investigating the effectiveness of a lower starting dose of cabozantinib in progressive medullary thyroid cancer. Patients are randomised to either 60 or 140 mg daily in an attempt to improve toxicity without sacrificing significant efficacy. This trial is currently recruiting.

The VERIFY study (NCT01876784) aims to assess the efficacy and safety of vandetanib in DTC. Recruitment is complete and full results are awaited.

Apatinib (DTC) and anlotinib (DTC and MTC) are currently under evaluation in phase III studies.

Although not yet in phase III, there is increasing interest in the use of the MEK inhibitor selumetinib in DTC. Phase II studies failed to reach significance when selumetinib was used as a single agent [23]; however, there is now an increasing body of evidence suggesting that it may be used to re-sensitise iodine refractory DTC to radioactive iodine. Work on mouse models [24] found that use of MEK inhibitors in BRAF mutant mice could convert previously

iodine-resistant tumours into tumours responsive to further radioactive iodine treatments. A pilot study in humans has subsequently been performed [25] where of the 20 evaluable patients, 12 showed increased uptake of ^{124}I following 4 weeks of selumetinib, with 8 patients reaching the dosimetric threshold for further radioiodine treatment. In those patients, five had a partial response on imaging and three had stable disease. All patients demonstrated a decrease in thyroglobulin levels. Genetic analysis of the patients' tumours showed greatest response in those with NRAS mutations. Toxicity levels were acceptable. Based on these results, phase II trials are currently recruiting in the UK (SELI-METRY) and the USA (NCT02393690).

Conclusion

The treatment of advanced thyroid cancer has developed with evidence of efficacy of multikinase inhibitors in improving progression-free survival. However an impact on overall survival has yet to be proven. An increased understanding of predictive biomarkers including histopathological, molecular and demographic factors has the potential to guide the most effective treatment selection for individual patients. Continued enrolment of patients into clinical trials is encouraged to further improve the outcomes for patients with advanced thyroid cancer.

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Thyroglobulin and Tg Antibodies

Ulla Feldt-Rasmussen and Luca Giovanella

Introduction

Thyroid malignancies are rare endocrine cancers consisting of on the one hand very rare and highly malignant tumours with poor prognosis and on the other the more prevalent differentiated thyroid carcinomas of papillary, follicular or mixed papillary–follicular forms where treatment globally leaves a high number of survivors for follow-up [1]. In differentiated thyroid cancers, the general 10-year survival exceeds 95%, while papillary forms alone have a better prognosis with a 10-year survival of more than 99% [2].

Thyroglobulin (Tg) is a large protein, which forms the backbone for production and storage of thyroid hormones, and thus specific for the thyroid gland. It is released into the bloodstream together with thyroid hormones upon both physiological and pathophysiological stimulations but also upon destruction of the thyroid gland (Table 1) [3]. Thyroglobulin in serum is therefore a specific and useful marker of thyroid tissue in, e.g. differentiated thyroid cancer (residual thyroid tissue or recurrent tumour tissue) in patients having had total thyroid ablation (total thyroidectomy and ^{131}I ablation therapy). Measurement of serum Tg has thus become the cornerstone in the follow-up algorithms in current guidelines for management of thyroid carcinomas after successful treatment [4, 5].

The presence of antithyroglobulin antibodies (TgAb), which are markers of thyroid autoimmune disease, however, interferes with Tg measurements in vitro and gives rise to false results [6, 7]. The prevalence of these antibodies has been described in at least 10% of most female populations and more prevalent, 15–25%, in patients

Table 1 Factors increasing release of thyroglobulin (Tg) into serum

• Thyrotropin stimulation
• Other stimulation of the thyrotropin receptor (e.g. thyrotropin receptor antibodies, human choriongonadotropic hormone)
• Thyroid surgery
• Radioactive iodine therapy
• Destructive thyroiditis (e.g. subacute thyroiditis De Quervain, postpartum thyroiditis, silent thyroiditis, Hashitoxicosis)
• Fine needle biopsy of thyroid nodule
• Other serious manipulation of the thyroid gland (e.g. strangulation)

Feldt-Rasmussen, et al. Cancer 1983, Spencer, JCEM 2011 [13], Clark and Franklyn, ACB 2012 [81]

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with differentiated thyroid cancer [7, 8]. The current management guidelines for using serum Tg as tumour marker have not taken these aspects into account, although recent publications have indicated that quantifying TgAb may act as surrogate tumour markers on their own [9–13].

Finally, the increased incidence of diagnosed thyroid cancers over the past decades, with mainly small papillary thyroid cancers adding to the increased incidence, also puts a greater challenge on the efficacy of both Tg and TgAb as biomarkers. The current chapter will only deal with the most incident and prevalent differentiated thyroid cancers and not all other thyroid cancer types such as anaplastic and medullary cancers, lymphomas and metastases.

Characteristics of Thyroid Cancer

The annual incidence of thyroid cancer varies considerably in different registries, ranging from 1.2 to 2.6 per 100,000 individuals in men and from 2.0 to 3.8 per 100,000 in women (reviewed in [14–16]). It is particularly elevated in Iceland and Hawaii (reviewed in [16, 17]). In Hawaii, the incidence rate of thyroid cancer in each ethnic group is higher than that registered in their country of origin. Ethnic or environmental factors (such as spontaneous volcanic background radiation) or dietary habits [16] probably play a role, but different healthcare systems may also be important in the efficiency of cancer detection and thus earlier treatment. In 2003, the American Cancer Society indicated an incidence in the USA of nearly 10/100,000 population, and the reported incidence has been increasing by more than 5%/year for a decade [16]. In Denmark, the age-standardized incidence of papillary thyroid cancer from 1943 to 2008 increased in both sexes, in men from 0.41 to 1.57 per 100,000 and from 0.90 to 4.11 per 100,000 in women, corresponding to a significant average annual percentage change of 1.7 and 1.8%, respectively. Iodine supplementation started in Denmark in the year 2000, which might be one of the explanations for the increased incidence, but small cancers accounted for most of the increase, and therefore the increased incidence was more likely to arise from higher ascertainment due to frequent ultrasound and other imaging performances discovering small nonclinically significant malignancies [14]. This might comply with the prevalence of thyroid carcinoma found in autopsy series or screening programmes. Autopsy studies indicate a much higher frequency ranging from 0.01 to over 2.0% [16, 18] and a survey of consecutive autopsies found as high as 2.7% of thyroids to harbour unsuspected thyroid cancer [18]. This high prevalence may be attributed to careful examination of the gland but probably also reflects a highly selected group of older patients dying in a hospital. Up to 6% of thyroid glands in autopsied adults in the United States, and over 20% in Japan, also harbour microscopically detectable foci of thyroid carcinoma, which are believed to be of no biologic significance but sometimes discovered as incidentalomas. Altogether autopsy studies suggest that thyroid cancer is in most instances an incidental finding, not diagnosed during life and often not the cause of death. The annual mortality from thyroid cancer in 2003 was 5 per million for men and 6 per million for women [16]. The discrepancy between incidence and mortality is reflected by the good prognosis for most differentiated thyroid cancers. Recent statistics suggest about six deaths/million in the USA, and the cause-specific survival for papillary cancers was recently described nationwide in Denmark to be 99.5% [19], which is in keeping with previous reports [2]. Above circumstances pose big challenges both to the treatment strategies for differentiated thyroid cancers, since many of the incidentally discovered microcarcinomas would never proceed to clinical disease, and treating those along the lines of the previous international guidelines for thyroid cancer would cause serious overtreatment of these patients. Accordingly, guidelines for treatment are changing towards more conservative ablation in cases of carcinomas classified of low risk for recurrence or relapse at diagnosis [4, 5]. However, the evidence may not be quite clear on this procedure yet, as one recent large study indicated excellent follow-up results when using conservative ablation [20], while results of a recent meta-analysis were not equally optimistic [21]. The choice of ablative

procedure, on the other hand, has a major influence on the optimal follow-up strategy of long-term survivors after thyroid carcinoma therapy, which will be described below. While total ablation for thyroid carcinoma of any thyroid tissue by surgery and radioiodine should leave no thyroid cells to produce Tg, this is not the case of, e.g. lobectomy, where one normal lobe is left in situ. This remaining lobe will thus produce Tg both in the basal situation but in particular if stimulated with endogenous or recombinant (Thyrogen®) TSH as part of the follow-up procedure.

Serum Thyroglobulin as Tumour Marker in Differentiated Thyroid Cancer

Tg is a very large glycoprotein of 660 kD, normally stored in the follicular colloid of the thyroid gland where it acts as a substrate for thyroid hormone biosynthesis. It consists of two identical helices and folded in a three-dimensional structure [22]. Immunoassay has been the main analytical technique used for the measurement of serum Tg, at first by competitive radioimmunoassay (RIA). Over the last few decades, immunometric assays (IMAs) have widely replaced radioimmunoassays for measuring peptides and proteins such as Tg. Immunometric assays (with either radioactive or non-radioactive tracers) are consistently more sensitive than radioimmunoassays and, additionally, have a shorter incubation time, wider working range and a more stable labelled antibody reagent that is less prone to labelling damage [23]. More recently fully automated non-radioactive immunometric assays become available, further improving the sensitivity of the Tg measurement with a fast "turnaround time".

Analytical Issues

Analytical Sensitivity

The sensitivity of the assay is critical when using serum Tg measurement to detect small amounts of thyroid tissue and small changes in concentration over long time periods. The analytical sensitivity of an assay should not be confused with the clinical sensitivity, i.e. the probability that a test will correctly identify an illness when present. Analytical sensitivity can be defined as the lowest concentration that can be reliably distinguished from zero [24, 25] and determined experimentally in a number of ways, each with advantages and limitations [26–29]. In the first instance, analytical sensitivity has often been determined by repeat analysis of the zero calibrator and determination of the apparent concentration equal to the zero plus (for immunometric assays) 2 or 3 standard deviations of the signal (i.e. minus for competitive RIA assays), when it is known as the limit of blank (LOB). There are significant limitations to this approach. In the majority of cases, the measured sensitivity will be below the concentration of the lowest concentration calibrator. The assumption is often made that the fitted standard curve is close to the measured dose response curve. This may not be the case and can be difficult to assess for automated immunoassays leading to a misleading estimate of the LOB [30]. Although of limited use in understanding the precision of low-concentration samples, the LOB can be useful when optimizing assay conditions during assay development.

The limit of detection (LOD) is defined as the lowest analyte concentration that can be distinguished from the LOB using replicate analysis of a sample of known low concentration. It has similar limitations to those of LOB. Currently, functional sensitivity (FS) is widely used to define the clinical utility of Tg assays. It is a measure of the imprecision of an assay at low analyte concentration and involves variation due to measurement imprecision and not to biological variations. In essence, it is the variation that would be observed in many repeated measures of a single biological sample under unchanging conditions and is defined as the concentration resulting in a coefficient of variation of 20%. The difference in FS between Tg assays has created a "generational" nomenclature system with each subsequent generation exhibiting a substantial improvement (i.e. tenfold). According to the recommendations of National Academy of Clinical Biochemistry

(NACB), FS should be determined from between batch precision of measurement of patient pools, in the same test mode (singleton or duplicate) as patient samples over the clinically relevant concentration range over two different lots of reagents and calibrators and over a period of 6 months [26].

The patient pools should be TgAb-negative and should cover the clinically relevant concentration range with three different concentration ranges. However, this method is difficult and demanding, and the data cited by the manufacturer for different Tg assays may not follow this definition. In addition, assays may be adapted over time (e.g. through reagent changes or recalibrations) even though they keep the same brand name [31].

Lastly, the limit of quantification (LOQ) is an alternative method for determining the characteristics of an assay at low analyte concentration, and it is increasingly used as a measure of sensitivity for Tg assays [32]. Basically, LOQ is similar to the FS but does have an additional requirement for predefined goals for bias and imprecision, such as the total allowable error (i.e. often defined as ≤30%) as determined by regulatory authorities and national guidelines (i.e. Clinical and Laboratory Standards Institute EP17-A2) [29]. Anyway, since FS and LOQ are not the same, laboratories and clinicians should be aware of how the analytical sensitivity of the assay they use was assessed [32, 33]. Laboratories should, therefore, verify these parameters as part of their evaluation process [31].

Standardization and Harmonization

Circulating Tg is heterogeneous in serum due to differential splicing of Tg mRNA and both carbohydrate and iodide heterogeneity [22]. Moreover, Tg biosynthesis may become deregulated in thyroid tumour cells resulting in differences in the structure of circulating Tg protein. These changes can lead to exposure or masking of epitopes and hence differences in Tg immunoreactivity [34]. Different Tg assays employ a number of antibodies against Tg with varying specificity for different epitopes, potentially resulting in a variable measurement of different Tg isoforms in the patient's specimen and ultimately to differences in Tg concentration reported by the assays. The introduction and use of the international reference material BCR® 457 has significantly reduced inter-method variability to about 30%, but has not eliminated it completely [35–37]. Then, a change in Tg assay still has the potential to disrupt serial monitoring and prompt inappropriate clinical decisions. In clinical practice, these between-method biases necessitate that postoperative Tg monitoring be made using the same manufacturer's method and preferably the same laboratory. If an assay change is unavoidable, a new baseline of a patient's serum Tg concentrations should be established through parallel Tg measurements using both the old and the new assay [32, 38]. Internal and external quality control programmes, including samples at low and very low Tg concentrations, are of pivotal importance for checking the precision, reproducibility (internal quality control) and accuracy (e.g. lack of bias of analytical results) of assays to ensure optimal patient care. Thus, laboratories providing Tg measurement are required to participate in a certified national or international programme of quality assurance [22, 32, 38].

Clinical Role of Tg Measurement

Tg is an important, sensitive method for monitoring DTC patients for the presence of residual or recurrent disease after total thyroidectomy and ^{131}I remnant ablation. For a long time, all guidelines recommended TSH stimulation in order to achieve optimal sensitivity. It is generally assumed that "negative" TSH-stimulated Tg measurement in combination with a negative clinical examination, negative neck US and, when indicated, negative additional imaging procedures predicts a very low risk of recurrence in both low-risk and high-risk patients [5, 39]. Once these findings have been established, routine DTC follow-up should therefore consist of periodic clinical examination combined with neck ultrasound and Tg measurement on thyroxine

(LT4) medication [5, 32, 33, 39]. Using Tg assays with a functional sensitivity of about 0.5 μg/L, different authors showed limited additional value of stimulated Tg when basal levels are not measurable [40–43]. More recently, Tg assays with a functional sensitivity of about 0.1–0.2 μg/L (i.e. highly sensitive or 2nd-generation assays) have been developed and are now commercially available. Spencer and colleagues have pointed out that there may be a direct proportional relationship between the basal and stimulated Tg levels measured by a highly sensitive assay [44]. Indeed, a number of studies were performed to investigate the diagnostic performance of new highly sensitive Tg measurements in the follow-up of patients with DTC, mostly using the Access Tg assay (Beckmann Coulter, Fullerton, CA, USA; functional sensitivity 0.1 μg/L) [45–51]. In a systematic meta-analysis of the available literature, including 9 studies and 3178 DTC patients, Giovanella et al. [52] confirmed the very high negative predictive value (98–100%) of an undetectable basal Tg (e.g. <0.1 μg/L). Nonetheless, these assays also have an adequate sensitivity for detection of recurrent disease (88–98%). However, the improved sensitivity is associated with an unsatisfactory clinical specificity and positive predictive value using FS as cutoff value, and TSH stimulation was proposed to clarify basal Tg levels ranging from 0.1 to 1 μg/L [53]. In these cases, patients are generally considered as "disease-free" after a negative TSH-stimulated Tg measurement. However, diagnostic specificity of basal serum Tg measurements can also be improved by using optimized thresholds and evaluating Tg kinetic. While FS and LOQ are analytical parameters, different clinical decision limits may be defined by appropriate methods. Using ROC curves analysis, Schlumberger et al. [47] found a higher clinical threshold of 0.27 μg/L and 0.22 μg/L for basal Tg measured by the Access Tg assay and the EIASON TgCa assay (Iason GmbH, Graz-Seisberg, Austria; functional sensitivity 0.02 μg/L), respectively. In a further extension of this study, Brassard et al. found that 32 of 715 DTC patients had a recurrence during the median follow-up of 6.2 years [54]. Assuming a cutoff level at 1.4 μg/L for stimulated Tg measured by the Access method, sensitivity, specificity and positive and negative predictive values were 78%, 90%, 26% and 99%, respectively. Similarly, using the cutoff level at 0.27 μg/L, sensitivity, specificity and predictive positive and negative values for basal Tg were 72%, 86%, 20% and 99%, respectively. Overall, including a stimulated Tg measurement does not provide further information when basal Tg levels <0.27 μg/L are found at early follow-up [54]. Similar results were obtained by Malandrino et al. using the same assay: when post-ablation basal Tg was <0.15 μg/L, a very low risk of recurrence occurred, even in patients with intermediate- or high-risk DTC [51].

Measuring basal Tg trend, with TSH at constant level, should reflect changes in thyroid tissue mass, providing a sensitive variable for disease detection [27, 55]. This is also supported by a growing number of studies showing the prognostic utility of monitoring the basal Tg trend and doubling time [56–58].

Importantly, limitations to the available evidence are that most patients who were enrolled in the studies were affected by low-risk DTC and were treated by thyroidectomy and subsequent radioiodine ablation. Indeed, data on patients with intermediate- and high-risk tumours are less robust, indicating that the above-described approach should be restricted to low-risk DTC patients while according to current guidelines, early follow-up should be based on stimulated Tg and diagnostic whole-body scan in addition to neck US in patients with intermediate- and high-risk DTC [6]. Finally, Tg may be a significantly less useful marker in patients treated by lobectomy and in those not receiving radioiodine after a total thyroidectomy [22, 59].

Current guidelines incorporate the four response-to-therapy categories firstly described by Tuttle et al. [60] and modified in Vaisman et al. [61] (Table 2). These clinical outcomes originally described the best response to initial therapy during the first 2 years of follow-up, but they are now being used to describe the clinical status at any point during follow-up.

However, as mentioned above, both basal and stimulated Tg thresholds were obtained, and

Table 2 Response to treatment in DTC patients: assessment criteria

Excellent response: no clinical, biochemical or structural evidence of disease. Definition: negative imaging and *either* suppressed Tg <0.2 μg/L *or* stimulated Tg <1 μg/L
Biochemical incomplete response: abnormal Tg or rising anti-Tg antibody (see the specific section) levels in the absence of localizable disease. Definition: negative imaging and suppressed Tg <1 μg/L *or* stimulated Tg <10 μg/L *or* rising TgAb levels Structural incomplete response: persistent or newly identified loco-regional or distant metastases
Indeterminate response: nonspecific biochemical or structural findings that cannot be confidently classified as either benign or malignant. This includes patients with stable or declining anti-Tg antibody levels without definitive structural evidence of disease

response to therapy assessment for dynamic risk stratification was validated in patients with DTC treated with total thyroidectomy and radioiodine ablation. Accordingly, current clinical guidelines provide unclear guidance with regard to serum Tg measurements in patients whose initial treatment comprised a less extensive ablative procedure than total thyroidectomy, or who did not receive radioiodine ablation.

Serum Tg Levels in DTC Patients Treated by Lobectomy

Momesso et al. [62] found an excellent outcome in 187 patients treated with lobectomy and having a post-surgery Tg <30 μg/L after lobectomy. However, the criteria to define Tg thresholds were not reported, and, notably, serum Tg levels are likely not dependent on the presence or absence of tumour foci but, instead, on the remaining thyroid lobe volume, current iodine status and TSH concentration. Thus, fine variations in Tg levels will be easily masked by basal Tg secretion from cells forming the remnant lobe, and measuring Tg is essentially useless in these cases. The only options for DTC follow-up in patients treated by lobectomy alone are to perform cervical US and, if recurrence or metastasis are suspected, to secure the diagnosis through a fine-needle biopsy [26].

Serum Tg Levels in DTC Patients Treated by Total Thyroidectomy Without Radioiodine Ablation

Durante et al. [63] compared the evolution of Tg levels over time in 290 low-risk patients with DTC treated by total or near-total thyroidectomy without ^{131}I ablation and 495 matched patients treated by additional ^{131}I ablation. After a median follow-up of 5 years, the final Tg levels were < 1 μg/L in 274 out of 290 non-ablated patients (95%) and 492 out of 495 ablated patients (99%). In a subgroup of 78 patients, serum Tg levels were measured serially, and 47 patients (60%) had a serum Tg <0.4 μg/L at the first postoperative examination (3–12 months). In 77 cases (98.7%), Tg concentrations remained stable or declined spontaneously over time, and patients remained disease-free; the remaining patient was the only one to develop recurrent disease. Similarly, Nascimento et al. [64] found unstimulated Tg level <0.3 and ≤2 μg/L in 86% and 96% of 86 low-risk DTC patients non-ablated after total thyroidectomy. However, they also emphasized that the results were strictly dependent on the completeness of surgery by a dedicated surgeon in a referral centre. More recently, Momesso et al. [62] evaluated 320 DTC patients treated with thyroidectomy without radioiodine ablation RAI and found an excellent outcome in patients having a Tg <0.2 μg/L after thyroidectomy. However, the abovementioned studies were retrospective with significant selection biases. In fact the mean largest diameter of primary DTC was 4 and 12 mm in non-ablated and ablated patients evaluated by Durante and colleagues, and 208 of 507 patients evaluated by Momesso and colleagues harboured primary carcinomas <10 mm in largest diameter (i.e. pT1a). Therefore, serum Tg results should be interpreted with caution in non-ablated patients taking into account both the TSH concentration and remnant thyroid volume (Table 3). Indeed, more sophisticated Tg reference intervals will have to be established for patients treated by conservative surgery and no radioiodine ablation. Mathematical normalization of Tg levels to TSH level and residual thyroid tissue tailored to individual patients should

Table 3 Factors influencing use and interpretation of serum Tg as tumour marker

• Ablative procedure (total vs. non-total)
• Substitution therapy
• Adequacy of substitution (concentration of serum thyrotropin)
• Time after destructive therapy (surgery, radioiodine)
• Presence of thyroglobulin antibodies
• Methods for measurement of thyroglobulin and thyroglobulin autoantibodies

Feldt-Rasmussen, et al. Cancer 1983, Spencer, JCEM 2011 [13], Clark and Franklyn, ACB 2012 [81]

be useful for the purpose, but it is extremely challenging to have highly precise and reproducible Tg and TSH assays and to standardize estimation of residual thyroid mass [22].

General Confounding Factors in Interpretation of Tg Measurements

The use of serum Tg as a tumour marker in DTC is hampered by a number of confounding factors (Table 3), i.e. the ablative procedure (total vs. non-total ablation) and thus amount of remaining normal thyroid tissue, as mentioned previously; the adequacy of the subsequent substitution therapy (serum TSH not elevated); time after destructive therapy, which releases Tg into the circulation and stays high for a long period due to the long half-life of the protein [65]; and not least presence of TgAb [3, 8, 66].

The important general demands to assays measuring Tg in serum are the following: first, the accuracy and sensitivity of serum Tg essential can be improved by using the CRM-457 international standard [35, 36, 59] as also mentioned previously. Furthermore, a high inter-assay precision across monitoring intervals up to 12 months is important, and "hook effects" should be diminished (an excessive amount of antigen overwhelming the binding capacity of the capture antibody) in order to avoid false lowering of very high Tg concentrations. Finally and very importantly, quantification of Tg can only be performed correctly in the absence of TgAb, which should therefore be measured in each individual sample. The analytical interference takes place "in vitro" (i.e. in the tube), and the direction of interference by presence of TgAb can give rise to either falsely low or falsely high serum Tg concentrations (see below for details) [3, 7, 59].

Influence of Thyroglobulin Antibodies on Serum Tg Measurements

Together with thyroperoxidase antibodies, TgAb are important pathogenic markers of thyroid autoimmune disease, present in approximately 10% of most female populations, depending on, e.g. the iodine intake [4, 8, 9, 67]. In differentiated thyroid carcinoma, on the other hand, TgAb are detected in 15–40% of patients, i.e. roughly twice or more as often as in the general population [4, 8, 9, 34]. Epitope recognition patterns of TgAb were recently shown to be restricted to immunodominant clusters in 58% of patients with different thyroid cancer, whereas the rest were either broadly heterogeneous (16%) or non-reactive (26%). However, median Tg recovery did not differ between sera with restricted and unrestricted specificities (69% vs. 80%; $P > 0.05$). Tg recovery in these sera was inversely correlated with the total number of epitopes recognized by sera ($r = -0.66$; $P < 0.001$). TgAbs with both restricted and broad specificities were present in patients with differentiated thyroid cancer. TgAb interference was related to the number of epitopes recognized by sera rather than the pattern of epitope recognition [68]. In an earlier study, Ruf et al. [69] showed that Tg epitope specificity of thyroid cancer TgAbs was similar to that of normal persons with low TgAb concentrations and unlike in that of patients with overt thyroid autoimmune thyroid diseases such as Graves' disease and Hashimoto's thyroiditis. This might also have a consequence for the TgAb interference in Tg assays of serum from different patient groups.

So, independently on whether the presence of thyroglobulin antibodies is due to true autoimmune disease or not, the challenge of potentially

compromising serum Tg measurements as tumour marker in differentiated thyroid carcinoma is not negligible, although TgAb together with the other thyroid autoantibodies do decrease after removal of the thyroid tissue by total ablation [70] while probably not disappearing after only lobectomy, since autoimmunity will be continuously stimulated by presence of thyroid autoantigens as well as by intra-thyroid lymphocytes. Notably, it took several years before TgAb disappeared after ablation, a fact which has to be kept in mind when interpreting Tg and TgAb results.

The initially established radioimmunoassays for measurement of serum Tg used double antibody techniques which could result in either falsely high or falsely low serum Tg quantification depending on the nature of the second antibody in the assay [7, 34, 59, 71]. Current assays for Tg measurement, however, generally use immunometric designs, where the influence from presence of TgAb in the sample will always be unidirectional resulting in a false lowering of the Tg concentration [7, 10, 34, 59].

Before mentioning the requirements for TgAb methods to reveal amounts liable to hamper Tg results, it must be mentioned that heterophilic antibodies can also give rise to interference and false results of serum Tg measurements [10, 72–74]. Both falsely elevated and decreased serum Tg measurement in the current assays can be seen in presence of heterophilic antibodies in current assays [10, 71–73], but the effect is usually eliminated by the manufacturers by adding blocking agents to the assay [73] and is anyway rare [75].

Interference in the serum Tg measurements by, e.g. TgAb leading to misinterpretation of the serum Tg concentrations will lead to errors with different consequences. A *false-positive* result will result in unnecessary further investigations and/or treatment for thyroid cancer on the basis of an inappropriately high serum Tg caused by TgAb interference measured by radioimmunoassay and cause unnecessary anxiety for the patient. Conversely, a *false-negative* result will lead to failure to recognize recurrent or metastatic disease in a thyroid cancer patient because serum thyroglobulin is inappropriately low or undetectable due to interference from TgAb causing delay in the detection of recurrent disease [59]. The term "undetectable" refers here to below the limit of sensitivity whether or not functional sensitivity or limit of quantification is used for the Tg assay, depending on local laboratory practice [4, 10, 59]. The impact from the TgAb method on the quality of the interpretation will be described further below.

Thyroglobulin Antibody Methods to Assess Interference in Tg Assays

To measure TgAb in serum of patients with differentiated thyroid carcinoma in order to avoid misinterpretation of Tg results in the long-term follow-up puts more demand on the TgAb methods than has previously been effective, nor recognized to be necessary [5]. A variety of different means to detect possible interference have been suggested and tested over the years. Some companies and researchers have previously been mostly devoted to a Tg recovery test, one reason being that it is simple and can easily be included in the Tg measurement kit. However, since the reliability of Tg recovery depends very much on the amounts of each of the components in serum (Tg and TgAb), which is largely unknown, the method is not universally reliable at any concentration range of added Tg [7]. Furthermore, it has become more clear from newer studies that a quantification of TgAb is necessary in the follow-up of thyroid cancer patients, also to reveal interference from TgAb, although some studies have indicated an additional value from different types of recovery tests [66, 76–78]. Finally, the recovery test may capture heterophilic antibodies [10, 23, 72–74], but most companies have taken precautions against interference from these antibodies by adding blocking reagents [73], and therefore this interference is far less prevalent than the one from presence of TgAb [75].

The second approach to assessing TgAb interference has been to apply assessment of discordant results between radioimmunoassay and immunometric assay results for serum Tg measurement [13, 34, 59, 67]. This method has recently been reappraised [70]. Only 3 of the

group of 433 patients included in the study were TgAb positive without demonstrating evidence of Tg assay discordance. No definitive histological evidence of tumour recurrence was reported in any of the three cases, although equivocal FNA of a neck node with positive Thyrogen® stimulation was obtained in one. Irrespectively, in all cases TgAb titre subsequently declined to undetectable concentrations. Even though this approach seems to demonstrate both high sensitivity and accuracy and therefore could be very appealing, it is impractical in modern laboratory settings [10, 13, 34, 79]. Since more evidence is pointing towards the use also of TgAb as surrogate markers for recurrence/relapse on its own, it may be more convenient and cost-effective in the future to rely entirely on sensitive Tg and TgAb methods.

The third approach to reveal interference in the Tg assays is direct measurement of TgAb in serum [13, 80–83]. In the early days of discovery of thyroid autoimmunity, the diagnosis of the condition relied on very crude methods and often only semiquantitative for measurement of thyroid autoantibodies, including TgAb (reviewed in [3]). Modern techniques have improved the technology for measuring these autoantibodies, and both increased sensitivity, precision and accuracy [34]. Current strategies to measure TgAb in differentiated thyroid cancer, but also in clinical management of modern thyroid autoimmunity unrelated to cancer, have increased the demand for sufficiently sensitive TgAb methods (Table 4). In thyroid cancer management, the sensitivity is particularly important, since even very low levels of TgAb may induce false results in the thyroglobulin assays [7, 10, 34, 68]. It is furthermore important for the TgAb methods to cover a broad epitope specificity in order to avoid missing interfering antibodies not captured by a more restricted method [7, 10, 59].

In a recent position paper, which will be mentioned in more detail later, it was stated that TgAb concentrations, like Tg concentrations, are best assessed by immunometric assays [10], although very little evidence is available on this issue [80], and other studies did not support this [81]. It was also recommended to use an assay that is standardized against the international reference preparation 65/93, which is the only globally available reference preparation, but it has to be realized that the preparation is very old, and most assays use local reference preparations for the kits and only calibrate their own standard towards the IRP 65/93 [34]. For longitudinal consistency of clinical care, consecutive measurements of TgAb concentrations should be performed in the same laboratory and consistently using the same assay [10, 34]. Furthermore, it cannot be overemphasized that the TgAb concentration must be measured in *all* patient sera prior to Tg analysis, because the TgAb of the patient can change over time, with treatment or with progression of disease (see later). Finally, no current Tg method used in general clinical routine overcomes interference from TgAb antibodies that result in both sufficient accuracy and sensitivity of Tg measurements for clinical use [10, 34].

However, a promising new methodology for Tg quantification has become available [78], and this liquid chromatography-tandem mass spectrometry method (LC-MS/MS) seemed to be superior to immunoassays in terms of avoiding interference from TgAb [84, 85]. Although these methods have been described to be more accurate and may have improved in functional sensitivity compared to the first ones, it still remains to be proven in a larger context [10]. The LC-MS/MS

Table 4 Analytical requirements to thyroglobulin antibody (TgAb) measurements in differentiated thyroid cancer

• TgAb methods should be sensitive immunoassays, as very low levels may induce false results in the serum thyroglobulin (Tg) assays
• Extremely high serum Tg concentrations as in metastatic thyroid carcinoma may compromise correct quantification of TgAb
• The TgAb concentration should be measured in all patient sera prior to thyroglobulin analysis, i.e. TgAb status of the patient can change
• The epitope specificity of TgAb methods should be broad
• Changes in serum TgAb concentrations can be used as a surrogate marker for remaining or relapsing thyroid tissue

Feldt-Rasmussen, et al. Hormones 2010 [9]; www.nacb.org; Feldt-Rasmussen et al. 1986 [92]

methodology is thus very promising, but not widely available partly due to the lack of evidence for its clinical efficacy but also due to the technical requirements and costs.

Serum Antithyroglobulin Antibodies as Surrogate Biomarkers in Patients with Differentiated Thyroid Carcinoma

Recent studies have found that patients with thyroid cancer, who are positive for TgAb before thyroid ablation, retain detectable TgAb if they have persistent malignancy after treatment [4, 8, 10, 34, 86–91], although TgAb concentrations do not correlate with the tumour load [87]. Thus, serial measurements of TgAb seem to have prognostic significance for monitoring the outcome response after treatment for differentiated thyroid carcinoma, i.e. a rise or de novo occurrence of TgAb is often the first sign of recurrence in such patients [87, 90]. Therefore, changes in serum TgAb concentrations can be used as a surrogate, yet imprecise, marker of residual benign or malignant thyroid tissue [9, 34, 59, 86–91]. A few caveats should be mentioned: (1) a very abrupt and extreme rise in serum thyroglobulin as in rapid development of metastatic thyroid carcinoma may compromise correct quantification of TgAb measurements by immune complex formation and lowering of the measured concentrations through tumour release of Tg [92]; (2) a rapid decrease in measured TgAb concentration is also seen shortly after thyroidectomy of patients with prior positive TgAb, due to the acute release of thyroglobulin [93]; (3) a similar but slower reaction is seen after radioactive iodine treatment [94, 95].

Unlike using serum Tg as tumour marker, which has been the subject of many publications over the past several decades, using TgAb concentrations is in its beginning of capturing evidence-based information as to the usefulness, and it will require many more long-term prospective studies on TgAb changes in relation to thyroid cancer outcome. There is no good evidence to advice on a specific "rising" or "falling" serum thyroglobulin antibody concentration, fraction or percentage. Some authors have suggested that disease-free patients typically display a 50% drop in thyroglobulin antibodies in the first postoperative year [34, 87]. However, this figure is very likely to depend on the initial pretreatment concentration, and more studies and evidence are thus required to be able to use this as early risk assessment in clinical practice in order to avoid uncertainty and expensive imaging. The study by Chiovato et al. [70] demonstrated very convincingly that the concentration of TgAb after thyroid ablation for thyroid carcinoma of 182 patients with thyroid autoimmune manifestations before treatment had a mean disappearance time of 3 years, indicating that the actual TgAb concentration is not very useful during that period for outcome prediction, and only lack of decrease or increasing concentrations could indicate persistent disease. Since all of these patients were subjected to total thyroidectomy and radioiodine ablation, they must all have started out with an immune stimulation from released antigens, acutely after surgery but prolonged after radioiodine, which might also explain the prolonged disappearance of TgAb [93, 94], which then does not necessarily indicate persistence of thyroid cancer.

It is also important to agree upon how to define a "positive" TgAb concentration in patients with differentiated thyroid cancer, which is a completely different diagnostic challenge compared to diagnosing benign thyroid autoimmune diseases. In the mentioned position paper [10], it is recommended that laboratories should report two reference ranges for TgAb: one based on TgAb in a population free of thyroid disease meant for diagnosis of autoimmune thyroid disorders, so-called manufacturer cut-off, while the limit of quantification should be regarded as the upper limit of normal in patients with differentiated thyroid carcinoma [4], while some would prefer to use functional sensitivity [34, 59]. Importantly, laboratories should verify the limit of detection, limit of quantification and reference range in their own population, and they should provide the information to clinicians on the various cut-offs and collaborate with clinicians to establish own reference ranges and cut-offs based on own population-based references. Using TgAb measurements as surrogate tumour marker, the trend is

more important than the absolute level, such that a consistent reduction in the serum TgAb concentration seems to indicate that the patient is likely free of disease, a consistent rise or de novo appearance of serum thyroglobulin antibodies raises suspicion of recurrence, while an unchanged serum TgAb concentration must be regarded as indeterminate [10, 81].

It should be emphasized that the recommendation to use serial TgAb concentrations as a surrogate tumour marker necessitates continuity of the method in the laboratory. Different methods for TgAb measurements report different numeric values despite claiming standardization against the same international reference preparation. Thus, changing methods disrupts TgAb monitoring. In this context it is worth noting that despite numeric differences between methods, the ratio between any two different TgAb methods appears constant for a given patient but different for different patients—reflecting TgAb heterogeneity. Establishing the ratio between an old and proposed new method on a specimen from the patient can be used to rebaseline to the new method [13, 34, 96], which is an important approach to avoid misinterpretation of the long-term outcomes but rarely done in clinical practice, which is a problem in many laboratories.

It also has to be realized that currently the data on TgAb use for outcome of thyroid cancer, like serum Tg, is based on an initial treatment strategy of total ablation, since insufficient ablation from a follow-up point of view will hamper both Tg and TgAb as tumour markers. For TgAb the important issue is continued presence of autoantigen as long as remnant thyroid cells are still present [10].

Remaining Controversies Using Serum Tg and TgAb as Combined Biomarkers in the Follow-Up of Differentiated Carcinoma Patients

Current ideal long-term management of differentiated thyroid carcinoma patients would in view of the above be a strategy of continuous follow-up measurement of both Tg and TgAb as biomarkers. However, there are no evidence-based guidelines and thus no defined strategy. Furthermore, there are several unsolved evidence issues to be solved before guidelines can be decided upon. One of them is whether or not LC-MSS can solve the methodological biases of TgAb interference in the Tg assays and if its performance can be sufficiently sensitive as well as cost-effective.

The current literature does not provide sufficient data for the provision of definite and conclusive answers and recommendations in the care of TgAb-positive patients with differentiated thyroid cancer. In the assessment of Tg and TgAb assays, the optimal strategy is likely to be dependent on the available assays and the feasibility and costs involved [59, 71, 80]. In the paper by Clark and Franklyn, it was emphasized that clinicians, laboratory specialists and assay manufacturers should collaborate, in agreement with previous statements [59]. The consensus statement in the position paper [10] provided an overview of the available evidence, and thus, the resulting consensus' expert opinion and the 25 clinical recommendations were soundly based on a meticulous discussion of the evidence by a group of experts.

Based on available evidence, a flow diagram was suggested for management of TgAb-positive patients with differentiated thyroid carcinoma, displayed as Fig. 1 in a modified version. This resulted in suggestions for personalized tailored follow-up of patients with differentiated thyroid cancer and TgAb after thyroid gland ablation.

There is very limited evidence to support whether or not to base serum TgAb as tumour marker on basal levels or concentrations after TSH/Thyrogen stimulation. In one study Nam et al. [97] looked at TgAb in totally ablated patients with differentiated cancer before and after rTSH, and they concluded that the changes in serum TgAb concentrations after TSH stimulation were different in patients with recurrence compared to those without evidence of residual disease. The number of patients was however low (n = 53) and the study thus inconclusive and needs confirmation from larger case control studies.

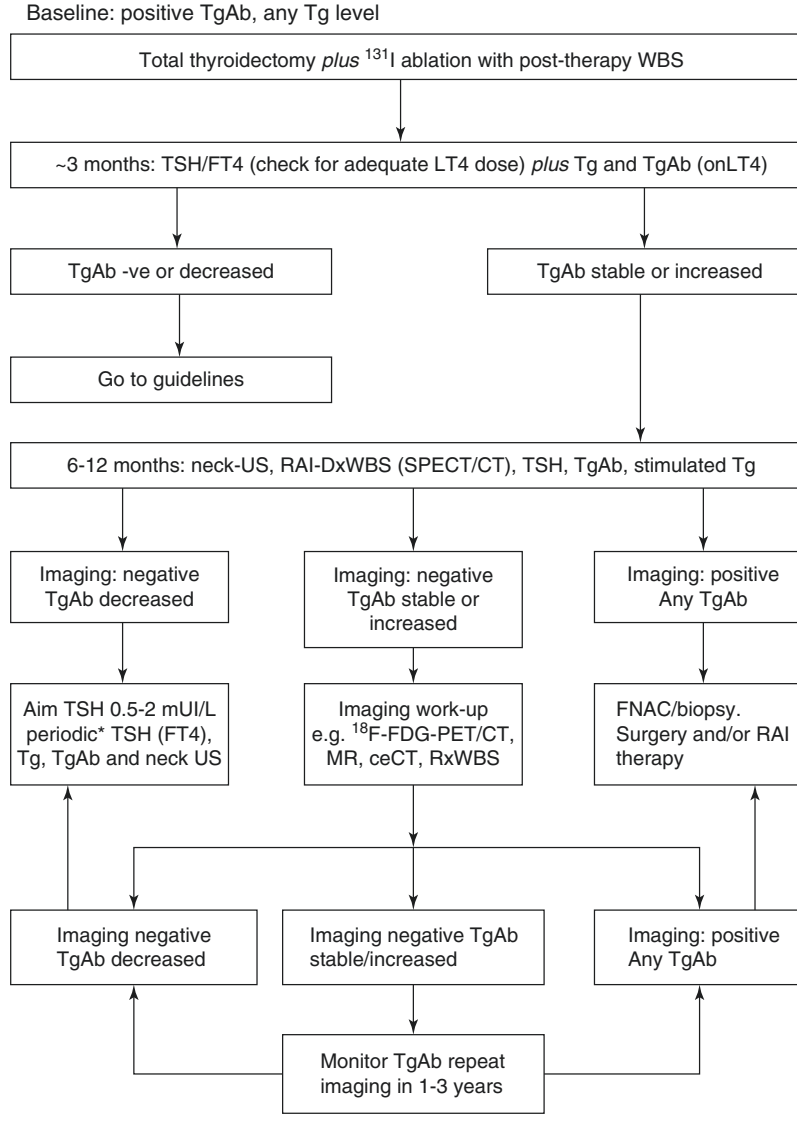

Fig. 1 Proposed algorithm for management of antithyroglobulin-antibody-positive patients with differentiated thyroid carcinoma after total thyroid ablation. *WBS* whole-body scintigraphy, *LT_4* levothyroxine, *TSH* thyrotropin, *FT4* free thyroxine estimate, *TgAb* antithyroglobulin antibody, *Tg* thyroglobulin, *dxWBS* diagnostic radioiodine whole-body scintigraphy, *US* ultrasound, *PET/CT* positron emission tomography combined with computed X-ray tomography (adapted from Thyroid [10], and CMC [4]

However, some of the suggestions in this paper are liable to change in the near future depending on future methodology and evidence. In general, fewer patients will have had a total thyroidectomy and radioablation, and more will receive limited surgery and no radioablation due to the increase in low-risk thyroid carcinomas; fewer patients will need TSH-stimulated Tg/TgAb measurements and whole-body scan, if sensitive measurements of serum thyroglobulin are available [26, 52]. The mainstay will probably still be serum Tg and TgAb measurements, but further follow-up studies of low-risk patients and TgAb-positive patients are needed to verify the evidence for new guidelines.

Hopefully, these suggestions will stimulate clinicians to collaborate closer with their local laboratories and subsequently stimulate laboratories to collaborate closer with assay manufacturers—to the long-term benefit of the patients.

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Medullary Thyroid Cancer

Rossella Elisei and Cristina Romei

Introduction

Medullary thyroid cancer (MTC) is a well-differentiated thyroid tumor which maintains the biological and pathological features of the parafollicular C cells which represent 0.1% of all thyroid cells. These cells are dispersed in the thyroid and characteristically located at the periphery of the thyroid follicles (Fig. 1). At variance with thyroid follicular cells, which derive from the endoderm, C cells are neuroendocrine cells since they originate from the neural crest and migrate to their final location along with the ultimobranchial body, during the embryonic development. However, recent genetic studies in mice indicate that the real progenitors to C cells, or at least to some of them, arise in the endoderm. According to these experiments, the neural crest theory fails in justifying the evolution and development of C cells in the chordate family [1–3]. Independently from the theories about the C cell origin, it is a matter of fact that there are at least four features that make the C cells different from follicular cells: (a) the prevalent distribution at the junction of the upper third and the lower two-thirds as well as along the central vertical axis of each thyroid lobe; (b) the thyrotropin-stimulating hormone (TSH) independent growth and function; (c) the inability to take up and concentrate iodine; and (d) the production and secretion of calcitonin (Ct), a biogenic amine which is almost exclusively produced by both normal and malignant C cells. The MTC origin from parafollicular C cells makes it a biological, pathological, and clinical different entity from the other differentiated thyroid tumors.

This chapter is aimed to give an overview of all biological, pathological, and clinical aspects of this rare tumor which still represents a medical challenge for its diagnosis, management, and therapy.

Epidemiology and Risk Factors

MTC is a rare tumor and it accounts for only 3–5% of all thyroid cancer and 0.4–1.5% of all thyroid nodules. When considering that, according to the Surveillance, Epidemiology, and End Results (SEER) program of the National Cancer Institute, the thyroid tumors prevalence among all human tumors is 3.8%, that one of MTC is less than 0.2% (*https://seer.cancer.gov/*). Because of its rarity, the overall frequency is unknown, but autoptic studies show an average prevalence of 0.14% in thyroids of subjects died for other reasons [4]. MTC is sporadic in about 75% of cases and familial in the other 25%. The familial cases

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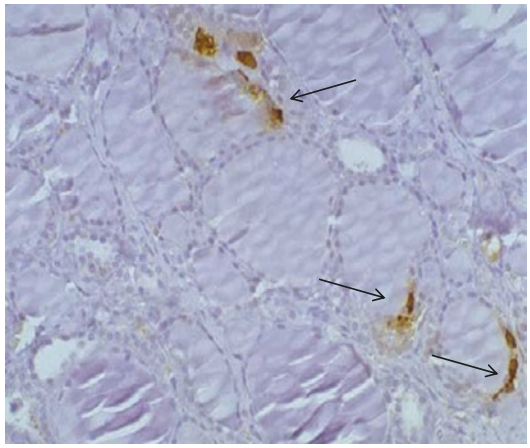

Fig. 1 Normal C cells (here indicated with the arrows) can be detected only by immunohistochemistry for calcitonin. They are rare (0.1% of all thyroid cells) and located at the periphery of the thyroid follicles

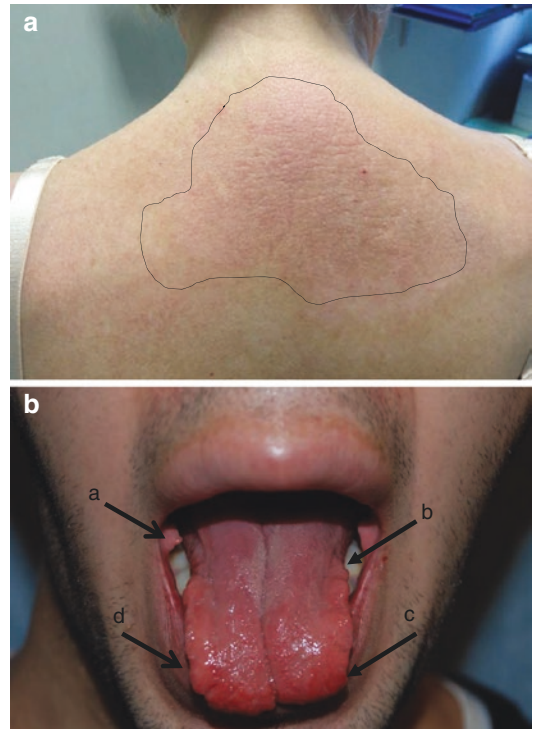

Fig. 2 Non-endocrine diseases associated to endocrine neoplasia in MEN II. Panel (**a**) cutaneous lichen amyloidosis, which is characteristically located in the interscapular region, is an itchy lesion of the skin typical of MEN IIA present in about 10% of cases; panel (**b**) neurinomas of the buccal mucosa (arrow a) and of the tongue (arrows b, c, and d) are typical of MEN IIB and present in 100% of cases. Similar lesions can be present also in the conjunctiva. Tick lips are also characteristic of the phenotype of MEN IIB patients

are inherited as an autosomal dominant trait, and they can be classified into three different phenotypes: (a) the multiple endocrine neoplasia type IIA (MEN IIA) in which the MTC can be associated with pheochromocytoma (PHEO) (50% of cases) and/or parathyroid adenomas (PTHomas) (30% of cases) and also, in a 10% of cases, with a cutaneous lichen amyloidosis (CLA) (Fig. 2a); (b) the multiple endocrine neoplasia type II B (MEN IIB) in which the MTC can be associated with a PHEO (45% of cases) and almost invariably accompanied by a marfanoid habitus, intestinal neurinomas and/or megacolon, scheletric abnormalities, corneal hypertrophy, and mucosal neurinomas (in the tongue and in the buccal mucosa and/or in the congiuntivas) (Fig. 2b); and (c) the familial medullary thyroid cancer (FMTC) in which the hereditary MTC is the only disease present in the family. While in the past it was reported that MEN IIA was the most frequent form of MEN II, nowadays, as effect of the introduction of the genetic screening for *RET* germline mutation, it has been demonstrated [5] that the FMTC is the most prevalent form, followed by MEN IIA and MEN IIB (Fig. 3).

At variance with the other well-differentiated thyroid cancer, papillary (PTC) and follicular (FTC), women and men are equally affected, both in the sporadic and hereditary form. Although the mean age at diagnosis is 45–50 years, a wide range in age at onset is present, but children are affected only in the hereditary form.

Up to date, neither environmental factors nor life or food styles have been demonstrated to be associated with the development of sporadic MTC. Nevertheless, associations with preexisting thyroid diseases and other disorders such as hypertension, allergies, and gallbladder disease have been reported in a pooled analysis of epidemiological studies [6]. The hypothesis that the development of MTC could be correlated to sunshine exposure has been postulated several years ago [7] but never confirmed in other series [8].

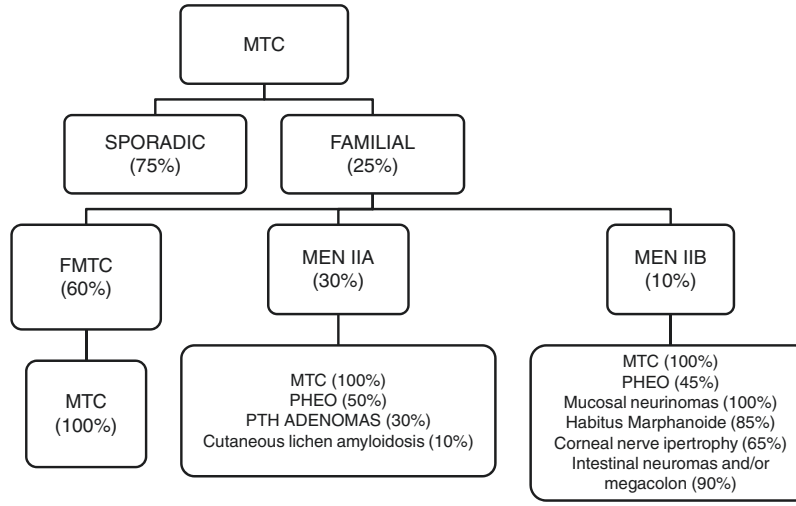

Fig. 3 Prevalences of the different forms of medullary thyroid cancer (MTC) and of the different endocrine (pheochromocytoma (PHEO) and parathyroid (PTH) adenomas) and non-endocrine diseases associated in the multiple endocrine neoplasia (MEN II) syndromes

The biological behavior of MTC is much less favorable when compared with that of PTC and FTC, but not as unfavorable as that of anaplastic thyroid cancer (ATC). A 10-year survival of about 50% for patients with MTC is reported in several series, and it is evident that both the cure and survival are positively affected by an early diagnosis [9]. Recently, an improvement of the survival of these patients, likely correlated with a better performance of both the diagnosis and the therapy of the disease, has been reported [10, 11].

Histological Diagnosis

At histology, the macroscopic appearance of MTC is of a hard and firm, red or chalky-white nodule. MTC microscopic appearance is pleiomorphic with spindle-shaped or rounded cells typically organized in a nested pattern (Fig. 4). Mitoses are uncommon, nuclei are usually uniform and characteristic secretory granules are present in the cytoplasm. Typically, amyloid substance is present among MTC cells, and when present it allows the specific diagnosis. MTC diagnosis can be confused with that of an ATC, or a Hürthle cell or an insular DTC, especially when pseudopapillary elements or giant cells are present. The positive immunohistochemistry for Ct, and also that for chromogranin A and carcinoembryonic antigen (CEA), is diagnostic of MTC [12]. The histological description of MTC should include the number and distribution of tumoral foci as well as the concomitant presence of C cell hyperplasia (CCH). C cell hyperplasia can be diffuse or focal and is considered the histological hallmarks of the hereditary forms even if nowadays it is known that about 30% of sporadic cases are also accompanied by CCH [13, 14]. The distinction between "neoplastic" and "benign/reactive" CCH is still undefined [15]. Nevertheless, a recent multicentric study demonstrated that sporadic MTC are multifocal (i.e., with >1 focal lesion) and bilateral in 17% and 5.6% of cases, respectively [16]. With the exception of the prevalence of CCH, no other major differences have been observed at histological level between the sporadic and the hereditary forms of MTC (authors' institutional observation).

Pathogenesis

In 1994, after discovering the transforming role of *RET* oncogene, it was demonstrated that germline activating point mutations of *RET* were present in the vast majority of a big series of hereditary MTCs [17]. In the same context, somatic *RET* mutations were found in about 40% of sporadic MTCs [18]. In vitro and in vivo studies confirmed the driver role of *RET* mutations in the development of MTC [19, 20].

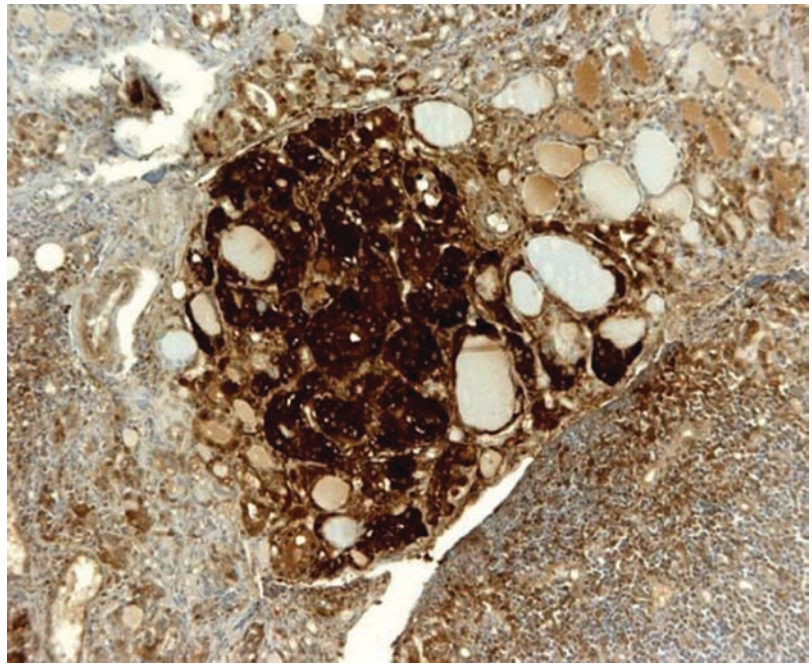

Fig. 4 Typical nested pattern of malignant C cells in a medullary thyroid cancer (immunohistochemistry for calcitonin)

Table 1 Genotype/phenotype correlation for the most common RET germline mutations

MEN IIB	MEN IIA	FMTC	MEN IIA/FMTC
M918	C634	G533	C609
A883	Y635	C630	C611
V804 + Y806		D631	C618
V804 + S904		K666	C620
		V804	L790
		R844	S891
		R912	

Germline *RET* point mutations are mainly localized in exons 10–11, 13–16, but other rare mutations have been reported also in other exons such as 5 and 8 [21, 22]. A significant genotype-phenotype correlation has been reported over the years with some *RET* mutation almost exclusively associated with a specific MEN II syndrome [23] (Table 1). Somatic *RET* mutations are mainly concentrated in exon 16, while only few cases have been reported in other exons [24]. Moreover, *RET* somatic mutation prevalence is significantly higher in bigger MTC tumors than in smaller [25], and particularly high in advanced and progressive MTC [26]. With a few exceptions, named "variant of unknown significance" (VUS) [27], the penetrance of *RET* mutations is complete in terms that all gene carriers sooner or later will develop the disease. At variance, the level of expressivity is different in terms that MTC due to different *RET* mutations can have different degree of aggressiveness and it is related to the transforming ability which is the highest for M918T mutation [27]. According to these differences, three risk levels (i.e., moderate, high, highest) have been suggested in the recently published "American Thyroid Association guidelines for the diagnosis and treatment of MTC" to classify the different *RET* mutations and to time surgical treatment of *RET* gene carrier [28].

Although not yet introduced in the routine clinical practice, it would be worth to know if a sporadic MTC is carrying a *RET* somatic mutation, since the mutated cases have a worse prognosis [29]; thus, a more aggressive therapeutic strategy or a more stringent follow-up could be reserved to these patients. Moreover, all cases of apparently sporadic MTC should be screened for a germline *RET* mutation, since about 5–10% of them are misdiagnosed hereditary forms [18].

RAS oncogene mutations, in particular *H-RAS* and *K-RAS*, are present in about 10–20% of sporadic MTC, and they are mutually exclusive with *RET* mutations [30]. No other driver mutations have been discovered in *RET*- and *RAS*-negative MTC even when tissues were analyzed with next generation sequencing techniques [31–33]. Nevertheless this approach allowed the identification of two MTC cases with a somatic rearrangement of *ALK* [33] and *RET* [34]. Some very rare mutations have been described, but their prevalence is so low to be considered as "private" mutation likely relevant only in that specific case [35]. A similar finding has been reported for hereditary cases: the *ESR2* gene, which encodes the beta subunit of the estrogen receptor (ERβ), has been found to be mutated in members with MTC but not in their unaffected relatives in one familial case. However, the same *ESR2* mutation was not found when it was looked for in other *RET*-negative familial cases, thus suggesting that it was a "private mutation" of the first family analyzed [36].

The challenge of the next future is to find the driver mutations of both the *RET*-negative familial cases and *RET*- and *RAS*-negative sporadic cases. This research is fundamental in the era of the new developing therapies which require a well-characterized molecular profile to be targeted [37].

Presurgical Diagnosis

From 0.4 to 1.5% of all thyroid nodules can be an MTC, and the challenge is to make both an early diagnosis and a presurgical correct diagnosis to plan the right surgical treatment. At neck ultrasound the thyroid nodule can be suspicious for malignancy but no specific echographic features for MTC have been identified so far [38].

Serum Ct is the most specific and sensitive marker for MTC both before and after thyroidectomy. It is a small polypeptide hormone (32 amino acids) physiologically produced almost exclusively by the parafollicular C cells. Ten years after the recognition of MTC as a distinct histological type of thyroid cancer, high levels of Ct were demonstrated to be present both in the tumoral tissue and in the serum of patients with MTC [39, 40]. Elevated baseline serum levels of Ct are strongly suggestive for MTC, especially when >100 pg/mL. Routine measurement of serum Ct in patients with nodular thyroid disease allows the preoperative diagnosis of unsuspected sporadic MTC [41]. In this regard, assaying serum Ct facilitates the early diagnosis of MTC, usually when the tumor is still at stage I, thus favoring successful surgical treatment [9]. Despite these evidences, there are still controversial opinions about the routine measurement of serum Ct in all cases of thyroid nodules. Two are the major concerns: (a) the false-positive cases and (b) the cost-benefit. As far as the false positivity of serum Ct is concerned, it should be taken into account that there are some rare clinical conditions in which serum Ct can be detectable such as in some neuroendocrine tumors, renal failure, hyperparathyroidism (hyperPTH), and some advanced carcinoma (i.e., lung and breast) which can be rather easily ruled out. Moreover, hypercalcitoninemia may be observed in isolated CCH surrounding either lymphocytic thyroiditis or microPTC [42]. Finally, the possibility to have false-positive results for the presence of heterophilic antibodies or low specificity of the Ct assay should be discussed with the referral laboratory. In any case, these confounding conditions can be ruled out with a Ct stimulation test since the peak Ct is much lower or even absent respect to that observed in the presence of MTC. Pentagastrin stimulation was used for many years in the clinical practice, but nowadays pentagastrin is not more available and the Ct stimulation is performed by a rapid e.v. infusion of 2.5 mg/Kg of calcium. Although high-dose calcium is a more

potent and better-tolerated Ct stimulator than pentagastrin [43, 44], some doubts about the specificity of this test have been arisen recently [45]. Regarding the cost-benefit concern, which is mainly due to the low prevalence of MTC among thyroid nodules, an American study clearly demonstrated that the benefit of this procedure can be compared to that obtained with the mammography and colonoscopy for breast and colon cancer, respectively [46], thus eliminating any doubts on this regard.

The problem of the presurgical diagnosis of MTC is still a matter of discussion. If the serum Ct routine screening of all thyroid nodules is not widely accepted and even not recommended by several international guidelines, the measurement of serum Ct should be performed at least in those cases with nodules suspicious for malignancy at neck ultrasound and in those cases for which the surgical treatment has been already planned. The reason to suggest this practice is related to the low sensitivity of the fine needle aspiration cytology (FNAC) for MTC. An international multicentric study has unequivocally demonstrated that the FNAC sensitivity for MTC is rather low since only 46% of MTC were revealed as MTC by FNAC and thus submitted to the right surgical treatment [47]. Another practice that can be useful in reaching a presurgical diagnosis of MTC is the measuring of Ct concentration in the washout of the needle used for FNAC of a suspected thyroid nodule [48]. This approach is of particular diagnostic utility also to ascertain the nature of enlarged neck lymph nodes, especially before thyroidectomy, in order to better plan the surgical approach or the most appropriate therapeutic strategies [48].

The presurgical diagnosis is similar in sporadic cases and in the index case of the familial forms while it is based on genetic screening for the relatives of index cases. In fact, after the identification of the germline *RET* mutation, all first-degree relatives should undergo the *RET* genetic screening for the same mutation [49]. This screening will allow the early identification of those relatives who will develop the MTC. The challenge is "when" to submit the gene carriers to surgical treatment. Nowadays we are aware that not all *RET* mutations have the same degree of aggressiveness and transforming ability and the age of development of MTC is strictly correlated with this degree. On this regard it is useful to recall that serum Ct is a very sensitive and specific marker of MTC and a periodic monitoring of this marker in gene carriers, especially in those with "moderate" *RET* mutation [50], will allow the early discovery of the tumor and the possibility to perform the thyroidectomy when the disease is still curable [51]. Moreover, the level of serum Ct can also guide the extension of thyroidectomy; in fact gene carriers with serum Ct < 30–40 pg/mL, according to the local cutoff, may avoid central node neck dissection [50–52] which represents the biggest risk factor for post-surgical hypoparathyroidism, especially in children [53].

Initial Treatment

It is worldwide recognized that total thyroidectomy and central neck nodes compartment dissection is the treatment of choice for MTC; therefore, an accurate presurgical diagnosis of MTC is fundamental for planning the correct surgical approach [41]. The opportunity or necessity to perform an elective ipsi- or bilateral neck dissection is still controversial. Nowadays this extensive surgery is better suggested in cases with serum Ct > 200 pg/mL because of the high risk to have neck node metastases [54]. However, since an accurate neck ultrasound can identify suspicious lymph nodes in the laterocervical compartment(s) [55], many surgeons prefer to perform the laterocervical dissection only in the presence of well-documented lymph node metastases. As matter of fact, when the lymph nodes of the neck are already metastatic, the biochemical cure of the disease is very unlikely to be obtained despite an extensive surgical treatment [56].

To avoid the risk of an unexpected hypertensive crisis during the surgical treatment, all MTC patients who should undergo thyroidectomy must be screened for the presence of PHEO by measuring plasma-free levels of metanephrine and normetanephrine [57]. The only possibility to

avoid this procedure is to have a negative result of the genetic screening before the thyroidectomy which is confirmatory that the case is sporadic.

The only recognized poor prognostic factors for both the cure and survival of patients with MTC are the advanced stage at diagnosis and the presence of a somatic *RET* mutation [29]. Although a positive correlation of preoperative serum levels of Ct has been correlated with the extension of the disease, there are cases less differentiated in which this correlation is lost and Ct values can be very low despite the spread of the disease [58]. At variance, preoperative elevated serum CEA levels are correlated with a bigger tumor size and greater number of lymph node metastases, thus reflecting the burden of disease. Moreover, serum CEA may be used as a surrogate tumor-associated marker in those MTCs that do not secrete Ct [59]. In cases with high levels of serum CEA, associated or not to elevated serum Ct, a computerized tomography (CT) scan with iodine contrast medium can be indicated to guide the extent of surgery.

Follow-Up

MTC patients are followed by monitoring the serum Ct and CEA levels and by neck US examination. Evaluating these diagnostic parameters every 6–12 months allows the clinicians to assess the clinical status of the patients and to early discover those who need medical treatment.

Because of the half-live(s) of serum Ct [60], the first monitoring after surgery is indicated at 3 months when the basal serum Ct levels can be reliable, not more affected by the presurgical values and reflecting the real clinical status of the patient. A postoperative elevated serum level of Ct is diagnostic of the persistence of the MTC, which occurs in 30–55% of cases after primary surgical treatment, particularly when the tumor is already extrathyroidal [61]. At variance, undetectable postoperative basal values of serum Ct indicate the cure of the disease with a risk of recurrence of 10%. This risk is even lower (about 3.0%) if also stimulated Ct is undetectable [62]. Cured patients can be followed up with 12–18 months clinical and biochemical controls, while those with detectable levels of serum Ct must be checked every 6 months with a clinical and biochemical control and at least a neck ultrasound since the neck is the most likely region for recurrence. When the value of basal serum Ct is less than 150 pg/mL also CEA level is low, likely normal, and it is very uncommon to find the lesion(s) producing and secreting Ct even with CT scan and other imaging techniques. No major efforts should be done to find these lesions, and an active surveillance with clinical and biochemical controls every 6–12 months is the most appropriate attitude (Fig. 5). However, if serum Ct is detectable, independently from its value, a total body CT scan with iodine contrast medium is indicated for both making a "starting point" for the follow up and discovering those cases, but existing, with low levels of serum Ct and advanced disease because of the dedifferentiation of the tumoral C cells [63].

The most sensitive biomarkers of MTC progression are the doubling times (DT) of serum Ct and CEA values, and when shorter than 1 year they represent poor prognostic factors for both the rapid progression of the disease and patient's death [64, 65]. There are some new evidences that elevated levels of serum Ca 19.9 in advanced MTC patients may represent a very bad prognostic factor for survival [66, 67].

The accurate identification of sites of recurrences is essential for patient management as well as their growth monitoring. The most appropriate diagnostic imaging work-up for both the localization and growth rate of the metastatic lesions includes US of the cervical region, CT scan of the chest, magnetic resonance imaging (MRI) for the liver and brain, and bone scintiscan [68]. These imaging techniques are preferred in clinical practice since, at variance with radionuclide imaging, they allow the correct identification and measurement of the lesions whose growth will be calculated according to the response evaluation criteria in solid tumors (RECIST) [69]. The schedule of their execution should take into account the Ct and CEA DT to avoid unnecessary radiation exposure to those

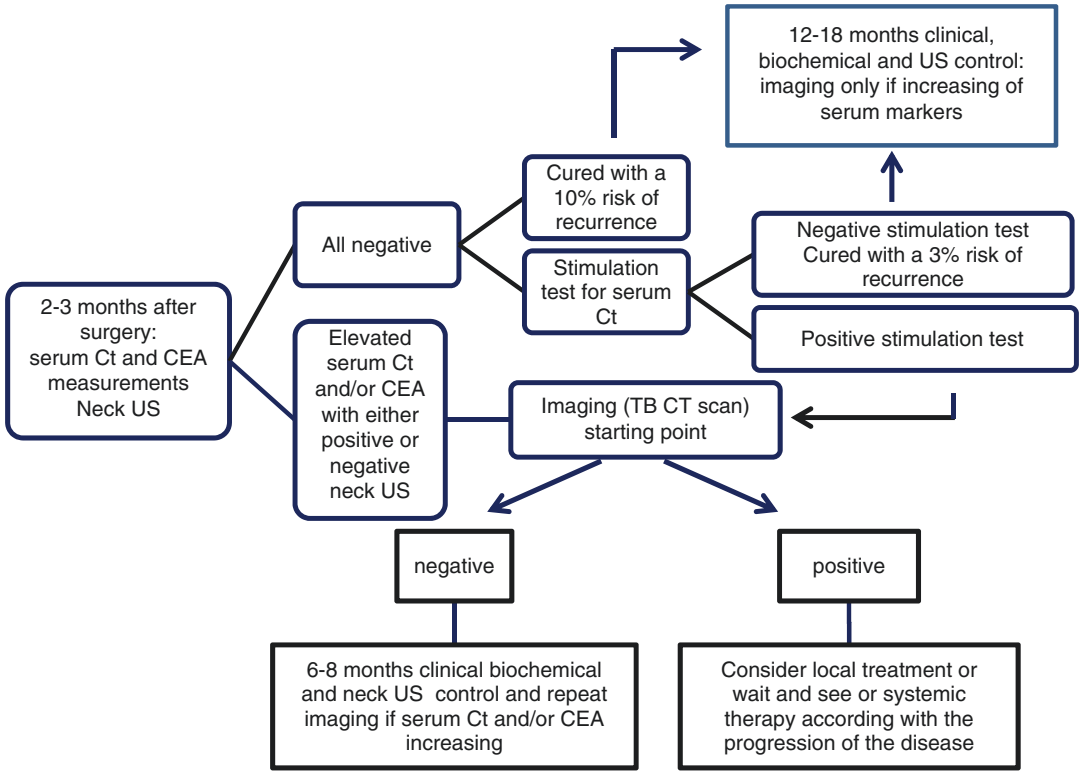

Fig. 5 Algorithm of follow-up of MTC patients after initial surgical treatment: measurement of serum calcitonin (Ct) and carcinoembryonic antigen (CEA) together with neck ultrasound (US) are the basic management tools, and their results represent the guide for both the schedule and the type of controls to be performed thereafter

patients with a long DT and, at the same time, to early intercept the tumor growth (Fig. 5).

Radionuclide imaging (RNI) techniques can play a role in recurrent MTC especially to detect residual or recurrent tumor that might be treated with radiopharmaceuticals. The most conventional RNI is the scintigraphy with the somatostatin analog ^{111}In-Pentetreotide (Octreoscan®), but nowadays its use for the evaluation of MTC patients is very limited, because the inhomogeneous distribution of somatostatin (SMS) receptors among different tumor lesions of the same patient are generally lower than in other neuroendocrine tumors [70]. The highest sensitivity is reported in MTC patients with neck and mediastinal lymph node metastases, while it is lower for distant metastases [71]. The real practical indication for this RNI is the evaluation of the presence of SMS receptors in patients candidate to therapy with radiolabeled SMS analogs.

^{123}I-metaiodobenzylguanidine (^{123}I-MIBG, structurally similar to norepinephrine) accumulates in tumors deriving from the neural crest. Scintigraphy with ^{123}I-MIBG can be positive in patients with MTC [72], but its sensitivity is rather low (38%) in patients with medium-low levels of serum Ct and no lesions detected with conventional imaging techniques [73].

Immunoscintigraphy with radiolabeled monoclonal anti-CEA antibody shows high sensitivity (75–100%) to detect MTC metastatic lesions in patients with aggressive and rapidly growing tumors [74]. However, this radiopharmaceutical is not commercially available.

Ligands for the gastrin/cholecystokinin B receptor (CCK-BR) constitute a new class of peptides that can be radiolabeled and utilized for diagnosis or therapy of CCK-BR-expressing tumors; among these, MTCs express the CCK-BR in almost 90% of the cases [75]. ^{111}In-labeled

derivatives of gastrin (^{111}In-DTPA-D-Glu^{1}-minigastrin) showed excellent targeting of CCK-BR-expressing tumors, with 94% sensitivity [76, 77]. However, the technique is restricted to very specialized centers, and its clinical usefulness is not yet demonstrated [78].

At variance with many other types of human tumors, [^{18}F] fludeoxyglucose (FDG) positron emission tomography (PET) suffers from the fact that the metabolic activity of MTC cells is only slightly increased with respect to normal cells. Indeed, ^{18}F-FDG-PET sensitivity in patients with MTC is correlated with Ct serum levels higher than 500 pg/mL, Ki-67 scores higher than 2.0%, and shorter Ct DT [79]. In particular, sensitivity of ^{18}F-FDG-PET is less than 20%, in patients with Ct levels <500 pg/mL and about 80% in patients with high levels of serum Ct (>1000 pg/mL) [80]. PET with ^{18}F-dihydroxyphenylalanine (DOPA) or with ^{68}Ga-labeled SMS analogs has more recently been proposed for patients with MTC. Indeed, PET with ^{18}F-DOPA is more accurate than other RNI techniques in patients with MTC, in particular for detecting local recurrent disease and lymph node involvement with a sensitivity of about 80% even in those cases with a long Ct DT and a low growth rate [81–83]. Other promising PET agents to image neuroendocrine tumors include SMS analogs labeled with ^{68}Ga through the DOTA chelator (e.g., ^{68}Ga-DOTA-TOC, ^{68}Ga-DOTA-TATE, ^{68}Ga-DOTA-NOC) [84]. However, the diagnostic role of ^{68}Ga-DOTA-peptides is still under investigation and validation, because of controversial reports probably due to the fact that MTCs show a variable and often low SMS-receptor subtype expression [84–86].

Treatment of Persistent/Recurrent and Metastatic Disease

After the initial surgical treatment, only 5–35% of MTCs, mainly those cases with intrathyroidal tumor, are cured also at biochemical level (i.e., normalization of serum Ct and/or CEA levels). Among the others, three groups of patients can be distinguished from a clinical point of view: (a) those with a biochemical persistent disease (i.e., detectable levels of serum markers Ct and CEA but no evidence of structural disease); (b) those with a structural disease, both local and/or distant, but with low Ct and CEA DT and a null or very slow growth rate; and (c) those with a structural disease and a rapid progression as assessed by both a short DT and a rapid growth of the lesions according to RECIST.

While the first and second group must be submitted to an "active surveillance" by following the algorithm above described (Fig. 5), the third group requires to be treated with the intent to control the disease growth or to reduce the symptoms that sometimes are related to the disease localization and growth. The type of treatment to be used is very much dependent on both the site and size of the metastatic lesion. Whenever possible a local treatment should be preferred to a systemic therapy that should be reserved to cases with multiple lesions in multiple organs and clearly progressing according to RECIST [87]. Local or regional recurrence of MTC in the neck and mediastinum can be effectively treated with a second surgical treatment. External beam radiotherapy (EBRT) should follow this surgery especially if the neck is extensively involved or if this surgical treatment was the last of several others previously performed [88]. ERBT can also be used for bone lesions, in particular if painful, and brain metastases for their stabilization. In patients with a predominant metastasis in the liver, transarterial chemoembolization (TACE) has proven effective in reducing the tumor mass [89]. TACE is usually well tolerated and determines both clinical improvement and tumor response for relatively long periods of time in the majority of patients. This therapeutic option should always be taken into consideration even when extrahepatic metastases are present [90, 91]. Several types of percutaneous thermal ablation techniques are entering in the clinical management of metastatic lesions especially bone, lung, and liver lesions. Radiofrequency ablation (RFA), microwave ablation (MWA), laser ablation, high-intensity focused ultrasound (HIFU), and cryoablation can be used with curative and/or palliative pur-

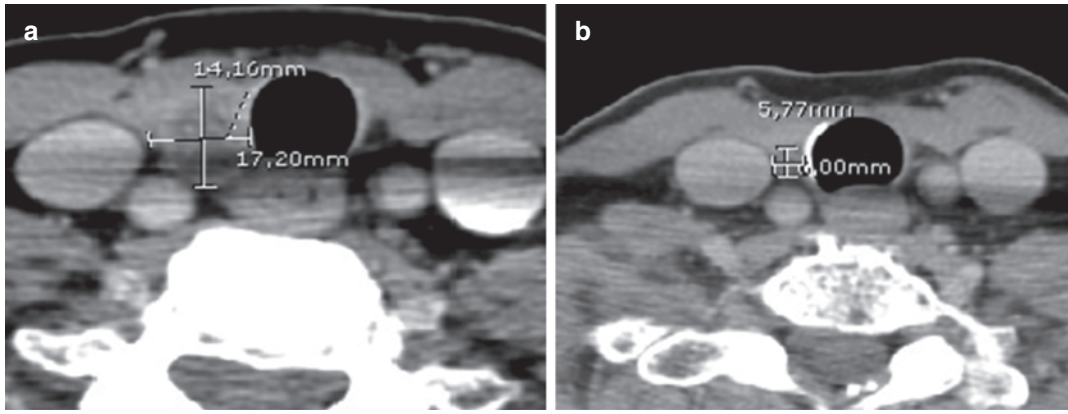

Fig. 6 Local paratracheal MTC recurrence before (panel **a**) and 3 months after (panel **b**) local treatment with thermoablation: a reduction of about 60% of the biggest diameter was observed

poses [92]. In thyroid cancer RFA is of particular usefulness for the treatment of local recurrences particularly when surgery cannot be performed because of a contralateral cord palsy or anesthesia contraindications (Fig. 6).

The main cause of MTC-related death is represented by distant metastases, which affect more frequently the lungs, bones, and liver, more rarely the brain and skin [93]. The 5-year survival rate of patients with distant metastases at diagnosis is 25% [94], with low response rates elicited by any of the therapeutic options available. Conventional chemotherapy, both mono drug and combined, induces a temporary clinical benefit in terms of disease stabilization (SD) and/or partial remission (PR) in only 20% of cases [95, 96], but it cannot be considered a valid therapeutic option especially when considering the cost-benefits between the very severe side effects and the poor results [97]. Other systemic therapies have been tested in the past such as interferon-α and SMS analogs, but they are able to only induce transient improvement of the symptoms and Ct level reduction with any proved benefit in disease stabilization or remission [98]. Anti-CEA pretargeted radioimmunotherapy (pRAIT) has been used for treatment of metastatic MTC and has been shown to induce long-term disease stabilization and significantly prolonged survival in high-risk MTC patients with manageable hematologic toxicity [99–101]. More recently, radionuclide therapy with the radiolabeled SMS analog ^{90}Y-DOTA-Tyr^{3}-octreotide (^{90}Y-DOTATOC) has been tested in metastatic MTC. This therapy has resulted in favorable biochemical response in about one-third of the patients (irrespective of the result of the pre-therapeutic scintigraphy), associated with a long-term survival benefit [102]. Patients with smaller tumors and higher uptake of the radiopeptide tended to respond better [103]. Further studies are in progress to investigate the effect of other radiolabeled SMS analogs, including ^{177}Lu-DOTA-TATE and other radiopeptides, among which gastrin-like ligands, on the survival of patients with advanced metastatic MTC [75, 102].

Bone metastases are present in about 20% of MTC patients, and about 50% of them suffer from skeletal-related events (SREs), less frequently from spinal cord compression [104]. Patients with bone metastatic lesions, independently from the origin of the carcinoma, are usually treated with bisphosphonates e.v. particularly with zoledronic acid or denosumab to reduce the risk of SREs, including bone fracture, spinal cord compression, and hypercalcemia. Positive results of this treatment have been reported for differentiated thyroid carcinoma [105], but no data are available for MTC. An accurate evaluation of the dental and buccal cavity status should be always performed before starting bisphosphonates for the high risk to develop the osteonecrosis of the jaw (ONJ) [106].

Despite all the above mentioned tentative of treatment, metastatic MTC has been orphan of a real therapy until recently. Starting from 2005, molecular targeted therapies became the new frontiers for treating patients with metastatic MTC [107]. In particular, the *RET* gene immediately represented a promising target for therapy in patients with familial MTC as well as for the majority of patients with advanced sporadic MTC [26]. Several inhibitors of the *RET* kinase activity which are active also against other tyrosine kinases (TKs), named tyrosine kinase inhibitors (TKIs), have been evaluated in the last decade [108–111]. They are small molecules able to simultaneously block several kinases (multitargeted TKI) including the vascular endothelial growth factor receptor (VEGF-R) which is also overexpressed in MTC [112] and in particular in those cases with a *RET* mutation [113]. A complex network among *RET* and *RAS* mutations and the overexpression of other TKs has been recently demonstrated suggesting a TK-inhibiting role of TKI through the inhibition of the driver mutations [114, 115]. Because of their modality of action, the TKI are cytostatic and not cytotoxic thus the expected effect is to stop the tumor growth. However, since they act also against VEGF-R, they have also an antiangiogenic effect which ends up in shrinking the tumoral lesions as a consequence of their devascularization (Fig. 7). Two of these drugs, vandetanib and cabozantinib, have been approved by both the Food and Drug Administration (FDA) and European Medical Agency (EMA) for the treatment of advanced

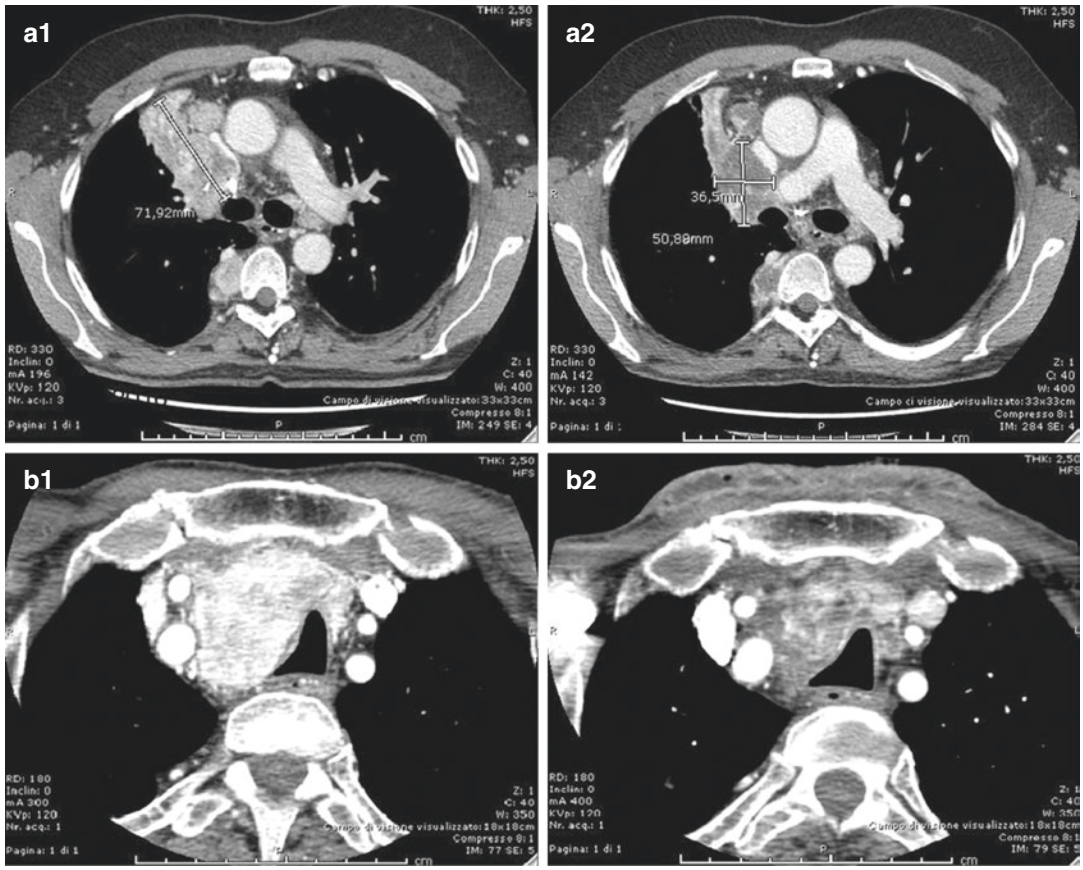

Fig. 7 Shrinkage of MTC metastatic lesions after 6 months of treatment with vandetanib (panel A) and cabozantinib (panel B). In both cases, the difference in both the size and vascularization before (panel A1 and panel B1) and after (panel A2 and panel B2) 6 months of the therapy is visible

and progressive MTC (Table 2). The approvals for the clinical use of the two drugs arrived after the positive results of the two phase III studies, ZETA and EXAM study, in which the effect of vandetanib and cabozantinib on the progression-free survival (PFS) of patients with advanced and progressive MTC, were tested against placebo [116, 117]. In both studies, the PFS of patients treated with the drug was significantly longer than that of patients treated with placebo. In both studies it was also observed a significant objective response rate (ORR) since many targeted lesions showed either a stabilization of the growth (SD) or a partial remission (PR) according to RECIST. Unfortunately, until now no advantages in terms of overall survival has been reported with the exception of a subgroup of patients with tumors positive for the M918T *RET* mutation and treated with cabozantinib [118]. The subgroup analysis, performed according to age, sex, different metastatic lesions, presence of *RET* mutations, and other parameters, was unable to identify a better responder group since in all cases an advantage from the drug treatment was observed. Finally, cabozantinib has been found to be active also in cases previously treated with another TKI thus demonstrating that cabozantinib can be used both as first- and second-line treatment. The major concerns about the use of these drugs are related to (a) the adverse events (AEs) that they can induce (Table 2), (b) the fact that once they have started they should be continued long life or at least until the evidence of clinical benefits, and (c) the appearance, sooner or later, of the escape phenomenon. The AE can be managed in the majority of cases either with the introduction of other drugs to treat the specific AE, such as angiotensin-converting-enzyme (ACE) inhibitors for hypertension, or reducing the daily dosage of the drug as in case of a severe fatigue or anorexia. Patients must be alerted of the possible AE and instructed to refer immediately the appearance of AE-related symptoms to give the doctor the possibility to rapidly intervene with the appropriate clinical attitude. As far as the problem of the escape phenomenon is concerned, so far what we can do is to plan an algorithm of drugs administration. At the present vandetanib and cabozantinib are the only two approved drugs, but unfortunately not all countries have the same access to the two drugs. However, supposing that both drugs are equally available, we would better start with vandetanib since we know that cabozantinib can be active also in second line [116] and reserve this latter at the time of the escape from vandetanib treatment. Among other drugs that could be used as following lines of treatment, lenvatinib has been tested in a phase II study and showed very promising results [111]. Lenvatinib, but also sorafenib and sunitinib, can be eventually used as "off-label" drugs in patients surviving to both vandetanib and cabozantinib treatment [119].

After the results of a phase I/II study performed in children and adolescents affected by metastatic MTC, mainly by MEN IIB, vandetanib has been approved also for the treatment of patients <18 years of age, although with a different schedule of drug administration [120].

Table 2 Similarities and differences between the two TKIs approved for the treatment of advanced medullary thyroid cancer

	Vandetanib	Cabozantinib
Phase III study	ZETA study	EXAM study
N of enrolled patients	331	330
Median PFS (months)	30.5 (drug) vs. 19.3 (placebo)	11.2 (drug) vs. 4.0 (placebo)
Objective response rate (%)	45	28
Overall survival	No improvement	No improvement
Half-life	19 days	55 h
Starting dosage	300 mg/day	140 mg/day
Content of capsules	300 mg or 100 mg	80 mg or 20 mg
Most frequent adverse events %		
Hypertension	32	32
Diarrhea	56	63
Skin rash	45	19
Anorexia	21	45
Nausea	33	43
Weight loss	10	47
Fatigue	24	40
QTc prolongation	14	NE

Vandetanib can be prescribed also for the treatment of Cushing's syndrome due to ectopic production of ACTH [120–123]. Although the prevalence of this ectopic syndrome is rather rare in MTC, varying from 1.9 to 11.6% [124], it represents a very poor prognostic factor for survival. For this reason Cushing's ectopic syndrome is per se an indication to therapy independently from the evidence of disease progression. On this regard it is useful to say that also other TKIs have been demonstrated to be useful for the treatment of this syndrome [125, 126] and, although they have not been approved for the treatment of MTC, an "off-label" use could be appropriate in cases that, for any reason, could not be treated with vandetanib.

Conclusions

The early diagnosis and successful treatment of MTC still remain the most important clinical challenges both for doctors and patients. The introduction of the *RET* genetic screening, serum Ct measurement, and neck ultrasound in the clinical practice certainly allowed to perform earlier diagnosis thus positively impacting on the survival of the patients. TKIs, particularly vandetanib and cabozantinib, have been also demonstrated to improve the management of advanced and progressive MTC. Nevertheless, it is highly desirable that further therapeutic strategies would be explored in the next future to overcome the occurrence of the drug resistance which represents, at the moment, the major limit of the new targeted drugs.

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Anaplastic Thyroid Carcinoma

Robert C. Smallridge and Keith C. Bible

Presentation

Although patients of 15 years old have rarely been described, patients with ATC are generally older, with a median (mean) age of 69 (66.5) years. Women are affected more often than men (~1.9:1) [1]. In the United States, there is a small variation among racial/ethnic groups, occurring in 1.8% of blacks, 1.3% American Indian native, 1.1% non-Hispanic white, 1.0% Asian-Pacific Islander, and 0.6% Hispanic white as a percentage of total thyroid cancer cases [2].

Anaplastic thyroid carcinoma may develop de novo, or in patients with a history of benign goiter or differentiated thyroid cancer, usually presenting as a rapidly growing neck mass. In most patients, the tumor extends beyond the confines of the thyroid gland to involve nearby structures including the recurrent laryngeal nerves, trachea, esophagus, and great vessels of the neck (carotid arteries and jugular veins). Such invasion commonly produces hoarseness/dysphonia, dysphagia, and dyspnea. Less commonly, patients may have pain, cough, hemoptysis, or superior vena cava syndrome [1].

Distant metastases are common at time of presentation, reported in two of every five patients. Most common sites of metastases are the lung, followed by the mediastinum, liver, and bone, with the heart, adrenal glands, kidneys, soft tissue, and brain less commonly involved [1]. One autopsy report identified the same general anatomic distribution, but an occurrence rate of metastases 2–4 times greater than noted while patients were alive [3], emphasizing the fact that ATC is most commonly distantly metastatic at diagnosis, even if not initially recognized.

Immediate assessment and protection of airway and esophageal/nutritional status are paramount, as will be further discussed, as there is great risk of fatal locoregional complications in ATC. Death in ATC stems primarily from distant metastases (~60%), from distant and locoregional disease (25%), or (less commonly) from complications of local infiltration (15%) [4].

Diagnosis

Guidelines for management of patients with ATC have been published by the American Thyroid Association [5] and the National Comprehensive Cancer Network (NCCN) [6], and an orderly approach is summarized in Table 1. It is critical to make the correct histopathological diagnosis

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Table 1 Approach to patients with anaplastic thyroid carcinoma

Diagnosis
Tissue (cytology; core biopsy)
Evaluation
Clinical assessment
Laboratory
Imaging
Staging
IVA/IB (resectable)
IVB (unresectable)
IVC—metastatic
Establish goals
Disclose status (risks/benefits of therapy)
Discuss patient's values/preferences
Patient makes informed decision

Adapted from Smallridge et al. [5]

quickly. These tumors are undifferentiated and often contain necrotic areas, so fine-needle aspiration cytology may be nondiagnostic. Moreover, sampling error is also an issue due to tumor heterogeneity in patients with mixed differentiated and anaplastic thyroid cancers. Hence, ultrasound-guided core biopsy should be performed to obtain sufficient representative material.

ATC has several histologic subtypes, including spindle cell, giant cell, squamoid, and paucicellular. Several other tumors may mimic ATC, such as poorly differentiated thyroid cancer, squamous cell carcinoma of the head and neck, medullary thyroid cancer, lymphoma, sarcoma, and metastases (e.g., arising from renal cell carcinoma, melanoma). Immunohistochemical markers such as pankeratin, thyroglobulin, chromogranin, calcitonin, E-cadherin, CD45, and others can help the pathologist define tumor origin—but it is critical to remember that ATCs are so dedifferentiated to have lost immunohistochemical reactivity for thyroglobulin and TTF-1. From the pathologist's perspective, ATC represents a "poorly differentiated carcinoma," leading to confusion, as ATC must be distinguished from "poorly differentiated thyroid carcinoma," wherein immunohistochemical reactivity for thyroglobulin and TTF-1 remains. If both FNA and core biopsy are insufficient, then an open biopsy should be performed [5].

ATC patients often have a prior history of thyroid disease, including goiter (reported in >80% of patients), and/or differentiated thyroid cancer (DTC, particularly more aggressive variants such as tall cell). The frequent coexistence of ATC and DTC suggests that the latter may precede the development of ATC; in some patients, ATC even arises in metastatic DTC deposits long after thyroidectomy, bolstering the contention that ATC may represent dedifferentiated DTC. The identification of an area of well- or poorly differentiated thyroid carcinoma in the biopsy or surgical specimen helps affirm that the observed undifferentiated tumor is ATC. There are limited data suggesting that survival is improved when the anaplastic component comprises only a small percentage (<10%) of the tumor, so the pathologist should provide some description of how much of the tumor is ATC vs. a more differentiated cancer [7]. Long-term survival is also improved if the tumor has lymphocytic, but not neutrophilic infiltration [8].

ATC is well known for its high genomic instability, with numerous mutations of both oncogenes and tumor suppressor genes, as well as epigenetic abnormalities, observed [9–15]. While the ATA Guidelines did not indicate that molecular studies were necessary for diagnosis or management of ATC [5], recent reports of molecular abnormalities [10–13, 15] (Table 2) and limited reports of patient responses to targeted agents

Table 2 Anaplastic thyroid carcinoma: prevalence of gene mutations

Gene (No. articles)	Median % (range)
TERT promoter (7)	46% (12–73)
TP53 (9)	67% (0–86)
BRAF (10)	27% (8–46)
RAS (11)	22% (8–62)
PIK3CA (7)	12% (4–23)
PTEN (5)	11% (0–16)
EIF1AX (2)	12% (9–14)
CTNNB1 (5)	4% (0–66)
AXIN1 (3)	3% (0–82)
APC (3)	3% (0–9)
AKT1 (4)	0% –
RET/PTC (3)	0% –

Adapted from Landa et al. [11]

[14, 16–29] support mutational interrogation of tumors from patients who wish aggressive treatment, with potential to use this information in designing later salvage therapies.

Evaluation

While the pathologist is confirming the diagnosis, the patient should have a rapid, but thorough, clinical assessment. History and physical exam should carefully define the patient's aerodigestive status (including vocal cord exam), as well as symptoms or signs suggesting the presence of distant metastases. Laboratory studies should include a complete blood count (leukocytosis has been implicated as an adverse predictor of outcome), comprehensive chemistry profile, TSH, and coagulation studies. Imaging studies should include a neck ultrasound for initial estimate of extent of local tumor invasion, followed promptly by a contrast-enhanced CT of the neck for more detailed assessment of locoregional involvement and to provide the surgeon with information critical to assessing the potential resectability of the tumor. The presence of distant metastases should be defined either by CT scans of the chest/abdomen/pelvis and a bone scan or preferably via ^{18}F-FDG-PET/CT scan. In the presence of any neurologic symptoms, contrast-enhanced MRI of the brain is also imperative. As in non-small cell lung carcinoma, brain MRI is best included in initial staging, best performed even in asymptomatic individuals prior to prescribing any systemic therapies.

Staging

Once an ATC diagnosis is confirmed and initial evaluation completed, ideally within just a few days, stage is defined. Stage IVA ATC is confined to the thyroid gland and is seen in only a small minority of patients; stage IVB is limited to the neck and occurs in ~40% of patients; stage IVC refers to those with distant metastases (~45%). Stage IVB is further divided into patients with potentially resectable disease (RO/R1 resections) vs. those with unresectable tumors (those for which only biopsy or debulking can be performed, with up-front surgery generally not advisable).

Establish Goals of Care

A multidisciplinary team involving an experienced surgeon, radiation oncologist, medical oncologist, and often an endocrinologist and also a palliative care physician should identify and prioritize treatment options and discuss these options with the patient, family, and other caregivers. The risks and benefits of available approaches (preservation of the airway; nutrition and potential need for feeding tube, surgery, external beam radiotherapy, systemic therapies, directed ablations) need to be explained in the context of the patient's estimated, often brief, life expectancy. In general, factors that favor longer survival include the ability to achieve complete surgical resection of the primary tumor, intensity-modulated radiation therapy to the neck/mediastinum, and absence of distant metastases. Traditionally, chemotherapy has often not been deemed of benefit, possibly due to more advanced disease historically noted at diagnosis. However, several recent reports have shown improved outcomes in select patients who undergo initial aggressive multimodal therapy [30–33].

Patients need to understand not only risks/benefits of therapy but their own expected outcomes based on TNM stage. For instance, Akaishi et al. [34] reported median survivals of 33.5, 6.1, and 2.5 months for Stages IVA, IVB, and IVC disease, respectively. Survival at 6 months was 100, 49.6, and 22.4%; at 1 year was 72.7, 24.8, and 8.2%; and at 2 years was 62.3, 10.6, and 0%. Thus, patients with Stage IVA disease are presumably more likely to benefit from an aggressive treatment regimen and therefore might reasonably be more willing to endure the considerable associated side effects and complications. Patients with IVC disease may, alternatively, be wise to select a palliative care approach from the beginning.

Supportive and Palliative Care

ATC patients need strong and continued physical and emotional support from the outset. Patients (and their families) face daunting stresses and challenges. Most often they were perfectly healthy just a few weeks earlier and suddenly are told that they will likely die, perhaps fairly soon. The ATA Guidelines [5] has a particularly comprehensive discussion of the approach to discussing patient's values and preferences and helping the patient make an informed decision, emphasizing the critical importance of first establishing that the patient has adequate decision-making capacity to render informed consent. If not, then a psychiatric or clinical ethics consultation may be needed including determination as to whether a surrogate decision-maker is needed. Patients should understand how each option may affect his/her quality of life, and palliative care/hospice should be included as an option. Formalizing advanced directives should also be encouraged and fully vetted with family members.

Comprehensive and proactive supportive and palliative care is a critical aspect of the management of all ATC patients, regardless of their election of more vs. less intensive treatment approaches. Providing the required palliative and supportive services can be challenging and time-intensive and may thus benefit from involvement of a dedicated palliative care team. Important issues include airway protection, palliation of swallowing difficulties and maintenance of adequate nutrition, pain control, and psychosocial/emotional support. Some ATC patients will require tracheostomy due to airway invasion or compression or bilateral vocal cord paralysis. Patients undergoing intensive radiotherapy often (in our experience about half) incur such great difficulties swallowing and/or painful swallowing so as to require temporary percutaneous endoscopic gastrostomy (PEG) tube placement—and all patients undergoing intensive radiation therapy are best provided nutritional counseling and support. Most ATC patients also require assistance with pain control at some point(s) in their disease course. All patients additionally require strong and ongoing emotional/psychological support.

Initiation of Therapy

Locoregional Disease (Table 3)

Patients with Stage IVA or resectable IVB disease should consider intensive multimodal therapy, assuming that their health is otherwise reasonably good and, after counseling, that they desire an aggressive approach. Initial treatment for these patients should best start with surgery, providing that imaging suggests that a complete (RO) resection, or grossly negative surgical margin (R1 resection), may be achievable. In the setting of extrathyroidal invasion (IVB disease), en bloc removal is advised, but not if total laryngectomy is required. Patients may present with hoarseness from ipsilateral recurrent laryngeal nerve damage, so special care must be taken to avoid damaging the contralateral nerve in such instances. Some patients present with tumor invasion into upper airway, tracheal compression, or bilateral vocal cord paralysis—but if not, elective tracheostomy is not advised [5].

Shortly after surgery, the highest priority is to sustain locoregional control of tumor, either with high-dose or palliative radiotherapy; without these additions, the benefits of surgery are expected to be transient and minimal. In the SEER database study of 516 patients, the combi-

Table 3 Algorithm management for ATC (Stages IVA-B)

	Initial	Response	Follow-up
Stage IVA/IVB (resectable)	Surgery RT+/− chemo	NED	Observe
			Adjuvant therapy
		Local recurrence	RT
			Surgery
			Chemo/clinical trial
		Systemic disease	Palliative RT
			Chemo/clinical trial
IVB (unresectable)	RT +/− chemo? Surgery after RT	Same as above	Hospice Same as above

Adapted from Smallridge et al. [5]

nation of surgery and external beam radiation therapy independently predicted survival [35], while a review of 2742 ATC patients in the National Cancer Database showed marginal treatment benefit [36]. If definitive therapy is elected, the preferred radiotherapeutic modality is intensity-modulated radiation therapy (IMRT), but palliative approaches such as Quad Shot may be appropriate in patients who wish a more palliative approach [37–39]. Several reports have shown that radiation or chemoradiation may alternatively be given before surgery [40, 41], but surgery is made much more difficult in the setting of prior neck radiotherapy, and tumor progression due to delayed surgery can lead to unresectability.

Critically, most ATC patients who undergo surgery and neck radiotherapy succumb to metastatic, as opposed to locoregional, disease alone. Hence, the important issue arises as to whether early intervention with systemic therapy might further contribute to improved overall survival. Several retrospective studies suggest that this may be the case, especially for IVA and IVB ATC [31–35], but prospective randomized trial data are lacking. Radiosensitizing, followed by adjuvant, chemotherapy may improve response rate but may also increase treatment-related morbidity [5]. Systemic chemotherapy can begin even sooner than radiotherapy, possibly as early as 1 week postoperatively, as less healing of the surgical wound is required to safely administer systemic chemotherapy as compared to definitive radiotherapy.

After completion of initial treatment with surgery followed by radiotherapy +/− chemotherapy, patients are reevaluated (restaged) to help define next steps. If there is no evidence of disease upon restaging, then observation with frequent cross-sectional imaging (perhaps combined with adjuvant systemic therapy) is favored. Recovery from the considerable toxicities from neck IMRT is a slow process, such that many patients (in our experience less than half) are not in sufficient physical or psychological condition to immediately consider additional superimposed therapy when assessed 1 month after IMRT completion.

If disease recurs locally, then consideration should include additional surgery or radiation (if maximal dose has not been given), additional systemic therapy (either cytotoxic or a clinical trial), or hospice. Although many ATC recurrences involve widely distributed metastatic deposits, some patients develop "oligometastatic" recurrences, wherein only one or a few sites of metastases are observed. In such cases, the best initial salvage therapeutic approach may represent focal directed therapies (e.g., thermal ablation, stereotactic radiosurgery), rather than systemic therapy, in efforts to provide more robust palliation of limited lesions—especially if symptomatic. Palliative locoregional approaches are critical in optimizing symptom control, especially in the case of bone or brain metastases. For those who experience rapidly progressive systemic disease, options include palliative local therapies, chemotherapy/clinical trial, or hospice care [5]. For Stage IVB patients who subsequently present with unresectable neck disease, several studies have shown responses to radio- and/or chemotherapy, with some patients subsequently being candidates for surgical resection [40].

Systemic Disease (Table 4)

To date, studies evaluating salvage systemic therapies have yielded almost universally disappointing results in patients with widely metastatic (Stage IVC) ATC [17, 29]. Consequently, it is extremely important that patients and their caregivers thoughtfully establish treatment goals and embrace the expected low chances of substantive benefit and the significant potential for side effects (and even harm) that may accompany systemic therapy. Most IVC patients who wish aggressive therapy should immediately receive neck/locoregional radiotherapy and chemotherapy. One report indicated that "maximal debulking" neck surgery may be appropriate in selected patients with Stage IVC disease, with potential for improved survival and quality of life if followed by adjuvant systemic therapy [42], but another report yielded contrary results

Table 4 Management algorithm for ATC (Stage IVC)

Establish goals	Initial therapy
Aggressive therapy	Neck/locoregional RT
	Palliative neck/locoregional RT
	Systemic therapy
	Cytotoxics
	Clinical trials
Supportive care	Palliative neck/locoregional RT
	Focal lesion control
	Focal palliative RT
	Palliative ablation/ embolization
	Hospice/palliative care

Adapted from Smallridge et al. [5]

Table 5 Targeted therapies in anaplastic thyroid carcinomas

Drug	Putative relevant therapeutic target(s)
Imatinib	PDGFR
Gefitinib; erlotinib	EGFR
Sorafenib	VEGFR; PDGFR; RAF
Pazopanib; lenvatinib	VEGFR
Vemurafenib; dabrafenib	Mutated BRAF
Everolimus	mTOR
Crizotinib	Mutated or translocated ALK
Fosbretabulin (combretastatin)	Microtubules; vascular supply
Efatutazone	PPAR-gamma

when many patients who underwent surgery did not receive adjuvant systemic therapy [43]. Patients may alternatively prefer supportive care, either because of poor overall health or upon recognizing the currently dismal response rates and considerable potential toxicities from any aggressive treatment in the setting of metastatic ATC.

First-line systemic therapy might be cytotoxic (a taxane, doxorubicin, or platin) either as single agent or in combination [5]. Alternatively, several clinical trials are usually available (see www.clinicaltrials.gov) and should also be considered. There is abundant preclinical literature describing the landscape of ATC at a molecular level [9–15]. Table 5 highlights many potential therapeutic molecular targets. Clinically, there have been a few trials, small series, and case reports that may help direct new clinical trials during the next few years [26, 28, 29, 44]. Vascular disrupting agents (combretastatin and fosbretabulin) have shown a trend toward prolonging survival [16, 17], and disease-modifying activity has been seen in response to therapy with multikinase inhibitors such as sorafenib [18], imatinib [19], EGFR antagonists [20], and everolimus [45]. Pazopanib as a single agent was ineffective [21] but is being examined in combination with IMRT and paclitaxel (www.clinicaltrials.gov identifier: NCT01236547). Efatutazone, a PPAR-γ agonist, showed activity in a phase 1 trial [46]. In $BRAF^{V600}$-mutated ATC, dabrafenib produced temporary disease regression in two patients [47], while vemurafenib demonstrated a response in a single patient [22], as did the ALK inhibitor crizotinib in one patient with an ALK mutation [23]. Targeted agents also can, however, have significant side effects, as described in another patient who had extensive tracheal necrosis after treatment with a VEGF monoclonal antibody [48]; moreover, attained responses in widely metastatic ATC are generally brief.

Improving outcomes in anaplastic thyroid carcinoma will require improved understanding of the key interactions among the multiple dysregulated genes and signaling pathways in ATC [9–15], as well as the contributions of the tumor microenvironment [24, 28, 49] and tumor immunology to tumor progression. On this basis, there is hope that combinatorial therapeutics [50–52], preferably selected based upon each patient's individual tumor characteristics, can lead to further therapeutic progress in ATC.

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Primary Thyroid Lymphoma

Stephanie Aleskow Stein

Introduction

Primary thyroid lymphoma (PTL) is a rare cause of malignancy, accounting for <5% of thyroid malignancies [1] and < 2% of extranodal lymphomas [2, 3] with an annual estimated incidence of 2 per one million [4]. PTL most commonly presents in females with chronic Hashimoto's thyroiditis in their sixth to seventh decade of life with a rapidly enlarging neck mass [3, 5–8]. Patients with Hashimoto's thyroiditis have a relative risk of developing PTL of 67 compared to those without thyroiditis [9]. Most thyroid lymphomas are non-Hodgkin's lymphomas (NHLs) of B-cell origin. The recognition and diagnosis of PTL can be challenging due to its rarity and difficulty distinguishing it from other thyroid diseases due, for example, to overlapping imaging characteristics and often co-occurrence in the same gland with thyroiditis. Despite the rarity of PTL, it is important to diagnose accurately as its management differs from that of other thyroid neoplasms. Treatment and prognosis ultimately depend upon the histology and stage of the tumor, but unlike most thyroid cancers, surgery is not the preferred treatment for most cases of PTL.

Histologic Subtypes

Lymphomas occur when there is malignant transformation of normal lymphocytes that can reside in both lymphoid and nonlymphoid tissues including the thyroid gland. The 2008 classification of lymphomas by the World Health Organization categorizes lymphomas by their dominant cell type including B-cell lymphomas, T-cell lymphomas, natural killer-cell lymphomas, and Hodgkin's lymphomas [10]. Lymphomas of the thyroid are almost exclusively of the non-Hodgkin's B-cell type. Diffuse large B-cell lymphoma (DLBCL) is most prevalent, accounting for more than 50% of cases, followed by the more indolent mucosa-associated lymphoid tissue (MALT) lymphoma which represents about 10–23% of cases [3, 7, 11, 12]. MALT lymphomas typically arise in the thyroid glands of patients with Hashimoto's thyroiditis [9, 13–15]. Many believe it is the chronic antigenic stimulation of lymphocytes in autoimmune disorders that leads to this malignant transformation [16]. Rarer subtypes of PTL include follicular (10%), small lymphocytic (3%), and Hodgkin's lymphoma (2%), along with Burkitt's, T-cell, mantle cell, and lymphoblastic lymphomas each accounting for <1% of cases [7].

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Clinical Presentation

PTL presents as an enlarging anterior neck mass that is, about 1/3 of the time, associated with compressive symptoms such as dyspnea, dysphagia, stridor, and hoarseness [5, 11, 12, 15]. Although duration of symptoms prior to diagnosis can range from a few days to 36 months, those with the more aggressive DLBL tend to present more acutely [3, 5, 8, 12]. Approximately 10% of patients experience B symptoms such as fever, night sweats, and weight loss [11].

On physical examination, the neck mass in patients with thyroid lymphoma is hard with a smooth surface and can be unilateral or bilateral [8]. Hypothyroidism is reported in approximately 1/3 of patients [6, 8, 15]. The majority of patients display elevated levels of thyroid antibodies, such as anti-Tg or anti-TPO antibodies. The tumor size at diagnosis is on average 7 cm (0.5–19.5 cm) [5].

Diagnosis

Imaging

Ultrasound is the initial diagnostic modality used in the workup of anatomic thyroid abnormalities. Distinguishing PTL from thyroid cancer and Hashimoto's thyroiditis on ultrasound is difficult given their similar appearance. Certain sonographic features that are seen with PTL but are not specific include enhanced posterior echoes, hypoechogenicity, and asymmetric enlargement (Fig. 1) [17, 18]. Ota classified the ultrasound features of PTL into nodular, diffuse, and mixed types [17]. Nodular-type PTL is confined to a unilateral lobe with internal echoes that are hypoechoic, homogeneous, and pseudocystic [18]. Diffuse-type PTL usually involves two lobes and is hypoechoic, with indistinct borders between the lymphomatous and non-lymphomatous tissues. Mixed-type PTL is characterized by multiple patchy lesions with hypoechoic regions in the thyroid. The positive predictive value of US in nodular and mixed type is similar and significantly higher (64.9 and 63.2%) than that in diffuse type (33.7%).

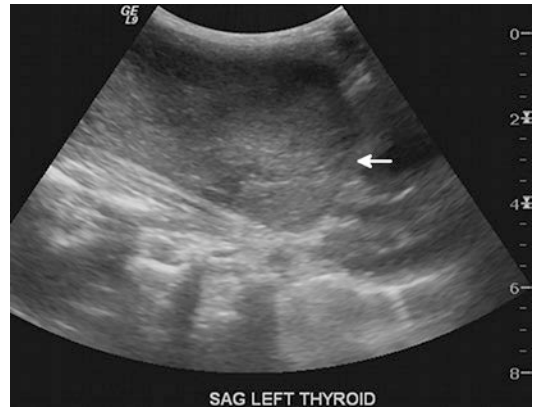

Fig. 1 Sagittal ultrasound scan of the left thyroid. The arrow indicates the location of the thyroid lymphoma, presenting as a homogeneous hypoechoic mass

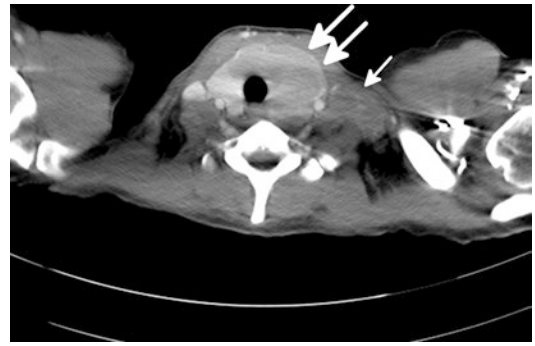

Fig. 2 Axial contrast-enhanced CT image of the neck. The double arrow indicates the location of the thyroid lymphoma. The single arrow indicates left-sided cervical lymphadenopathy

Xia et al. later simplified this sonographic characterization of PTL into diffuse and non-diffuse disease [19]. The non-diffuse appearance was found to be more common (63% vs. 37%) and features included being hypoechoic, multifocal, and hypervascular with an absence of calcification. Sonography alone was quite likely to miss diffuse-type PTL.

Recently, multi-slice computed tomography (MSCT) has been investigated as another imaging modality for PTL (Fig. 2) [20]. The pattern of PTL on MSCT has been categorized into three types: type 1, solitary nodule; type 2, multinodular; and type 3, homogeneous, bilateral enlargement [21]. Of 22 DLBCL and 5

MALT lymphoma cases studied, type 3 was most common. Type 1 pattern was only observed in patients with DLBCL. Most lesions showed homogeneous attenuation equal to that of surrounding muscles on plain MSCT images. No calcifications were observed in any of the tumors. Cervical lymph node involvement was observed in 12 cases of DLBCL and 3 cases of MALT, and invasion of cervical vessels, trachea, and esophagus was diagnosed in 11 cases. MSCT was found to be useful over ultrasound in detection of metastatic lymph nodes in areas that are poorly assessed with ultrasound and in the evaluation of tumor extension into adjacent structures.

Biopsy and Fine Needle Aspiration (FNA)

Once PTL is suspected by clinical presentation and ultrasound, the next step in diagnosis is biopsy. Accurate diagnosis is again challenging due to its histological similarities to thyroiditis as well as the co-occurrence of thyroiditis and PTL in the same gland which can result in sampling error. Therefore, clinicians should consider PTL in patients presenting with an enlarging thyroid mass even when the FNA cytology suggests thyroiditis. Traditionally open surgical biopsy was felt to be necessary, but with recent advances in immunophenotypic analysis, including immunohistochemistry and flow cytometry, the accuracy of FNA has improved, although is still variable. These advances in diagnosis of PTL mirror that of systemic lymphomas with a reported accuracy rate of FNA of 80–100% [22–24].

On cytological review, DLBCL appears as a relatively uniform population of large, abnormal lymphoid cells with the presence of lymphoepithelial lesions and decreased or absent colloid [1, 25]. Nuclear abnormalities such as segmentation or micronucleoli can be seen. DLBCL is easier to recognize than MALT lymphomas, which can appear similar to lymphocytic thyroiditis. Fine needle aspirate smears of MALT lymphoma can have a heterogeneous appearance but are generally highly cellular with a prominent population of intermediate-sized lymphoid cells, lymphoepithelial lesions, reactive lymphoid follicles, as well as a large plasma cell component [1, 26]. Slightly irregular nuclei, small nucleoli, and moderate slightly basophilic cytoplasm ("centrocyte-like cells") are often seen. A frequency of irregularly shaped nuclei with prominent nucleoli above 20% can help distinguish MALT lymphoma from Hashimoto's thyroiditis [27]. A frequency of large-sized cells above 15% distinguishes DLBCL from MALT lymphoma. Mixed cytology DLBCL and MALT lymphoma are seen in about 1/3 of cases [5]. The cytologic findings in Hashimoto's thyroiditis include mall lymphocytes, Hürthle cells, florid lymphoid hyperplasia with expanded germinal centers, and increased interstitial connective tissue [28].

Immunophenotyping in PTL assists in confirming a B-cell lineage to the lymphoid cells with expression of pan-B-cell antigens including CD19 and CD20 [1, 29]. CD3, CD5, CD10, and CD23 are usually negative in both DLBCL and MALT lymphoma [30]. The majority of DLBCLs are B-cell lymphoma (Bcl)-6 positive, and approximately half are Bcl-2 positive [31]. Monotypic surface immunoglobulin is often detected by flow cytometry [29]. Seen with MALT lymphomas are the presence of immunoglobulin light chains and Bcl-2. Patients with MALT lymphoma are typically CD5+/CD25+, while patients with non-MALT are CD5-/CD25- [30]. Immunoglobulin-M heavy chain staining in the plasma cell component also is a clue to the diagnosis of MALT lymphoma [26]. In Hashimoto's thyroiditis, both B- and T-cells are demonstrated by immunohistochemistry [5].

Early studies of FNA not combined with immunohistochemistry or flow cytometry were disappointing. In two smaller studies of patients with PTL, FNA without immunophenotyping was suggestive of the diagnosis in only 56% and 33% of patients in each study, respectively [6, 32]. Matsuzuka et al. found that among 83 patients with PTL who underwent FNA without immunophenotyping, 65 patients (78.3%) were definitively diagnosed by FNA, while another 10 patients (12.0%) had borderline cytological results leaving 8 patients (9.6%) with false-negative results [8]. This led to the recommendation

for open biopsy on all patients being worked up for PTL.

More recent studies have shown improvement in the accuracy of FNA when combined with immunophenotyping, particularly in the case of DLBCL which is more easily diagnosed given its high density of large monotonous atypical cells. In a study of 17 patients with PTL in which immunocytochemistry was combined with FNA, of those with DLBCL, 6/7 (85.7%) were correctly diagnosed by FNA [1]. On the other hand, only 4/10 (40%) MALT lymphoma cases were correctly diagnosed, and another three cases were misdiagnosed as Hashimoto's thyroiditis. In another study examining patients with PTL diagnosed at Johns Hopkins Hospital (JHH), 0/4 patients who had FNA without immunophenotyping were successfully diagnosed, while 7/8 (88%) who had FNA with immunophenotyping were diagnosed solely by FNA [33].

Although core needle or surgical biopsies are currently needed less for the diagnosis of PTL, they still have a role such as in distinguishing thyroiditis from low-grade MALT lymphoma and to ensure that aggressive histologies are not missed such as in glands with mixed MALT lymphoma and DLBCL. In the study by Sangalli et al., 6/10 MALT lymphoma cases required open surgical biopsy for definitive diagnosis [1]. In the study at JHH, of the eight patients with PTL who underwent FNA with immunohistochemical and flow cytometric analysis, one patient needed open biopsy for definitive diagnosis, which demonstrated low-grade MALT lymphoma [33]. Matsuzuka et al. found that 78.3% of patients had a correct diagnosis with FNA but open surgical biopsy was performed on all patients for confirmation of the final diagnosis [8].

A definitive position on whether core needle or open surgical biopsy is necessary is difficult to propose as there are no randomized or prospective trials that address this question, and so insight must be gained from retrospective studies. It is important to interpret these earlier studies in light of whether flow cytometry or immunohistochemistry was incorporated on FNA samples. Some variables that should be considered when interpreting FNA results are the expertise level of the physician performing the procedure and whether adequate tissue was obtained by sampling, including several passes from different areas of the lesion. Moreover, it is important to let the pathologist know whenever there is suspicion for PTL, so appropriate immunophenotypic analysis can be done. The proficiency of the pathologist in interpreting FNA results and performing immunophenotyping is also a key factor in determining the accuracy of FNA. In any clinical setting in which there is doubt about the quality of any of these variables to effect accurate diagnosis, open biopsy should be done.

Staging and PreTreatment Evaluation

Once the diagnosis of PTL is made, the next step prior to treatment is staging. Staging is according to the Ann Arbor system and defines whether the lymphoma is limited to the thyroid gland (stage IE), spreads to regional lymph nodes (IIE), has spread to lymph nodes on both sides of the diaphragm (IIIE), or has systemic dissemination (IVE) [34]. Most patients present with either stage IE (30–66%) or IIE (25–66%) disease [5–7, 12, 15]. Stage IIIE and IVE disease is seen about 2–7% of the time.

Comprehensive imaging to determine the stage of disease at presentation is important to establish a treatment plan and prognosis. CT scans of the head, neck, chest, abdomen, and pelvis are performed as the primary technique for staging. CT scan has been found to be superior to ultrasound in defining local extent of disease [35]. Bone marrow biopsy should also be performed to rule out marrow involvement.

Recently there has been interest as to the utility of fluorodeoxyglucose positron emission tomography (FDG-PET) scanning at initial diagnosis and in monitoring therapeutic response (Fig. 3). In a series of five patients with PTL, there was avid FDG uptake in the two patients with untreated PTL that was seen subsequently to decrease with treatment [36]. The response to treatment was detected earlier by FDG-PET compared to CT in one patient. In another two

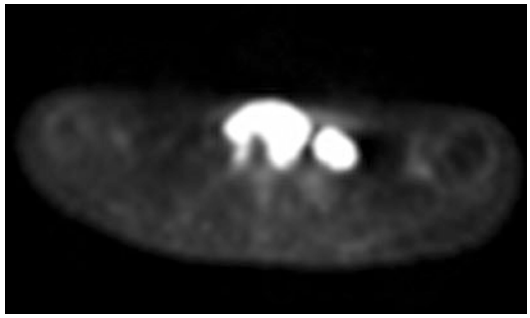

Fig. 3 FDG-PET/CT showing FDG uptake in the thyroid lymphoma as well as FDG-avid, left-sided cervical lymphadenopathy

patients, disease recurrence after treatment also was detected earlier by FDG-PET than by CT. Limiting the use of FDG-PET, however, is its low diagnostic specificity and its relatively higher cost. Focal FDG accumulation can be seen in thyroid adenomas and thyroid carcinomas of all types, and diffuse thyroid uptake is seen with Hashimoto's thyroiditis. However, it can be a useful tool for detecting regional and distant disease and response to treatment [37, 38].

Treatment

Traditionally, surgery and radiation therapy (RT) were considered the standard treatment for PTL. However, with high relapse rates, low survival rates, and the realization that thyroid lymphomas are sensitive to chemotherapy and radiation, surgery now plays a limited role [39, 40]. Surgery is mainly used when treating localized, indolent disease with thyroidectomy, for alleviation of compressive symptoms, and tracheostomy for relief of airway compromise. The mainstay of treatment for disseminated disease and lymphoma with aggressive histology is chemotherapy, with the most common regimen including cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP), as well as radiation. Rituximab, an anti-CD20 monoclonal antibody, was approved in 2006 by the Food and Drug Administration as first-line therapy for NHL. An increased antitumor effect without a substantial increase in toxicity is seen when rituximab is combined with traditional combination therapy (R-CHOP). When determining the treatment plan, the histology of the tumor and the stage need to be considered. There are no randomized, controlled trials evaluating the efficacy of different treatment modalities in PTL, so insight must be gained from retrospective studies and from studies of extranodal NHLs.

Localized, Indolent Disease

RT is effective for local neck disease, particularly for MALT lymphoma [41, 42]. Doria et al. found similar outcomes in subjects with PTL with disease confined to the neck who received RT both to the neck and mediastinum as in those who received combined modality treatment (CMT) with chemotherapy and RT [43]. Similar results by Vigliotti et al. indicated that patients with stage IE and IIE disease without mediastinal involvement responded equally well to RT alone and to CMT [44]. RT alone resulted in a 5-year disease-free survival (DFS) rate of 83% for stage IE patients and 100% for stage IIE patients without mediastinal involvement. Both RT alone and CMT had better survival rates than chemotherapy or surgery alone.

Laing et al. concluded that it is both the stage and the histology that is important in determining the efficacy of localized treatment alone [45]. The cause specific survival was 88% at 5 and 10 years for those with localized MALT lymphoma treated with RT alone compared with 55% for those with non-MALT lymphoma subtypes ($p = 0.003$). Cure from initial RT was approximately 70% for those with MALT lymphoma compared to approximately 55% at 5 years for those without MALT origin. Tsang et al. also demonstrated excellent results from RT alone in localized MALT lymphoma patients [46]. A complete response without relapse was seen in 100% of the patients with a median follow-up time of 4.9 years. Series of patients such as these with low-grade localized MALT lymphomas appear to do very well with RT alone.

Studies of surgery alone for treatment of PTL are limited to small series of patients that have

generally shown surgery alone to be effective for patients with localized MALT lymphoma. However, it does not seem that surgery plus RT results in better outcomes than RT alone. Pyke et al. demonstrated no difference in remission rates or relapse-free survival between patients with localized disease who had surgical debulking plus RT vs. those who had RT alone for treatment [41]. On the other hand, there were two patients with stage IE PTL who underwent surgery alone for treatment and were able to obtain a complete remission and remain disease-free with a median follow-up of 50.5 months. This encouraging outcome from surgery alone also was seen by Thieblemont et al. in five MALT lymphoma patients with localized disease who were followed for a median of 4.6 years postoperatively with no patients experiencing relapse [3].

Thyroidectomy does not seem to be of benefit in patients with aggressive histologic subtypes such as DLBCL. An overall 5-year survival of only 33% was seen among the three patients with stage IE and IIE disease treated with surgery alone followed by Vigliotti et al. [44]. Pathology of these tumors was predominantly of the diffuse large cell type.

Without availability of randomized controlled trials, inferences drawn from small studies and retrospective studies indicate that the overall benefit of thyroidectomy is limited to stage IE MALT lymphoma patients but may not provide any extra benefit over RT alone. With the limitations of surgery for treatment of PTL realized over recent years, the frequency of surgical approaches has declined. During the time period from 1973 to 1987, 81% of patients underwent surgery for PTL, whereas from 1997 to 2005, only 61% had surgery [7].

Disseminated, Aggressive Disease

Because of its aggressive nature and propensity for systemic recurrence, patients with DLBCL as well as those with non-localized indolent subtypes should be treated with both chemotherapy and RT [44, 47]. Evidence supporting the use of CMT is again confounded by the lack of randomized controlled trials. There is also a lack of stratification in most studies by histologic subtype making meaningful deductions limited.

Matsuzuka et al. demonstrated the general efficacy of CHOP plus RT in a report of 119 patients with the majority having intermediate grade, stage IIE PTL [8]. Those treated with six courses of CHOP combined with RT had a survival rate of 100% at 8 years, while those treated with an older regimen of one or two courses of chemotherapy plus RT had a survival rate of 75%. Both groups, however, had better outcomes compared with a 35% death rate from older studies prior to the introduction of chemotherapy.

Onal et al. examined treatment outcomes among 60 patients with aggressive PTL, and 27 patients with indolent disease, all with either stage IE or IIE disease [12]. Among those with aggressive lymphoma, CMT significantly improved overall survival (OS), DFS, and local control (LC) over RT or chemotherapy alone. CMT also significantly improved DFS and LC but not OS for those with indolent lymphomas. The addition of rituximab to CHOP improved OS compared to CHOP alone (92% vs. 71%, $p = 0.06$).

Further supporting the benefit of CMT are the findings of Doria et al. [43]. Among 211 patients with stage IE and IIE PTL of various histologic subtypes, CMT significantly decreased distant and overall relapse rates with overall relapse rates being 7.7%, 37.1%, and 43% for CMT, RT alone, and chemotherapy alone, respectively ($p = 0.004$). The only group in which a difference between treatment modalities was not seen was in those patients receiving RT to the neck and mediastinum with disease confined to the neck.

In the retrospective review by Ha et al., the majority of his patients had DLBCL and were treated either with surgery alone, surgery followed by RT, chemotherapy alone, or CMT [48]. The failure-free survival rates at 5 and 10 years were 76%, 50%, and 91% for those treated with surgery plus RT, CT alone, and CMT, respectively, supporting the role for CMT in those with aggressive histologic subtypes ($p = 0.15$). Of the four patients treated with surgery alone, three developed a recurrence.

Although the tumors treated were not of the primary thyroid subtype, extrapolation can be made from prospective, randomized studies of NHL such as the one by Miller et al. [47]. In this study, of 401 patients with stage I or II NHL of various histologic subtypes, subjects were randomized to either treatment with CHOP alone or CHOP plus RT. Those treated with CHOP plus RT had significantly better progression-free survival than patients treated with CHOP alone (77% vs. 64%, respectively, $p = 0.03$) as well as better OS (82% vs. 72%, respectively, $p = 0.02$).

Thieblemont et al. used an approach, whereby treatment modality was based on histology and stage with localized, indolent subtypes treated with surgery alone and disseminated or aggressive subtypes treated with chemotherapy [3]. Overall, complete remission was achieved in 19 of 25 (76%) available patients and partial remission in 6 patients (5 with DLBCL, 1 with follicular lymphoma) with 3 of these patients eventually relapsing (1 follicular lymphoma, 1 DLBCL, 1 Hodgkin's lymphoma). None of the patients with MALT lymphoma treated with surgery alone relapsed. The 5-year probability rate of OS was 44% for DLBCL patients and 100% for other lymphoma subtypes.

Rituximab has more recently been added to chemotherapy regimens for treatment of lymphoma, and there are only a few small studies that assessed its efficacy in PTL. In a small case series of three elderly patients with DLBCL of the thyroid who were considered inoperable or poor surgical candidates, treatment with rituximab plus cyclophosphamide, mitoxantrone, vincristine, and prednisolone resulted in a complete remission without disease recurrence 16–25 months after therapy completion [49]. These patients tolerated the therapy well with toxicities predominantly being hematologic. Of 43 patients 60 years old and older with stage IE and IIE DLBCL treated with R-CHOP studied by Watanabe et al., 42 patients (98%) responded to the treatment [50]. Five-year overall survival and event-free survival were 87% and 74%, respectively. R-CHOP has been studied in larger series of patients with nodal DLBCL. In a study of 1222 elderly patients with nodal DLBCL, 6 cycles of R-CHOP significantly improved event-free, progression-free, and OS over 6 cycles of CHOP alone [51].

Surgery in disseminated or aggressive disease is used for the alleviation of compressive symptoms or protection of the airway. In a retrospective study of 27 patients who underwent total thyroidectomy or lobectomy for compressive symptoms, five of whom also had a tracheostomy, there were no operative mortalities, no complications of hypoparathyroidism or nerve injury [52]. The mean symptom-free survival of patients was 10 years when chemotherapy and RT were also used. It is possible, however, that chemotherapy, RT, and the less invasive tracheal stents can alleviate compressive symptoms and/or impending airway obstruction without the need for more invasive surgery [53].

Prognosis

The prognosis of PTL patients is largely dependent on the stage and histologic subtype. In a population-based study of 1408 patients with PTL over 32 years of follow-up from the SEER database, the median OS for all cases was 9.3 years, 5-year OS was 66%, and the disease-specific survival was 79% [7]. When stratified by stage, the 5-year disease-specific survival was 86%, 81%, and 64% for stage I, II, and III/VI disease, respectively. Stratified by histologic subtype, the 5-year disease-specific survival rate was 75% for DLBCL, 96% for MALT lymphoma, 87% for follicular lymphoma, 86% for small lymphocytic lymphoma, and 83% for other NHLs. Patients with stage IV disease were 2.2 times more likely to die than those with stage I disease ($p < 0.001$), those with DLBCL were nearly 5 times more likely to die than those with MALT lymphoma ($p < 0.01$), and those with follicular lymphoma were more than 3.5 times more likely to die than those with MALT lymphoma ($p < 0.05$). Recently, Chai et al. found the 5-year survival to be 100% for MALT lymphoma and mixed MALT and DLBCL patients and 87.5% for DLBCL patients [54].

Table 1 Overview of DLBCL and MALT lymphoma

	DLBCL	MALT lymphoma
Prevalence [3, 7, 11, 12]	>50%	10–23%
Clinical presentation	Aggressive	More indolent
	Enlarging neck mass, dyspnea, dysphagia, stridor, hoarseness, fever, night sweats, weight loss	
Ultrasound features	Enhanced posterior echoes, hypoechogenicity, asymmetric enlargement	
Cytology	Large, monotonous lymphoid cells, lymphoepithelial lesions, decreased or absent colloid, frequency of large-sized cells >15%	Intermediate-sized cells, lymphoepithelial lesions, reactive lymphoid follicles, plasma cell component, frequency of large-sized cells <15%
Preferred treatment	Combined modality treatment with radiation + chemotherapy	Localized—surgery or radiation Disseminated—radiation + chemotherapy

In another study of 171 cases of PTL in which patients were treated primarily based on stage (RT alone for stage IE and CMT for stages IIE and higher), the 5-year OS was 89% for stage I disease and 83% for stage II disease [15]. Based on histologic subtype, Derringer found disease-specific 5-year survival rates for MALT lymphoma, mixed lymphoma, and DLBCL of 100%, 78%, and 71%, respectively, and 5-year OS of 77%, 47%, and 54%, respectively [5]. Poor prognostic factors include advanced age and stage, presence of DLBCL, lack of treatment with radiation or surgery, greater tumor size, mediastinal involvement, rapid clinical growth, presence of B symptoms, dysphagia, or stridor [5, 7, 12]. Table 2 provides an overview of DLBCL and MALT lymphoma.

Conclusion

PTL is a rare cause of thyroid malignancy and extranodal lymphomas. A summary of the two most common subtypes of PTL is displayed in Table 1. It should be suspected when a patient presents with an enlarging neck mass, especially in a patient with a history of Hashimoto's thyroiditis. Certain ultrasound features can suggest the diagnosis of PTL such as enhanced posterior echoes and hypoechogenicity, but diagnosis is ultimately made by biopsy. With improvement in immunophenotypic techniques, FNA can be used as the initial diagnostic test in centers with experienced physicians performing the procedure and interpreting the cytology. However, if optimal procedural technique or correct interpretative skills are not available, open surgical biopsy should be performed for confirmation. The most common subtype of PTL is DLBCL, which can be distinguished by its more aggressive presentation and specific findings on FNA. The second most common subtype is MALT lymphoma which is more difficult to diagnose based on its more indolent nature and possible similar appearance on ultrasound and FNA to that of chronic thyroiditis. Treatment and outcomes are based on both stage and histology. Localized, indolent lymphomas can be treated with radiation or surgery alone, while disseminated indolent lymphoma or aggressive histologic subtypes should be treated with CMT. There is a need for larger, prospective randomized studies to make more definite conclusions on diagnosis and treatment although this is difficult to do given the rarity of PTL.

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The Role of TSH Suppression in the Management of Differentiated Thyroid Cancer

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The Role of TSH Suppression in the Management of Differentiated Thyroid Cancer

The routine therapy of differentiated thyroid cancer (DTC) consists of (1) surgical treatment either lobectomy or total/near total thyroidectomy with or without lymph node dissection and (2) therapy with radioactive iodine (RAI), if clinically indicated [1]. The long-term management of thyroid cancer consists of therapy with levothyroxine aiming at appropriate thyroid hormone replacement in thyroidectomized patients but also at suppressing thyroid stimulating hormone (TSH) release from the pituitary gland via a negative feedback loop.

The concept of TSH suppression by supraphysiologic levothyroxine doses was first introduced in the 1937, when Sir Thomas Dunhill reported two cases of papillary thyroid cancer that regressed after treatment with thyroid extracts [2]. Later, W H Balme in 1954 reported a case of a 40-year-old female with metastatic differentiated thyroid carcinoma that responded favorably to prolonged therapy with thyroxin [3]. These observations led to the hypothesis that TSH may be a growth stimulus for thyroid cancer, and thus its suppression should lead to decreased growth of neoplastic cells expressing TSH receptor. There is a convincing body of evidence that DTC cells maintain TSH receptor expression, although its intensity is variable and usually lower compared with the normal thyroid tissue [4–12]. However, despite attempts to define the growth regulatory effects of TSH by in vitro and in vivo models, the results remain controversial to date. One of the explanations of the discrepancies observed in thyroid cancer models in vitro is a biphasic growth response curve—with TSH being predominantly a differentiation stimulus at physiologic concentrations and at higher concentrations acting as thyroid cancer growth stimulus [13]. The other potential reason for the discordant results of in vitro studies is lack of the TSH receptor expression in the majority of well-established human thyroid cancer cell lines [14, 15]. Moreover, there is evidence that marked stimulation of proliferation by TSH is observed only in models using human fetal thyrocytes, while mitogenic effects of TSH in other models depend on the presence of IGF-1 or insulin signaling, while TSH alone does not stimulate proliferation [16]. Ex vivo studies utilizing cell

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cultures derived from thyroid cancer patients incubated for 48 h with different TSH concentrations revealed that the proliferation rate was not influenced by TSH levels [17]. In addition, in vivo transgenic mouse models indicate that TSHR signaling is not sufficient to induce carcinogenesis but is involved in goitrogenesis [18].

Clinicians routinely apply a well-documented phenomenon of TSH stimulatory effects on cancer cell differentiation, specifically induction of sodium iodine symporter expression. In clinical practice, the short-term endogenous or exogenous TSH stimulation is utilized to enhance radioactive iodine uptake for diagnostic and therapeutic purposes [1]. However, the concept of long-term TSH suppression as a management strategy for thyroid cancer patients is based on equivocal preclinical evidence. Therefore, it is important to analyze the association between the TSH suppression and patients' outcome documented by the available clinical studies.

The Association Between TSH Suppression and Overall Survival (OS) and Disease-Specific Survival (DS)

The clinical evidence behind the association between TSH suppression and mortality is either weak or moderate as being derived predominantly from retrospective cohort studies. These studies are suffering from lack of randomization and appropriate controls, absence of blinding, and lack of exclusive focus on the effect of TSH suppression on clinical outcomes. One of the first meta-analyses summarizing the evidence behind the association between TSH suppression and patient mortality was based on case series and cohort studies published between 1980 and 1998 [19]. This study, based on the analysis of more than 4000 patients among which 69% received therapy with suppressive doses of levothyroxine, followed for 4.5–19.5 years, revealed a significant 27% risk reduction of combined mortality rate and disease recurrence rate (RR = 0.73; CI = 0.60 ± 0.88; $P < 0.05$) in patients treated with TSH suppression [19]. However, there was a significant heterogenicity among ten studies included in this meta-analysis [20–29]—with two of them showing increased adverse outcome associated with TSH suppression [26, 29] and one study revealing no effect of TSH suppression whatsoever [22]. Moreover, this meta-analysis does not provide information about the other risk factors associated with increased mortality such as age, histology type, stage, completeness of the surgical resection, or therapy with radioactive iodine. The discrepant results across analyzed studies could be related to inclusion of very heterogeneous groups of patients, characterized by different stages of disease and as such different risks of 10-year mortality. Therefore, it is important to analyze the role of TSH suppression in low-risk patients with small tumors confined to the thyroid gland separately from the patients with either local or distant metastases. Several studies have uniformly documented that TSH suppression does not change the outcome in stage I low-risk differentiated thyroid cancer patients [1, 22, 25, 30–36].

In contrast, there is a significant discrepancy in the studies focused on the role and degree of TSH suppression in patients with higher risk tumors—thyroid cancer with locoregional and/or distant metastases. Some studies have proven beneficial effects of TSH suppression in this group of patients [21, 22, 30, 37], while others failed to document any association between aggressive TSH suppression and overall survival and/or disease-specific survival [32, 33, 35, 38] (Table 1). Majority of these studies are characterized by a retrospective design; relatively small number of patients included and a short duration of follow-up. The two largest studies with relatively long duration of follow-up of 7 years [35, 38] revealed no difference in the mortality rate between patients treated with full TSH suppression (TSH < 0.1 mIU/mL) and the ones characterized by moderately suppressed or low normal TSH levels (0.1–2 mIU/mL). However, TSH levels exceeding 2 mIU/mL were associated with shorter overall survival.

Interestingly, there is only one prospective randomized controlled open-label study focused on the role of TSH suppression in thyroid cancer

Table 1 The effect of TSH suppression on overall survival (OS) and disease-specific survival (DSS) in intermediate- and high-risk thyroid cancer patients

Study	Number of intermediate/high-risk[a] patients enrolled	Predictors of OS and DSS	TSH suppression as independent factor associated with better outcome?	Average number of TSH measurements during follow-up	Duration of follow-up
Retrospective studies					
McGriff et al. [19]	4174—no data on baseline risk characteristics	TSH suppression	Yes—combined outcome of disease progression and death	No data	No data
Jonklaas et al. [30]	483	Stage, extent of surgery, RAI treatment, TSH	Yes—in univariate model and multivariate model for OS No—in multivariate model for DSS	2.3	3 years
Carhill et al. [35]	1813	Stage, extent of surgery, RAI treatment, TSH	No—in univariate and multivariate model for OS TSH normal/elevated associated with worse prognosis	6	6 years
Klubo-Gwiezdzinska et al. [38]	597	Age, gender, distant metastases, TSH	No—in univariate and multivariate model for OS TSH > 2 mIU/mL associated with worse prognosis	12	7.1 years
Diessl et al. [37]	157	TSH, freeT3	Yes—in univariate and multivariate model for DSS	>3	8 years
Hovens et al. [33]	291	Extra-thyroid extension, age, distant metastases, TSH	No—in univariate and multivariate model, TSH > 2 associated with worse outcome	>4	8.8 years
Prospective randomized controlled trials					
Sugitani et al. [32]	296	n/a	No effect on 5 years DSS	15	6.9 years

[a]Intermediate/high-risk patients—patients with tumors larger than 4 cm and/or with microscopic or gross extrathyroidal extension and/or with lymph nodes or distant metastases

recurrence rate and mortality. Although the majority of the patients enrolled in this study were characterized by low-risk thyroid cancer, there were 296 patients with lymph node metastases and 50 patients with high-risk features [32]. This study did not reveal any differences in the outcome in patients treated with suppressive levothyroxine doses leading to average TSH level of 0.07 ± 0.13 mIU/mL compared with patients who underwent therapy with physiologic levothyroxine doses resulting in TSH of 3.2 ± 1.7 mIU/mL [32]. The mortality rate in this study was overall very low as 9 patients died of thyroid cancer and 23 patients died from other diseases, which could have affected the statistical power to detect potential differences.

The Association Between TSH Suppression and Thyroid Cancer Recurrence Rate and Progression-Free Survival

There is an evidence that low-risk thyroid cancer patients do not benefit from aggressive TSH suppression, as it does not affect the recurrence rate

and progression-free survival [1]. Most published studies suggest that there is no difference between moderate and aggressive TSH suppression in preventing relapse of thyroid cancer in intermediate- and high-risk patients (Table 2). The risk of cancer progression increases with TSH exceeding low normal values (Table 2). Again, age and disease stage are consistently the most important factors predicting and affecting progression-free survival in multivariate models. The results of studies summarized in Table 2 are not uniformly consistent, as bias associated with retrospective design and heterogeneous patients' population is inevitable.

To summarize, there is a convincing body of evidence that TSH suppression does not affect the outcome in low-risk thyroid cancer patients. However, there is a significant uncertainty associated with the evaluation of the effect of TSH suppression on overall survival, disease-specific survival, and recurrence rate in intermediate- and high-risk thyroid cancer patients, warranting further investigation in well-designed long-term prospective studies. The optimal TSH goals for

Table 2 The effect of TSH suppression on thyroid cancer recurrence and progression-free survival (PFS)

Study	Number of intermediate/high-risk[a] patients enrolled	Predictors of disease recurrence/ PFS	TSH suppression as independent factor associated with better outcome?	Average number of TSH measurements during follow-up	Duration of follow-up
Retrospective studies					
Mazzaferri et al. [20]	1355, only 33 with distant metastases, no data on the number of patients with intermediate-risk features	Size, local tumor invasion, lymph node metastases, RAI	Yes—in univariate model for cancer recurrence, No—in multivariate model	No data	15.7 years
Cooper et al. [22]	378	Stage, age, RAI	Yes—in univariate model for PFS No—in multivariate model for PFS	2.6	4.5 years
Pujol et al. [21]	76	Stage, age, TSH	Yes—in univariate and multivariate model for PFS	No data	No data
Jonklaas et al. [30]	483	Stage, extent of surgery, RAI treatment	No—in univariate and multivariate model for PFS	2.3	3 years
Carhill et al. [35]	1813	Stage, extent of surgery, RAI treatment,	No—in univariate and multivariate model for PFS	6	6 years
Klubo-Gwiezdzinska et al. [38]	597	Age, gender, distant metastases	No—in univariate and multivariate model for PFS	12	7.1 years
Hovens et al. [33]	291	Age, extrathyroidal extension, distant metastases	No—in univariate and multivariate model for relapse, TSH > 2 mIU/mL associated with increased risk of relapse	>4	8.8 years
Prospective randomized controlled trials					
Sugitani et al. [32]	296	n/a	No effect on PFS	15	6.9 years

[a]Intermediate/high-risk patients—patients with tumors larger than 4 cm and/or with microscopic or gross extrathyroidal extension and/or with lymph nodes or distant metastases

individual patients must balance the potential benefit of TSH suppression with the possible harm from subclinical thyrotoxicosis especially in patients with medical conditions that can be exacerbated with aggressive TSH suppression.

The Risks Associated with Therapy with Suppressive Levothyroxine Doses

Subclinical hyperthyroidism has been associated with increased cardiac mortality and is an independent predictor of cardiac death in patients with preexisting cardiac conditions [39–43]. A recent observational study demonstrated increased risk of all-cause and cardiovascular mortality in DTC patients with suppressed TSH, independent of age, sex, and cardiovascular risk factors [44]. Interestingly, in this study, the TSH level was a predictor of cardiac mortality—with threefold increased risk of death per each tenfold decrease in geometric mean of serum TSH level [44]. Some studies suggest that the absolute risk of death in patients with subclinical hyperthyroidism is age and gender dependent with highest risk observed in older men [41]. The elderly patients with TSH values of <0.3 mU/L have increased cardiac mortality; moreover higher levels of FT4 in this group of patients are associated with an increased risk of cardiovascular and all-cause mortality [45]. The older patients are also at a significantly greater risk of development of atrial fibrillation, even with moderate TSH suppression (0.1–0.4 mU/L) [46–49].

Therapy with suppressive doses of levothyroxine has been also found to negatively affect bone health [50]. Sugitani et al. in a prospective randomized controlled study documented significant reduction of bone density 1-year post-thyroidectomy in women over 50 years old who underwent TSH suppression therapy compared with the patients treated with non-suppressive levothyroxine doses [51]. Suppressive levothyroxine doses led to threefold increased likelihood of development of osteoporosis in women with thyroid cancer [52]. Moreover, there is an evidence that in postmenopausal women diagnosed with and treated for osteoporosis, therapy with bisphosphonates is less effective in patients who are concomitantly on suppressive doses of levothyroxine [53]. Suppressed TSH is also associated with increased risk of major osteoporotic fractures, and the extended duration of suppression is increasing this risk exponentially even further [54]. The detrimental effects of supraphysiologic doses of levothyroxine on the bone health leading to acceleration to bone turnover and decreased bone density are seen predominantly in postmenopausal women, but not in men and premenopausal women [55–57].

Therefore, it is extremely important to take into consideration a balance between the cardiovascular and bone health-related risks of TSH suppression against the risks of tumor recurrence, progression, and mortality [1, 31]. Currently available guidelines focused on management of patients with thyroid cancer recommend "one size does not fit all" approach to decision-making about the TSH suppression.

Comparison of Different Guidelines Focused on the Role of TSH Suppression in Thyroid Cancer Management

The levothyroxine dose is tailored toward certain TSH goals—either full suppression with TSH goal of less than 0.1 mIU/mL, moderate suppression with TSH goal of 0.1–0.5 mIU/mL, or low normal TSH values of 0.5–2 mIU/mL [1]. The decision-making about the degree of TSH appropriate suppression required for a patient's management is based on risk stratification assessing the likelihood of cancer-related death and persistent/recurrent disease and response to routine therapy as well as to presence of comorbidities which may increase the risk of serious complications of therapy with levothyroxine [1].

American Thyroid Association guidelines recommend targeting low normal TSH values of 0.5–2 mIU/mL in patients with low-risk thyroid cancer defined as intrathyroidal DTC with no evidence of extrathyroidal extension, vascu-

lar invasion, or metastases and in patients who have an excellent response to therapy (Table 3) [1]. In patients with intermediate-risk features such as thyroid cancer exceeding 4 cm in size, tumors with microscopic extrathyroidal extension, presence of lymph node metastases, or aggressive tumor histology such as tall cell, hobnail variant or columnar cell carcinoma, as well as for the patients who have either indeterminate or incomplete biochemical response to treatment, the TSH goal is targeted at 0.1–0.5 mIU/mL. High-risk patients with thyroid cancer and with tumors characterized by gross extrathyroidal extension or distant metastases as well as the patients with structural incomplete response to standard therapy should have fully suppressed TSH levels with a goal of <0.1 mIU/mL. This goal could be modified to a less stringent one if there are cardiovascular comorbidities, particularly atrial fibrillation or osteoporosis (Table 3) [1].

The British Thyroid Association guidelines recommend a very similar approach [58]. Low-risk patients or patients with excellent response to treatment should be treated with physiologic doses of levothyroxine with a TSH goal of 0.2–3 mIU/mL. The TSH goal for the patients characterized by intermediate-risk features or indeterminate response to treatment is targeted at 0.1–0.5 mIU/mL for 5–10 years. Levothyroxine dose for high-risk patients or patients with either biochemical or structural incomplete response should be tailored toward suppressed TSH values of less than 0.1 mIU/mL indefinitely (Table 3) [58].

The European Society of Medical Oncology and the European Thyroid Association guidelines apply a similar paradigm for the management of thyroid cancer, introducing a risk stratification-based TSH goal. However, the system of risk stratification is slightly different in this model compared with the American and British one, as patients are categorized into (1) a very low risk if they present with a unifocal microcarcinoma less than 1 cm in size, without extrathyroidal extension and lymph node metastases; (2) low-risk tumors exceeding 1 cm but confined to thyroid, without extrathyroidal extension or local and/or distant metastases; and (3) high-risk patients with incomplete surgical resection, presenting with tumors characterized by extrathyroidal extension, lymph node and distant metastases, vascular invasion, and aggressive histology (Table 3) [59]. Patients characterized by very low- and low-risk features or individuals who have an excellent response to treatment with no evidence of disease can be targeted to achieve a TSH goal of 0.5–2 mIU/mL. High-risk patients or patients with evidence of persistent/recurrent structural disease should be treated with suppressive levothyroxine doses with a TSH goal of less than 0.1 mIU/mL (Table 3) [59, 60].

The Latin American Thyroid Association guidelines are similar to the European risk stratification system and recommend TSH suppression for high-risk thyroid cancer patients and a TSH goal of 0.4–1 mIU/mL for very low- and low-risk thyroid cancer patients [61]. Importantly these guidelines identify the contraindications for TSH suppression, namely, atrial fibrillation, ischemic cardiovascular disease, and severe osteoporosis (Table 3) [61].

The Japanese Society of Thyroid Surgeons and Japanese Association of Endocrine Surgeons guidelines significantly differ from the guidelines from Western societies [62, 63]. The main reason for this discrepancy lies in the different therapeutic approaches implemented in Japan—with a watchful waiting strategy—consisting of active surveillance with imaging, without thyroidectomy for microcarcinoma confined to the thyroid gland and hemithyroidectomy with prophylactic central neck lymph node dissection for tumors showing progression. Radioactive iodine treatment and therapy with suppressive doses of levothyroxine are not routinely recommended in Japan [62, 63].

To summarize, therapy with levothyroxine should be individualized based on the anticipated biological behavior of thyroid cancer in each patient and the patients' age and presence of comorbidities, particularly cardiovascular and bone disease.

Table 3 TSH goals recommended per different international guidelines

	Full TSH suppression (<0.1 mIU/mL)	Moderate TSH suppression (0.1–0.5 mIU/mL)	Low normal TSH level (0.5–2 mIU/mL[a])
American Thyroid Association guidelines [1]	High-risk patients • Tumor with gross extrathyroidal extension • Incomplete resection of the tumor • Distant metastases • Lymph node metastases exceeding 3 cm in size • Follicular thyroid cancer with >4 foci of vascular invasion Structural incomplete response[b] to treatment No atrial fibrillation	Intermediate-risk patients • Tumor exceeding 4 cm • Tumor with microscopic extrathyroidal extension • Presence of lymph node metastases not exceeding 3 cm • Tumor 1–4 cm in sized with BRAFV600E mutation • Papillary thyroid cancer with vascular invasion Indeterminate response[c] to treatment in patients <60 years old, without osteoporosis and atrial fibrillation Biochemical incomplete[d] response to treatment in patients not suffering from atrial fibrillation	Low-risk patients • Tumor less than 4 cm confined to the thyroid • No lymph nodes metastases or • <5 microscopic metastases to central neck lymph nodes • No distant metastases • Follicular thyroid cancer with less than 4 foci of vascular invasion Excellent response[e] to treatment Indeterminate response[c] to treatment if patient >60 years old, with atrial fibrillation or osteoporosis Biochemical incomplete response[d] to treatment in patients with atrial fibrillation
British Thyroid Association guidelines[a] [58]	High-risk patients • Extrathyroidal invasion • Incomplete resection • Distant metastases Structural or biochemical incomplete response to treatment	Intermediate-risk patients • Tumor with microscopic invasion • Cervical lymph node metastases • Tumor with aggressive histology or angioinvasion Indeterminate response to treatment	Low-risk patients • Tumor confined to the thyroid • No lymph nodes or distant metastases • No aggressive histology Excellent response to treatment
European Thyroid Association guidelines [59, 60]	High-risk patients • Incomplete surgical resection • Extrathyroidal extension, • Lymph node and/or distant metastases • Vascular invasion • Aggressive histology		Very low-risk patients • Unifocal microcarcinoma <1 cm, without extrathyroidal extension and lymph node metastases Low-risk patients • Tumor >1 cm confined to thyroid, without extrathyroidal extension or local and/or distant metastases
Latin American Thyroid Association[a] [61]	High-risk patients • Incomplete surgical resection, • Extrathyroidal extension, • Lymph node and/or distant metastases • Vascular invasion • Aggressive histology No contraindications to TSH suppression		Very low-risk patients • Unifocal microcarcinoma <1 cm, without extrathyroidal extension and lymph node metastases Low-risk patients • Tumor >1 cm confined to thyroid, without extrathyroidal extension or local and/or distant metastases Patients with contraindications to TSH suppression-atrial fibrillation, ischemic cardiovascular disease or severe osteoporosis
Japanese Thyroid Association guidelines [62, 63]	No routine TSH suppression		

[a]Latin American Thyroid Association defines low normal TSH as 0.4–1 mIU/mL; British Thyroid Association defines low normal TSH as 0.2–3 mIU/mL
[b]Structural incomplete response—patients with structural or functional evidence of disease
[c]Indeterminate response—patients with non-specific findings on imaging studies, non-stimulated Tg detectable, but less than 1 ng/mL, stimulated Tg detectable, but less than 10 ng/mL or Tg antibodies stable or declining in the absence of structural or functional disease
[d]Biochemical incomplete response—negative imaging, suppressed Tg > 1 ng/mL, stimulated Tg > 10 ng/mL *or r*ising Tg Ab levels
[e]Excellent response—negative imaging, suppressed Tg <0.2 ng/mL, TSH stimulated Tg < 1 ng/mL

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Part IX

Miscellaneous Topics

Hyperthyroidism and Pregnancy

Jorge H. Mestman

Maternal, obstetrical, and neonatal complications are significantly increased in women with poorly controlled thyroid diseases. In 1975, Mujtaba and Burrow [1] reported on the outcome of 26 pregnancies in 21 hyperthyroid women treated with antithyroid drugs, both methimazole and propylthiouracil; the infant outcome showed high rate of complications: four of the infants had a goiter at birth, three of them born with hyperthyroidism, and in two of them diagnosis made a few days after birth, "because of maternal antithyroid therapy." Five children had congenital abnormalities. Forty years later, these outcomes are still reported in the medical literature. Prepregnancy patient education, including contraception, and a management team approach during pregnancy and postpartum period, avoids most of these abnormal outcomes. This chapter will be discussing clinical topics relevant to the care of women affected by Graves' disease during their reproductive age. The sections to be included are:

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Prepregnancy Counseling

Changes in thyroid economy, which could affect the well-being of the mother and offspring, occur early in pregnancy; therefore it is imperative to advise women with past or present history of hyperthyroidism during their reproductive age, to plan their pregnancies, and to contact their healthcare professionals before or as soon as the diagnosis of pregnancy is confirmed [2]. Graves' disease, an autoimmune disease, affects 0.5% to 1.3 of women between 20 and 45 years of age [3, 4]; the clinical course of thyroid disease is affected by the immunologic changes that occur in pregnancy and in the postpartum period [5]. Women with a history of hyperthyroidism due to Graves' disease, rendered hypothyroid by ablation therapy, either surgery or RAI therapy, on thyroid replacement therapy, usually need an increase in their levothyroxine dose early in pregnancy, because of a 30–50% increase in thyroxine demand in the first half of pregnancy [6, 7]. Some studies, but not all of them, have shown that an elevated serum TSH of over 2.5 mIU/L in the first trimester of pregnancy increase the incidence of miscarriages, premature labor, and intellectual deficiencies in the offspring later in life [8–10]). A serum TSH closed to 1 mIU/L before conception prevents the development of hypothyroidism after conception in almost 85% of hypothyroid women on thyroid replacement therapy [11]. Titers of serum TSH receptor antibodies

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(TRAbs) may remain elevated for some time after ablation therapy, particularly after ^{131}I therapy [12–14], with the risk of inducing fetal thyrotoxicosis if not promptly recognized. Surgical thyroid ablation should be considered in Graves' disease women with high TRAb titers considering pregnancy, although occasionally it has been reported after total thyroidectomy [15]. In 1 study [16], 42 women with active or previous history of Graves' hyperthyroidism delivered 42 babies; 9 infants were diagnosed with hyperthyroidism at birth; in 4 out of 9 infants born from euthyroid mothers, 3 mothers were treated with surgery and 1 with ^{131}I ablation before pregnancy.

Inadvertent treatment with radioiodine during pregnancy has been studied, with risks to the fetus dependent upon timing of exposure and radiation dose [17]. In one systematic review, spontaneous miscarriage was more likely when exposure occurred during the first 2 weeks after fertilization, prior to implantation [18].

In summary, women with past or present history of Graves' disease and their partners should be offered proper information and education in planning future pregnancies; contraception; therapeutic options for hyperthyroidism; avoiding conception while having active thyroid disease; frequent medical and obstetric visits during pregnancy; need for serial and frequent thyroid tests; potential fetal risk of antithyroid drug (ATD) therapy, among them fetal teratogenicity; possible recurrence of the disease early in gestation in the postpartum period; and breastfeeding recommendations.

Physiologic Thyroid Changes Through Pregnancy

In early pregnancy, the maternal thyroid gland is challenged with an increased demand for thyroid hormone production [19, 20], due to (a) increase in blood thyroxine-binding globulin (TBG), secondary to the effect of estrogens on the liver; (b) stimulatory effect of human chorionic gonadotropin (hCG) on the thyroid-stimulating hormone (TSH) receptor; (c) high concentrations of type 3 iodothyronine deiodinase (D3), which degrades thyroxine and triiodothyronine to inactive compounds and is compensated for by an increase in T4 synthesis and secretion [21]; and (d) supply of iodine available to the thyroid gland [22]. In the United States, the iodine content in the diet appears to be insufficient in only about 10–20% of pregnancies. The suggested total daily iodine ingestion for pregnant women is 229 μg/day and 289 μg/day for lactating women; prenatal vitamins should contain 150 μg of iodine in the form of potassium iodine, complementing the daily dietary iodine intake [23].

As the result of the above, early in pregnancy there is a slight increase secretion of FT4, albeit within normal range, and a decrease in serum TSH levels, with 10–20% of women having subnormal TSH levels, sometimes as low as 0.03 mIU/L [24]. The normal thyroid gland is able to compensate for this increase in thyroid hormone demands; however, in those situations in which there is a subtle pathologic abnormality of the thyroid gland, such as chronic autoimmune thyroiditis, or in women on thyroid hormone replacement therapy, the normal increase in the production of thyroid hormones is not met, and hypothyroidism ensues.

The fetal thyroid gland starts secreting thyroid hormones at about 10–12 week's gestation with significant fetal thyroid hormone production by 20 weeks [19, 25]. Fetal TSH level starts progressively to increase at approximately 18 weeks of gestation to a peak value of 10 mU/L at term. At the same time, fetal T4 levels begin to increase steadily until the end of gestation. Fetal serum T3 remains low until the 30th week of gestation and then slowly increases until birth. In the iodine uptake by the fetal gland occurring between 10 and 14 weeks, TSH receptor responds to the stimulation by TSI (thyroid-stimulating immunoglobulin) by 18 weeks' gestation [26].

Interpretation of Thyroid Tests

Measurement of serum TSH is the most practical, simple, and economic screening test for detection and diagnosis of thyroid dysfunction in the

outpatient setting, and it is complemented by an assessment of thyroxine levels in cases of abnormal TSH values. Uncommon exceptions include patients with pituitary hypothyroidism and those with resistance to thyroid hormone syndrome. The most common and available commercial tests in clinical practice for the determination of free thyroxine (FT4) are automated immunoassays by nonequilibrium methods. Interpretation of FT4 levels during pregnancy can be challenging, and higher thyroid-binding globulin and lower albumin levels decrease the accuracy of immunoassays, causing falsely low FT4 levels in the second and third trimester of pregnancy [27, 28]. A study comparing total T4 (TT4), FT4 index (FT4I), and two different FT4 analog immunoassays found that total T4 levels and FT4I more accurately demonstrated actual temporal changes in T4 levels throughout gestation [27]. Determination of TT4 in pregnancy needs to be adjusted, since its blood concentration is elevated by the effect of circulating thyroxine-binding protein (TBG). TT4 serum values start increasing by 50% after the 4–6 weeks of conception reaching a peak at about 20 weeks' gestation and plateau after until deliver. It is suggested to adjust TT4 (adjusted TT4, AFT4) reference range in pregnancy, by a factor of 1.5 times that of the nonpregnant values [27] (e.g., normal reference range in nonpregnancy, 4.0–12.0; multiplying by a factor of 1.5, the TT4 reference range for pregnancy should be 6.0–18.0); the next option is the calculation of serum FT4 by the FT4 index (FT4I), with the use of serum TT4 and an indirect assessment of TBG, such as the resin uptake (RU) or similar tests [27, 29]. In this review FT4, FT4I and adjusted (ATT4) will be used interchangeable (FT4/FT4I/ATT4). Other laboratory tests for the determination of FT4 include equilibrium dialysis technique and tandem mass spectrometry [30]; although more accurate, these tests are more expensive and not commonly available in clinical practice. In summary, several options for the assessment of serum-free T4 include (a) FT4, (b) adjusted TT4, (c) FT4I, or (d) a more expensive and less available equilibrium dialysis technique and tandem mass spectrometry. Routine use of serum FT3 or TT3 is reserved for the especial situation in which serum TSH is undetectable and FT4 is normal, such as in the occasional patient with an autonomous functionally thyroid nodule or the patient ingesting inappropriate doses of triiodothyronine (T3). Another clinical situation in whom serum TT3 or FT3 may be of clinical application is the woman with severe hyperthyroidism where serum T3 levels are disproportionally higher than serum T4 levels. It was reported in a series of hyperthyroid women, in whom the serum FT4 was normalized by ATD therapy, that newborn serum TSH were above reference range, suggesting state of fetal hypothyroidism [31]. Since the reason to limit the routine determination of FT3 in pregnancy.

A suppressed or undetectable serum TSH in the presence of an elevated FT4 or adjusted TT4 confirmed the diagnosis of clinical hyperthyroidism, while a serum FT4 within normal limits is consistent with subclinical hyperthyroidism. This is an important clinical and therapeutic distinction since antithyroid therapy is not indicated in subclinical hyperthyroidism [32].

TSH receptor antibodies (TRAbs) play a significant role in the differential etiologic diagnosis of hyperthyroidism and in assessing the risk of fetal and neonatal hyperthyroidism (Table 1) [33, 34]. These antibodies, binding to the TSH receptor (TSHR), are classified as stimulating antibodies, the cause of Graves' disease, and blocking antibodies, occasionally responsible for fetal hypothyroidism. Two assays are commercially

Table 1 Indications for maternal determination of thyroid receptor antibodies (TRAbs) (18–22 weeks gestation)

• Fetal or neonatal hyperthyroidism in previous pregnancies
• Graves' disease
– Active
– Post-ablation therapy (surgery, ^{131}I)
• Thyroidectomy during pregnancy
• Fetal monitoring findings of
– Fetal tachycardia (>160/min)
Intrauterine growth restriction
Fetal goiter
– Poly-oligohydramnios
Accelerated bone maturation (>28 weeks)

available, competition-based assays, thyroid-binding inhibitory immunoglobulins (TBII), and assays that detect cAMP production, thyroid-stimulating immunoglobulin (TSI) assay. Crossing the placenta barriers, the stimulating antibodies, if present in significant titers, are responsible for the development of fetal and/or neonatal hyperthyroidism. Therefore, testing for TRAbs is an important component in the diagnosis and management of Graves' disease. The specificity of TBII methods is lower because they cannot differentiate between stimulating and blocking antibodies. From a clinical practical point of view, presence of blocking antibodies is unusual; it was reported in 9 out of 788 neonates in which neonatal screening tests were suggestive of neonatal hypothyroidism [35].

Maternal-Placental-Fetal Interactions (Fig. 1)

Studies over the past few decades have shown an important role of maternal thyroid hormones in embryogenesis [36, 37]. Maternal T4 crosses the placenta early in pregnancy at a time when the fetal thyroid gland is not functional, and maternal TSH does not cross the placenta barrier. Thyrotropin-releasing hormone (TRH) does cross the placental barrier, but its physiologic significance is unknown. Methimazole (MMI), propylthiouracil (PTU), and carbimazole (CMZ)—a drug that is metabolized to methimazole—do cross the placenta barrier and if given in inappropriately high doses may produce fetal goiter and

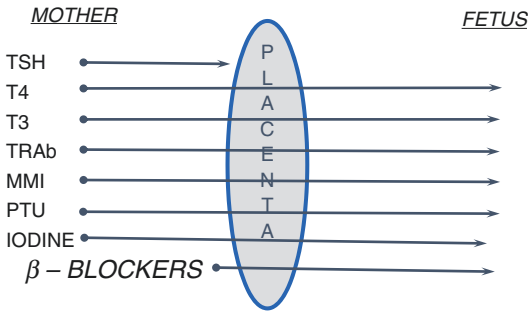

Fig. 1 Maternal-fetus transfer of hormones, immunoglobulins, and drugs

hypothyroidism [38]. Preparations that contain iodine given in large doses or for prolonged periods are contraindicated in pregnancy because accumulation by the fetal thyroid may induce goiter, cretinism, and hypothyroidism [39]. As mentioned above, TRAb crosses the placenta, and maternal blood concentrations have a tendency to decrease with progression of pregnancy [40], suggested as the reason for spontaneous hyperthyroid symptom improvement. In hyperthyroid women with persistently elevated TRAb levels, the risk of caring a fetus with hyperthyroidism is significant. It has been estimated that TBII levels of above three times normal reference range have a sensitivity of 100% and specificity of 43% in predicting neonatal hyperthyroidism; in the same report, mothers with serum TSI below 400% (reference range below 140%) gave birth to no hyperthyroid infant [41].

Etiology of Hyperthyroidism (Table 2)

Graves' hyperthyroidism diagnosed during pregnancy needs to be differentiated from other etiologies, grouped as transient non-autoimmune hyperthyroidism of early pregnancy [42] affecting 1–5% of all pregnant women, more often than

Table 2 Management of Graves' hyperthyroidism in pregnancy

Control of maternal hyperthyroidism
(a) Indication, choice, and timing of ATD therapy
(b) Proper ATD dosage and adjustment with the goal to keep serum FT4/ATT4/FT4I in the upper limits of reference range
(c) Detection of early medical complications
(d) Postpartum follow-up
Early detection of fetal and neonatal dysfunction
(a) A medical team approach, including obstetrician, maternal-fetal medicine specialist, endocrinologist, neonatologist, anesthesiologist, and pediatric endocrinologist
(b) Timing and interpretation of maternal serum TRAb levels
(c) Detection of fetuses at risk, in need of intensive fetal monitoring management
(d) Neonatal thyroid evaluation in the first week of life
(e) Long-term follow-up

Graves' disease. It presents with hyperthyroid symptoms occurring after 6–8 weeks' gestation, coinciding with increasing levels of hCG secretion by the placenta, stimulating the TSH receptor [43]. Hyperemesis gravidarum (HG) (Fig. 2), the most common cause of thyrotoxicosis in the first trimester of pregnancy [44, 45], is characterized by severe nausea, vomiting, and weight loss, with onset between 6 and 8 weeks' gestation, requiring frequent visits to the emergency room and inpatient care for IV hydration. Serum TSH measured by a sensitive assay is consistently undetected or suppressed, with elevation in serum FT4. Symptoms of hyperthyroidism are mild, with exception of tachycardia, due most likely to dehydration. Absence of prepregnancy hyperthyroid symptoms, ophthalmopathy and goiter, distinguished them clinically from Graves' disease patients. Spontaneous normalization of hyperthyroxinemia parallels the improvement in vomiting and coincides with a decrease in serum hCG values by 12–16 weeks' gestation. Suppressed serum TSH may lag for a few more weeks after normalization of free thyroid hormone levels (Fig. 2). Antithyroid medications are not therapeutically indicated. Occasionally, severe vomiting and hyperthyroidism may require parenteral nutrition. In 67 patients studied by Goodwin and colleagues [44], liver and electrolyte abnormalities were routinely found in women with worse symptoms, including severe vomiting, weight loss of at least 5 kg, and significant dehydration. Other causes of GTH include twin pregnancies and trophoblastic disease.

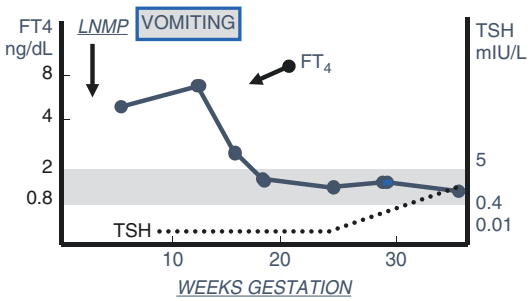

Fig. 2 Transient hyperthyroidism of hyperemesis gravidarum (THHG)

Two cases [46] have been reported of severe hyperemesis, hCG levels were not elevated, and the symptoms persisted through gestation. Thyrotoxicosis and hyperemesis gravidarum were due to a mutation of the TSH receptor, providing thyroid hypersensitivity to hCG.

Graves' Disease

The clinical presentation of Graves' hyperthyroidism in pregnancy varies: (a) first time diagnosis, (b) patient under treatment with ATD therapy, or (c) recurrence of hyperthyroidism in a patient on remission after previous course of ATD therapy or after ablation therapy. Stimulation of the thyroid gland by placenta hCG in the first trimester and the elevation of serum TRAb titers, with stimulating activity, from prepregnancy values, have been suggested as the cause of recurrent disease [47]. It needs to be kept in mind that women rendered hypothyroid post-ablation therapy, either ^{131}I therapy or surgery, on levothyroxine replacement therapy may present with laboratory tests consistent with hypothyroidism, either subclinical (elevated serum TSH and normal FT4/ATT4) or clinical (high serum TSH and low FT4/ATT4), as a result of the inability to the residual thyroid gland to compensate for the increase in thyroid hormone demands usually seen early in pregnancy [5]. Prompt correction of hypothyroidism is necessary to prevent potential maternal and fetal complications. In most patients in whom the diagnosis of Graves' hyperthyroidism is made for the first time during pregnancy, hyperthyroid symptoms antedate conception. The clinical diagnosis of thyrotoxicosis may present difficulties during gestation because many hypermetabolic symptoms and signs are commonly seen in normal pregnancy, such as mild palpitations, heart rate between 90 and 100 beats/min, mild heat intolerance, shortness of breath on exercise, and warm skin. However, some clinical clues increase the likelihood of Graves' hyperthyroidism such as the presence of a goiter, orbitopathy, proximal muscle weakness, tachycardia with a consistent pulse rate of more than 100 beats/min, frequent daily bowel movements, and weight loss

or inability to gain weight despite a good appetite. Occasionally, the patient may be seen for the first time in congestive heart failure, with physical findings suggestive of cardiac valve disease, particularly mitral insufficiency or stenosis [48]. Other common symptoms of hyperthyroidism include nervousness, increased sweating, insomnia, irritability, changes in personality, decreased tolerance to exercise, eye irritation, frequent lacrimation, and pruritus. Not all symptoms are present in a given patient; therefore physicians should be aware of subtle complaints, particularly in the presence of weight loss or inability to gain weight. In one study, reduction in peripheral vascular resistance and higher cardiac output were still present despite normalization of T4 levels [49]. This is an important finding with significant clinical implications. Left ventricular decompensation in controlled hyperthyroid pregnant women may develop in the presence of superimposed preeclampsia, at the time of delivery [50], or with undercurrent complications such as anemia or infection [51].

In the first trimester of pregnancy, Graves' hyperthyroidism should be clinically differentiated from other causes of "transient non-autoimmune hyperthyroidism of early pregnancy" syndrome [42] as mentioned before.

Maternal and Obstetric Complications

Prompt recognition and treatment of hyperthyroidism avoids most pregnancy complications. In patients with poorly controlled hyperthyroidism through pregnancy, one of the most common maternal complications is pregnancy-induced hypertension (PIH), with a risk for severe preeclampsia five times greater than in patients with controlled disease. Millar and coworkers [52] reported on 181 pregnant hyperthyroid women, 34 remained euthyroid through pregnancy, 90 become euthyroid before the third trimester, and 57 remained hyperthyroid throughout gestation. Uncontrolled hyperthyroidism was associated with a ninefold greater incidence of low birth weight (LBW) and five times greater incidence of pregnancy-induced hypertension (PIH), as compared to controlled disease women. The incidence of LBW infants was almost 2.5 times greater in those whose hyperthyroidism was treated during pregnancy but who became euthyroid at some time during gestation. In mothers who achieved a euthyroid state before or early in pregnancy, the incidence of LBW infants was no different from the control population. Other obstetrical complications in uncontrolled hyperthyroidism include preterm delivery, placental abruption, prematurity, stillbirth, and miscarriage. These data were recently confirmed by a retrospective analysis from the US Consortium on Safe Labor from 223.512 singleton pregnancy delivered between 2002 and 2008, information obtained from electronic medical records, without information about type of treatment or control of disease. The authors reported increased odds of preeclampsia, preterm delivery, labor induction, and ICU admissions [50]. Similar results were obtained in a study from India [53, 54]. Mitsuada et al. [55] reported on the risk for small for gestational age (SGA) infants born to Graves' hyperthyroid mothers; risk factors included presence of maternal thyrotoxicosis lasting more than 30 weeks of pregnancy, duration of Graves' disease of approximately 10 years, and the onset of Graves' disease before age 20 years.

Infants of hyperthyroid uncontrolled mothers are at risk for the development of neonatal central hypothyroidism, some of these infants recovered normal thyroid function in a few weeks, whereas another group had long-standing hypopituitary-thyroid dysfunction [56–58].

Maternal Management

Control of hyperthyroidism through pregnancy and early detection of fetal thyroid dysfunction are the two most important aspects in the management of women affected by Graves' disease (Table 3). Control of hyperthyroidism includes (a) indication, choice, and timing of ATD therapy, (b) proper ATD dosage and adjustment with the goal to keep serum FT4/ATT4/FT4I in the

Table 3 Etiology of hyperthyroidism in pregnancy

Immune thyroid disease
Graves' disease
Chronic thyroiditis
Sporadic silent thyroiditis
Nonautoimmune thyroid disease
Multinodular goiter
Toxic adenoma
Subacute thyroiditis
Transient non-autoimmune hyperthyroidism of early pregnancy
Gestational thyrotoxicosis
Multiple gestations
Nausea and vomiting
Hyperemesis gravidarum
Trophoblastic tumor
Hydatidiform mole
Choriocarcinoma
Iatrogenic
Excessive levothyroxine intake
Overtreatment
Factitious
Iodine induced

upper limits of reference range, and (c) detection of early medical complications. Early detection of fetal and neonatal dysfunction includes (a) a medical team approach, including obstetrician, maternal-fetal medicine specialist, endocrinologist, neonatologist, anesthesiologist, and pediatric endocrinologist, (b) timing and interpretation of maternal serum TRAb levels, and (c) detection of fetuses at risk, in need of intensive fetal monitoring management. Although the incidence of fetal/neonatal Graves' disease is relatively low (5–10%), the medical consequences of an unidentified newborn with the disease are very serious [13, 58].

Medical therapy is the cornerstone management of hyperthyroidism in pregnancy. In the United States, the two available ATDs are propylthiouracil (PTU) and methimazole (MMI); in other countries such as the UK, carbimazole, a methimazole precursor, is used. The three drugs are effective in controlling the disease. PTU is associated with a risk of maternal liver failure [59], and its use is limited to (a) first trimester of pregnancy (Fig. 3), (b) allergy to MMI/carbimazole, and (c) thyroid decompensation or crisis [60]. Taylor and Vaidya [61] reported six cases of PTU-induced liver failure, two of them required liver transplantation, and one patient died. MMI can also induce liver toxicity, but these effects are milder, confined to liver cholestasis, not associated with liver failure, and seen more frequently in older patients [62].

Because of the risk of more severe congenital abnormalities with MMI than PTU (see Section "Congenital Malformations"), PTU is used in the first trimester, switching MMI to PTU in women planning pregnancy or as soon as gestation is diagnosed. MMI is reassumed after 12 week's gestation (Fig. 3). The initial dose of propylthiouracil or methimazole is guided by the severity of hyperthyroid symptoms. An initial dose of 150–450 mg daily of propylthiouracil in three divided doses or 5–20 mg of methimazole, given as a single daily dose, is usually recommended. Very seldom a larger initial dose is required; such in the presence of a large goiter, drug resistance is extremely unusual (if really existed as a medical entity), in most cases due to patient inconsistence in taking the medication. In patients with minimal symptoms, an initial dose of 5–10 mg of MMI daily or PTU 50 mg two or three times a day may be initiated. Thyroid test (TSH and FT4/FT4I/ATT4) are obtained every 2–4 weeks as clinically indicated. In the majority of patients, clinical improvement is seen in 2–3 weeks, and improvement in thyroid tests occurs within the first 2 weeks of therapy, with normalization to serum FT4/FT4I/ATT4 in 3–7 weeks, while serum TSH will remain suppressed or undetectable sometimes throughout the duration of pregnancy. Because of the immunologic changes that occur with progression of pregnancy, requirement for antithyroid medications decreases after the second half of gestation along with a gradual reduction of blood TRAb titers in the vast majority of women. Once there is an improvement in serum FT4/FT4I/ATT4, the ATD dose is decreased with the aim to keep the FT4/ATT4 in the upper limits of normal with the minimum dose of ATD (Fig. 3). Another indication to decrease ATD dose is when serum TSH values become detectable. Blood TRAb titers play a crucial role in adjusting the dose of ATD; as

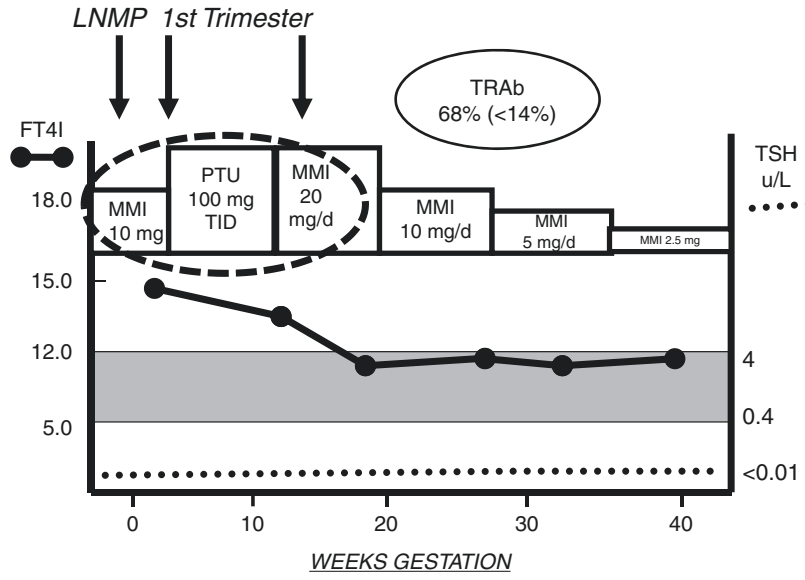

Fig. 3 Medical management of Graves' hyperthyroidism in pregnancy: (1) PTU use in the first trimester, (2) minimum ATD dose to keep the FT4 (FT4I, ATT4) in the upper limit of normal, and (3) maintain ATD therapy until delivery in the presence of maternal TRAb titers three times above reference range. After Patil-Sisodia K & Mestman JM. Endocrine Practice 2010;16:118

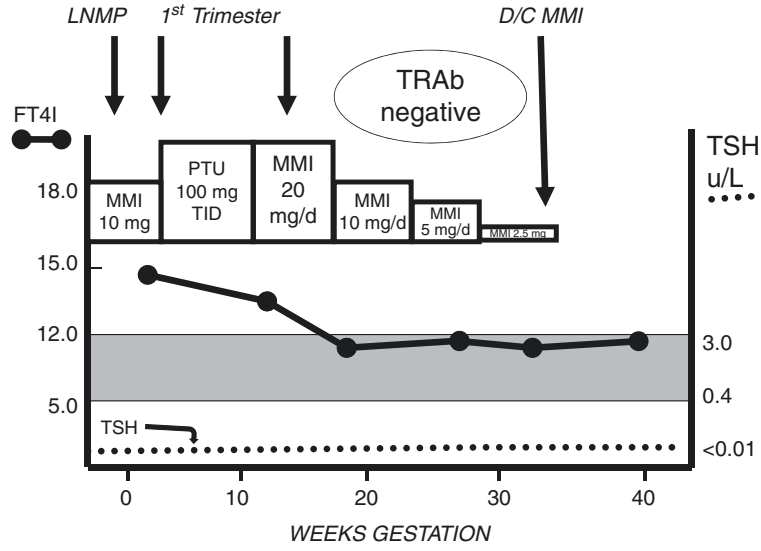

Fig. 4 Discontinuation of ATD therapy at about 34 weeks in hyperthyroid women on minimum dose of ATD and negative TRAb titers. After Patil-Sisodia K & Mestman JM. Endocrine Practice 2010;16:118

mentioned above serum TRAb titers tend to decrease after the 20 weeks of gestational age, becoming negative or slightly elevated. In some patients with small goiters, short duration of symptoms, low or negative serum TRAb titers, and minimal amounts of antithyroid medication (MMI 2.5–5 mg, PTU 50–100 mg daily) are able to discontinue ATDs by 34 weeks' gestation or beyond (Fig. 4). It is estimated that 30–40% of women are able to remain euthyroid without antithyroid therapy in the last few weeks of pregnancy. In a study of 44 women in 46 pregnancies, the correlation among TRAb activity, the dose of antithyroid therapy, and neonatal outcome was studied [63]. Medication was discontinued in 30 pregnancies 3–18 weeks before delivery. Neonatal thyrotoxicosis was seen in four infants whose mothers' TRAb levels exceeded 70% (normal, <15%). Interestingly, of the infants born with elevated serum TSH, maternal TRAb was less than 30% in most, suggesting that in Graves' disease-associated hyperthyroid pregnancies, a

low TRAb titer is an indication to use the minimal amount of antithyroid therapy to avoid the development of fetal hypothyroidism.

β-Adrenergic blocking agents (propranolol 10–40 mg every 6 h or atenolol 25–50 mg/day) are very effective in controlling hyperdynamic symptoms and are used for a short period of time, decreasing the dose with symptoms improvement. We preferred short-acting drugs such as propranolol since it is more convenient to adjust and decrease the dose according to patient's symptoms. Occasionally women may require small doses such as 10 mg at bedtime, even after achieving normalization in serum FT4/ATT4 levels. Long-term use of beta-blocking agent drugs has been reported to induce bradycardia, intrauterine birth restriction, and neonatal hypoglycemia [64]. One publication reported an increased incidence of spontaneous abortion with the combined use of propranolol and methimazole vs. antithyroid drugs alone [65]. One situation in which β-adrenergic blocking agents may be very effective is in the treatment of severe hyperthyroidism during labor. In a case reported in which both mother and fetus were affected, labetalol was infused at a rate of 2 mg/min and controlled maternal and fetal tachycardia within 45 min [66].

Subtotal thyroidectomy in pregnancy is reserved for uncommon cases of large goiters causing compressing symptoms, patients not responding to large doses of ATD, unusual case of allergy to ATDs [67], and poor drug consistency; the timing of surgery is between 18 and 24 weeks' gestation. In a population-based study [68], maternal complications were higher during pregnancy as compared to nonpregnant control group. Beta-blockers should be used before surgery for symptom control and continued for the first few days after surgery; potassium iodide (KI), to be given for 8–14 days before surgery, is useful in decreasing thyroid blood flow as well as improvement of maternal thyroid function. A few days before surgery, it is essential to obtain maternal serum TRAb to assess fetal hyperthyroidism risks; a titer three times above referral range is a predictor of fetal hyperthyroidism and an indication of close obstetrical monitoring.

Thyroid Storm in Pregnancy

Thyroid storm is a rare and serious complication of uncontrolled hyperthyroidism, as a result of undiagnosed disease, patient inconsistency with medication, or discontinuation of ATD during pregnancy due to concern for teratogenicity, occurring in 1–2% of pregnant hyperthyroid women. It is triggered by a precipitating event, such as infection, eclampsia, labor, a surgical procedure, or cesarean section. It is associated with a high maternal and fetal morbidity and mortality. Clinical presentation varies, but fever, altered mentation status, and a precipitating event are the most likely signs that would alert the physician of the possibility of thyroid storm in a patient with hyperthyroidism [60]. These symptoms are a manifestation of the abrupt onset of a hypermetabolic state that can lead to multi-organ failure, including heart failure, liver dysfunction, nausea, vomiting, and diarrhea [69]. Although thyroid storm is a clinical diagnosis, presenting symptoms should be accompanied by thyroid function tests indicative of hyperthyroidism, values that are indistinctive from other forms of hyperthyroidism. There are several published score systems to assist the physician in the diagnosis and severity thyroid storm [70, 71].

Patients should be immediately admitted to an intensive care unit; fetal monitoring may show concerning findings; however, these may improve as the mother is adequately treated. Delivery can exacerbate and worsen maternal status [72] and should be avoided if possible. Management includes (a) supportive therapy such as fluids and correction of electrolyte abnormalities, oxygen therapy as needed, and control of hyperpyrexia. Acetaminophen is the drug of choice because aspirin may increase serum-free thyroid hormones, (b) congestive heart failure may require large doses of digoxin, (c) proper antibiotic therapy is instituted in case of infection, and (d) control of hyperadrenergic symptoms, β-adrenergic blocker therapy propranolol 60–80 mg every 4 h orally or 1 mg/min intravenously. Esmolol, a short-acting β-acting antagonist, can be given intravenously with a loading dose of 250–500 μg/kg of body weight followed by continuous infusion at 50–100 μg/kg/

min, (d) MMI 30 mg or PTU 300 mg every 6 h is initiated as soon as the diagnosis is entertained, and PTU is preferred because it blocks conversion of serum T4 toT3. If the patient is unable to take oral medications, a nasogastric tube may be needed; (e) one hour after the administration of thioamides, iodine is administered to block the secretion of thyroid hormones from the thyroid gland; Lugol's solution, ten drops three times a day, or sodium iodide is given intravenously 1 g every 12 h; (d) glucocorticoids are also helpful because they reduce the peripheral conversion of serum T4 to T3. Hydrocortisone, 50–100 mg every 8 h or equivalent amounts of other glucocorticoids, such as dexamethasone 2–4 mg every 8 h. In summary, thyroid storm is a life-threatening condition with a mortality rate of 20–30%, 50 and it requires early recognition and aggressive treatment.

Congenital Malformations

Congenital malformation induced by antithyroid drugs are known to produce specific birth defects. The first case was described in 1972 [73]; the authors reported an infant born of a mother that took methimazole in the first trimester of gestation, with a localized lesion in the parietal area of the scalp characterized by congenital absence of the skin and punched-out ulcer-like lesion, known as aplasia cutis. Since then, only one case of this lesion has been reported with the use of PTU [74]. Since the first publication, several studies have described a specific embryopathy in infants born to mothers treated with MMI/carbimazole in the first trimester of pregnancy [75, 76]. It is known as methimazole/carbimazole embryopathy, and it includes choanal atresia (failure of the nasal passages to develop), tracheoesophageal fistula, esophageal atresia, omphalocele, hypothelia and athelia (failure of the nipples to develop), minor dysmorphic features, and developmental delay. The prevalence of these malformations in the general population is 1 in 2500 for esophageal atresia and 1 in 1000 for choanal atresia. Congenital heart defects were recently recognized in children whose mothers were exposed to MMI or carbimazole (MMI-CMZ) early in pregnancy [77]. In one study, echocardiography was performed in 60 of 68 neonates born of mothers with Graves' disease, and four cases of congenital heart defects were diagnosed (two atrial septal defects, one ventricular septal defect, and one tetralogy of Fallot) [78]. Andersen et al. [79] studied congenital malformations in the Danish population and estimated that fetal exposure to ATD early in pregnancy may affect ~ 1/30 of children. The authors also showed for the first time that neonates exposed to PTU in early pregnancy had a significant incidence of congenital malformations ~1/40, as compared to infants not exposed to ATD. The PTU congenital malformations tended to be less severe than the ones observed with MMI and affected mainly the face and neck area (preauricular and branchial sinus fistula/cyst) and the urinary system (single cyst of the kidney and hydronephrosis). Some of these malformations were detected within 2 years after birth and some of the children needed corrective surgery. According to Yoshihara et al. [80] in a Japanese population of newborns exposed to ATD in the first trimester of pregnancy, an overall rate of major anomalies in the MMI group was 4.1%, a rate significantly higher than the 2.1% in the control group and the PTU-treated mothers. A recent meta-analysis demonstrated an increased risk of congenital anomalies with exposure to all thioamides in pregnancy [81].

Ishikawa [82] made a very interesting observation; he reported a high incidence of dysplasia of the hip in infants of mothers affected with hyperthyroidism in the first trimester of pregnancy: 3 out of 12 with Graves' disease (20%, $P < 0.0001$) and 5 of 34 with severe gestational thyrotoxicosis (12.8%, $P < 0.0001$) as compared to 13 out of 2070 normal pregnancies (0.63%); all infants were female.

The significant finding of congenital malformations in children exposed to PTU in the first trimester opens a new dilemma in the management of hyperthyroidism in women planning pregnancy (see Section "Prepregnancy Counseling"), and in all women of reproductive age, since unplanned pregnancy is reported to be over 50% in the US population [83]. Guidelines

from the Endocrine Society [84], American Thyroid Association [85], and European Thyroid Association [86] favored the use of PTU in hyperthyroid women planning a pregnancy or switching from MMI to PTU as soon as the diagnosis of pregnancy is confirmed. Laurberg and Andersen [87] reviewed the literature on the association between weeks gestation in early pregnancy and ATD fetal exposure with the development of birth defects. They concluded that high risk for malformations was confined to gestational weeks 6 through 10, the major period of organogenesis, suggesting that the risk of birth defects could be minimized if pregnant women stop ATD intake before gestational week 6. Their recommendation for fertile women on drug therapy is to receive written instructions: [1] a pregnancy test within a few days after a missed menstrual period; [2] if the test is positive, to contact their physician; and [3] if feasible, to discontinue ATD therapy and follow with weekly thyroid function tests. They added that PTU is the drug of choice if therapy is needed, because congenital defects are less severe as compared to methimazole/carbimazole. A recent prospective cohort study suggested that high serum FT4 concentrations in the first half of pregnancy may have negative effects on brain development in offspring [88], with a statistically significant 1.4–3.7 point reduction in mean child IQ, and MRI findings of reduction of total gray matter and cortex volume, findings suggesting a clinical impact of hyperthyroidism in the first half of gestation. This finding could incline the physician to maintain euthyroidism in the first trimester of pregnancy with the use of PTU, at a risk of a lower incidence and severity of congenital malformations.

Fetal-Neonatal Care

As mentioned previously, a team approach in the management of hyperthyroidism in pregnancy offers the best results for pregnancy outcome. Fetal hypo- and hyperthyroidism are severe conditions with high fetal and neonatal morbidity and mortality if not diagnosed and treated properly. Women at risk for having affected outcomes are well defined: for fetal hyperthyroidism, (a) a previous pregnancy with an affected infant and (b) uncontrolled hyperthyroid women and those with Graves' disease treated with ablation therapy before pregnancy, with TRAb titers over three times reference range by 18 weeks' gestation (Fig. 5), and for fetal hypothyroidism maternal inappropriate high ATD dosage in the second half of gestation.

Early signs of fetal hyperthyroidism include fetal tachycardia (consistent fetal heart rate

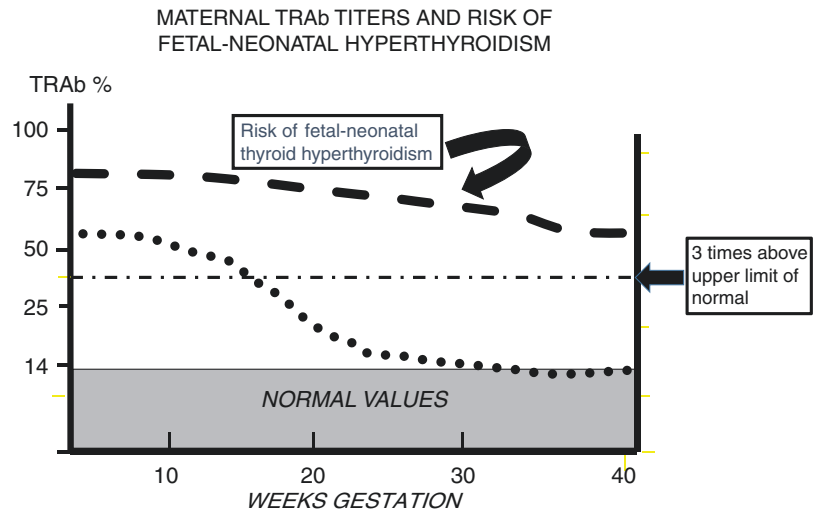

Fig. 5 Titers of serum TRAb decrease after the second half of gestation; pregnancies with titers three times above upper limit of normal are at risk of fetal and neonatal hyperthyroidism

>160 per minute for at least 5 min) (a typical fetal monitoring pattern has been described [89]), inappropriate fetal growth, thyroid gland enlargement (reported as the first sign of fetal hyperthyroidism), presence of oligo-polyhydramnios, and advanced bone age (this last complication detected later in gestation by 30–32 weeks) [90]. The size of the normal fetal gland according to gestational age was reported [91]. Less commonly, fetal hyperthyroidism can lead to heart failure and subsequently, increase risk of intrauterine fetal demise [25].

Serial fetal ultrasounds are an invaluable tool in expert hands to diagnose and assess fetal signs of thyroid dysfunction. It should be started at about 18 weeks gestation in women at risk, at the time of full maturation of the fetal hypothalamic pituitary thyroid axis. The use of ultrasonography for monitoring the size of the fetal thyroid gland as an indicator of thyroid dysfunction and possibility for therapeutic intervention was evaluated by Luton and associates in France [90]; the authors studied 41 hyperthyroid women considered to be at high risk (presence of high titers of TRAb) and on antithyroid therapy and detected fetal goiter in 11 of them. They considered the detection of fetal goiter as the earliest sonographic sign of fetal dysfunction. Of the 11 fetus, four of them were hyperthyroid, and seven were hypothyroid secondary to high doses of maternal antithyroid drug treatment; all of them benefited from adjusting drug therapy. The authors concluded that ultrasonography of the fetal thyroid gland by an experienced ultrasonographer is an excellent diagnostic tool, in conjunction with close teamwork, to ensure normal fetal thyroid function. Cordocentesis, an invasive technique to assess fetal thyroid function, is associated with high morbidity and mortality, and it should be performed in centers with experience [92].

Inappropriate high doses of ATD during management of hyperthyroidism may induce fetal hypothyroidism with or without fetal goiter [93]. To prevent it, it is recommended to keep maternal serum FT4/ATT4 in the upper limit of reference range with the minimum ATD dose, particular in women with negative or low TRAb titers. Neonatal hypothyroidism diagnosis is made at the time of routine neonatal hypothyroid screening; occasionally a goiter is detected at birth, rarely nowadays requiring maternal cesarean section.

There are a small subgroup of babies born from Graves' disease mothers on ATD therapy diagnosed with hyperthyroidism at birth [94] or within 48–96 h after birth. Maternal TRAb titers in the third trimester are over three times reference [15] (Fig. 5); this high titer of TRAb crossing the placenta may produce fetal hyperthyroidism, controlled during pregnancy by the placenta transfer of maternal ATD; most of these infants are born euthyroid, but in the first 2–4 days of life, they develop hyperthyroidism once the protective effects of ATD disappear. Since, the importance to follow these newborns with frequent determination of FT4 in the first 7 days of life. Their hyperthyroidism may last for a few months since TRAb half-life up to 3 months [26]. It has been suggested that obtaining a determination of TRAb in cord blood at birth may predict neonates at risk of developing hyperthyroidism [26]. In a very interesting study [58], 28 children born of hyperthyroid mothers were seen before 1 month of age in a pediatric clinic; they were divided into three groups: (1) 9 born with neonatal hyperthyroidism, eight of them from hyperthyroid mothers and one from a euthyroid thyroidectomized mother; (2) 11 with primary hypothyroidism, ten of them from treated mothers and three of them needed levothyroxine treatment; and (3) 5 infants with hypothalamic-pituitary hypothyroidism.

Maternal Postpartum Care

Maternal follow-up, in women with Graves' disease, during the first postpartum year is strongly recommended [95]. Postpartum aggravation of autoimmune thyroid disease was elegantly described by Amino et al. [96]. They classified postpartum thyroid dysfunctions into five categories: (1) persistent thyrotoxicosis, (2) transient thyrotoxicosis, (3) destructive thyrotoxicosis followed by transient hypothyroidism, (4) transient hypothyroidism, and (5) persistent hypothyroid-

ism. The syndrome could be the first manifestation of thyroid dysfunction in women with euthyroid chronic or Hashimoto's thyroiditis or could manifest as a recurrence of hyperthyroidism in women with remission during pregnancy or as the first manifestation of Graves' hyperthyroidism. Tada et al. [97] reported from Japan that at least 40% of Graves' women aged 20–39 years developed their disease during the postpartum period. In another study it was concluded that women with a family history of Graves' disease and those older than 35 at the time of pregnancy are at increased risk for postpartum Graves' disease [98], in the vast majority after 6 months postpartum.

In postpartum thyroiditis syndrome, the hyperthyroid phase occurs during the first 1–4 months after delivery, with typical hyperthyroid symptoms that could be mild or severe; the differential diagnosis is between an episode of recurrent or new Graves' hyperthyroidism (persistent) and transient thyrotoxicosis, a distinction of clinical significance since management and long-term follow-up differ. In the case of transient destructive thyrotoxicosis (postpartum thyroiditis), hyperthyroid symptoms are less severe and resolve spontaneously in a few weeks, leading to a euthyroid state or developing onto hypothyroidism by the third or fourth month postpartum, followed in 3–4 months by euthyroidism in the majority of women [96], although in one study permanent hypothyroidism was reported in 50% of women [99]. The differential diagnosis between destructive thyrotoxicosis and Graves' hyperthyroidism is based on clinical findings and ancillary tests (laboratory and radiologic). Determination of serum TRAb titers is very useful, since they are positive in Graves' disease and negative in the destructive thyroiditis form; a higher serum ratio FT3/FT4 is helpful but may overlap in some cases. A 4–6 hour ^{123}I thyroid nuclear uptake, high in Graves' and low or absent in destructive thyrotoxicosis, is very helpful; however, in lactating women, this test is contraindicated, unless breastfeeding is suspending for several days. Thyroid volume and blood flow were measured quantitatively by color flow Doppler ultrasonography in one study [100]. The authors studied 42 women with newly developed hyperthyroidism after delivery. Eighteen patients had Graves' disease and 24 had thyroiditis. Twelve of 14 patients who developed thyrotoxicosis in the first 3 months postpartum had postpartum thyroiditis; all patients who developed thyrotoxicosis after 6.5 months postpartum had Graves' disease. TRAbs were positive in all women with Graves' hyperthyroidism and were negative in those with PPT. The authors concluded that a positive TRAb titer and a high TBF >4.0% are indicators of postpartum onset of Graves' disease.

Yoshihara et al. [101] reported on the incidence of postpartum thyrotoxicosis (PT) in three groups of Graves' hyperthyroid women: (1) women treated with ATD, (2) those ablated with radioactive iodine (RI), and (3) post-subtotal thyroidectomy. The overall incidence of PT was 2.1% (4/188) in the RI group, 23.6% (35/148) in the subtotal thyroidectomy group, and 55.1% (59/107) in the ATD group.

From the above studies, it is recommended to educate and alert women with previous or present history of Graves' hyperthyroidism about the high probability of disease recurrence in the first year after delivery; thyroid tests should be considered every 3 months in the first year after delivery or at any time a patient develops symptoms suggestive of thyroid dysfunction.

Breast Feeding

This is a frequent concern of mothers in need of drug therapy during lactation [102]. ^{131}I therapy is absolutely contraindicated and surgery is really indicated during lactation. Several studies have shown that ATD concentration in breast milk is very low, as shown by normal thyroid function in the babies and normal long-term outcomes [103, 104]. For reasons already discussed, the ATD of choice is methimazole, in daily doses up to 20 mg. It is recommended to split the total dose, every 8–12 h, preferably given after the baby feeding. In situations in which the mother is allergic to methimazole/carbimazole, PTU is used in divided doses up to 450 mg a day.

Resistance to Thyroid Hormone Syndrome

Described by Refetoff and colleagues in 1967 [105], resistance to thyroid hormone (RTH) is a syndrome of reduced end-organ responsiveness to thyroid hormone caused primarily by mutations in the thyroid hormone receptor β-gene, characterized by elevated free thyroid hormones with nonsuppressed TSH and with signs of hyperthyroidism in some tissues and hypothyroidism in others [106]. The clinical manifestations include goiter and tachycardia, and the prevalence is about 1/40,000 live births. Unaffected fetuses of mothers with RTH syndrome and affected fetuses from normal mothers are at risk for poor obstetric outcome. Anselmo and associates [107] reported 36 couples with 9 mothers and 9 fathers affected by the disease and with 18 unaffected relatives. The rates of miscarriage were 23.7% when the mother was affected, 6.7% when the father was affected, and 8.8% with unaffected first-degree relatives, with a rate of 8.1%in the general population. The birth weights of unaffected infants born to affected mothers were lower than those of affected newborns, who in addition had a lower serum TSH at birth. This finding suggests that high maternal thyroid hormone levels produced fetal thyrotoxicosis and had a direct toxic effect on the fetus. The approach to a pregnant patient with the RTH syndrome would depend on the genotype of the fetus [106]. This requires obtaining the genotype of the fetus from DNA through amniocentesis or chorionic villus sampling, a history of the course and outcome of previous pregnancies, and information about other family members with RTH syndrome.

Conclusions

Pregnancies of mothers with a history of Graves' hyperthyroidism, previously treated, and those diagnosed at time of pregnancy are at higher risk of maternal and obstetrical complications as compared to a euthyroid population. Preconception patient education and contraception are imperative in women with thyroid disease, during the reproductive age. A medical team including endocrinologists, obstetricians, medical fetal-maternal physicians, anesthesiologists, neonatologist, and pediatric endocrinologists should be available from the time pregnancy is diagnosed. Antithyroid drug (ATD) therapy is the treatment of choice; thyroid surgery is indicated in selective cases. Selection of drug, timing of administration, and close follow-up with proper thyroid tests are essential for a good maternal and fetal outcome. Potential complications of ATD therapy should be considered and discussed with potential parents, as well as the interpretation of serum TRAb titers in detecting and treating thyroid fetal and neonatal disease. Breastfeeding recommendations and postpartum follow-up for a year following delivery is part of the care of a woman with a history of Graves' hyperthyroidism.

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Thyroid Hormone Resistance Syndromes

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Introduction

The term resistance to thyroid hormone (RTH) has traditionally been used to describe a subset of patients with defects in the action of thyroid hormone (TH). Recently a broader description of syndromes with impaired sensitivity to TH has been proposed which includes not only the classic RTH syndromes but also patients with defects in TH transport into the cell (thyroid hormone cell membrane transport defect, THCMTD) and a defect in TH metabolism (thyroid hormone metabolism defect, THMD) [1]. Among the RTH syndromes, there are three types: the "classic" RTHβ, in subjects with mutation in the *thyroid hormone receptor beta* (*THRB*) gene; RTHα, due to a mutation in the *thyroid hormone receptor alpha* (*THRA*) gene; and nonTR-RTH, in subjects expressing the RTHβ phenotype but having no *THRB* gene mutations. The latter may be due to *THRB* gene mosaicism or a defect in an unidentified cofactor involved in TH action (Fig. 1). The thyroid function tests and other clinical manifestations of these individuals with impaired sensitivity to TH are summarized in Table 1.

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The overall goal of this chapter is to discuss the treatment options for patients with various syndromes of impaired sensitivity to TH. However, given that these conditions are relatively uncommon, there is little or no evidence-based information using multicenter trials and on the "best" treatment of these conditions. The first step toward treatment of all of these syndromes is making an accurate diagnosis based on a combination of the clinical presentation and laboratory tests that ultimately requires genetic confirmation. Recognizing the etiology leads to a more logical approach to treatment. This assertion becomes most obvious in cases where a misdiagnosis results in inappropriate irreversible treatment, for example, a patient with RTHβ misdiagnosed with Graves' disease who undergoes thyroidectomy or radioactive iodide ablation.

Thyroid Hormone Cell Membrane Transport Defect (THCMTD)

Clinical Diagnosis

Patients with THCMTD have been demonstrated to have a defect in the *monocarboxylate transporter 8* (*MCT8 or SLC16A2*) gene and present with profound neuropsychomotor defects and a characteristic combination of thyroid function test (TFT) abnormalities (Table 1). MCT8, one of several TH cell membrane transporters, plays an

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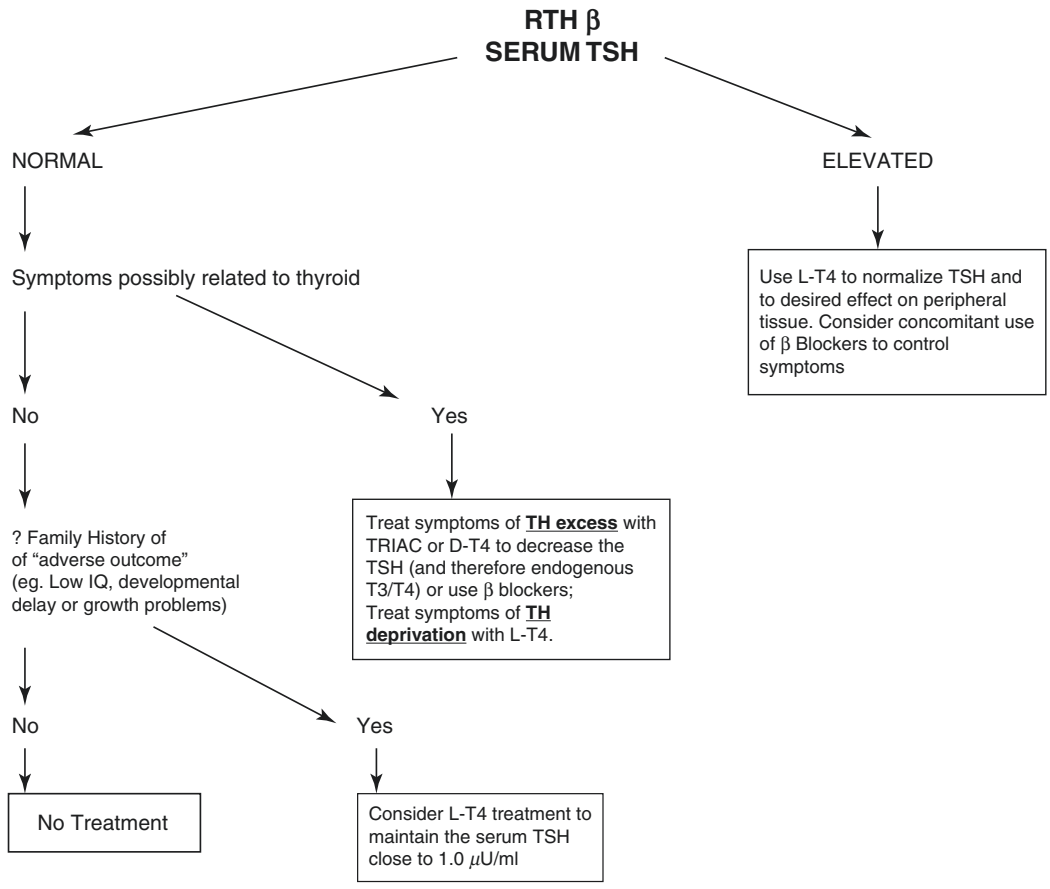

Fig. 1 Proposed scheme for treatment of patients with RTHβ

Table 1 Classification of syndromes of impaired sensitivity to thyroid hormone

Syndrome	Gene	FT4	FT3	rT3	TSH	# Families	Hallmark clinical features
Thyroid hormone cell transport defect (THCMTD)	MCT8 (SLC16A2)	↓	↑↑	↓	N, Sl↓	72	Severe psychomotor retardation Peripheral tissue hyperthyroidism
Thyroid hormone metabolism defect (THMD)	SBP2 (SECISBP2)	↑↑	↓	↑↑	N, Sl↑	5	Growth delay, muscular dystrophy, hearing impairment azoospermia
Thyroid hormone action defects							
RTH β	TRβ (THRB)	↑↑	↑,N	↑↑	N, Sl↑	372	Goiter, tachycardia, attention deficit disorder
NonTR-RTH	Unknown*	↑↑	↑,N	↑↑	N, Sl↑	(approx.15% of RTH Beta	Same as RTHβ
RTH α alpha	TRα (THRA)	↓	N, sl↑	↓	N	14	Delayed skeletal development, GI dysmotility

RTH resistance to thyroid hormone, *TR* thyroid hormone receptor, *sl* slight, *N* normal, ↑ increased, ↓ decreased
*The mechanism for non-TR RTH is unknown but probably involved abnormal cofactors or substances that interfere with the action of thyroid hormone on its receptor, or may represent a THRbeta mutation mosaicism
After [30]

important role in the supply of TH to the brain and, therefore, on brain development. Therefore, despite adequate synthesis and secretion of TH, and because of the variable tissue distribution of TH transporters, patients with MCT8 defects have evidence of TH deprivation in the brain and at the same time symptoms of TH excess in peripheral tissue. Located on the X-chromosome, *MCT8* gene mutations cause in males severe inability to talk or walk and increased metabolism with poor weight gain. The incidence MCT8 deficiency is not known. A sex-linked form of mental retardation with motor abnormalities was described in 1944 by Allan Herndon and Dudley. Its etiology was recognized in 2004 when the same defect, associated with characteristic TFT abnormalities, was found to be caused by *MCT8* gene mutations [2, 3]. Since then, 253 individuals belonging to 135 families with *MCT8* defects were identified. De novo *MCT8* gene mutations can be maintained in the population because carrier females have no symptoms; thus no negative selection takes place. Brain defects have been observed in human fetal life suggesting that the damage from lack of TH transport occurs in utero. The natural history of the disease depends on the severity of TH transport defect. Most subjects die in childhood due to aspiration or complications of their poor neurologic dysfunction.

Treatment

Symptomatic Therapy: Current treatment options for patients with *MCT8* gene mutations are limited to symptomatic and supportive measures. Intense physical, occupational, and cognitive therapy has been shown to have a positive influence on the degree of neurologic impairment. Braces can be used to prevent contractures. A feeding tube is often used to prevent aspiration and provide sufficient caloric intake as one of the consequences of the THCMTD is visceral hyperthyroidism. Dystonia is treated with anticholinergics, L-DOPA, carbamazepine, and baclofen. Drooling may respond to treatment with glycopyrrolate or scopolamine. Many patients have been reported to have seizures which are treated with standard anticonvulsants.

L-T_4 and PTU Treatment: Treatment of the low serum T_4 concentration with physiological doses of levothyroxine has been ineffective, presumably because of the impaired uptake of the hormone in MCT8-dependent tissues including the central nervous system. In addition to impaired T_4 transport into cells, there is an overexpression of deiodinases which converts the serum T_4 to high levels of T_3. This, in part, accounts for the peripheral tissue hyperthyroidism and failure to thrive, seen in these children. Treatment with propylthiouracil (PTU) to reduce the amount of T_3 generated, along with supraphysiologic doses of L-T_4, more readily available to the brain than T_3 is effective in improving nutrition and may prevent seizure development. The dose of L-T_4 is titrated to bring the serum T_4 concentration just above the upper limit of normal. PTU is given three times a day to reduce the serum T_3 concentration to the upper limit of normal for the age of the patient. Doses can be two- to threefold higher than those used in the treatment of thyrotoxicosis. Serum TSH concentration is usually undetectable.

DITPA Treatment: The use of TH analogues that may bypass the molecular defect by using alternative transporters has been studied in Mct8-deficient mice, and one of them, 3,5-diiodothyropropionic acid (DITPA), is able to ameliorate the brain TH deficit in these mice without causing thyrotoxic effect in the liver [4]. Results from a small trial in which DITPA treatment was given on a compassionate basis have been published [5]. In this study the youngest patient treated was 8.5 months old. The dose of DITPA was between 1.0 and 2.4 mg/kg/day, given in three divided oral doses. This dose was able to normalize the serum TSH and T_3 levels and to increase the serum T_4 and rT_3 to levels just in the lower normal range. The predominant beneficial effect of treatment was in the state of nutrition and weight gain with little improvement in neurocognitive function. A decrease in the frequency of seizures has anecdotally been reported by the parents of several children. The lack of significant neuropsychological effect is not surprising as these children are all

treated at an age after neurologic damage had occurred. The goal of therapy would be to treat in utero male-affected fetuses (see below).

TRIAC and TETRAC Treatment: Based on recent studies, triiodothyroacetic acid (TRIAC, T3A), a metabolite of T_3 given to 1- and 12-day-old Mct8/Oatp1c1 double knockout mice, restored T_3-dependent neural differentiation in the cerebral and cerebellar cortex [6]. For humans, this corresponds to prenatal and early postnatal period. In another study using young adult Mct8-deficient mice, TRIAC was able to restore serum T_3 levels but severely decreased T_4 levels [7]. Analysis suggested that TRIAC treatment resulted in relative brain hypothyroidism due to lower tissue levels of T_3. Although there are no published results in humans, there is an ongoing clinical protocol to investigate the treatment of MCT8 deficiency with TRIAC [8].

Tetraiodothyronine (TETRAC, T4A), a metabolite of T_4, has been also used in the first week of life in Mct8-deficient mice [9]. It produced TH-dependent neuronal differentiation in the cerebellum, cerebral cortex, and striatum but was ineffective in suppressing hypothalamic TRH expression. None of the TH analogues presented above are commercially available or approved by FDA as drugs in the USA.

Special considerations during pregnancy—In women with an affected child and a known mutation of *MCT8* gene, male fetuses carried in subsequent pregnancies can be assessed in utero for the presence of the mutation. If the pregnancy with an affected male embryo is allowed to progress, treatment with intra-amniotic high doses of TH or treatment with DITPA, although not reported in the literature, could be considered, but further studies are needed to determine whether this treatment can prevent the neurologic sequelae of this condition. Preliminary data in mice indicates that the DITPA will cross the placenta [10] and therefore would be logical to consider this compound as a potential therapy in women who refuse termination of pregnancy. We have no experience to date in treating fetuses with MCT8 deficiency.

Thyroid Hormone Metabolism Defect (THMD)

Clinical Diagnosis

The major TH secreted from the thyroid gland is T_4. It circulates in serum to be delivered to all tissues by active transport into cells (see THCMTD above). However T_4 is a precursor or "prohormone" that is activated by 5′-deiodination yielding the active hormone T_3 (deiodinase type 1, D1, or type 2, D2). T_4 is also inactivated by 5-deiodination to form reverse T_3, (rT_3) through D1 or type 3, D3. Each tissue adjusts the amount of T_3 or rT_3 in the intracellular compartment by regulation of the D1, D2, and D3. Deficiency in deiodinases alters the formation of the active hormone and results in the syndrome of TH metabolism defect. While only 12 families have been described with THMD, their TFTs are rather classic with elevated serum levels of free T_4 and nonsuppressed TSH with low serum T_3 values and high rT_3. These subjects have mutations the *selenocysteine-binding protein 2* (*SBP2*, *SECISBP2*) gene [11]. While the clinical phenotype can be mild or severe, the thyroid function tests are usually consistent. Incorporation of selenocysteine into proteins are dependent on SBP2. There appears to be a hierarchy as to which selenoproteins are most crucial for survival and preserved during states of SBP2 deficiency. Also many *SBP2* gene mutations allow for the synthesis of isoforms from alternative translation start sites [12]. The most common clinical feature is growth retardation which usually prompts thyroid testing. Other findings are photosensitivity, muscle weakness, impaired hearing, hypoglycemia, and azoospermia.

Treatment

Since the defect in THMD is the inability to convert T_4 to the active hormone T_3, this is the one thyroid condition in which treatment with L-T_3 is recommended. Usually normal replacement doses of T_3 are sufficient to normalize the serum TSH and most observed tissue responses to TH

(e.g., linear growth). In general for adults and children over the age of 15 years, we recommend treatment with oral L-T_3 50 μg q12 h. For children less than 15, L-T_3 dose is adjusted as follows: 12.5 μg for ages 1–3 years (8–15 kg body weight), 25 μg for ages 4–9 years (16–25 kg body weight), and 37.5 μg for ages 10–14 years (26–45 kg body weight). We have shown that this regimen achieves similar serum levels of T_3 as those in adults receiving the corresponding higher L-T_3 dose.

Also it would be rational to consider supplementation with selenium, given that the molecular defect is caused by the inability of selenocysteine to be incorporated in the protein structure and serum selenium concentration is low. Whereas administration of up to 400 μg of both selenomethionine and sodium selenite normalized the serum selenium concentration, they had no effect on selenoprotein deiodinase D2 activity and glutathione peroxidase concentration and failed to correct the abnormalities of serum iodothyronine levels [13].

Resistance to Thyroid Hormone β(RTHβ) and NonTR-RTH

Clinical Diagnosis

RTHβ is characterized by elevated levels of serum TH and nonsuppressed TSH [14]. Some degree of thyroid gland enlargement is universally found in these patients. The thyroid status of the peripheral tissue varies among tissues depending on the relative expression of the TRβ. For example, because TH action in the heart is predominately mediated by the TRα receptor, patients with TRβ mutations have tachycardia secondary to the high levels of TH effecting the tissue not opposed the mutant TRβ. Diagnosis is usually detected in children who present with symptoms of hyperthyroidism (goiter, overactivity, growth disturbance). Tachycardia is more common in adults. TFTs are analyzed, and the characteristic elevation of serum TH concentrations and nonsuppressed or elevated TSH levels are detected.

The differential diagnosis includes patients with elevated TH levels such as binding protein abnormalities (e.g., dysalbuminemia, TBG excess) interfering substances falsely elevating the TH or TSH levels (e.g., autoimmune thyroid disease, anti-T4 antibodies). These diagnoses are usually ruled out by measurement of free TH levels by equilibrium dialysis or measurement of specific interfering substances. TSH-secreting pituitary adenomas (TSHomas) should also be considered in the differential diagnosis of RTHβ. Unlike TSHomas, RTHβ is inherited (although there are de novo mutations of the *THRB* gene), and family analysis shows other affected family members, unlike TSHomas. Detection of a mutation in the *THRB* gene also rules out the diagnosis of TSHoma. However, 15% of patients presenting with clinical RTHβ have no detectable mutation in the *THRB* gene, such patients behave as RTHβ patients but are classified as NonTR-RTH (Fig. 2).

The mechanism for impaired TH action in RTHβ can be due to a mutation that affects the ligand-binding domain of the receptor impairing T_3 binding. These patients have a normal TRβ allele, but due to interference of mutant allele with the normal allele (dominant negative activity), impaired action of TH ensues. However it should be noted that the most severely affected individuals are those that are homozygous for point mutations or complete absence of both alleles. Secondly, an abnormal cofactor could impair activation of the receptor ligand complex.

Treatment

Thyroid hormone: There is no treatment that will correct the defect of TRβ function in subjects with RTHβ. Fortunately, treatment is not needed in most patients because the hyposensitivity to TH seems to be adequately compensated by the increase in secretion of T_4 and generation of T_3. This is not the case in patients with limited thyroid reserve due to prior destructive therapy directed to the thyroid gland. In general, treatments that attempt to lower the TH levels to normal and especially thyroid gland ablation should

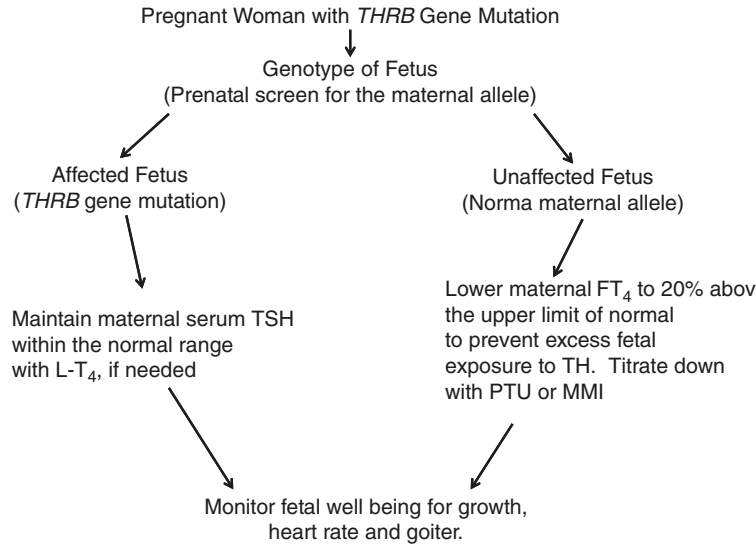

Fig. 2 Proposed scheme for treatment of RTHβ patients during pregnancy

not be carried out. Patients with reduced thyroid reserve should be treated with a sufficient amount of levothyroxine to reduce their serum TSH concentrations to normal or near normal. This may require as much as 1000 μg of L-T_4 daily.

In some patients with RTHβ, several peripheral tissues may be relatively more resistant than the thyrotrophs. Thus, the compensation for the hormonal resistance in these tissues is incomplete, and judicious administration of a dose of T_4 higher than that needed to restore TSH secretion to normal may be indicated. The dose must be individually determined by assessing tissue responses. In children, this should be done by regular assessment of growth, bone maturation, and mental development. L-T_4 should be given in incremental doses, and the basal metabolic rate, nitrogen balance, and serum sex hormone-binding globulin should be measured after treatment for 4–6 weeks before the dose is changed; bone age and growth should be followed on a longer-term basis. Development of a catabolic state is an indication of overtreatment.

Patients may have local symptoms caused by the goiter size, which is commonly present in RTHβ. Thyroid size can be reduced by the administration of a single high dose of L-T_3 given every other day [15]. The dose of L-T_3 should be titrated but can be as high as to 250 μg.

Beta-blocker: Subjects with RTHβ having symptoms of thyrotoxicosis, more specifically tachycardia and tremor, usually respond to the administration of the beta adrenergic blocker, atenolol (25–100 mg daily). We have also successfully used atenolol to treat symptoms of hyperdefecation in adults and children.

TRIAC is a TH analogue with low biological effect but in in vitro systems has three times the affinity for TRβ compared to T_3. Interestingly it has equal affinity for TRα as does T_3 in vitro. TRIAC has been used in several patients, and, together with reduction in the TSH and T_4 levels, there is some decrease in heart rate as well as effects on peripheral tissue (such as increase in cholesterol and in sex hormone-binding globulin). However, these effects were minimal and not consistent. Doses used were between 1.2 and 2.8 mg/day. TRIAC is not available in North America but can be obtained from laboratories ANA (Neuilly-sur-Seine, France).

Dextrothyroxine (D-T_4): D-T_4 had been thought to be variably useful in lowering serum cholesterol levels without producing adverse thyromimetic effects. There are three reports in the literature of such treatments with contradicting results. Therefore, we are unable to recommend it. Doses used have been 0.075 mg/kg or 2 mg per day in adults. D-T_4 is available as Dynothel from

Henning (Berlin, Germany) or Choloxin from Abbott in the USA.

Other drugs: Corticosteroids, dopamine, and somatostatin analogues have the theoretical advantage of lowering TSH production without causing thyromimetic effects. They have been used in several RTHβ patients before 2000 with variable effects and unacceptable side effects.

Patients with symptoms of ADD should be treated using standard regimens for ADD, independently from RTH. Some beneficial effects from treatment with L-T_3 have been observed [16]. This is attributed to a decrease in serum T_4, more available to the brain, which expresses predominantly TR. L-T_3 continues to maintain eumetabolism in peripheral tissues in face of lower T_4.

Special considerations during pregnancy—Management of TH levels during pregnancy in a mother with RTHβ or a normal mother carrying a fetus with *THRB* gene mutation is not straightforward. A pregnant woman with RTHβ is protected from adverse effects of her high TH levels, but fetal thyrotoxicosis may occur in offspring that do not carry the mutation.

A retrospective study of a large family with RTHβ, due to *THRB* R243Q, demonstrated that the adverse effect of TH on the fetus was independent of that on the pregnant woman [17]. The prevalence of early pregnancy loss was increased by threefold in affected mothers, but not in couples with an affected father and unaffected mother. Two-thirds of infants born to affected mothers carried the *THRB* gene mutation, which suggests that nearly all miscarried fetuses had no mutation and thus, a normal response to TH. Furthermore, unaffected infants born to affected mothers had lower birth weights and suppressed serum TSH concentrations. These adverse pregnancy outcomes are similar to those for infants with excess TH, caused by gain-of-function TSH receptor mutations, who are born prematurely and have low birth weights [18]. Management of pregnancies in mothers with RTH who are carrying unaffected fetuses may warrant judicious use of antithyroid medication, depending on the well-being of the fetus [19]. In such mothers, free T_4 should be maintained not higher than 20% above the upper limit of normal. This can be achieved by judicious use of PTU. There is no basis for regular treatment of normal mothers carrying affected fetuses (inherited from the father, or due to de novo mutation) unless the fetus is found to have a large goiter or be in distress. In such case the treatment with intra-amniotic infusion of L-T_4, should be considered, although this was reported in a single case [20]. Further studies are needed before a recommendation can be made.

Resistance to Thyroid Hormone α (RTHα)

Clinical Diagnosis

Mutations in the *thyroid hormone receptor alpha* (*THRA*) gene have now been described in 14 patients due to 8 different mutations [21–27]. Four mutations are frameshifts with early termination resulting in a truncated receptor, and the other four are point mutations in the ligand-binding domain and in the C-terminal helix. As in the case of *THRB* gene mutations, this results in three different mechanisms causing functional impairment: (1) there is reduced affinity for T_3; (2) the mutant TRα interferes with the normal TRα allele, resulting in a dominant negative effect; and (3) defective coactivator recruitment to the liganded receptor. Because TRα is not involved in the feedback regulation of the hypothalamic-pituitary-thyroid axis, the TFTs are different from those of the RTHβ phenotype due to *THRB* gene mutations. Patients with *THRA* gene mutations have low serum T_4, borderline high T_3, and very low rT_3, with normal to minimally elevated TSH concentrations. A high ratio of free T_3 to free T_4 serum concentration seems to be a common finding in all the cases described to date.

The phenotype varies in severity but is consistent with the manifestations of untreated congenital hypothyroidism in peripheral tissues. Due to the dominant role of TRα in mediating TH action in the bone, intestine, heart, and brain, signs of hypothyroidism in these issues are dominant. There are significant bony abnormalities includ-

ing reduced bone age, short stature, femoral epiphyseal dysgenesis, wormian cranial sutures, and macrocephaly. Other major clinical findings affect the gastrointestinal tract (constipation to megacolon), heart (bradycardia), striated muscles, and the central nervous system (ranging from autism to mental retardation).

Treatment

Treatment with L-T_4 was reported in two cases [28, 29]. In one of the cases, the hypothalamic-pituitary axis responded to exogenous TH, but the skeletal, gastrointestinal, and myocardial tissues were resistant [28], consistent with the greater resistance to TH in tissues expressing the TRα isoform. In a third case report [21], L-T_3 treatment was given which reduced the serum TSH and consequently the T_4 level, but also an increase in heart rate was observed. The experience is too preliminary to make definitive recommendations regarding treatment of patients with RTHα due to mutations in the *THRA* gene.

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