Endocrinology
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Paolo Vitti
Laszlo Hegedüs *Editors*

Thyroid Diseases

Pathogenesis, Diagnosis, and Treatment

Springer

Endocrinology

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Within the health sciences, Endocrinology has an unique and pivotal role. This old, but continuously new science is the study of the various hormones and their actions and disorders in the body. The matter of Endocrinology are the glands, i.e., the organs that produce hormones, active on the metabolism, reproduction, food absorption and utilization, growth and development, behavior control, and several other complex functions of the organisms. Since hormones interact, affect, regulate, and control virtually all body functions, Endocrinology not only is a very complex science, multidisciplinary in nature, but is one with the highest scientific turnover. Knowledge in the Endocrinological sciences is continuously changing and growing. In fact, the field of Endocrinology and Metabolism is one where the highest number of scientific publications continuously flourishes. The number of scientific journals dealing with hormones and the regulation of body chemistry is dramatically high. Furthermore, Endocrinology is directly related to genetics, neurology, immunology, rheumatology, gastroenterology, nephrology, orthopedics, cardiology, oncology, gland surgery, psychology, psychiatry, internal medicine, and basic sciences. All these fields are interested in updates in Endocrinology. The aim of the MRW in Endocrinology is to update the Endocrinological matter using the knowledge of the best experts in each section of Endocrinology: basic endocrinology, neuroendocrinology, endocrinological oncology, pancreas with diabetes and other metabolic disorders, thyroid, parathyroid and bone metabolism, adrenals and endocrine hypertension, sexuality, reproduction, and behavior.

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Paolo Vitti • Laszlo Hegedüs
Editors

Thyroid Diseases

Pathogenesis, Diagnosis, and Treatment

With 117 Figures and 101 Tables

Springer

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Series Preface

Is there an unmet need for a new MRW series in Endocrinology and Metabolism? It might not seem so! The vast number of existing textbooks, monographs and scientific journals suggest that the field of hormones (from genetic, molecular, biochemical and translational to physiological, behavioral, and clinical aspects) is one of the largest in biomedicine, producing a simply huge scientific output. However, we are sure that this new Series will be of interest for scientists, academics, students, physicians and specialists alike.

The knowledge in Endocrinology and Metabolism almost limited to the two main (from an epidemiological perspective) diseases, namely hypo/hyperthyroidism and diabetes mellitus, now seems outdated and closer to the interests of the general practitioner than to those of the specialist. This has led to endocrinology and metabolism being increasingly considered as a subsection of internal medicine rather than an autonomous specialization. But endocrinology is much more than this.

We are proposing this series as the *manifesto* for **"Endocrinology 2.0"**, embracing the fields of medicine in which hormones play a major part but which, for various historical and cultural reasons, have thus far been "ignored" by endocrinologists. Hence, this MRW comprises "traditional" (but no less important or investigated) topics: from the molecular actions of hormones to the pathophysiology and management of pituitary, thyroid, adrenal, pancreatic and gonadal diseases, as well as less common arguments. Endocrinology 2.0 is, in fact, the science of hormones, but it is also the medicine of sexuality and reproduction, the medicine of gender differences and the medicine of wellbeing. These aspects of Endocrinology have to date been considered of little interest, as they are young and relatively unexplored sciences. But this is no longer the case. The large scientific production in these fields coupled with the impressive social interest of patients in these topics is stimulating a new and fascinating challenge for Endocrinology.

The aim of the **MRW in Endocrinology** is thus to update the subject with the knowledge of the best experts in each field: basic endocrinology, neuroendocrinology, endocrinological oncology, pancreatic disorders, diabetes and other metabolic disorders, thyroid, parathyroid and bone metabolism, adrenal and endocrine

hypertension, sexuality, reproduction and behavior. We are sure that this ambitious aim, covering for the first time the whole spectrum of Endocrinology 2.0, will be fulfilled in this vast Springer MRW in Endocrinology Series.

Andrea Lenzi, M.D.
Series Editor
Emmanuele A. Jannini, M.D.
Series Co-Editor

Volume Preface

Despite the availability of a number of endocrine textbooks, we believe that there is a need for a comprehensive volume on the thyroid, which can be used by trainees, general endocrinologist, and experts in the field alike. The present one, available as a book, but also electronically, and intended to be updated online whenever developments warrant this, in our minds fills a void. Covering the majority of clinically relevant phenotypes, and thyroid physiology as well as pathogenesis, diagnosis, and treatment, we have been fortunate to profit from the dedicated service of a number of recognized and authoritative experts. Challengingly, being the first in a series of volumes intended to eventually cover all of endocrinology, there has been little precedence to learn from and to use as a *point de référence*.

The original idea for this volume was nurtured in the garden of the Italian Society of Endocrinology and was then developed together with another European as co-editor. The cutting-edge information is provided by a bouquet of mainly European colleagues but also some from the USA, Canada, and Australia. With this in mind, we can only hope that the many instruments, or at least their players, orchestrated a tune that to the readers appears as one harmonious melody and not a cacophony of contradictions and overlaps. The two conductors have agreed and followed the same score, and a strong friendship has developed over the nearly 3 years from conception to delivering a final product.

The first of seven sections covers regulation of thyroid function, synthesis of thyroid hormones, and their mechanism of action. To be followed by thyroid tests and imaging and moving into thyroid diseases highlighting basic concepts, clinical diagnoses, and management in greater detail. The solitary thyroid nodule and multinodular goiter constitute the second part, while the next covers Hashimoto's and the other types of thyroiditis. The fourth section deals with different types of hypothyroidism, while thyrotoxicosis and hyperthyroidism, autoimmune and non-autoimmune, are covered in the fifth section. Here, also the newest development in the diagnosis and management of the very challenging and complex Graves' ophthalmopathy is given much attention. The section on thyroid carcinomas, in addition to describing the various histiotypes, discusses pathogenesis and the newest medical therapies as well as the most recently elucidated molecular mechanisms and genetic defects. In the last section, a number of other conditions influencing thyroid function, or inducing thyroid dysfunction, come in focus. Pregnancy, nonthyroidal

illness, and the ever expanding and complicated effects of drugs and other substances that influence and interfere with thyroid function are offered competent attention. A separate pharmacopeia has not been deemed necessary. However, pharmacological treatments are included in the chapters where relevant.

Just a few weeks after delivering his chapter on "Regulation of Thyroid Function, Synthesis and Function of Thyroid Hormone" the chocking message of the untimely passing away of professor Theo J. Visser reached us. It is with sadness, but also great affection and respect, that we dedicate this book to the memory of a gentle, humorous giant of a researcher and colleague.

Needless to say, we are indebted to all our colleagues who have kindly and generously dedicated time and enthusiasm to contribute so competently to this text. As ever, our thanks go to our families for their patience and support during our absence with this endeavor at mind.

Pisa and Odense
April 2018

Paolo Vitti, M.D., Ph.D.
Laszlo Hegedüs, M.D., D.M.Sc.

Contents

About the Editors

Professor Laszlo Hegedüs, 65, is trained in Copenhagen, Denmark. In 1992, having defended his thesis, he became a consultant physician at the Department of Endocrinology in Odense, Denmark. Here he built up a thyroid research group and became a Full Professor in 2006.

Publications, more than 480, include chapters in leading endocrine textbooks and include diagnostic imaging; radioiodine therapy; ultrasound-guided diagnosis and treatment of thyroid diseases; environmental and hereditary aspects of the regulation of thyroid function and size; environmental and genetic background for thyroid disorders utilizing Danish twins; and late effects of thyroid diseases. Developing and utilizing ThyPRO to evaluate thyroid-related quality of life and the role of selenium in thyroid diseases are also in focus.

Dr. Hegedüs has served as editor of *Clinical Endocrinology* and as editorial board member of a number of journals. He has mentored around 50 individuals defending their academic theses. He is President-Elect of the European Thyroid Association and a former President of the Danish Thyroid Association. He is extensively used at endocrine and thyroid teaching courses and has given more than 200 invited talks. Professor Hegedüs is the recipient of several prizes, including the George Murray lecture and the Pitt-Rivers lecture from the British Thyroid Association and the Frontiers in Science Award from the American Association of Clinical Endocrinologists.

Paolo Vitti is Full Professor of Endocrinology and Director of the School of Endocrinology at the Medical School, University of Pisa, Italy, and Chief of Endocrinology at the University Hospital of Pisa. Dr. Vitti is a past member of the Executive Committee of the European Thyroid Association and a past Secretary of the Italian Thyroid Association. He was appointed with the European Thyroid Association – Merck Serono Prize 2014. He has been a member of the International Council for the Control of Iodine Deficiency Disorders (ICCIDD) since 1996 and an ICCIDD board member since 2003. He has also been Deputy-Regional Coordinator for the ICCIDD in West Central Europe since 2001 and a member of expert WHO/ICCIDD teams for external reviews in Kenya, Macedonia, and Peru. He is currently the President of Italian Society of Endocrinology. He is the author of over 150 articles, most of which have been published in authoritative international journals, and of a number of chapters of international and national endocrine textbooks.

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Part I

Thyroid Physiology and Laboratory Evaluation of the Thyroid

Regulation of Thyroid Function, Synthesis, and Function of Thyroid Hormones

1

Theo J. Visser

Contents

This chapter is adapted from Visser (2011).

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Abstract

Thyroid hormone (TH) is a common name for the two products secreted by thyroid follicles, namely the prohormone thyroxine (T4) as the major product and the active hormone triiodothyronine (T3) as the minor product. Most T3 is produced by deiodination of T4 in peripheral tissues. TH is essential for the development of different tissues, in particular the central nervous system, and for the function of the tissues throughout life. The secretion of thyroid hormone is regulated within the hypothalamus-pituitary thyroid axis, but the biological activity of thyroid hormone is largely regulated at the level of the target tissues.

This chapter covers various aspects of (a) the neuroendocrine regulation of thyroid function, (b) the biosynthesis of thyroid hormone, in particular T4, (c) the local regulation of bioactive TH in target tissues, and (d) the mechanisms by which T3 exerts its biological activities.

Keywords

Thyroid hormone · Thyroxine (T4) · Triiodothyronine (T3) · Thyrotropin-releasing hormone (TRH) · Thyroid-stimulating hormone (TSH) · Hypothalamus · pituitary · Thyroid gland · Serum thyroid hormone-binding proteins · Transporters · Deiodinases · Nuclear receptors

Introduction

Normally, human thyroid follicular cells produce predominantly the prohormone thyroxine (3,3′,5,5′-tetraiodothyronine; T4), which is converted in peripheral tissues to the bioactive hormone 3,3′,5-triiodothyronine (T3). The bioavailability of thyroid hormone (TH) in target tissues largely depends on the supply of plasma T4 and T3, the activity of transporters mediating the cellular uptake and/or efflux of these hormones, as well as the activity of deiodinases catalyzing the activation or inactivation of TH. Thyroid function is under positive control of thyroid-stimulating hormone (TSH), also called thyrotropin. In turn, TSH secretion from the anterior pituitary is stimulated by the hypothalamic factor thyrotropin-releasing hormone (TRH). TSH secretion is downregulated by negative feedback action of TH on the hypothalamus and the pituitary. The contribution of locally produced T3 versus uptake of plasma T3 is much greater for some tissues such as the brain and the pituitary than for other tissues. Although serum TSH is an important parameter for the diagnosis of thyroid dysfunction, it is not representative for the thyroid state of all tissues. In this chapter various aspects will be discussed of (a) the neuroendocrine regulation of thyroid function, (b) the biosynthesis of TH (i.e., the prohormone T4), (c) the local regulation of bioactive TH in target tissues, and (d) the mechanisms by which T3 exerts its biological activities. A schematic overview of the hypothalamus-pituitary-thyroid-periphery axis is presented in Fig. 1.

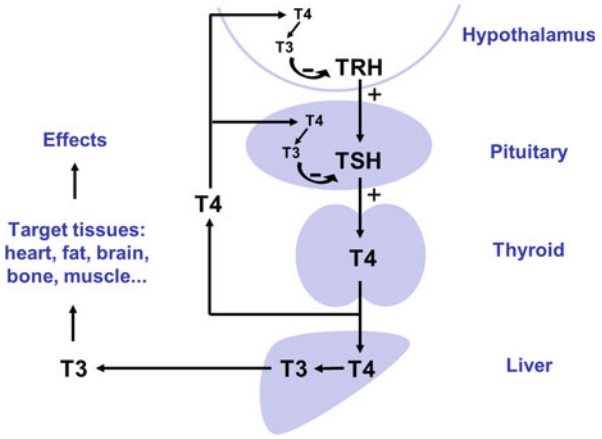

Fig. 1 Schematic of the regulation of the production and metabolism of thyroid hormone in the hypothalamus-pituitary-thyroid-periphery axis, showing the liver as a major T3-producing tissue

Regulation of Thyroid Function

TRH

TRH is a tripeptide, pyroglutamyl-histidyl-prolineamide (pGlu-His-ProNH_2) in which the C-terminal carboxyl group is blocked by amidation and the N-terminal α-amino group is blocked by cyclization (Joseph-Bravo et al. 2015). Besides stimulating TSH secretion, TRH also stimulates prolactin secretion from the anterior pituitary. TRH is not only produced in the hypothalamus but is widely distributed throughout the central nervous system. It is also detected in the posterior pituitary and in different peripheral tissues, such as the heart, adrenal, ovary, testis, uterus, and placenta.

Hypophysiotropic TRH is produced in neurons, the cell bodies of which are located in the paraventricular nucleus (PVN) of the hypothalamus (Fliers et al. 2006). The synthesis of TRH involves the production of a large precursor protein (proTRH). Human proTRH consists of 242 amino acids and contains 6 copies of the TRH progenitor sequence Gln-His-Pro-Gly, flanked at both sides by pairs of basic amino acids (Arg, Lys). Cleavage of proTRH at the basic amino acids by prohormone convertases and further removal of remaining basic residues by carboxypeptidases result in the liberation of the progenitor sequences. A specific glutaminyl cyclase catalyzes the formation of the pGlu ring, and peptidylglycine-α-amidating-monooxygenase (PAM) converts Pro-Gly to ProNH_2, ultimately yielding mature TRH (Perello and Nillni 2007). The processing of proTRH takes place in vesicles which transport mature TRH and intervening peptides along the axons of the TRH neurons to the median eminence (ME), where they are released into the portal vessels of the hypophyseal stalk. Some of the intervening peptides may have biological activities (Perello and Nillni 2007).

TRH is transported through the hypophyseal stalk to the anterior lobe of the pituitary, where it stimulates the production and secretion of TSH (and prolactin). These actions of TRH are initiated by its binding to the TRH receptor (TRHR) which

is expressed on both the thyrotroph (TSH-producing cell) and the lactotroph (prolactin-producing cell) (Sun et al. 2003). This receptor belongs to the family of G protein-coupled receptors, characteristically containing seven transmembrane domains. Human TRHR is a protein consisting of 398 amino acids, and binding of TRH induces a change in its interaction with the trimeric G protein, resulting in the stimulation of phospholipase C (PLC) activity. The activated PLC catalyzes the hydrolysis of phosphatidylinositol 4,5-diphosphate to the second messengers inositol 1,4,5-trisphosphate and diacylglycerol, which initiate a cascade of reactions, including an increase in cellular Ca^{2+} levels and protein kinase C (PKC) activity, that ultimately stimulates the synthesis as well as the release of TSH (and prolactin) (Joseph-Bravo et al. 2016). Stimulation of TSHβ gene expression by TRH depends on the presence of the pituitary-specific transcription factor POU1F1, previously known as Pit-1.

In addition to the anterior pituitary, TRHR is also expressed in different peripheral tissues, including the brain, heart, ovary, uterus, and thyroid. Some of these tissues also express TRH, suggesting that TRH has also actions outside the anterior pituitary. The expression of TRH and TRHR in different brain regions underlies the function of TRH as a neurotransmitter or neuromodulator. Centrally mediated actions of TRH include neurobehavioral, hemodynamic, and gastrointestinal effects. In rats and mice, these central activities are mediated by a separate TRH receptor, Trhr2. Rat and mouse Trhr1 corresponds with human TRHR (Sun et al. 2003).

TRH is rapidly degraded in blood and in different tissues, in particular the brain, pituitary, liver, and lung. Although multiple enzymes are involved, an important role is played by the TRH-degrading ectoenzyme TRHDE, also called pyroglutamyl peptidase II, which catalyzes the cleavage of the pGlu-His bond in TRH (Heuer et al. 1998). This enzyme has been characterized as a zinc-containing metalloproteinase which in humans consists of 1024 amino acids. It has a single transmembrane domain and is inserted in the plasma membrane such that most of the protein is exposed on the cell surface (ectopeptidase). Enzymatic cleavage of the protein close to the cell membrane releases most of the protein in a soluble and enzymatically active form into the circulation. Plasma TRHDE is derived mostly from the liver. In the brain and the pituitary, TRHDE is located in close vicinity of the TRH receptor, where it plays an important role in the local regulation of TRH bioavailability. Interestingly, TRHDE activity in the pituitary and in plasma is increased in hyperthyroidism and decreased in hypothyroidism which may contribute to the negative feedback control of TSH secretion by TH (Heuer et al. 1998).

The synthesis and release of hypothalamic TRH is importantly regulated by negative feedback action of TH (Joseph-Bravo et al. 2015; Mariotti and Beck-Peccoz 2016). Since TH stimulates energy expenditure and thermogenesis, it is not surprising that cold exposure induces hypothalamic TRH secretion with consequent stimulation of the HPT axis. The activity of the HPT axis is also regulated by feeding, with an important role for leptin. Fasting is associated with a central downregulation of the HPT axis, which is prevented in animal studies by administration of leptin (Joseph-Bravo et al. 2015; Mullur et al. 2014).

Bi-allelic inactivating mutations in TRH or TRHR are obvious causes of congenital central hypothyroidism, but no patients have been identified with central

hypothyroidism caused by mutations in TRH, and only three families have been reported with central hypothyroidism caused by mutations in TRHR (Bonomi et al. 2009; Koulouri et al. 2016; Garcia et al. 2017).

TSH

TSH is a glycoprotein produced by the thyrotropic cells of the anterior pituitary. Like the other hypophyseal hormones, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), it is composed of two subunits. The α subunit is identical and the β subunit is homologous among the three hormones (Mariotti and Beck-Peccoz 2016). Although hormone specificity is determined by the β subunit, heterodimerization with the α subunit is required for biological activity. Human TSH consists of 205 amino acids, 92 in the α subunit and 113 in the β subunit. It has a molecular weight of 28 kDa, 20% of which is contributed by three complex carbohydrate groups: two on the α subunit and one on the ß subunit. The structure of these carbohydrate groups is important for the biological activity of TSH and is dependent on the stimulation of the thyrotroph by TRH (Mariotti and Beck-Peccoz 2016).

In addition to the stimulation by TRH and negative feedback by TH, TSH production and secretion is also subject to negative regulation by hypothalamic somatostatin and dopamine and by cortisol. The inhibitory effect of cortisol is exerted at both the hypothalamic and pituitary level (Mariotti and Beck-Peccoz 2016).

TSH binds to a specific TSH receptor (TSHR) located in the plasma membrane of the follicular cell. Like the TRH receptor, this is also a G protein-coupled receptor which, in humans, is a protein consisting of 764 amino acids with an exceptionally long extracellular N-terminal domain (Kleinau et al. 2017). TSHR is preferentially coupled to a $G_s\alpha$ subunit of the trimeric G protein. Binding of TSH to its receptor induces the dissociation of the G protein subunits, resulting in the activation of the membrane-bound adenylate cyclase and, thus, in the stimulation of cAMP formation as second messenger. The increased cAMP levels activate several cellular processes, ultimately resulting in an increased production and secretion of TH (Maenhaut et al. 2015). In particular, the expression of genes coding for key players in TH production (e.g., the iodide transporter, thyroglobulin, and thyroid peroxidase) is increased through mechanisms which also involve different thyroid-specific transcription factors such as TTF1 (NKX2-1), TTF2 (FOXE1), and PAX8. At high TSH concentrations, TSHR also couples to the $G_q\alpha$ subunit, resulting in the activation of the phosphoinositide pathway, which is also involved in the regulation of thyroid function and growth (Maenhaut et al. 2015).

As discussed elsewhere in this volume, hyperthyroidism is often caused by an autoimmune process in which TSHR-stimulating antibodies play an important role. Hyperthyroidism may also be caused by a hyper-functioning adenoma. In most patients with a toxic adenoma, somatic mutations have been identified in TSHR, which result in the constitutive activation of this receptor (Davies et al. 2005). In other patients, somatic mutations have been found in the $G_s\alpha$ subunit which result

in the constitutive activation of the G protein in the absence of TSH. Together, mutations in TSHR and $G_s\alpha$ account for the majority of toxic thyroid adenomas. Also, germline activating TSHR mutations have been identified in patients with congenital, non-autoimmune hyperthyroidism. Conversely, germline inactivating TSHR mutations have been described in patients with TSH resistance (Persani et al. 2010). Often, TSH resistance is partial, and patients are clinically euthyroid as the diminished TSHR function is compensated by increased plasma TSH levels. However, complete absence of functional TSHR causes severe congenital hypothyroidism associated with thyroid hypoplasia (Persani et al. 2010).

Several patients have been reported with congenital central hypothyroidism caused by bi-allelic inactivating mutations in TSHβ (Nicholas et al. 2017). Since TSHR has some basal constitutive activity in the absence of TSH, one would expect that patients who lack functional TSH are only moderately hypothyroid. However, this is refuted by the identification of patients with severe congenital hypothyroidism caused by bi-allelic inactivating TSHβ mutations (Nicholas et al. 2017).

Thyrostimulin

Thyrostimulin was discovered in 2002 and consists of a second α subunit (glycoprotein hormone A2, GPHA2) and a fifth β subunit (GPHB5) which are homologous to the GPHA1 and GPHB1–4 subunits of TSH, LH, FSH, and hCG (Hsu et al. 2002; Nakabayashi et al. 2002). In evolutionary terms, it is the most ancient glycoprotein hormone which is expressed in vertebrates and invertebrates, displaying various biological activities (Karponis and Ananth 2017). Bioactive human thyrostimulin is produced by co-transfection of cells with GPHA2 and GPHB5, and appears to be even more active than TSH in stimulating TSHR, whereas it does not stimulate the LH and FSH receptors (Okada et al. 2006). In mice, overexpression of GPHB5 induces hyperthyroidism, but deletion of GPHB5 has little effect on serum TH levels (Okada et al. 2006). In humans, GPHA2 and GPHB5 are expressed in various tissues, where GPHA2 mRNA levels are usually much higher than GPHB5 mRNA levels, and by far the highest GPHA2 expression is observed in the pancreas. In the human anterior pituitary, GHPA2 and GHPB5 are co-expressed in corticotrophs (Okada et al. 2006). In mice, thyrostimulin may have a biological function in bone development, but the physiological role of thyrostimulin in humans remains to be established.

Biosynthesis of Thyroid Hormone

The functional unit of the thyroid gland is the follicle, composed of a single layer of epithelial cells surrounding a colloidal lumen in which TH is produced and stored. This section is a brief overview of the steps involved in the production and secretion of TH, schematically presented in Fig. 2. An extensive overview has been published recently (Carvalho and Dupuy 2017).

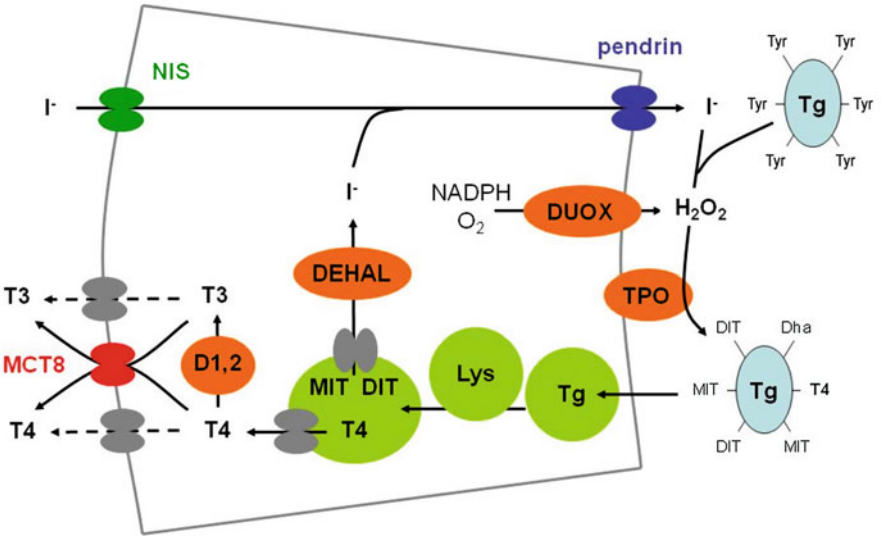

Fig. 2 Schematic of a thyroid follicular cell and important steps in the synthesis of thyroid hormone

Iodide Uptake

Iodine is an essential trace element required for the synthesis of TH. It is not surprising, therefore, that the basolateral membrane of the follicular cell contains an active transporter that mediates uptake of I^- together with Na^+. The human Na/I symporter (NIS, SLC5A5) has been characterized as a protein consisting of 618 amino acids and 13 transmembrane domains (Ravera et al. 2017; Carvalho and Dupuy 2017; Targovnik et al. 2017). NIS transports I^- and Na^+ in a stoichiometry of 1:2, indicating that I^- transport is electrogenic and driven by the Na^+ gradient across the plasma membrane. TSH stimulates the expression of the *NIS* gene to such an extent that the intracellular iodide concentration may be up to 500-fold higher than its extracellular level. The activity of NIS also strictly requires its interaction with the KCNQ1-KCNE2 K^+ channel in thyroid cells, but the exact mechanism of this interaction is unknown (Ravera et al. 2017). Various NIS mutations have been identified in patients with congenital hypothyroidism (Targovnik et al. 2017; Ravera et al. 2017; Carvalho and Dupuy 2017).

NIS is not completely specific for iodide but also binds other anions, some of which are even transported. An important example is perchlorate (ClO_4^-) which potently inhibits iodide uptake by NIS, an effect utilized in the perchlorate discharge test used for the diagnosis of an organification defect, i.e., impaired incorporation of iodine in Tg (Targovnik et al. 2017). Perchlorate inhibits the uptake but not the release of iodide from the thyroid. Therefore, if perchlorate is administered after a dose of radioactive iodide, it will provoke a marked release of radioactivity from the thyroid in case of an organification defect but not from a normal thyroid gland. Importantly, perchlorate is also an environmental pollutant with potential thyroid disrupting activity (Leung et al. 2014).

Pertechnetate (TcO_4^-) is another anion transported by NIS, and this observation is utilized in the scanning of the thyroid gland using radioactive $^{99m}TcO_4^-$. Of course, the latter is not incorporated into thyroglobulin and, thus, cannot be used to test the hormone production capacity of the thyroid.

It is not sufficient that iodide is transported across the basolateral plasma membrane of thyroid cells. Since TH production takes place at the luminal surface of the apical membrane, iodide also has to pass this membrane, and this involves at least two proteins: pendrin (PDS, SLC26A4) and anoctamin (ANO1, TMEM16A) (Silveira and Kopp 2015). Pendrin is the protein mutated in patients with Pendred's syndrome (Wemeau and Kopp 2017), which comprises deafness due to a cochlear defect and hypothyroidism due to an organification defect (positive perchlorate discharge test). Pendrin functions as a chloride-iodide exchanger in the thyroid and as a bicarbonate-chloride exchanger in the cochlea (Silveira and Kopp 2015). ANO1 is a Ca^{2+}-activated chloride (iodide) channel. Efflux of iodide from thyroid cells is acutely stimulated by TSH, which may involve recruitment and/or activation of these apical iodide exporters.

Thyroid Hormone Synthesis

Thyroglobulin (Tg) is an exceptionally large glycoprotein consisting of two identical subunits. Each mature subunit in human Tg contains 2748 amino acids and has a molecular weight of ~330 kDa (Targovnik et al. 2017; Carvalho and Dupuy 2017). The *Tg* gene is located on human chromosome 8q24.2-q24.3; it covers about 300 kb of genomic DNA and consists of 48 exons. Many Tg mutations have been identified in patients with congenital hypothyroidism (Targovnik et al. 2017).

DUOX2 is a large and complex glycoprotein embedded in the apical membrane of the thyrocyte (Muzza and Fugazzola 2017; Carvalho and Dupuy 2017). Mature human DUOX2 contains 1527 amino acids and has 7 transmembrane domains, an NADPH-binding domain, an FAD-binding domain, a heme-binding domain, 2 calcium-binding EF hands, and a peroxidase domain. It catalyzes the oxidation of NADPH from the cytoplasm and delivers its product H_2O_2 to the luminal surface of the membrane. The heme group is the site of H_2O_2 generation, and its location within transmembrane domains fits with the vectorial (enzyme/transport) function of DUOX2. Functional expression of DUOX2 requires the presence of the maturation factor DUOXA2, a protein consisting of 320 amino acids and 5 putative transmembrane domains (Carvalho and Dupuy 2017; Muzza and Fugazzola 2017). The *DUOX2* and *DUOXA2* genes are clustered together with the homologous *DUOX1* and *DUOXA1* genes on human chromosome 15q15. A large number of DUOX2 mutations and a small number of mutations in DUOXA2 have been reported in patients with congenital hypothyroidism. DUOX1 and DUOXA1 are expressed at lower levels in the thyroid and do not (fully) compensate for the loss of function mutations in DUOX2 and DUOXA2 (Muzza and Fugazzola 2017).

Mature TPO is a glycoprotein consisting of 919 amino acids and featuring a single transmembrane domain. A short C-terminal domain is located in the cytoplasm, but most of the protein is exposed on the luminal surface of the apical membrane which

also contains a heme-binding domain, the active center of the enzyme (Targovnik et al. 2017; Carvalho and Dupuy 2017). Functional TPO exists as a homodimer where the subunits are linked through a disulfide bond. The human *TPO* gene covers about 150 kb on chromosome 2p25, distributed over 17 exons. In addition to full-length TPO (TPO1), other isoforms are generated by alternative splicing (TPO2–5), some of which retain enzyme activity. Over 100 TPO mutations have been identified in patients with congenital hypothyroidism (Targovnik et al. 2017).

Formation of Iodothyronines

TH synthesis takes place at the luminal surface of the apical membrane within the scaffold of the Tg molecule and occurs in two steps which are both catalyzed by TPO: (1) the iodination of Tyr residues and (2) the coupling of iodotyrosines with formation of iodothyronines (Carvalho and Dupuy 2017; Taurog et al. 1996). The prosthetic heme group of TPO undergoes a two-electron oxidation by H_2O_2 (supplied by DUOX2) to generate "compound I." Compound I may perform a one-electron oxidation reaction, by which it is converted to compound II, or a two-electron oxidation by which native TPO is regenerated. TPO catalyzes the two-electron oxidation of I^- to I^+ by H_2O_2 with subsequent electrophilic substitution of Tyr residues in Tg, producing 3-iodotyrosine (monoiodotyrosine, MIT). Substitution of MIT residues with a second iodine produces 3,5-diiodotyrosine (DIT).

Coupling of two suitably positioned iodotyrosines results in the formation of an iodothyronine residue at the site of the acceptor iodotyrosine and a dehydroalanine residue at the site of the donor iodotyrosine. This involves the one-electron oxidation of each donor and acceptor iodotyrosine residue, generating radicals that rapidly combine to produce an iodothyronine residue. T4 is generated by the reaction of two DIT residues, and T3 is generated by reaction of an acceptor DIT with a donor MIT residue. MIT does not seem to function as an acceptor residue, since thyroidal production of rT3 (and 3,3′-T2) is negligible (Carvalho and Dupuy 2017).

Although Tyr is the building block of TH, the Tyr content of Tg is not greater than that of most other proteins. Of the 67 Tyr residues per Tg subunit, ≈20–25 are available for iodination, but the capacity for iodothyronine formation is limited. Each Tg subunit has only four hormonogenic acceptor Tyr residues that can ultimately be transformed into iodothyronines, localized at positions 5, 1291, 2554, and 2747 of mature Tg (Carvalho and Dupuy 2017; Targovnik et al. 2017). However, at normal levels of iodination, the average yield is 1–1.5 molecule of T4 and ≈0.1 molecule of T3 per Tg subunit. They are stored within the Tg scaffold in the follicular lumen until their secretion is required.

Release of Thyroid Hormone

In response to TSH stimulation, Tg is resorbed from the lumen by both macro- and micropinocytosis (Carvalho and Dupuy 2017; Botta et al. 2017). Macropinocytosis or

fluid endocytosis involves the formation of large pseudopodia which engulf Tg-containing colloid, resulting in the formation of large cytoplasmic vesicles (colloid droplets). Micropinocytosis concerns the receptor-mediated endocytosis of Tg, which may involve different receptor proteins such as megalin, a very large (≈600 kDa) cargo protein located in the apical membrane of different cell types, including thyrocytes. It is believed that Tg-containing vesicles generated by receptor-mediated endocytosis largely undergo transcytosis or recycling, whereas vesicles generated by fluid endocytosis fuse with lysosomes, generating so-called phagolysosomes (Botta et al. 2017). In these vesicles, Tg is hydrolyzed by lysosomal proteases, cathepsins (Friedrichs et al. 2003), resulting in the liberation of T4, a small amount of T3, as well as excess MIT and DIT molecules. The iodotyrosines are exported from the phagolysomes by a so-called system h amino acid transporter, which may very well represent the L-type amino acid transporter LAT1 and/or LAT2 (Andersson et al. 1990; Zevenbergen et al. 2015; Krause and Hinz 2017). This provides the access of MIT and DIT to iodotyrosine dehalogenase (DEHAL1 or IYD) located in the endoplasmic reticulum which catalyzes their deiodination by NADH (Gnidehou et al. 2004; Moreno et al. 2008). The iodide is reutilized for iodination of Tg.

Human IYD is a homodimer of a 289-amino acid protein containing an N-terminal membrane anchor and a conserved nitroreductase domain with an FMN-binding site (Gnidehou et al. 2004; Moreno et al. 2008). The *IYD* gene is located on chromosome 6q24-q25 and consists of five exons. Since IYD lacks an NADH-binding sequence, iodotyrosine deiodinase activity requires the involvement of a reductase, which has not yet been identified. Low levels of IYD are also expressed in the liver and kidney.

Little is yet known about the exact mechanism of T4 (and T3) secretion. Possibly, this also involves their release form the phagolysosomes through the system h (L-type) transporter(s). Subsequently, T4 and T3 are secreted via transporters located in the basolateral membrane, and recent evidence suggests an important role for the TH transporter MCT8 herein. Some T4 is converted before secretion to T3 by iodothyronine deiodinases present in the thyrocyte (see below).

In an average human subject, T4 and T3 are secreted in a ratio of about 15:1, i.e., about 100 μg (130 nmol) T4 and 6 μg (9 nmol) T3 per day. The latter represents ≈20% of daily total T3 production. Hence, most T3 is produced by deiodination of T4 in peripheral tissues (Bianco et al. 2002).

Inhibitors of Thyroid Hormone Production and/or Secretion

Administration of a large amount of iodide usually results in an acute but transient decrease in TH secretion (Maenhaut et al. 2015). The mechanism of this inhibition of TH secretion by excess iodide is not understood. Excess iodide also results in the inhibition of TH synthesis, a phenomenon known as the Wolff-Chaikoff effect (Maenhaut et al. 2015). The mechanism appears to involve, among other things, the formation of an iodinated lipid (iodolactone) that inhibits several steps in TH synthesis. This includes the inhibition of NIS, resulting in a decrease in the intracellular iodide concentration and, thus, a decrease in iodolactone formation,

relieving the inhibited hormone synthesis. This escape from the Wolff-Chaikoff effect occurs despite the continued administration of excess iodide.

Thiourea derivatives have been known since the pioneering work of Astwood in the 1940s as potent inhibitors of TH synthesis (Astwood 1984). Two of these, methimazole (MMI) and 6-propyl-2-thiouracil (PTU), are widely used in the medical treatment of patients with hyperthyroidism. Their antithyroid activity is based on the potent inhibition of TPO, the mechanism of which depends on the available iodide concentration (Taurog 2000). In the presence of iodide, the thiourea inhibitors compete with the Tyr residues in Tg for the TPO-I^+ iodination complex, preventing the formation of TH.

MMI is a more potent inhibitor of TPO than PTU (Taurog 2000), and lower doses of MMI (or the prodrug carbimazole) are required for the treatment of hyperthyroidism compared with PTU. Besides inhibiting TH synthesis by TPO, PTU also inhibits conversion of T4 to T3 by the type 1 iodothyronine deiodinase (D1) located not only in the thyroid but also in the liver and kidney (see below). In contrast, MMI does not affect D1 activity.

Transport of Thyroid Hormone

Plasma Transport

In plasma, TH is bound to three proteins, thyroxine-binding globulin (TBG), transthyretin (TTR), and albumin (Benvenga 2012; Refetoff 2015a, b). Human TBG is a 54 kDa glycoprotein produced in the liver, consisting of 395 amino acids and 4 carbohydrate residues. The *TBG (SERPINA7)* gene is located on human chromosome Xq22.3, spans ≈5.5 kb, and contains five exons. Of all plasma TH transport proteins, TBG has the highest affinity for T4 (K_d ≈0.1 nM) but also the lowest serum concentration (≈15 mg/l) (Benvenga 2012; Refetoff 2015a, b).

TTR is a non-glycosylated protein composed of 4 identical subunits, consisting each of 127 amino acids. The *TTR* gene is located on human chromosome 18q12.1, covers ≈7 kb, and contains four exons (Richardson 2007; Benvenga 2012; Refetoff 2015a, b). TTR has a cigar-shaped structure with two identical binding channels, each formed by two symmetrically positioned subunits, with ligand entry sites at opposite ends of the TTR molecule. TTR binds the first T4 molecule with a higher affinity (K_d ≈10 nM) than the second T4 molecule, and its plasma concentration amounts to ≈250 mg/l. Plasma TTR is produced in the liver, but the protein is also expressed in the choroid plexus and the placenta, where it may be involved in plasma-cerebrospinal fluid and maternal-fetal T4 transfer, respectively. TTR also binds retinol-binding protein and thus also plays an important role in vitamin A transport (Richardson 2007).

Albumin has multiple low-affinity binding sites for TH, with K_d values for T4 of 1–10 μM, but it has by far the highest plasma concentration (≈40 g/l) (Benvenga 2012; Refetoff 2015a, b). Iodothyronines also bind to lipoproteins, in particular high-density lipoprotein (HDL). Although the proportion of plasma T4 and T3 bound to lipoproteins is low compared with the other plasma transport proteins, it may be important to target TH specifically to lipoprotein receptor-expressing tissues (Benvenga 2012).

The consequence of the different concentrations and affinities of the TH-binding proteins is that in healthy humans ≈75% of plasma T4 is bound to TBG, ≈15% to albumin, and ≈10% to TTR (Benvenga 2012; Refetoff 2015a, b). The total binding capacity of these proteins is so high that only ≈0.02% of plasma T4 is free (nonprotein-bound). The affinity of T3 for the different proteins is one-tenth of that of T4. Therefore, the free T3 fraction in plasma amounts to ≈0.2%. Although mean normal plasma total T4 (≈100 nmol/l) and T3 (≈2 nmol/l) levels differ about 50-fold, the difference in mean normal FT4 (≈20 pmol/l) and FT3 (≈5 pmol/l) levels is only about fourfold. rT3 binds with intermediate affinity to the plasma proteins (Benvenga 2012; Refetoff 2015a, b).

Since the plasma FT4 and FT3 concentrations determine the tissue availability of TH, they are more important indices of thyroid state than total plasma T4 and T3 levels. Both concentration and TH-binding affinity of the different plasma proteins are influenced by a variety of (patho)physiological factors (Benvenga 2012). Since it binds most TH in plasma, variations in TBG concentration are more important than variations in TTR or albumin concentrations. Inherited TBG excess is a rare phenomenon caused by *TBG* gene duplication. Inherited TBG deficiency is caused by mutations in the *TBG* gene, resulting in a decreased T4 affinity or protein stability. More severe *TBG* gene defects result in a complete lack of serum TBG in affected males (Refetoff 2015a, b). Plasma TBG levels are also influenced by various endogenous and exogenous factors. Notably, plasma TBG levels are increased by estrogens, whereas they are decreased by androgens. In addition, different endogenous factors, such as free fatty acids, and drugs, such as salicylates, competitively inhibit T4 binding to TBG (Benvenga 2012; Refetoff 2015a, b).

A large number of mutations have also been identified in the TTR gene, some of which cause a decrease in T4 binding affinity, whereas others (e.g., Ala109Thr, Thr119Met) result in an increased T4 affinity (Saraiva 2001). More importantly, TTR mutations often cause familial amyloidotic polyneuropathy (FAP), involving the deposition of insoluble TTR fibrils in nerves or the heart (Saraiva 2001).

Finally, also binding of TH to albumin is subject to genetic variation. Especially, a specific increase in the binding of T4 to albumin occurs in some otherwise healthy subjects, which often leads to the false diagnosis of hyperthyroidism if inadequate methods for analysis of plasma FT4 are used (Benvenga 2012; Refetoff 2015a, b). This "familial dysalbuminemic hyperthyroxinemia" (FDH) is caused by polymorphisms in the albumin gene, in particular p.R218H, resulting in a marked increase in T4 affinity.

Perturbation of plasma iodothyronine binding provokes an adaptation of the hypothalamus-pituitary-thyroid axis to maintain normal FT4 and FT3 concentrations. Therefore, measurement of plasma TSH and FT4 rather than total T4 levels is the cornerstone of the diagnosis of thyroid disorders.

Tissue Transport

The biological activity of TH is importantly regulated at the level of the target tissues, involving plasma membrane transporters which facilitate the cellular uptake and/or efflux of iodothyronines as well as deiodinases which catalyze the activation and/or inactivation of the hormone (Fig. 3).

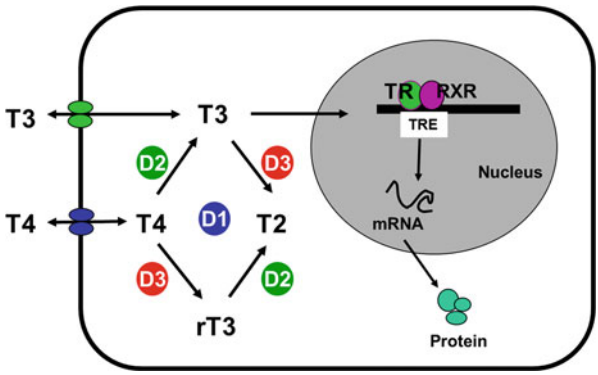

Fig. 3 Pathways of thyroid hormone metabolism

Despite decades of research and evidence published by different laboratories suggesting the involvement of carrier-mediated mechanisms, it was believed for a long time that TH crosses the plasma membrane by passive diffusion. A detailed review of these early studies of cellular TH transport was published in 2001 (Hennemann et al. 2001). Since then several transporters from different protein families were identified which are capable of transporting iodothyronines. This includes several members of the organic anion transporting polypeptide (OATP) family (Abe et al. 2002; Hagenbuch 2007), the Na-taurocholate cotransporting polypeptide (NTCP) (Friesema et al. 1999), the L-type amino acid transporters LAT1 and LAT2 (Taylor and Ritchie 2007; Zevenbergen et al. 2015; Krause and Hinz 2017), and the monocarboxylate transporters MCT8 (Friesema et al. 2003, 2006) and MCT10 (Friesema et al. 2008). Detailed reviews on TH transporters have been published recently (Visser et al. 2011; Schweizer et al. 2014; Bernal et al. 2015).

Of the above transporters, only NTCP (SLC10A1) transports its substrates in an Na^+-dependent manner (Friesema et al. 1999; Anwer and Stieger 2014). It is exclusively expressed in the liver and plays an important role in hepatic uptake of (conjugated) bile acids (Slijepcevic et al. 2017). Human NTCP is a 349-amino acid glycoprotein containing nine transmembrane domains. The *SLC10A1* gene comprises five exons and is located on chromosome 14q24.1. The SLC10 family contains seven members, but only NTCP is capable of transporting (sulfated) iodothyronines (Visser et al. 2011). NTCP may be important for the liver targeting of TH analogues such as eprotirome (Kersseboom et al. 2017). Interestingly, NTCP also plays an important role as a receptor for the entry of hepatitis B and D virus in liver cells (Li and Urban 2016).

The human OATP family consists of 11 members, most of which have been shown to transport iodothyronine derivatives (Hagenbuch 2007; Stieger and Hagenbuch 2014; van der Deure et al. 2010). In general they are multi-specific, transporting a variety of ligands, not only anionic but also neutral and even cationic compounds. OATPs are glycoproteins containing ~700 amino acids and 12 transmembrane domains. The human OATP1 subfamily contains four members (OATP1A2, 1B1, 1B3, 1C1) with interesting properties. They are encoded by genes clustering on chromosome12p12, each containing 14–15 exons. OATP1B1 and 1B3 are expressed only in the liver and show preferential transport of sulfated over non-sulfated iodothyronines. OATP1A2 also effectively transports non-sulfated

T4 and T3 and is expressed in different tissues, including the liver, kidney, intestine, and brain. OATP1C1 is the most interesting transporter in this subfamily, showing a high preference for T4 as the ligand and almost exclusive expression in the brain, in particular in astrocytes and the choroid plexus (blood-CSF barrier) (Pizzagalli et al. 2002; Bernal et al. 2015). In astrocytes, OATP1C1 is crucial for the conversion of T4 to T3 by the type 2 deiodinase expressed in these cells (see below).

T4 and T3 are also transported by two members of the heterodimeric amino acid transporters, LAT1 and LAT2 (Taylor and Ritchie 2007; Zevenbergen et al. 2015; Krause and Hinz 2017). These transporters are glycoproteins consisting of two subunits: a heavy chain and a light chain. In humans, there are two possible heavy chains (SLC3A1, SLC3A2) and 13 possible light chains (SLC7A1–11, SLC7A13, SLC7A14). The heavy chains contain a single transmembrane domain, and the light chains contain 12–14 transmembrane domains. LAT1 is composed of the SLC3A2–SLC7A5 and LAT2 of the SLC3A2–SLC7A8 subunits. These transporters are expressed in various tissues, and the expression of LAT1 is particularly stimulated in activated immune and cancer cells. Both LAT1 and LAT2 are obligate exchangers, facilitating the bidirectional transport of a variety of aliphatic and aromatic amino acids as well as iodothyronines across the plasma membrane (Taylor and Ritchie 2007).

Two important TH transporters belong to the monocarboxylate transporter (MCT) family, named such since the first four transporters characterized in this family (MCT1–4) were found to transport monocarboxylates such as lactate and pyruvate (Halestrap 2013). The MCT (SLC16) family contains 14 members; in addition to MCT1–4, MCT7 (Hugo et al. 2012) and MCT11 (Rusu et al. 2017) have also been identified as monocarboxylate transporters, while carnitine is the physiological substrate for MCT9 (Kolz et al. 2009) and creatine for MCT12 (Abplanalp et al. 2013). MCT10 facilitates the transport of aromatic amino acids and iodothyronines (Kim et al. 2002; Friesema et al. 2008), while MCT8 (SLC16A2) only transports iodothyronines (Friesema et al. 2003, 2006).

The prevalent form of human MCT8 consists of 539 amino acids and MCT10 consists of 515 amino acids. Like the other MCTs, they contain 12 transmembrane domains. However, unlike most other MCTs, they are not glycosylated, and they also do not require ancillary proteins for functional expression. MCT8 and MCT10 are highly homologous proteins, in particular in their transmembrane domains, explaining their similar substrate specificities. They have identical gene structures; the *MCT8* gene is located on human chromosome Xq13.2, and the *MCT10* gene is located on chromosome 6q21-q22. Both consist of six exons and five introns, with a large ~100 kb first intron. MCT8 and MCT10 show wide but different tissue distributions.

MCT8 and MCT10 are the most active and specific TH transporters known to date (Friesema et al. 2006, 2008). MCT8 is importantly expressed in the brain, where it is localized in the endothelial cells of the blood-brain barrier, in the choroid plexus, and in neurons in different brain regions. Males with hemizygous MCT8 mutations suffer from severe psychomotor retardation, known as the Allan-Herndon-Dudley syndrome (AHDS), associated with low (F)T4 and elevated (F)T3 levels (Heuer and Visser 2009; Visser et al. 2007, 2008). As TH is crucial for brain development, AHDS is thought to be caused by impaired transport of T4 (and T3) into the brain

during important stages of development. However, MCT8 is also expressed in the thyroid and in peripheral tissues. In the thyroid, MCT8 is involved in TH secretion, and inactivation of MCT8 results in an increased residence time of T4 in the thyroid, with a consequent increase in local conversion of T4 to T3. There is also evidence for increased T4 to T3 conversion in the liver and kidney.

Metabolism of Thyroid Hormone

Deiodination

The thyroid gland of a healthy human subject normally produces predominantly the prohormone T4 and only a small amount of the bioactive hormone T3. It is generally accepted that in humans ≈80% of circulating T3 is produced by enzymatic outer ring deiodination (ORD) of T4 in peripheral tissues (Peeters and Visser 2017; Gereben et al. 2008; van der Spek et al. 2017). Alternatively, inner ring deiodination (IRD) of T4 produces the inactive metabolite rT3, thyroidal secretion of which is negligible (Figs. 3 and 4). Deiodination is also an important pathway by which T3 and rT3 are further metabolized. T3 largely undergoes IRD to the inactive compound 3,3′-T2, which is also produced by ORD of rT3. Thus, the bioactivity of TH is determined to an important extent by the enzyme activities responsible for the ORD (activation) or IRD (inactivation) of iodothyronines.

Three iodothyronine deiodinases (D1–3) are involved in the reductive deiodination of TH (Figs. 3 and 4) (Gereben et al. 2008; Peeters and Visser 2017; van der Spek et al. 2017). They are homologous proteins consisting of ~250–300 amino acids, with a single transmembrane domain located at the N-terminus. The

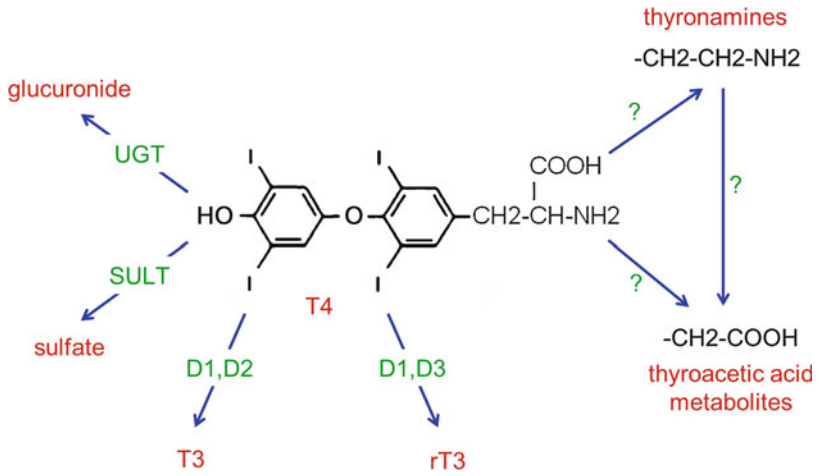

Fig. 4 Schematic of the regulation of the nuclear availability of thyroid hormone in a target cell by transporters and deiodinases

deiodinases are inserted in cellular membranes such that the major part of the protein is exposed to the cytoplasm. This is consistent with the reductive nature of the cytoplasmic compartment required for the deiodination process. However, some controversy exists regarding the topography of D3 as some studies suggest that its active site is exposed on the cell surface (Wajner et al. 2011). The different deiodinases require thiols as cofactor. Although reduced glutathione (GSH) is the most abundant intracellular thiol, its activity is very low compared with the unnatural thiol dithiothreitol (DTT) which is often used in in vitro studies. Alternative endogenous cofactors include dihydrolipoamide, glutaredoxin, and thioredoxin. Probably all three deiodinases are functionally expressed as homodimers (Gereben et al. 2008).

The most remarkable feature of all three deiodinases is the presence of a selenocysteine (Sec) residue in the center of the amino acid sequence. As in other selenoproteins, this Sec residue is encoded by a UGA triplet which in mRNAs for non-selenoproteins functions as a translation stop codon. The translation of the UGA codon into Sec requires the presence of a particular stem-loop structure in the 3′-untranslated region of the mRNA, termed Sec insertion sequence (SECIS) element, Sec-tRNA, and a number of cellular proteins, including SECIS-binding protein (SBP2). A SECIS element has been identified in the mRNA of all deiodinases (Gereben et al. 2008).

The *DIO1* gene coding for human D1 is located on chromosome 1p32.3 and consists of four exons. D1 is expressed predominantly in the liver, kidneys, and thyroid (Gereben et al. 2008; van der Spek et al. 2017; Peeters and Visser 2017). It catalyzes the ORD and/or IRD of a variety of iodothyronine derivatives with a preference for rT3 and iodothyronine sulfates. In the presence of the artificial cofactor DTT, D1 displays high K_m and V_{max} values. D1 is rapidly inactivated by iodoacetate and gold thioglucose due to reaction with the reactive Sec residue. D1 activity is also potently and uncompetitively inhibited by PTU. Together with the ping-pong-type cofactor-dependent enzyme kinetics, these findings suggest that the catalytic mechanism of D1 involves the transfer of an I^+ ion from the substrate to the selenolate (Se^-) group of the enzyme, generating a selenenyl iodide (SeI) intermediate which is reduced back to native enzyme by thiols or converted into a dead-end complex by PTU.

Hepatic and renal D1 contribute importantly to the production of plasma T3 and the clearance of plasma rT3. D1 activity in the liver and kidney is increased in hyperthyroidism and decreased in hypothyroidism, representing the regulation of D1 activity by T3 at the transcriptional level (Zhang et al. 1998).

The *DIO2* gene coding for human D2 is located on chromosome 14q31.1 and consists of two exons. D2 is expressed primarily in the brain, anterior pituitary, brown adipose tissue, and thyroid and to some extent also in skeletal muscle (Gereben et al. 2008; Larsen 2009). In the brain, D2 mRNA has been localized in astrocytes and in particular also in tanycytes lining the third ventricle in the hypothalamic region (Werneck de Castro et al. 2015). D2 is a low-K_m, low-capacity enzyme possessing only ORD activity, with a preference for T4 over rT3 as the substrate (van der Spek et al. 2017; Peeters and Visser 2017; Gereben et al. 2008). The amount of T3 in the brain, pituitary, and brown adipose tissue is derived to a large extent from local conversion of T4 by D2 and to a minor extent from plasma

T3. The enzyme located in the anterior pituitary and the hypothalamus is very important for the negative feedback regulation of TSH and TRH secretion by T4.

In general, D2 activity is increased in hypothyroidism and decreased in hyperthyroidism. This is explained in part by substrate-induced inactivation of the enzyme by T4 and rT3 involving the ubiquitin-proteasome system (Gereben et al. 2008). However, inhibition of D2 mRNA levels by T3 has also been demonstrated in the brain and pituitary. The substrate (T4, rT3) and product (T3)-dependent down-regulation of D2 activity is important to maintain brain T3 levels in the face of changing plasma TH levels. However, D2 in the hypothalamus is largely protected from substrate-induced enzyme inactivation, allowing proper negative feedback regulation of the HPT axis at the hypothalamic level (Werneck de Castro et al. 2015).

In mammals, D2 mRNA contains a second UGA codon just upstream of a UAA stop codon (Gereben et al. 2008). It is unknown to what extent this second UGA codon specifies the incorporation of a second Sec residue or acts as a translation stop codon. The amino acid sequence downstream of this second Sec is not required for enzyme activity (Salvatore et al. 1999).

The *DIO3* gene coding for human D3 is located on chromosome 14q32.31 and consists of a single exon. D3 activity has been detected in different human tissues, i.e., brain, skin, liver, and intestine, where activities are much higher in the fetal than in the adult stage (Gereben et al. 2008). D3 is also abundantly expressed in the placenta and the pregnant uterus. D3 has only IRD activity, catalyzing the inactivation of T4 and T3 with intermediate K_m and V_{max} values. D3 in tissues such as the brain is thought to play a role in the regulation of intracellular T3 levels, while its presence in placenta, pregnant uterus, and fetal tissues may serve to protect developing organs against undue exposure to active TH. Indeed, fetal plasma contains low T3 (and high rT3) concentrations. However, local D2-mediated T3 production from T4 is crucial for brain development. Also in adult subjects, D3 appears to be an important site for clearance of plasma T3 and production of plasma rT3. In the brain, but not in placenta, D3 activity is increased in hyperthyroidism and decreased in hypothyroidism, which at least in the brain is associated with parallel changes in D3 mRNA levels (Gereben et al. 2008).

Since D1–3 are selenoproteins, selenium deficiency would be expected to result in reduced D1–3 activities in different tissues, but this is only observed for D1 in the liver and kidney and not for D2 or D3 activities in other tissues (Kohrle 2005). This may be explained by findings that the selenium state of different tissues varies greatly in Se-deficient animals. In addition, the efficiency of the SECIS element to facilitate read-through of the UGA codon may differ among selenoproteins, which could result in the preferred incorporation of Sec into D2 or D3 over other selenoproteins.

Substitution of Sec by Cys in the three deiodinases results in a large reduction of enzyme activity, and replacement with Leu or Ala completely inactivates the enzymes, indicating that Sec is indeed the catalytic center of the deiodinases (Gereben et al. 2008; Peeters and Visser 2017). However, the catalytic mechanisms appear to differ between the deiodinases. In contrast to the ping-pong-type enzyme kinetics of D1, both D2 and D3 show sequential-type enzyme kinetics. D2 and D3

are also much less sensitive to inhibition by iodoacetate, gold thioglucose, and in particular, PTU (Gereben et al. 2008). Interestingly, the amino acid two positions downstream of the catalytic Sec residue (Ser in D1, Pro in D2 and D3) plays an important role in determining the reactivity of the catalytic Sec residue and its sensitivity for these inhibitors (Gereben et al. 2008; Peeters and Visser 2017).

Patients with mutations in any one of the deiodinases have not been reported so far. However, patients have been identified with high serum T4, low T3, and mostly elevated TSH levels and various other symptoms, associated with mutations in SBP2 (Dumitrescu et al. 2005; Schoenmakers et al. 2010). The changes in serum T4 and T3 levels suggest that impaired SBP2 function has a greater impact on tissue ORD than on tissue IRD activity, although it is not clear which deiodinase is most affected by SBP2 deficiency. The relatively high serum TSH despite elevated T4 levels suggest that at least D2 activity is diminished in these patients. Patients with SBP2 mutations suffer from a multisystem disorder, reflecting the impaired synthesis of various other important selenoproteins. In total 25 selenoproteins are encoded by the human genome, many of which play an important role in tissue antioxidant defense. Recently, another patient has been reported with a mutation in the Sec-tRNA, showing a similar phenotype as patients with SBP2 mutations (Schoenmakers et al. 2016).

Alanine Side Chain Modification

Triiodothyroacetic acid (Triac, TA3) and tetraiodothyroacetic acid (Tetrac, TA4) are metabolites with interesting biological properties (Groeneweg et al. 2017; Davis et al. 2016). TA3 shows equally high affinity for the nuclear T3 receptors (and in the invertebrate amphioxus even much higher affinity (Holzer et al. 2017)) as T3 itself, and TA4 has anti-tumor activity by blocking αvβ3 integrin. TA3 and TA4 are produced by metabolism of the alanine side chain of T3 and T4, respectively, although TA3 is also generated by enzymatic ORD of TA4. These metabolites have been identified in early studies after administration of ^{125}I-labeled T4 or T3 to human subjects or experimental animals, as well as in vitro after incubation with tissue preparations, in particular kidney (Groeneweg et al. 2017). However, exactly how iodothyronines are converted to the acetic acid metabolites has not been settled, although two possible pathways have been suggested (Fig. 4).

The first pathway implies the decarboxylation of iodothyronines to the corresponding iodothyronamines with subsequent conversion by monoamine oxidase-like enzymes to the iodothyroacetic acid derivatives. In consideration of the iodothyronine structure, it is logical to hypothesize that the first reaction is catalyzed by aromatic amino acid decarboxylase (AADC) also known as DOPA decarboxylase (DDC). However, studies utilizing recombinant AADC have failed to observe decarboxylation of any iodothyronine (Hoefig et al. 2012). A subsequent study indicated relatively slow conversion of 3,5-T2 to 3,5-diiodothyronamine (3,5-T2AM) by ornithine decarboxylase (ODC) but decarboxylation of other iodothyronines is negligible (Hoefig et al. 2015). 3-Iodothyronamine (3T1AM) has received much attention recently as it exerts highly interesting pharmacological

effects, including bradycardia and hypothermia (Scanlan et al. 2004; Hoefig et al. 2016). The physiological relevance of the iodothyronamines, however, remains to be established. Although iodothyronamines are converted to the acetic acid metabolites by monoamine oxidase-like enzyme(s) (Wood et al. 2009), the relevance of this pathway for the conversion of T4 to TA4 and of T3 to TA3 is uncertain as there is no evidence that T4 and T3 actually undergo decarboxylation.

The alternative route for production of TA4 and TA3 involves a number of steps, the first of which concerns the conversion of T4 and T3 to their pyruvate metabolites, TK4 and TK3, respectively. This reaction has been documented using tyrosine amino transferase (TAT) in the presence of α-ketoglutarate as the co-substrate and pyridoxal-5′-phosphate as the cofactor (Nakano 1967). It has been suggested early on that further conversion of the pyruvate metabolites TK4 and TK3 to the acetic acid metabolites TA4 and TA3 may proceed via other intermediates with lactate and/or acetaldehyde side chains (Wilkinson 1957), but this remains to be established.

Sulfation

Iodothyronines also undergo conjugation of the phenolic hydroxyl group with sulfate or glucuronic acid (Fig. 4). Sulfation and glucuronidation increase the water solubility of substrates, facilitating their biliary and/or urinary clearance. However, iodothyronine sulfate levels are normally very low in plasma, bile, and urine, as these conjugates are rapidly degraded by D1, suggesting that sulfate conjugation is a primary step leading to the irreversible inactivation of TH (Kester and Visser 2005; Wu et al. 2005). Plasma levels (and biliary excretion) of iodothyronine sulfates are increased if D1 activity is inhibited by drugs such as PTU, and during fetal development, non-thyroidal illness and fasting. Under these conditions, T3S may function as a reservoir of inactive hormone from which active T3 may be recovered by action of tissue sulfatases and bacterial sulfatases in the intestine.

Sulfotransferases represent a family of enzymes with a monomer molecular weight of ≈34 kDa, located in the cytoplasm of different tissues, in particular the liver, kidney, intestine, and brain. They catalyze the transfer of sulfate from 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to usually a hydroxyl group of the substrate. Different phenol sulfotransferases have been identified with significant activity toward iodothyronines, including human SULT1A1, SULT1A2, SULT1A3, SULT1B1, and SULT1C2 (Kester and Visser 2005). They show substrate preference for 3,3′-T2 > T3 > rT3 > T4. Surprisingly, iodothyronines, in particular rT3 and T4, are also sulfated by human estrogen sulfotransferase (SULT1E1) (Kester and Visser 2005). Different human SULTs also catalyze the sulfation of iodothyronamines (Pietsch et al. 2007).

Glucuronidation

In contrast to the sulfates, iodothyronine glucuronides are rapidly excreted in the bile. However, this is not an irreversible pathway of hormone disposal, since after

hydrolysis of the glucuronides by bacterial ß-glucuronidases in the intestine, part of the liberated iodothyronines is reabsorbed, constituting an enterohepatic cycle (Wu et al. 2005; Peeters and Visser 2017). Nevertheless, about 20% of daily T4 production appears in the feces, probably via biliary excretion of glucuronide conjugates. Glucuronidation is catalyzed by UDP-glucuronyltransferases (UGTs) using UDP-glucuronic acid (UDPGA) as cofactor. UGTs are localized in the endoplasmic reticulum of predominantly the liver, kidney, and intestine.

Glucuronidation of T4 and T3 is catalyzed by different members of the UGT1A family, i.e., UGT1A1, UGT1A3, and UGT1A7–10. Usually, this involves the glucuronidation of the hydroxyl group (Fig. 4), but human UGT1A3 also catalyzes the glucuronidation of the side-chain carboxyl group, with formation of so-called acyl glucuronides (Kato et al. 2008). Interestingly, TA4 and TA3 are glucuronidated in human liver much more rapidly than T4 and T3, and this occurs largely by acyl glucuronidation (Moreno et al. 1994).

In rodents, metabolism of TH is accelerated through induction of T4-glucuronidating UGTs by different classes of compounds, including barbiturates, fibrates, and PCBs (Visser et al. 1993; Hood et al. 2003). This may result in a hypothyroid state when the thyroid gland is not capable of compensating for the increased hormone loss. In humans thyroid function may be affected by induction of T4 glucuronidation by antiepileptics, but overt hypothyroidism is rare (Benedetti et al. 2005). Administration of such drugs to LT4-substituted hypothyroid patients may necessitate an increase in the LT4 substitution dose.

Thyroid Hormone Actions

TH is critical for the development of different tissues, such as brain (Bernal 2015), intestine (Sirakov and Plateroti 2011), bone (Bassett and Williams 2016), skeletal muscle (Salvatore et al. 2014), and the auditory system (Ng et al. 2013). However, TH is also important for the maintenance of tissue function throughout life and is a crucial factor in the regulation of protein, carbohydrate, and lipid metabolism as well as for thermogenesis (Mullur et al. 2014; Bianco and McAninch 2013; Vaitkus et al. 2015). The interested reader is referred to the cited literature for extensive reviews of these TH actions. We will focus here on the most important mechanism of TH action mediated by binding of T3 to its nuclear receptors.

Mechanism of T3 Action

Most biological actions of T3 are initiated by its binding to nuclear T3 receptors (TRs) (Yen 2001; Mullur et al. 2014; Brent 2012; Mendoza and Hollenberg 2017). These proteins are members of the superfamily of ligand-dependent transcription factors, which also includes the receptors for steroids (e.g., cortisol, estradiol, testosterone), 1,25-dihydroxyvitamin D3, retinoic acid, and 9-cis-retinoic acid. The latter retinoid X receptor (RXR) is an important member of this gene family, as it

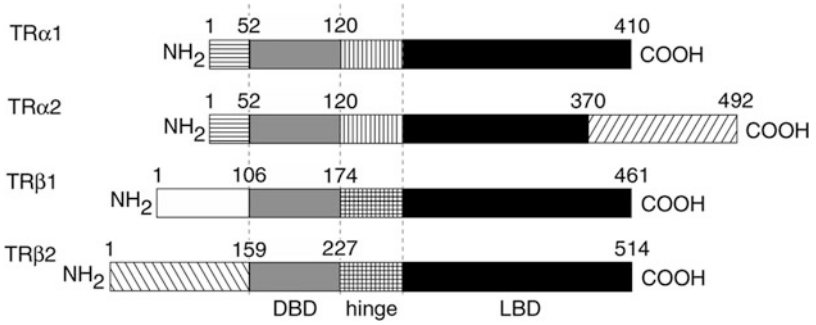

Fig. 5 Domain structures of the different T3 receptor (TR) isoforms. The TRα2 variant is incapable of binding T3. *DBD* DNA-binding domain, *LBD* ligand-binding domain

forms functional heterodimers with a number of other nuclear receptors, including TRs. Two TR genes have been identified; the *THRA* gene is located on human chromosome 17q21.1 and the *THRB* gene on human chromosome 3p24.2. By alternative exon utilization of both genes, four major receptor isoforms, i.e., TRα1, TRα2, TRβ1, and TRβ2, are generated which consist of 410–514 amino acids (Fig. 5). Although the *THRB* gene (≈150 kb) is much larger than the *THRA* gene (≈30 kb), their coding sequences show a high degree of homology (Yen 2001; Brent 2012; Mullur et al. 2014; Mendoza and Hollenberg 2017).

As in the other members of the nuclear receptor family, functional key domains have been recognized in the TRs, in particular the DNA-binding domain (DBD), which is ≈100 amino acids long, and the ligand-binding domain (LBD) which is ≈250 amino acids in length. The amino acid sequences of the TRα and TRβ subtypes are most homologous in their DBD and LBD and least homologous at their N terminus. The latter contains the ligand-independent activation function 1 (AF1) domain, while an AF2 domain necessary for homo- and heterodimerization and ligand-dependent activation is located at the C-terminus. The short sequence between the DBD and the LBD is referred to as the hinge region.

The structural difference between TRα1 and TRα2 is located at the C-terminus of the proteins, with completely different sequences for the last 40 and 122 amino acids, respectively. The alteration in the LBD of TRα2 is associated with a complete loss of T3 binding. Therefore, this splice variant is not a bona fide T3 receptor although it is referred to as TRα2. TRα2 may have a weak negative effect on the action of T3 mediated by the other TRs but, more importantly, its production signifies a negative effect on T3 action as it goes at the expense of TRα1. The N-terminal 106 amino acids of TRβ1 and 159 amino acids of TRβ2 differ almost completely due to utilization of alternative promoters and transcription start sites. This is important in relation to the tissue-specific expression of TRβ2 vs TRβ1 (see below), but it is not clear to what extent the different N-terminal domains of TRβ2 are associated with distinct molecular mechanisms of action.

The different TR isoforms show distinct tissue distributions (8112-114). TRα1 is the predominant T3 receptor expressed in the brain, heart, and bone, whereas TRβ1

is the predominant receptor in the liver and kidney. TRβ2 is preferentially expressed in the anterior pituitary, hypothalamus, cochlea, and retina. The presence of TRβ2 in these tissues suggests an important function in the negative feedback of TH at the level of the hypothalamus and pituitary as well as in the development and function of the sensory organs. However, evidence from mouse models suggests that both TRβ1 and TRβ2 are required for the negative feedback action of TH and for the development of cones in the retina (Ng et al. 2015).

The high homology between the LBDs of TRα1 and TRβ1/TRβ2 explains their very similar ligand specificity, with affinities decreasing in the order TA3 ≈ T3 > T4 > rT3. T3 is the major endogenous iodothyronine occupying the nuclear TH receptors, which are thus true T3 receptors. Recently, several TRβ-selective agonists have been developed with pharmacologically interesting and selective effects on the liver, resulting in lowering of body weight, lipid, and cholesterol levels without detrimental effects on the heart (Meruvu et al. 2013; Coppola et al. 2014). The tissue-selective effects of these compounds are not only determined by their differential affinities for TR isoforms but also by their preferential uptake by tissue-specific transporters. One such analogue, eprotirome, is effectively transported by the liver-specific bile acid transporter NTCP, and this may contribute to the liver-selective effects of this compound (Kersseboom et al. 2017).

Regulation of the expression of T3-responsive genes involves the binding of the TRs to so-called T3 response elements (TREs) in regulatory regions of these genes (Brent 2012; Mullur et al. 2014; Yen 2001; Mendoza and Hollenberg 2017). TREs usually consist of two half-sites arranged as repeats, inverted repeats, or palindromes. The consensus TRE half-site sequence is AGGTCA, and the most prevalent TRE is a direct repeat of this half-site spaced by four nucleotides (DR4). However, some TREs show marked deviation from this "consensus" half-site sequence which, moreover, may also be recognized by other nuclear receptors. This may be the basis for "cross talk" between different nuclear receptors and their target genes. Although TRs may bind as homodimers to the TREs, T3 effects on gene expression are usually mediated by TR/RXR heterodimers.

Binding of the TR/RXR heterodimer to TRE does not require T3 or 9-cis-retinoic acid, the ligand for RXR. The DBDs of these (and other) nuclear receptors contain two "zinc fingers" (peptide loops that chelate Zn^{2+}) which fit in the grooves of the DNA and are thus very important for the specificity of the receptor-response element interaction. In the absence of T3, the TR/RXR heterodimer is associated with corepressor proteins such as NCoR (nuclear corepressor) or SMRT (silencing mediator of retinoid and TH receptors), which suppress the basal transcription machinery (Fig. 6).

Binding of T3 induces a conformational change in the TR, resulting in the release of the corepressors and the recruitment of coactivator proteins such as SRC1 (steroid receptor coactivator-1) and CBP (cAMP response element-binding protein (CREB)) (Yen 2001; Brent 2012; Mullur et al. 2014; Mendoza and Hollenberg 2017). The AF2 domain, a highly conserved 9-amino acid sequence located at the C-terminus of the different nuclear receptors, plays an important role in the binding of the coactivators which stimulate the activity of the basal transcription machinery. One mechanism by which transcription is stimulated involves the association of

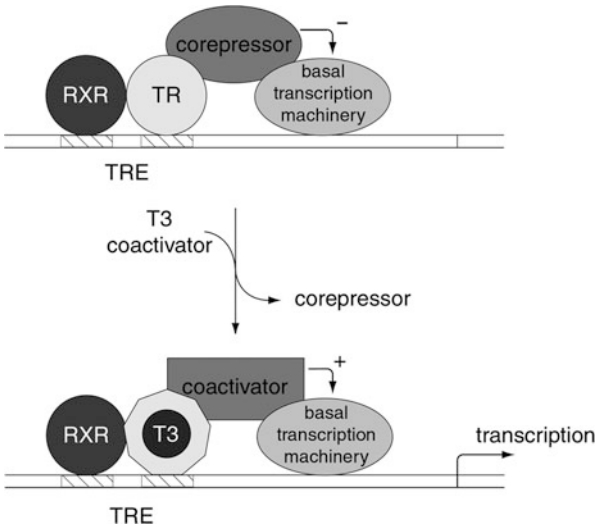

Fig. 6 Simplistic model of the regulation of gene transcription by T3. *TRE* T3 response element in the promoter of a T3-responsive gene, *RXR* retinoid X receptor, *TR* T3 receptor

coactivators with histone acetyltransferases (HATs). Acetylation of histones loosens the chromatin structure and thus facilitates interaction of the transcription machinery with the DNA. Conversely, corepressors are associated with histone deacetylases.

T3 Inhibition of TSH and TRH Gene Expression

The above discussion of the mechanism of action of T3 concerns the expression of genes which are under positive control of TH. However, a number of genes are negatively regulated by T3/TR, in particular those involved in the negative feedback regulation of the hypothalamus-pituitary-thyroid axis, in particular the *TSHβ* and *TRH* genes. This could involve the presence of negative half-site TREs in regulatory regions of these genes (Shibusawa et al. 2003). Binding of TRβ2 to these negative TREs in the absence of T3 would result in the activation of gene transcription, whereas in the presence of T3 transcription is inhibited. However, binding of TRs to the promoter region of negatively regulated genes may also be mediated by other transcription factors. Therefore, the exact mechanism for the negative regulation of gene expression by T3 still remains to be established.

TSHβ gene transcription is also strongly inhibited by 9-cis-retinoic acid, the effect of which is mediated by the pituitary-specific RXRγ1 subtype, and involves both TRE-dependent and TRE-independent interactions with the *TSHβ* gene promoter. The clinical relevance of this effect is underscored by studies showing that treatment of patients with bexarotene, another RXR ligand, induces central hypothyroidism (Sharma et al. 2006).

In addition to the regulation of TSH α and β subunit gene expression, T3 also acutely inhibits TSH secretion, the exact mechanism of which is not fully understood (Bargi-Souza et al. 2017). Although T3 is the active hormone exerting the inhibition of

TSH production and secretion, serum T4 appears to be a major player in the negative feedback regulation of the hypothalamus-pituitary-thyroid axis by acting as a precursor for local D2-mediated generation of T3 at these central sites (Fonseca et al. 2013).

Much knowledge regarding the molecular mechanisms of TR/T3 action has been gained from studies in patients with resistance to TH (RTH) associated with heterozygous mutations in *THRA* or *THRB* and in corresponding mouse models. The clinical phenotypes differ between patients with RTHα or RTHβ, largely reflecting the different tissue distributions of the receptor isoforms (Dumitrescu and Refetoff 2013; Moran et al. 2017; van Gucht et al. 2017). As TRβ1/TRβ2 is the predominant TR expressed in the anterior pituitary and hypothalamus, RTHβ is associated with an impaired negative feedback action of TH, resulting in elevated serum T4 and T3 and non-suppressed TSH levels. Tissues with predominant TRβ expression (liver, kidney) may be in a hypothyroid state, whereas tissues with predominant TRα expression may be in a hyperthyroid state as their unaffected TR is exposed to elevated TH levels. RTHβ patients may present with goiter, tachycardia, atrial fibrillation, mild mental retardation, hyperactivity, and increased resting energy expenditure (Dumitrescu and Refetoff 2013).

Patients with RTHα show a variable phenotype, depending on the severity of the mutation, delayed mental and motor development, growth retardation, macrocephaly, anemia, constipation, and skin tags (Moran et al. 2017; van Gucht et al. 2017). Most of these symptoms reflect the expression of *THRA* in the brain, bone, and intestine. Although THRA is also the predominant TR expressed in the heart, patients with RTH do not display bradycardia. The anemia in RTHα patients suggests a role for TRα1 in the differentiation of erythrocyte precursor cells. RTHα patients usually have low serum T4 and high T3 levels; the underlying mechanism may involve a decreased TRα1-dependent stimulation of D3 expression by T3.

References

Abe T, Suzuki T, Unno M, Tokui T, Ito S. Thyroid hormone transporters: recent advances. Trends Endocrinol Metab. 2002;13:215–20.

Abplanalp J, Laczko E, Philp NJ, Neidhardt J, Zuercher J, Braun P, Schorderet DF, Munier FL, Verrey F, Berger W, Camargo SM, Kloeckener-Gruissem B. The cataract and glucosuria associated monocarboxylate transporter MCT12 is a new creatine transporter. Hum Mol Genet. 2013;22:3218–26.

Andersson HC, Kohn LD, Bernardini I, Blom HJ, Tietze F, Gahl WA. Characterization of lysosomal monoiodotyrosine transport in rat thyroid cells. Evidence for transport by system h. J Biol Chem. 1990;265:10950–4.

Anwer MS, Stieger B. Sodium-dependent bile salt transporters of the SLC10A transporter family: more than solute transporters. Pflugers Arch. 2014;466:77–89.

Astwood EB. Landmark article May 8, 1943: treatment of hyperthyroidism with thiourea and thiouracil. By E.B. Astwood. JAMA. 1984;251:1743–6.

Bargi-Souza P, Goulart-Silva F, Nunes MT. Novel aspects of T3 actions on GH and TSH synthesis and secretion: physiological implications. J Mol Endocrinol. 2017;59:R167–R78.

Bassett JH, Williams GR. Role of thyroid hormones in skeletal development and bone maintenance. Endocr Rev. 2016;37:135–87.

Benedetti MS, Whomsley R, Baltes E, Tonner F. Alteration of thyroid hormone homeostasis by antiepileptic drugs in humans: involvement of glucuronosyltransferase induction. Eur J Clin Pharmacol. 2005;61:863–72.

Benvenga S. Thyroid hormone transport proteins and the physiology of hormone binding. In: Braverman LE, Utiger RD, editors. Werner & Ingbar's the thyroid. Philadelphia: Lippincott Williams & Wilkins; 2012.

Bernal J. Thyroid hormones in brain development and function. In: DeGroot L, editor. Thyroid disease manager; 2015. www.endotext.org.

Bernal J, Guadano-Ferraz A, Morte B. Thyroid hormone transporters-functions and clinical implications. Nat Rev Endocrinol. 2015;11:690.

Bianco AC, McAninch EA. The role of thyroid hormone and brown adipose tissue in energy homoeostasis. Lancet Diabetes Endocrinol. 2013;1:250–8.

Bianco AC, Salvatore D, Gereben B, Berry MJ, Larsen PR. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. Endocr Rev. 2002;23:38–89.

Bonomi M, Busnelli M, Beck-Peccoz P, Costanzo D, Antonica F, Dolci C, Pilotta A, Buzi F, Persani L. A family with complete resistance to thyrotropin-releasing hormone. N Engl J Med. 2009;360:731–4.

Botta R, Lisi S, Rotondo Dottore G, Vitti P, Marino M. Binding of thyroglobulin (Tg) to the low-density lipoprotein receptor-associated protein (RAP) during the biosynthetic pathway prevents premature Tg interactions with sortilin. J Endocrinol Investig. 2017;40:991–7.

Brent GA. Mechanisms of thyroid hormone action. J Clin Invest. 2012;122:3035–43.

Carvalho DP, Dupuy C. Thyroid hormone biosynthesis and release. Mol Cell Endocrinol. 2017;458:6–15.

Coppola M, Glinni D, Moreno M, Cioffi F, Silvestri E, Goglia F. Thyroid hormone analogues and derivatives: actions in fatty liver. World J Hepatol. 2014;6:114–29.

Davies TF, Ando T, Lin RY, Tomer Y, Latif R. Thyrotropin receptor-associated diseases: from adenomata to Graves disease. J Clin Invest. 2005;115:1972–83.

Davis PJ, Goglia F, Leonard JL. Nongenomic actions of thyroid hormone. Nat Rev Endocrinol. 2016;12:111–21.

Dumitrescu AM, Refetoff S. The syndromes of reduced sensitivity to thyroid hormone. Biochim Biophys Acta. 2013;1830:3987–4003.

Dumitrescu AM, Liao XH, Abdullah MS, Lado-Abeal J, Majed FA, Moeller LC, Boran G, Schomburg L, Weiss RE, Refetoff S. Mutations in SECISBP2 result in abnormal thyroid hormone metabolism. Nat Genet. 2005;37:1247–52.

Fliers E, Alkemade A, Wiersinga WM, Swaab DF. Hypothalamic thyroid hormone feedback in health and disease. Prog Brain Res. 2006;153:189–207.

Fonseca TL, Correa-Medina M, Campos MP, Wittmann G, Werneck-de-Castro JP, Arrojo e Drigo R, Mora-Garzon M, Ueta CB, Caicedo A, Fekete C, Gereben B, Lechan RM, Bianco AC. Coordination of hypothalamic and pituitary T3 production regulates TSH expression. J Clin Invest. 2013;123:1492–500.

Friedrichs B, Tepel C, Reinheckel T, Deussing J, von Figura K, Herzog V, Peters C, Saftig P, Brix K. Thyroid functions of mouse cathepsins B, K, and L. J Clin Invest. 2003;111:1733–45.

Friesema EC, Docter R, Moerings EP, Stieger B, Hagenbuch B, Meier PJ, Krenning EP, Hennemann G, Visser TJ. Identification of thyroid hormone transporters. Biochem Biophys Res Commun. 1999;254:497–501.

Friesema EC, Ganguly S, Abdalla A, Manning Fox JE, Halestrap AP, Visser TJ. Identification of monocarboxylate transporter 8 as a specific thyroid hormone transporter. J Biol Chem. 2003;278:40128–35.

Friesema EC, Kuiper GG, Jansen J, Visser TJ, Kester MH. Thyroid hormone transport by the human monocarboxylate transporter 8 and its rate-limiting role in intracellular metabolism. Mol Endocrinol. 2006;20:2761–72.

Friesema EC, Jansen J, Jachtenberg JW, Visser WE, Kester MH, Visser TJ. Effective cellular uptake and efflux of thyroid hormone by human monocarboxylate transporter 10. Mol Endocrinol. 2008;22:1357–69.

Garcia M, Gonzalez de Buitrago J, Jimenez-Roses M, Pardo L, Hinkle PM, Moreno JC. Central hypothyroidism due to a TRHR mutation causing impaired ligand affinity and transactivation of Gq. J Clin Endocrinol Metab. 2017;102:2433–42.

Gereben B, Zavacki AM, Ribich S, Kim BW, Huang SA, Simonides WS, Zeold A, Bianco AC. Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. Endocr Rev. 2008;29:898–938.

Gnidehou S, Caillou B, Talbot M, Ohayon R, Kaniewski J, Noel-Hudson MS, Morand S, Agnangji D, Sezan A, Courtin F, Virion A, Dupuy C. Iodotyrosine dehalogenase 1 (DEHAL1) is a transmembrane protein involved in the recycling of iodide close to the thyroglobulin iodination site. FASEB J. 2004;18:1574–6.

Groeneweg S, Peeters RP, Visser TJ, Visser WE. Triiodothyroacetic acid in health and disease. J Endocrinol. 2017;234:R99–R121.

Hagenbuch B. Cellular entry of thyroid hormones by organic anion transporting polypeptides. Best Pract Res Clin Endocrinol Metab. 2007;21:209–21.

Halestrap AP. The SLC16 gene family – structure, role and regulation in health and disease. Mol Asp Med. 2013;34:337–49.

Hennemann G, Docter R, Friesema EC, de Jong M, Krenning EP, Visser TJ. Plasma membrane transport of thyroid hormones and its role in thyroid hormone metabolism and bioavailability. Endocr Rev. 2001;22:451–76.

Heuer H, Visser TJ. Minireview: pathophysiological importance of thyroid hormone transporters. Endocrinology. 2009;150:1078–83.

Heuer H, Schafer MK, Bauer K. The thyrotropin-releasing hormone-degrading ectoenzyme: the third element of the thyrotropin-releasing hormone-signaling system. Thyroid. 1998;8:915–20.

Hoefig CS, Renko K, Piehl S, Scanlan TS, Bertoldi M, Opladen T, Hoffmann GF, Klein J, Blankenstein O, Schweizer U, Kohrle J. Does the aromatic L-amino acid decarboxylase contribute to thyronamine biosynthesis? Mol Cell Endocrinol. 2012;349:195–201.

Hoefig CS, Wuensch T, Rijntjes E, Lehmphul I, Daniel H, Schweizer U, Mittag J, Kohrle J. Biosynthesis of 3-iodothyronamine from T4 in murine intestinal tissue. Endocrinology. 2015;156:4356–64.

Hoefig CS, Zucchi R, Kohrle J. Thyronamines and derivatives: physiological relevance, pharmacological actions, and future research directions. Thyroid. 2016;26:1656–73.

Holzer G, Roux N, Laudet V. Evolution of ligands, receptors and metabolizing enzymes of thyroid signaling. Mol Cell Endocrinol. 2017;459:5–13.

Hood A, Allen ML, Liu Y, Liu J, Klaassen CD. Induction of T(4) UDP-GT activity, serum thyroid stimulating hormone, and thyroid follicular cell proliferation in mice treated with microsomal enzyme inducers. Toxicol Appl Pharmacol. 2003;188:6–13.

Hsu SY, Nakabayashi K, Bhalla A. Evolution of glycoprotein hormone subunit genes in bilateral metazoa: identification of two novel human glycoprotein hormone subunit family genes, GPA2 and GPB5. Mol Endocrinol. 2002;16:1538–51.

Hugo SE, Cruz-Garcia L, Karanth S, Anderson RM, Stainier DY, Schlegel A. A monocarboxylate transporter required for hepatocyte secretion of ketone bodies during fasting. Genes Dev. 2012;26:282–93.

Joseph-Bravo P, Jaimes-Hoy L, Uribe RM, Charli JL. 60 years of neuroendocrinology: TRH, the first hypophysiotropic releasing hormone isolated: control of the pituitary-thyroid axis. J Endocrinol. 2015;226:T85–T100.

Joseph-Bravo P, Jaimes-Hoy L, Charli JL. Advances in TRH signaling. Rev Endocr Metab Disord. 2016;17:545–58.

Karponis D, Ananth S. The role of thyrostimulin and its potential clinical significance. Endocr Regul. 2017;51:117–28.

Kato Y, Ikushiro S, Emi Y, Tamaki S, Suzuki H, Sakaki T, Yamada S, Degawa M. Hepatic UDP-glucuronosyltransferases responsible for glucuronidation of thyroxine in humans. Drug Metab Dispos. 2008;36:51–5.

Kersseboom S, van Gucht ALM, van Mullem A, Brigante G, Farina S, Carlsson B, Donkers JM, van de Graaf SFJ, Peeters RP, Visser TJ. Role of the bile acid transporter SLC10A1 in liver targeting of the lipid-lowering thyroid hormone analog eprotirome. Endocrinology. 2017;158:3307–18.

Kester MHA, Visser TJ. Sulfation of thyroid hormones. In: Pacifici GM, Coughtrie MWH, editors. Human cytosolic sulfotransferases. Boca Raton: CRC Press; 2005.

Kim DK, Kanai Y, Matsuo H, Kim JY, Chairoungdua A, Kobayashi Y, Enomoto A, Cha SH, Goya T, Endou H. The human T-type amino acid transporter-1: characterization, gene organization, and chromosomal location. Genomics. 2002;79:95–103.

Kleinau G, Worth CL, Kreuchwig A, Biebermann H, Marcinkowski P, Scheerer P, Krause G. Structural-functional features of the thyrotropin receptor: a class A G-protein-coupled receptor at work. Front Endocrinol (Lausanne). 2017;8:86.

Kohrle J. Selenium and the control of thyroid hormone metabolism. Thyroid. 2005;15:841–53.

Kolz M, Johnson T, Sanna S, Teumer A, Vitart V, Perola M, Mangino M, Albrecht E, Wallace C, Farrall M, Johansson A, Nyholt DR, Aulchenko Y, Beckmann JS, Bergmann S, Bochud M, Brown M, Campbell H, Eurospan Consortium, Connell J, Dominiczak A, Homuth G, Lamina C, McCarthy MI, Engage Consortium, Meitinger T, Mooser V, Munroe P, Nauck M, Peden J, Prokisch H, Salo P, Salomaa V, Samani NJ, Schlessinger D, Uda M, Volker U, Waeber G, Waterworth D, Wang-Sattler R, Wright AF, Adamski J, Whitfield JB, Gyllensten U, Wilson JF, Rudan I, Pramstaller P, Watkins H, Procardis Consortium, Doering A, Wichmann HE, Kora S, Spector TD, Peltonen L, Volzke H, Nagaraja R, Vollenweider P, Caulfield M, Wtccc TI, Gieger C. Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations. PLoS Genet. 2009;5:e1000504.

Koulouri O, Nicholas AK, Schoenmakers E, Mokrosinski J, Lane F, Cole T, Kirk J, Farooqi IS, Chatterjee VK, Gurnell M, Schoenmakers N. A novel thyrotropin-releasing hormone receptor missense mutation (P81R) in central congenital hypothyroidism. J Clin Endocrinol Metab. 2016;101:847–51.

Krause G, Hinz KM. Thyroid hormone transport across L-type amino acid transporters: what can molecular modelling tell us? Mol Cell Endocrinol. 2017;458:68–75.

Larsen PR. Type 2 iodothyronine deiodinase in human skeletal muscle: new insights into its physiological role and regulation. J Clin Endocrinol Metab. 2009;94:1893–5.

Leung AM, Pearce EN, Braverman LE. Environmental perchlorate exposure: potential adverse thyroid effects. Curr Opin Endocrinol Diabetes Obes. 2014;21:372–6.

Li W, Urban S. Entry of hepatitis B and hepatitis D virus into hepatocytes: basic insights and clinical implications. J Hepatol. 2016;64:S32–40.

Maenhaut C, Christophe D, Vassart G, Dumont J, Roger PP, Opitz R. Ontogeny, anatomy, metabolism and physiology of the thyroid. In: DeGroot L, editor. Thyroid disease manager; 2015. www.endotext.org.

Mariotti S, Beck-Peccoz P. Physiology of the hypothalamic-pituitary-thyroid axis. In: DeGroot L, editor. Thyroid disease manager. 2016. www.endotext.org.

Mendoza A, Hollenberg AN. New insights into thyroid hormone action. Pharmacol Ther. 2017;173:135–45.

Meruvu S, Ayers SD, Winnier G, Webb P. Thyroid hormone analogues: where do we stand in 2013? Thyroid. 2013;23:1333–44.

Moran C, Agostini M, McGowan A, Schoenmakers E, Fairall L, Lyons G, Rajanayagam O, Watson L, Offiah A, Barton J, Price S, Schwabe J, Chatterjee K. Contrasting phenotypes in resistance to thyroid hormone alpha correlate with divergent properties of thyroid hormone receptor alpha1 mutant proteins. Thyroid. 2017;27:973–82.

Moreno M, Kaptein E, Goglia F, Visser TJ. Rapid glucuronidation of tri- and tetraiodothyroacetic acid to ester glucuronides in human liver and to ether glucuronides in rat liver. Endocrinology. 1994;135:1004–9.

Moreno JC, Klootwijk W, van Toor H, Pinto G, D'Alessandro M, Leger A, Goudie D, Polak M, Gruters A, Visser TJ. Mutations in the iodotyrosine deiodinase gene and hypothyroidism. N Engl J Med. 2008;358:1811–8.

Mullur R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism. Physiol Rev. 2014;94:355–82.
Muzza M, Fugazzola L. Disorders of H2O2 generation. Best Pract Res Clin Endocrinol Metab. 2017;31:225–40.
Nakabayashi K, Matsumi H, Bhalla A, Bae J, Mosselman S, Hsu SY, Hsueh AJ. Thyrostimulin, a heterodimer of two new human glycoprotein hormone subunits, activates the thyroid-stimulating hormone receptor. J Clin Invest. 2002;109:1445–52.
Nakano M. Purification and properties of halogenated tyrosine and thyroid hormone transaminase from rat kidney mitochondria. J Biol Chem. 1967;242:73–81.
Ng L, Kelley MW, Forrest D. Making sense with thyroid hormone – the role of T(3) in auditory development. Nat Rev Endocrinol. 2013;9:296–307.
Ng L, Cordas E, Wu X, Vella KR, Hollenberg AN, Forrest D. Age-related hearing loss and degeneration of cochlear hair cells in mice lacking thyroid hormone receptor beta1. Endocrinology. 2015;156:3853–65.
Nicholas AK, Jaleel S, Lyons G, Schoenmakers E, Dattani MT, Crowne E, Bernhard B, Kirk J, Roche EF, Chatterjee VK, Schoenmakers N. Molecular spectrum of TSHbeta subunit gene defects in central hypothyroidism in the UK and Ireland. Clin Endocrinol. 2017;86:410–8.
Okada SL, Ellsworth JL, Durnam DM, Haugen HS, Holloway JL, Kelley ML, Lewis KE, Ren H, Sheppard PO, Storey HM, Waggie KS, Wolf AC, Yao LY, Webster PJ. A glycoprotein hormone expressed in corticotrophs exhibits unique binding properties on thyroid-stimulating hormone receptor. Mol Endocrinol. 2006;20:414–25.
Peeters RP, Visser TJ. Metabolism of thyroid hormone. In: DeGroot L, editor. Thyroid disease manager. 2017. www.endotext.org.
Perello M, Nillni EA. The biosynthesis and processing of neuropeptides: lessons from prothyrotropin releasing hormone (proTRH). Front Biosci. 2007;12:3554–65.
Persani L, Calebiro D, Cordella D, Weber G, Gelmini G, Libri D, de Filippis T, Bonomi M. Genetics and phenomics of hypothyroidism due to TSH resistance. Mol Cell Endocrinol. 2010;322:72–82.
Pietsch CA, Scanlan TS, Anderson RJ. Thyronamines are substrates for human liver sulfotransferases. Endocrinol. 2007;148:1921–7.
Pizzagalli F, Hagenbuch B, Stieger B, Klenk U, Folkers G, Meier PJ. Identification of a novel human organic anion transporting polypeptide as a high affinity thyroxine transporter. Mol Endocrinol. 2002;16:2283–96.
Ravera S, Reyna-Neyra A, Ferrandino G, Amzel LM, Carrasco N. The Sodium/Iodide Symporter (NIS): molecular physiology and preclinical and clinical applications. Annu Rev Physiol. 2017;79:261–89.
Refetoff S. Defects of thyroid hormone transport in serum. In: DeGroot L, editor. Thyroid disease manager. 2015a. www.endotext.org.
Refetoff S. Thyroid hormone serum transport proteins. In: DeGroot L, editor. Thyroid disease manager. 2015b. www.endotext.org.
Richardson SJ. Cell and molecular biology of transthyretin and thyroid hormones. Int Rev Cytol. 2007;258:137–93.
Rusu V, Hoch E, Mercader JM, Tenen DE, Gymrek M, Hartigan CR, DeRan M, von Grotthuss M, Fontanillas P, Spooner A, Guzman G, Deik AA, Pierce KA, Dennis C, Clish CB, Carr SA, Wagner BK, Schenone M, Ng MCY, Chen BH, Media Consortium, Sigma T. D. Consortium, Centeno-Cruz F, Zerrweck C, Orozco L, Altshuler DM, Schreiber SL, Florez JC, Jacobs SBR, Lander ES. Type 2 diabetes variants disrupt function of SLC16A11 through two distinct mechanisms. Cell. 2017;170:199–212 e20.
Salvatore D, Harney JW, Larsen PR. Mutation of the Secys residue 266 in human type 2 selenodeiodinase alters 75Se incorporation without affecting its biochemical properties. Biochimie. 1999;81:535–8.
Salvatore D, Simonides WS, Dentice M, Zavacki AM, Larsen PR. Thyroid hormones and skeletal muscle – new insights and potential implications. Nat Rev Endocrinol. 2014;10:206–14.

Saraiva MJ. Transthyretin mutations in hyperthyroxinemia and amyloid diseases. Hum Mutat. 2001;17:493–503.

Scanlan TS, Suchland KL, Hart ME, Chiellini G, Huang Y, Kruzich PJ, Frascarelli S, Crossley DA, Bunzow JR, Ronca-Testoni S, Lin ET, Hatton D, Zucchi R, Grandy DK. 3-Iodothyronamine is an endogenous and rapid-acting derivative of thyroid hormone. Nat Med. 2004;10:638–42.

Schoenmakers E, Agostini M, Mitchell C, Schoenmakers N, Papp L, Rajanayagam O, Padidela R, Ceron-Gutierrez L, Doffinger R, Prevosto C, Luan J, Montano S, Lu J, Castanet M, Clemons N, Groeneveld M, Castets P, Karbaschi M, Aitken S, Dixon A, Williams J, Campi I, Blount M, Burton H, Muntoni F, O'Donovan D, Dean A, Warren A, Brierley C, Baguley D, Guicheney P, Fitzgerald R, Coles A, Gaston H, Todd P, Holmgren A, Khanna KK, Cooke M, Semple R, Halsall D, Wareham N, Schwabe J, Grasso L, Beck-Peccoz P, Ogunko A, Dattani M, Gurnell M, Chatterjee K. Mutations in the selenocysteine insertion sequence-binding protein 2 gene lead to a multisystem selenoprotein deficiency disorder in humans. J Clin Invest. 2010;120:4220–35.

Schoenmakers E, Carlson B, Agostini M, Moran C, Rajanayagam O, Bochukova E, Tobe R, Peat R, Gevers E, Muntoni F, Guicheney P, Schoenmakers N, Farooqi S, Lyons G, Hatfield D, Chatterjee K. Mutation in human selenocysteine transfer RNA selectively disrupts selenoprotein synthesis. J Clin Invest. 2016;126:992–6.

Schweizer U, Johannes J, Bayer D, Braun D. Structure and function of thyroid hormone plasma membrane transporters. Eur Thyroid J. 2014;3:143–53.

Sharma V, Hays WR, Wood WM, Pugazhenthi U, Germain DLS, Bianco AC, Krezel W, Chambon P, Haugen BR. Effects of rexinoids on thyrotrope function and the hypothalamic-pituitary-thyroid axis. Endocrinology. 2006;147:1438–51.

Shibusawa N, Hollenberg AN, Wondisford FE. Thyroid hormone receptor DNA binding is required for both positive and negative gene regulation. J Biol Chem. 2003;278:732–8.

Silveira JC, Kopp PA. Pendrin and anoctamin as mediators of apical iodide efflux in thyroid cells. Curr Opin Endocrinol Diabetes Obes. 2015;22:374–80.

Sirakov M, Plateroti M. The thyroid hormones and their nuclear receptors in the gut: from developmental biology to cancer. Biochim Biophys Acta. 2011;1812:938–46.

Slijepcevic D, Roscam Abbing RLP, Katafuchi T, Blank A, Donkers JM, van Hoppe S, de Waart DR, Tolenaars D, van der Meer JHM, Wildenberg M, Beuers U, Oude Elferink RPJ, Schinkel AH, van de Graaf SFJ. Hepatic uptake of conjugated bile acids is mediated by both sodium taurocholate cotransporting polypeptide and organic anion transporting polypeptides and modulated by intestinal sensing of plasma bile acid levels in mice. Hepatology. 2017;66:1631–43.

Stieger B, Hagenbuch B. Organic anion-transporting polypeptides. Curr Top Membr. 2014;73:205–32.

Sun Y, Lu X, Gershengorn MC. Thyrotropin-releasing hormone receptors – similarities and differences. J Mol Endocrinol. 2003;30:87–97.

Targovnik HM, Citterio CE, Rivolta CM. Iodide handling disorders (NIS, TPO, TG, IYD). Best Pract Res Clin Endocrinol Metab. 2017;31:195–212.

Taurog A. Hormone synthesis: thyroid iodine metabolism. In: Braverman LE, Utiger RD, editors. Werner & Ingbar's the thyroid. Philadephia: Lippincott Williams & Wilkins; 2000.

Taurog A, Dorris ML, Doerge DR. Mechanism of simultaneous iodination and coupling catalyzed by thyroid peroxidase. Arch Biochem Biophys. 1996;330:24–32.

Taylor PM, Ritchie JW. Tissue uptake of thyroid hormone by amino acid transporters. Best Pract Res Clin Endocrinol Metab. 2007;21:237–51.

Vaitkus JA, Farrar JS, Celi FS. Thyroid hormone mediated modulation of energy expenditure. Int J Mol Sci. 2015;16:16158–75.

van der Deure WM, Peeters RP, Visser TJ. Molecular aspects of thyroid hormone transporters, including MCT8, MCT10, and OATPs, and the effects of genetic variation in these transporters. J Mol Endocrinol. 2010;44:1–11.

van der Spek AH, Fliers E, Boelen A. The classic pathways of thyroid hormone metabolism. Mol Cell Endocrinol. 2017.

van Gucht ALM, Moran C, Meima ME, Visser WE, Chatterjee K, Visser TJ, Peeters RP. Resistance to thyroid hormone due to heterozygous mutations in thyroid hormone receptor alpha. Curr Top Dev Biol. 2017;125:337–55.

Visser TJ. Biosynthesis, transport, metabolism, and actions of thyroid hormone. In: Wass JAH, Stewart PM, editors. Oxford textbook of endocrinology and diabetes. Oxford: Oxford University Press; 2011.

Visser TJ, Kaptein E, Gijzel AL, de Herder WW, Ebner T, Burchell B. Glucuronidation of thyroid hormone by human bilirubin and phenol UDP-glucuronyltransferase isoenzymes. FEBS Lett. 1993;324:358–60.

Visser WE, Friesema EC, Jansen J, Visser TJ. Thyroid hormone transport by monocarboxylate transporters. Best Pract Res Clin Endocrinol Metab. 2007;21:223–36.

Visser WE, Friesema EC, Jansen J, Visser TJ. Thyroid hormone transport in and out of cells. Trends Endocrinol Metab. 2008;19:50–6.

Visser WE, Friesema EC, Visser TJ. Minireview: thyroid hormone transporters: the knowns and the unknowns. Mol Endocrinol. 2011;25:1–14.

Wajner SM, Goemann IM, Bueno AL, Larsen PR, Maia AL. IL-6 promotes nonthyroidal illness syndrome by blocking thyroxine activation while promoting thyroid hormone inactivation in human cells. J Clin Invest. 2011;121:1834–45.

Wemeau JL, Kopp P. Pendred syndrome. Best Pract Res Clin Endocrinol Metab. 2017;31:213–24.

Werneck de Castro JP, Fonseca TL, Ueta CB, McAninch EA, Abdalla S, Wittmann G, Lechan RM, Gereben B, Bianco AC. Differences in hypothalamic type 2 deiodinase ubiquitination explain localized sensitivity to thyroxine. J Clin Invest. 2015;125:769–81.

Wilkinson JH. Recent work on thyroid hormones. Postgrad Med J. 1957;33:333–7.

Wood WJ, Geraci T, Nilsen A, DeBarber AE, Scanlan TS. Iodothyronamines are oxidatively deaminated to iodothyroacetic acids in vivo. Chembiochem. 2009;10:361–5.

Wu SY, Green WL, Huang WS, Hays MT, Chopra IJ. Alternate pathways of thyroid hormone metabolism. Thyroid. 2005;15:943–58.

Yen PM. Physiological and molecular basis of thyroid hormone action. Physiol Rev. 2001;81:1097–142.

Zevenbergen C, Meima ME, Lima de Souza EC, Peeters RP, Kinne A, Krause G, Visser WE, Visser TJ. Transport of iodothyronines by human L-type amino acid transporters. Endocrinology. 2015;156:4345–55.

Zhang CY, Kim S, Harney JW, Larsen PR. Further characterization of thyroid hormone response elements in the human type 1 iodothyronine deiodinase gene. Endocrinology. 1998;139:1156–63.

Tests of Thyroid Function

2

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Contents

Abstract

Thyroid function testing is the most used diagnostic evaluation in endocrine practice and is used as a screening tool, to verify the clinical diagnosis of hyper and hypothyroidism, to assess adequacy of medical treatment, and in the follow up of differentiated thyroid cancer. A small fraction of T4 and T3 are present in the circulation in free or unbound form but only the free thyroid hormone

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fractions (FT4 and FT3) can enter cells and employ their physiological actions. Most thyroid function tests are performed on serum and are based on automated assays: currently total T4 and T3 (TT4 and TT3) concentrations are measured by competitive immunoassay methods employing immunofluorescence or chemiluminescence, but since several conditions (drugs, pregnancy, nonthyroidal illness, genetic alterations) determine binding protein abnormalities, the measurements of TT4 and TT3 as stand-alone thyroid tests is limited. The assessment of FT4 and FT3 avoids most artifacts due to abnormalities of binding proteins but is technically challenging; direct and indirect methods of measurement have been developed, and their advantages and pitfalls are discussed in this chapter. The sensitivity of the latest generation of TSH assays rarely results in false or artifactual measurements. From a clinical perspective, serum TSH is the first-line test for detecting thyroid dysfunction in ambulatory patients while FT3 measurement is usually unnecessary, since FT4 together with TSH is adequate to diagnose both overt and subclinical hypo- and hyperthyroidism. The measurement of serum thyroglobulin (Tg) in the context of the management of differentiated thyroid carcinoma and other dynamic testing and indicators of thyroid status such as urinary iodine, radioactive iodine uptake, and perchlorate discharge test are also discussed in this chapter.

Keywords

Triiodothyronine (T3) · Thyroxine (T4) · TT4 · TT3 · Thyroid hormone-binding proteins · FT4 measurement · FT3 · Measurement · Free thyroid hormone tests · Serum thyrotropin (TSH) · Thyrotropin-releasing hormone (TRH) stimulation test · Thyroglobulin (Tg) measurement · Urinary iodine · Perchlorate discharge test · Equilibrium dialysis · Ultrafiltration · Free thyroid hormone index (FT4I) · Radioimmunoassays (RIAs) · Immunoradiometric assay · Immunoenzymatic assay · Chemiluminescent assay · "Hook" effect · Tg antibody interference · Familial dysalbuminemic hyperthyroxinemia · Transthyretin-associated hyperthyroxinemia · Heterophilic antibodies · Interference · TSH reflex

Introduction

Thyroid function testing is the most used diagnostic evaluation in endocrine practice. A profound understanding of the indications and limitations of thyroid testing is essential for an adequate clinical diagnosis and the management of a wide range of thyroid disorders (Bartalena et al. 1996).

Thyroid function tests are used as a screening tool, to verify the clinical diagnosis of hyper and hypothyroidism, to assess adequacy of medical treatment, and in the follow up of differentiated thyroid cancer.

These tests cover assessment of the hypothalamic-pituitary-thyroid axis, radio-iodine uptake tests, determination of urinary iodide and serum thyroglobulin, as well as investigation of variables suggestive of thyroid autoimmunity (Table 1). In this chapter we will discuss the tests belonging to the first group while those investigating the presence of thyroid autoimmunity will be examined elsewhere.

Table 1 Classification of the most important thyroid function tests

Tests that asses the hypothalamic-pituitary-thyroid axis	Serum total thyroxine (TT4) Serum total triiodothyronine (TT3) Serum free thyroxine (FT4) Serum free triiodothyronine (FT3) Serum thyrotropin (TSH) TRH test
Indicators of iodine intake and utilization by the thyroid	Urinary iodine Radioactive iodine uptake test (RAIU)
Other tests related to thyroid function	Serum thyroglobulin (Tg) Perchlorate discharge test
Tests for the evaluation of thyroid autoimmunity	Thyroid peroxidase antibody (TPOAb) Thyroglobulin antibody (TgAb) TSH receptor antibody (TRAb)

Triiodothyronine (T3), the biologically active hormone, and Thyroxine (T4) are the major secretory products of the thyroid; more precisely T4 is synthetized exclusively in the thyroid gland, whereas approximately 80% of T3 is produced by peripheral conversion of T4, by monodeiodination mediated by 5′-monodeiodinase operating at different tissue levels.

Thyroid hormone synthesis and secretion are regulated by intrathyroidal and extrathyroidal control, the most important factor being pituitary thyrotropin (TSH). In turn, TSH production and secretion is under the regulation of a feedback inhibition via thyroid hormones, and stimulated by the hypothalamic tri-peptide thyrotropin-releasing hormone (TRH).

T4 and T3, in the circulation, are mostly bound to a set of plasma proteins which differ widely in their concentration and affinity for these hormones. The three major transport proteins are T4-binding globulin (TBG), transthyretin (TTR or T4-binding pre-albumin, TBPA) and albumin (ALB) (Bartalena and Robbins 1993).

Protein-bound thyroid hormones do not cross plasma membranes and are therefore considered to be biologically inactive and functioning as a *reservoir* for circulating thyroid hormones. Biologically active free thyroid hormone fractions (FT4 and FT3) can enter cells by specific membrane transport mechanisms. In the pituitary, the negative feedback of thyroid hormone on TSH secretion is mediated primarily by T3 produced locally from deiodination of T4.

Most thyroid function tests are performed on serum and are based on automated immunoassays. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has recently been introduced in this field, in order to measure total and free thyroid hormones (Soldin and Soldin 2011) or urinary iodine. Technically, it is easier to develop methods to measure total thyroid hormone (TT4 and TT3) concentrations, as compared to tests that estimate free concentrations, because the former are measured at nanomolar levels whereas the latter are measured in the picomolar range and require assays that have to be free from interference by the much higher

total hormone concentration (Baloch et al. 2003). Over the years, sensitivity and specificity of the assays have been improved but lack of uniformity of the methodologies and reference standards of the different thyroid function tests are responsible for persistent and significant variability between methods. Reference measurement systems have already been put in place for serum concentrations of TT4, TT3, and thyroglobulin, while measurement of TSH, free thyroid hormones, and urinary iodine still represents a major challenge (Faix and Miller 2016).

Total Thyroid Hormone Measurement

In man, approximately 0.04% of the total serum T4 and 0.4% of the total serum T3 are present in the circulation in free or unbound form. Among the three main binding proteins, serum albumin (ALB) is present at approximately 100-fold the molar concentration of transthyretin (TTR) and 2000-fold that of thyroxine-binding globulin (TBG). However, from the view point of the association constants for T4, TBG has the highest affinity, about 50-fold higher than that of TTR and 7000-fold higher than that of ALB. As a result, TBG binds 75% of serum T4, while TTR and ALB bind 20% and 5%, respectively (Pappa et al. 2015) (Table 2).

Several other serum proteins, in particular high density lipoproteins, bind to T4 and T3, but their contribution is considered negligible in both physiological and pathological conditions.

TT4 and TT3 assays have evolved through a variety of measurement technologies over the past four decades.

In the 1950s, tests estimated the TT4 concentration as "protein-bound iodide." In the 1960s, the first competitive protein-binding methods were developed and were later replaced by radioimmunoassay (RIA) methods (Chopra et al. 1971). Currently, serum TT4 and TT3 concentrations are measured by competitive immunoassay methods employing immunofluorescence or chemiluminescence, but high-performance liquid chromatography (HPLC), gas chromatography, and mass spectrometry have also been employed in the research field (Burman et al. 1981; Tai et al. 2002; Thienpont et al. 1994).

Table 2 Affinity characteristics and concentrations of thyroid hormone-binding proteins in serum

	TBG	TTR	Albumin
Association constant			
KT_4 KT_3	1×10^{10} 4.6×10^8	7×10^5 / 10^7 7×10^5 / 1.5×10^7	5×10^4 / 7×10^5 7×10^3 / 1×10^5
Concentration			
mg/dl mmol/L	1.5 0.27	25 4.6	4200 640
Hormone distribution			
T4 (%) T3 (%)	68 80	11 9	20 11

Table 3 Reference ranges for serum thyroid hormones and TSH

Hormone	Reference range	Possible interfering factors
TT4	58–160 nmol/L (4.5–12.6 μg/dL)	Pregnancy, nonthyroidal illnesses, drugs, binding proteins concentration variations due to genetic mutations
TT3	1.2–2.7 nmol/L (80–180 ng/dL)	Nonthyroidal illnesses, drugs, nutritional status, binding proteins concentration variations due to genetic mutations
FT4	0.9–1.6 ng/dL (11.6–20.6 pmol/L)	Pregnancy, drugs
FT3	2.3–4.2 pg/mL (3.5–6.4 pmol/L)	Nonthyroidal illnesses, drugs, nutritional status
TSH	0.45–4.1 mU/L (1–15 pmol/L)	Drugs, aging, pulsatile secretion, heterophilic antibodies

In adults, normal serum TT4 concentrations range from 58 to 160 nmol/L (4.5–12.6 μg/dL), while TT3 concentrations range from 1.2–2.7 nmol/L (80–180 ng/dL), without significant gender difference (Table 3).

Since serum TT4 and TT3 concentrations reflect circulating hormones bound to proteins, abnormal serum TT4 and TT3 concentrations may be encountered as a result of binding protein abnormalities rather than as a result of thyroid dysfunction. Awareness of this is important in order to prevent thyroid treatment or unnecessary investigations. Congenital TBG deficiency is an X-linked genetic abnormality occurring in approximately 1:15,000 newborn males. Twenty-seven different gene mutations have been documented so far (Pappa et al. 2015). Usually this alteration goes unnoticed, unless TT4 is measured and the clinical suspicion can be confirmed by assessing TT4 levels in family members followed by confirmatory genetic testing.

An increase in serum TBG concentration may be genetically determined in 1:25,000 newborns due to gene duplication or triplication, occurs physiologically during pregnancy (due to increased estrogen levels) or may be caused by a variety of drugs or occurs during nonthyroidal illness (Table 4). For the above mentioned reasons, a pregnant woman could be diagnosed as hyperthyroid if only TT4 were to be determined, or a hypothyroid pregnant woman may remain undiagnosed if TT4 levels fall within the normal range due to increase in circulating TBG levels (Fig. 1). Mutations in TBG, TTR, and albumin can increase the binding affinity for T4 and T3 and cause a condition known as euthyroid hyperthyroxinemia. These rare mutations cause an increase in the total T4 and/or T3 levels, but the unbound concentrations remain normal and are unaffected. Additionally, some patient sera may contain other abnormal binding proteins, such as autoantibodies to thyroid hormones. Endogenous autoantibodies to T4 and/or T3 may sporadically be present in patients and invalidate the assay (Despres and Grant 1998; Li Calzi et al. 1988).

These binding protein abnormalities compromise the use of TT4 and TT3 measurements as stand-alone thyroid tests. Serum TT4 and TT3 measurements are typically requested, whenever possible, as part of a two-test panel that includes an assessment of binding protein status, either directly by TBG immunoassay or by a "T3 uptake" test.

Table 4 Conditions which may influence the serum concentrations of thyroid hormone-binding proteins (modified from references Bartalena et al. 1996; Pappa et al. 2015; Stockigt 2003; Kundra and Burman 2012). *TTR* transthyretin, *ALB* albumin, *AIDS* acquired immune deficiency syndrome, *TBG* thyroxine-binding globulin

	Increased TBG	Decreased TGB	TTR or ALB abnormalities
Drugs	Estrogens Tamoxifen 5-fluorouracil Heroin and methadone Clofibrate Perphenazine Raloxifene	Androgens and anabolic steroids Glucocorticoids L-asparaginase Nicotinic acid	
Physiologic and Pathologic conditions	Pregnancy Hypothyroidism Acute/chronic hepatitis Adrenal insufficiency AIDS Angioneurotic edema Acute intermittent porphyria	Hyperthyroidism Critical illness Sepsis Hepatic failure Nephrotic syndrome Diabetic ketoacidosis Chronic alcoholism Malnutrition Acromegaly Cushing's syndrome Extreme prematurity	Nonthyroidal illness Malnutrition Inflammation Pregnancy Nephrotic syndrome
Congenital Conditions	TBG excess (X-linked)	TBG gene defects. Partial deficiency (X linked) and complete deficiency (X linked) Carbohydrate-deficient glycoprotein syndrome type 1 (CDG1), autosomal recessive	Familial dysalbuminemic hyperthyroxinemia Transthyretin-associated hyperthyroxinemia

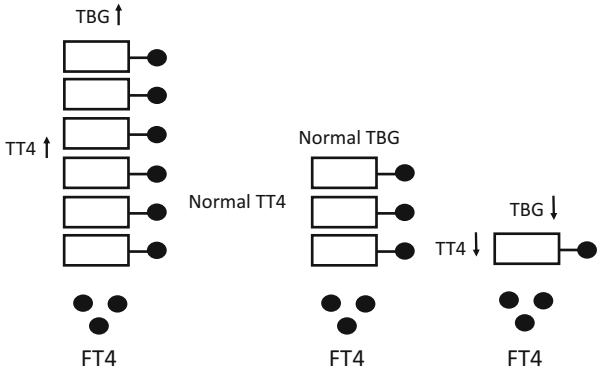

Fig. 1 Schematic presentation of the effect of serum TT4 variation due to changes in thyroxine-binding protein levels. The figure shows conditions in which free thyroid hormone levels are normal but determination of TT4 may be misleading in the presence of TBG concentration abnormalities

Free Thyroid Hormone Tests

The assessment of free thyroid hormones is critical in the evaluation of thyroid function, because the free hormone hypothesis states that physiological effects of T4 and T3 depend on free thyroid hormone levels and not on the total hormone concentration. The measurement of free thyroid hormones avoids the artifacts due to the high frequency of abnormalities of binding protein (Tables 3 and 4).

The methods for the assessment of free hormones belong to two major groups (Ekins 1990). Direct methods employ a physical separation (equilibrium dialysis or ultrafiltration) to separate the free hormone from the dominant protein-bound fraction (Fig. 2). These methods are technically challenging, expensive, usually employed for research purpose but also used as a reference for assigning calibrator values for indirect methods. Only radioimmunoassays (RIAs) have the sensitivity for measuring the picomolar hormone concentrations in dialysates and ultrafiltrates (Nelson and Tomei 1988). However, currently tandem MS can offer extremely high sensitivity and has become the routine method in some laboratories (Soldin and Soldin 2011). With direct methods the separation step that requires rigorous control of a number of variables (temperature, pH, buffer, dilutions), potentially interfering with the equilibrium between free and bound thyroid hormone fractions, is critical.

Estimate tests are based upon the assumption that an antibody may bind the free hormone without affecting the bound/unbound ratio (Fig. 3). The one step methods employ a labeled analog that does not interact with binding proteins and compete with the free fraction of thyroid hormone for a specific antibody adsorbed on a solid phase (Wilkins et al. 1985). An alternative one step method employs a labeled antibody, and the free hormone competes with exogenous thyroid hormone immobilized on a solid phase (Christofides and Sheehan 1995). The two step methods employ an antibody on a solid phase that binds the free fraction of the hormone in the first step (Nuutila et al. 1990). Then the bound hormone is washed away and the labeled thyroid hormone adsorbed on the solid phase is measured by a

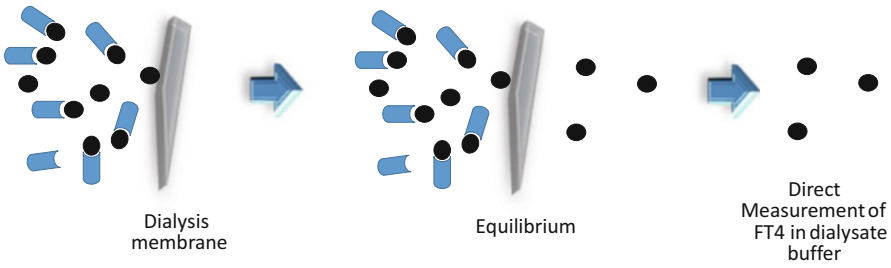

Fig. 2 Direct determination of FT4 levels using equilibrium dialysis. FT4 can freely diffuse in the dialysis buffer after application of a membrane that is impermeable to the binding proteins. After establishing equilibrium, free thyroid hormone fraction may be determined with sensitive immunoassays or by mass spectrometry

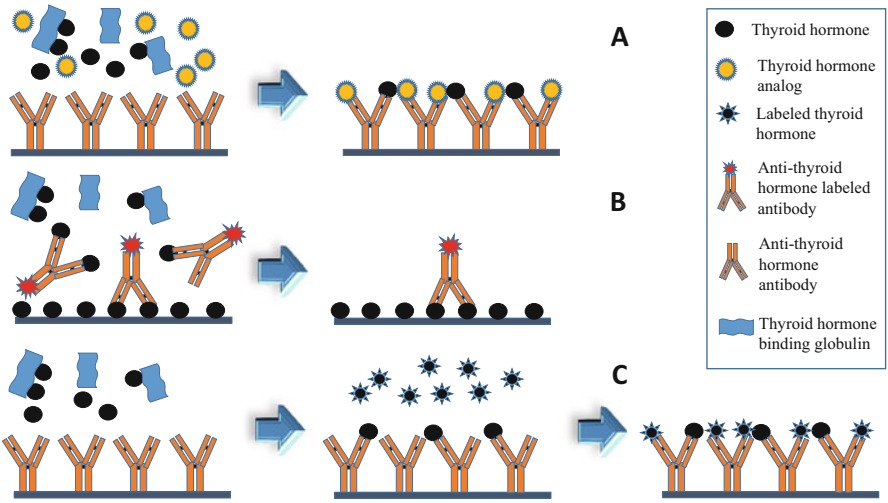

Fig. 3 Schematic presentation of indirect assays for determination of free thyroid hormone. The one step designs utilize a thyroid hormone analog (panel A) or antithyroid hormone labeled antibody (panel B). In the case of the two steps labeled hormone method (panel C), the labeled thyroid hormone never gets in contact with serum proteins, because of a preliminary washing step. In all three methods the concentration of the free hormone is inversely proportional to the labeled substrate bound to the solid phase at the end of the process

conventional immunoassay (Fig. 3), and concentrations are calculated by comparison to a standard curve. With all three methods the concentration of the free hormone is inversely proportional to the labeled substrate bound to the solid phase at the end of the process. At this time, no prediction of validity and performance of any assay can be made based only on the measurement design. Recommendation protocols and guidelines for validation of assays under controlled conditions are necessary.

Historically, the free thyroid hormone index (FT4I) calculation belonged to this category. This index correlated well with free thyroid hormone concentrations but has now become obsolete since it has a poorer diagnostic discrimination, and requires both the assessment of total thyroid hormone concentration combined with either an estimation of the free T4 fraction by the addition of a tracer necessary to determine the TBG-binding sites (T3 or T4 uptake test) or assessment of the concentration of T4-binding protein (TBG), an indicator of thyroid hormone-binding protein capacity. FT4I determination may be misleading in all the physiological and pathological conditions characterized by altered TBG and albumin concentrations, such as nonthyroidal illnesses. FT4 and FT3 values are 1.5 times higher when measurements are conducted at 37 °C compared to 25 °C. For this reason it is recommended to test free thyroid hormones at 37 °C.

Presently, measurement of thyroid hormones is conducted on automated systems (Costongs et al. 1995; Letellier et al. 1996), which is rapid and allows quality checks for hemolysis, icterus, and turbidity. FT4 and FT3 immunoassays are all protein-

dependent to some extent and are prone to under- or overestimate the real values in patients with significant abnormalities of thyroid hormone-binding proteins, especially TBG that has the highest affinity for thyroid hormones. When TBG levels are increased FT4 levels are usually underestimated due to reduced recovery. Furthermore, in patients with nonthyroidal illness, underestimation of FT4 concentration may occur because analogs used by some assays have residual binding activity to albumin. In such patients albumin levels are lower than those employed in the calibrators used to set up the assay. Moreover, they still appear sensitive to certain drugs (Stockigt and Lim 2009), high circulating free fatty acid levels**,** reduction of extracellular fluid volume after hemodialysis, and other factors which compete for binding sites on thyroid-binding proteins (Pappa et al. 2015; Dufour 2007). In euthyroid pregnant women, FT4 measurements show a progressive decrease in concentrations toward the end of the pregnancy. At this point it is worth mentioning that there is no agreement on the best method for the assessment of FT4 during pregnancy, but international guidelines recommend caution in the interpretation of the results and the use of trimester-specific reference ranges, independent of assay employed (Alexander et al. 2017). Instead of measuring free T4, total T4 measurement (with a pregnancy-adjusted reference range) is, by recent guidelines (Alexander et al. 2017), considered a reliable alternative for estimating hormone concentration during the last part of pregnancy. For a detailed outline of the advantages and potential limitations of free thyroid hormone determination methodologies see also Thienpont et al. (2013).

The measurement of free thyroid hormones from the same serum sample, using different methods, is going to provide discordant results (Thienpont et al. 2013, 2010), and this is the reason for why the results need to be compared to method-specific reference ranges.

The reference ranges of free thyroid hormones vary depending on the method and approximates 0.9–1.6 ng/dL (11.6–20.6 pmol/L) for FT4 and 2.3–4.2 pg/mL (3.5–6.4 pmol/L) for FT3 (Table 3).

Most of the imprecision introduced by commercial kits is due to the calibration protocols employed by manufacturers, which introduce biases that can be corrected using mathematical recalibration with regression analysis (Faix 2013). It is also recommended to test the performance of the chosen assay in different conditions, such as in euthyroid subjects, hyper- and hypothyroid patients, pregnancy, after serum dilution, sera-containing drugs potentially interfering with thyroid hormone-binding proteins, and to check for recovery after addition of albumin or TBG to the incubation mixture (Stockigt and Lim 2009).

From a clinical perspective, FT3 measurement is usually unnecessary, since FT4 together with TSH is adequate to assess thyroid hormone deficit or excess in most patients with thyroid dysfunction. This is valid with few exceptions characterized by discordant FT4/TSH levels and listed in Table 5. Furthermore, FT3 measurement may be relevant in peculiar clinical states characterized by peripheral T3 secretion as it may occur with toxic adenoma or Graves' disease (Smith and Hegedüs 2016).

Table 5 Summary of clinical conditions characterized by "discordant" FT4/TSH serum.↑ increased, *N* normal, ↓ decreased

Test results		Possible causes
TSH	FT4	
N or ↓	↑	Drugs (glucorticoids, dopamine, amiodarone)
N	↑	L-T4 treatment (frequent when blood sampling follows T4 ingestion in the morning) Antibody interfering activity (antibodies against T4, heterophilic antibodies interfering with FT4 measurement, rheumatoid factor)
N	↓	Drugs interfering with protein binding Pregnancy (third trimester)
↑	N	Hypothyroid subjects, first weeks after thyroid hormone administration Recovery from nonthyroidal illnesses Heterophilic antibodies or other agents interfering with TSH measurement
↓	N	First weeks after hyperthyroidism treatment with antithyroid drugs Drugs (glucorticoids, dopamine, amiodarone)
N or ↑	↑	TSH-secreting pituitary adenoma Peripheral resistance to thyroid hormone action
↓, N,↑	↓	Central hypothyroidism

Serum Thyrotropin (TSH)

Thyrotropin (TSH) is a 28 kD glycoprotein composed by α and β subunits that are noncovalently linked. Serum TSH measurement plays a critical role in the diagnosis of thyroid disease and is one of the most frequently requested tests in clinical medicine. This is mainly due to its wide availability, low cost, high sensitivity, and not least the fact that TSH secretion is extremely sensitive to plasma concentrations of free thyroid hormones and thus a valid indicator of thyroid function status.

In recent years, the sensitivity and accuracy of TSH measurements have dramatically improved. The routine measurement of TSH initially used a RIA technique. These first-generation assays had a sensitivity of 1 mU/L, which did not allow the discrimination between normal and reduced concentrations in the case of mild thyrotoxicosis. Due to the low sensitivity of the TSH assays, a TRH stimulation test was frequently performed in order to confirm the diagnosis of thyrotoxicosis. Furthermore, a major issue with early TSH RIAs was their cross reactivity with gonadotropins (luteinizing hormone, follicle-stimulating hormone) and hCG, which share a common α subunit with TSH (Pierce 1971). Currently, RIA techniques are only used for the determination of TSH on filter paper dry blood spots for the screening of neonatal hypothyroidism.

At present, serum TSH is measured with immunometric assays. These assays use two antibodies to produce a "sandwich"-type assay in which one antibody (usually directed against the α subunit) serves to anchor the TSH molecule on a solid matrix. The other one (usually a monoclonal antibody directed against the β subunit)

is radioiodinated (immunoradiometric assay) or conjugated with an enzyme (immunoenzymatic) or a chemiluminescent compound (chemiluminescent assay). In these assays, the signal generated is directly proportional to the concentration of TSH in the serum. This technique is considered more specific, sensitive, and rapid than a radioimmunoassay, but not all immunometric TSH assays are equally sensitive and specific. A useful functional categorization is based on the minimal detectable TSH concentration that can be quantified with a between-run coefficient of variation not exceeding 20%. The term *generation* has been employed to categorize each assay with respect to its sensitivity. Each successive generation offers a 10-fold improvement in sensitivity. The *first* generation (TSH radioimmunoassay) had a lower limit of detection of approximately 1 mU/L, whereas the *third* generation assays have a detection limit of 0.004–0.01 mU/L (Spencer et al. 1996a).

The sensitivity of the current generation of TSH assays rarely results in false or artifactual measurements of TSH. However, even these assays may be prone to interference. An uncommon source of artifactual measurement of TSH is the presence in the serum sample of heterophilic antibodies induced by immunization with materials contaminated with animal serum, or the presence of rheumatoid factor (Despres and Grant 1998). Discrepant results may be due to the presence of antibodies directed against the animal immunoglobulins used in the assay. These immunoglobulins act to bind the anchoring or detecting antibodies and lead to an overestimation of TSH. This effect can be blocked by the addition of an excess of nonspecific immunoglobulin from the same animal species (Brennan et al. 1987).

One of the first steps in assessing discrepancies between the clinical classification and thyroid function tests is to measure the thyroid hormones and/or TSH on different platforms to determine whether the abnormality can be reproduced. In considering the presence of an interfering substance in the measurement of TSH (or T4 and T3), it is helpful to examine the linearity of recovery in serial dilutions of the patient's serum with appropriate diluents.

In the presence of a normally functioning hypothalamic-pituitary axis, there is an inverse correlation between the serum concentration of FT4 and TSH. Changes in the serum concentration of TT4, as a result of TBG abnormalities or drugs competing with T4 binding to TBG, have no effect on the level of serum TSH. The pituitary is exquisitely sensitive to both minimal decreases and increases in thyroid hormone concentration, with a logarithmic change in TSH levels in response to changes in T4 (Fig. 4). Thus, serum TSH responses to changes in serum FT4 concentrations are amplified. TSH levels should be elevated in patients with primary hypothyroidism and low or undetectable in thyrotoxicosis. Indeed, in the absence of hypothalamic pituitary disease, systemic illness, or drug interference, TSH is an accurate indicator of thyroid hormone status and the adequacy of thyroid hormone replacement.

In normal subjects, serum TSH concentrations show a typical skewed distribution. In order to achieve an accurate estimate of the lower normal limit, the serum TSH reference range should be calculated after logarithmic transformation (Fig. 5). Circulating TSH shows a circadian rhythm with a peak between 11 pm and 5 am and a nadir between 5 pm and 8 pm (Brabant et al. 1987). Secretory pulses occur every 2–3 h and are interspersed with periods of tonic nonpulsatile TSH secretion.

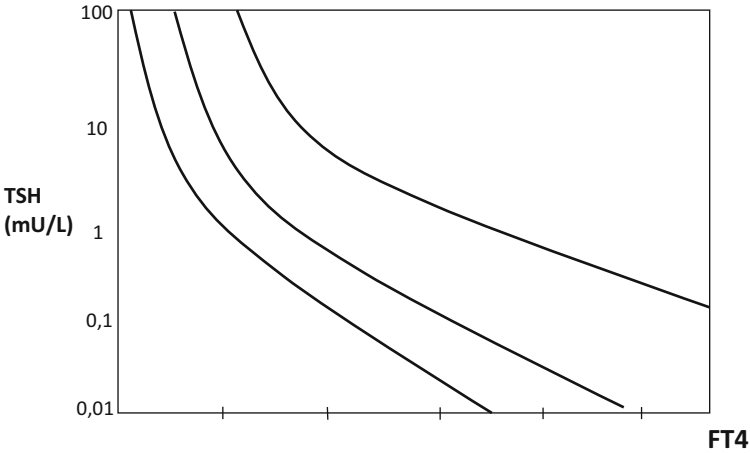

Fig. 4 Logarithmic correlation between serum TSH concentrations and FT4 levels. Curves from three representative subjects are depicted to show the considerable variability among normal subjects due to individual set points

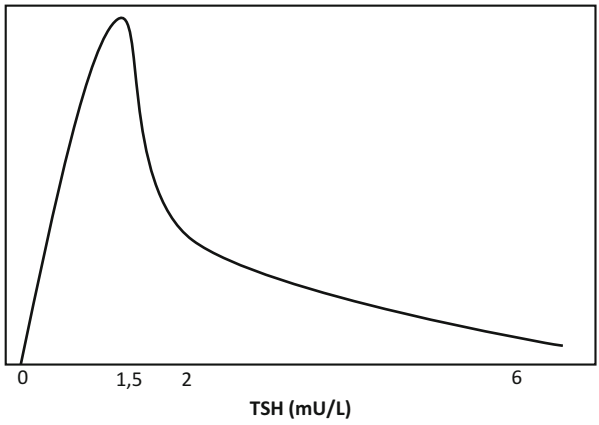

Fig. 5 Serum TSH concentrations in normal subjects show a typical skewed distribution. In order to achieve an accurate estimate of the lower normal limit, the serum TSH reference range should be calculated after logarithmic transformation

Although TSH secretion is pulsatile, the low amplitude of the pulses and the 30′ half-life of TSH results in only modest circulatory variations. These fluctuations are rarely critical for the clinical evaluation of thyroid function (Persani et al. 1995), but blood sampling for TSH assessment should be avoided during the night or early in the morning.

The largest (16.088 subjects) cross-sectional study of a normal population, the NHANES III study, using a chemiluminescence-based assay with a working range between 0.01 mIU/L and 50 mIU/L, has defined the 95% reference limit of TSH as 0.45–4.1 mU/L (Hollowell et al. 2002) (Table 3). Using current third-generation

immunoenzymatic methodology, the lower TSH reference limit has now been set between 0.3 and 0.4 mIU/L. It is worth remembering that in the first trimester maternal hCG stimulates the TSH receptor thereby increasing thyroid hormone production and secretion causing a reduction in serum TSH concentration (Glinoer 1997). Therefore, during pregnancy, women have lower serum TSH concentrations than before pregnancy, and a TSH below the nonpregnant lower limit of 0.4 mU/L is observed in as many as 15% of healthy women during the first trimester of pregnancy (Alexander et al. 2017).

In contrast, the upper TSH reference limit (97.5%) has become controversial with estimates ranging from 2.1 mIU/L to 7.5 mIU/L (Wartofsky and Dickey 2005). Multiple factors influence the calculation of the upper TSH reference limit in a population. These include population demographics (such as sex, ethnicity, iodine intake, BMI, smoking status, and age) as well as the failure to exclude with certainty the presence of subclinical autoimmune thyroid disease, goiter, or interfering medication. Complicating these aspects is the fact that current TSH immunometric assays differ in specificity for recognizing circulating TSH isoforms, accounting for up to a 1.0 mIU/L difference, or in some cases greater than the influence of many of the other variables. The TSH upper reference limit remains a contentious issue for both pregnant and nonpregnant subjects. This has led to guidelines proposing the adoption of an empiric TSH upper limit of 2.5–3.0 mIU/L, which is in agreement with the TSH interval found in selected groups with the lowest prevalence of thyroid antibodies.

Current guidelines recommend that serum TSH be used as the first-line test for detecting both overt and subclinical hypo- and hyperthyroidism in ambulatory patients with presumably intact hypothalamic/pituitary function (Garber et al. 2012; Ross et al. 2016). For this reason, laboratory algorithms have been developed to employ the automatic linking with free thyroid hormone measurements only if the initial TSH measurement is outside the normal range (TSH reflex). Importantly, it is worth remembering that TSH measurement alone often fails to detect the presence of pituitary and/or hypothalamic disease (central hypothyroidism or TSH-secreting pituitary tumors). In fact, in these conditions serum TSH concentrations are usually within the normal reference limits, and only the concomitant evaluation of FT4 and FT3 levels can unveil the underlying disease. The analysis of the factors which may influence serum TSH concentrations and the diagnostic criteria of hypothyroidism are discussed elsewhere (see ▶ Chap. 13, “Diagnosis and Treatment of Hypothyroidism” by Asharaff and Razvi).

Thyrotropin-Releasing Hormone (TRH) Stimulation Test

TRH is a tripeptide (pyroglutamyl-histidyl-prolineamide) physiologically synthesized by neurons localized in the supraoptic and supraventricular nuclei of the hypothalamus. TRH is stored in the median eminence of the hypothalamus and reaches the anterior pituitary where it controls the release of TSH (Anderson et al. 1971) via the portal venous system. TRH tonic action induces the transcription of TSH subunits and the posttranslational glycosylation of TSH which is critical for its

biological activity. The thyrotropin-releasing hormone (TRH) test is based on serial measurements of TSH concentrations (at 0, 15, 30, 60, and 120 min), after the administration of synthetic TRH (Hollander et al. 1972). TRH acts as an amplifier to exaggerate any underlying abnormality in TSH secretion by the pituitary. A bolus dose of 200–400 μg is usually administered as a single intravenous (IV) infusion. In normal individuals, the serum TSH concentration rises rapidly threefold to fivefold, reaches a peak in 20–30 min, and returns to basal values within 2–3 h. Normal increments range between 5 and 30 mU/L and average about 12 mU/L. The absence of a significant threefold to fivefold (or above 7 mU/L) rise in TSH concentration is compatible with pituitary or hypothalamic disease or thyrotoxicosis. With the introduction of super-sensitive TSH assays, the TRH dynamic testing has become unnecessary for the diagnosis of thyrotoxicosis. A delayed and stunted rise may be observed in the presence of hypothalamic disease. TRH-induced secretion of TSH is followed by the release of thyroid hormones that can be detected by direct measurement of their serum concentrations; peak levels are normally reached approximately within 4 h after the administration of TRH. The incremental rise in serum TT3 is relatively greater, and the peak is on average 50% above the basal level. Measurement of changes in serum thyroid hormone concentrations, after the administration of TRH, has been proposed as an indicator of the integrity of the thyroid gland or the bioactivity of endogenous thyroid hormones (Shenkman et al. 1972). Side effects related to the intravenous administration of TRH, in decreasing order of frequency, include nausea, flushing or a sensation of warmth, urge to urinate, peculiar taste, lightheadedness or headache, dry mouth, urge to defecate, and chest tightness. These symptoms are usually mild, begin within 1 min after the injection of TRH and last for a few seconds to several minutes. A transient rise in blood pressure has been observed on occasion, but no other changes are observed in vital signs, urine analysis, blood count, or routine blood chemistry tests (Anderson et al. 1971). There have been case reports describing pituitary apoplexy in patients who underwent TRH testing, in some cases with the co-administration of gonadotropin-releasing hormone (GnRH). In most of these patients a large pituitary tumor was present, and this condition, which may predispose to the complication, should therefore induce caution in performing the test (Masago et al. 1995).

Responses to TRH are decreased by treatment with glucocorticoids, levodopa, dopamine, somatostatin, or dopamine analogues, and responses are augmented by the dopaminergic antagonists metoclopramide and domperidone. The negative feedback inhibition of basal TSH secretion or TRH-induced TSH release is so exquisitely sensitive that doses of exogenous hormone, insufficient to increase the serum T4 or T3 concentrations above the normal range, can decrease the TSH response to TRH. Conversely, small decreases in serum T4 concentrations cause increased basal serum TSH concentrations and an increased response to TRH administration. An elevation in both serum TSH and free T4 is compatible with a TSH-producing pituitary tumor, resistance to thyroid hormone action (RTH), or hyperthyroidism with an artifactual increase in TSH levels. TRH does not increase falsely elevated TSH levels. A differentiation between hypothalamic and pituitary causes of central hypothyroidism by TRH testing is not possible. Other diagnostic procedures such as magnetic resonance imaging (MRI) are required for a definitive

evaluation of such patients, but the test is still used in the differential diagnosis between RTH and TSH-producing pituitary tumors.

Thyroglobulin (Tg)

Thyroglobulin (Tg) is a 660 kD glycoprotein containing 134 thyrosil residues, some of which are accessible for iodination and establishing the backbone for thyroid hormone synthesis. Tg, stored in colloid of the thyroid follicles, is detectable in the serum of most individuals when a sensitive method is used (Van Herle et al. 1973). In the serum of normal subjects Tg levels range from <1 to 25 ng/ml (<1.5–38 pmol/L) (Van Herle et al. 1973). Tg concentrations reflect three major factors: the mass of differentiated thyroid tissue, possible degrees of thyroid injury (which causes the release of Tg), and the extent of stimulation of the TSH receptor (Refetoff and Lever 1983). An elevated serum Tg is a very nonspecific indicator of thyroid dysfunction. Furthermore Tg determination may be inaccurate in case of the coexistence of TgAb (Spencer et al. 1998). Most patients with elevated serum Tg have benign thyroid conditions, such as endemic and sporadic nontoxic goiter. Serum Tg roughly correlates with the thyroid size. Transient elevation occurs in patients with subacute thyroiditis and after 131-I therapy.

Serum Tg concentration is increased in patients with benign as well as differentiated follicular cell–derived cancers, and are not employed diagnostically to distinguish between the two. After total thyroid ablation for papillary or follicular thyroid carcinoma, Tg should not be detectable, and its subsequent appearance typically signifies the presence of persistent or recurrent thyroid tissue.

A major clinical indication for measuring serum Tg is thus in the management, but not in the diagnosis, of differentiated thyroid carcinoma (DTC), since Tg is a sensitive marker of the possible persistence/recurrence of the neoplastic disease. Tg measurement is also an aid in the differential diagnosis of thyrotoxicosis with low thyroid radioiodine uptake. Very low or undetectable concentrations of Tg are found in patients with thyrotoxicosis factitia (Mariotti et al. 1982) while destructive forms such as subacute thyroiditis or postpartum thyroiditis are characterized by increased Tg (Smallridge et al. 1986).

In patients with DTC, serum Tg level is directly related to the mass of neoplastic tissue and secretion of Tg is TSH-dependent. Therefore, the serum Tg level may rise when thyroid hormone therapy is withdrawn or after injection of rhTSH, which will increase the sensitivity of the marker for the detection of persistent or recurrent thyroid carcinoma, even when 131-I scans are negative. Supersensitive assays of serum Tg with a functional sensitivity of less than 0.1 ng/mL improve the sensitivity during thyroid hormone treatment but at the expense of a decreased specificity (Francis and Schlumberger 2008). When the TSH level is stable during L-T4 therapy, any change in the serum Tg level will reflect a change in tumor mass. The pattern of serial serum Tg measurements, made when the patient has a stable TSH, is clinically more useful than an isolated Tg value (48). In TgAb positive patients, serial TgAb measurements (by immunoassay) are valuable as a surrogate tumor marker test (Spencer 2011).

Currently, immunometric assays (IMA) are more widespread than radioimmunoassays (RIA). This is because IMA offer the practical advantage of a shorter incubation time, an extended dynamic range for the assay, and a more stable labeled antibody reagent that is less prone to labeling damage. Laboratories can now choose from a range of both isotopic (immunoradiometric, IRMAs) and nonisotopic (primarily chemiluminescence, ICMA) IMA methods. However, IMA methods are more susceptible to interference by Tg autoantibodies (TgAb), which may cause an underestimation of serum Tg levels, although possible interference is not merely a reflection of TgAb titres. Novel methods based on liquid chromatography/mass spectrometry have been suggested to reliably measure Tg levels in the presence of TgAb (Clarke et al. 2012; Hoofnagle and Roth 2013; Kushnir et al. 2013), but their efficacy seems limited.

There are five methodologic problems that can impair the clinical utility of the Tg measurement: (a) between-method biases, (b) functional sensitivity, (c) "hook" effect (some IMA methods), and interference caused by (d) Human Anti-Mouse Antibody (HAMA) and (e) Tg autoantibodies (TgAb) (Spencer and Lopresti 2008).

Between-Method Biases and Functional Sensivity

The bias between different Tg methods may result from differences between the Tg-free matrix used to dilute standards and patient serum, or differences in the epitope recognition by the different Tg antibodies used by the different manufacturers. A recent collaborative effort, sponsored by the Community Bureau of Reference of the Commission of the European Communities, has developed a new international Tg reference preparation, CRM-457. The widespread adoption of the CRM-457 standard was projected to reduce the considerable method-to-method variability that exists with this procedure, and it is important that assays used in the follow up of DTC are calibrated against the CRM-457 international standard (Haugen et al. 2016). Currently, serum Tg levels determined by methods that use CRM-457 standards can differ by as much as fourfold. These method-to-method differences are greater than the goal for maximum imprecision required for monitoring individual patients and precludes the interchangeable use of different Tg methods for long-term follow up of thyroid cancer patients.

Methods that are unable to detect Tg in normal sera are usually too insensitive for monitoring DTC patients for recurrence. As pointed out in international guidelines (Haugen et al. 2016), it is critical that assay sensitivity is assessed in terms of *functional sensitivity* and not analytical sensitivity.

Tg Antibody Interference

TgAb are detected in a percentage of DTC patients which is higher than the general population (~20 vs. ~10%, respectively). All sera sent for Tg measurement should be prescreened for the presence of TgAb because even very low serum TgAb levels can interfere with Tg measurement (Haugen et al. 2016). IMA assays are the most

sensitive methods for Tg measurement currently available. In the presence of TgAb, Tg values are lower than expected or even undetectable when measured by IMAs. Tg RIA assays are more resistant to TgAb interference (Stanojevic et al. 2009). On the other hand Tg RIA assays can yield false positive Tg results (Stanojevic et al. 2009), are less widely available, and may be less sensitive than immunometric assays in detecting small amounts of residual tumor. Therefore, their role in the clinical care of cancer patients is uncertain (Haugen et al. 2016). Tandem mass spectrometry, the latest methodology proposed, also seems of limited efficacy. In clinical practice, TgAb are used as a surrogate marker for Tg measurement, because their levels correlate with the clinical status of DTC patients and their disappearance correlate with the complete ablation of thyroid tissue.

Human Anti-Mouse Antibodies Interference

As with other immunometric assays, the presence of circulating anti-mouse antibodies (heterophilic antibodies) interfere with Tg IMA but not Tg measurements made by RIA. Interference is thought to reflect inappropriate binding of the murine-derived monoclonal antibodies used in IMA methods, as opposed to the rabbit polyclonal antibodies typically used in RIAs. In most cases interference is characterized by a false positive result. However, false negative interference has also been reported.

"Hook" Effect

Falsely low Tg values due to a "hook effect" are especially problematic when Tg is utilized as a tumor marker. It is not unusual to encounter very high Tg values when patients have advanced metastatic disease. A hook effect occurs when an excessive amount of antigen overwhelms the binding capacity of the capture antibody. This results in an inappropriately low signal that translates into an inappropriately low or paradoxically normal range result for a patient with an excessively elevated serum Tg concentration (>1000 μg/L). The hook effect is principally encountered in IMA methods. Manufacturers of IMA methods attempt to overcome the hook effect problem by one of two approaches:

- Two-step assay design. The serum specimen is first reacted with the capture antibody before unbound constituents are washed away and the labeled antibody is introduced, followed by a second incubation.
- Two different dilutions (usually undiluted and 1/10) are made for each specimen to identify any "hook."

A "hook" is suspected when the dilution tube has a higher Tg-value than the undiluted specimen. Further dilutions are made until the result in the dilution tube decreases and the serum Tg concentrations of the two dilutions are in agreement (Spencer et al. 1996b).

Urinary Iodine

Urinary iodine excretion, at equilibrium, is the best indicator of dietary intake of this microelement. This measurement can be used to assess the intake of patients before treatment with radioactive iodine and in case of thyrotoxicosis, especially amiodarone-induced, or when studied at the population level, as an indicator of the iodine intake. In fact, urinary iodine excretion is one of the parameters used in epidemiology to classify populations with insufficient, adequate, or excessive iodine intake.

The most widely used method of measurement is the so-called Sandell-Kolthoff reaction, which is easy to perform, inexpensive, and only requires spot urine specimens. The Sandell-Kolthoff method is based on the reduction of yellow cerium ion +4 (Ce^{4+}) by arsenic ion (As^{3+}) to colorless cerium ion +3 (Ce^{3+}), a reaction which is usually very slow but catalized by the presence of trace amounts of iodide (Pierce 1971). Digestion of urine aliquots by ammonium persulfate is carried out at approximately 100 °C followed by the addition of arsenous acid and ceric ammonium sulfate. A change in color of the urine is observed and quantified by a spectrophotometer and a standard curve built with known amounts of iodine. Small amounts of urine (0.5–1 ml) are sufficient for an accurate measure, and samples may be frozen until assayed, if needed.

An alternative method implies the substitution of ammonium persulfate with chloric acid (but the chemical mixture can be explosive if residues dry in ventilating systems). The coefficient of variation of the assays is less than 10%.

Semiquantitative methods are also available and based on calorimetric reactions on paper strips indicating gross ranges of urinary iodine. This type of assay requires running samples through prepacked columns activated with charcoal. All the above methods recognize urinary iodine concentrations in the range of 50–200 μg/l, but samples can be diluted if necessary. At steady state, a urinary iodine concentration of 100 μg/l corresponds roughly to a daily iodine intake of about 150 μg. Correction of iodine for urinary creatinine level is unreliable and thus unnecessary.

A novel method of assessment is based on the use of inductively coupled plasma mass spectrometry (ICP-MS). Urine needs to be diluted due to the high concentration of salts, but the sample does not need other manipulations prior to the assay. ICP-MS requires expensive equipment and training of technicians, but the sensitivity of iodine measurements is higher than that for other techniques used so far and has a very broad dynamic range (Shelor and Dasgupta 2011).

Radioactive Iodine Uptake (RAIU)

This test is based on the administration (oral or IV) of radioactive isotopes (^{131}I or ^{123}I) of the stable form of iodide (^{127}I), to determine the level of uptake of the radiotracer by the thyroid tissue. When the radioactive tracer is given to the patient it mixes with the endogenous iodide pool and is taken up by the thyroid in the same manner as the stable isotope. After the radio-tracer administration the intrathyroidal content of it gradually increases over time while the extrathyroidal pool decreases until becoming undetectable. RAIU is an indicator of the rate of thyroid hormone

synthesis and release into the circulation. Among the factors that may influence the thyroid iodine uptake cardiac or kidney insufficiency, drugs or products containing iodine, thyroid hormone treatment or ingestion without a medical prescription, antithyroid drugs, and soybean ingestion are worth mentioning. Administration of radioisotopes during pregnancy or breastfeeding is contraindicated because the tracer is transported across the placenta and excreted into the milk.

The two radioactive isotopes are both gamma radiation emitters, but the half-life is longer for ^{131}I and the radiation delivered by ^{123}I much less, rendering this form preferable especially for tests conducted in children (Quimby et al. 1970).

The uptake is usually measured few hours after the administration (3–6 h) and again after 20–28 h. Since the value at 24 h is considered at plateau, usually measurements of RAIU are made at this time point, which is also convenient in the clinical setting. Measuring RAIU at plateau of isotope accumulation is critical since it increases the sensitivity of this measurement. Normal uptake values are in the range of 5–25%, based on the iodine intake level of the individual subjects. Lower normal uptake levels are recorded in individuals and populations with higher dietary iodine intakes. In populations with low iodine intake the normal RAIU at 24 h may reach 50%.

Higher levels may be measured in patients with thyroid hyperfunction or sometimes after recovery from thyroid function suppression. Lower than normal values may be observed in case of thyroid hormone biosynthesis defects, primary hypothyroidism, iodide contamination, and painless or subacute thyroiditis.

Perchlorate Discharge Test

Perchlorate discharge test is a rarely used dynamic test, which assesses the efficiency of iodide organification by the thyroid. The perchlorate discharge test is performed by administering a radioiodine (I^{123}) tracer dose and measuring the uptake after 3–4 h. Thereafter, in adults, 1 g (500 mg in children younger than 6 years of age) of perchlorate ($KClO_4$) is administered orally and the radioiodine uptake repeated after 1 h. Perchlorate acutely blocks the sodium-iodide symporter (NIS), allowing nonorganified iodide, present inside the follicular cell, to leak outside. If the radioiodine uptake, conducted after perchlorate administration, shows a reduction of 15% or more, the test is considered positive and a thyroid organification defect is very likely. A radioiodine uptake value between 10 and 15%, after perchlorate administration, is considered borderline and may be due to a partial organification defect. A positive perchlorate discharge test is seen in patients with point or frame-shift mutations in the TPO gene, in Pendred's Syndrome (goitrous hypothyroidism and sensorineural hearing impairment), and in defects of the H_2O_2 generating system dual oxidase 2 (DUOX2).

Conclusions

Nowadays the assessment of thyroid function allows the diagnosis of subclinical alterations with high precision.

However, even the most sensitive free thyroid hormone and TSH assays may be prone to interference and artifactual measurement due to the presence in the serum sample of interfering factors. For this reason the lucid analysis of inexplicable discrepancies in thyroid function tests should lead to the reanalysis of such cases employing alternative platforms to determine whether the abnormality can be verified. When considering the presence of possible interfering factors, it is frequently helpful to examine the linearity of recovery in serial dilutions of the patient's serum with appropriate diluents. At this time, no prediction of validity and performance of any assay can be made based only on the measurement design. Recommendation protocols and guidelines for validation of assays under controlled conditions are necessary.

Cross-References

► Diagnosis and Treatment of Hypothyroidism

References

Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, Grobman W, Laurberg P, Lazarus JH, Mandel SJ, Peeters R, Sullivan S. 2017 guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and the postpartum. Thyroid. 2017;27(3):315–89.

Anderson MS, Bowers CY, Kastin AJ, Schalch DS, Schally AV, Snyder PJ, Utiger RD, Wilber JF, Wise AJ. Synthetic thyrotropin-releasing hormone. A potent stimulator of thyrotropin secretion in man. N Engl J Med. 1971;285(23):1279–83.

Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, LiVosli VA, Niccoli-Sire P, John R, Ruf J, Smyth PP, Spencer CA, Stockigt JR. Laboratory medicine practice guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid. 2003;13:1–126.

Bartalena L, Robbins J. Thyroid hormone transport proteins. Clin Lab Med. 1993;3:583–98.

Bartalena L, Bogazzi F, Brogioni S, Burelli A, Scarcello G, Martino E. Measurement of serum free thyroid hormone concentrations: an essential tool for the diagnosis of thyroid dysfunction. Horm Res. 1996;45(3–5):142–7.

Brabant G, Brabant A, Ranft U, Ocran K, Köhrle J, Hesch RD, von zur Mühlen A. Circadian and pulsatile thyrotropin secretion in euthyroid man under the influence of thyroid hormone and glucocorticoid administration. J Clin Endocrinol Metab. 1987;65(1):83–8.

Brennan MD, Klee GG, Preissner CM, Hay ID. Heterophilic serum antibodies: a cause for falsely elevated serum thyrotropin levels. Mayo Clin Proc. 1987;62(10):894–8.

Burman KD, Bongiovanni R, Garis RK, Wartofsky L, Boehm TM. Measurement of serum T4 concentration by high performance liquid chromatography. J Clin Endocrinol Metab. 1981;53(5):909–12.

Chopra IJ, Nelson JC, Solomon DH, Beall GN. Production of antibodies specifically binding triiodothyronine and thyroxine. J Clin Endocrinol Metab. 1971;32(3):299–308.

Christofides ND, Sheehan CP. Enhanced chemiluminescence labeled-antibody immunoassay (Amerlite-MAB) for free thyroxine: design, development, and technical validation. Clin Chem. 1995;41(1):17–23.

Clarke NJ, Zhang Y, Reitz RE. A novel mass spectrometry-based assay for the accurate measurement of thyroglobulin from patient samples containing antithyroglobulin autoantibodies. J Investig Med. 2012;60(8):1157–63.

Costongs GM, van Oers RJ, Leerkes B, Janson PC. Evaluation of the DPC IMMULITE random access immunoassay analyser. Eur J Clin Chem Clin Biochem. 1995;33(11):887–92.

Despres N, Grant AM. Antibody interference in thyroid assays: a potential for clinical misinformation. Clin Chem. 1998;44(3):440–54.

Dufour DR. Laboratory tests of thyroid function: uses and limitations. Endocrinol Metab Clin North Am. 2007;36:155–69.

Ekins R. Measurement of free hormones in blood. Endocr Rev. 1990;11(1):5–46.

Faix JD. Principles and pitfalls of free hormone measurements. Best Pract Res Clin Endocrinol Metab. 2013;27(5):631–45.

Faix JD, Miller WG. Progress in standardizing and harmonizing thyroid function tests. Am J Clin Nutr. 2016;104(Suppl 3):913S–7S.

Francis Z, Schlumberger M. Serum thyroglobulin determination in thyroid cancer patients. Best Pract Res Clin Endocrinol Metab. 2008;22(6):1039–46.

Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, Pessah-Pollack R, Singer PA, Woeber KA, American Association of Clinical Endocrinologists, American Thyroid Association Taskforce on Hypothyroidism in Adults. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. Thyroid. 2012;22(12):1200–35.

Glinoer D. The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology. Endocr Rev. 1997;18:404–33.

Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L. 2015 American Thyroid Association Management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: The American Thyroid Association Guidelines Task Force on thyroid nodules and differentiated thyroid cancer. Thyroid. 2016;26(1):1–133.

Hollander CS, Mitsuma T, Shenkman L, Woolf P, Gershengorn MC. Thyrotropin-releasing hormone: evidence for thyroid response to intravenous injection in man. Science. 1972;175(4018):209–10.

Hollowell JG, Staehling NW, Flanders WD, et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab. 2002;87:489–99.

Hoofnagle AN, Roth MY. Improving the measurement of serum thyroglobulin with mass spectrometry. J Clin Endocrinol Metab. 2013;98(4):1343–52.

Kundra P, Burman KD. The effect of medications on thyroid function tests. Med Clin North Amer. 2012;96:283–95.

Kushnir MM, Rockwood AL, Roberts WL, Abraham D, Hoofnagle AN, Meikle AW. Measurement of thyroglobulin by liquid chromatography-tandem mass spectrometry in serum and plasma in the presence of antithyroglobulin autoantibodies. Clin Chem. 2013;59(6):982–90.

Letellier M, Lévesque A, Daigle F, Grant A. Performance evaluation of automated immunoassays on the Technicon Immuno 1 system. Clin Chem. 1996;42(10):1695–701.

Li Calzi L, Benvenga S, Battiato S, Santini F, Trimarchi F. Autoantibodies to thyroxin and triiodothyronine in the immunoglobulin G fraction of serum. Clin Chem. 1988;34(12):2561–2.

Mariotti S, Martino E, Cupini C, Lari R, Giani C, Baschieri L, Pinchera A. Low serum thyroglobulin as a clue to the diagnosis of thyrotoxicosis factitia. N Engl J Med. 1982;307(7):410–2.

Masago A, Ueda Y, Kanai H, Nagai H, Umemura S. Pituitary apoplexy after pituitary function test: a report of two cases and review of the literature. Surg Neurol. 1995;43(2):158–64.

Nelson JC, Tomei RT. Direct determination of free thyroxin in undiluted serum by equilibrium dialysis/radioimmunoassay. Clin Chem. 1988;34(9):1737–44.

Nuutila P, Koskinen P, Irjala K, Linko L, Kaihola HL, Eskola JU, Erkkola R, Seppälä P, Viikari J. Two new two-step immunoassays for free thyroxin evaluated: solid-phase radioimmunoassay and time-resolved fluoroimmunoassay. Clin Chem. 1990;36(7):1355–60.

Pappa T, Ferrara AM, Refetoff S. Inherited defects of Thyroid hormone binding proteins. Best Pract Res Clin Endocrinol Metab. 2015;29(5):735–47.

Persani L, Terzolo M, Asteria C, Orlandi F, Angeli A, BeckPeccoz P. Circadian variations of thyrotropin bioactivity in normal subjects and patients with primary hypothyroidism. J Clin Endocrinol Metab. 1995;80:2722–8.

Pierce JG. Eli Lilly lecture. The subunits of pituitary thyrotropin – their relationship to other glycoprotein hormones. Endocrinology. 1971;89(6):1331–44.

Quimby EH, Feitelberg S, Gross W. Radioactive nuclides in medicine and biology. 3rd ed. Philadelfia: Lea & Febiger; 1970.

Refetoff S, Lever EG. The value of serum thyroglobulin measurement in clinical practice. JAMA. 1983;250(17):2352–7.

Ross DS, Burch HB, Cooper DS, Greenlee MC, Laurberg P, Maia AL, Rivkees S, Samuels M, Sosa JA, Stan MN, Walter M. 2016 American Thyroid Association guidelines for diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. Thyroid. 2016;26(10):1343–421.

Shelor CP, Dasgupta PK. Review of analytical methods for the quantification of iodine in complex matrices. Anal Chim Acta. 2011;702(1):16–36.

Shenkman L, Mitsuma T, Suphavai A, Hollander CS. Triiodothyronine and thyroid-stimulating hormone response to thyrotrophin-releasing hormone. A new test of thyroidal and pituitary reserve. Lancet. 1972;1(7742):111–2.

Smallridge RC, De Keyser FM, Van Herle AJ, Butkus NE, Wartofsky L. Thyroid iodine content and serum thyroglobulin: cues to the natural history of destruction-induced thyroiditis. J Clin Endocrinol Metab. 1986;62(6):1213–9.

Smith TJ, Hegedüs L. Graves' Disease. New England J Med. 2016;375:1552–66.

Soldin OP, Soldin SJ. Thyroid hormone testing by tandem mass spectrometry. Clin Biochem. 2011;44:89–94.

Spencer C. Clinical utility of thyroglobulin antibody (TgAb) measurements for patients with differentiated thyroid cancers (DTC). J Clin Endocrinol Metab. 2011;96:3615–27.

Spencer CA, Lopresti JS. Measuring thyroglobulin and thyroglobulin autoantibody in patients with differentiated thyroid cancer. Nat Clin Pract Endocrinol Metab. 2008;4(4):223–33.

Spencer CA, Takeuchi M, Kazarosyan M. Current status and performance goals for serum thyrotropin (TSH) assays. Clin Chem. 1996a;42(1):140–5.

Spencer CA, Takeuchi M, Kazarosyan M. Current status and performance goals for serum thyroglobulin assays. Clin Chem. 1996b;42(1):164–73.

Spencer CA, Takeuchi M, Kazarosyan M, Wang CC, Guttler RB, Singer PA, Fatemi S, LoPresti JS, Nicoloff JT. Serum thyroglobulin autoantibodies: prevalence, influence on serum thyroglobulin measurement, and prognostic significance in patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab. 1998;83(4):1121–7.

Stanojevic M, Savin S, Cvejic D, Dukic A, Zivancevic SS. Correlation of thyroglobulin concentrations measured by radioimmunoassay and immunoradiometric assay and the influence of thyroglobulin antibody. J Immunoassay Immunochem. 2009;30:197–207.

Stockigt J. Assessment of thyroid function: towards an integrated laboratory-clinical approach. Clin Biochem Rev. 2003;24(4):109–22.

Stockigt JR, Lim CF. Medications that distort in vitro tests of thyroid function, with particular reference to estimates of serum free thyroxine. Best Pract Res Clin Endocrinol Metab. 2009;23(6):753–67.

Tai SS, Sniegoski LT, Welch MJ. Candidate reference method for total thyroxine in human serum: use of isotope-dilution liquid chromatography-mass spectrometry with electrospray ionization. Clin Chem. 2002;48(4):637–42.

Thienpont LM, De Brabandere VI, Stöckl D, De Leenheer AP. Development of a new method for the determination of thyroxine in serum based on isotope dilution gas chromatography mass spectrometry. Biol Mass Spectrom. 1994;23(8):475–82.

Thienpont LM, Van Uytfanghe K, Van Houcke S, IFCC Working Group for Standardization of Thyroid Function Tests (WG-STFT). Standardization activities in the field of thyroid function tests: a status report. Clin Chem Lab Med. 2010;48(11):1577–83.

Thienpont LM, Van Uytfanghe K, Poppe K, Velkeniers B. Determination of free thyroid hormones. Best Pract Res Clin Endocrinol Metab. 2013;27(5):689–700.

Van Herle AJ, Uller RP, Matthews NI, Brown J. Radioimmunoassay for measurement of thyroglobulin in human serum. J Clin Invest. 1973;52(6):1320–7.

Wartofsky L, Dickey RA. The evidence for a narrower thyrotropin reference range is compelling. J Clin Endocrinol Metab. 2005;90(9):5483–8.

Wilkins TA, Midgley JE, Barron N. Comprehensive study of a thyroxin-analog-based assay for free thyroxin ("Amerlex FT4"). Clin Chem. 1985;31(10):1644–53.

Thyroid Autoantibodies

3

R. A. Ajjan and A. P. Weetman

Contents

Abstract

Thyroid autoantibodies are directed predominantly against thyroglobulin, thyroid peroxidase and the TSH receptor, although other components of the thyroid cell

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may also be autoantigenic targets in a small proportion of patients. In terms of pathogenesis, any role for thyroglobulin or thyroid peroxidase antibodies is confined to the secondary phase of autoimmune destruction. In contrast, stimulating antibodies against the TSH receptor cause Graves' disease, and, in a small proportion of patients with autoimmune hypothyroidism, blocking antibodies against the TSH receptor are responsible for the thyroid dysfunction. This chapter reviews the pathogenic role of thyroid antibodies in autoimmune thyroid disorders, describes the various methods used to detect these antibodies, and describes their clinical utility in diagnosis and patient management, including the use of thyroid antibody measurement in relation to pregnancy and the postpartum period. It also reviews the effect of thyroglobulin antibodies on the measurement of serum thyroglobulin and the consequences for the management of patients with differentiated thyroid cancer.

Keywords
Thyroglobulin antibody · Thyroid peroxidase antibody · TSH receptor antibody · Graves' disease · Autoimmune hypothyroidism · Pregnancy · Postpartum thyroiditis · Pendrin antibody · Sodium iodide symporter antibody · Thyroid hormone antibody

Introduction

Autoimmune disease is characterized by activation of humoral and cellular immune responses against a self-antigen. The production of antibodies is the hallmark of autoimmune conditions, which can be used for diagnosis and monitoring of disease progression and may even have a future role in developing immune-based therapies. Autoimmune thyroid disease (ATD) encompasses an array of organ-specific autoimmune conditions in which antibodies can be detected in sera against various thyroid antigens. Thyroid antibodies may contribute to propagation of the autoimmune disease and can also have a functional role, directly modulating thyroid hormone synthesis. In particular, antibodies against the thyroid-stimulating hormone receptor (TSHR) can activate or block the function of the receptor directly causing hyper- or hypothyroidism, respectively.

A prerequisite for the synthesis of thyroid hormones is the interaction of the pituitary thyroid-stimulating hormone (TSH) with its receptor, consequently triggering hormone production by thyroid follicular cell. The first step in this process is the uptake of iodine by thyroid cells, mediated by the sodium iodide symporter (NIS). Pendrin is responsible for efflux of iodine into the thyroid follicles, which is subsequently incorporated into thyroglobulin (TG). Protein iodination within TG is a process mediated by thyroid peroxidase (TPO), thereby forming monoiodotyrosine and diiodotyrosine and later the thyroid hormones T3 and T4. All of these molecules have been shown to represent thyroid autoantigens and have a variable role in thyroid autoimmunity. This chapter reviews the role of thyroid

autoantibodies in the pathogenesis of ATD and describes the clinical use of these antibodies in the management of thyroid dysfunction, extrathyroidal complications of autoimmune thyroid disease, and thyroid malignancy.

Thyroglobulin Antibodies (TG-Ab)

The Antigen

TG was the very first autoantigen to be described; the now classic experiments undertaken by Rose and Witebsky in 1956 (Rose and Witebsky 1956) showed that immunization of rabbits with thyroid extract led to the production of TG-Ab and a lymphocytic thyroiditis (around three-quarters of thyroid protein is TG). Later the same year, Roitt and Doniach identified TG-Ab in the circulation of patients with Hashimoto's thyroiditis, using the classical immunoprecipitation method (Roitt et al. 1956). The failure of these antibodies to fix complement was also demonstrated very early on Roitt et al. (1958).

TG is a homodimeric 660 kD glycosylated iodoprotein (0.1–1.1% iodine) which is synthesized in thyroid cells and accumulates in the thyroid follicular lumen where it is the major component of thyroid colloid. The major function of TG is in the synthesis of T3 and T4, which are produced as a result of TPO-mediated iodination and coupling of several specific, hormonogenic tyrosine residues within TG, once the molecule is within the follicular lumen (Targovnik 2013). Iodination status and the activity of the thyroid gland determine the exact composition of TG within the lumen, with multimeric and aggregate forms of TG being predominant in the center. Follicular TG is subsequently endocytosed, and thyroid hormones are released by this mechanism into the circulation. Some TG is also able to enter the circulation under normal circumstances: levels rise with thyroid overactivity or excess thyroid tissue (as in functional thyroid cancer metastases) and decline with underactivity.

TG was cloned and sequenced in 1987 (Malthiery and Lissitzky 1987), leading to description of four domains within the protein; domain D has homology with acetylcholinesterase, leading to speculation that immunological cross-reactivity with the latter protein in the orbit may account for the association between thyroid disease and Graves' ophthalmopathy (GO) (Ludgate et al. 1989). However, although acetylcholinesterase antibodies can be detected in some patients with thyroid autoimmunity, these antibodies show no correlation with TG-Ab and are not associated with clinical ophthalmopathy (Weetman et al. 1988). A range of TG polymorphisms has been defined and some of these, in exons 10–12 and exon 33, have been associated with an increased risk of thyroid autoimmunity (Hasham and Tomer 2012).

Immunization of animals with xenogeneic TG leads to the production of antibodies to many epitopes, but true autoantibodies are rather species restricted (Roitt et al. 1958), and, in man, initial studies showed that each TG subunit has two major epitopes and one minor epitope for autologous autoantibodies (Nye et al. 1980). TG epitopes recognized by human autoantibodies are usually conformational and have

Table 1 Characteristics of the main thyroid autoantigens

	TSHR	TPO	TG
Chromosomal location	14	2	8
Amino acids	743	933	2748
Protein	G protein-coupled receptor	Hemoprotein enzyme	Iodinated glycoprotein
Molecular weight (kDa)	85	105–110	660
Function	Receptor for TSH	Iodination and coupling of tyrosine to form thyroid hormones	Storage of T3 and T4
Homologies	FSH and LH receptor	Myeloperoxidase	Acetylcholinesterase

been further investigated by using panels of monoclonal antibodies to inhibit the binding of autoantibodies to TG (Fukuma et al. 1991; Estienne et al. 1998). Using such approaches, a number of overlapping epitopes have been identified, and although there is no difference in epitope recognition between sera from patients with Graves' disease (GD) and Hashimoto's thyroiditis, sera from healthy subjects who incidentally have TG-Ab do have a different pattern (Latrofa et al. 2008). The main characteristics of TG are summarized in Table 1.

Role of TG-Ab in Disease Pathogenesis

There is less evidence for a critical role for TG-Ab in disease pathogenesis than there is for antibodies against the TSHR or TPO. TG-Ab in ATD are predominantly IgG class, distributed across all four IgG subclasses. The proportion which is of the non-complement-fixing IgG4 subclass is insufficient to explain the failure of these antibodies to fix complement (Adler et al. 1984). Using enzyme-linked immunosorbent assay (ELISA)-based methods, a distinct IgG4 subclass preponderance of TG-Ab has been noted, but using the more quantitative method of affinity purification of IgG subclasses, it has been found that while IgG4 subclass antibodies are overrepresented, their levels are exceeded by those of the IgG1 and IgG2 subclasses (Weetman et al. 1989). It appears that TG-Ab do not bind complement because the widely scattered epitopes on TG do not permit antibody cross-linking.

Although non-complement fixing, a pathogenic role for TG-Ab has been postulated via antibody-dependent cell-mediated cytotoxicity (ADCC), in which antigen-coated target cells incubated with antibody are destroyed by natural killer (NK) cells and macrophages, which bind to the target cells via Fc receptors (Suzuki et al. 1980). Such assay systems are an in vitro construct, and it is unclear whether TG-Ab could even access follicular TG in vivo or how such complexes could be surface bound. It is noteworthy that transplacental passage of maternal TG (or TPO) autoantibodies has no detectable cytotoxic effect on the fetal thyroid.

Healthy individuals often have low affinity, mainly IgM class TG-Ab (so-called "natural" autoantibodies) which are able to bind TG and complement. These opsonized complexes have been shown to be readily taken up by B cells, and this allows efficient autoantigen presentation to T cells, which could be critical in initiating disease (Nielsen et al. 2001). Moreover, in animal models of autoimmune thyroiditis, TG-Ab appear first, and only much later do antibodies against TPO appear (if they occur at all), suggesting that tolerance at the B cell and presumably T cell level is broken first for TG and then spreads to TPO (Chen et al. 2010).

TG-Ab in Clinical Management

Thyroid Autoimmunity

The first method to detect antibodies to TG used the Ouchterlony agar gel diffusion precipitation assay. More sensitive methods quickly followed – initially the tanned red cell hemagglutination test and then solid-phase ELISA (Ewins and Wilkin 1983) and radioimmunoassay (RIA) (Beever et al. 1989). Most recently, both competitive and noncompetitive immunometric assays (IMAs) have been developed, which are automated and usually rely on chemiluminescence for detection. However, despite attempts to standardize the results against the International Reference Preparation MRC 65/93, these IMAs vary considerably in their sensitivity and specificity, resulting from (i) methodologic differences between specific epitopes on endogenous TG and the TG used in the assay and (ii) patient-specific TG-Ab heterogeneity, which at least ensures that the same assay will give the same result when measured serially in a patient (Spencer et al. 2005).

TG-Ab are rarely found in healthy children, but thereafter the prevalence increases with age until extreme old age when prevalence declines, and so low levels are frequently present in normal adults. For example, a population-based survey in the USA which used a sensitive RIA found that over 10% of healthy adults had TG-Ab, with a higher frequency in women (Hollowell et al. 2002). In this study, 70% of those with TG-Ab also had TPO antibodies, whereas 55% of those with TPO antibodies were TG-Ab positive. The presence of autoantibodies against TG or TPO in healthy individuals is strongly associated with the presence of focal lymphocytic thyroiditis (Yoshida et al. 1978).

Over 90% of patients with autoimmune hypothyroidism have TG-Ab, and somewhat lower levels are found in around half of patients with GD. Using the hemagglutination assay, TG-Ab were only rarely found in patients in the absence of TPO antibodies, leading to the proposal that screening for thyroid autoimmunity could be based on measurement of TPO antibodies alone (Nordyke et al. 1993). On the other hand, TG-Ab detected by RIA have been found to predict the histological diagnosis of Hashimoto's thyroiditis more strongly than TPO antibodies (Kasagi et al. 1996) and to be diagnostically superior to TPO antibodies for autoimmune thyroiditis in adolescents (Lindberg et al. 2001). Further evidence for a dichotomy in the behavior of TG and TPO antibodies is the appearance of the former but not the latter in cancer patients after immunotherapy with GVAX, a vaccine of whole

tumor cells transfected to secrete granulocyte-macrophage colony-stimulating factor; in this setting, unique epitopes on TG appear to be recognized by these antibodies (De et al. 2015). Iodization has been associated with the development of thyroid autoimmunity, and in one study TG but not TPO antibodies were more frequent in iodized salt users; these TG-Ab are predominantly directed against the B epitope on TG and may be an example of iodine unmasking a cryptic epitope (Latrofa et al. 2013).

Pregnancy

Most studies of the impact of thyroid autoimmunity on fertility and postpartum thyroiditis have utilized TPO antibodies as a marker of preexisting autoimmune thyroiditis in pregnant women (see section "TPO-Ab in Clinical Management"), based on the presumption that the presence of TG-Ab adds little as a categorical variable. As described above, however, TG-Ab occurring in isolation from TPO antibodies are more commonly detected using RIA and IMA, and in a cross-sectional study of infertile women using IMA, 5% were found to have isolated TG-Ab and this group also had higher TSH levels (Unuane et al. 2013). Another study has found a higher rate of miscarriage in euthyroid women with TG-Ab compared to TPO antibodies (Ticconi et al. 2011). It would clearly be worth evaluating the role of TG-Ab in pregnancy and the postpartum period further using IMA, especially with temporal changes in population dietary iodine intake, which impacts on TG-Ab prevalence and epitope specificity.

Thyroid Cancer

TG (and TPO) antibodies occur in around 20% of patients with differentiated thyroid cancer (DTC), and the presence of TG-Ab complicates the follow-up of these patients because TG-Ab interfere with the assay of serum TG (Spencer et al. 2005). A clinical position statement has been published recently on the treatment and follow-up of such patients (Verburg et al. 2013). This statement covers the methodological aspects of TG-Ab measurement mentioned above and in addition discusses the assay methods used for TG itself which may overcome such interference, as well as the possibility that TG-Ab levels could act as a surrogate tumor marker.

The most sensitive method for TG measurement is IMA, but this method is also the most prone to interference from TG-Ab, in the presence of which TG is underestimated; although TG RIA is more resistant to TG-Ab interference, it has limited availability and lower sensitivity (Spencer 2013). The difficulty is compounded by variations in the methods and cutoffs used for determining the presence of TG-Ab, leading to misclassification of sera as false-negative or false-positive (Spencer et al. 2014). Recovery tests are not a reliable way to discern the presence of TG-Ab due to variability in the epitopes of the added TG. Liquid chromatography-mass spectrometry is an alternative method for TG measurement that is not impeded by TG-Ab, but its availability is limited, it requires considerable sample preparation, and it fails to detect TG in a substantial proportion of patients due to lower functional sensitivity than IMA (Netzel et al. 2015).

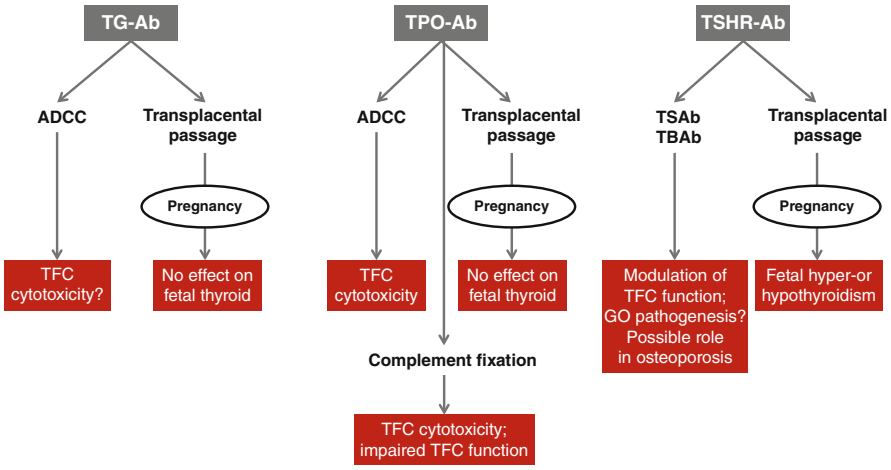

Fig. 1 The pathogenic role of the main thyroid autoantibodies. Thyroglobulin (*TG*) and thyroid peroxidase (*TPO*) antibodies (*Ab*) can participate in antibody-dependent cell-mediated cytotoxicity (*ADCC*), and TPO-Ab can additionally fix complement resulting in thyroid follicular cell death or dysfunction. Thyroid-stimulating hormone receptor (*TSHR*)-Ab directly modulate the function of thyroid follicular cells and may play a role in the pathogenesis of extrathyroidal complications as well as Graves' disease-induced osteoporosis. In pregnancy, TG-Ab and TPO-Ab do not appear to affect the fetus, whereas TSH-Ab result in fetal hyper- or hypothyroidism depending on their TSHR-stimulating (TSAb) or TSHR-blocking activity (TBAb). TFC: thyroid follicular cells, GO: Graves' ophthalmopathy

Hashimoto's thyroiditis is probably associated with DTC for reasons which are not clearly established, and many studies have sought to examine whether thyroid lymphocytic infiltration or the levels of thyroid antibodies in DTC have prognostic significance. In a review of data up to 2011, Spencer has pointed out that TG-Ab in DTC could arise either from an underlying autoimmune thyroiditis or from tumor-induced autoimmunity: there is some evidence that TG-Ab per se may be an independent risk factor for DTC, and there are differences in TG epitope recognition by antibodies in these patients in the presence or absence of thyroiditis (Spencer 2011). More recent evidence has found that the presence of TG-Ab is not an independent prognostic factor for disease-free survival from DTC, nor do trends in antibody levels predict disease status (McLeod et al. 2014; Smooke-Praw et al. 2014). However, patients with TG-Ab in the year after treatment seem more likely to have persistent or recurrent disease, whereas conversion from TG-Ab positivity to negativity after treatment in patients with DTC is associated with a favorable outcome (Durante et al. 2014). Some of the variability in these reports may be explained by TG epitope heterogeneity; around 40% of DTC patients with TG-Ab have restricted epitope recognition, similar to patients with autoimmune thyroiditis, and also have a higher rate of persistent or recurrent disease (Lupoli et al. 2015). The pathogenic role and clinical use of TG-Ab are summarized in Figs. 1 and 2, respectively.

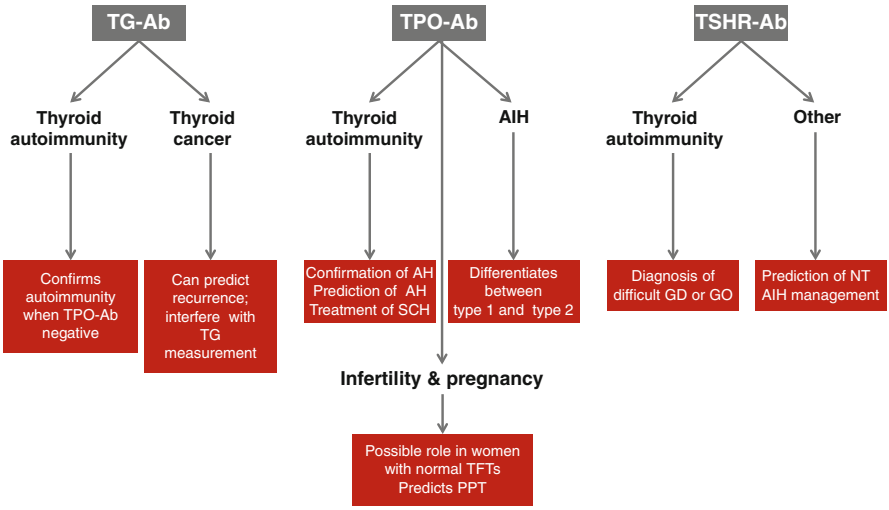

Fig. 2 The role of thyroid autoantibodies in clinical management. Thyroglobulin antibodies (*TG-Ab*) are markers of thyroid autoimmunity, but their main clinical use is in thyroid cancer. Detection of TG-Ab in patients with thyroid cancer previously treated with surgery indicates that TG measurement is not a reliable marker for disease recurrence. Moreover, appearance of TG-Ab following surgery in antibody-negative individuals suggests recurrence of thyroid cancer. Thyroid peroxidase antibodies (*TPO-Ab*) represent a marker of thyroid autoimmunity and therefore help to confirm autoimmune hypothyroidism (*AH*) and aid in the management of subclinical hypothyroidism (*SCH*). Moreover, TPO-Ab help in differentiating type 1 from type 2 amiodarone-induced hyperthyroidism (AIH), but there are caveats to such an approach (see text). In pregnancy, TPO-Ab may indicate the need for thyroxine therapy even in those with normal thyroid function although definitive results of clinical trials are currently awaited. TPO-Ab during pregnancy can additionally predict the development of postpartum thyroiditis (*PPT*). Thyroid-stimulating hormone receptor antibodies (*TSH-Ab*) are used in the diagnosis of difficult cases of Graves' disease (*GD*) or Graves' ophthalmopathy (*GO*). Furthermore, TSHR-Ab measurement is useful to predict neonatal thyrotoxicosis (*NT*) and helps in the differentiation between the two types of AIH

Thyroid Peroxidase Antibodies (TPO-Ab)

The Antigen

TPO was initially described as the thyroidal microsomal antigen by Belyavin and Trotter more than half a century ago (Belyavin and Trotter 1959). Twenty-six years later, two groups simultaneously characterized this autoantigen as TPO (Czarnocka et al. 1985; Portmann et al. 1985). TPO belongs to the family of mammalian peroxidases and has a high sequence homology with myeloperoxidase. It is the primary enzyme involved in iodination of tyrosine residues on TG and in their coupling to form thyroid hormones (McLachlan and Rapoport 1992). TSH stimulates TPO expression, whereas antithyroid drugs inhibit the action of this enzyme.

In man, TPO is a membrane-associated protein of 933 amino acids with five potential glycosylation sites and is encoded by a single gene on chromosome 2, spanning 17 exons. An alternatively spliced variant of TPO mRNA, resulting in a smaller protein (876 amino acids), has been termed TPO-2, which coexists with the full-length protein. TPO-2 lacks enzymatic activity due, at least in part, to the absence of Asn 579 and is believed to have different antibody reactivity to full-length protein secondary to altered conformation (Niccoli et al. 1997). The exact role of TPO-2 in normal physiology or autoimmune disease pathology remains largely unclear. It has been proposed that TPO-2 is expressed early during embryogenesis, and hence tolerance occurs to TPO-2 more effectively than full-length TPO, which may have a role in initiation of thyroid autoimmunity later in life (Gardas et al. 1997).

Human TPO-Ab and monoclonal TPO antibodies recognize multiple conformational epitopes on TPO which map to two major, closely associated domains, A and B, within an immunodominant conformational region on the protein located in an area that shares homology with myeloperoxidase (McLachlan and Rapoport 2007). The multiple discontinuous amino acid residues critical for the two immunodominant regions have been described (Dubska et al. 2006). Although TPO-Ab primarily recognize this immunodominant region, cloning techniques have also demonstrated the existence of TPO-Ab which lie outside this region (Pichurin et al. 2001); the pathological relevance of these is not yet clear. The main characteristics of TPO are summarized in Table 1.

Role of TPO-Ab in Disease Pathogenesis

TPO-Ab are of the IgG class and, like TG-Ab, are distributed across all four IgG subclasses (Adler et al. 1984). There is no evidence to suggest that TPO-Ab initiate thyroid autoimmunity, as they cannot penetrate the tight junctions between thyroid cells to reach their apically situated autoantigen, although they appear to have some role in the autoimmune reaction once the tight junctions have been disrupted through T cell-mediated damage.

A direct inhibitory effect of TPO-Ab on enzyme activity has been described in vitro, but this does not appear to translate into a significant in vivo effect (Song et al. 1996). It is more likely that TPO-Ab induce tissue destruction through the complement pathway and by mediating ADCC. TPO-Ab can fix complement which in turn could result in complement-medicated thyroid cell destruction. On the other hand, expression of regulatory proteins by thyroid cells, most importantly CD59, limits C3-mediated tissue injury (Tandon et al. 1994). Sublethal complement attack can impair thyrocyte function, however, by suppression of the cAMP response to TSH, and this may have a role in clinical thyroid dysfunction in the early stage of the disease process (Weetman 2003). Importantly, complement-attacked thyroid cells release proinflammatory molecules, which may contribute to tissue destruction and augmentation of the inflammatory reaction (Weetman and McGregor 1984). TPO-Ab may also mediate ADCC through interaction of NK cells with the Fc portion of antibodies attached to thyroid follicular cells, resulting in cell lysis (Metcalfe et al. 1997).

TPO-Ab in Clinical Management

Thyroid Immunity

Early tests for TPO-Ab detection relied on complement-fixing properties of these antibodies (Trotter et al. 1957). Similarly to TG, TPO-Ab were also detected using hemagglutination with microsome-coated red blood cells agglutinating in the presence of these antibodies (Cayzer et al. 1978). TPO-Ab testing was subsequently replaced by RIA or IMA assays using purified or recombinant TPO and standardized against Medical Research Council (MRC) reference preparation 66/38 (Beever et al. 1989; Ruf et al. 1988). The currently used third-generation assays show high accuracy and greater harmonization of results between the different kits available, although cutoff rates continue to be debated, and there are still pronounced differences in the threshold values considered as positive by different manufacturers (D'Aurizio et al. 2015; Tozzoli et al. 2016).

The prevalence of TPO-Ab in healthy children and adolescent is low, but this increases with age and displays a female predominance (Roti et al. 1992; Kabelitz et al. 2003). Antibodies against TPO are detected in the vast majority of patients with autoimmune hypothyroidism and around 70% of GD patients. Although the properties of TPO antibodies from patients with AH and GD are largely similar, some minor differences have been described, such as different IgG heavy chain usage, but have no known clinical significance (Chardes et al. 2002; Ajjan and Weetman 2001).

Autoimmune Hypothyroidism

In hypothyroid individuals, the presence of TPO-Ab helps to confirm the autoimmune nature of thyroid failure. TPO-Ab status can also be utilized to make treatment decisions in those with "borderline" thyroid function. For instance, it is known that the risk of developing overt hypothyroidism is highest in subjects with the highest TPO-Ab levels, while the presence of TPO-Ab at levels up to three times normal has no predictive value (Walsh et al. 2010). Moreover, the presence of TPO-Ab, particularly at a younger age, can predict the development of future thyroid dysfunction even in individuals with normal TSH and thyroid hormone levels. In the Wickham survey and over 20 years of follow-up, the odds ratio of developing hypothyroidism in TPO-Ab-positive individuals with normal thyroid function was 8 (95% CI 5–15) in women and 25 (10–63) in men. In cases in whom the TSH was also elevated, the risk of hypothyroidism rose to 28 (22–65) in women and 173 (81–370) in men. The risk in individuals with raised TSH alone was comparable to those with positive TPO antibodies and normal thyroid function at 8 (3–20) and 44 (19–104) for women and men, respectively (Vanderpump et al. 1995).

Current guidelines are rather equivocal about the utility of measuring thyroid antibodies, including their use in patients with subclinical hypothyroidism. Although levothyroxine replacement is recommended if the TSH is greater than 10 mIU/L, irrespective of thyroid antibody levels, the need for replacement when an elevated TSH is below this cutoff is based on individual factors, including the presence of TPO-Ab; a significant proportion of individuals with TPO-Ab do not develop thyroid dysfunction (Garber et al. 2012).

The emergence of antibodies against TPO has a clear genetic predisposition (Hansen et al. 2006), and a genome-wide association analysis has identified five loci associated with TPO-Ab positivity; three of these were also associated with thyroid dysfunction, indicating an interaction between genetic predisposition, TPO-Ab status, and clinical thyroid disease, as well as illustrating that in some cases, TPO-Ab positivity may be independent of the presence of overt disease (Medici et al. 2014). Further evidence suggests that antibody status can determine the clinical course of autoimmune hypothyroidism. Rotondi and colleagues have shown that TG-Ab- and TPO-Ab-negative autoimmune hypothyroidism runs a milder clinical course, with patients having lower TSH levels, higher FT4 levels, and smaller goiters (Rotondi et al. 2014). This is an unusual group of patients, as the great majority of patients will be thyroid antibody positive, but they have a strong family history of thyroid autoimmunity and a hypoechoic pattern on thyroid ultrasound indicating lymphocytic thyroiditis; the fact that their age of presentation is similar to the antibody-positive group suggests that this is a true subset rather than one in which circulating thyroid antibodies have not yet developed (a significant initial source of thyroid antibodies is within the thyroid itself).

Infertility and Pregnancy

The prevalence of TPO-Ab in women in the reproductive age ranges between 5% and 20% rising to 14–33% in those with recurrent miscarriages or infertility (Poppe et al. 2008). TPO-Ab positivity is associated with increased risk of pregnancy complications, with 1.7–2.5-fold increased risk of premature delivery, spontaneous premature delivery, and very premature delivery, independent of TSH or thyroxine levels (Korevaar et al. 2013). Moreover, the presence of TPO-Ab has also been associated with in vitro fertilization (IVF) failure, with one prospective study of 234 women showing a doubling of the failure rate in TPO-Ab-positive women (50% vs. 23% failure rate in positive and negative TPO-Ab status, respectively) (Poppe et al. 2003). Also, the detection of TPO-Ab during pregnancy has been linked to psychomotor delay in the offspring (Pop et al. 1995). The mechanisms for the association of TPO-Ab with subfertility are unclear with some suggesting that presence of TPO-Ab is a marker of very early thyroid dysfunction, whereas others attributing the association to the presence of a state of autoimmunity adversely affecting fertility (Vissenberg et al. 2015).

It is universally agreed that treatment of pregnant women with subclinical hypothyroidism and positive TPO-Ab is warranted, with the majority requiring long-term thyroxine therapy (Shields et al. 2013). However, the best management strategy for those with detectable TPO-Ab and normal thyroid function is not entirely clear. A meta-analysis involving 187 women in two studies suggests that thyroxine replacement therapy is associated with a reduction in miscarriages and preterm delivery in mothers with detectable TPO antibodies and normal thyroid function (Thangaratinam et al. 2011). Two larger studies [the TABLET (UK) and T4Life (Netherlands) trials] are currently ongoing to conclusively determine the clinical benefit of such an approach. Current guidelines recommend levothyroxine treatment in TPO-Ab-positive women who wish to conceive or who are pregnant if

the TSH level is above 2.5 (Garber et al. 2012). TPO-Ab have also been implicated in non-autoimmune conditions that are associated with reduced fertility. Polycystic ovary syndrome (PCOS) is a clinical entity characterized by insulin resistance and difficulty to conceive. The presence of TPO-Ab can determine response to therapy with clomiphene or metformin with antibody-positive patients demonstrating an inferior response by mechanisms that remain unclear (Ott et al. 2010).

Postpartum Thyroiditis (PPT)

PPT occurs in around 5% in the general population, and the detection of TPO-Ab during pregnancy can predict the development of this condition (Stagnaro-Green 2012). However, the timing of the test appears to be important given amelioration of the immune response in the later stages of pregnancy. A recent Australian study demonstrated that for best predictive accuracy of PPT, TPO-Ab and/or TG-Ab should be checked in the first trimester of pregnancy [OR = 7.8 (95% CI: 2.2–27.6)], while testing is best avoided in the second and third trimesters due to low predictive value [OR = 1.2 (0–8.9) and 2.0 (0–16.8), respectively] (Ekinci et al. 2015). In another study involving 400 women, more than 80% who developed postpartum thyroiditis had detectable TPO-Ab at early pregnancy (8–12 weeks) (Bhattacharyya et al. 2015), further emphasizing the relative sensitivity of these antibodies for the prediction of PPT. Despite these results, current guidelines do not recommend a universal screening program for TPO-Ab status in pregnant women (De et al. 2012), and a recent Cochrane analysis found no overall evidence from existing trials for a benefit to the mother or neonate, despite the increased diagnosis rate (Spencer et al. 2015).

Other Clinical Scenarios

Some evidence indicates that TPO-Ab positivity is associated with a feeling of ill-health and poor quality of life irrespective of TSH level (Winther et al. 2016; Watt et al. 2012), possibly due to the effects of cytokines from the underlying autoimmune process on the brain and other tissues (Ott et al. 2011). Preexistent TPO-Ab are a risk factor for the development of overt hypothyroidism or destructive thyrotoxicosis with certain drugs, especially newer oncological therapies. The best known of these is interferon-α; being TPO-Ab positive prior to treatment is associated with a fourfold increased risk of thyroid dysfunction (Costelloe et al. 2010). Treatment with the kinase inhibitor sunitinib of 27 patients with metastatic carcinoma caused hypothyroidism in 60% within 1–4 months of initiating therapy and a decrease in thyroid volume in 90% of patients at 1 year, consistent with a role for kinases in maintaining thyroid function and growth. TPO-Ab became detectable in 25% of patients, which correlated with more profound hypothyroidism and larger reduction in thyroid volume. Intriguingly, patients who developed TPO-Ab had almost double the survival compared with those with negative antibodies (10.8 and 5.8 months, respectively). However, given the small sample size and the absence of a mechanism for increased survival in TPO-Ab-positive patients, further work is needed in this area (Pani et al. 2015). Other examples of novel cancer treatments which have been variably implicated in autoimmune thyroid dysfunction are reviewed elsewhere

(Torino et al. 2013). TPO-Ab have also been used for the management of amiodarone-induced hyperthyroidism, which is further discussed below. The pathogenic role and clinical use of TPO-Ab are summarized in Figs. 1 and 2, respectively.

TSHR in Thyroid Autoimmunity

The Antigen

TSHR, composed of 764 amino acids, is the key autoantigen in GD and plays a role in some patients with autoimmune hypothyroidism. The TSHR belongs to the G protein-coupled receptor family and is composed of three extracellular loops containing the amino terminus, seven transmembrane segments, and three intracellular loops ending with a carboxyl terminus. The gene encoding TSHR, located on chromosome 13, is composed of ten exons, the first nine of which encode the extracellular domain (ECD). The ECD has multiple TSH binding sites as well as TSHR-stimulating antibody (TSAb) binding sites. The receptor undergoes important modifications during synthesis including glycosylation, which is essential for the proper folding of the receptor and for its antigenic properties. TSHR also undergoes a posttranslational cleavage unique among G protein-coupled receptors, which results in the formation of two moieties, a 53 kD extracellular A subunit and a transmembrane and intracellular B subunit, which are coupled by disulfide bridges.

The A subunit is shed from the cell surface, which may play a role in generating an autoimmune response to the TSHR (Rapoport et al. 1998). This concept is supported by experimental animal models showing that a more stable mouse model of GD occurs when immunization is performed with the A subunit rather than the whole molecule (Chen et al. 2004), and other data indicate that the affinity maturation of TSAb in GD is driven by shed A-subunit multimers, rather than monomers (Rapoport et al. 2015). The B cell epitopes on TSHR are conformational. TSAb recognize predominantly epitopes on the TSHR N terminus A subunit rather than the holoreceptor, due either to TSHR dimerization or steric hindrance from the remainder of the receptor (Chazenbalk et al. 2002). Thyroid-blocking antibodies (TBAb) recognize the holoreceptor more efficiently, and epitopes are more focused on the C terminus than those for TSAb, but there is significant overlap. The main characteristics of TSHR are summarized in Table 1.

Role of TSHR-Ab in Disease Pathogenesis

TSHR-Ab are IgG class, and TSAb are mainly IgG1 subclass, with some evidence of light chain restriction as well (Morshed and Davies 2015). The crucial aspect of TSHR-Ab, compared to TG-Ab and TPO-Ab, is related to their functional role that determines disease presentation. TSAb stimulate the receptor and are dominant in patients with GD. On the other hand, TBAb result in hypothyroidism and are detected with variable frequency, ranging from 9% to 90%, in patients with

autoimmune hypothyroidism and deranged thyroid function (Chiovato et al. 1990; Bryant et al. 1995). Also, TBAb are found in some GD patients, in whom the overall degree of thyroid activity is determined by the sometimes fluctuant levels of TSAb and TBAb (McLachlan and Rapoport 2013). Certain TSHR-Ab bind the receptor without altering function and are termed "neutral" (Ajjan and Weetman 2008).

Considerable efforts have been concentrated on isolating TSAb monoclonal antibodies in order to determine exactly how these antibodies bind to the receptor and modulate its function. In 2002, three groups independently reported the generation of mouse monoclonal antibodies having TSAb activity (Ando et al. 2002; Costagliola et al. 2002; Sanders et al. 2002), followed a year later by the isolation of a human monoclonal antibody after screening that involved more than 16,000 hybridomas derived from a patient with GD (Sanders et al. 2003). These developments have allowed for a comprehensive analysis of TSAb/TSHR interactions. These antibodies recognize conformationally intact glycosylated TSHR and almost none binding to linear TSHR epitopes (Sanders et al. 2003; Ando et al. 2004; McLachlan and Rapoport 2004). The crystal structure of the monoclonal TSAb and TSHR ectodomain complex has been reported, clearly demonstrating binding to the leucine-rich domain of the receptor (Sanders et al. 2007). This corresponds to the TSH binding site, explaining the relatively high sensitivity of assays which utilize binding inhibition for the detection of TSHR antibodies (further discussed below). In contrast, TBAb have at least two binding sites on TSHR, one on the A subunit and one on the B subunit. Similarly to TSAb, TBAb on the A subunit are conformational, but linear interactions can be detected on the B subunit (Ando et al. 2004; Morgenthaler et al. 2003; Oda et al. 2000; Minich et al. 2004).

In addition to direct modulation of thyroid function by TSHR-Ab, immature thymocytes express TSHR which can be stimulated by TSAb; this interaction could be implicated in propagation of the immune reaction by breaking immune tolerance in the thymus (Gimenez-Barcons et al. 2015). Moreover, the detection of TSHR gene and protein in thymocytes and the functional role of the receptor in these cells may explain the thymic enlargement that sometimes occurs in GD.

TSHR-Ab in Clinical Management

Assays for TSHR-Ab Detection

The very first method to establish the presence of a TSHR-Ab used the ability of a subset of these antibodies to stimulate in vivo the release of radioiodine-labeled thyroid hormones from guinea pigs that had been given radioiodine (Adams and Purve 1956). Since then a huge variety of assays has been developed, leading occasionally to confusion over terminology, as many different names have been used, not always with accuracy, for these antibodies. A summary is provided in Table 2. Assays for the detection of TSHR-Ab can be broadly divided into binding and functional assays. The most widely used test clinically is based on the detection of what were originally termed TSH-binding inhibitory immunoglobulins (TBII), and this method detects the range of TSHR antibodies, without an indication as to

Table 2 Summary of the main types of assays which have been used to measure thyroid-stimulating hormone receptor antibodies (*TSHR-Ab*) in Graves' disease

TSHR-Ab type	Assay method
Long-acting thyroid stimulator (LATS), LATS protector (LATS-P)	In vivo release of radioiodine from pre-labeled guinea pig or mouse thyroid; LATS-P has also been measured using murine thyroidal intracellular colloid droplet formation
Thyroid-stimulating antibody (TSAb)	Stimulation of cAMP release by thyroid slices, follicles or cells (including cell lines such as FRTL-5), or CHO cells transfected with the TSHR
Thyroid-blocking antibodies (TBAb)	Inhibition of TSH-stimulated cAMP release by thyroid cells or cells transfected with the TSHR
TSH-binding inhibitory immunoglobulins (TBII)	Inhibition of labeled TSH binding to the TSHR in solubilized or recombinant form measured by radioassay, chemiluminescence, or ELISA; electrochemiluminescence immunoassay measures inhibition of the binding of a human thyroid-stimulating monoclonal antibody (M22) to TSHR
TSHR-binding antibodies	Immunoprecipitation or flow cytometry of TSH receptor-transfected cells or ELISA-based methods using cell extracts

CHO Chinese hamster ovary, *cAMP* cyclic adenosine monophosphate, *ELISA* enzyme-linked immunosorbent assay

functional nature of the antibodies, although this can of course be inferred by the clinical and biochemical features of the patient at the time of testing. Discrete TSAb and TBAb activities can only be determined by functional bioassays.

Binding Assays

The first-generation TBII assays, developed in the 1970s, typically used porcine thyroid membranes as a source of TSHR and relied on detecting the inhibition of radio-labeled TSH binding to its receptor (Southgate et al. 1984). The assay has subsequently undergone major refinement using recombinant TSHR, rather than receptor expressed on cell membranes (Kakinuma et al. 1999). The assay was further simplified by using solid-phase ELISA (rather than liquid phase) and employing fluorescent techniques to replace radio-labeled assays (Ajjan and Weetman 2008). These assays are commonly referred to as second-generation assays and use recombinant porcine or human TSHR. The last decade has seen the introduction of third-generation assays which rely on the inhibition of binding of a labeled monoclonal TSHR antibody rather than TSH to increase assay accuracy. Although the increased sensitivity using monoclonal antibody offers theoretical advantages, studies have failed to conclusively demonstrate superiority for the monoclonal antibody approach (Zophel et al. 2009; Liu et al. 2008; Pedersen et al. 2010).

Functional Assays

Stimulation of the TSHR by TSH or TSAb results in the generation of cAMP, and most functional tests rely in measuring cAMP levels after incubating the receptor with

patient sera. The first generation of TSAb used animal or human thyroid slices or cells and tested cAMP production after incubation with sera from patients with suspected GD (Toccafondi et al. 1980; Kasagi et al. 1987; Vitti et al. 1988). To improve sensitivity and minimize interference caused by plasma proteins, ammonium sulfate-precipitated IgG was used instead of sera (Ochi et al. 2002), and the application of hypotonic media in the assay further increased sensitivity (Kasagi et al. 1982). TBAb can be measured in a similar setup in which TSH-stimulated cAMP production is inhibited by the addition of sera or IgG containing such antibodies.

Cloning of the TSHR enabled the development of sensitive bioassays by generating a CHO cell line stably expressing the receptor. Production of cAMP in these cells was measured after adding patient sera, which improved sensitivity to around 90%. However, the assay was labor intensive and required specialized laboratories equipped with cell culture facilities and the ability to undertake radioactive immunoassays (Morgenthaler et al. 1998; Vitti et al. 1993). In order to simplify the technique, third-generation assays have been created that employ a luciferase reporter to detect increased cAMP production, without the need for radioactivity (Watson et al. 1998). The luciferase reporter assay was further modified using CHO cells expressing a chimeric human TSHR/rat LH receptor, although it is not entirely clear whether this approach offers increased sensitivity (Lytton et al. 2010). Cutoff values for TSAb measurements remain laboratory specific, although attempts have recently been made at standardization of the various TSHR-Ab bioassays (Diana et al. 2015).

While developing a reliable TSAb assay is certainly an attractive scientific proposition, the clinical need for such an assay is relatively limited. Combining the clinical presentation with TBII data will enable the physician to predict whether the patient has stimulating or blocking TSHR antibodies. However, a number of rare clinical scenarios remain where a functional assay may be of use. A prime example is a pregnant woman with a history of GD previously treated with radioactive iodine or surgery, where the exact nature of TSHR-Ab might help to decide on the appropriate clinical management (further discussed below). The main features of the two types of TSHR-Ab assays relevant to clinical practice are summarized in Table 3.

TSHR Antibodies in Hyperthyroidism

Around 80% of patients with hyperthyroidism have GD, although this percentage is influenced by iodine status and geographical variations. The diagnosis of autoimmune hyperthyroidism can be made clinically in those with a typical history, a diffuse goiter, and extrathyroidal complications. In a significant number of patients, however, the cause of hyperthyroidism cannot be determined with certainty clinically, not least because the widespread use of thyroid function testing means that presentation is now often at an early stage and detection of TSHR-Ab confirms the autoimmune nature of the hyperthyroidism (Ajjan and Weetman 2008). Theoretically all patients with GD should be positive for TSHR-Ab and especially TSAb, but in practice this is never achievable due to assay insensitivity or the purely intrathyroidal synthesis of these antibodies. The overall detection level in newly diagnosed GD with the second-generation TBII is 95%, and those patients who are TBII negative have less severe disease and no orbital involvement (Vos et al. 2008). There is no clear consensus on

Table 3 Clinical utility of thyroid-stimulating hormone receptor antibody (*TSHR-Ab*) measurement

	TBII assay	Bioassay
Principle	Inhibition of TSH (or monoclonal TSHR-Ab) binding to its receptor by patient sera (or immunoglobulin)	Detection of cAMP production by TSAb (or blocking of TSH-induced cAMP production by TBAb)
Platform	Solid-phase assay	Cells transfected with the TSHR
Sensitivity	95–98%	Up to 99%
Advantages	Relatively easy to perform; no more expensive than TPO-Ab or TG-Ab measurement	Differentiates between TSAb and TBAb; more sensitive but this is dependent on the specific assay used
Disadvantages	Unable to differentiate TSAb from TBAb (this disadvantage is offset by using the patient's biochemical status at the time of measurement as an indicator of functional ability)	Labor intensive and requires cell culture facilities; prone to assay variability and so standardization is complex; expensive; not widely available
Clinical use	Diagnosis of GD or GO Prediction of NT in pregnant women with a history of previous or current GD	Prediction of NT in pregnant women with GD previously treated with RAI or thyroidectomy

TSAb thyroid-stimulating antibody, *TBAb* thyroid-blocking antibody, *NT* neonatal thyrotoxicosis, *RAI* radioactive iodine, *GD* Graves' disease, *GO* Graves' ophthalmopathy, *TS/TBAb* thyroid-simulating/thyroid-blocking antibody

which patients to test, with some form of TSHR-Ab measurement being used by only 58% of endocrinologists in one North American survey which gave an index case of uncomplicated GD as an example (Burch et al. 2012). The 2011 American Thyroid Association guidelines on the management of thyrotoxicosis state that "a radioactive iodine uptake should be performed when the clinical presentation of thyrotoxicosis is not diagnostic of GD" and advocate the measurement of TSHR-Ab when a scintiscan is not available (Bahn Chair et al. 2011). The high sensitivity of TBII assays and the convenience for the patient make such an approach questionable, and our impression is that practice is changing to greater use of TSHR-Ab measurement as the primary diagnostic tool, especially in Europe. Attempts have also been made to use TSHR-Ab to predict relapse of GD after a course of antithyroid drugs. While elevated levels of TSHR-Ab both before and after treatment are associated with an increased likelihood of short-term relapse, the assay is insufficiently sensitive or specific to predict medium- or long-term relapse, and therefore routine clinical use for guiding antithyroid drug therapy is not recommended (Massart et al. 2009; Carella et al. 2006).

In addition to their role in diagnosis, TSHR-Ab could in the future provide new therapeutic tools for the management of difficult clinical cases, including contraindications to antithyroid drugs or the presence of significant extrathyroidal complications. For example, the development of TBAb that compete with TSAb for TSHR binding could help in controlling hyperthyroidism. Alternatively, if we could pinpoint the exact epitopes recognized by TSAb, small molecules could be synthesized that resemble these epitopes, consequently neutralizing the effects of TSAb (Gershengorn and Neumann 2012).

Extrathyroidal Complications

Although at least 90% of GD patients have abnormalities in their orbit when examined by imaging techniques, only 25–50% show clinical signs of this condition, with the majority of these only having mild disease that requires little or no treatment (Bartalena 2013; Bartalena and Fatourechi 2014). Studies so far have failed to identify the reasons for the large variability of GO presentation, although some environmental factors have been implicated (Prabhakar et al. 2003). Smoking has a key role in disease pathogenesis, a concept supported by amelioration in disease activity in those who stop smoking (Pfeilschifter and Ziegler 1996; Villanueva et al. 2000). Another is related to treatment of GD with radioactive iodine, which can result in worsening of GO (or even the development of new disease), particularly in smokers (Prabhakar et al. 2003; Bonnema and Hegedus 2012).

The close association between GD and GO suggests a common pathogenesis, probably related to one or more shared autoantigens. Of the major thyroid autoantigens, TPO does not seem to have a role, but TG received some interest as it is expressed in retrobulbar tissue from GO patients (Marino et al. 2004). However, the low incidence of GO in autoimmune hypothyroidism and the fact that TG-immunized mice do not develop eye changes argue against a key role for TG in disease pathogenesis.

On the other hand, a role for the TSHR has been implicated in GO given (i) the close association between GD and GO, (ii) the expression of TSHR (both mRNA and protein) in retrobulbar-derived cells including fibroblasts and preadipocytes (Bahn 2004), (iii) the development of orbital changes in some animal models immunized with TSHR (Many et al. 1999), and (iv) the correlation between TSHR-Ab levels and GO activity/severity (Gerding et al. 2000; Khoo and Bahn 2007; Morris et al. 1988). Clinically, the detection of TSHR-Ab helps in the diagnosis of difficult cases of GO, particularly in the rare instances of patients with normal thyroid function (Khoo et al. 2000). The use of TSHR-Ab has also been evaluated for predicting GO progression. Although high or low TSHR-Ab titers are associated with relapse and remission of GO, respectively, around half of all such individuals are in a "gray area" in which there is no utility in measurement, and therefore the routine clinical use of antibody measurement for prediction of disease outcome is not advised (Eckstein et al. 2006).

A number of other antibodies against eye muscle antigens (55, 64 and 95 kDa antigens) and fibroblasts (23 and 66 kDa antigens) have been detected with higher frequency in patients with GO compared with controls (Prabhakar et al. 2003; Mizokami et al. 2004; Bahn 2003). The 64 kDa reactivity comprises different proteins including D1 protein, calsequestrin, and succinate dehydrogenase, whereas the 55 kDa protein is thought to be G2s protein. However, only a fraction of GO patients display reactivity against these antigens, and any correlation with disease activity is weak and inconsistent. The insulin-like growth factor 1 receptor (IGFR) has been proposed as an orbital autoantigen, particularly as autoantibodies against this protein may have a functional role by stimulating the receptor. Moreover, in vitro studies demonstrated that inhibition of IGFR ameliorates the inflammatory profile of fibroblasts, making this molecule a possible therapeutic target (Smith 2003; Chen et al. 2014). However, other studies have failed to show a correlation between IGFR antibodies and clinical activity of GO with a similar prevalence of these antibodies in GO patients and controls (Khong et al. 2016).

Largely similar conclusions can be drawn for antibodies against collagen XIII, which are detected in two thirds of patients with active GO but also in up to half the patients with GD and no sign of active eye disease (De Bellis et al. 2005). Other candidate autoantigens have been described but all have equally low specificity for active GO, and these include actin, tubulin, and acetylcholine receptor (Prabhakar et al. 2003; Mizokami et al. 2004; Bahn 2003). In summary, none of these additional antigens appear to be the main autoantigen in GO, and the described antibodies are likely to have arisen as a result of orbital tissue injury.

TSHR-Ab in Pregnancy

Around 1% of neonates born to mother with current or previous history of GD develop hyperthyroidism (Cooper and Laurberg 2013). It has been known for many years that this complication occurs in mothers with high TSHR-Ab levels in the third trimester (Munro et al. 1978). A recent retrospective study examined the value of second-generation TBII in predicting neonatal hyperthyroidism in 47 neonates born to 42 mothers (Abeillon-du et al. 2014). A total of nine neonates had hyperthyroidism, with five cases described as severe and requiring medical therapy. All hyperthyroid neonates were born to mothers with TBII $>$ 5 IU/L (measured in the second trimester), indicating a sensitivity of 100%, although the specificity was low at 43%. Bioassays were conducted in 20 of the cases, and the mothers of all neonates with hyperthyroidism had TSAb activity of >400%. Therefore, the second-generation TBII offers a sensitive tool for the detection of neonatal hyperthyroidism. However, the low specificity of this test suggests that mothers with positive TBII could also be tested using functional assays to predict accurately neonatal hyperthyroidism, depending on assay availability and expense.

The fetal thyroid becomes responsive to TSH and TSAb by week 20, and this, together with increased transplacental passage of immunoglobulin in the last trimester of pregnancy, justifies testing TSHR-Ab at weeks 20–24 of pregnancy in a woman with GD, with values three times above the upper limit of normal being an indication for close follow-up of the fetus (Fisher 1997). Other proposals have included an assessment of TSHR-Ab in the first trimester and at a slightly later stage at 24–28 weeks: the optimal time is not yet established because there is uncertainty on when to measure in relation to the normal decline in TSHR-Ab levels during pregnancy. Contrary to some routine clinical practice, TSHR-Ab assessment is not required in euthyroid pregnant patients in successful remission after a course of an antithyroid drug, as the mother's euthyroid status indicates that TSAb must be absent.

In women whose hypothyroidism is due to the presence of TBAb, the transplacental passage of these antibodies may cause neonatal hypothyroidism, and this situation can be more complex still with alterations in the ratio of TBAb to any coexisting TSAb occurring during the course of pregnancy itself (McLachlan and Rapoport 2013). If suspected in advance (e.g., from previous pregnancies), screening the mother for TBAb in the last trimester could be useful clinically, but this is not routine. Most cases of transient hypothyroidism caused by TBAb are suspected by the absence of thyroid agenesis or dysgenesis on ultrasonography and ^{123}I scintigraphy: isotope scanning alone may incorrectly suggest athyreosis in the presence of maternal

TRAb (Leger et al. 2014). In cases of suspected transient hypothyroidism due to TBAb, levothyroxine treatment usually is continued until the child is aged 3, with repeat thyroid imaging and a trial of levothyroxine withdrawal at that stage.

Other Clinical Scenarios

The measurement of TBAb in adults with suspected autoimmune hypothyroidism is not indicated. As discussed earlier, TBAb detection is variable in patients with autoimmune hypothyroidism (Chiovato et al. 1990; Bryant et al. 1995), which may be related to assay sensitivity. More recent work has shown that 90–100% of patients with hypothyroidism secondary to Hashimoto's thyroiditis tested positive for TBAb in bioassays or binding assays, indicating that the prevalence of these antibodies in autoimmune hypothyroidism is high (Diana et al. 2016). Spontaneous fluctuations in antibody levels may result in variable levothyroxine requirements or remission of the hypothyroidism, but the full duration of such remissions is unclear, and thus trials of levothyroxine withdrawal are not advocated in routine practice (McLachlan and Rapoport 2013; Takasu and Matsushita 2012).

Detection of TSHR-Ab can help to differentiate between the two types of amiodarone-induced hyperthyroidism (AIH). A positive TSHR-Ab is consistent with type 1 AIH (due to incipient GD), and therefore treatment with an antithyroid drug is justified (Bogazzi et al. 2010). However, there are two main caveats for the use of TSHR-Ab to guide management decisions. Firstly, type 1 AIH can coexist with the type 2 variant (inflammatory), and therefore some antibody-positive patients may receive suboptimal therapy if treated with an antithyroid drug alone. Secondly, failure to detect TSHR-Ab does not necessarily rule out type 1 AIH. Therefore, it is not uncommon to treat for a mixed type until the clinical picture becomes clearer and appropriate therapy can then be tailored accordingly. TPO-Ab have also been used for differentiating type 1 from type 2 AIH with largely similar limitations to TSHR-Ab. Recent work has demonstrated that presence of TPO-Ab does not exclude the diagnosis of type II AIH (Tomisti et al. 2016), further limiting the use of antibody tests to guide management strategies.

A recent study in 93 GD patients implicated TSAb in increased bone turnover markers, potentially predisposing to osteoporosis. In contrast, TBAb (present in ten patients) appeared to have a protective effect (Cho et al. 2015). Although this is an interesting observation, concrete conclusions cannot be made given the limited number studied and the failure to evaluate bone mass in these patients.

The pathogenic role and clinical use of TSHR-Ab are summarized in Figs. 1 and 2, respectively.

Other Thyroid Autoantibodies

Sodium Iodide Symporter Antibodies

The human NIS, mediating iodine uptake by the thyroid gland, was cloned two decades ago and found to have a complex structure of 12 transmembrane domains, comprising 643 amino acids with three potential glycosylation sites. The coding

region of *hNIS* is interrupted by 14 introns, and the chromosome location is mapped to chromosome 19p (Portulano et al. 2014). Autoantibodies which inhibited the function of NIS in cultured dog thyrocytes were first described in 1995 in one of 148 sera taken from patients with a mix of autoimmune diseases (Raspe et al. 1995). A higher frequency of NIS antibodies was reported using an assay which used a truncated version of the NIS expressed in CHO cells, with inhibitory activity being found in 31% of GD sera (Ajjan et al. 1998), but others have detected NIS inhibitory activity in only 3% of GD patients (8 of 256 sera) (Chin et al. 2000). One possible explanation for these discrepancies is variability in iodine uptake of the cell lines used; very high initial uptake in some lines may fail to detect sera with weak NIS inhibitory activity.

Given the vagaries of functional assays, radioligand binding assays to measure NIS antibodies have been developed, and these appear to be more consistent, with 11–22% of IgGs from GD patients showing binding to the NIS (Ajjan et al. 2000; Seissler et al. 2000; Brix et al. 2014). NIS-binding antibodies have also been detected in AH sera at a similar frequency, indicating that these antibodies are not GD specific and the clinical significance of these antibodies is not yet established.

Pendrin Antibodies

Pendrin is a glycoprotein composed of 780 amino acids and acts as a sodium-independent chloride/iodide transporter apically in thyroid cells where it mediates iodide efflux into the follicular lumen; it is also found in the kidney and inner ear. Antibodies to pendrin were first described using an immunoblotting assay; 81% of patients with ATD, especially Hashimoto's thyroiditis, were found to be positive using this technique, as were 9% of controls (Yoshida et al. 2009). However, a subsequent study using an ELISA was unable to detect any pendrin antibodies in patients with thyroid autoimmunity (Belguith-Maalej et al. 2010). Using a radioligand binding assay, pendrin antibodies were detectable in around 9% of patients with ATD; healthy controls were negative (Kemp et al. 2013). These results have been subsequently confirmed in a second cohort (Brix et al. 2014). At present it is unclear what role, if any, these antibodies have in disease pathogenesis.

Thyroid Hormone Antibodies

Antibodies against thyroid hormones are found in around 10–40% of patients with Hashimoto's thyroiditis and 5–20% of those with GD, depending on assay method, as well as in a number of other conditions and in healthy subjects (Sakata et al. 1985). Their impact clinically has been in their potential for interference in RIAs for thyroid hormones, which have now been superseded by IMAs. Recently it has been reported that over 90% of patients with type 1 diabetes mellitus have thyroid hormone antibodies, directed especially against T3, and there was an association between these antibodies and the development and progression of diabetes-related complications (Benvenga et al. 2015). A similarly high frequency of T3 and T4

antibodies has been found in vitiligo (Colucci et al. 2014). Although both disorders are well known to be associated with thyroid autoimmunity, the frequencies of TG- and TPO-Ab are much lower, and so the reasons for these associations are unclear and warrant further study.

Summary

There have been considerable developments in the methods used to detect thyroid antibodies since their first description six decades ago. Current IMAs for TG- and TPO-Ab are sensitive and generally reliable, although uncertainty still remains with establishing exact cutoffs for positivity (Jensen et al. 2006). This reflects a fundamental philosophical issue, which is that very low levels of such antibodies, particularly of low affinity, may well be a normal consequence of the failure to delete all autoreactive lymphocytes during ontogeny. Nonetheless measurement of these antibodies is useful in determining the cause of thyroid dysfunction and can be used to anticipate the development of such dysfunction in those who are currently euthyroid. Second- and third-generation assays for TBII are both sensitive and, when taken together with the clinical and biochemical features, specific; they are increasingly useful in determining the etiology of thyrotoxicosis and in the management of women with GD who are pregnant, to predict the likelihood of neonatal GD.

Other thyroid antibodies, directed against NIS, pendrin, and thyroid hormones, have no clinical utility at present. However, future developments in proteomic technology may allow simultaneous measurement of a number of autoantibodies based on addressable microbeads or nanobarcoded particles. This would increase the potential for the discovery of new disease subsets based on autoantibody pattern, as the phenomenon of sequential development of multiple autoantibodies ("autoimmune escalation") predicts that particular antibody clusters could mark the potential for temporary or permanent tissue damage or for the development of extrathyroidal complications such as GO. As an initial example of such an approach, IgG4 subclass thyroid antibody levels can be used to generate different heritability plots in juveniles with thyroid autoimmunity, with the potential to identify those at greatest risk of more severe fibrotic disease (Outschoorn et al. 2014).

References

Abeillon-du PJ, Chikh K, Bossard N, Bretones P, Gaucherand P, Claris O, et al. Predictive value of maternal second-generation thyroid-binding inhibitory immunoglobulin assay for neonatal autoimmune hyperthyroidism. Eur J Endocrinol. 2014;171(4):451–60.

Adams DD, Purve HD. Abnormal responses in the assay of thyrotropin. Proc Univ Otago Med Sch. 1956;34:11–2.

Adler TR, Beall GN, Curd JG, Heiner DC, Sabharwal UK. Studies of complement activation and IgG subclass restriction of anti-thyroglobulin. Clin Exp Immunol. 1984;56(2):383–9.

Ajjan RA, Weetman AP. Techniques to quantify TSH receptor antibodies. Nat Clin Pract Endocrinol Metab. 2008;4(8):461–8.

Ajjan RA, Findlay C, Metcalfe RA, Watson PF, Crisp M, Ludgate M, et al. The modulation of the human sodium iodide symporter activity by Graves' disease sera. J Clin Endocrinol Metab. 1998;83(4):1217–21.

Ajjan RA, Kemp EH, Waterman EA, Watson PF, Endo T, Onaya T, et al. Detection of binding and blocking autoantibodies to the human sodium-iodide symporter in patients with autoimmune thyroid disease. J Clin Endocrinol Metab. 2000;85(5):2020–7.

Ajjan RA, Weetman AP. Autoimmune thyroid disease and autoimmune polyglandular syndrome. In: Austin KF, Frank MM, Canton HI, Atkinson JP, Samter M, editors. Samter's immunological diseases. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 605–26.

Ando T, Latif R, Pritsker A, Moran T, Nagayama Y, Davies TF. A monoclonal thyroid-stimulating antibody. J Clin Invest. 2002;110(11):1667–74.

Ando T, Latif R, Daniel S, Eguchi K, Davies TF. Dissecting linear and conformational epitopes on the native thyrotropin receptor. Endocrinology. 2004;145(11):5185–93.

Bahn RS. Clinical review 157: pathophysiology of Graves' ophthalmopathy: the cycle of disease. J Clin Endocrinol Metab. 2003;88(5):1939–46.

Bahn RS. TSH receptor expression in orbital tissue and its role in the pathogenesis of Graves' ophthalmopathy. J Endocrinol Invest. 2004;27(3):216–20.

Bahn Chair RS, Burch HB, Cooper DS, Garber JR, Greenlee MC, Klein I, et al. Hyperthyroidism and other causes of thyrotoxicosis: management guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists. Thyroid. 2011;21(6):593–646.

Bartalena L. Diagnosis and management of Graves disease: a global overview. Nat Rev Endocrinol. 2013;9(12):724–34.

Bartalena L, Fatourechi V. Extrathyroidal manifestations of Graves' disease: a 2014 update. J Endocrinol Invest. 2014;37:691.

Beever K, Bradbury J, Phillips D, McLachlan SM, Pegg C, Goral A, et al. Highly sensitive assays of autoantibodies to thyroglobulin and to thyroid peroxidase. Clin Chem. 1989;35(9):1949–54.

Belguith-Maalej S, Hadj-Kacem H, Rebuffat SA, Mnif-Feki M, Nguyen B, Abid M, et al. Absence of anti-pendrin auto-antibodies in the sera of Tunisian patients with autoimmune thyroid diseases. Clin Lab. 2010;56(7–8):335–43.

Belyavin G, Trotter WR. Investigations of thyroid antigens reacting with Hashimoto sera; evidence for an antigen other than thyroglobulin. Lancet. 1959;1(7074):648–52.

Benvenga S, Pintaudi B, Vita R, Di VG, Di BA. Serum thyroid hormone autoantibodies in type 1 diabetes mellitus. J Clin Endocrinol Metab. 2015;100(5):1870–8.

Bhattacharyya R, Mukherjee K, Das A, Biswas MR, Basunia SR, Mukherjee A. Anti-thyroid peroxidase antibody positivity during early pregnancy is associated with pregnancy complications and maternal morbidity in later life. J Nat Sci Biol Med. 2015;6(2):402–5.

Bogazzi F, Bartalena L, Martino E. Approach to the patient with amiodarone-induced thyrotoxicosis. J Clin Endocrinol Metab. 2010;95(6):2529–35.

Bonnema SJ, Hegedus L. Radioiodine therapy in benign thyroid diseases: effects, side effects, and factors affecting therapeutic outcome. Endocr Rev. 2012;33(6):920–80.

Brix TH, Hegedus L, Weetman AP, Kemp HE. Pendrin and NIS antibodies are absent in healthy individuals and are rare in autoimmune thyroid disease: evidence from a Danish twin study. Clin Endocrinol (Oxf). 2014;81(3):440–4.

Bryant WP, Bergert ER, Morris JC. Identification of thyroid blocking antibodies and receptor epitopes in autoimmune hypothyroidism by affinity purification using synthetic TSH receptor peptides. Autoimmunity. 1995;22(2):69–79.

Burch HB, Burman KD, Cooper DS. A 2011 survey of clinical practice patterns in the management of Graves' disease. J Clin Endocrinol Metab. 2012;97(12):4549–58.

Carella C, Mazziotti G, Sorvillo F, Piscopo M, Cioffi M, Pilla P, et al. Serum thyrotropin receptor antibodies concentrations in patients with Graves' disease before, at the end of methimazole treatment, and after drug withdrawal: evidence that the activity of thyrotropin receptor antibody and/or thyroid response modify during the observation period. Thyroid. 2006;16(3):295–302.

Cayzer I, Chalmers SR, Doniach D, Swana G. An evaluation of two new haemagglutination tests for the rapid diagnosis of autoimmune thyroid diseases. J Clin Pathol. 1978;31(12):1147–51.

Chardes T, Chapal N, Bresson D, Bes C, Giudicelli V, Lefranc MP, et al. The human anti-thyroid peroxidase autoantibody repertoire in Graves' and Hashimoto's autoimmune thyroid diseases. Immunogenetics. 2002;54(3):141–57.

Chazenbalk GD, Pichurin P, Chen CR, Latrofa F, Johnstone AP, McLachlan SM, et al. Thyroid-stimulating autoantibodies in Graves' disease preferentially recognize the free A subunit, not the thyrotropin holoreceptor. J Clin Invest. 2002;110(2):209–17.

Chen CR, Aliesky H, Pichurin PN, Nagayama Y, McLachlan SM, Rapoport B. Susceptibility rather than resistance to hyperthyroidism is dominant in a thyrotropin receptor adenovirus-induced animal model of Graves' disease as revealed by BALB/c-C57BL/6 hybrid mice. Endocrinology. 2004;145(11):4927–33.

Chen CR, Hamidi S, Braley-Mullen H, Nagayama Y, Bresee C, Aliesky HA, et al. Antibodies to thyroid peroxidase arise spontaneously with age in NOD.H-2h4 mice and appear after thyroglobulin antibodies. Endocrinology. 2010;151(9):4583–93.

Chen H, Mester T, Raychaudhuri N, Kauh CY, Gupta S, Smith TJ, et al. Teprotumumab, an IGF-1R blocking monoclonal antibody inhibits TSH and IGF-1 action in fibrocytes. J Clin Endocrinol Metab. 2014;99(9):E1635–40.

Chin HS, Chin DK, Morgenthaler NG, Vassart G, Costagliola S. Rarity of anti- Na+/I- symporter (NIS) antibody with iodide uptake inhibiting activity in autoimmune thyroid diseases (AITD). J Clin Endocrinol Metab. 2000;85(10):3937–40.

Chiovato L, Vitti P, Santini F, Lopez G, Mammoli C, Bassi P, et al. Incidence of antibodies blocking thyrotropin effect in vitro in patients with euthyroid or hypothyroid autoimmune thyroiditis. J Clin Endocrinol Metab. 1990;71(1):40–5.

Cho SW, Bae JH, Noh GW, Kim YA, Moon MK, Park KU, et al. The presence of thyroid-stimulation blocking antibody prevents high bone turnover in untreated premenopausal patients with Graves' disease. PLoS One. 2015;10(12):e0144599.

Colucci R, Lotti F, Dragoni F, Arunachalam M, Lotti T, Benvenga S, et al. High prevalence of circulating autoantibodies against thyroid hormones in vitiligo and correlation with clinical and historical parameters of patients. Br J Dermatol. 2014;171(4):786–98.

Cooper DS, Laurberg P. Hyperthyroidism in pregnancy. Lancet Diabetes Endocrinol. 2013;1(3):238–49.

Costagliola S, Franssen JD, Bonomi M, Urizar E, Willnich M, Bergmann A, et al. Generation of a mouse monoclonal TSH receptor antibody with stimulating activity. Biochem Biophys Res Commun. 2002;299(5):891–6.

Costelloe SJ, Wassef N, Schulz J, Vaghijiani T, Morris C, Whiting S, et al. Thyroid dysfunction in a UK hepatitis C population treated with interferon-alpha and ribavirin combination therapy. Clin Endocrinol (Oxf). 2010;73(2):249–56.

Czarnocka B, Ruf J, Ferrand M, Carayon P, Lissitzky S. Purification of the human thyroid peroxidase and its identification as the microsomal antigen involved in autoimmune thyroid diseases. FEBS Lett. 1985;190(1):147–52.

D'Aurizio F, Tozzoli R, Villalta D, Pesce G, Bagnasco M. Immunoassay of thyroid peroxidase autoantibodies: diagnostic performance in automated third generation methods. A multicentre evaluation. Clin Chem Lab Med. 2015;53(3):415–21.

De Bellis A, Sansone D, Coronella C, Conte M, Iorio S, Perrino S, et al. Serum antibodies to collagen XIII: a further good marker of active Graves' ophthalmopathy. Clin Endocrinol (Oxf). 2005;62(1):24–9.

De GL, Abalovich M, Alexander EK, Amino N, Barbour L, Cobin RH, et al. Management of thyroid dysfunction during pregnancy and postpartum: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2012;97(8):2543–65.

De RA, de Gruijl TD, Uram JN, Tzou SC, Iwama S, Talor MV, et al. Development of thyroglobulin antibodies after GVAX immunotherapy is associated with prolonged survival. Int J Cancer. 2015;136(1):127–37.

Diana T, Kanitz M, Lehmann M, Li Y, Olivo PD, Kahaly GJ. Standardization of a bioassay for thyrotropin receptor stimulating autoantibodies. Thyroid. 2015;25(2):169–75.

Diana T, Wuster C, Kanitz M, Kahaly GJ. Highly variable sensitivity of five binding and two bio-assays for TSH-receptor antibodies. J Endocrinol Invest. 2016;39(10):1159–65.

Dubska M, Banga JP, Plochocka D, Hoser G, Kemp EH, Sutton BJ, et al. Structural insights into autoreactive determinants in thyroid peroxidase composed of discontinuous and multiple key contact amino acid residues contributing to epitopes recognized by patients' autoantibodies. Endocrinology. 2006;147(12):5995–6003.

Durante C, Tognini S, Montesano T, Orlandi F, Torlontano M, Puxeddu E, et al. Clinical aggressiveness and long-term outcome in patients with papillary thyroid cancer and circulating anti-thyroglobulin autoantibodies. Thyroid. 2014;24(7):1139–45.

Eckstein AK, Plicht M, Lax H, Neuhauser M, Mann K, Lederbogen S, et al. Thyrotropin receptor autoantibodies are independent risk factors for Graves' ophthalmopathy and help to predict severity and outcome of the disease. J Clin Endocrinol Metab. 2006;91(9):3464–70.

Ekinci EI, Chiu WL, Lu ZX, Sikaris K, Churilov L, Bittar I, et al. A longitudinal study of thyroid autoantibodies in pregnancy: the importance of test timing. Clin Endocrinol (Oxf). 2015;82(4):604–10.

Estienne V, McIntosh RS, Ruf J, Asghar MS, Watson PF, Carayon P, et al. Comparative mapping of cloned human and murine antithyroglobulin antibodies: recognition by human antibodies of an immunodominant region. Thyroid. 1998;8(8):643–6.

Ewins DL, Wilkin TJ. A clinical comparison of the enzyme-linked immunosorbent assay (ELISA) and haemagglutination (TRC) in the routine detection of antithyroglobulin antibodies. Acta Endocrinol (Copenh). 1983;103(2):216–22.

Fisher DA. Fetal thyroid function: diagnosis and management of fetal thyroid disorders. Clin Obstet Gynecol. 1997;40(1):16–31.

Fukuma N, Petersen VB, McLachlan SM, Pegg CA, Rees SB. Human monoclonal thyroglobulin autoantibodies of high affinity. I. Production, characterisation and interaction with murine monoclonal thyroglobulin antibodies. Autoimmunity. 1991;10(4):291–5.

Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, et al. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. Thyroid. 2012;22(12):1200–35.

Gardas A, Lewartowska A, Sutton BJ, Pasieka Z, McGregor AM, Banga JP. Human thyroid peroxidase (TPO) isoforms, TPO-1 and TPO-2: analysis of protein expression in Graves' thyroid tissue. J Clin Endocrinol Metab. 1997;82(11):3752–7.

Gerding MN, van der Meer JW, Broenink M, Bakker O, Wiersinga WM, Prummel MF. Association of thyrotrophin receptor antibodies with the clinical features of Graves' ophthalmopathy. Clin Endocrinol (Oxf). 2000;52(3):267–71.

Gershengorn MC, Neumann S. Update in TSH receptor agonists and antagonists. J Clin Endocrinol Metab. 2012;97(12):4287–92.

Gimenez-Barcons M, Colobran R, Gomez-Pau A, Marin-Sanchez A, Casteras A, Obiols G, et al. Graves' disease TSHR-stimulating antibodies (TSAbs) induce the activation of immature thymocytes: a clue to the riddle of TSAbs generation? J Immunol. 2015;194(9):4199–206.

Hansen PS, Brix TH, Iachine I, Kyvik KO, Hegedus L. The relative importance of genetic and environmental effects for the early stages of thyroid autoimmunity: a study of healthy Danish twins. Eur J Endocrinol. 2006;154(1):29–38.

Hasham A, Tomer Y. Genetic and epigenetic mechanisms in thyroid autoimmunity. Immunol Res. 2012;54(1–3):204–13.

Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab. 2002;87(2):489–99.

Jensen EA, Petersen PH, Blaabjerg O, Hansen PS, Brix TH, Hegedus L. Establishment of reference distributions and decision values for thyroid antibodies against thyroid peroxidase (TPOAb), thyroglobulin (TgAb) and the thyrotropin receptor (TRAb). Clin Chem Lab Med. 2006;44(8):991–8.

Kabelitz M, Liesenkotter KP, Stach B, Willgerodt H, Stablein W, Singendonk W, et al. The prevalence of anti-thyroid peroxidase antibodies and autoimmune thyroiditis in children and adolescents in an iodine replete area. Eur J Endocrinol. 2003;148(3):301–7.

Kakinuma A, Morimoto I, Kuroda T, Fujihira T, Eto S, McLachlan SM, et al. Comparison of recombinant human thyrotropin receptors versus porcine thyrotropin receptors in the thyrotropin binding inhibition assay for thyrotropin receptor autoantibodies. Thyroid. 1999;9(9):849–55.

Kasagi K, Konishi J, Iida Y, Ikekubo K, Mori T, Kuma K, et al. A new in vitro assay for human thyroid stimulator using cultured thyroid cells: effect of sodium chloride on adenosine 3′,5′-monophosphate increase. J Clin Endocrinol Metab. 1982;54(1):108–14.

Kasagi K, Konishi J, Iida Y, Tokuda Y, Arai K, Endo K, et al. A sensitive and practical assay for thyroid-stimulating antibodies using FRTL-5 thyroid cells. Acta Endocrinol (Copenh). 1987;115(1):30–6.

Kasagi K, Kousaka T, Higuchi K, Iida Y, Misaki T, Alam MS, et al. Clinical significance of measurements of antithyroid antibodies in the diagnosis of Hashimoto's thyroiditis: comparison with histological findings. Thyroid. 1996;6(5):445–50.

Kemp EH, Sandhu HK, Watson PF, Weetman AP. Low frequency of pendrin autoantibodies detected using a radioligand binding assay in patients with autoimmune thyroid disease. J Clin Endocrinol Metab. 2013;98(2):E309–13.

Khong JJ, McNab AA, Ebeling PR, Craig JE, Selva D. Pathogenesis of thyroid eye disease: review and update on molecular mechanisms. Br J Ophthalmol. 2016;100(1):142–50.

Khoo TK, Bahn RS. Pathogenesis of Graves' ophthalmopathy: the role of autoantibodies. Thyroid. 2007;17(10):1013–8.

Khoo DH, Eng PH, Ho SC, Tai ES, Morgenthaler NG, Seah LL, et al. Graves' ophthalmopathy in the absence of elevated free thyroxine and triiodothyronine levels: prevalence, natural history, and thyrotropin receptor antibody levels. Thyroid. 2000;10(12):1093–100.

Korevaar TI, Schalekamp-Timmermans S, de Rijke YB, Visser WE, Visser W, de Muinck Keizer-Schrama SM, et al. Hypothyroxinemia and TPO-antibody positivity are risk factors for premature delivery: the generation R study. J Clin Endocrinol Metab. 2013;98(11):4382–90.

Latrofa F, Ricci D, Grasso L, Vitti P, Masserini L, Basolo F, et al. Characterization of thyroglobulin epitopes in patients with autoimmune and non-autoimmune thyroid diseases using recombinant human monoclonal thyroglobulin autoantibodies. J Clin Endocrinol Metab. 2008;93(2):591–6.

Latrofa F, Fiore E, Rago T, Antonangeli L, Montanelli L, Ricci D, et al. Iodine contributes to thyroid autoimmunity in humans by unmasking a cryptic epitope on thyroglobulin. J Clin Endocrinol Metab. 2013;98(11):E1768–74.

Leger J, Olivieri A, Donaldson M, Torresani T, Krude H, van Vliet G. European Society for Paediatric Endocrinology consensus guidelines on screening, diagnosis, and management of congenital hypothyroidism. Horm Res Paediatr. 2014;81(2):80–103.

Lindberg B, Svensson J, Ericsson UB, Nilsson P, Svenonius E, Ivarsson SA. Comparison of some different methods for analysis of thyroid autoantibodies: importance of thyroglobulin autoantibodies. Thyroid. 2001;11(3):265–9.

Liu C, Hermsen D, Domberg J, Graeber C, Hautzel H, Duan Y, et al. Comparison of M22-based ELISA and human-TSH-receptor-based luminescence assay for the measurement of thyrotropin receptor antibodies in patients with thyroid diseases. Horm Metab Res. 2008;40(7):479–83.

Ludgate M, Dong Q, Dreyfus PA, Zakut H, Taylor P, Vassart G, et al. Definition, at the molecular level, of a thyroglobulin-acetylcholinesterase shared epitope: study of its pathophysiological significance in patients with Graves' ophthalmopathy. Autoimmunity. 1989;3(3):167–76.

Lupoli GA, Okosieme OE, Evans C, Clark PM, Pickett AJ, Premawardhana LD, et al. Prognostic significance of thyroglobulin antibody epitopes in differentiated thyroid cancer. J Clin Endocrinol Metab. 2015;100(1):100–8.

Lytton SD, Ponto KA, Kanitz M, Matheis N, Kohn LD, Kahaly GJ. A novel thyroid stimulating immunoglobulin bioassay is a functional indicator of activity and severity of Graves' orbitopathy. J Clin Endocrinol Metab. 2010;95(5):2123–31.

Malthiery Y, Lissitzky S. Primary structure of human thyroglobulin deduced from the sequence of its 8448-base complementary DNA. Eur J Biochem. 1987;165(3):491–8.

Many MC, Costagliola S, Detrait M, Denef F, Vassart G, Ludgate MC. Development of an animal model of autoimmune thyroid eye disease. J Immunol. 1999;162(8):4966–74.
Marino M, Chiovato L, Lisi S, Altea MA, Marcocci C, Pinchera A. Role of thyroglobulin in the pathogenesis of Graves' ophthalmopathy: the hypothesis of Kriss revisited. J Endocrinol Invest. 2004;27(3):230–6.
Massart C, Gibassier J, d'Herbomez M. Clinical value of M22-based assays for TSH-receptor antibody (TRAb) in the follow-up of antithyroid drug treated Graves' disease: comparison with the second generation human TRAb assay. Clin Chim Acta. 2009;407(1–2):62–6.
McLachlan SM, Rapoport B. The molecular biology of thyroid peroxidase: cloning, expression and role as autoantigen in autoimmune thyroid disease. Endocr Rev. 1992;13(2):192–206.
McLachlan SM, Rapoport B. Thyroid stimulating monoclonal antibodies: overcoming the road blocks and the way forward. Clin Endocrinol (Oxf). 2004;61(1):10–8.
McLachlan SM, Rapoport B. Thyroid peroxidase as an autoantigen. Thyroid. 2007;17(10):939–48.
McLachlan SM, Rapoport B. Thyrotropin-blocking autoantibodies and thyroid-stimulating autoantibodies: potential mechanisms involved in the pendulum swinging from hypothyroidism to hyperthyroidism or vice versa. Thyroid. 2013;23(1):14–24.
McLeod DS, Cooper DS, Ladenson PW, Ain KB, Brierley JD, Fein HG, et al. Prognosis of differentiated thyroid cancer in relation to serum thyrotropin and thyroglobulin antibody status at time of diagnosis. Thyroid. 2014;24(1):35–42.
Medici M, Porcu E, Pistis G, Teumer A, Brown SJ, Jensen RA, et al. Identification of novel genetic Loci associated with thyroid peroxidase antibodies and clinical thyroid disease. PLoS Genet. 2014;10(2):e1004123.
Metcalfe RA, Oh YS, Stroud C, Arnold K, Weetman AP. Analysis of antibody-dependent cell-mediated cytotoxicity in autoimmune thyroid disease. Autoimmunity. 1997;25(2):65–72.
Minich WB, Lenzner C, Morgenthaler NG. Antibodies to TSH-receptor in thyroid autoimmune disease interact with monoclonal antibodies whose epitopes are broadly distributed on the receptor. Clin Exp Immunol. 2004;136(1):129–36.
Mizokami T, Salvi M, Wall JR. Eye muscle antibodies in Graves' ophthalmopathy: pathogenic or secondary epiphenomenon? J Endocrinol Invest. 2004;27(3):221–9.
Morgenthaler NG, Pampel I, Aust G, Seissler J, Scherbaum WA. Application of a bioassay with CHO cells for the routine detection of stimulating and blocking autoantibodies to the TSH-receptor. Horm Metab Res. 1998;30(3):162–8.
Morgenthaler NG, Minich WB, Willnich M, Bogusch T, Hollidt JM, Weglohner W, et al. Affinity purification and diagnostic use of TSH receptor autoantibodies from human serum. Mol Cell Endocrinol. 2003;212(1–2):73–9.
Morris III JC, Hay ID, Nelson RE, Jiang NS. Clinical utility of thyrotropin-receptor antibody assays: comparison of radioreceptor and bioassay methods. Mayo Clin Proc. 1988;63(7):707–17.
Morshed SA, Davies TF. Graves' disease mechanisms: the role of stimulating, blocking, and cleavage region TSH receptor antibodies. Horm Metab Res. 2015;47(10):727–34.
Munro DS, Dirmikis SM, Humphries H, Smith T, Broadhead GD. The role of thyroid stimulating immunoglobulins of Graves's disease in neonatal thyrotoxicosis. Br J Obstet Gynaecol. 1978;85(11):837–43.
Netzel BC, Grebe SK, Carranza Leon BG, Castro MR, Clark PM, Hoofnagle AN, et al. Thyroglobulin (Tg) testing revisited: Tg assays, TgAb assays, and correlation of results with clinical outcomes. J Clin Endocrinol Metab. 2015;100(8):E1074–83.
Niccoli P, Fayadat L, Panneels V, Lanet J, Franc JL. Human thyroperoxidase in its alternatively spliced form (TPO2) is enzymatically inactive and exhibits changes in intracellular processing and trafficking. J Biol Chem. 1997;272(47):29487–92.
Nielsen CH, Leslie RG, Jepsen BS, Kazatchkine MD, Kaveri SV, Fischer E. Natural autoantibodies and complement promote the uptake of a self antigen, human thyroglobulin, by B cells and the proliferation of thyroglobulin-reactive CD4(+) T cells in healthy individuals. Eur J Immunol. 2001;31(9):2660–8.

Nordyke RA, Gilbert Jr FI, Miyamoto LA, Fleury KA. The superiority of antimicrosomal over antithyroglobulin antibodies for detecting Hashimoto's thyroiditis. Arch Intern Med. 1993;153(7):862–5.
Nye L, Pontes de Carvalho LC, Roitt IM. Restrictions in the response to autologous thyroglobulin in the human. Clin Exp Immunol. 1980;41(2):252–63.
Ochi Y, Kajita Y, Takasu N, Nagata A. Sensitive thyroid stimulating antibody (TSAb) assay using polyethylene glycol (PEG) – a review. J Immunoassay Immunochem. 2002;23(4):461–70.
Oda Y, Sanders J, Evans M, Kiddie A, Munkley A, James C, et al. Epitope analysis of the human thyrotropin (TSH) receptor using monoclonal antibodies. Thyroid. 2000;10(12):1051–9.
Ott J, Aust S, Kurz C, Nouri K, Wirth S, Huber JC, et al. Elevated antithyroid peroxidase antibodies indicating Hashimoto's thyroiditis are associated with the treatment response in infertile women with polycystic ovary syndrome. Fertil Steril. 2010;94(7):2895–7.
Ott J, Promberger R, Kober F, Neuhold N, Tea M, Huber JC, et al. Hashimoto's thyroiditis affects symptom load and quality of life unrelated to hypothyroidism: a prospective case–control study in women undergoing thyroidectomy for benign goiter. Thyroid. 2011;21(2):161–7.
Outschoorn IM, Talor MV, Burek CL, Hoffman WH, Rose NR. Heritability analysis of IgG4 antibodies in autoimmune thyroid disease. Autoimmunity. 2014;47(5):320–6.
Pani F, Atzori F, Baghino G, Boi F, Tanca L, Ionta MT, et al. Thyroid dysfunction in patients with metastatic carcinoma treated with sunitinib: is thyroid autoimmunity involved? Thyroid. 2015;25(11):1255–61.
Pedersen IB, Handberg A, Knudsen N, Heickendorff L, Laurberg P. Assays for thyroid-stimulating hormone receptor antibodies employing different ligands and ligand partners may have similar sensitivity and specificity but are not interchangeable. Thyroid. 2010;20(2):127–33.
Pfeilschifter J, Ziegler R. Smoking and endocrine ophthalmopathy: impact of smoking severity and current vs lifetime cigarette consumption. Clin Endocrinol (Oxf). 1996;45(4):477–81.
Pichurin P, Guo J, Yan X, Rapoport B, McLachlan SM. Human monoclonal autoantibodies to B-cell epitopes outside the thyroid peroxidase autoantibody immunodominant region. Thyroid. 2001;11(4):301–13.
Pop VJ, de Vries E, van Baar AL, Waelkens JJ, de Rooy HA, Horsten M, et al. Maternal thyroid peroxidase antibodies during pregnancy: a marker of impaired child development? J Clin Endocrinol Metab. 1995;80(12):3561–6.
Poppe K, Glinoer D, Tournaye H, Devroey P, van Steirteghem A, Kaufman L, et al. Assisted reproduction and thyroid autoimmunity: an unfortunate combination? J Clin Endocrinol Metab. 2003;88(9):4149–52.
Poppe K, Velkeniers B, Glinoer D. The role of thyroid autoimmunity in fertility and pregnancy. Nat Clin Pract Endocrinol Metab. 2008;4(7):394–405.
Portmann L, Hamada N, Heinrich G, DeGroot LJ. Anti-thyroid peroxidase antibody in patients with autoimmune thyroid disease: possible identity with anti-microsomal antibody. J Clin Endocrinol Metab. 1985;61(5):1001–3.
Portulano C, Paroder-Belenitsky M, Carrasco N. The Na+/I- symporter (NIS): mechanism and medical impact. Endocr Rev. 2014;35(1):106–49.
Prabhakar BS, Bahn RS, Smith TJ. Current perspective on the pathogenesis of Graves' disease and ophthalmopathy. Endocr Rev. 2003;24(6):802–35.
Rapoport B, Chazenbalk GD, Jaume JC, McLachlan SM. The thyrotropin (TSH) receptor: interaction with TSH and autoantibodies. Endocr Rev. 1998;19(6):673–716.
Rapoport B, Aliesky HA, Chen CR, McLachlan SM. Evidence that TSH receptor A-subunit multimers, not monomers, drive antibody affinity maturation in Graves' disease. J Clin Endocrinol Metab. 2015;100(6):E871–5.
Raspe E, Costagliola S, Ruf J, Mariotti S, Dumont JE, Ludgate M. Identification of the thyroid Na+/I- cotransporter as a potential autoantigen in thyroid autoimmune disease. Eur J Endocrinol. 1995;132(4):399–405.
Roitt IM, Doniach D, Campbell PN, Hudson RV. Auto-antibodies in Hashimoto's disease (lymphadenoid goitre). Lancet. 1956;271(6947):820–1.

Roitt IM, Campbell PN, Doniach D. The nature of the thyroid auto-antibodies present in patients with Hashimoto's thyroiditis (lymphadenoid goitre). Biochem J. 1958;69(2):248–56.
Rose NR, Witebsky E. Studies on organ specificity. V. Changes in the thyroid glands of rabbits following active immunization with rabbit thyroid extracts. J Immunol. 1956;76(6):417–27.
Roti E, Gardini E, Minelli R, Bianconi L, Braverman LE. Prevalence of anti-thyroid peroxidase antibodies in serum in the elderly: comparison with other tests for anti-thyroid antibodies. Clin Chem. 1992;38(1):88–92.
Rotondi M, de Martinis ML, Coperchini F, Pignatti P, Pirali B, Ghilotti S, et al. Serum negative autoimmune thyroiditis displays a milder clinical picture compared with classic Hashimoto's thyroiditis. Eur J Endocrinol. 2014;171(1):31–6.
Ruf J, Czarnocka B, Ferrand M, Doullais F, Carayon P. Novel routine assay of thyroperoxidase autoantibodies. Clin Chem. 1988;34(11):2231–4.
Sakata S, Nakamura S, Miura K. Autoantibodies against thyroid hormones or iodothyronine. Implications in diagnosis, thyroid function, treatment, and pathogenesis. Ann Intern Med. 1985;103(4):579–89.
Sanders J, Jeffreys J, Depraetere H, Richards T, Evans M, Kiddie A, et al. Thyroid-stimulating monoclonal antibodies. Thyroid. 2002;12(12):1043–50.
Sanders J, Evans M, Premawardhana LD, Depraetere H, Jeffreys J, Richards T, et al. Human monoclonal thyroid stimulating autoantibody. Lancet. 2003;362(9378):126–8.
Sanders J, Chirgadze DY, Sanders P, Baker S, Sullivan A, Bhardwaja A, et al. Crystal structure of the TSH receptor in complex with a thyroid-stimulating autoantibody. Thyroid. 2007;17(5):395–410.
Seissler J, Wagner S, Schott M, Lettmann M, Feldkamp J, Scherbaum WA, et al. Low frequency of autoantibodies to the human Na(+)/I(−) symporter in patients with autoimmune thyroid disease. J Clin Endocrinol Metab. 2000;85(12):4630–4.
Shields BM, Knight BA, Hill AV, Hattersley AT, Vaidya B. Five-year follow-up for women with subclinical hypothyroidism in pregnancy. J Clin Endocrinol Metab. 2013;98(12):E1941–5.
Smith TJ. The putative role of fibroblasts in the pathogenesis of Graves' disease: evidence for the involvement of the insulin-like growth factor-1 receptor in fibroblast activation. Autoimmunity. 2003;36(6–7):409–15.
Smooke-Praw S, Ro K, Levin O, Ituarte PH, Harari A, Yeh MW. Thyroglobulin antibody levels do not predict disease status in papillary thyroid cancer. Clin Endocrinol (Oxf). 2014;81(2):271–5.
Song YH, Li Y, Maclaren NK. The nature of autoantigens targeted in autoimmune endocrine diseases. Immunol Today. 1996;17(5):232–8.
Southgate K, Creagh F, Teece M, Kingswood C, Rees SB. A receptor assay for the measurement of TSH receptor antibodies in unextracted serum. Clin Endocrinol (Oxf). 1984;20(5):539–48.
Spencer CA. Clinical review: clinical utility of thyroglobulin antibody (TgAb) measurements for patients with differentiated thyroid cancers (DTC). J Clin Endocrinol Metab. 2011;96(12):3615–27.
Spencer C. Commentary on: implications of thyroglobulin antibody positivity in patients with differentiated thyroid cancer: a clinical position statement. Thyroid. 2013;23(10):1190–2.
Spencer CA, Bergoglio LM, Kazarosyan M, Fatemi S, Lopresti JS. Clinical impact of thyroglobulin (Tg) and Tg autoantibody method differences on the management of patients with differentiated thyroid carcinomas. J Clin Endocrinol Metab. 2005;90(10):5566–75.
Spencer C, Petrovic I, Fatemi S, LoPresti J. Serum thyroglobulin (Tg) monitoring of patients with differentiated thyroid cancer using sensitive (second-generation) immunometric assays can be disrupted by false-negative and false-positive serum thyroglobulin autoantibody misclassifications. J Clin Endocrinol Metab. 2014;99(12):4589–99.
Spencer L, Bubner T, Bain E, Middleton P. Screening and subsequent management for thyroid dysfunction pre-pregnancy and during pregnancy for improving maternal and infant health. Cochrane Database Syst Rev. 2015;9:CD011263.
Stagnaro-Green A. Approach to the patient with postpartum thyroiditis. J Clin Endocrinol Metab. 2012;97(2):334–42.

Suzuki S, Mitsunaga M, Miyoshi M, Hirakawa S, Nakagawa O, Miura H, et al. Cytophilic antithyroglobulin antibody and antibody-dependent monocyte-mediated cytotoxicity in Hashimoto's thyroiditis. J Clin Endocrinol Metab. 1980;51(3):446–53.

Takasu N, Matsushita M. Changes of TSH-stimulation blocking antibody (TSBAb) and thyroid stimulating antibody (TSAb) over 10 years in 34 TSBAb-positive patients with hypothyroidism and in 98 TSAb-positive Graves' patients with hyperthyroidism: reevaluation of TSBAb and TSAb in TSH-receptor-antibody (TRAb)-positive patients. J Thyroid Res. 2012;2012:182176.

Tandon N, Yan SL, Morgan BP, Weetman AP. Expression and function of multiple regulators of complement activation in autoimmune thyroid disease. Immunology. 1994;81(4):643–7.

Targovnik HM. Thyroglobulin structure, function and biosynthesis. In: Braverman LE, Cooper DS, editors. The thyroid – a fundamental and clinical text. Philadelphia: Lippincott, Williams and Wilkins; 2013. p. 74–92.

Thangaratinam S, Tan A, Knox E, Kilby MD, Franklyn J, Coomarasamy A. Association between thyroid autoantibodies and miscarriage and preterm birth: meta-analysis of evidence. BMJ. 2011;342:d2616.

Ticconi C, Giuliani E, Veglia M, Pietropolli A, Piccione E, Di SN. Thyroid autoimmunity and recurrent miscarriage. Am J Reprod Immunol. 2011;66(6):452–9.

Toccafondi RS, Aterini S, Medici MA, Rotella CM, Tanini A, Zonefrati R. Thyroid-stimulating antibody (TSab) detected in sera of Graves' patients using human thyroid cell cultures. Clin Exp Immunol. 1980;40(3):532–9.

Tomisti L, Urbani C, Rossi G, Latrofa F, Sardella C, Manetti L, et al. The presence of anti-thyroglobulin (TgAb) and/or anti-thyroperoxidase antibodies (TPOAb) does not exclude the diagnosis of type 2 amiodarone-induced thyrotoxicosis. J Endocrinol Invest. 2016;39:585.

Torino F, Barnabei A, Paragliola R, Baldelli R, Appetecchia M, Corsello SM. Thyroid dysfunction as an unintended side effect of anticancer drugs. Thyroid. 2013;23(11):1345–66.

Tozzoli R, D'Aurizio F, Ferrari A, Castello R, Metus P, Caruso B, et al. The upper reference limit for thyroid peroxidase autoantibodies is method-dependent: a collaborative study with biomedical industries. Clin Chim Acta. 2016;452:61–5.

Trotter WR, Belyavin G, Waddams A. Precipitating and complement-fixing antibodies in Hashimoto's disease. Proc R Soc Med. 1957;50(11):961–2.

Unuane D, Velkeniers B, Anckaert E, Schiettecatte J, Tournaye H, Haentjens P, et al. Thyroglobulin autoantibodies: is there any added value in the detection of thyroid autoimmunity in women consulting for fertility treatment? Thyroid. 2013;23(8):1022–8.

Vanderpump MP, Tunbridge WM, French JM, Appleton D, Bates D, Clark F, et al. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clin Endocrinol (Oxf). 1995;43(1):55–68.

Verburg FA, Luster M, Cupini C, Chiovato L, Duntas L, Elisei R, et al. Implications of thyroglobulin antibody positivity in patients with differentiated thyroid cancer: a clinical position statement. Thyroid. 2013;23(10):1211–25.

Villanueva R, Inzerillo AM, Tomer Y, Barbesino G, Meltzer M, Concepcion ES, et al. Limited genetic susceptibility to severe Graves' ophthalmopathy: no role for CTLA-4 but evidence for an environmental etiology. Thyroid. 2000;10(9):791–8.

Vissenberg R, Manders VD, Mastenbroek S, Fliers E, Afink GB, Ris-Stalpers C, et al. Pathophysiological aspects of thyroid hormone disorders/thyroid peroxidase autoantibodies and reproduction. Hum Reprod Update. 2015;21(3):378–87.

Vitti P, Chiovato L, Lopez G, Lombardi A, Santini F, Mammoli C, et al. Measurement of TSAb directly in serum using FRTL-5 cells. J Endocrinol Invest. 1988;11(4):313–7.

Vitti P, Elisei R, Tonacchera M, Chiovato L, Mancusi F, Rago T, et al. Detection of thyroid-stimulating antibody using Chinese hamster ovary cells transfected with cloned human thyrotropin receptor. J Clin Endocrinol Metab. 1993;76(2):499–503.

Vos XG, Smit N, Endert E, Tijssen JG, Wiersinga WM. Frequency and characteristics of TBII-seronegative patients in a population with untreated Graves' hyperthyroidism: a prospective study. Clin Endocrinol (Oxf). 2008;69(2):311–7.

Walsh JP, Bremner AP, Feddema P, Leedman PJ, Brown SJ, O'Leary P. Thyrotropin and thyroid antibodies as predictors of hypothyroidism: a 13-year, longitudinal study of a community-based cohort using current immunoassay techniques. J Clin Endocrinol Metab. 2010;95(3):1095–104.

Watson PF, Ajjan RA, Phipps J, Metcalfe R, Weetman AP. A new chemiluminescent assay for the rapid detection of thyroid stimulating antibodies in Graves' disease. Clin Endocrinol (Oxf). 1998;49(5):577–81.

Watt T, Hegedus L, Bjorner JB, Groenvold M, Bonnema SJ, Rasmussen AK, et al. Is thyroid autoimmunity per se a determinant of quality of life in patients with autoimmune hypothyroidism? Eur Thyroid J. 2012;1(3):186–92.

Weetman AP. Autoimmune thyroid disease: propagation and progression. Eur J Endocrinol. 2003;148(1):1–9.

Weetman AP, McGregor AM. Autoimmune thyroid disease: developments in our understanding. Endocr Rev. 1984;5(2):309–55.

Weetman AP, Tse CK, Randall WR, Tsim KW, Barnard EA. Acetylcholinesterase antibodies and thyroid autoimmunity. Clin Exp Immunol. 1988;71(1):96–9.

Weetman AP, Black CM, Cohen SB, Tomlinson R, Banga JP, Reimer CB. Affinity purification of IgG subclasses and the distribution of thyroid auto-antibody reactivity in Hashimoto's thyroiditis. Scand J Immunol. 1989;30(1):73–82.

Winther KH, Cramon P, Watt T, Bjorner JB, Ekholm O, Feldt-Rasmussen U, et al. Disease-specific as well as generic quality of life is widely impacted in autoimmune hypothyroidism and improves during the first six months of levothyroxine therapy. PLoS One. 2016;11(6):e0156925.

Yoshida H, Amino N, Yagawa K, Uemura K, Satoh M, Miyai K, et al. Association of serum antithyroid antibodies with lymphocytic infiltration of the thyroid gland: studies of seventy autopsied cases. J Clin Endocrinol Metab. 1978;46(6):859–62.

Yoshida A, Hisatome I, Taniguchi S, Shirayoshi Y, Yamamoto Y, Miake J, et al. Pendrin is a novel autoantigen recognized by patients with autoimmune thyroid diseases. J Clin Endocrinol Metab. 2009;94(2):442–8.

Zophel K, Wunderlich G, Kotzerke J, von Landenberg LP, Roggenbuck D. M22 based (manual) ELISA for TSH-receptor antibody (TRAb) measurement is more sensitive than 2nd generation TRAb assays. Clin Chim Acta. 2009;403(1–2):266.

Nonisotopic Thyroid Imaging

4

E. Papini, R. Guglielmi, G. Bizzarri, and A. Frasoldati

Contents

Abstract

Thyroid ultrasonography (US) is a reliable diagnostic tool for the evaluation of focal and diffuse thyroid disease. US accurately identifies the size and location of

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thyroid nodules and defines their risk of malignancy. Irregular margins, microcalcifications, taller-than-wide shape, deep hypoechogenicity and extrathyroid extension are clear-cut signs of malignancy while the predictive role of color-doppler and elastography findings is still controversial. The use of the recently proposed US classifications systems decreases the possible inconsistency of US descriptive reports, provides a reproducible tool to select lesions that should undergo aspiration biopsy, and improves the communication between sonographers, clinicians and pathologists. Additionally, US examination may suggest an altered thyroid function or an inflammatory condition or confirm size abnormalities and changes of thyroid structure.

The expensive cross-sectional imaging techniques (computed tomography or magnetic resonance imaging) are less sensitive and specific tools for the diagnosis of thyroid diseases. These imaging modalities should be used in the presurgical staging of thyroid malignancies at risk of extra-thyroidal spread to demonstrate the involvement of cervical structures and planning the extension of surgery. Their use in the postsurgical surveillance of cervical cancer recurrences appears limited and is indicated mainly in patients with increasing thyroglobulin (or calcitonin) levels and inconclusive neck US.

The role of functional imaging methods (thyroid scintiscan and PET/CT) is addressed in the dedicated section of this text.

Keywords
Thyroid imaging · Thyroid ultrasound · Computerized tomography · Magnetic resonance imaging · Thyroid nodules

A Rationale for the Use of Thyroid Imaging

Currently, most physicians who commonly deal with thyroid diseases in their clinical practice use thyroid imaging as a major diagnostic tool for the management of their patients (American Institute of Ultrasound in Medicine et al. 2013; Haugen et al. 2016; Gharib et al. 2016). Thyroid imaging is primarily based on neck ultrasound (US) examination, and, in many countries, thyroid US evaluation is directly performed by endocrinologists in their offices.

Notably, endocrine consultations are increasingly due to newly diagnosed thyroid lesions revealed by neck imaging studies (e.g., CT, MRI, color-Doppler carotid evaluation, PET/CT scan) performed for reasons not related to thyroid disease (Jin and McHenry 2012). Conversely, only in specific cases are these more expensive and less accessible technologies needed for a correct categorization of thyroid diseases.

The widespread use of cervical US is probably the leading cause of the "epidemic" of thyroid nodules and the increased incidence of small-size thyroid carcinomas (Davies and Welch 2006; Leenhardt et al. 2004; Hegedüs 2016). The cost-benefit ratio of this trend is debated in light of the limited aggressiveness of most nonpalpable thyroid tumors and the consequent cost for society (Davies and Welch 2006;

Leenhardt et al. 2004; Hegedüs 2016). For all these reasons, the endocrinologist should have an adequate knowledge of the various thyroid imaging techniques, should use them appropriately, and should evaluate their results according to a cost-effective strategy.

The clinical settings in which thyroid imaging plays a relevant role are the following:

- Patients with thyroid enlargement or tenderness or with the finding of a cervical lump at physical examination
- Patients with laboratory abnormalities indicating a thyroid disease or first-degree young relatives of patients with autoimmune thyroid disease
- Patients at high risk for thyroid malignancy (history of familial thyroid carcinoma, previous neck irradiation)
- Stratification of the risk of malignancy of thyroid nodules
- Guidance for thyroid nodule fine-needle aspiration (FNA) or for minimally invasive procedures
- Presurgical staging of patients who undergo surgery for thyroid malignancy or symptomatic goiter
- Postsurgical surveillance of neck recurrences after resection of thyroid carcinoma

Thyroid US examination with high-frequency transducers provides an unmatched spatial and time resolution for the detection of focal lesions, the stratification of the risk of malignancy, and the assessment of diffuse structural abnormalities. Thyroid radioisotope scan is of use for the characterization of the gland's functional status in hyperthyroid patients, especially if candidate for radioiodine treatment, and for the detection of hyperfunctioning nodules that usually do not deserve FNA, in toxic multinodular goiter (Bonnema and Hegedüs 2012). Computed tomography (CT) and magnetic resonance imaging (MRI) offer valuable information in patients with a large goiter or an invasive tumor in the presurgical evaluation for classification of whether eligible or not for surgery. These cross-sectional imaging techniques provide a detailed definition of the relationship of the thyroid with the anatomical structures located in the neck or the mediastinum.

In the following paragraphs, the details of these thyroid imaging techniques and their clinical indications will be concisely reported.

Techniques

Ultrasonography

The normal thyroid is a bilobed gland, located in the lower anterior neck, with two pear-shaped lobes connected anteriorly by a central rim of tissue defined as the isthmus. The left and right lobes are located immediately to the left and right of the trachea, respectively. The internal carotid arteries and internal jugular veins are located posterolaterally to the thyroid lobes, whereas the strap muscles of the neck are located anteriorly (Solbiati et al. 2001; Arun et al. 2014). Usually, the thyroid gland size is

20 mm or less in both the transverse and the anteroposterior diameter and is 40–60 mm in its longitudinal diameter. The isthmus typically has an anteroposterior thickness of less than 3–5 mm (Arun et al. 2014). Thyroid volume may be estimated with the ellipsoid formula (Papini et al. 2014) or, more precisely, with a dedicated tridimensional software. At US evaluation, normal thyroid echotexture appears fairly homogeneous with a slight ground-glass appearance and is more echogenic with respect to strap muscles (Leenhardt et al. 2011). Cervical US investigation is performed on patients in the supine position and with their neck in hyperextension over a pad (Solbiati et al. 2001). The thyroid gland is initially 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 the 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 (Baskin 2008; Chaudhary and Bano 2013). 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. The whole thyroid should then be carefully evaluated by transverse scans of the upper, middle, and lower part of each lobe. In presence of thyroid or cervical lesions, the detected nodules or lymph nodes should be examined along several planes by carefully revolving the probe over the area of interest. All the US findings that may be helpful for the clinical management of diffuse or nodular thyroid disease should be assessed and the characters, dimensions, and location clearly reported. The US images of focal thyroid disease and the graphic representation of regional lymph node involvement should be part of the US report (Leenhardt et al. 2011; Frasoldati et al. 2016).

Computed Tomography and Magnetic Resonance

US examination is the primary imaging modality for thyroid disease, but other cross-sectional techniques used for the evaluation of neck or chest disease may commonly reveal thyroid abnormalities (Frasoldati et al. 2016). The diagnostic accuracy of CT and MRI for thyroid malignancy is low, and these imaging methods are of no use in the diagnostic work-up of thyroid nodules. In contrast, they are useful tools for the presurgical assessment of the intrathoracic growth of large goiters as well as for the evaluation of the extension of the disease in patients with aggressive thyroid malignancy (Binder et al. 1980; Park et al. 2016)

Today, multidetector CT (MDCT) allows multiplanar reconstructions (axial, sagittal, coronal planes) with slice thickness of 2 mm or less. Acquisition should include a volume from the skull base to the tracheal bifurcation (Baskin 2008). At the CT evaluation, when without contrast medium, the thyroid appears as a homogeneous and mildly hyperattenuating gland when compared to the surrounding neck muscles and has an average attenuation of 80–100 HU. After the injection of contrast material, the gland shows an intense and homogeneous enhancement.

For large goiters, a study without contrast injection may disclose a relevant intrathoracic growth that should best be approached in a thoracic surgery department. For invasive thyroid carcinomas, the use of CT contrast media is usually needed to reliably rule out an infiltrative growth or the presence of nodal metastasis in cervical regions (e.g., the retrotracheal area) that cannot be reliably visualized by US examination (Arun et al. 2014; Frasoldati et al. 2016).

In such patients with aggressive disease who are candidates for rapid radioiodine ablation and post-dose whole-body scintigraphy, MRI with gadolinium should be used as the first choice cross-sectional imaging method because the iodide load of CT contrast media may interfere with thyroid iodide uptake for a few months (Park et al. 2016). MRI examination is commonly performed with axial and coronal T1-weighted and with fat-saturated T2-weighted images, followed by post-contrast axial and coronal T1-weighted images. When compared with the cervical muscles, the thyroid gland is faintly hyperintense on T1-weighted MR images and iso- to slightly hyperintense on T2-weighted images. Analogously to contrast-enhanced CT imaging, gadolinium-enhanced MRI shows the thyroid gland as characterized by an intense and homogeneous enhancement (Arun et al. 2014). MRI offers special opportunity by functional imaging capability as diffusion-weighted imaging (DWI). DWI was recently proposed in the detection and characterization of thyroid cancer and neck recurrences and is reported as being highly specific, but having a low-sensitivity, diagnostic procedure for thyroid nodule evaluation (Noda et al. 2015). Both MRI and CT imaging provide precise anatomic information regarding the position of the thyroid relative to adjacent vascular, respiratory, and muscular structures. Still, in spite of the better contrast resolution of MR imaging and the elevated spatial resolution of CT, B-mode US examination remains the best option for the evaluation of thyroid nodules and cervical lesions.

Thyroid Imaging for the Assessment of Diffuse Thyroid Disease

Ultrasound

Diffuse structural changes as well as marked alterations of thyroid volume may be observed in a number of different clinical conditions, most frequently diffuse or multinodular goiter (MNG); autoimmune inflammatory disorders, i.e., Hashimoto's thyroiditis and Graves' disease; and de Quervain's subacute thyroiditis (Papini et al. 2014).

Multinodular goiter (MNG) is characterized by a marked, symmetric enlargement of both thyroid lobes or, less frequently, with an asymmetric growth of the thyroid lobes (Hegedüs et al. 2016; Bahn and Castro 2011). The US examination may demonstrate presence of a variable number of nodules of different size, sometimes strictly contiguous or truly confluent (Chaudhary and Bano 2013; Frasoldati et al. 2016; Binder et al. 1980; Park et al. 2016; Noda et al. 2015; Hegedüs et al. 2016; Bahn and Castro 2011; Rago and Vitti 2014). The sonographic appearance of the gland is clearly inhomogeneous, with conglomerates of solid nodules, anechoic

colloid collections sometimes exhibiting comet-tail artifacts, and hypoechoic degenerative or hemorrhagic areas without normal thyroid parenchyma (Ahuja et al. 1996). Eggshell-like or isolated macrocalcifications may be present, due to previous inflammatory and/or necrotic changes. This clinical and US picture reliably indicates a benign condition, even if the occurrence of small occult neoplastic foci (mostly MPTC) cannot be completely excluded as evinced by autopsy data (Kovács et al. 2005). In the absence of suspicious features, FNA should be performed only on large (>20 mm) and in size increasing nodules. Close attention should be devoted to the appearance, over time, of large, solid, and marked hypoechoic areas, with intrinsic vascularity and necrotic foci, which may suggest development of follicular or anaplastic thyroid carcinomas (Hoang et al. 2007).

Hashimoto's thyroiditis and Graves' disease are clinical conditions that share common pathologic changes. Diffuse lymphocytic infiltration of the thyroid gland, markedly increased vascularity, and later appearance of fibrotic changes are commonly observed in both conditions (American Institute of Ultrasound in Medicine et al. 2013; Walfish 1997; Langer et al. 2001). Thus, overt hyperthyroid and hypothyroid patients may present with rather similar US images, characterized by a pronounced hypoechoic gland with hyperechoic fibrotic streaks, pseudonodular organization of the parenchyma, and lobulated posterior margins (Yamashiro et al. 2007; Lupo and Levine 2014). Especially, in Graves' disease the vascularity is strongly increased, and in the active phase of the inflammatory process, color-Doppler imaging reveals intense and diffuse vascular signals, defined as the "thyroid inferno" pattern (Ralls et al. 1988). In the majority of patients with autoimmune thyroiditis in its active stage, vascularity is also increased, yet less dramatically than in Graves' disease. Initially, the hypoechogenicity may be mild, and the thyroid gland may still appear as rather homogeneous (Fig. 1). Subsequently, the gland becomes frankly inhomogeneous, due to the presence of

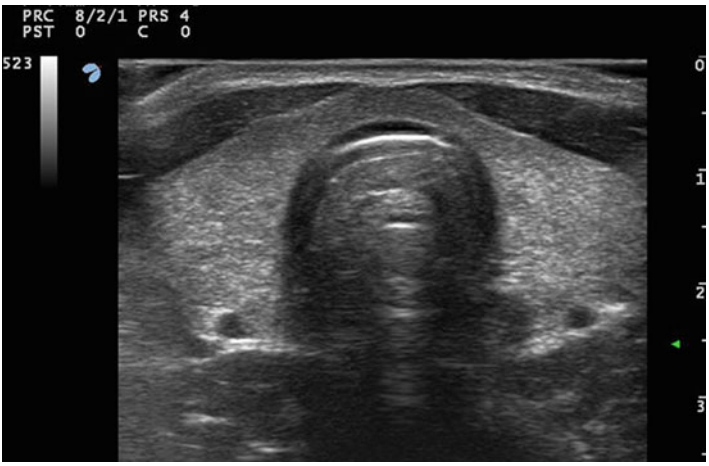

Fig. 1 US scan of the thyroid gland in a 16-year-old patient with initial lymphocytic thyroiditis. The size is within normal limits, but a slight inhomogeneous texture may be appreciated

multiple confluent small hypoechoic areas ("salt-and-pepper" pattern), which may be difficult to differentiate from the picture in subacute thyroiditis (Bennedbaek and Hegedüs 1997), and its volume may decrease, preluding to a progressive atrophic involution (Yamashiro et al. 2007).

In patients with subclinical hypothyroidism, who present with a "normal" thyroid gland at physical examination and no detectable serum antithyroid antibodies, US evaluation may often reveal the typical US changes of chronic thyroiditis (Rago et al. 2001). Thus, in these patients with subclinical autoimmune thyroiditis, thyroid US findings may justify the control of thyroid function over time (Pedersen et al. 2000; Raber et al. 2002; Rosário et al. 2009; Tam et al. 2015).

Both in Hashimoto's thyroiditis and in Graves' disease, inflammatory lymph nodes are usually revealed in the central neck compartment, either near the lower pole of the lobes or close to the anterior capsule of the isthmus. This finding may be helpful for the clinical diagnosis, because it usually precedes the onset of major changes of thyroid function, but may be misleading in the case of papillary carcinomas arising in chronic thyroiditis as they can be confused with metastatic lymph nodes. Notably, both benign and malignant nodules may coexist with chronic autoimmune thyroiditis, but the detection of small hypoechoic nodules may be particularly difficult due to the presence of the diffuse or focal inflammatory hypoechoic changes (Anderson et al. 2010a; Moon et al. 2009; Nam et al. 2015). Solid nodules with round shape, ill-defined smooth margins, and hyperechoic texture, as compared to the surrounding parenchyma, are frequently observed in chronic thyroiditis and do not generally require cytologic examination due to their benign nature (Anderson et al. 2010b). Instead, pronounced hypoechoic nodules, even if hardly distinguishable from the hypoechoic inflammatory pattern of the thyroid gland, should always be carefully evaluated (Moon et al. 2009; Nam et al. 2015).

The diagnosis of de Quervain's subacute thyroiditis is usually clear-cut from the clinical picture, characterized by neck pain or tenderness, fever, and general malaise. The US presentation is, in most instances, distinctive with one or more areas of the gland involved by the appearance of highly hypoechoic, cloudy, or fingerlike areas (Yamashiro et al. 2007; Bennedbaek and Hegedüs 1997). These findings, caused by inflammation, typically show ill-defined margins and are nearly always devoid of vascular signals at color-Doppler examination. Subacute thyroiditis may initially affect only a portion of one lobe and then develop into a confluent pattern, eventually with patchy bilateral extension. Thus, the US texture of the gland is asymmetrically inhomogeneous, and, in some cases, a dominant part of one lobe may appear highly hypoechoic, while the contralateral lobe is often unaffected. The complete recovery of the thyroid tissue is to be expected, usually within 3–6 months, along with but delayed compared with the remission of clinical symptoms (Bennedbaek and Hegedüs 1997).

Other Imaging Techniques

Currently, the only indication for CT or MR imaging in diffuse, nonneoplastic, thyroid disease is the presurgical staging of large-size goiters that are associated

with local pressure symptoms. Large goiters may variably affect the trachea and esophagus, and this may result in stenosis of the upper airway and/or swallowing dysfunction that may be clinically ill-defined unless appropriately evaluated (Sorensen et al. 2014). In these patients presurgery cross-sectional imaging should be performed for a better definition of the indication and timing of surgical resection and for an appropriate planning of the surgical approach (Binder et al. 1980; Park et al. 2016; Noda et al. 2015; Hegedüs et al. 2016; Bahn and Castro 2011; Rago and Vitti 2014; Ahuja et al. 1996; Kovács et al. 2005; Hoang et al. 2007; Walfish 1997; Langer et al. 2001; Yamashiro et al. 2007; Lupo and Levine 2014; Ralls et al. 1988; Bennedbaek and Hegedüs 1997; Rago et al. 2001; Pedersen et al. 2000; Raber et al. 2002; Rosário et al. 2009; Tam et al. 2015; Anderson et al. 2010a, b; Moon et al. 2009; Nam et al. 2015; Sorensen et al. 2014). Indeed, both CT and MRI may accurately define the extent of the intrathoracic growth of the goiter, the displacement of the vascular structures of the neck, and the severity of the airway lumen reduction (Arun et al. 2014; Cooper et al. 1991).

Thyroid Imaging for the Assessment of Risk of Malignancy in Focal Thyroid Lesions

Thyroid Ultrasound

According to the definition provided by the recent guidelines for the clinical management of thyroid lesions, a thyroid nodule is a lesion "radiologically distinct from the surrounding parenchyma" (Haugen et al. 2016; Gharib et al. 2016). Thus, thyroid nodules are more precisely defined by means of US imaging than by the traditional physical examination. Additionally, thyroid nodules that are incidentally found during carotid duplex studies and other imaging techniques, like CT, MRI, and 2–18 [F] fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET) (Giovannella et al. 2014; Davies et al. 2016), should be accurately evaluated with thyroid US examination based on the greater diagnostic accuracy of this technique. Therefore, irrespective of the road leading to the detection of thyroid nodules, a neck US examination should be performed with the evaluation of the following characteristics: (a) size and number, (b) texture and echogenicity, (c) shape and margins, (d) presence and type of calcifications, and (e) vascularization and stiffness pattern (Haugen et al. 2016; Gharib et al. 2016; Kim et al. 2002; Papini et al. 2002, 2016; Mandel 2004; Moon et al. 2011). Each of the abovementioned features will be separately discussed hereafter.

All the collected information should be used for rating of the probability of malignancy (or benignity) of the nodule, a critical factor for the subsequent management of the patient. The US TIRADS categorization of thyroid nodules, proposed in 2009 for this purpose (Horvath et al. 2009), has gained growing interest, as demonstrated by the attention for this issue in all recent guidelines (Haugen et al. 2016; Gharib et al. 2016; Shin et al. 2016; Perros et al. 2014).

Size and number. Presently, about 40% of cytological diagnoses of thyroid malignancy relate to papillary microcarcinomas (PTMC), defined as tumors with a largest diameter of 1 cm or less (Davies and Welch 2014). While the size of a nodule scarcely predicts its benign or malignant nature (Papini et al. 2002; McHenry et al. 2008), a modest increase in the risk of malignancy may be expected in nodules larger than 4 cm in diameter (Haugen et al. 2016; Gharib et al. 2016). Importantly, larger thyroid tumors are often associated with more advanced local disease (Verburg et al. 2009).

The long-term surveillance of thyroid nodules has traditionally been based on the detection of variations in their size. Yet, nodule growth does not indicate malignancy because benign hyperplastic nodules may exhibit a slow but progressive growth (Alexander et al. 2003; Durante et al. 2015), whereas the majority of PTMC may remain stable for years (Ito and Miyauchi 2015). Rather than an independent sign of malignancy, a steady nodule growth is relevant for the decision-making in the management of nodular thyroid disease, eventually suggesting an active treatment rather than a further wait-and-see strategy. US monitoring of nodule size is also critical for the evaluation of the objective response to any medical or nonsurgical treatment. For this purpose, the measurement of the major nodule diameter is frequently employed, but this is an unreliable index (Haugen et al. 2016; Gharib et al. 2016). As a nearly 20% interobserver variability in the measurement of any thyroid nodule diameter has been reported (Brauer et al. 2005), the determination of the volume of thyroid nodules with the ellipsoid formula (longitudinal diameter x transverse diameter x anteroposterior diameter $\times$ $\pi/6$) should preferentially be used. The volume of the nodule is, e.g., used to assess the efficacy of US-guided interventional procedures, such as laser thermal ablation (LTA) or radiofrequency (RF) ablation (Gharib et al. 2016). This parameter may also be affected by up to a nearly 50% interobserver variability (Brauer et al. 2005; Choi et al. 2010; Park et al. 2010). Thus, a 50% increase in thyroid volume should be considered as a reasonable threshold for significant growth of thyroid lesions (Haugen et al. 2016). Three-dimensional (3-D) US imaging has been proposed to achieve a higher accuracy of volume measurement, as compared to planar images (McQueen and Bathia 2015), but it is still infrequently used in clinical practice.

Similarly to the size, the number of thyroid nodules (solitary vs. multiple) seems to be irrelevant for the prediction of malignancy in the individual patient (Papini et al. 2002; Campanella et al. 2014). As a general rule, the number of the detected nodules should be included in the US report only when the lesions are clearly defined and can be reliably monitored. In multinodular goiters, with diffuse coalescent nodules, and in diffuse colloid cystic hyperplasia, with multiple anechoic or mixed lesions, the actual number of thyroid nodules may be difficult to assess, and detailed reporting is of limited clinical value (Gharib et al. 2016).

Structure, echogenicity, and texture. At US examination the majority of thyroid nodules show a slightly inhomogeneous structure and may present either with a mixed, fluid/solid, pattern or a nonuniform solid texture due to degenerative or inflammatory changes. A complete homogeneous appearance is typical of a minority of lesions, specifically the anechoic cysts, exhibiting a totally fluid content, or the

solid "isoechoic" nodules, showing a texture quite similar to that of perinodular thyroid parenchyma. In chronic autoimmune thyroiditis, several homogeneous hyperechoic solid areas are frequently observed within the hypoechoic gland (the "white knight" sign) (Mandel 2004; Moon et al. 2008; Wienke et al. 2003).

Most thyroid carcinomas present as hypoechoic solid nodules at US examination. Unfortunately, about half of the benign nodules share this characteristic. Thus, hypoechogenicity in a solid thyroid nodule is a quite sensitive, yet poorly specific, predictor of malignancy (Mandel 2004; Moon et al. 2008). On the other hand, in case of marked hypoechogenicity, defined as an US texture that appears darker than that of the pre-thyroid muscles, the risk of malignancy is reported to rise up to nearly 90% (Cooper et al. 1991; Moon et al. 2008). These data are based on robust evidence, but it should be underlined that the assessment of echogenicity is associated with a significant interobserver variability. Besides, the coexistence of chronic thyroiditis in the extranodular thyroid tissue may cause major problems in the interpretation of the actual nodule echogenicity (Choi et al. 2010; Park et al. 2010; Wienke et al. 2003).

Thyroid nodules with a predominant cystic content only occasionally correspond to a thyroid carcinoma (Gharib et al. 2016) (Fig. 2). However, thyroid malignancy cannot be completely ruled out by the presence of a cystic component, since a 4–6% prevalence of thyroid malignancy in partially cystic nodules has been reported in several series (Chan et al. 2003; Lee et al. 2009). Mixed nodules with a large (≥50% of the volume) irregular solid component, which is eccentrically located and vascularized at color-Doppler examination, are at greater risk of being malignant

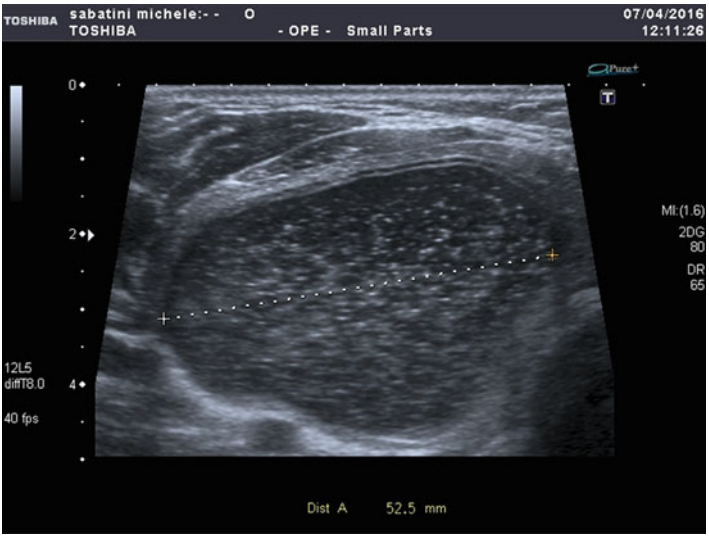

Fig. 2 Transverse US scan of the thyroid gland. A large hemorrhagic cyst with regular margins and no solid internal lump is present in the right lobe. The aspect of the content is typical of bloody fluid. Benign lesion at histological examination

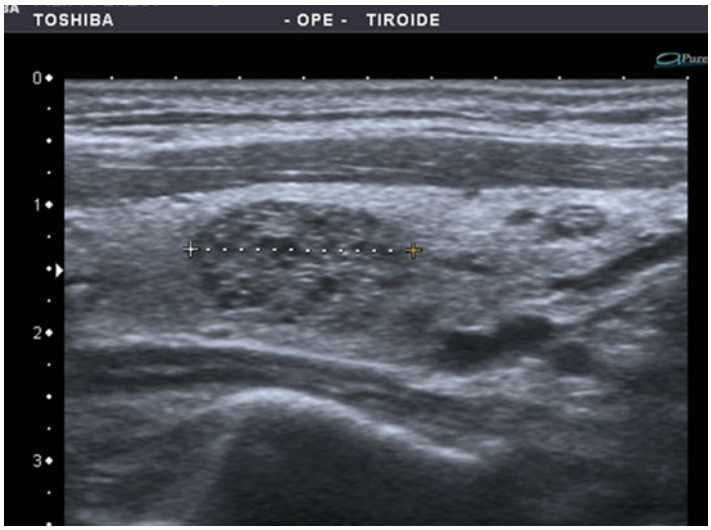

Fig. 3 Longitudinal US scan of the thyroid gland. A benign isoechoic nodule with oval shape, spongiform appearance, and regular margins is seen

(Chan et al. 2003; Lee et al. 2009). The coexistence of other suspicious features, such as microcalcifications and/or irregular margins or shape, is of aid in not overlooking this potentially insidious lesion. A category of echoic lesions which has recently gained interest as highly predictive of a benign thyroid nodule is the so-called spongiform nodule (Fig. 3). This kind of lesion, characterized by the aggregation of multiple microcystic components in more than 50% of the nodule volume, is consistently reported as associated with a nearly 100% benignity rate (Haugen et al. 2016; Gharib et al. 2016; Moon et al. 2008; Chan et al. 2003).

Shape and margins. The vast majority of benign nodules have a round to oval profile (Kim et al. 2002; Moon et al. 2008) (Fig. 4), while malignant lesions sometimes exhibit a taller-than-wide shape (Fig. 5). This US sign (Kim et al. 2002) is based on a ≥ 1 ratio between the anteroposterior (AP) and the transverse diameter of the nodule, which is likely to reflect a centrifugal pattern of growth of the lesion. The above reported definition has been revised to include all nodules with an AP diameter longer than the transverse one, either measured in transverse or longitudinal planes (Moon et al. 2008). For clinical purposes, a simple and user-friendly categorization of nodule shape is the following: (a) oval to round, (b) taller than wide, and (c) irregular (Shin et al. 2016). The "taller-than-wide" sign is highly specific for malignancy but unfortunately has poor sensitivity (Campanella et al. 2014). Of note, when the contour of the nodule is highly irregular, the calculation of the AP/transverse (longitudinal) diameter ratio may be difficult and unreliable.

Ill-defined margins have been indicated as signs of malignancy (Kim et al. 2002; Papini et al. 2002). Actually, this definition has been used to encompass different US patterns, with two consequences: this sign has variable accuracy across studies and

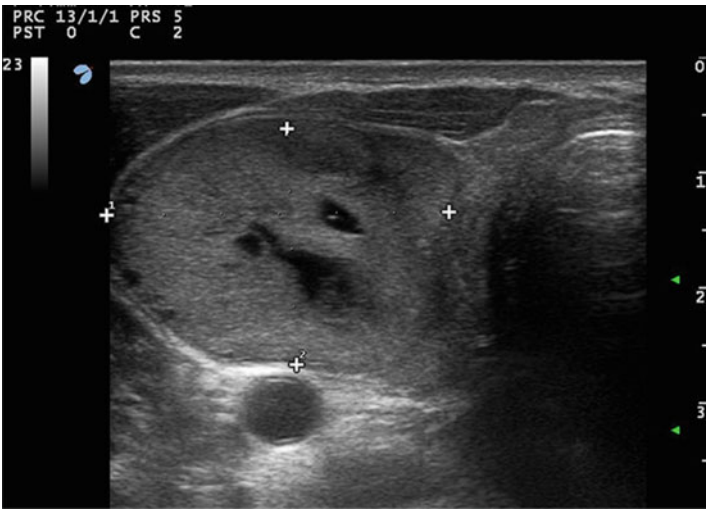

Fig. 4 Tranverse US scan of the thyroid gland. A large isoechoic nodule with oval shape, small cystic changes, and regular margins is seen in the right lobe. The nodule was benign at histological examination

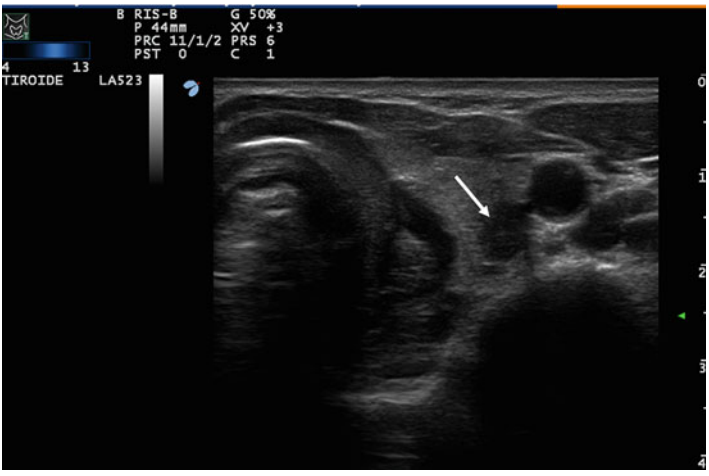

Fig. 5 Tranverse US scan of the thyroid gland. A suspicious nodule with taller-than-wide shape, irregular margins, and intranodular microcalcifications is depicted in the left thyroid lobe. The nodule was a papillary carcinoma, tall cell variant, at histological examination

its interobserver reproducibility has been rather low (Campanella et al. 2014; Wienke et al. 2003). For practical purposes, two questions should be separately addressed by the US examiner: (a) Is the nodule clearly demarcated from the extranodular tissue? (b) Is the nodule contour regular or not? Nodule margins may be ill-defined and yet show a regular and smooth profile, as it is often observed in

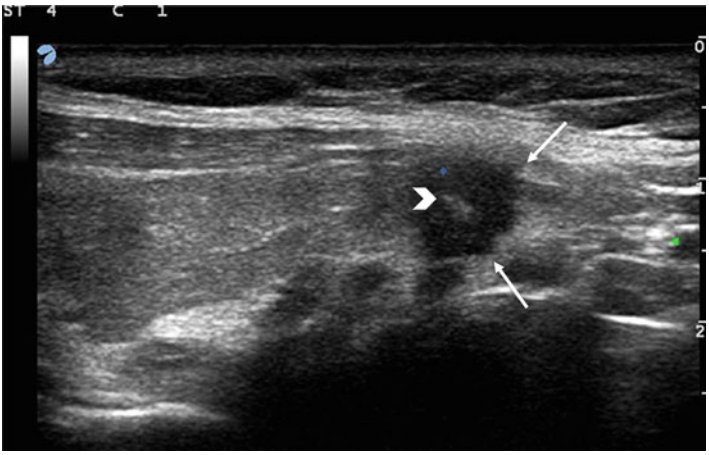

Fig. 6 US scan of the thyroid gland. A markedly hypoechoic nodule with round shape, spiculated margins (*arrows*), and intranodular hyperechoic spots (*arrowhead*) is clearly depicted. The nodule was a papillary carcinoma, classic variant, at histological examination

mildly hypoechoic benign nodules (Reading et al. 2005). Notably, the lack of echogenicity difference between the nodule and the surrounding tissue (i.e., the absence of interface contrast) may be influenced by the technical performance of the US equipment and by the settings of the examination. Instead, spiculated margins, even when exhibiting a clear-cut separation from the surrounding parenchyma, is a highly specific sign of malignancy (Moon et al. 2008; Fig. 6).

The so-called peripheral hypoechoic halo is easily detectable in a large number of nodules. In most instances, the halo corresponds to a peripheral arrangement of the nodule vascularity and is typically seen in benign hyperplastic nodules presenting with an even, smooth profile and a peripheral color-Doppler or power-Doppler flow mapping (Levine 2008). An unevenly thickened or incomplete hypoechoic halo, due to fibrotic pseudo-capsular structures or to inflammatory and/or necrotic changes, may be observed in a minority (about 10%) of papillary thyroid carcinomas and in many follicular carcinomas (Kobayashi et al. 2005; Youan et al. 2006; Jeh et al. 2007). In addition, the association of peripheral eggshell calcifications, with hypoechoic growth outside the calcification rim, may suggest malignancy (Moon et al. 2008; Kim et al. 2008). Although the majority of thyroid cancers are devoid of a peripheral halo, the absence of the halo has poor specificity as a predictor of malignancy. In conclusion, while the detection of a regular peripheral hypoechoic halo strengthens the judgment of benignity in regularly shaped and profiled nodules, an irregular hypoechoic halo in suspiciously looking nodules does not rule out malignancy, but rather makes it more probable.

Calcifications. In clinical practice, three types of calcifications may be distinguished on the basis of their size and localization: (a) microcalcifications; (b) macrocalcifications, also referred to as "coarse" calcifications; and (c) peripheral (or "rim") calcifications (Khoo et al. 2002; Taki et al. 2004; Yoon et al. 2007). Microcalcifications appear as tiny (<1 mm) hyperechoic punctate spots, usually

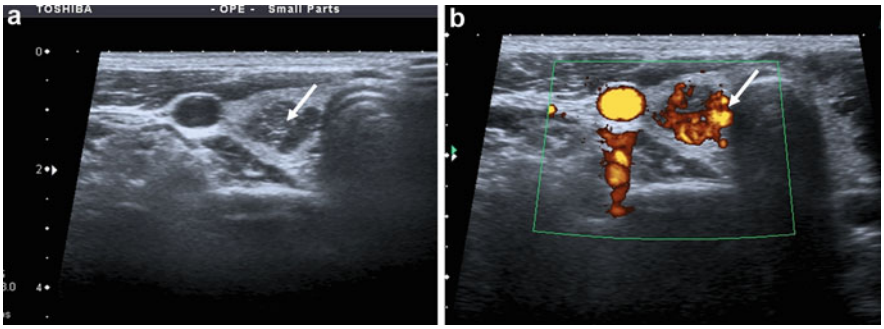

Fig. 7 (**a**) Tranverse US scan of the thyroid gland. A suspicious nodule with irregular margins and intranodular microcalcifications (*arrow*) is present in the right lobe. (**b**) Intense chaotic vascular signals (*arrow*) are revealed by color-Doppler examination. The nodule was a papillary carcinoma, follicular variant, at histological examination

devoid of posterior shadowing, and may correspond to psammoma bodies, a typical histological finding of papillary thyroid carcinoma (Wang et al. 2006; Chammas et al. 2008) (Figs. 6 and 7a). Microcalcifications need to be distinguished from the hyperechoic spots defined as "comet-tail" artifacts. The latter are usually due to solid drops of colloid or to small interfaces within a mixed lesion and typically observed in benign nodules (Moon et al. 2008; Levine 2008; Beland et al. 2011). Intranodular hyperechoic spots which cannot be clearly identified either as microcalcifications or as comet-tail artifacts should best be described in the US report as "indeterminate hyperechoic spots" (Gharib et al. 2016; Perros et al. 2014).

The specificity of microcalcifications as a sign of malignancy is high (85–95%), but its sensitivity remains quite low (Campanella et al. 2014). Macrocalcifications are usually the result of necrotic or hemorrhagic changes and are frequently encountered in large benign goiters. However, when found in solitary solid nodules, they should be considered as a potential sign of malignancy (Khoo et al. 2002; Taki et al. 2004; Lee and Rho 2009). This rule does not apply to nodules previously treated by US-guided percutaneous minimally invasive procedures, such as percutaneous ethanol injection (PEI) or laser ablation (LA) and radiofrequency thermoablation, as coarse intranodular calcifications are frequently observed in these settings (Gharib et al. 2016; Guglielmi et al. 2004). Peripheral "rim" calcifications have traditionally been considered as a typical sign of "old" hyperplastic nodules. This issue has been questioned in recent studies as the discontinuity of the eggshell structure, especially if associated with extrusive growth of hypoechoic tissue, has been reported as predictive of malignancy (Haugen et al. 2016; Moon et al. 2008; Yoon et al. 2007).

Vascularity. Color- and power-Doppler examinations provide useful information about the vascular architecture of thyroid nodules. Three types of color-flow mapping may be identified (Solbiati et al. 2001; Yuan et al. 2006; Moon et al. 2010):

– Peripheral: vascular signals are predominantly revealed around the nodule. Scanty blood flow may be occasionally detected in the central part of the nodule, but the prevalent blood distribution is clearly perinodular.

– Intranodular: marked vascular signals are detected in the central part of the nodule.
– Absent: absence of color-flow mapping, both in the peripheral and in the central part of the nodule.

The value of this parameter in the differentiation between benign and malignant nodules is controversial (Shin et al. 2016; Tamsel et al. 2007). The majority of malignant nodules show an intranodular vascular pattern (Fig. 7b), but this pattern is shared by a non-negligible percentage of benign solid nodules as well. On the other hand, peripheral flow mapping is the pattern most often observed in benign nodules; yet, about 20% of thyroid cancers may also show a peripheral vascular ring (Rago et al. 2007). The complete absence of vascularity has also been suggested as a sign predictive of benignity, but the consistency of this finding is questionable. In light of this evidence, some authors do not recommend the routine use of color- or power-Doppler examination for thyroid nodules (Haugen et al. 2016). Thus, vascularity should represent only ancillary information in the diagnostic work-up of thyroid lesions (Gharib et al. 2016; Shin et al. 2016).

Elastography pattern. Elastography provides a visual modeling based on a color scale of the degree of stiffness shown by the nodular tissue under the mechanical force applied by the US probe (Rago et al. 2007). The stiffer the nodule, the more likely that it is malignant (Fig. 8). The combination of elastography with B-mode US has been reported to increase the diagnostic performance of US evaluation (Rago et al. 2007; Trimboli et al. 2012; Azizi et al. 2013). Therefore, elastography may be

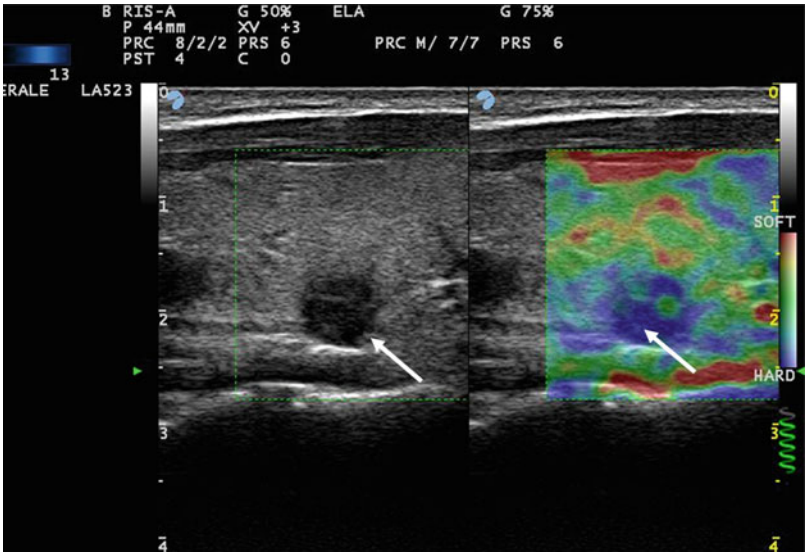

Fig. 8 US scan and elastographic assessment of the thyroid gland. A hypoechoic nodule with irregular margins and posterior extracapsular growth (*arrow*) is present in the left thyroid lobe. Elastography demonstrates elevated stiffness of the nodule (*blue color*) (*arrow*). The nodule was a papillary carcinoma, classical variant, at histological examination

of use in those nodules that show ambiguous features at US evaluation, in order to establish if they should be referred for FNA assessment (Russ et al. 2013). However, some limitations need to be taken into account. Primarily, elastography is an operator-dependent technique, and a consistent and universally validated reporting system is not yet available (Ghajarzadeh et al. 2014). Furthermore, coexisting diffuse alterations in the perinodular tissue, as in chronic thyroiditis, and the presence of macrocalcifications or large fluid areas greatly decrease elastography's reliability (Trimboli et al. 2012). Recent technological developments [e.g., strain index, acoustic radiation force impulse, supersonic shear wave (Zhang et al. 2014; Park et al. 2015; Liu et al. 2015)] are reported as valuable tools to overcome these limitations. Presently, elastography, as is the case for color-Doppler evaluation, provides only complementary information in the diagnostic work-up of thyroid lesions (Gharib et al. 2016; Shin et al. 2016).

Ultrasound classification systems for thyroid nodules. The information collected with US evaluation of thyroid nodules can be used to stratify their risk of malignancy, in analogy with the imaging reporting system that was developed for breast lesions (BI-RADS) (D'Orsi et al. 2003). The adoption of a standardized US reporting system may reduce the inconsistency of US "descriptive" reports, providing a reproducible tool to select nodules for FNA and improving the communication between sonographers, clinicians, and pathologists. The first US classification system with a worldwide dissemination was defined by the acronym TIRADS (Thyroid Imaging Reporting and Data System) (Horvath et al. 2009). This classification is based on an analytical and detailed categorization of US patterns, organized in ten different classes and subclasses of thyroid nodules. Later, the BTA system (Perros et al. 2014) proposed a conceptual correlation of the US findings with the tiered cytological categories. US results were subdivided into four categories according to increasing risk of malignancy, from U2 (benign thyroid nodule) to U4-5 (suspicious and malignant lesion, respectively). Notably, this system also oriented the suspicious US findings toward specific types (papillary, follicular, or medullary) of thyroid malignancies. The 2015 ATA system (Haugen et al. 2016) is slightly more complex as it rates five US classes of thyroid nodules: 1 (benign), 2 (likely benign), 3 (indeterminate), 4 (likely malignant), and 5 (malignant). The 2016 AACE-ACE-AME system is aimed at offering a simpler and more practical tool. This rating system, easy for use in clinical practice, is based on a 3-class categorization with a risk of malignancy ranging from $<1\%$ for the US class 1 (low risk) to $>50–70\%$ for the US class 3 (high risk) (Gharib et al. 2016). More recently, the Korean Imaging-Based Management of Thyroid Nodules document has recommended a categorization of thyroid nodules into four classes: high, intermediate, and low suspicion nodules and definitely benign nodules (Shin et al. 2016). The probability of malignancy is stratified, partly at variance with the other current systems, on the basis of three major criteria: solid appearance, echogenicity, and suspicious US features. Intranodular vascularity and the elastography findings are recognized as potentially useful but complementary (Shin et al. 2016) (Tables 1, 2, 3, and 4).

All these risk stratification systems seem quite effective for defining the clinical relevance of thyroid nodules and the rating of the risk of malignancy. Therefore, they

Table 1 American Thyroid Association Thyroid Nodule and Cancer Guidelines. Sonographic patterns and estimated risk of malignancy

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
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

should always be added to the descriptive US report for a clear communication of the probability of a neoplastic thyroid lesion. Ideally, a single risk stratification system should be implemented and endorsed by all the international scientific societies in order to improve communication, including, but not limited to, the interpretation of scientific literature.

CT and MRI

Computed tomography and magnetic resonance imaging are less accurate than US for the evaluation of risk of malignancy in focal thyroid lesions. Small size nodules may be undetectable by CT and/or MRI evaluation, and the presence of microcalcifications may easily be overlooked (Shin et al. 2016). The value of diffusion-weighted (DW) MRI in the characterization of thyroid nodules is reported as promising but requires further study (Wu et al. 2014) Thus, after US characterization, no further imaging is needed for thyroid lesions that are not associated with signs of infiltrative growth of the cervical structures.

Table 2 AACE-ACE-AME 2016 Clinical Practice Guidelines. Thyroid ultrasound features and risk of malignancy

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

Table 3 South Korean K-TIRADS. Thyroid ultrasound features and risk of malignancy

Category	US feature	Malignancy risk (%)
5. High suspicion	Solid hypoechoic nodule with any of three suspicious features	>60
4. Intermediate suspicion	Solid hypoechoic nodule without any of three suspicious US features Partially cystic with any of three US suspicious features	15–50%
3. Low suspicion	Partially cystic or isohyperechoic nodule without any of three suspicious US features	15–20
2. Benign	Spongiform Partially cystic nodule with comet-tail artifact Pure cyst	<3%
1. No nodule		<1

Primary thyroid lymphoma, mostly the mucosa-associated lymphoid tissue lymphoma (MALT), is a rare neoplasia that is usually diagnosed in elderly subjects with a long history of nodular goiter or Hashimoto's thyroiditis (Solbiati et al. 2001). Patients with lymphoma characteristically present with compressive symptoms of the airways

Table 4 British Thyroid Association U-score

U1. *Normal*
U2. *Benign*
(a) Halo, isoechoic/mildly hyperechoic
(b) Cystic change +/- ring-down sign (colloid)
(c) Microcystic/spongiform
(d and e) Peripheral eggshell calcification
(f) Peripheral vascularity
U3. *Indeterminate/equivocal*
(a) Homogeneous, hyperechoic (markedly), solid, halo (follicular lesion)
(b) Hypoechoic, equivocal echogenic foci, cystic change
(c) Mixed/central vascularity
U4. *Suspicious*
(a) Solid, hypoechoic (cf thyroid)
(b) Solid, very hypoechoic (cf strap muscle)
(c) Disrupted peripheral calcification, hypoechoic
(d) Lobulated outline
U5. *Malignant*
(a) Solid, hypoechoic, lobulated/irregular outline, microcalcification (? papillary carcinoma)
(b) Solid, hypoechoic, lobulated/irregular outline, globular calcification (? medullary carcinoma)
(c) Intranodular vascularity
(d) Shape (taller > wide) (AP > TR)
(e) Characteristic-associated lymphadenopathy

Note: *U-score* ultrasound score, *AP* anteroposterior diameter, *TR* transverse diameter

and esophagus, and the disease commonly manifests as a spreading mass that is hypoattenuating at CT examination (Sharma et al. 2016). A diffuse replacement of the thyroid parenchyma is frequently associated with narrowing of the tracheal lumen and displacement of the cervical vascular structures. Both in cases of thyroid lymphoma and anaplastic carcinoma, abnormal regional lymph nodes are commonly seen and may be coalescent with the pathologic thyroid tissue (Aiken 2012).

Thyroid Imaging for the Presurgical Staging and Follow-Up of Thyroid Malignancy

Ultrasonography

US examination plays a critical role for planning of surgery in patients referred for thyroid carcinoma surgery (Haugen et al. 2016; Perros et al. 2014; King 2008; Lew and Solorzano 2010; Yeh et al. 2015). Specifically, US assessment should aim at obtaining the following information, needed to plan the appropriate surgical approach:

(a) Size, location, and extension of the primary tumor
(b) Description of potential cervical lymph node involvement

These data are of relevant interest because local recurrence of differentiated thyroid carcinoma is in most cases due to lymph node metastases overlooked during the initial surgery. On a topographical basis, metastatic cervical lymph nodes may be detected either in the lateral (right and left) or in the central neck compartments (Robbins et al. 2008; Carty et al. 2009; Stack et al. 2012; Randolph et al. 2013). The lateral neck compartments approximately correspond, on each side, to the neck regions covered by the sternocleidomastoid muscles and are subdivided into levels from II to V. The margins of the central neck compartment are the carotid arteries, laterally, and the trachea, medially. This compartment extends, cranio-caudally, from the hyoid bone to the horizontal plane of the innominate artery (Robbins et al. 2008; Carty et al. 2009; Stack et al. 2012) and is further subdivided into the levels VI and VII. The central compartment houses the peri-thyroidal and para- and pre-tracheal lymph nodes, usually the first nodes that are involved when thyroid malignancies spread. About 60–65% of lymph node metastases from thyroid carcinoma occur in the central compartment, mostly in the pre-tracheal and the ipsilateral paratracheal lymph nodes (Machens et al. 2002; Wada et al. 2003; Roh et al. 2008; Kupferman et al. 2004). In this area, unfortunately, the sensitivity of the presurgical US examination in detecting suspicious lymph nodes is suboptimal (Yeh et al. 2015).

The lateral neck compartments are usually involved after the metastatic spread to the central compartment has already occurred. "Skip metastases," defined as lateral lymph node metastases not associated with the involvement of the central neck compartment, is a possible but rare occurrence (Machens et al. 2002; Wada et al. 2003; Roh et al. 2008; Kupferman et al. 2004).

As for the level subdivision of the lateral neck compartments, level II extends cranio-caudally from the skull base to the hyoid bone, level III from the hyoid bone to the cricoid cartilage, and level IV from the cricoid to the clavicle. Along the sagittal plane, level II is limited anteriorly by the stylohyoid muscles and levels III and IV by the sternohyoid muscles, while the posterior margin of the sternocleidomastoid muscles represents the posterior limit of all the abovementioned levels (Carty et al. 2009; Stack et al. 2012). Level II lymph nodes (upper jugular nodes) are rarely affected by tumors, usually only by those found in the upper third of the thyroid lobes. In contrast, the lymph nodes in levels III–IV, also defined as mid- and lower jugular nodes, are those most frequently affected by thyroid cancer metastases (Machens et al. 2002; Wada et al. 2003; Roh et al. 2008; Kupferman et al. 2004). Level V corresponds to the posterior triangle of the neck, and its anterior and posterior boundaries are the sternocleidomastoid and the trapezius muscles, respectively, while the clavicle is its inferior limit. Level V metastases are present in a minority of patients with thyroid cancer, usually only when levels II to IV are clearly involved (Machens et al. 2002; Wada et al. 2003; Roh et al. 2008; Kupferman et al. 2004). Evidence of LNM in the lateral compartment requires a lateral neck dissection. Therefore, the results of the presurgical US examination of the lateral neck compartments are critical for surgical planning, and any suspicious findings should be referred for cytological examination with FNA (Haugen et al. 2016; Gharib et al. 2016; Shin et al. 2016).

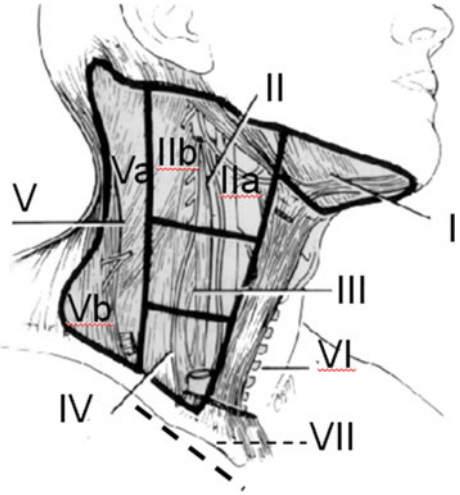

Fig. 9 Topographic classification of neck lymph nodes according to the American Joint Committee on Cancer and the American Academy of Otolaryngology – Head and Neck Surgery, now endorsed by the pTNM classification of thyroid cancer
The central neck compartment (levels VI–VII) extends longitudinally from the hyoid bone to the plane of the innominate artery and mediolaterally from the carotid arteries and the trachea
The lateral neck compartments (levels II–V) are limited medially by the carotid arteries and laterally by the trapezius muscle (Modified from Robbins et al. Arch Otolaryngol Head Neck Surg, vol 117: 601–605, 1991)

Preoperative US examination of the neck should cover multiple transverse planes from the carotid arteries to the midline (to investigate the central compartment) and to the posterior triangle (lateral compartment). At the same time, the probe should be guided along a cranio-caudal direction first in the central compartment (level I to levels VI–VII) and then in both lateral compartments (levels II–V) (King 2008; Lew and Solorzano 2010). By offering a general view of the region, transverse planes allow the targeting of major abnormalities, while US examination along the longitudinal planes is useful for a closer inspection (Fig. 9).

Lymph node evaluation should always be preceded by the US examination of the primary tumor. The precise location and size of the malignant lesion are relevant for the surgical treatment and need to be detailed in the US report. Although the presurgical T (tumor) staging by US has limited accuracy, any sign of thyroid capsule infiltration or extracapsular invasion should be reported along with the presence of any suspicious foci (King 2008; Lew and Solorzano 2010).

The assessment of possible metastatic involvement of cervical lymph nodes is the major endpoint of preoperative US examination, due to its critical role for planning extension of the surgical resection (Leenhardt et al. 2013). The sensitivity of US for metastatic disease is clearly lower in the central as compared to the lateral neck compartments (reported range: 25–50% vs. 75–90%, respectively) (Park et al. 2009; Choi et al. 2009a; Frasoldati and Valcavi 2004). Thus, US is reliable in the assessment of

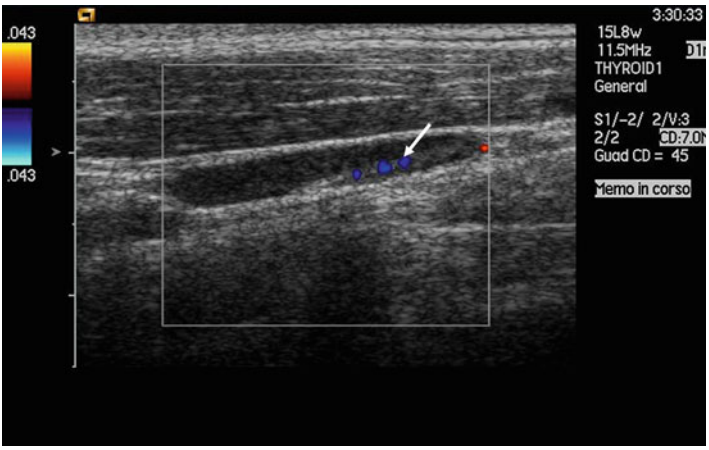

Fig. 10 Normal lymph node. The shape is elongated, the hilum is clearly visualized as a hyperchoic streak, and regular central vascular signals are revealed by color-Doppler examination. A hyperechoic border limits the mildly hypoechoic structure

metastatic disease in the lateral neck lymph nodes, while a false negative US examination may frequently occur in the central compartment, even in experienced hands. Additionally, the coexistence of chronic autoimmune thyroiditis may be associated with the presence of enlarged lymph nodes in the central neck compartment, which can lead to false positive US findings (Frasoldati and Valcavi 2004; Sousa do Rosario et al. 2005). In the lateral cervical compartment, the US diagnosis of nodal metastases from thyroid carcinoma may be difficult due to the frequent finding of enlarged benign lymph nodes of inflammatory origin in this area. Notably, also malignant lymph nodes due to other neck tumors and the less frequent benign neck lesions, such as parathyroid adenomas, neuromas, or lymphangiomas, may mimic malignant lymph nodes and deceive the US examiner (Frasoldati and Valcavi 2004).

The US assessment of cervical lymph nodes is based on the following parameters: size, shape, texture, hilum, and vascularity (Sousa do Rosario et al. 2005; Ahuja and Ying 2005; Kuna et al. 2006). In analogy with the US evaluation of thyroid nodules, no single US finding is characterized by an absolute specificity and an elevated specificity. Thus, in most cases only the methodical evaluation of the US features of all the lymph nodes will allow evaluation of the actual risk of malignancy (Lee et al. 2010; Leboulleux et al. 2007).

Shape. Benign lymph nodes usually show an oval form with regular margins, a slightly hypoechoic homogeneous texture, and a recognizable hyperechoic hilum (Fig. 10). The interest of the US examiner is usually attracted to enlarged lymph nodes (e.g., $\geq$15–20 mm maximum diameter), but the size offers poor specificity for malignancy because inflammatory lymph nodes, especially in the lateral cervical compartments, may be substantially enlarged. The measurement of the short axis (i.e., the minimum diameter) of the lymph node provides a more specific parameter, as a short diameter $\geq$6 or 7 mm was reported to be significantly associated with malignancy

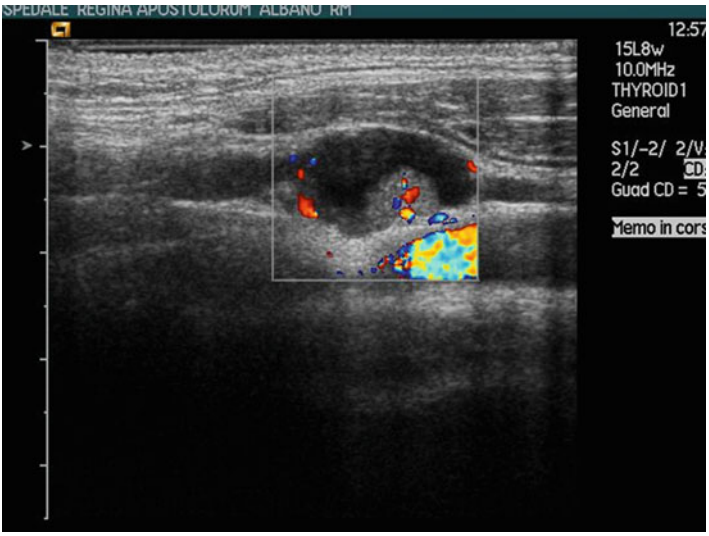

Fig. 11 Neck nodal metastasis of papillary carcinoma. The lymph node is rounded, with peripheral vascular signals and with highly suspicious cystic changes

(Leenhardt et al. 2013; Kuna et al. 2006; Lee et al. 2010; Leboulleux et al. 2007). A more reliable index of malignancy, as compared to single diameters, is the increase of the shortest-to-longest axis ratio (S:L $\geq$0.5) or the reduction of the longitudinal-to-transverse diameter ratio to $\leq$2. Thus, lymph nodes exhibiting a round, globular shape should be considered as suspicious. This statement, however, is strongly influenced by the lymph node location because the specificity for malignancy of the "round shape sign" was reported as high as 80% in the lateral compartments but as low as 11% in cervical level VI (Kuna et al. 2006; Lee et al. 2010; Leboulleux et al. 2007).

Central hilum. The presence of a clearly defined hyperechoic hilum structure is relevant because it is highly suggestive of a benign condition and nearly completely rules out the risk of malignancy. Importantly, the absence of a sonographically detectable hilum is a nonspecific clue of malignancy, because this finding is shared also by many benign lymph nodes (Kuna et al. 2006; Lee et al. 2010; Leboulleux et al. 2007).

Structure. The examination of the lymph node structure is diagnostically relevant, and any change from the solid, mildly hypoechoic pattern that is typical for benign reactive nodes should alert the US examiner. A hyperechoic, "thyroid-like" texture or a cystic appearance may be detected in part of malignant lymph nodes (Kessler et al. 2003; Landry et al. 2010). In particular, the presence of cystic changes is strongly predictive of metastatic lymph nodes from papillary thyroid carcinoma (Fig. 11). Of note, in patients with a sonographically normal thyroid gland or with benign FNA cytology of the coexisting thyroid nodules, the possibility of an undetected tuberculosis infection, of lymphomatous involvement (Fig. 12), or of benign congenital neck cysts should be considered as differential diagnostic options (Choi et al. 2009b; Ahuja et al. 2001).

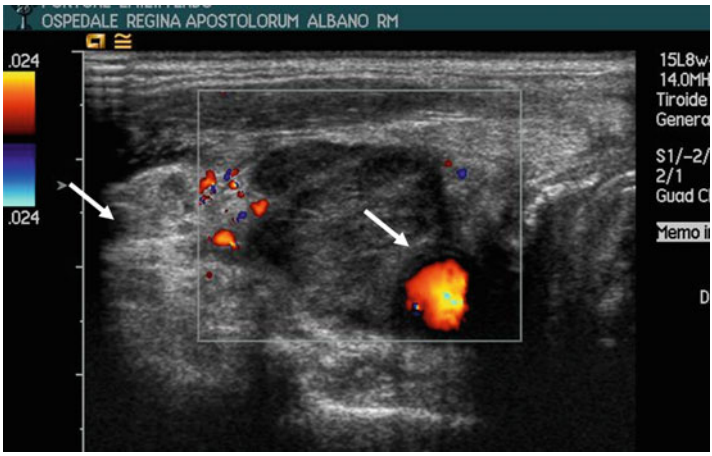

Fig. 12 US scan of a thyroid lymphoma. The presence of a pathologic lymph node with inhomogeneous structure, irregular shape, and lobulated margins in the carotid space. The lesion results in an incomplete carotid encasement (*arrow*)

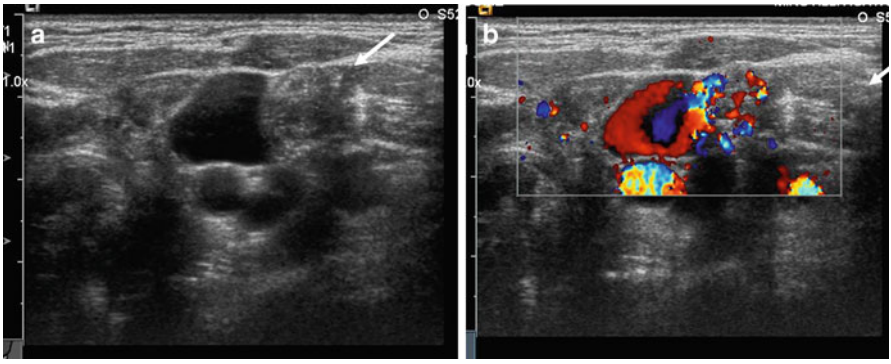

Fig. 13 (**a**) Neck nodal metastasis of papillary carcinoma abutting the jugular vein. The lymph node is rounded, irregularly shaped, and with characteristic microcalcifications (*arrow*). (**b**) Multiple central and peripheral vascular signals are demonstrated by color-Doppler examination (*arrow*)

Calcifications. Lymph node microcalcifications, usually characterized by tiny intranodal hyperechoic spots that are most frequently devoid of posterior shadowing, are an additional US sign highly predictive of malignancy (Fig. 13a). Unfortunately, this very specific finding has poor sensitivity, because it is detectable only in a minority of metastatic cervical lymph nodes (Kuna et al. 2006; Lee et al. 2010; Leboulleux et al. 2007).

Vascularity. Color-Doppler examination may be useful in the differentiation of benign vs. malignant lymph nodes, especially when the findings of B-mode US evaluation are inconclusive. Irrespective of its variable location (central or polar),

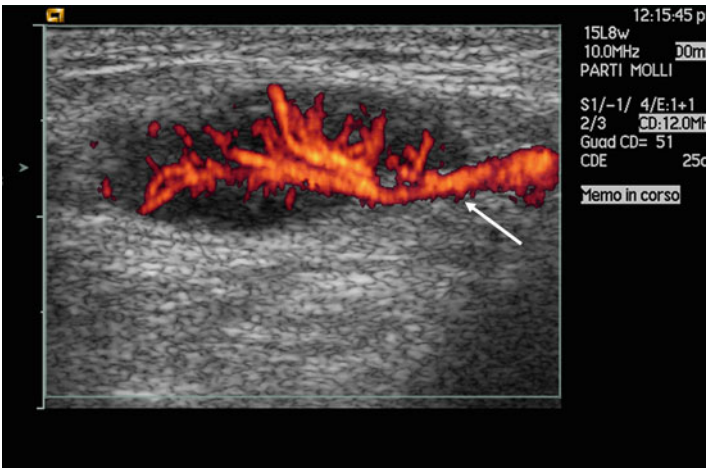

Fig. 14 Inflammatory lymph node. The lymph node is enlarged but the shape is still oval with a dominant longitudinal diameter. The vascular signals are intense but regularly arranged as fern leaves along the central hilum axis

appearance (elongated or dot-like), and intensity, the vascular hilum in benign lymph nodes reveals a geometrical and regular pattern (Fig. 14). This is at variance with the chaotic and anarchic vascularity of malignant lymph nodes, sometimes characterized by a dominant peripheral component due to the neoplastic colonization of the cortical area (Kuna et al. 2006; Lee et al. 2010; Leboulleux et al. 2007) (Fig. 13b).

CT and MR Imaging

CT and/or MR are not indicated in the presurgical staging of biopsy-proven malignant thyroid nodules in the absence of clinical or US signs of infiltrative growth or a cytologic diagnosis of aggressive histology (Papini et al. 2016). On the other hand, these cross-sectional imaging modalities are required to define the full extent of large thyroid carcinomas as well as the presence of extrathyroidal spread in aggressive malignancies (poorly differentiated or anaplastic carcinomas and thyroid lymphomas) (Arun et al. 2014) (Figs. 15 and 16). MRI and contrast-enhanced CT are reported to have similar diagnostic accuracy for the assessment of the infiltrative growth of thyroid carcinomas into the adjacent structures (Wang et al. 2001, 2003; Seo et al. 2010). The invasion of the tracheal wall, esophagus, or prevertebral fascia at presurgical staging usually requires major reconstructive surgery (as in case of laryngectomy or tracheal resection) or may classify the patient as inoperable.

The visualization of neoplastic growth in the lumen or the inner wall of the trachea is diagnostic of malignant invasion (Wang et al. 2001). However, infiltration of the esophageal wall is more difficult to demonstrate due to the absence of air distension within the lumen. The contact of a thyroid tumor with more than 180° of

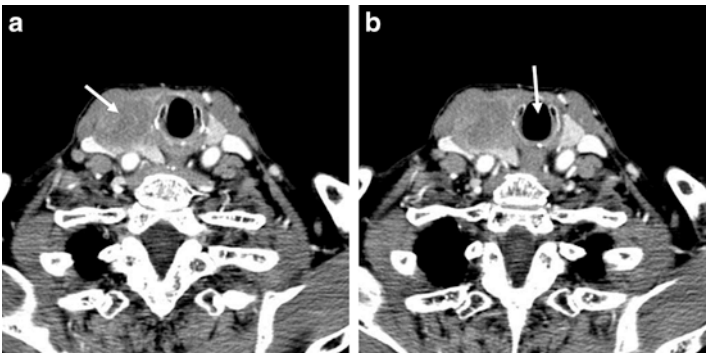

Fig. 15 (**a**) CT scan. Large cervical mass due to a thyroid lymphoma (*arrow*). (**b**) The trachea is displaced toward the left side but the airway lumen is only minimally restricted (*arrow*). Several pathologic lymph nodes are also present

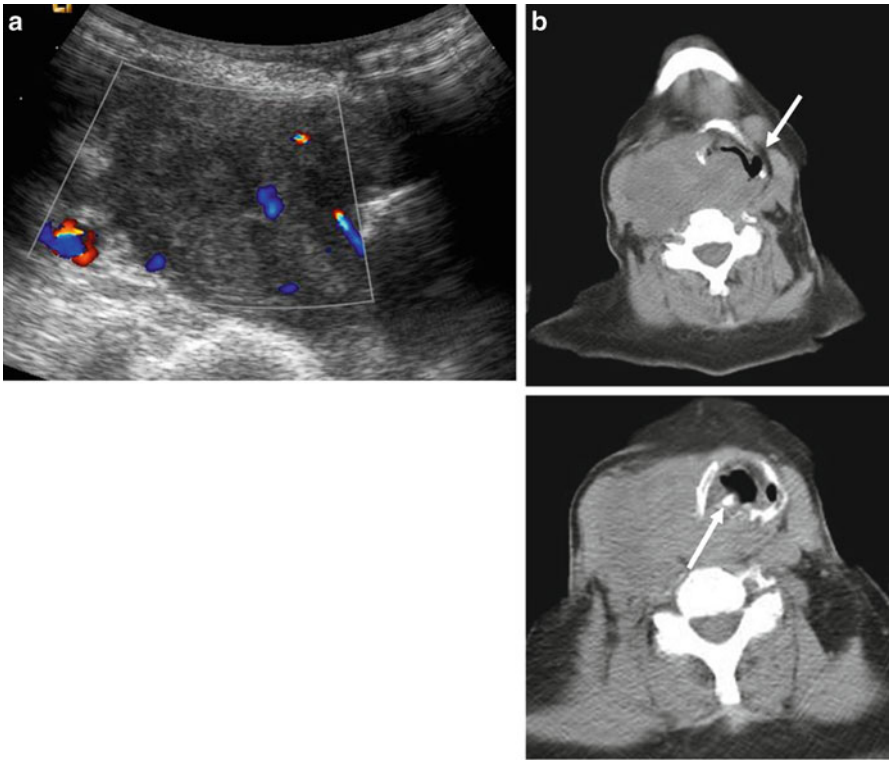

Fig. 16 US (**a**) and CT (**b**) scans of the neck in a patient with anaplastic thyroid carcinoma. (**a**) US evaluation confirms the physical finding of a large cervical mass. (**b**) The severe stenosis of the trachea and the neoplastic infiltration of the upper airway (*arrows*) are clearly only demonstrated by the CT assessment

the circumference of the trachea, or the esophagus, is suggestive of neoplastic infiltration into these structures and warrant endoscopic examination (Wang et al. 2003). Complicating the presurgical classification, the majority of patients with an initial involvement of the vital structures do not have clear signs, by presurgical imaging, suggestive of invasion. Suspicious signs for airway infiltration, which are characterized by a low diagnostic accuracy, are distortion of the trachea or the focal thickening of the mucosa at CT examination and the presence of abnormal focal signals in the outer tracheal wall by MRI. The loss of fatty tissue signals in the tracheoesophageal groove is predictive of the involvement of the recurrent laryngeal nerve (Seo et al. 2010).

CT and MRI are also useful for the assessment of neoplastic involvement of the major cervical vessels. US and color-Doppler findings suggestive of invasion of the jugular veins are confirmed and better defined in their cranio-caudal extension by cross-sectional techniques, pointing at the need of surgical vein resection. The evidence of "encasement" (the presence of malignant tissue that surrounds $> 260^{\circ}$ of the arterial vessel circumference) of the carotid artery or mediastinal vessels is strongly predictive of an incomplete resectability of the thyroid carcinoma and requires major reconstructive surgery. Finally, the invasion of the prevertebral fascia may be suspected but not clearly demonstrated by presurgical imaging. The effacement of the retropharyngeal fat and the presence of hyperintensity or contour abnormalities of the muscles at MRI evaluation are compatible with infiltration of the prevertebral muscles, which may prohibit curative surgery (Seo et al. 2010).

Neck US examination remains the procedure of choice for the assessment of metastatic involvement of cervical lymph nodes in patients with thyroid malignancy. US evaluation of the neck can demonstrate, even in sub-centimetric nodes, discrete abnormalities of their shape, architecture, and echogenicity (Chaudhary and Bano 2013; King 2008; Lew and Solorzano 2010). Conversely, CT and MR imaging are characterized by low sensitivity and specificity. Besides the demonstration of nodal enlargement and intense post-contrast enhancement, CT reveals the specific intranodal microcalcifications with lower accuracy than US. MRI shows suspicious cystic changes of lymph nodes as high T1-weighted signals, due to the elevated iodine content of colloid (Fig. 17). However, in thyroid malignancies with clinical or US evidence of extrathyroidal spread, these cross-sectional techniques are needed before surgery for the imaging of possible metastatic involvement in blind spots at US examination. Paratracheal nodes in the lower neck and in the cervicothoracic junction may be difficult to visualize sonographically even by directing the US probe downward. Similarly, the retropharyngeal and retro-esophageal nodal compartments cannot be reliably assessed by US. Thus, in patients with extensive central compartment involvement, in carcinomas invading the superior mediastinum, and in cases with aggressive histology, presurgical imaging of the neck and chest with CT or MRI is warranted (Arun et al. 2014; King 2008).

Finally, in patients with aggressive histotypes of thyroid carcinoma, relevant information may be provided by the assessment with 18FDG PET/CT in order to appropriately plan the extent of the initial treatment.

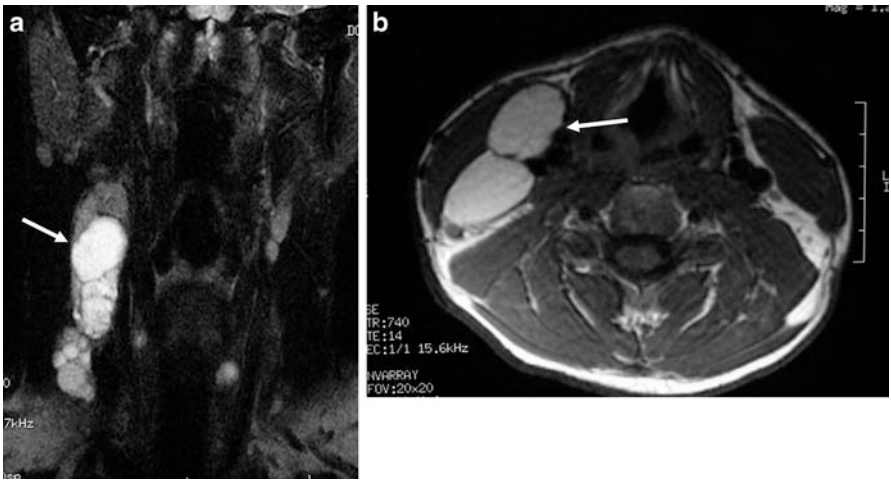

Fig. 17 MR imaging of the neck. Cystic nodal metastases from occult papillary thyroid carcinoma. The typical cystic changes with hyperintensity on both T2 (**a**) and T1 (**b**) sequences are well depicted (*arrows*)

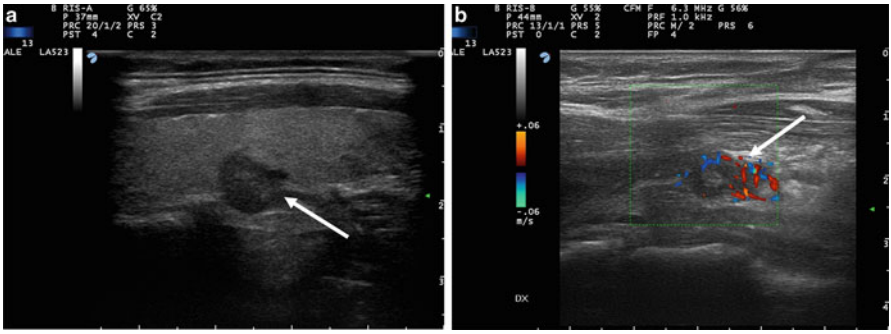

Fig. 18 (**a**) Thyroid US scan: Papillary thyroid carcinoma with posterior extracapsular extension (*arrow*). (**b**) Eight months after total thyroidectomy, a neck US scan demonstrates a persistence of disease within the thyroid bed (*arrow*)

Postsurgical Thyroid Imaging for Follow-Up of Thyroid Carcinoma

Cervical US examination is the cornerstone, together with the determination of serum thyroglobulin (or calcitonin in medullary thyroid carcinoma), for the follow-up of differentiated thyroid tumors (Arun et al. 2014; King 2008; Lew and Solorzano 2010). The local persistence, or recurrence, of disease is reliably detected by experienced sonographers. The presence of suspicious sub-centimetric lesions in the thyroid bed or cervical lymph nodes is usually well visualized and may be easily confirmed by US-guided FNA (Frasoldati et al. 2016) (Fig. 18a and b).

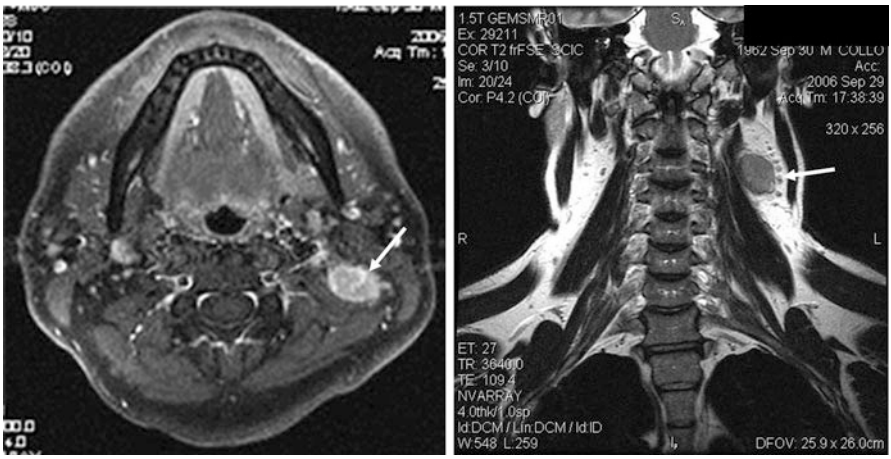

Fig. 19 MRI scan. Metastatic lymph node in the left posterior cervical space. The pathologic lymph node, which is hyperintense in the T1 and T2 sequences (*arrows*), is located in an "US blind spot" and was not revealed by a previous US examination

The role of cross-sectional imaging for the evaluation of recurrences of thyroid tumors in the posttreatment neck is limited. These techniques should be used in selected cases only, as a second step approach, together with functional imaging techniques (18FDG PET/CT or whole-body radioisotope scan) (Arun et al. 2014; King 2008). Specifically, CT or MRI should be performed in those patients with increasing levels of serum markers of thyroid carcinoma that are negative at neck US examination, in order to rule out the presence of undisclosed metastatic disease in the retropharyngeal compartment or in other cervical blind spots at US assessment (Fig. 19).

The use of imaging techniques for the assessment of distant metastasis of thyroid malignancy is beyond the scope of this chapter.

Summary

Thyroid imaging offers a relevant diagnostic and therapeutic contribution to the management of patients with thyroid disease. Ultrasonography (US) is a sensitive tool for the evaluation of diffuse and focal thyroid disease and of cervical masses. Thyroid US defines the risk of malignancy in thyroid nodules and aids in decision-making about which nodules need to undergo fine-needle aspiration biopsy. Additionally, US examination confirms the presence of size abnormalities and/or possible changes of thyroid structure in patients with clinical findings that suggest an altered thyroid function or an inflammatory condition. Thus, all patients under investigation for clinical suspicion of thyroid disease or malignancy should undergo a dedicated cervical US.

Cross-sectional imaging techniques (computed tomography or magnetic resonance imaging) are less sensitive and specific tools for the diagnosis of thyroid disease, as compared to US. In addition, they are far more expensive and less accessible. Their role is limited to a minority of cases as a second step diagnostic procedure. These imaging modalities should be used mainly in the pre-operation staging of thyroid malignancies with US evidence of extrathyroidal spread and for the surveillance of cervical recurrences of thyroid carcinomas in patients with inconclusive neck US associated with increasing serum thyroglobulin (or calcitonin, in case of medullary thyroid carcinoma).

The role of functional imaging methods (PET/CT and radioisotope scintiscan) is beyond the scope of this chapter, and we refer the reader to the dedicated section of this text.

References

Ahuja AT, Ying M. Sonographic evaluation of cervical lymph nodes. Am J Roentgenol. 2005;184:1691–9.

Ahuja A, Chick W, King W, Metreweli C. Clinical significance of the comet-tail artifact in thyroid ultrasound. J Clin Ultrasound. 1996;24:129–33.

Ahuja AT, Ying M, Yuen HY, Metreweli C. 'Pseudocystic' appearance of non-Hodgkin's lymphomatous nodes: an infrequent finding with high-resolution transducers. Clin Radiol. 2001;56:111–5.

Aiken AH. Imaging of thyroid cancer. Semin Ultrasound CT MR. 2012;33:138–49.

Alexander EK, Hurwitz S, Heering JP, et al. Natural history of benign solid and cystic nodules. Ann Intern Med. 2003;138:315–8.

American Institute of Ultrasound in Medicine, American College of Radiology, Society for Pediatric Radiology, Society of Radiologists in Ultrasound. AIUM practice guideline for the performance of a thyroid and parathyroid ultrasound examination. J Ultrasound Med. 2013;32:1319–29.

Anderson L, Middleton WD, Teefey SA, et al. Hashimoto thyroiditis: part 1. Sonographic analysis of the nodular form of Hashimoto thyroiditis. Am J Roentgenol. 2010a;195:208–15.

Anderson L, Middleton WD, Teefey SA, et al. Hashimoto thyroiditis: part 2. Sonographic analysis of benign and malignant nodules in patients with diffuse Hashimoto thyroiditis. Am J Roentgenol. 2010b;195:216–9.

Arun C, Nachiappan AC, Metwalli ZA, et al. The thyroid: review of imaging features and biopsy techniques with radiologic-pathologic correlation. Radiographics. 2014;34:276–93.

Azizi G, Keller J, Lewis M, Puett D, Rivenbark K, Malchoff C. Performance of elastography for the evaluation of thyroid nodules: a prospective study. Thyroid. 2013;23:734–40.

Bahn RS, Castro MR. Approach to the patient with nontoxic multinodular goiter. J Clin Endocrinol Metab. 2011;96:1202–12.

Baskin HJ. Anatomy and anomalies. In: Baskin HJ, Duick DS, Levine RA, editors. Thyroid ultrasound and ultrasound-guided FNA. 2nd ed. New-York: Springer; 2008. p. 45–6.

Beland MD, Kwon L, Delellis RA, Cronan JJ, Grant EG. Nonshadowing echogenic foci in thyroid nodules: are certain appearances enough to avoid thyroid biopsy? J Ultrasound Med. 2011;30:753–60.

Bennedbaek FN, Hegedüs L. The value of ultrasonography in the diagnosis and follow-up of subacute thyroiditis. Thyroid. 1997;7:45–50.

Binder RE, Pugatch RD, Faling LJ, Kanter RA, Sawin CT. Diagnosis of posterior mediastinal goiter by computed tomography. J Comput Assist Tomogr. 1980;4:550–2.

Bonnema SJ, Hegedüs L. Radioiodine therapy in benign thyroid diseases: effects, side effects, and factors affecting therapeutic outcome. Endocr Rev. 2012;33:920–80.
Brauer VFH, Miehle K, Wiesner TD, et al. Interobserver variation for ultrasound determination of thyroid nodule volumes. Thyroid. 2005;15:1169–75.
Campanella P, Ianni F, Rota CA, Corsello SM, Pontecorvi A. Quantification of cancer risk of each clinical and ultrasonographic suspicious feature of thyroid nodules: a systematic review and meta-analysis. Eur J Endocrinol. 2014;170:203–11.
Carty SE, Cooper DS, Doherty GM, et al. Consensus statement on the terminology and classification of central neck dissection for thyroid cancer. Thyroid. 2009;19:1153–8.
Chammas MC, de Araujo Filho VJ, Moysés RA, et al. Predictive value for malignancy in the finding of microcalcifications on ultrasonography of thyroid nodules. Head Neck. 2008;30:1206–61.
Chan BK, Desser TS, McDougall IR, et al. Common and uncommon sonographic features of papillary thyroid carcinoma. J Ultrasound Med. 2003;22:1083–90.
Chaudhary V, Bano S. Thyroid ultrasound. Indian J Endocrinol Metab. 2013;17:219–27.
Choi JS, Kim J, Kwak JY, et al. Preoperative staging of papillary thyroid carcinoma: comparison of ultrasound imaging and CT. Am J Roentgenol. 2009a;193:871–8.
Choi EC, Moon WJ, Lim YC. Case report. Tuberculous cervical lymphadenitis mimicking metastatic lymph nodes from papillary thyroid carcinoma. Br J Radiol. 2009b;82:208–11.
Choi SH, Kim E-K, Kwak JY, Kim MJ, Son EJ. Interobserver and intraobserver variations in ultrasound assessment of thyroid nodules. Thyroid. 2010;20:167–72.
Cooper JC, Nakielny R, Talbot CH. The use of CT in the evaluation of large multinodular goiter. Ann R Coll Surg Engl. 1991;73:32–5.
D'Orsi CJ, Sickles EA, Mendelson EB, et al. BI-RADS® Atlas, breast imaging reporting and data system. 4th ed. Reston: American College of Radiology; 2003.
Davies L, Welch HG. Increasing incidence of thyroid cancer in the United States. JAMA. 2006;295:2164–7.
Davies L, Welch HG. Current thyroid cancer trends in the United States. JAMA Otolaryngol Head Neck Surg. 2014;140:317–22.
Davies L, Pichiotino E, Black WC, Tosteson AN. Developing a registry for thyroid incidentalomas: lessons learned and the path forward. Thyroid. 2016;26:650–6.
Durante C, Costante G, Lucisano G, et al. The natural history of benign thyroid nodules. JAMA. 2015;313:926–35.
Frasoldati A, Valcavi R. Challenges in neck ultrasonography: lymphadenopathy and parathyroid glands. Endocr Pract. 2004;10:261–8.
Frasoldati A, Pacella CM, Papini E, Hegedüs L. In: Wartoksky L, Van Nostrand D, editors. Thyroid cancer: a comprehensive guide to clinical management. 3rd ed. New York: Springer; 2016.
Ghajarzadeh M, Sodagari F, Shakiba M. Diagnostic accuracy of sonoelastography in detecting malignant thyroid nodules: a systematic review and meta-analysis. Am J Roentgenol. 2014;202:379–89.
Gharib H, Papini E, Garber JR, et al. American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinology Guidelines for clinical practice for the diagnosis and management of thyroid nodules – 2016 Update. Endocr Pract. 2016;22:622–39.
Giovannella L, Ceriani L, Treglia G. Role of isotope scan, including PET/CT, in nodular goitre. Best Pract Res Clin Endocrinol Metab. 2014;28:507–18.
Guglielmi R, Pacella CM, Bianchini A, et al. Percutaneous ethanol injection treatment in benign thyroid lesions: role and efficacy. Thyroid. 2004;14:125–31.
Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: The American Thyroid Association guidelines task force on thyroid nodules and differentiated thyroid cancer. Thyroid. 2016;26:1–133.
Hegedüs L. Down-sizing the overzealous search for low-risk thyroid malignancy. Endocrine. 2016;52:408–10.

Hegedüs L, Paschke R, Krohn K, Bonnema SJ. MultinodularGoiter. In: Jameson JL, De Groot LJ, editors. Endocrinology: adult and pediatric. 7th ed. Philadelphia: Elsevier; 2016. p. 1570–83.
Hoang JK, Lee WK, Lee M, Johnson D, Farrell S. US features of thyroid malignancy: pearls and pitfalls. Radiographics. 2007;27:847–60.
Horvath E, Majlis S, Rossi R, et al. An ultrasonogram reporting system for thyroid nodules stratifying cancer risk for clinical management. J Clin Endocrinol Metab. 2009;94:1748–51.
Ito Y, Miyauchi A. Nonoperative management of low-risk differentiated thyroid carcinoma. Curr Opin Oncol. 2015;27:15–20.
Jeh SK, Jung SL, Kim BS, Lee YS. Evaluating the degree of conformity of papillary carcinoma and follicular carcinoma to the reported ultrasonographic findings of malignant thyroid tumor. Korean J Radiol. 2007;8:192–7.
Jin J, McHenry CR. Thyroid incidentaloma. Best Pract Res Clin Endocrinol Metab. 2012;26:83–96.
Kessler A, Rappaport Y, Blank A, et al. Cystic appearance of cervical lymph nodes is characteristic of metastatic papillary thyroid carcinoma. J Clin Ultrasound. 2003;31:21–5.
Khoo MLC, Asa SL, Witterick IJ, Freeman JL. Thyroid calcification and its association with thyroid carcinoma. Head Neck. 2002;24:651–5.
Kim EK, Park CS, Chung WY, et al. New sonographic criteria for recommending fine-needle aspiration biopsy of nonpalpable solid nodules of the thyroid. Am J Roentgenol. 2002;178:687–91.
Kim BM, Kim MJ, Kim EK, et al. Sonographic differentiation of thyroid nodules with eggshell calcifications. J Ultrasound Med. 2008;27:1425–30.
King AD. Imaging for staging and management of thyroid cancer. Cancer Imaging. 2008;8:57–69.
Kobayashi K, Fukata S, Miyauchi A. Diagnosis of follicular carcinoma of the thyroid: role of sonography in preoperative diagnosis of follicular nodules. J Med Ultrasonics. 2005;32: 153–8.
Kovács GL, Gonda G, Vadász G, Ludmány E, Uhrin K, Görömbey Z, Kovács L, Hubina E, Bodó M, Góth MI, Szabolcs I. Epidemiology of thyroid microcarcinoma found in autopsy series conducted in areas of different iodine intake. Thyroid. 2005;15(2):152–7.
Kuna SK, Bracic I, Tesic V, et al. Ultrasonographic differentiation of benign from malignant neck lymphadenopathy in thyroid cancer. J Ultrasound Med. 2006;25:1531–7.
Kupferman ME, Patterson M, Mandel SJ, et al. Patterns of lateral neck metastasis in papillary thyroid carcinoma. Arch Otolaryngol Head Neck Surg. 2004;130:857–60.
Landry CS, Grubbs EG, Busaidy NL, et al. Cystic lymph nodes in the lateral neck as indicators of metastatic papillary thyroid cancer. Endocr Pract. 2010;17:240–4.
Langer JE, Khan A, Nisenbaum HL, et al. Sonographic appearance of focal thyroiditis. AJR. 2001;176:751–4.
Leboulleux S, Girard E, Rose M, et al. Ultrasound criteria of malignancy for cervical lymph nodes in patients followed up for differentiated thyroid cancer. J Clin Endocrinol Metab. 2007;92:3590–4.
Lee SK, Rho BH. Follicular thyroid carcinoma with an eggshell calcification. J Ultrasound Med. 2009;28:801–6.
Lee MJ, Kim EK, Kwak JY, Kim MJ. Partially cystic thyroid nodules on ultrasound: probability of malignancy and sonographic differentiation. Thyroid. 2009;19:341–6.
Lee K, Kawata R, Nishikawa S, et al. Diagnostic criteria of ultrasonographic examination for lateral node metastasis of papillary thyroid carcinoma. Acta Otolaryngol. 2010;130:161–6.
Leenhardt L, Grosclaude P, Chérié-Challine L. Increased incidence of thyroid carcinoma in France: a true epidemic or thyroid nodule management effects? Report from the French Thyroid Cancer Committee. Thyroid. 2004;14:1056–60.
Leenhardt L, Tranquart F, Tramalloni J. In: Carpi A, Mechanick JI, editors. Thyroid cancer. Boca Raton: CRC Press; 2011. p. 99–116.
Leenhardt L, Erdogan MF, Hegedus L, et al. European Thyroid Association guidelines for cervical ultrasound scan and ultrasound-guided techniques in the postoperative management of patients with thyroid cancer. Eur Thyroid J. 2013;2:147–59.

Levine RA. Thyroid ultrasound physics. In: Baskin HJ, Duick DS, Levine RA, editors. Thyroid ultrasound and ultrasound-guided FNA. New York: Springer; 2008. p. 9–26.
Lew JI, Solorzano CC. Use of ultrasound in the management of thyroid cancer. Oncologist. 2010;15:253–8.
Liu BJ, Xu HX, Zhang YF, Xu JM, Li DD, Bo XW, et al. Acoustic radiation force impulse elastography for differentiation of benign and malignant thyroid nodules with concurrent Hashimoto's thyroiditis. Med Oncol. 2015;32:50.
Lupo M, Levine RA. Ultrasound tissue enlargement: thyroiditis. In: Baskin HJ, Duick DS, Levine RA, editors. Thyroid ultrasound and ultrasound-guided FNA. 3rd ed. New York: Springer; 2014. p. 63–76.
Machens A, Hinze R, Thomusch O, et al. Pattern of nodal metastasis for primary and preoperative thyroid cancer. World J Surg. 2002;26:22–8.
Mandel SJ. Diagnostic use of ultrasonography in patients with nodular thyroid disease. Endocr Pract. 2004;10:246–52.
McHenry CR, Huh ES, Machekano RN. Is nodule size an independent predictor of malignancy? Surgery. 2008;144:1062–9.
McQueen AS, Bathia KSS. Thyroid nodule ultrasound: technical advances and future horizons. Insights Imaging. 2015;6:173–88.
Moon WJ, Jung SL, Lee JH, et al. Benign and malignant thyroid nodules: US differentiation – multicenter retrospective study. Radiology. 2008;247:762–70.
Moon HJ, Kim EK, Kim MJ, Kwak JY. Lymphocytic thyroiditis on fine-needle aspiration biopsy of focal thyroid nodules: approach to management. Am J Roentgenol. 2009;193:345–9.
Moon HJ, Kwak JY, Kim MJ, Son EJ, Kim EK. Can vascularity at power Doppler US help predict thyroid malignancy? Radiology. 2010;255:260–9.
Moon WJ, Baek JH, Jung SL, Kim DW, Kim EK, Kim JY, Korean Society of Thyroid Radiology (KSThR), Korean Society of Radiology, et al. Korean Society of Radiology Ultrasonography and the ultrasound-based management of thyroid nodules: consensus statement and recommendations. Korean J Radiol. 2011;12:1–14.
Nam SY, Shin JH, Ko EY, Hahn SY. A comparison of lymphocytic thyroiditis with papillary thyroid carcinoma showing suspicious ultrasonographic findings in a background of heterogeneous parenchyma. Ultrasonography. 2015;34:45–50.
Noda Y, et al. MRI of the thyroid for differential diagnosis of benign thyroid nodules and papillary carcinomas. Am J Roentgenol. 2015;204:332–5.
Papini E, Guglielmi R, Bianchini A, et al. Risk of malignancy in nonpalpable thyroid nodules: predictive value of ultrasound and color-Doppler features. J Clin Endocrinol Metab. 2002;87:1941–6.
Papini E, Pacella CM, Hegedus L. Thyroid ultrasound and ultrasound-assisted procedures: from shadows into an array of applications. Eur J Endocrinol. 2014;170:133–44.
Papini E, Pacella CM, Frasoldati A, Hegedus L. Ultrasonic imaging of the thyroid gland. In: Wartofski L, Van Nostrand D, editors. Thyroid cancer. A comprehensive guide to clinical management. 3rd ed. New York: Springer; 2016.
Park JS, Son KR, Na DG, et al. Performance of preoperative sonographic staging of papillary thyroid carcinoma based on the sixth edition of the AJCC/UICC TNM classification system. Am J Roentgenol. 2009;192:66–72.
Park CS, Kim SH, Jung SL, et al. Observer variability in the sonographic evaluation of thyroid nodules. J Clin Ultrasound. 2010;38:287–93.
Park AY, Son EJ, Han K, Youk JH, Kim JA, Park CS. Shear wave elastography of thyroid nodules for the prediction of malignancy in a large scale study. Eur J Radiol. 2015;84:407–12.
Park HS, Chung EJ, Park MW, et al. Usefulness of radiologic examinations for diagnosing level VI lymph node metastasis in patients with laryngohypopharyngeal carcinoma. Eur Arch Otorhinolaryngol. 2016;273:3959: Epub ahead of print.
Pedersen OM, Aardal NP, Larssen TB, Varhaug JE, Myking O, Vik-Mo H. The value of ultrasonography in predicting autoimmune thyroid disease. Thyroid. 2000;10:251–9.

Perros P, Boelaert K, Colley S, et al. British Thyroid Association Guidelines for the management of thyroid cancer. Clin Endocrinol (Oxf). 2014;81 Suppl 1:1–122.
Raber W, Gessl A, Nowotny P, Vierhapper H. Thyroid ultrasound versus antithyroid peroxidase antibody determination: a cohort study of four hundred fifty-one subjects. Thyroid. 2002;12:725–31.
Rago T, Vitti P. Diagnostic role of ultrasound and elastosonography in nodular goiter. Best Pract Res Clin Endocrinol Metab. 2014;28:519–29.
Rago T, Chiovato L, Grasso L, Pinchera A, Vitti P. Thyroid ultrasound as a tool for detecting thyroid autoimmune disease and predicting thyroid dysfunction in apparently healthy subjects. J Endocrinol Invest. 2001;24:763–9.
Rago T, Santini F, Scutari M, Pinchera A, Vitti P. Elastography: new developments in ultrasound for predicting malignancy in thyroid nodules. J Clin Endocrinol Metab. 2007;92:2917–22.
Ralls PW, Mayekawa DS, Lee KP, et al. Color-flow Doppler sonography in Graves disease: "thyroid inferno". Am J Roentgenol. 1988;150:781–4.
Randolph G, Sacks B, Baskin HJ. Ultrasound and mapping of the neck lymph nodes. In: Baskin HJ, Duick DS, Levine RA, editors. Thyroid ultrasound and ultrasound-guided FNA. 3rd ed. New York: Springer; 2013. p. 149–77.
Reading CC, Charboneau JW, Sebo TJ. Sonography of thyroid nodules: a classic pattern diagnostic approach. Ultrasound Q. 2005;21:157–65.
Robbins KT, Shaha AR, Medina JE, et al. Consensus statement on the classification and terminology of neck dissection: committee for neck dissection classification, American Head and Neck Society. Arch Otolaryngol Head Neck Surg. 2008;134:536–8.
Roh JL, Kim JM, Park C. Lateral cervical lymph node metastases from papillary thyroid carcinoma: pattern of nodal metastases and optimal strategy for neck dissection. Ann Surg Oncol. 2008;15:1177–82.
Rosário PW, Bessa B, Valadão MM, Purisch S. Natural history of mild subclinical hypothyroidism: prognostic value of ultrasound. Thyroid. 2009;19:9–12.
Russ G, Royer B, Bigorgne C, Rouxel A, Bienvenu-Perrard M, Leenhardt L. Prospective evaluation of thyroid imaging reporting and data system on 4550 nodules with and without elastography. Eur J Endocrinol. 2013;168:649–55.
Seo YL, Yoon DJ, Lim KJ, et al. Locally advanced thyroid cancer: can CT help in prediction of extrathyroidal invasion to adjacent structures? Am J Roentgenol. 2010;195:240–4.
Sharma A, Jasim S, Reading CC, et al. Clinical presentation and diagnostic challenges of thyroid lymphoma: a cohort study. Thyroid. 2016;26:1061–7.
Shin JH, Baek JH, Chung J, et al. Ultrasonography diagnosis and imaging-based management of thyroid nodules: revised Korean Society of thyroid radiology consensus statement and recommendations. Korean J Radiol. 2016;17:370–95.
Solbiati L, Osti V, Cova L, Tonolini M. Ultrasound of thyroid, parathyroid glands and neck lymph nodes. Eur Radiol. 2001;11:2411–24.
Sorensen JR, Hegedus L, Kruse-Andersen S, Godballe C, Bonnema SJ. The impact of goiter and its treatment on the trachea, air-flow, oesophagus and swallowing. A systematic review. Best Pract Res Clin Endocrinol Metab. 2014;28:481–94.
Sousa do Rosario PW, de Faria S, Bicalho L, et al. Ultrasonographic differentiation between metastatic and benign lymph nodes in patients with papillary thyroid carcinoma. J Ultrasound Med. 2005;24:1385–9.
Stack Jr BC, Ferris RL, Goldenberg D, et al. American Thyroid Association consensus review and statement regarding the anatomy, terminology, and rationale for lateral neck dissection in differentiated thyroid cancer. Thyroid. 2012;22:501–8.
Taki S, Terahata S, Yamashita R, et al. Thyroid calcifications: sonographic patterns and incidence of cancer. Clin Imaging. 2004;28:368–71.
Tam AA, Kaya C, Üçler R, Dirikoç A, Ersoy R, Çakır B. Correlation of normal thyroid ultrasonography with thyroid tests. Quat Imaging Med Surg. 2015;5:569–74.

Tamsel S, Dermipolat G, Erdogan M, et al. Power Doppler US patterns of vascularity and spectral Doppler US parameters in predicting malignancy in thyroid nodules. Clin Radiol. 2007;62:245–51.

Trimboli P, Guglielmi R, Monti S, et al. Ultrasound sensitivity for thyroid malignancy is increased by real-time elastography: a prospective multicenter study. J Clin Endocrinol Metab. 2012;97:4524–30.

Verburg FA, Mader U, Luster M, Reiners C. Primary tumour diameter as a risk factor for advanced disease features of differentiated thyroid carcinoma. Clin Endocrinol (Oxf). 2009;71:291–7.

Wada N, Duh QY, Sugino K, et al. Lymph node metastasis from 259 papillary thyroid microcarcinomas: frequency, pattern of occurrence and recurrence, and optimal strategy for neck dissection. Ann Surg. 2003;237:399–407.

Walfish PG. Thyroiditis. Curr Ther Endocrinol Metab. 1997;6:117–26.

Wang JC, Takashima S, Takajama F, et al. Tracheal invasion by thyroid carcinoma: prediction using RM imaging. Am J Roentgenol. 2001;177:929–36.

Wang JC, Takashima S, Matsushita T, et al. Esophageal invasion by thyroid carcinomas: prediction using MR imaging. J Comput Assist Tomogr. 2003;27:18–25.

Wang N, Xu Y, Ge C, Guo R, Guo K. Association of sonographically detected calcification with thyroid carcinoma. Head Neck. 2006;28:1077–83.

Wienke JR, et al. Sonographic features of benign thyroid nodules: interobserver reliability and overlap with malignancy. J Ultrasound Med. 2003;22:1027.

Wu LM, Chen XX, Li YL, Hua J, Chen J, Hu J, Xu JR. On the utility of quantitative diffusion-weighted MR imaging as a tool in differentiation between malignant and benign thyroid nodules. Acad Radiol. 2014;21:355–63.

Yamashiro I, de Cassio SO, Chammas MC, Cerri GG. Ultrasound findings in thyroiditis. Radiol Bras. 2007;40:75–9.

Yeh MW, Bauer AJ, Bernet VA, et al. American Thyroid Association statement on preoperative imaging for thyroid cancer surgery. Thyroid. 2015;25:3–14.

Yoon DY, Lee JW, Chang SK, et al. Peripheral calcification in thyroid nodules: ultrasonographic features and prediction of malignancy. J Ultrasound Med. 2007;26:1349–55.

Youan WH, Chiou HJ, Chou YH, et al. Gray-scale and color Doppler ultrasonographic manifestations of papillary thyroid carcinoma: analysis of 51 cases. Clin Imaging. 2006;30:394–401.

Yuan WH, Chiou HJ, Chou YH, et al. Gray-scale and color Doppler ultrasonographic manifestations of papillary thyroid carcinoma: analysis of 51 cases. Clin Imaging. 2006;30:394–401.

Zhang YF, Liu C, Xu HX, et al. Acoustic radiation force impulse imaging: a new tool for the diagnosis of papillary thyroid microcarcinoma. Biomed Res Int. 2014;2014:41–69.

Part II

Goiter and Thyroid Nodule

Nontoxic Goiter

5

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Abstract

Nontoxic goiter is a common condition related to deficiency in the iodine intake. Other etiological factors include genetic susceptibility, female gender, age, and tobacco smoking. Since the thyroid tissue has preponderance for nodular degeneration, most goiters harbor nodules of different size and texture. In some patients with long-standing goiter, hyperthyroidism will emerge gradually due to functional autonomy of one or more nodules. Although the risk of malignancy in nodular goiter is low in unselected patients, evaluation by thyroid ultrasonography, and by fine-needle aspiration biopsy of nodules with suspicious features, is crucial for determining the nature of the goiter.

In reaching a treatment decision, a range of factors should be taken into account. Asymptomatic patients with a benign goiter most often need no treatment. Thyroid surgery, typically in the form of a hemi- or a total thyroidectomy, can rapidly remove the target tissue and is the treatment of choice if the goiter is very large, if it compromises the upper airways, or if there is suspicion of thyroid malignancy. Specific risks, correlating with goiter size, include vocal cord paralysis and hypoparathyroidism, and these risks increase in cases of reoperation for recurrent goiter.

The noninvasive nature is the major advantage of radioiodine (^{131}I) therapy, resulting in a goiter volume reduction of 35–50% within 2 years. However, a low thyroid ^{131}I uptake (RAIU) is a hindrance for ^{131}I therapy efficacy in some patients. In such cases, recombinant human TSH (rhTSH) stimulation can augment the goiter shrinkage, which reduces the need for additional treatment due to goiter recurrence. Alternatively, rhTSH stimulation allows a reduction of the administered ^{131}I, while achieving a goiter reduction comparable to that obtained by ^{131}I therapy given without rhTSH stimulation.

In selected patients, noninvasive interventional treatment may be an option in solitary thyroid lesions.

Keywords

Goiter · Thyroid Nodule · Iodine Deficiency · Functional Autonomy · Quality of Life · Neck Compression · Thyroid Ultrasound · Risk Assessment · Fine-Needle Aspiration Biopsy · Thyroidectomy · Radioiodine Therapy · Recombinant Human Thyrotropin · Noninvasive Interventional Therapy

Introduction

Nontoxic goiters are caused by excessive growth of the thyroid tissue leading to an enlargement of the thyroid gland. Serum levels of the thyroid hormones must be normal according to the definition. Ideally, the presence of goiter should be established by applying the reference values for the thyroid volume, obtained ultrasonographically, in healthy individuals residing in an iodine-sufficient area. Thus, a thyroid gland should not be larger than 18 mL in women and 25 mL in men (Hegedüs et al. 2003; Knudsen et al. 2000). Since the thyroid tissue intrinsically has preponderance for nodular degeneration, at least in susceptible individuals, long-standing goiters most often harbor nodules of different size and texture in one or several areas of the thyroid parenchyma (Brix et al. 1999; Carle et al. 2014; Fiore et al. 2014). The nodules can be solid, cystic, or complex (combination of solid and cystic components). If one or more nodules are found embedded in a normal-sized thyroid gland, the term "thyroid nodularity" rather than "goiter" should be applied. All thyroid nodules may be regarded as tumors (or adenomas), but histologically this term applies only to encapsulated nodules, whereas those without a capsule represent thyroid hyperplasia. However, a histological differentiation is not of major importance from a clinical point of view.

Clinically, goiter is categorized into diffuse, solitary nodular, or multinodular thyroid disease, the latter being the most prevalent phenotype (Hegedüs et al. 2003). It can be very difficult to assess whether a person has a goiter, merely by clinical examination. Therefore, thyroid imaging is necessary, and in most cases mandatory, to explore the size and nature of the thyroid gland. Also, it is crucial to rule out thyroid cancer, although the ratio of malignant versus benign nodules is very low in unselected patients.

Initially, a nodular goiter is nontoxic. However, some nodules gain functional autonomy with detachment from the normal TSH regulation. Such lesions constitute approximately 10% of all thyroid nodules and appear scintigraphically warm (or hot), in contrast to cold nodules that more or less have lost their ability to synthesize and secrete thyroid hormones (Hegedüs et al. 2003). Thus, many nontoxic multinodular goiters are in fact a mix of nodules with different functionality. As functional autonomy progresses, the nontoxic goiter eventually becomes a toxic goiter, and this process constitutes a continuum (Hegedüs et al. 2003; Vanderpump et al. 1995). The two entities cannot histologically be differentiated from each other, and a distinction is in principle arbitrary and based only on biochemical parameters.

A goiter usually develops slowly, and it may have been present for years without drawing the attention of the patient due to lack of symptoms. There are numerous reasons, covering various symptoms of compression and/or cosmetic complaints, for why a patient is referred for evaluation of goiter. However, far from all patients need treatment as this often includes thyroid ablation, either of the entire gland or part of it. Thus, many oligosymptomatic patients would undoubtedly benefit from refraining from, or at least having treatment delayed.

Etiology and Epidemiology

Nodular goiter is caused by intrinsic and external factors in a complex interaction. Genetic susceptibility, female gender, and age are important, but non-modifiable, etiological factors for the development of goiter, whereas modifiable factors include body weight, smoking habits, alcohol consumption, and not least the iodine intake (Carle et al. 2014; Hegedüs et al. 2009; Knudsen and Brix 2014). Clustering of goiter within families, and results from twin studies, strongly support that genetic predisposition, with a dominant pattern of inheritance, plays a central role in goiter development (Hegedüs et al. 2009). A higher incidence of goiter in females than in men emphasizes that genes are of major importance, whereas the impact of sex hormones is more elusive (Knudsen et al. 2002).

The effect of smoking on goiter development is probably mediated by thiocyanate, which competitively inhibits the iodide (the ionized state of iodine) transport into the thyroid gland (Brix et al. 2000). In contrast, the mechanisms behind the observed inverse correlation between alcohol consumption and thyroid size, leading to thyroid fibrosis remain obscure (Hegedüs et al. 1988; Knudsen et al. 2001). These and other environmental factors probably exert their effects in an additive or a synergistic manner and in an interaction with individual genetic determinants (Hegedüs et al. 2009; Knudsen and Brix 2014). At the individual level, it is impossible to estimate the relative contribution of genes versus known and unknown environmental factors (Knudsen and Brix 2014). Potential gene-gene, gene-environment, and environment-environment interactions underline that the pathogenesis of nodular goiter is very complex (Hegedüs et al. 2009; Knudsen and Brix 2014). As for the solitary thyroid nodule, clonal expansion due to a somatic mutation of one or more genes seems to be the central cause in most cases. For further details, see ► Chap. 6, "Thyroid Nodule" by Ralf Paschke.

Iodine is crucial for normal thyroid function, but in superfluous amounts, it counteracts the response of the thyroid to TSH and may even lead to apoptosis of the thyroid cells (Burikhanov and Matsuzaki 2000). In hyperthyroid patients, this can be used therapeutically, as treatment with iodine causes involution of thyroid hyperplasia and decreases vascularity, at least temporarily (Eng et al. 1999). In the context of endemic multinodular goiter, iodine deficiency is worldwide the most important environmental factor (Carle et al. 2014; Fiore et al. 2014). As a plausible mechanism, long-standing iodine-deficiency, even when mild, may result in an increase of serum TSH above the genetically determined normal set-point for the individual (Hansen et al. 2004). The higher level of TSH, as a major growth factor, results with time in an enlargement of the thyroid gland. In parallel with regional variations in the iodine status, there are considerable differences in the prevalence of goiter between populations. Comparisons of epidemiologic studies of goiter are hampered by differences in selection criteria, the influence of confounding factors such as smoking habits, and the methods employed for evaluation of the thyroid. Nevertheless, a clear inverse correlation exists across studies (Fiore et al. 2014) between the iodine intake (normally measured as the urinary iodine excretion) and the prevalence of goiter (Fig. 1). Regarding the solitary thyroid nodule, there seems to be no clear linkage to the iodine status.

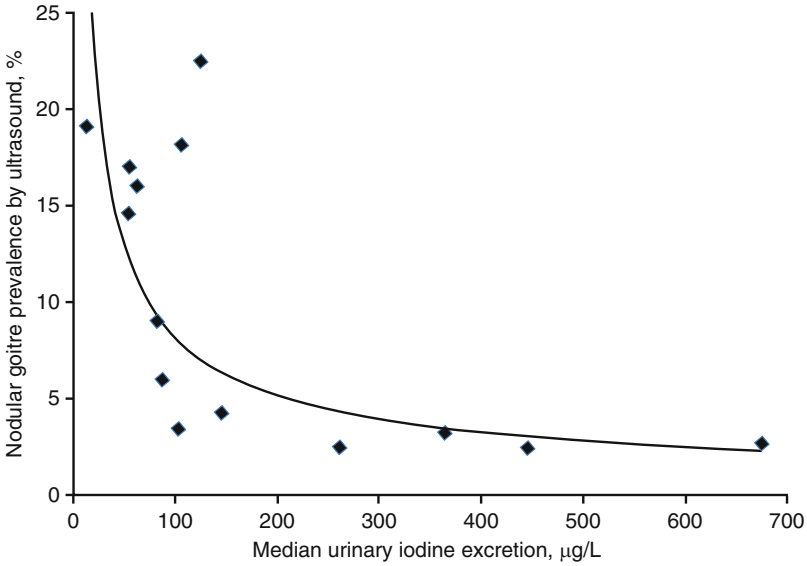

Fig. 1 Population-based studies showing the inverse correlation between nodular goiter and the iodine status, as assessed by the urinary iodine excretion. Each dot represents an individual study (From Carle et al. 2014, with permission)

Contrasting cross-sectional studies, longitudinal population-based cohort studies provide more valid data on incidence, prevalence, natural history, and the interaction with etiologic risk factors. In the population-based Whickham survey (Vanderpump et al. 1995), conducted in the United Kingdom, the prevalence of goiter was assessed clinically on two occasions 20 years apart and in the same individuals. Twenty-three percent of women and 5% of men had goiter at the first investigation, but these numbers had declined to 10% and 2%, respectively, 20 years later. Since, according to previous observations, thyroid volume correlates positively with age and body weight (Hegedüs 1990), the lower prevalence of goiter with advancing age may therefore be related to a decline of the lean body mass.

Ultrasound is much more accurate and precise than palpation for estimation of thyroid size. This applies in particular to the detection of thyroid nodules, realizing that 70% of these are smaller than 10 mm (Tan et al. 1995). The importance of iodine as an etiological factor is supported by longitudinal studies showing that the prevalence of goiter is reduced by iodine fortification programs in iodine-deficient areas. In the Pescopagano study (Fiore et al. 2014; Aghini-Lombardi et al. 1999) of an Italian population living in an iodine-deficient area, the prevalence of goiter was 46%, as assessed by ultrasound. This number decreased to 26% after 15 years of iodine fortification, by which time the mean urinary iodine excretion had increased from a median of 55 ug/L to 98 ug/L. In the Dan-Thyr study (Laurberg et al. 2006), the Danish population was monitored by thyroid ultrasound and biochemical tests, before and after iodine fortification was initiated in the year 2000 (Fig. 2). Before iodine fortification, the prevalence of goiter was 25–33% among women

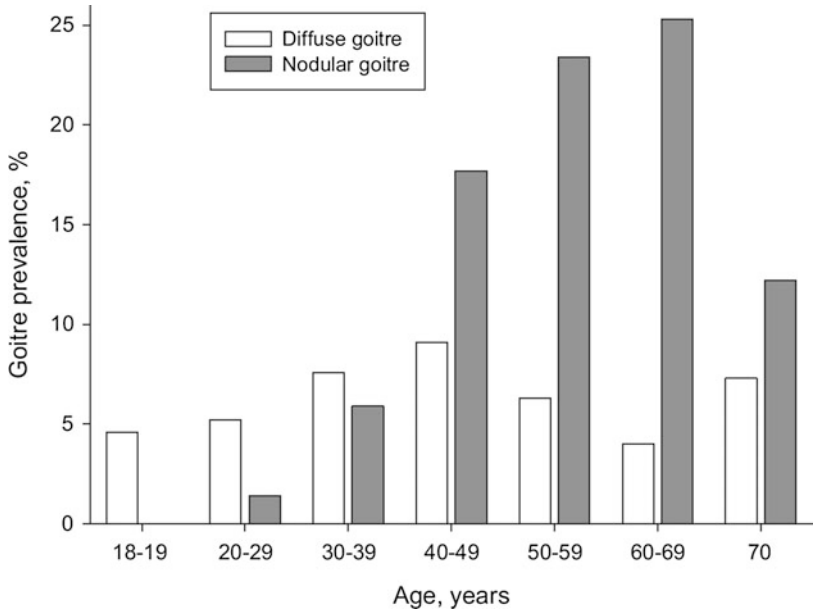

Fig. 2 Prevalence of goiter among females in Denmark, a region of mild to moderate iodine deficiency, before iodine fortification was established in 2000. Thyroid nodularity as well as the prevalence of goiter increases with age up to 70 years (From Carle et al. 2014, with permission)

40–65 years of age and highest in the western part of Denmark, where the iodine intake was lowest. The majority of goiters were multinodular. After 5–11 years of iodine fortification, the prevalence of goiter had clearly decreased, while the overall prevalence of thyroid nodules remained unchanged (Vejbjerg et al. 2007; Krejbjerg et al. 2014). In both the Pescopagano and the Dan-Thyr studies, the prevalence of goiter increased with age up to 70–75 years, where after it decreased. In most individuals, it seems unlikely that a thyroid nodule should vanish spontaneously once it has developed. Therefore, it probably takes a period of time, corresponding to a generation, before iodine fortification of a population residing in an iodine-deficient area results in a significant decline in thyroid nodularity.

Natural History

The natural history of nodular goiter varies and is difficult to predict in the individual patient. The spontaneous growth rate of a benign thyroid nodule, whether solitary or located in a multinodular goiter, can be up to 20% yearly (Berghout et al. 1990) but is usually much lower. In addition, thyroid nodularity increases with age. In old people with goiter, the thyroid parenchyma typically harbors many nodules of different size and texture (Hegedüs et al. 2003; Carle et al. 2014). The risk of malignancy in thyroid nodules located within a multinodular goiter is considered to be similar to the risk in solitary

thyroid nodules (Hegedüs et al. 2003) and around 2–8% depending on patient selection. It is debated whether a thyroid nodule, once diagnosed as being benign, can degenerate into malignancy. However, currently such a transformation is considered to be an uncommon phenomenon. Therefore, lifelong monitoring with serial biopsies of untreated thyroid nodules, although debated, seems to be unnecessary as a routine (Lee et al. 2013). In support, nodular growth is not predictive of malignancy (Singh et al. 2016).

Autonomous function of one or more nodules is a common finding in multinodular goiters. Although reversal of the autonomy may occur in a few cases, a high proportion of patients become hyperthyroid with time as a consequence of continuous nodule growth and progression of the autonomy. In fact, functional autonomy is often present at the initial evaluation of patients with nodular goiter, reflected by low-normal or subnormal levels of serum TSH (Fast et al. 2008). Furthermore, longitudinal studies have shown that hyperthyroidism emerges in 1% per year of patients with nodular goiter (Vanderpump et al. 1995). In parallel, nodular autonomy increased with age and reached 15% in the elderly in the Italian Pescopagano study (Fiore et al. 2014; Aghini-Lombardi et al. 1999). Hyperthyroidism can also result from a sudden high iodine load, as seen by the administration of, for example, amiodarone or radiographic contrast media (Kornelius et al. 2016). In such cases, a ^{131}I thyroid uptake measurement will show low values. The hyperthyroidism is often modest with few symptoms, and it remits spontaneously within weeks or a few months.

Whereas progression towards hyperthyroidism is part of the natural history in many patients with multinodular goiter, spontaneous development of hypothyroidism is only occasionally seen, most likely as the results of coexisting autoimmune thyroiditis.

Symptoms and Impact of Goiter

Complaints by patients with a goiter cover a broad spectrum and correlates poorly with goiter size (Hegedüs et al. 2003). Some nodular goiters are detected incidentally during consultation at the physician or by neck imaging for non-thyroid purposes, and most of these goiters are non-symptomatic at diagnosis. This contrasts the observation that quality of life, covering a range of domains, is reduced in goiter patients referred to surgery or ^{131}I therapy (Cramon et al. 2015; Watt et al. 2014). Symptoms comprise globus sensation, dysphagia, cough, voice alterations, respiratory distress, and choking sensation. For some patients, cosmetic complaints or cancrophobia are the most important concerns (Watt et al. 2014; Abdul-Sater et al. 2011). Pain from the thyroid gland is uncommon in patients with nodular goiter but may occur as the result of hemorrhage into a nodule or a cyst embedded in the goiter.

On account of its location in the lower part of the neck and at the thoracic inlet, a nodular goiter may affect vital structures, such as the trachea, esophagus, nerves, and blood vessels, but huge individual variations exist due to differences in goiter size, structure, configuration, and growth rate. Patient-related factors, such as vulnerability and susceptibility to symptoms in general, are also important in this respect. Unfortunately, most studies investigating how goiters affect adjacent organs and quality of life are small and heterogeneous and conducted in selected patients.

Impact on the Trachea and the Respiratory Function

A goiter may cause upper airway obstruction by displacing or narrowing the trachea, but respiratory symptoms can be few or even absent due to the slow progression. As the air flow is fourfold proportional to the radius of the trachea, according to Poiseuille's law, even minor changes in the tracheal lumen can have a pronounced impact on the airflow. In case of tracheal stenosis, the inspiration is more prone to be compromised than the expiration (Bonnema 2008). This is due to the fact that the inspiratory airflow through a narrowed lumen induces a negative transmural pressure gradient across the tracheal wall. In a clinical context, this gives rise to a stridorous respiration, whereby the tracheal cartilage rings collapse during inspiration – as opposed to the prolonged expiration typically seen in asthma or chronic obstructive pulmonary disease affecting the smaller airways. To some extent, the severity of tracheal compression correlates with goiter volume. In patients referred to ^{131}I therapy, the smallest cross-sectional area of the trachea, as measured by CT or MRI, was smaller in patients with goiter volumes larger than 100 mL than in those with a goiter smaller than 100 mL (Bonnema et al. 1999, 2008; Graf et al. 2011). However, also the configuration of the goiter may be of importance. Thus, a goiter that encircles most of the trachea is probably more critical than one that merely causes deviation of the trachea from the midline, although the significance of such a distinction remains to be investigated.

Deviation of the trachea from the midline can be detected by conventional X-ray, is found in 34–73% of patients referred for surgery, and does not differ between cervically and substernally located goiters (Sørensen et al. 2014). Tracheal compression is less common than tracheal deviation, although the validity of conventional X-ray for the detection of tracheal compression can be questioned due to the poor agreement with CT findings (Sørensen et al. 2014). In two small studies, no correlation could be demonstrated between flow volume loop parameters and the tracheal diameter assessed by conventional X-ray (Albareda et al. 2010; Torchio et al. 2003), while such a relationship in fact was found in a study using MRI (Bonnema et al. 2008). Thus, CT or MRI should be regarded as the methods of choice for assessment of tracheal dimensions.

The clinical consequence of tracheal compression is a reduction of the respiratory capacity. Indeed, several studies report that a significant fraction of goiter patients suffer from upper airway obstruction, but the degree of respiratory complaints show large variations (Sørensen et al. 2014). Only a few studies have used objective criteria, in terms of the dynamic expiratory and inspiratory functions. Reduction in one or more of these parameters was found in 14–31% of patients referred for evaluation of goiter (Menon et al. 2011; Miller et al. 1990) and was even more common, 26–60%, among those considered for thyroidectomy (Albareda et al. 2010; Jauregui et al. 1977).

Impact on the Esophagus

The swallowing function may also be affected in patients with a goiter. Questionnaire studies have shown that swallowing difficulties may be present in up to 83% of

patients referred for goiter surgery and negatively affect quality of life (Sørensen et al. 2014). Results from objective examinations are in line with the perception of the patients, but the mechanisms causing swallowing difficulties are incompletely understood. Radiological studies have demonstrated esophageal compression and/or deviation in 8–27% of patients referred for goiter surgery (Sørensen et al. 2014; Netterville et al. 1998). The anatomical dislocation may lead to motility disturbances and a prolonged passage through the esophagus. Indeed, functional studies, employing scintigraphical methods, found a prolongation by 2–7 sec of the esophageal transit time in 39% of patients with a large goiter (Glinoer et al. 1987; Jorgensen et al. 1989). Using water manometry, 50% of patients with goiter may have disturbance of the esophageal motility, with affection of the upper, the lower, or both esophageal sphincters (Scerrino et al. 2013). Other factors, unrelated to the esophagus, may be involved. Thus, up to 90% of goiter patients may suffer from dysmotility of the hyoid bone, tilting of the epiglottis, or bolus retention in the pharynx (Fiorentino et al. 2011).

Impact on the Voice

In goiter patients, studies of phonatory symptoms, such as hoarseness, vocal fatigue, and vocal straining, and their impact on quality of life, are few and small. In 40 female patients with goiter the most common symptom was vocal fatigue, while vocal straining was the only symptom significantly more frequent among patients, as compared with control subjects (Hamdan et al. 2016). In one of six patients, phonatory symptoms had a negative impact on quality of life, as measured by the Voice Handicap Index (Hamdan et al. 2016). To be noted, a patient can occasionally present with unilateral vocal cord paresis, caused by a benign goiter, without this causing any phonetic complaints (Lang et al. 2014).

Another study examined the auditory perceptual voice characteristics of 96 individuals with various thyroid diseases, including benign goiters (Bone et al. 2012). Many patients had a mild degree of abnormal auditory voice perception, but this was clinically significant in only 8% of the subjects. The perception of high pitch was the only variable which differed significantly, when comparing goiter patients with and without symptoms of neck compression.

Diagnosis

Clinical Examination

A normal thyroid gland is often neither visible nor palpable until it has reached at least double size. Large goiters in elderly patients may have descended into the mediastinum, with little or no palpable cervical thyroid tissue remaining (Hegedüs and Bonnema 2010). The thyroid is preferably examined with the patient's head tilted slightly backward and with the clinician standing behind the patient. Apart from the

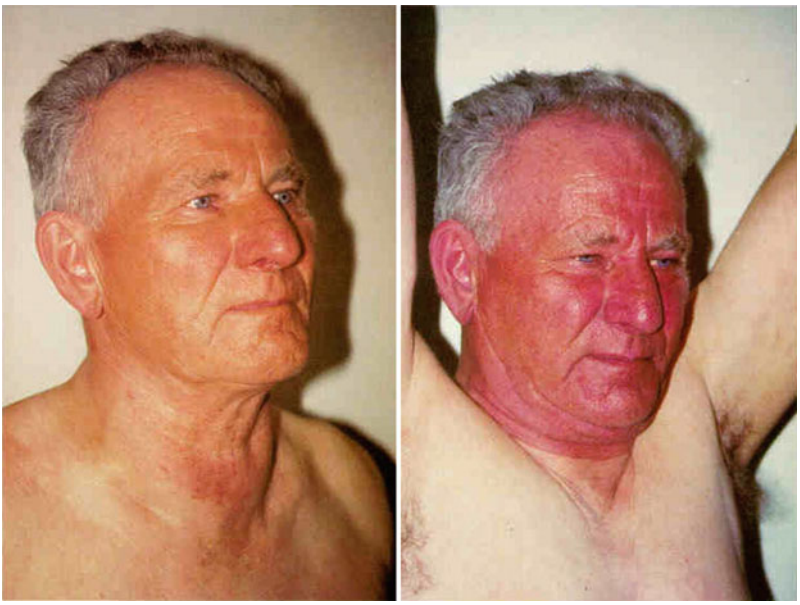

Fig. 3 Pemberton's sign. By elevating both arms the goiter will "cork off" the thoracic inlet, resulting in facial congestion and cyanosis (From Wallace and Siminoski 1996, with permission)

size and detection of nodules embedded in each thyroid lobe and in the midline of the neck, also the consistency of the gland and possible intrathoracic extension should be registered. However, detection of a goiter by clinical examination, and especially a detailed determination of its size and structure, can be difficult. Anatomy of the patient's neck, the thickness of the strap muscles, and the training of the physician are also important factors in this respect. Indeed, clinical examination of the thyroid is associated with a high inter- and intra-observer variation (Jarløv et al. 1991).

A large goiter located in the upper mediastinum may result in the superior vena cava syndrome, which can be confirmed by the presence of Pemberton's sign (Fig. 3). This maneuver is achieved by having the patient elevate both arms until they touch the sides of the face. In case the goiter "corks off" the thoracic inlet, this results in facial congestion, cyanosis, and respiratory distress after approximately 1 min (Wallace and Siminoski 1996).

Disseminated thyroid cancer should be suspected if the clinical examination discloses enlarged lymph nodes in the neck, hoarseness due to vocal cord paralysis, or Horner's syndrome (unilateral miotic pupil). This also applies if the goiter is adherent to adjacent structures or has a very firm consistency. A stridorous respiration reflects a significant compression of the trachea and supports the presence of a large goiter. However, this is not specific for thyroid cancer as it can be seen also in patients with a benign goiter.

It should be underlined that a goiter may represent other benign conditions than multinodular degeneration in an enlarged thyroid gland; these are Hashimoto's autoimmune thyroiditis, Graves' disease, De Quervain's subacute thyroiditis, or

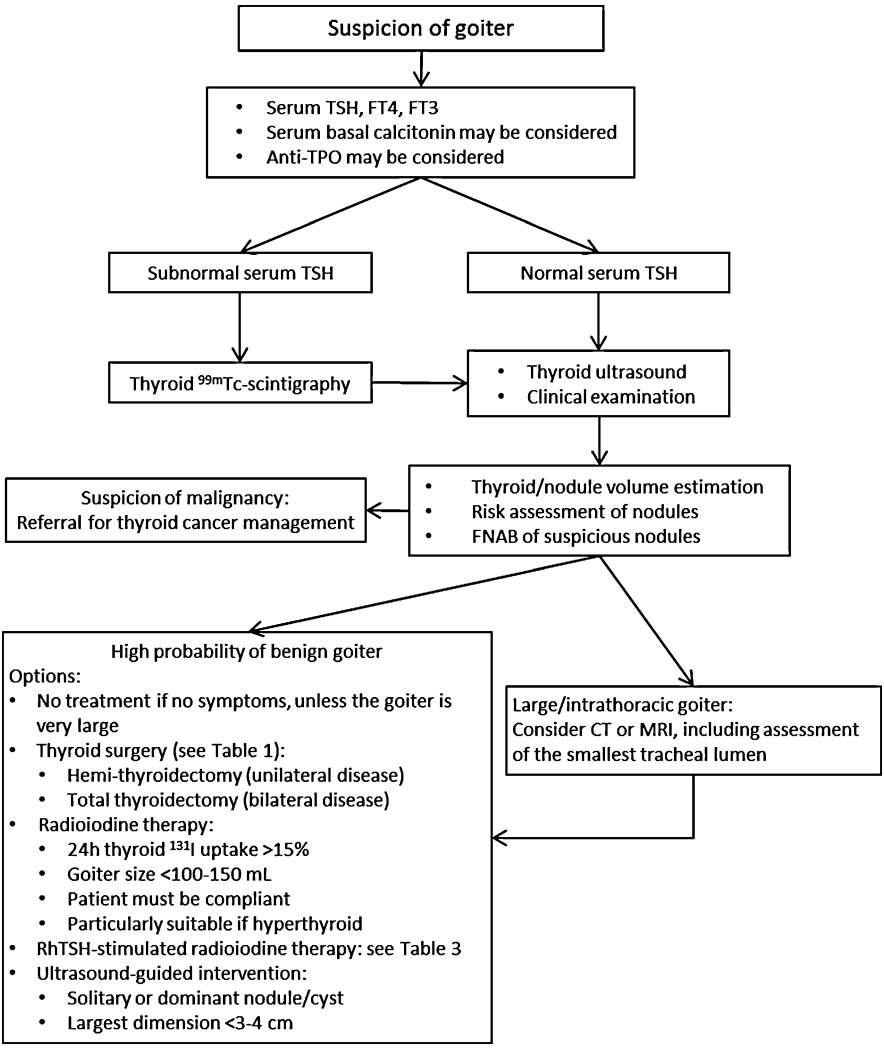

Fig. 4 Proposal for work-flow including diagnostic strategy and therapeutic options in patients referred with goiter

Riedel's thyroiditis. Biochemical and serological tests as well as thyroid imaging help the clinician in differentiating between these diseases.

Signs and symptoms of thyrotoxicosis may be present if the goiter harbors autonomously functioning nodules. Clinical examination may reveal shivering of the hands, warm moist skin, and increased pulse rate. However, such objective signs can be subtle or even absent since the hyperthyroidism typically is mild and often has been long-standing.

A suggestion for a diagnostic and therapeutic work-up of a patient with goiter is shown in Fig. 4.

Biochemical and Serological Tests

Thyroid function tests: Obviously, measurement of serum TSH should be the initial biochemical test, and if the level of this hormone is outside the reference range, assessments of serum free T4 and free T3 should follow. Transition from a nontoxic to a toxic nodular goiter is part of the natural history of this disease, and a serum TSH value below the normal range merely reflects that the hyperfunctioning nodules no longer can be counterbalanced by a suppression of the paranodular TSH-responsive thyroid tissue. With progression of the autonomy, overt hyperthyroidism will ensue (Vanderpump et al. 1995). In consequence, serum TSH should be monitored, at least annually, if the patient is left untreated. If serum TSH is subnormal at diagnosis, independent of the serum levels of the thyroid hormones, this will strengthen the indication for treatment of the goiter, even in the absence of symptoms (Surks et al. 2004). Although randomized trials are lacking, large scale epidemiological studies have demonstrated that morbidity (Brandt et al. 2013a) as well as mortality (Laulund et al. 2014) is significantly associated with a subnormal serum TSH.

Markers of thyroid autoimmunity: Thyroid-peroxidase (TPO), thyroglobulin, and TSH-receptor antibodies in serum should be considered in the work-up of all patients with goiter. Both Hashimoto's autoimmune thyroiditis and Graves' disease may be superimposed on a classic multinodular goiter, a phenomenon not uncommonly seen (Pedersen et al. 2001). Thus, if serum TSH is increased above the normal range in a patient with newly discovered nodular goiter, this should raise suspicion of coexisting Hashimoto's autoimmune thyroiditis and anti-TPO. Anti-thyroglobulin should be measured in case anti-TPO is negative. In addition, these antibodies are markers of an increased risk for hypothyroidism, in case the goiter subsequently is treated with ^{131}I (Nygaard et al. 1999a). TSH-receptor antibodies are markers of Graves' disease (Smith and Hegedus 2016), which can emerge in a nodular goiter that may have been present for years. The hyperthyroidism resulting from Graves' disease is usually much more severe than that seen in a toxic nodular goiter, but mild hyperthyroidism may occur in the former condition. Therefore, measurement of TSH-receptor antibodies can be justified in any patient, in whom serum TSH is below the normal range. If positive, the hyperthyroidism is probably due to immune stimulation of the thyroid gland, mediated by the TSH-receptor antibodies rather than caused by autonomously functioning nodules.

Serum thyroglobulin: This is a marker of the thyroid tissue mass and is primarily used for postsurgical monitoring of thyroid cancer and in epidemiological studies. Although serum thyroglobulin correlates positively with thyroid size, it has little place in the diagnostic work-up of benign nodular goiter due to lack of accuracy at the individual level.

Calcitonin is a marker of medullary thyroid cancer (MTC), and it is also used for monitoring of this disease. However, it is debatable whether calcitonin determination should be done routinely in patients with nodular goiter, and no consensus exists on this issue (Gharib et al. 2016; Haugen et al. 2016). Measurement of the basal or stimulated calcitonin level is generally more sensitive than fine-needle aspiration biopsy in detecting MTC (Hegedüs et al. 2003). In contrast to the high sensitivity of

this test, the specificity is low (Hasselgren et al. 2010). As the prevalence of MTC among patients with nodular goiter is low, in the range 0.4–1.4%, serum calcitonin determination in all goiter patients will result in many false-positive values and subsequently many unnecessary thyroidectomies.

Imaging

Accepting that clinical examination of the thyroid gland is unreliable and heavily influenced by observer variation (Jarløv et al. 1991), thyroid imaging is mandatory in order to obtain more robust information of the nature and size of the goiter. Several technologies are available, each carrying their advantages and limitations. Thyroid imaging is crucial not only in the diagnostic work-up of patients with nodular goiter but may also be of great value during follow-up. Some of the methods may be used in a complementary fashion.

Ultrasonography is easily accessible, noninvasive, and cost-effective and should be a key method for imaging of thyroid nodules. By ultrasonography, the texture of the thyroid tissue can be assessed in great detail, and it allows an evaluation of regional lymph nodes. Being a three-dimensional technology, volume calculation of individual nodules or the entire thyroid gland is a very useful feature of ultrasound. The calculation is done either by planimetry or, more commonly, by employing the ellipsoid formula. Each principle offers a fairly high level of accuracy. Of further benefit, ultrasound is of great help in diagnostic and therapeutic procedures, e.g., fine-needle aspiration biopsy, cyst punctures, or nonsurgical ablation of solitary nodules (Hegedüs et al. 2003; Hegedüs 2001). Disadvantages of thyroid ultrasonography include its operator dependency, the inability to access substernal goiters, and inaccurate volume estimation of very large goiters.

Ultrasound can detect even minute nodules embedded in the thyroid parenchyma (Fig. 5). Depending on the patient population and the discriminatory level for

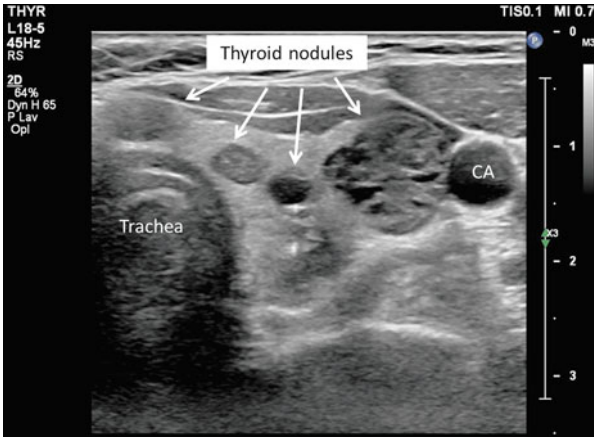

Fig. 5 Ultrasound picture (transverse section) of a thyroid gland with multinodular degeneration. Several thyroid nodules are seen, of which two are partly cystic (*dark areas*). *CA* carotid artery

defining thyroid tissue as a nodule, at least five times as many nodules are diagnosed by ultrasound, as compared with thyroid palpation alone (Hegedüs et al. 2003; Tan and Gharib 1997). Most of these nodules are without clinical relevance and can be considered as thyroid incidentalomas. However, it may impact the choice of treatment if ultrasonography reveals a multinodular goiter; disclosing that the patient's problem may not be confined to a solitary nodule.

Certain ultrasound features of the thyroid nodule are predictive of malignancy, but the high sensitivity of each of these characteristics is opposed by low rates of specificity. A high observer-variation in the assessment of some of the ultrasound characteristics is another shortcoming (Choi et al. 2010). Several meta-analyses have evaluated the accuracy of a range of ultrasound features in the prediction of cancer in thyroid nodules (Brito et al. 2014; Remonti et al. 2015; Campanella et al. 2014). The highest diagnostic odds ratios for cancer prediction apply to features like "taller than wide" shape, microcalcifications, and irregular margins, while spongiform or cystic appearance most reliably predict a benign nature of the nodule. Neither nodule size *per se* nor growth (by serial measurements) has any significant predictive value (Singh et al. 2016; Brito et al. 2014).

Realizing that no single characteristic of the nodule, as assessed by ultrasound, can differentiate between a benign and a malignant nodule reliably, standardized systems for reporting ultrasound features have been developed in order to describe sets of characteristics associated with specific risk levels for malignancy. Based on systems used for reporting breast imaging data, a tool for ultrasonographic classification of thyroid nodules was launched in 2009 (Horvath et al. 2009). This is called the Thyroid Imaging Reporting And Data System (TIRADS) and consists of a six-point scale for risk stratification based on a range of ultrasound patterns. Several international recommendations regarding ultrasound risk classification of thyroid nodules, and following the TIRADS principle, have been issued in recent years (Gharib et al. 2016; Haugen et al. 2016; Na et al. 2016; Russ et al. 2017). There is only slight variation between these recommendations, and none of them have been validated in large-scale prospective studies.

The various TIRADS risk classifications are primarily based on nodule characteristics obtained by gray scale ultrasound. Other ultrasound features include nodule vascularity, determined by Doppler, and nodule stiffness, determined by real-time or shear wave elastography (Rago and Vitti 2014). However, the utility of these technologies in this context is controversial, and it is questionable whether they improve the risk classification beyond what is achieved by the gray scale assessments (Swan et al. 2016).

Thyroid scintigraphy is a rapid, relatively low-cost method, and it inflicts only a low radiation burden to the body and the surroundings. Access to nuclear medicine facilities is naturally a prerequisite. ^{99m}Tc is most often used as tracer for practical reasons. The method allows a determination of hyperfunctioning (hot/warm) and hypofunctioning (cold) thyroid areas (Fig. 6). It also provides a rough estimate of the size and configuration of the goiter and its extension into the mediastinum, if present. Additionally, scintigraphy aids in prioritizing which nodules to offer fine-needle aspiration biopsy (FNAB), based on the fact that thyroid tissue showing a high isotope uptake, with rare

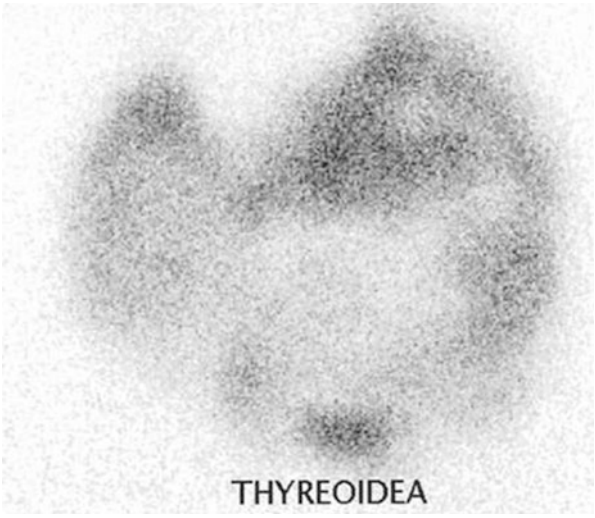

Fig. 6 ^{99m}Tc scintigram of a large multinodular goiter, showing a mix of low and high isotope uptake areas

exceptions, never represents a carcinoma. For these reasons, scintigraphy is of some use in the evaluation of the multinodular goiter (Giovanella et al. 2014). However, its low image resolution does not allow a detailed morphological evaluation of the thyroid and its texture, nor for an accurate estimation of the goiter volume.

At many centers, thyroid scintigraphy is not part of the initial evaluation of patients with nontoxic nodular goiter (Bonnema et al. 2000). Scintigraphy is recommended, however, in patients with a subnormal serum TSH level, in order to visualize hot nodules suitable for ^{131}I therapy. Furthermore, if the FNAB shows follicular neoplasia (see below), a scintigraphy demonstrating the nodule to be warm is reassuring.

Positron emission tomography (*PET*), most often using [18F]-2-deoxy-2-fluoro-D-glucose as tracer, has gained no place in the routine work-up of patients with benign nodular goiter. However, PET is highly sensitive for detection of malignant tissue, and, therefore, it may be useful in the evaluation and monitoring of patients with thyroid cancer. The widespread use of PET for non-thyroid reasons results, incidentally, in the detection of focal thyroid uptake in around 1.6% (Soelberg et al. 2012). These lesions almost always represent a thyroid nodule, the existence of which was unknown to both patient and physician. Importantly, up to 35% of such nodules detected incidentally harbor thyroid cancer (Soelberg et al. 2012). The overwhelming majority are papillary carcinomas, and many of these patients subsequently undergo thyroid cancer surgery and year-long monitoring. It remains to be established whether such an active approach saves lives and to what extent it loads an unnecessary burden on many patients.

Computed tomography (CT) and magnetic resonance imaging (MRI) are useful methods if the major part of the goiter is displaced into the mediastinum, where access by ultrasound is impossible (Fig. 7) (Bonnema et al. 2002a). In addition, if a large goiter is suspected of causing tracheal compression, as commonly seen, CT and

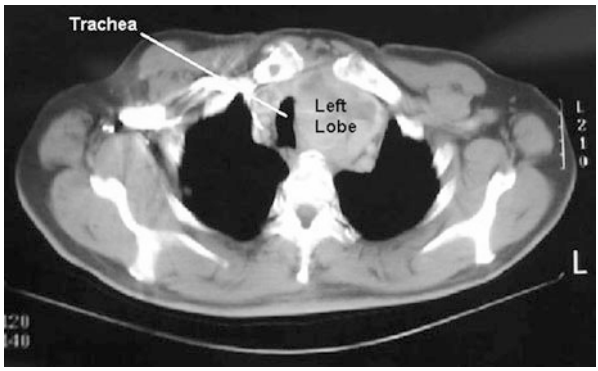

Fig. 7 CT scan of the thorax, showing an intrathoracic extention of the left thyroid lobe with compression of the trachea

MRI allow an assessment of the smallest cross-sectional tracheal area. Routine radiography of the trachea, e.g., conventional X-ray of the thorax, can show deviation of the trachea from the midline, but it cannot reliably quantify the degree of tracheal obstruction (Melissant et al. 1994). MRI may be marginally more precise than CT in the evaluation of the goiter volume and the tracheal dimensions (Bonnema et al. 2002a), but no greater differences exist in this respect between the two technologies. Thus, whether MRI or CT should be preferred is mostly a matter of availability and patient compliance.

Other Tests

Thyroid ^{131}I uptake measurement is rarely part of routine work-up of patients with goiter. If performed, a low uptake indicates either iodine contamination or exacerbation of an inflammatory or autoimmune thyroiditis. If such conditions are suspected, thyroid scintigraphy is more relevant to perform for practical reasons. In the patient with nontoxic goiter, indication for measurement of the thyroid ^{131}I uptake is foremost to assess whether ^{131}I therapy is feasible and, if so, to allow calculation of the ^{131}I dose.

Flow volume loop is a method by which the inspiratory and expiratory pulmonary capacities can be quantified. This may be considered in patients with a very large goiter, in whom tracheal compression is suspected. Most often the inspiratory part of the respiration is compromised, with stridor being the clinical equivalence in severe cases. Many patients do not have respiratory complaints due to the slow progression of the goiter enlargement, and mild forms of upper airway obstruction may therefore be overlooked during a routine examination (Sørensen et al. 2014). On the other hand, if doubt exists as to whether dyspnea is due to a large goiter, or is caused by another condition unrelated to the thyroid, flow volume loops may have the potential to differentiate between some of the conditions causing respiratory symptoms. However, flow volume loops are cumbersome to perform, and lack of precision of the method also limits its utility in the individual patient (Sørensen et al. 2014).

Fine-Needle Aspiration Biopsy (FNAB)

The possibility of thyroid malignancy should be considered in all patients with a nodular goiter. The prevalence of clinically important thyroid cancer in patients referred for goiter evaluation is in the range of 2–8% but is highly dependent on the population and the selection of patients (Hegedüs et al. 2003). The risk of thyroid cancer seems inversely correlated with the serum TSH level according to some studies (Haymart et al. 2008), but selection bias may have influenced the results. Nevertheless, mild or overt hyperthyroidism due to one or more autonomously functioning nodules does not exclude presence of malignancy in nodules found in other areas of the multinodular goiters.

Ultrasound assessment of the risk of thyroid malignancy is crucial, in order to select those nodules that should undergo FNAB (Fig. 8). A cytologically benign result cannot rule out malignancy, but the risk of overlooking a thyroid cancer is reduced to a few per cent, depending on the skills of the ultrasonographer and the pathologist (Baloch and LiVolsi 2014). As most thyroid cancers show little aggressiveness and slow growth, a false-negative FNAB has in many cases little or no influence on the prognosis but will only delay the diagnosis. Also, many thyroid microcarcinomas, found incidentally, show no signs of progression during long-term follow-up. How such microcarcinomas should be managed is still unsettled, but a

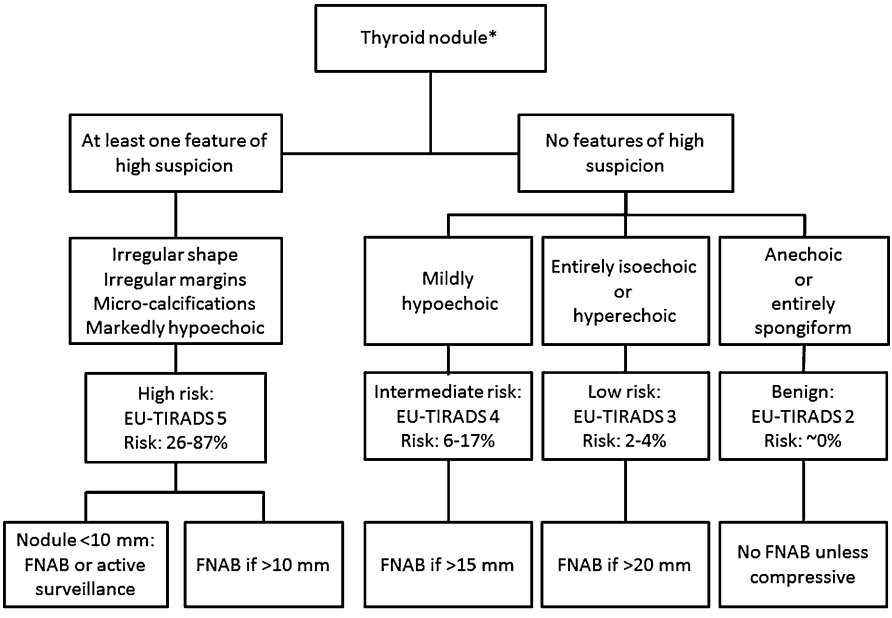

Fig. 8 Algorithm for malignancy risk stratification and fine-needle aspiration biopsy decision-making, according to the ETA EU-TIRADS guidelines (Redrawn from Russ et al. 2017)

strategy involving active surveillance rather that thyroid surgery may be justified (Leboulleux et al. 2016).

In a multinodular goiter harboring several bilateral lesions, FNAB should be performed in all suspicious nodules, or at least those with the highest risk features on ultrasound. The execution of the FNAB procedure should be guided by ultrasound to lower the risk of sampling error (Baloch and LiVolsi 2014). Warm nodules demonstrated by thyroid scintigraphy, if performed, are with rare exceptions never malignant, and FNAB of such lesions is therefore not indicated.

According to international recommendations on ultrasound-based scoring systems – as, for example, the EU-TIRADS (Russ et al. 2017) – FNAB should be performed if the nodule is categorized in the intermediate or high-risk group, and if larger than 15 mm and 10 mm, respectively. However, it is questionable whether a strict size criterion is advisable in all cases, since the risk of thyroid carcinomas may be similar in nodules below and above 10 mm in diameter (Papini et al. 2002). Thus, it is at present unclarified whether patients with a nodule less than 10 mm, but with highly suspicious ultrasound features, are better served by active surveillance or immediate FNAB.

If the initial test is non-diagnostic due to limited cellularity in the specimen, FNAB should be repeated (Baloch and LiVolsi 2014), since the cytological result is a decisive factor in directing management strategy. FNAB will show a benign result in 50–70% of unselected patients with nodular goiter (Hegedüs et al. 2003). It should be noted, however, that if the suspicion of thyroid malignancy is high, based on the patient history or the results from the clinical examination, the patient should be offered surgery, independent of the results of FNAB.

In 10–15% of cases, the cytology shows "follicular neoplasia." This is an ambiguous result, as the majority (~80%) of such nodules will be benign, whereas 10–20% will represent a thyroid cancer (Hegedüs et al. 2003). Usually, diagnostic hemithyroidectomy is performed to verify the nature of the nodule histologically. To avoid unnecessary surgery in oligo- or asymptomatic patients who appear to have a benign nodule, the application of a gene-expression classifier, in order to differentiate benign from malignant nodules, has gained much interest in recent years (Eszlinger et al. 2014). However, the validity of these tests remains to be confirmed in large prospective studies, and cost is also a barrier for the implementation in the routine setup.

Treatment

Many goiters are found incidentally, typically by imaging performed for other purposes. Under such circumstances, goiter symptoms are often sparse or absent. The majority of nontoxic goiters harbor one or more thyroid nodules, which can be solid, cystic, or complex in composition. When malignancy has been ruled out by FNAB of nodules with suspicious features on ultrasound, many patients do not need treatment. In case the patient is left untreated, there is no evidence that lifelong monitoring of the goiter by palpation or thyroid imaging is cost-effective (Singh

et al. 2016; Jarhult and Vedad 2014). Annual measurement of serum TSH seems reasonable to detect emerging autonomy of the thyroid nodules. Obviously, the patient should be reexamined in case of symptoms of goiter growth and related incipient symptoms.

There is poor correlation between goiter size and symptoms (Bonnema et al. 2004), and no thyroid volume threshold can be given above which intervention is absolutely indicated. Nevertheless, in case of a very large goiter (approximately 200 mL or more), the patient should be recommended treatment, despite absence of symptoms, since the evidently high growth potential may eventually cause critical neck compression (Hegedüs et al. 2003). If treatment is indicated, several options exist, and the final choice must be made in a dialogue with the patient. In addition to the size of the goiter and severity of symptoms, a range of other factors must be taken into account before reaching a final choice of treatment. These include availability and competence of local expertise, age of the patient, comorbidity, previous neck surgery or ^{131}I therapy, coexisting hyperthyroidism, appearance on scintigraphy, cancrophobia, and fear of side effects of treatment.

Iodine Supplementation

Since long-standing iodine insufficiency is a major etiological factor in the development of nontoxic nodular goiter, it seems logical to consider iodine supplementation as a potential treatment for this condition. Indeed, this approach has been adopted by some clinicians, especially in regions with a relatively low dietary iodine intake (Bonnema et al. 2000).

In diffuse goiters of moderate size, iodine supplementation may have a small but significant effect. Thus, in a placebo-controlled trial (Kahaly et al. 1997), it was shown that the median volume of diffuse goiters was reduced from 29 to 18 ml but at the expense of thyroid dysfunction and appearance of thyroid antibodies in 10% of the patients. The efficacy of iodine supplementation, once a large nodular goiter has developed, is even more modest, and not better than levothyroxine suppression therapy in comparative trials. In disfavor of iodine treatment – besides the suboptimal goiter shrinkage – is also the well-known phenomenon that a sudden increase of iodine intake may induce thyrotoxicosis in predisposed individuals (Kornelius et al. 2016).

Thyroid Hormone Suppressive Therapy

Levothyroxine suppressive therapy for goiter shrinkage in euthyroid individuals has been used for many years. The rationale for this treatment is to decrease serum TSH and thereby to abolish a major trophic factor. In theory, this should be effective but evidence to support this treatment strategy is sparse. In diffuse nontoxic goiters, levothyroxine suppressive therapy may reduce the volume by up to 30% (Perrild et al. 1982). However, as for the nontoxic nodular goiter, there are few controlled

studies, in which ultrasonography has been applied for size monitoring. In one study (Berghout et al. 1990), 58% of patients had a significant (>13%) decrease in thyroid volume, but regrowth was seen after cessation of therapy. In another randomized trial (Wesche et al. 2001), the median goiter volume reduction was only 1% after 2 years and much less than observed in the ^{131}I treated group. In a placebo-controlled trial (Grussendorf et al. 2011), levothyroxine combined with elementary iodine supplementation resulted in a goiter volume reduction of only 7.9% and a nodule volume reduction of 17.3% within 1 year.

Levothyroxine suppressive treatment, which probably must be lifelong to avoid goiter recurrence, is often targeted towards a subnormal serum TSH level. This mimics subclinical hyperthyroidism, a condition known to have negative impact on the skeleton (Abrahamsen et al. 2014) and the cardiovascular system (Surks et al. 2004), and may even reduce life span (Laulund et al. 2014). Additionally, and in disfavor of levothyroxine, the natural history of a nodular goiter is progression towards hyperthyroidism due to autonomous function of the thyroid nodules. Adding all the above, and considering that levothyroxine treatment is not feasible in the majority of referred goiter patients (Fast et al. 2008), few arguments remain to support levothyroxine treatment for goiter reduction (Gharib et al. 2016).

Surgery

The goal of any therapy is to reduce the goiter to an extent that alleviates symptoms and hinders recurrence. Both goals can be achieved with surgery since patient satisfaction, as measured with a disease-specific quality-of-life instrument, is high (Cramon et al. 2015). The vast majority of goiters, also those with a partly intrathoracic extension, can be removed through a cervical approach (Kocher's incision). Both thyroid lobes are affected in the majority of patients referred for nodular goiter. Therefore, total thyroidectomy is often indicated. Until 10–15 years ago, bilateral subtotal resection was often done in this situation in anticipation of a lower risk of complications. However, such a strategy leads to a goiter recurrence rate of 15–40% (Hegedüs et al. 2003). Postoperative use of levothyroxine or iodine does not seem to offer protection from regrowth of thyroid tissue (Hegedüs et al. 1999). In fact, any thyroid tissue left in situ can give rise to goiter recurrence, and in such cases, a second thyroid operation is associated with a much higher risk of complications due to scar tissue (al Suliman et al. 1997). Similarly, a hemithyroidectomy should be complete without remnant thyroid tissue. In experienced centers, and with current surgical techniques, total thyroidectomy does not *per se* increase the risk of complications (Pappalardo et al. 1998).

Advantages and Disadvantages

Thyroid surgery is a focused treatment in the sense that a contralateral normal lobe can be left unharmed in case of unilateral nodular disease. Furthermore, it has the clear advantages that rapid relief of goiter symptoms is accomplished, while

Table 1 Factors favoring thyroid surgery for nontoxic goiter

Suspicion of cancer
Large goiter (approximately 150 mL or larger)
No or low ^{131}I uptake in the thyroid target tissue (e.g., cold nodules)
Unilateral thyroid lesion with a normal contralateral thyroid lobe[a]
Need of rapid relief of the goiter
Need of goiter treatment during pregnancy
Severe impact on the trachea with reduced respiratory capacity
Patient preference

[a] ^{131}I therapy should be favored if the contralateral lobe is functionally suppressed due to a solitary hot nodule located in the other lobe, as assessed by thyroid scintigraphy

histological examination of the specimen may assure the patient of the benign nature of the goiter. Other factors favoring thyroid surgery are listed in Table 1.

The perioperative mortality risk is less than 1%, whereas other disadvantages like pre- and postoperative bleeding and infection occur more commonly. Specific risks include vocal cord paralysis and hypoparathyroidism, being either transient or permanent. The incidence rates of these complications are in the range of 2–8% and correlate with the goiter size (al Suliman et al. 1997). The risk of hypoparathyroidism is virtually zero if only a hemithyroidectomy is performed. Complication rates increase several fold in cases of reoperation for recurrent goiter (al Suliman et al. 1997). Tracheomalacia, compromising respiration in the postoperative phase, is seen in approximately 5% of patients operated for a very large goiter (Abdel Rahim et al. 1999).

Lifelong levothyroxine substitution may be a consequence of thyroid surgery, depending on the extent of the procedure. After total thyroidectomy, 100 μg levothyroxine should be started on the day after the operation, followed by dose adjustment after 6 weeks, guided by thyroid function tests. After hemithyroidectomy, the remaining half of the thyroid gland is capable of ensuring sufficient thyroid hormone production in the vast majority of patients. A transient increase in serum TSH is often seen, but due to compensatory hypertrophy of the remaining lobe, serum TSH usually normalizes within a few months. According to meta-analysis, the risk of hypothyroidism after a hemithyroidectomy is 22%, of which many probably are transient (Verloop et al. 2012).

Some patients dislike the visible scar on the neck left by the classic surgical approach. For this reason, other surgical approaches have been developed, such as an axillary access. Novel techniques may also reduce the operation time as well as the length of the hospital stay (Dralle et al. 2014).

Effect of Thyroid Surgery on Adjacent Neck Structures

The swallowing function following goiter surgery is either unchanged or improved, according to several questionnaire studies (Sørensen et al. 2014). On the other hand, studies of the esophageal function, employing objective methods, are very few. One study found that the pressure of the upper esophageal sphincter decreased after thyroidectomy, without changes in esophageal motility and the

lower esophageal pressure (Scerrino et al. 2013). Other neck structures, like the position of the hyoid bone and the epiglottis, seem unaffected by thyroidectomy (Fiorentino et al. 2011).

Shown in several studies, thyroidectomy has a beneficial effect on the respiratory capacity, and this correlates with the mass of the removed goiter (Sørensen et al. 2014). Surgery significantly reduces or even eliminates upper airway obstruction caused by the goiter (Thusoo et al. 2000). This primarily has impact on inspiratory parameters, but also the expiratory component improves after surgical removal of the goiter (Sørensen et al. 2014).

The effects of thyroidectomy on the tracheal anatomy, and its dimensions have not been investigated in detail.

^{131}I Therapy

^{131}I therapy has been used since the 1940s for treatment of Graves' disease, but not until the last 30 years has it been an indication for nontoxic goiter (Bonnema and Hegedüs 2012). ^{131}I is usually administered orally in one single procedure. The treatment is given either in a hospital or in an outpatient setting, depending on local safety regulations that vary between countries. These regulations may dictate that proximity to other persons must be avoided for up to 1 week. The administered ^{131}I activity is usually in the range of 3.7–14 MBq/g (0.1–0.38 mCi/g) thyroid tissue corrected for the thyroid ^{131}I uptake (Bonnema and Hegedüs 2012). This corresponds to a thyroid dose of 100–175 Gray. It is unknown whether a lower dose, as compared with that commonly administered, leads to a similar goiter reduction or whether the goiter shrinkage can be improved by using higher doses.

There are large regional differences in the use of ^{131}I therapy for nontoxic goiter (Bonnema et al. 2000). This is partly due to differences in experience, traditions, and local procedures across countries and various centers. A prerequisite for ^{131}I therapy being effective is that the thyroid ^{131}I uptake is sufficiently high (24 h uptake >30–40%). This is rarely a problem in hyperthyroid disorders where the thyroid tissue is metabolically active. However, in nontoxic goiters, a low thyroid ^{131}I uptake may be a limiting factor, especially in areas with a high iodine intake, as this parameter is inversely correlated with the thyroid ^{131}I uptake. The problem of a low thyroid ^{131}I uptake can be overcome by use of recombinant human TSH (rhTSH) stimulation, as discussed in a subsequent section.

Efficacy of ^{131}I Therapy

Goiter Shrinkage and Patient Satisfaction

There are no large randomized controlled trials comparing the effect of ^{131}I therapy of nontoxic goiter versus no treatment or surgery. However, observational studies have consistently shown that ^{131}I therapy reduces the volume of nontoxic nodular goiters by 35–50% within 1 year (Bonnema and Hegedüs 2012), and further

reduction is achieved with longer follow-up. The reduction is even greater when treating nontoxic diffuse goiter. The magnitude of the relative goiter reduction shows large individual variations, but the majority of the patients are satisfied with the effect of ^{131}I therapy and experience an increase in quality of life (Bonnema and Hegedüs 2012). The goal of the treatment is relief of goiter symptoms, whether cosmetic or due to compression of adjacent structures. Some patients may experience an improvement in symptoms already after a goiter reduction of just 10–20% within 1 year (Bonnema and Hegedüs 2012). In such individuals no further treatment is needed. A second ^{131}I therapy can be given 6–12 months after the first treatment, if symptoms persist due to insufficient goiter reduction (Nygaard et al. 1993). Also substernal goiters can be treated with ^{131}I (Hegedüs and Bonnema 2010; Bonnema et al. 2002b), but such glands are often large, and the relative goiter reduction obtained by ^{131}I therapy is inversely correlated with the initial goiter volume, despite the application of equivalent radiation doses (Bonnema et al. 1999).

There are no data to support that ^{131}I therapy increases the risk of surgical complications, should a subsequent thyroidectomy become necessary.

Coexisting Autonomous Thyroid Nodules

If serum TSH is subnormal, this often reflects that one or more autonomously functioning nodules are embedded in the goiter. ^{131}I therapy to achieve euthyroidism is highly effective in this situation, and persistent subclinical hyperthyroidism will often strengthen the indication for ^{131}I therapy. The iodine uptake is high in the hyperactive thyroid tissue while the paranodular thyroid tissue receives much less irradiation during ^{131}I therapy. After therapy, most patients with subclinical hyperthyroidism achieve euthyroidism within 12 months, and the long-term risk of permanent hypothyroidism is much lower than seen after ^{131}I therapy of Graves' disease (Bonnema and Hegedüs 2012). Such a high cure rate and a low risk of thyroid failure support ^{131}I therapy as an almost ideal treatment in patients with nodular goiter and coexisting mild hyperthyroidism. ^{131}I may be a relevant choice of therapy even in large goiters, if the goal is to treat the autonomous nodules rather than to reduce the volume of an oligosymptomatic large goiter.

When there is coexisting subclinical hyperthyroidism, or even mild overt hyperthyroidism, there is no need for adjunctive antithyroid drugs in order to normalize serum TSH before ^{131}I therapy. The increase in TSH, as a result of antithyroid drug therapy, diverts the ^{131}I isotope from the autonomous nodules to the paranodular tissue. This may reduce the cure rate after ^{131}I therapy, increase the risk of hypothyroidism, and even attenuate the goiter reduction following therapy (Bonnema and Hegedüs 2012).

Adverse Effects of ^{131}I Therapy

The advantages of ^{131}I therapy should be held up against the disadvantages, as listed in Table 2. ^{131}I therapy of nontoxic goiter is generally well tolerated, but adverse effects may occur (Bonnema and Hegedüs 2012). A radiation-induced (actinic)

Table 2 ^{131}I therapy of nodular goiter

Advantages
Ease of treatment and outpatient setting in many countries
35–50% goiter reduction within 1–2 years
High degree of patient satisfaction
Few acute adverse events
Treats coexisting hyperthyroidism due to functional autonomy
Improves inspiratory capacity
Can be repeated, if necessary
No hindrance for later thyroid surgery
Minute risk of ^{131}I-induced malignancy
Disadvantages
No or insufficient effect with a low thyroid ^{131}I uptake
Less efficient in very large goiters
Safety regulations must be followed
Pregnancy not allowed for 4–6 months
Goiter reduction is slow (months)
Risk of hypothyroidism (10–50%)
1–5% risk of Graves'-like condition
Lifelong control of thyroid function
No histological specimen obtained

thyroiditis is occasionally seen a few days after the ^{131}I therapy, resulting in a painful goiter swelling. These symptoms disappear spontaneously and can usually be managed by nonsteroidal anti-inflammatory drugs.

Mild biochemical hyperthyroidism, due to the secretion of stored thyroid hormones, is often seen after ^{131}I therapy, but this rarely gives rise to significant symptoms. If the condition does not resolve within a few weeks and even aggravates, this should raise suspicion of a radiation-induced Graves'-like condition (which may include Graves' orbitopathy). The condition can be verified by the appearance of TSH-receptor antibodies in serum. This complication, seen in a few per cent of patients (Nygaard et al. 1999b), often resolves within 6–18 months, and it should be managed as any patient with *de novo* Graves' disease.

Permanent thyroid failure is the most prominent long-term side effect of ^{131}I therapy, and it is important to inform the patient about this risk. The incidence of hypothyroidism is approximately 20% within 5 years, and the risk is to some extent correlated to the goiter shrinkage (Bonnema and Hegedüs 2012). The risk of thyroid failure is, however, much lower than after ^{131}I therapy of Graves' disease (Nygaard et al. 1993, 1995), most probably because dormant paranodular thyroid tissue, receiving much less irradiation, regains function along with the hyperactive nodular tissue being eradicated. Hypothyroidism may occur many years after ^{131}I therapy, and the condition usually evolves slowly. Lifelong thyroid function testing is therefore of crucial importance. This should be done every 3 months within the first year after ^{131}I therapy and thereafter annually.

Sialoadenitis, acute as well as chronic, is a well described adverse effect of ^{131}I therapy, but mainly associated with the much larger amounts of radioactivity used in thyroid cancer patients. This complication is only rarely encountered after treating benign thyroid diseases, including nontoxic goiter (Bonnema and Hegedüs 2012).

Concerns with ^{131}I Therapy

Carcinogenicity

The potential risk of malignancy induced by thyroid and whole-body irradiation is an issue that may lead to abstaining from this treatment. Overwhelmingly, the data stem from observational studies of ^{131}I therapy of Graves' disease, with very little data on the risk of malignancy, thyroid or other, following therapy of nontoxic goiter. There are a few reports of increased mortality restricted to the first year after ^{131}I therapy for hyperthyroid disorders, unless hypothyroidism resulted from the treatment (Franklyn et al. 1998, 2005). Other studies have shown persistently higher death rates after ^{131}I therapy (Metso et al. 2007, 2008). Despite many years of experience with this treatment modality, it remains difficult to conclude whether any observed risk of cancer is due to the ^{131}I therapy, the thyroid disease *per se,* or to the influence of other factors such as smoking. In favor of a pronounced impact from confounding factors, population-based register data have shown significantly increased morbidity before as well as after the diagnosis of hyperthyroidism, with an excess mortality of around 30% (Brandt et al. 2012, 2013a). Furthermore, epidemiological studies have found that the association between hyperthyroidism and mortality is mainly due to cardiovascular diseases in case of Graves' disease, as opposed to cancer deaths among patients with toxic nodular goiter (Brandt et al. 2013b).

For several reasons, data obtained from Graves' patients treated with ^{131}I cannot uncritically be extrapolated to patients with nontoxic goiter. The amount of ^{131}I activity usually differs between Graves' disease and nontoxic goiter, due to differences in thyroid size, ^{131}I biokinetics, and thyroid function at the time of treatment. Nevertheless, the excess risk of developing cancer due to ^{131}I therapy for benign thyroid diseases, including nontoxic goiter, is most likely very low or none. Finally, the possibility exists that an undetected thyroid microcarcinoma is embedded in the goiter at the time of the ^{131}I therapy, thus being a coexisting phenomenon.

Teratogenicity

Pregnancy is an absolute contraindication for ^{131}I therapy, and conception should be postponed until at least 4 months after the therapy (Bonnema and Hegedüs 2012; Bahn et al. 2011). This applies also to men given ^{131}I therapy. ^{131}I administration at 10–12 weeks of gestation, or later during pregnancy, most likely results in fetal thyroid ablation, but apart from loss of the thyroid function, a normal pregnancy outcome has been described (Berg et al. 2008).

^{131}I therapy potentially affects the gonadal function, the ovaries marginally more than the testes (Hyer et al. 2002). In women treated with ^{131}I for thyroid cancer

(Sawka et al. 2008a), menopause occurs at a slightly younger age than in those untreated, but ^{131}I therapy is not in general associated with a significantly increased risk of long-term infertility, miscarriage, or congenital malformations (Sawka et al. 2008a; Garsi et al. 2008). ^{131}I therapy, given for hyperthyroidism in men, is associated with marginal and reversible decreases in testosterone levels (Ceccarelli et al. 2006). Importantly, in men treated for thyroid cancer, the rates of infertility and congenital malformations in the offspring do not seem to be elevated (Sawka et al. 2008b).

Considering that most studies are based on thyroid cancer patients treated with large amounts of ^{131}I, these results are reassuring in the context of treatment of benign thyroid disorders. Thus, ^{131}I therapy, from this perspective, may be given also to younger adult patients with nontoxic goiter.

^{131}I Therapy Augmented by Recombinant Human TSH (rhTSH) Stimulation

A major limitation of ^{131}I therapy is that the relative goiter reduction correlates inversely with the baseline goiter size, despite the administration of equivalent doses of ^{131}I (Bonnema et al. 1999). A low thyroid ^{131}I uptake (RAIU), inversely correlated to the dietary iodine content, is another hindrance for this therapy in many patients with nontoxic goiter. Thus, ^{131}I therapy for nontoxic goiter is rarely feasible if the thyroid RAIU is low (24 h thyroid ^{131}I uptake $<$15–20%), or if symptomatic and dominant nodules in a multinodular goiter are scintigraphically cold.

The problem with a low thyroid RAIU, seen in some patients with nontoxic goiter, may be overcome by injection of rhTSH 24–48 h before ^{131}I therapy. Stimulation with this compound increases the thyroid 24 h RAIU by 100% or more, even in iodine-loaded subjects (Nielsen et al. 2005; Braverman et al. 2008; Fast et al. 2009a; Bogazzi et al. 2010). This effect depends strongly on the baseline thyroid RAIU (Nielsen et al. 2005; Fast et al. 2009b). Thus, the patients with the lowest baseline RAIU are those who achieve the highest increase in the RAIU after rhTSH stimulation. Also, the increase in the thyroid 24 h RAIU by rhTSH stimulation correlates inversely with serum TSH (Fast et al. 2009b). A low or subnormal serum TSH, often seen in nontoxic goiter, reflects that one or more autonomously functioning nodules lead to suppression of the paranodular thyroid tissue. By rhTSH stimulation, these areas of the thyroid are reactivated, resulting in a more homogeneous distribution of ^{131}I, in addition to the increase in the overall thyroid RAIU (Nieuwlaat et al. 2001).

RhTSH is given as a single intramuscular injection 24–48 h before ^{131}I administration. Doses in the range of 0.005–0.9 mg have been used, but 0.1 mg or even less may be optimal, and a larger gain in the thyroid RAIU is not obtained by higher doses of rhTSH (Bonnema and Hegedüs 2012). A "modified-release rhTSH," which has a slightly delayed serum TSH peak after injection, has also been introduced (Graf et al. 2011). However, any clinically relevant difference, as compared to the old formulation of rhTSH, remains to be demonstrated.

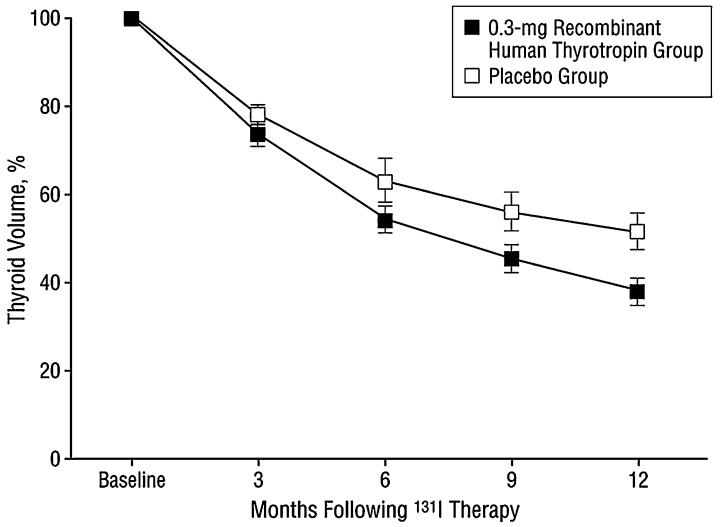

Fig. 9 The mean change (%) in thyroid volume following ^{131}I therapy of patients with goiter, randomized to prestimulation with either 0.3 mg of recombinant human thyrotropin (rhTSH) or placebo, 24 h prior to the therapy. The goiter reduction was significantly more pronounced in the rhTSH group at 6 months and thereafter (From Nielsen et al. 2006, with permission)

Beneficial Effects of rhTSH-Stimulated ^{131}I Therapy

RhTSH-stimulated ^{131}I therapy has been used for benign nontoxic goiter for more than 15 years, and several studies have demonstrated this therapeutic concept to be more effective than conventional ^{131}I therapy, in terms of goiter volume reduction (Bonnema and Hegedüs 2012). The applied thyroid dose has been in the range of 150–300 Gray, much more than the 100 Gray by conventional ^{131}I therapy, and is due to the thyroid RAIU being boosted by rhTSH. In double-blinded trials (Nielsen et al. 2006; Bonnema et al. 2007), comparing rhTSH with placebo stimulation before ^{131}I therapy, the relative goiter reduction was enhanced by up to 56% (Fig. 9). Most benefit, as compared to conventional ^{131}I therapy, was observed in goiters larger than 100 mL, supporting that goiters of this size may be eligible for ^{131}I therapy, if pre-stimulated with rhTSH.

Long-term follow-up studies have demonstrated that the enhanced goiter volume reduction is maintained several years after rhTSH-stimulated ^{131}I therapy, and accompanied by high patient satisfaction, despite a higher rate of permanent hypothyroidism (Fast et al. 2012).

Adverse Effects of rhTSH-Stimulated ^{131}I Therapy

RhTSH-stimulated ^{131}I therapy results in a higher risk of permanent hypothyroidism, which is to be expected considering the enhanced goiter reduction (Bonnema and

Hegedüs 2012). The fact that some patients with hypothyroidism have a reduced quality of life (Watt et al. 2006) and that patient satisfaction is generally high following conventional ^{131}I therapy (Nielsen et al. 2006; Bonnema et al. 2007) may disfavor rhTSH-stimulated ^{131}I therapy. However, long-term follow-up studies have shown that a second ^{131}I treatment and/or thyroid surgery – due to goiter recurrence – is less needed, when compared with conventional ^{131}I therapy (Fast et al. 2012). The above findings support rhTSH-stimulated ^{131}I therapy as a relevant therapeutic alternative in selected patients in whom surgery is unattractive, declined, or contraindicated.

Other issues with rhTSH stimulation need to be addressed. Stimulation by rhTSH of patients with an intact thyroid gland carries a potential risk of thyroid growth (see section below) and excess secretion of thyroid hormones from the thyroid gland (Bonnema and Hegedüs 2012). In the initial studies – using relatively high rhTSH doses – thyroid pain and a sense of neck swelling emerged within the first week after ^{131}I therapy (Bonnema and Hegedüs 2012). A temporary increase in serum levels of thyroid hormones was seen as well, but the magnitude of these inadvertent effects depends on the rhTSH dose administered (Bonnema and Hegedüs 2012). When this is kept in the range of 0.01–0.03 mg, stable thyroid hormone levels are obtained, and the therapy is well tolerated (Nieuwlaat et al. 2003). Patients with subclinical or mild overt toxic nodular goiter are those at highest risk of developing a surge of thyroid hormone release from the gland (Bonnema and Hegedüs 2012). Taken together, and balancing the positive effect on the thyroid RAIU with less desirable effects from the rhTSH stimulation, it seems that the optimal rhTSH dose for augmenting ^{131}I therapy is in the range of 0.03–0.1 mg (Bonnema and Hegedüs 2012).

Theoretically, rhTSH may provoke an autoimmune response by its effect on the thyroid gland. Such a side effect has been described in patients treated for thyroid cancer (Bonnema and Hegedüs 2012) but has only very sparsely been studied in patients with a benign goiter. A temporary rise of anti-TPO may occur, as shown in a small study (Rubio et al. 2005), but the clinical relevance of such a possible impact on the immune system by rhTSH remains to be clarified.

Improved Goiter Reduction or Lower Irradiation?

RhTSH-stimulated ^{131}I therapy, given in order to improve the goiter reduction, can be characterized as a "superiority approach," and was applied in the majority of previous studies (Bonnema and Hegedüs 2012). By another concept, the "equality approach," the amount of radioactivity is reduced equivalently to the increase in the thyroid RAIU (Fig. 10). Hereby, the goiter reduction is comparable to that obtained by conventional ^{131}I therapy (Nieuwlaat et al. 2003). Since the whole-body irradiation is considerably lower (Nieuwlaat et al. 2004) by this approach, some patients, and physicians, may be less reluctant to accept ^{131}I therapy. Moreover, some observations suggest that rhTSH may have a preconditioning effect on the thyroid gland, rendering the thyrocyte more radiosensitive. In a randomized study (Fast et al. 2010a), in which patients with nontoxic nodular goiter were given either conventional ^{131}I therapy, aiming at a thyroid dose of 100 Gray, or rhTSH-stimulated ^{131}I therapy aiming at 50 Gray, the

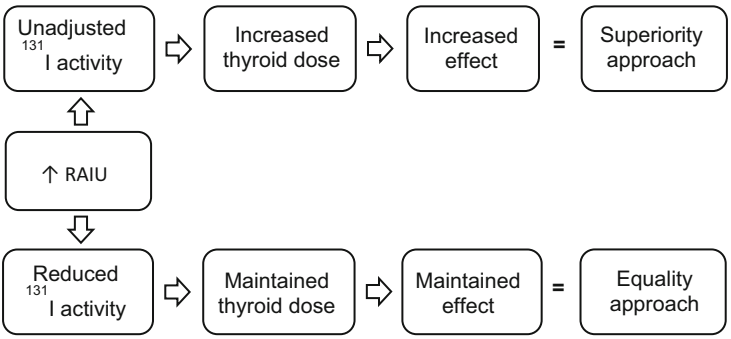

Fig. 10 One injection of recombinant human TSH increases the thyroid ^{131}I uptake (RAIU) and thereby amplifies the effect of ^{131}I therapy. Following a "superiority approach," the purpose is to improve the goiter reduction by the ^{131}I therapy. In contrast, following the "equality approach," the amount of radioactivity is reduced equivalently to the increase in the thyroid RAIU. Hereby, the goiter reduction is comparable to that obtained by conventional ^{131}I therapy without rhTSH stimulation, but the burden of radiation is much lower

goiter volume was equally reduced by 40% after 12 months in the two groups. However, patients receiving rhTSH-stimulated ^{131}I therapy were exposed to 70% less radioactivity than those in the conventionally treated group (Fast et al. 2010a).

Whether a "superiority approach" or an "equality approach" is to be employed depends on a range of factors, each of which has advantages and disadvantages (Bonnema and Hegedüs 2012). Given that rhTSH-stimulated ^{131}I therapy is an off-label treatment and that no consensus exists on patient selection, patient preparation, dose of rhTSH, or amount of radioactivity administered, a pragmatic regimen of rhTSH-stimulated ^{131}I therapy is given in Table 3.

Effect of ^{131}I Therapy on the Adjacent Neck Structures

No study has yet investigated the impact on the esophagus following ^{131}I therapy of goiter, whereas the effects on the respiratory system have been evaluated more thoroughly.

Early Effects

In the acute phase, thyroid swelling, caused by the irradiation, may lead to compression of the upper airways, especially when the goiter is very large and/or encircles the trachea. This problem may theoretically be of highest concern when the ^{131}I therapy is rhTSH pre-stimulated, since rhTSH *per se,* in doses above 0.3 mg, causes a temporary thyroid swelling (Braverman et al. 2008; Fast et al. 2010b). However, in a randomized trial (Bonnema et al. 2008), comparing placebo-stimulated versus rhTSH-stimulated ^{131}I therapy, no further acute compression of the trachea or deterioration of the respiratory function were seen, on average, in either group. Nevertheless, if a large goiter is known to severely compress the

Table 3 A pragmatic regimen for rhTSH-stimulated ^{131}I therapy of benign multinodular goiter in an outpatient setting

Patients particularly suitable for rhTSH-stimulated ^{131}I therapy:
Bilateral multinodular goiter without suspicion of malignancy
24 h thyroid ^{131}I–uptake <15–20%
Goiter volume > 50 mL up to ca. 300 mL. Patients with larger goiters should be encouraged to surgery
Euthyroid (serum TSH may be normal or below normal range)
Inform about radiation safety regulations. No particular precautions are needed in relation to the rhTSH prestimulation
Inform of the lifelong risk of permanent hypothyroidism and that this is higher with rhTSH prestimulation
Instruct the patient to contact the treating physician if respiratory distress should emerge following ^{131}I therapy. In case of known tracheal compression (lumen ca. 50 mm^2 or less, assessed by CT or MRI), consider prophylactic glucocorticoids to avoid thyroid swelling
0.1 mg rhTSH is injected intramuscularly, once, 24 h before ^{131}I therapy. Inform about low risk of thyroid swelling induced by rhTSH
The following day, administer the maximum amount of ^{131}I allowed in an outpatient setting. This maximum activity may vary between countries; is often around 600 MBq
Following rhTSH-stimulated ^{131}I therapy, monitor clinically and biochemically, as after conventional ^{131}I therapy without rhTSH stimulation

Note: This solution does not apply to countries, where ^{131}I therapy must be given inhospital. It should be emphasized that rhTSH-stimulated ^{131}I therapy is an off-label treatment

trachea, as detected by CT or MRI, glucocorticoids (25 mg prednisone daily for 14 days, starting on the day of ^{131}I administration) may be considered to prevent thyroid swelling following ^{131}I therapy.

Late Effects

In some earlier studies, the smallest cross-sectional area of the trachea increased by 18–36%, and the tracheal deviation was reduced by 20%, 1 year after high-dose ^{131}I therapy of patients with very large goiters (Bonnema et al. 1999; Huysmans et al. 1994). In a subsequent study (Bonnema et al. 2008), comparing placebo-stimulated versus rhTSH-stimulated ^{131}I therapy, the tracheal lumen increased by 31% at 12 months, in the rhTSH-stimulated group, while no significant changes were seen in the placebo-stimulated group. In parallel, the inspiratory capacity increased by 25% in the rhTSH group, and by only 9% in the placebo group. Thus, the enhanced goiter reduction following ^{131}I therapy with rhTSH stimulation, as compared to ^{131}I therapy alone, leads to an improved decompression of the trachea and improved respiratory function (Bonnema et al. 2008).

Other Noninvasive Interventional Treatments

Percutaneous ethanol injection therapy, laser and radiofrequency ablation, and more recently high-intensity focused ultrasound can be used for treatment of

thyroid nodules (Papini et al. 2014). Using these modalities, the volume of the thyroid nodule can be reduced by 30–60% within 3–6 months. Most studies of either of these techniques have been performed in patients suffering from a solitary solid thyroid lesion, but the treatment concepts might easily be extrapolated to the patient with a dominant nodule, solid or cystic, embedded in a multinodular goiter. However, it should be realized that smaller nodules left untreated in a multinodular goiter may have growth potential, which in turn may result in relapse of goiter symptoms.

In comparison with surgery, the major advantages of all these techniques are their minimally invasive nature and that they do not require general anesthesia. In contrast to ^{131}I therapy, noninvasive interventional therapy normally targets only the symptomatic nodule and does not result in any major damage to the remaining part of the thyroid gland. Also, there is no exposure to ionizing radiation. However, ethanol injection therapy, formerly much used, may result in leakage of the ethanol into the surrounding tissue, with necrosis and scarring of healthy tissue (including extrathyroidal structures) as a result (Gharib et al. 2013).

Far from all nodules are suitable for noninvasive interventional therapy, and selection for the various modalities must be based on nodule size and structure, location in the thyroid gland, result of the fine-needle aspiration biopsy, compliance of the patient, and obviously the local expertise. Adverse effects include pain, risk of recurrent laryngeal nerve damage, and the possibility of extrathyroidal fibrosis complicating subsequent surgery, if this is deemed necessary due to lack of treatment response after noninvasive interventional therapy (Papini et al. 2014).

Summary

Nontoxic goiter, being related to iodine deficiency, often harbors one or more nodules. The risk of thyroid malignancy, compression of vital structures in the neck, and gradual development of hyperthyroidism due to nodular autonomy are central issues related to goiter. Besides a clinical examination, the diagnostic work-up includes thyroid function tests, ultrasonography, FNAB of suspicious nodules, thyroid scintigraphy if serum TSH is subnormal, and CT or MRI in case of a dominant intrathoracic extension of the goiter. The optimal treatment strategy depends on a range of factors, here among goiter size, the degree of symptoms, the age and comorbidity of the patient, and the wish to maintain normal thyroid function. When treatment is warranted, the choice usually stands between thyroid surgery and ^{131}I therapy, but noninvasive interventional treatment may be an option in selected patients with a solitary nodule. The major advantages of surgery are that it rapidly and completely relieves the patient from the goiter and ensures histological specimens. A low thyroid ^{131}I uptake is a hindrance for ^{131}I therapy efficacy, but this may be overcome by use of rhTSH pre-stimulation.

References

Abdel Rahim AA, Ahmed ME, Hassan MA. Respiratory complications after thyroidectomy and the need for tracheostomy in patients with a large goitre. Br J Surg. 1999;86:88–90.

Abdul-Sater L, Henry M, Majdan A, et al. What are thyroidectomy patients really concerned about? Otolaryngol Head Neck Surg. 2011;144:685–90.

Abrahamsen B, Jorgensen HL, Laulund AS, et al. Low serum thyrotropin level and duration of suppression as a predictor of major osteoporotic fractures-the OPENTHYRO register cohort. J Bone Miner Res. 2014;29:2040–50.

Aghini-Lombardi F, Antonangeli L, Martino E, et al. The spectrum of thyroid disorders in an iodine-deficient community: the Pescopagano survey. J Clin Endocrinol Metab. 1999;84:561–6.

al Suliman NN, Ryttov NF, Qvist N, et al. Experience in a specialist thyroid surgery unit: a demographic study, surgical complications, and outcome. Eur J Surg. 1997;163:13–20.

Albareda M, Viguera J, Santiveri C, et al. Upper airway obstruction in patients with endothoracic goiter enlargement: no relationship between flow-volume loops and radiological tests. Eur J Endocrinol. 2010;163:665–9.

Bahn RS, Burch HB, Cooper DS, et al. Hyperthyroidism and other causes of thyrotoxicosis: management guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists. Thyroid. 2011;21:593–646.

Baloch ZW, LiVolsi VA. Current role and value of fine-needle aspiration in nodular goitre. Best Pract Res Clin Endocrinol Metab. 2014;28:531–44.

Berg G, Jacobsson L, Nystrom E, et al. Consequences of inadvertent radioiodine treatment of Graves' disease and thyroid cancer in undiagnosed pregnancy. Can we rely on routine pregnancy testing? Acta Oncol. 2008;47:145–9.

Berghout A, Wiersinga WM, Drexhage HA, et al. Comparison of placebo with L-thyroxine alone or with carbimazole for treatment of sporadic non-toxic goitre. Lancet. 1990;336:193–7.

Bogazzi F, Tomisti L, Ceccarelli C, et al. Recombinant human TSH as an adjuvant to radioiodine for the treatment of type 1 amiodarone-induced thyrotoxicosis: a cautionary note. Clin Endocrinol. 2010;72:133–4.

Bone SL, Vertigan AE, Eisenberg RL. Auditory-perceptual voice characteristics in pre-operative patients undergoing thyroid or parathyroid surgery. Folia Phoniatr Logop. 2012;64:87–93.

Bonnema SJ. Upper airway obstruction due to goiter: an overlooked problem! J Postgrad Med. 2008;54:82–3.

Bonnema SJ, Hegedüs L. Radioiodine therapy in benign thyroid diseases: effects, side effects, and factors affecting therapeutic outcome. Endocr Rev. 2012;33:920–80.

Bonnema SJ, Bertelsen H, Mortensen J, et al. The feasibility of high dose iodine 131 treatment as an alternative to surgery in patients with a very large goiter: effect on thyroid function and size and pulmonary function. J Clin Endocrinol Metab. 1999;84:3636–41.

Bonnema SJ, Bennedbæk FN, Wiersinga WM, et al. Management of the nontoxic multinodular goitre: a European questionnaire study. Clin Endocrinol. 2000;53:5–12.

Bonnema SJ, Andersen PB, Knudsen DU, et al. MR imaging of large multinodular goiters: Observer agreement on volume versus observer disagreement on dimensions of the involved trachea. AJR Am J Roentgenol. 2002a;179:259–66.

Bonnema SJ, Knudsen DU, Bertelsen H, et al. Does radioiodine therapy have an equal effect on substernal and cervical goiter volumes? Evaluation by magnetic resonance imaging. Thyroid. 2002b;12:313–7.

Bonnema SJ, Nielsen VE, Hegedüs L. Long-term effects of radioiodine on thyroid function, size and patient satisfaction in non-toxic diffuse goitre. Eur J Endocrinol. 2004;150:439–45.

Bonnema SJ, Nielsen VE, Boel-Jørgensen H, et al. Improvement of goiter volume reduction after 0.3 mg recombinant human thyrotropin-stimulated radioiodine therapy in patients with a very large goiter: a double-blinded, randomized trial. J Clin Endocrinol Metab. 2007;92:3424–8.

Bonnema SJ, Nielsen VE, Boel-Jørgensen H, et al. Recombinant human thyrotropin-stimulated radioiodine therapy of large nodular goiters facilitates tracheal decompression and improves inspiration. J Clin Endocrinol Metab. 2008;93:3981–4.

Brandt F, Almind D, Christensen K, et al. Excess mortality in hyperthyroidism: the influence of preexisting comorbidity and genetic confounding: a danish nationwide register-based cohort study of twins and singletons. J Clin Endocrinol Metab. 2012;97:4123–9.

Brandt F, Thvilum M, Almind D, et al. Morbidity before and after the diagnosis of hyperthyroidism: a nationwide register-based study. PLoS One. 2013a;8:e66711.

Brandt F, Thvilum M, Almind D, et al. Graves' disease and toxic nodular goiter are both associated with increased mortality but differ with respect to the cause of death: a Danish population-based register study. Thyroid. 2013b;23:408–13.

Braverman L, Kloos RT, Law Jr B, et al. Evaluation of various doses of recombinant human thyrotropin in patients with multinodular goiters. Endocr Pract. 2008;14:832–9.

Brito JP, Gionfriddo MR, Al NA, et al. The accuracy of thyroid nodule ultrasound to predict thyroid cancer: systematic review and meta-analysis. J Clin Endocrinol Metab. 2014;99:1253–63.

Brix TH, Kyvik KO, Hegedüs L. Major role of genes in the etiology of simple goiter in females: a population-based twin study. J Clin Endocrinol Metab. 1999;84:3071–5.

Brix TH, Hansen PS, Kyvik KO, et al. Cigarette smoking and risk of clinically overt thyroid disease: a population-based twin case-control study. Arch Intern Med. 2000;160:661–6.

Burikhanov RB, Matsuzaki S. Excess iodine induces apoptosis in the thyroid of goitrogen-pretreated rats in vivo. Thyroid. 2000;10:123–9.

Campanella P, Ianni F, Rota CA, et al. Quantification of cancer risk of each clinical and ultrasonographic suspicious feature of thyroid nodules: a systematic review and meta-analysis. Eur J Endocrinol. 2014;170:R203–11.

Carle A, Krejbjerg A, Laurberg P. Epidemiology of nodular goitre. Influence of iodine intake. Best Pract Res Clin Endocrinol Metab. 2014;28:465–79.

Ceccarelli C, Canale D, Battisti P, et al. Testicular function after 131I therapy for hyperthyroidism. Clin Endocrinol. 2006;65:446–52.

Choi SH, Kim EK, Kwak JY, et al. Interobserver and intraobserver variations in ultrasound assessment of thyroid nodules. Thyroid. 2010;20:167–72.

Cramon P, Bonnema SJ, Bjorner JB, et al. Quality of life in patients with benign nontoxic goiter: impact of disease and treatment response, and comparison with the general population. Thyroid. 2015;25:284–91.

Dralle H, Machens A, Thanh PN. Minimally invasive compared with conventional thyroidectomy for nodular goitre. Best Pract Res Clin Endocrinol Metab. 2014;28:589–99.

Eng PH, Cardona GR, Fang SL, et al. Escape from the acute Wolff-Chaikoff effect is associated with a decrease in thyroid sodium/iodide symporter messenger ribonucleic acid and protein. Endocrinology. 1999;140:3404–10.

Eszlinger M, Hegedus L, Paschke R. Ruling in or ruling out thyroid malignancy by molecular diagnostics of thyroid nodules. Best Pract Res Clin Endocrinol Metab. 2014;28:545–57.

Fast S, Bonnema SJ, Hegedüs L. The majority of Danish nontoxic goitre patients are ineligible for Levothyroxine suppressive therapy. Clin Endocrinol. 2008;69:653–8.

Fast S, Nielsen VE, Bonnema SJ, et al. Time to reconsider nonsurgical therapy of benign non-toxic multinodular goitre: focus on recombinant human TSH augmented radioiodine therapy. Eur J Endocrinol. 2009a;160:517–28.

Fast S, Nielsen VE, Grupe P, et al. Optimizing 131I uptake after rhTSH stimulation in patients with nontoxic multinodular goiter: evidence from a prospective, randomized, double-blind study. J Nucl Med. 2009b;50:732–7.

Fast S, Hegedüs L, Grupe P, et al. Recombinant human thyrotropin-stimulated radioiodine therapy of nodular goiter allows major reduction of the radiation burden with retained efficacy. J Clin Endocrinol Metab. 2010a;95:3719–25.

Fast S, Nielsen VE, Bonnema SJ, et al. Dose-dependent acute effects of recombinant human TSH (rhTSH) on thyroid size and function: comparison of 0.1, 0.3 and 0.9 mg of rhTSH. Clin Endocrinol. 2010b;72:411–6.

Fast S, Nielsen VE, Grupe P, et al. Prestimulation with recombinant human thyrotropin (rhTSH) improves the long-term outcome of radioiodine therapy for multinodular nontoxic goiter. J Clin Endocrinol Metab. 2012;97:2653–60.

Fiore E, Tonacchera M, Vitti P. Influence of iodization programmes on the epidemiology of nodular goitre. Best Pract Res Clin Endocrinol Metab. 2014;28:577–88.

Fiorentino E, Cipolla C, Graceffa G, et al. Local neck symptoms before and after thyroidectomy: a possible correlation with reflux laryngopharyngitis. Eur Arch Otorhinolaryngol. 2011;268:715–20.

Franklyn JA, Maisonneuve P, Sheppard MC, et al. Mortality after the treatment of hyperthyroidism with radioactive iodine. N Engl J Med. 1998;338:712–8.

Franklyn JA, Sheppard MC, Maisonneuve P. Thyroid function and mortality in patients treated for hyperthyroidism. JAMA. 2005;294:71–80.

Garsi JP, Schlumberger M, Rubino C, et al. Therapeutic administration of 131I for differentiated thyroid cancer: radiation dose to ovaries and outcome of pregnancies. J Nucl Med. 2008;49:845–52.

Gharib H, Hegedus L, Pacella CM, et al. Clinical review: nonsurgical, image-guided, minimally invasive therapy for thyroid nodules. J Clin Endocrinol Metab. 2013;98:3949–57.

Gharib H, Papini E, Garber JR, et al. American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi Medical Guidelines for Clinical Practice for the Diagnosis and Management of Thyroid Nodules – 2016 Update. Endocr Pract. 2016;22:622–39.

Giovanella L, Ceriani L, Treglia G. Role of isotope scan, including positron emission tomography/computed tomography, in nodular goitre. Best Pract Res Clin Endocrinol Metab. 2014;28:507–18.

Glinoer D, Verelst J, Ham HR. Abnormalities of esophageal transit in patients with sporadic nontoxic goitre. Eur J Nucl Med. 1987;13:239–43.

Graf H, Fast S, Pacini F, et al. Modified-release recombinant human TSH (MRrhTSH) augments the effect of ^{131}I therapy in benign multinodular goiter. Results from a multicenter international, randomized, placebo-controlled study. J Clin Endocrinol Metab. 2011;96:1368–76.

Grussendorf M, Reiners C, Paschke R, et al. Reduction of thyroid nodule volume by levothyroxine and iodine alone and in combination: a randomized, placebo-controlled trial. J Clin Endocrinol Metab. 2011;96:2786–95.

Hamdan AL, Dowli A, Jabbour J, et al. Phonatory symptoms and impact on quality of life in female patients with goiter. Ear Nose Throat J. 2016;95:E5–E10.

Hansen PS, Brix TH, Sorensen TI, et al. Major genetic influence on the regulation of the pituitary-thyroid axis: a study of healthy danish twins. J Clin Endocrinol Metab. 2004;89:1181–7.

Hasselgren M, Hegedus L, Godballe C, et al. Benefit of measuring basal serum calcitonin to detect medullary thyroid carcinoma in a Danish population with a high prevalence of thyroid nodules. Head Neck. 2010;32:612–8.

Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2016;26:1–133.

Haymart MR, Repplinger DJ, Leverson GE, et al. Higher serum thyroid stimulating hormone level in thyroid nodule patients is associated with greater risks of differentiated thyroid cancer and advanced tumor stage. J Clin Endocrinol Metab. 2008;93:809–14.

Hegedüs L. Thyroid size determined by ultrasound. Influence of physiological factors and non-thyroidal disease (thesis). Dan Med Bull. 1990;37:249–63.

Hegedüs L. Thyroid ultrasound. Endocrinol Metab Clin N Am. 2001;30:339–60.

Hegedüs L, Bonnema SJ. Approach to management of the patient with primary or secondary intrathoracic goiter. J Clin Endocrinol Metab. 2010;95:5155–62.

Hegedüs L, Rasmussen N, Ravn V, et al. Independent effects of liver disease and chronic alcoholism on thyroid function and size: the possibility of a toxic effect of alcohol on the thyroid gland. Metabolism. 1988;37:229–33.
Hegedüs L, Nygaard B, Hansen JM. Is routine thyroxine treatment to hinder postoperative recurrence of nontoxic goiter justified? J Clin Endocrinol Metab. 1999;84:756–60.
Hegedüs L, Bonnema SJ, Bennedbæk FN. Management of simple nodular goiter: current status and future perspectives. Endocr Rev. 2003;24:102–32.
Hegedüs L, Brix TH, Paschke R. Etiology of simple goiter. Thyroid. 2009;19:209–11.
Horvath E, Majlis S, Rossi R, et al. An ultrasonogram reporting system for thyroid nodules stratifying cancer risk for clinical management. J Clin Endocrinol Metab. 2009;94:1748–51.
Huysmans DA, Hermus AR, Corstens FH, et al. Large, compressive goiters treated with radioiodine. Ann Intern Med. 1994;121:757–62.
Hyer S, Vini L, O'Connell M, et al. Testicular dose and fertility in men following I(131) therapy for thyroid cancer. Clin Endocrinol. 2002;56:755–8.
Jarhult J, Vedad R. A conservative approach is reasonable in patients with non-toxic goitre: results from an observational study during 30 years. Eur Thyroid J. 2014;3:240–4.
Jarløv AE, Hegedüs L, Gjørup T, et al. Observer variation in the clinical assessment of the thyroid gland. J Intern Med. 1991;229:159–61.
Jauregui R, Lilker ES, Bayley A. Upper airway obstruction in euthyroid goiter. JAMA. 1977;238:2163–6.
Jorgensen F, Hesse B, Gronbaek P, et al. Abnormal oesophageal function in patients with non-toxic goiter or enlarged left atrium, demonstrated by radionuclide transit measurements. Scand J Gastroenterol. 1989;24:1186–92.
Kahaly G, Dienes HP, Beyer J, et al. Randomized, double blind, placebo-controlled trial of low dose iodide in endemic goiter. J Clin Endocrinol Metab. 1997;82:4049–53.
Knudsen N, Brix TH. Genetic and non-iodine-related factors in the aetiology of nodular goitre. Best Pract Res Clin Endocrinol Metab. 2014;28:495–506.
Knudsen N, Bülow I, Jørgensen T, et al. Goitre prevalence and thyroid abnormalities at ultrasonography: a comparative epidemiological study in two regions with slightly different iodine status. Clin Endocrinol. 2000;53:479–85.
Knudsen N, Bülow I, Laurberg P, et al. Alcohol consumption is associated with reduced prevalence of goitre and solitary thyroid nodules. Clin Endocrinol. 2001;55:41–6.
Knudsen N, Bulow I, Laurberg P, et al. Low goitre prevalence among users of oral contraceptives in a population sample of 3712 women. Clin Endocrinol. 2002;57:71–6.
Kornelius E, Chiou JY, Yang YS, et al. Iodinated contrast media-induced thyroid dysfunction in euthyroid nodular goiter patients. Thyroid. 2016;26:1030–8.
Krejbjerg A, Bjergved L, Pedersen IB, et al. Thyroid nodules in an 11-year DanThyr follow-up study. J Clin Endocrinol Metab. 2014;99:4749–57.
Lang BH, Chu KK, Tsang RK, et al. Evaluating the incidence, clinical significance and predictors for vocal cord palsy and incidental laryngopharyngeal conditions before elective thyroidectomy: is there a case for routine laryngoscopic examination? World J Surg. 2014;38:385–91.
Laulund AS, Nybo M, Brix TH, et al. Duration of thyroid dysfunction correlates with all-cause mortality. the OPENTHYRO Register Cohort. PLoS One. 2014;9:e110437.
Laurberg P, Jorgensen T, Perrild H, et al. The Danish investigation on iodine intake and thyroid disease, DanThyr: status and perspectives. Eur J Endocrinol. 2006;155:219–28.
Leboulleux S, Tuttle RM, Pacini F, et al. Papillary thyroid microcarcinoma: time to shift from surgery to active surveillance? Lancet Diabetes Endocrinol. 2016;4:933–42.
Lee S, Skelton TS, Zheng F, et al. The biopsy-proven benign thyroid nodule: is long-term follow-up necessary? J Am Coll Surg. 2013;217:81–8.
Melissant CF, Smith SJ, Perlberger R, et al. Lung function, CT-scan and X-ray in upper airway obstruction due to thyroid goitre. Eur Respir J. 1994;7:1782–7.

Menon SK, Jagtap VS, Sarathi V, et al. Prevalence of upper airway obstruction in patients with apparently asymptomatic euthyroid multi nodular goitre. Indian J Endocrinol Metab. 2011;15: S127–31.

Metso S, Jaatinen P, Huhtala H, et al. Increased cardiovascular and cancer mortality after radioiodine treatment for hyperthyroidism. J Clin Endocrinol Metab. 2007;92:2190–6.

Metso S, Auvinen A, Salmi J, et al. Increased long-term cardiovascular morbidity among patients treated with radioactive iodine for hyperthyroidism. Clin Endocrinol. 2008;68:450–7.

Miller MR, Pincock AC, Oates GD, et al. Upper airway obstruction due to goitre: detection, prevalence and results of surgical management. Q J Med. 1990;74:177–88.

Na DG, Baek JH, Sung JY, et al. Thyroid imaging reporting and data system risk stratification of thyroid nodules: categorization based on solidity and echogenicity. Thyroid. 2016;26:562–72.

Netterville JL, Coleman SC, Smith JC, et al. Management of substernal goiter. Laryngoscope. 1998;108:1611–7.

Nielsen VE, Bonnema SJ, Boel-Jørgensen H, et al. Recombinant human thyrotropin markedly changes the 131I kinetics during 131I therapy of patients with nodular goiter: an evaluation by a randomized double-blinded trial. J Clin Endocrinol Metab. 2005;90:79–83.

Nielsen VE, Bonnema SJ, Boel-Jørgensen H, et al. Stimulation with 0.3-mg recombinant human thyrotropin prior to iodine 131 therapy to improve the size reduction of benign nontoxic nodular goiter: a prospective randomized double-blind trial. Arch Intern Med. 2006;166:1476–82.

Nieuwlaat WA, Hermus AR, Sivro-Prndelj F, et al. Pretreatment with recombinant human TSH changes the regional distribution of radioiodine on thyroid scintigrams of nodular goiters. J Clin Endocrinol Metab. 2001;86:5330–6.

Nieuwlaat WA, Huysmans DA, Van Den Bosch HC, et al. Pretreatment with a single, low dose of recombinant human thyrotropin allows dose reduction of radioiodine therapy in patients with nodular goiter. J Clin Endocrinol Metab. 2003;88:3121–9.

Nieuwlaat WA, Hermus AR, Ross HA, et al. Dosimetry of radioiodine therapy in patients with nodular goiter after pretreatment with a single, low dose of recombinant human thyroid-stimulating hormone. J Nucl Med. 2004;45:626–33.

Nygaard B, Hegedüs L, Gervil M, et al. Radioiodine treatment of multinodular non-toxic goitre. Br Med J. 1993;307:828–32.

Nygaard B, Hegedüs L, Gervil M, et al. Influence of compensated radioiodine therapy on thyroid volume and incidence of hypothyroidism in Graves' disease. J Intern Med. 1995;238:491–7.

Nygaard B, Hegedüs L, Ulriksen P, et al. Radioiodine therapy for multinodular toxic goiter. Arch Intern Med. 1999a;159:1364–8.

Nygaard B, Metcalfe RA, Phipps J, et al. Graves' disease and thyroid associated ophthalmopathy triggered by 131I treatment of non-toxic goiter. J Endocrinol Investig. 1999b;22:481–5.

Papini E, Guglielmi R, Bianchini A, et al. Risk of malignancy in nonpalpable thyroid nodules: predictive value of ultrasound and color-Doppler features. J Clin Endocrinol Metab. 2002;87:1941–6.

Papini E, Pacella CM, Misischi I, et al. The advent of ultrasound-guided ablation techniques in nodular thyroid disease: towards a patient-tailored approach. Best Pract Res Clin Endocrinol Metab. 2014;28:601–18.

Pappalardo G, Guadalaxara A, Frattaroli FM, et al. Total compared with subtotal thyroidectomy in benign nodular disease: personal series and review of published reports. Eur J Surg. 1998;164:501–6.

Pedersen IB, Knudsen N, Perrild H, et al. TSH-receptor antibody measurement for differentiation of hyperthyroidism into Graves' disease and multinodular toxic goitre: a comparison of two competitive binding assays. Clin Endocrinol. 2001;55:381–90.

Perrild H, Hansen JM, Hegedüs L, et al. Triiodothyronine and thyroxine treatment of diffuse non-toxic goitre evaluated by ultrasonic scanning. Acta Endocrinol. 1982;100:382–7.

Rago T, Vitti P. Diagnostic role of ultrasound and elastosonography in nodular goiter. Best Pract Res Clin Endocrinol Metab. 2014;28:519–29.

Remonti LR, Kramer CK, Leitão CB, et al. Thyroid ultrasound features and risk of carcinoma: a systematic review and meta-analysis of observational studies. Thyroid. 2015;25:538–50.

Rubio IG, Perone BH, Silva MN, et al. Human recombinant TSH preceding a therapeutic dose of radioiodine for multinodular goiters has no significant effect in the surge of TSH-receptor and TPO antibodies. Thyroid. 2005;15:134–9.
Russ G, Bonnema SJ, Erdogan MF et al. European thyroid association guidelines for ultrasound malignancy risk stratification of thyroid nodules in adults: the EU-TIRADS. Eur Thyroid J. 2017 (in press).
Sawka AM, Lakra DC, Lea J, et al. A systematic review examining the effects of therapeutic radioactive iodine on ovarian function and future pregnancy in female thyroid cancer survivors. Clin Endocrinol. 2008a;69:479–90.
Sawka AM, Lea J, Alshehri B, et al. A systematic review of the gonadal effects of therapeutic radioactive iodine in male thyroid cancer survivors. Clin Endocrinol. 2008b;68:610–7.
Scerrino G, Inviati A, Di GS, et al. Esophageal motility changes after thyroidectomy; possible associations with postoperative voice and swallowing disorders: preliminary results. Otolaryngol Head Neck Surg. 2013;148:926–32.
Singh ON, Maraka S, Espinosa DA, et al. Diagnostic accuracy of thyroid nodule growth to predict malignancy in thyroid nodules with benign cytology: systematic review and meta-analysis. Clin Endocrinol. 2016;85:122–31.
Smith TJ, Hegedus L. Graves' disease. N Engl J Med. 2016;375:1552–65.
Soelberg KK, Bonnema SJ, Brix TH, et al. Risk of malignancy in thyroid incidentalomas detected by 18F-fluorodeoxyglucose positron emission tomography: a systematic review. Thyroid. 2012;22:918–25.
Sørensen JR, Hegedus L, Kruse-Andersen S, et al. The impact of goitre and its treatment on the trachea, airflow, oesophagus and swallowing function. A systematic review. Best Pract Res Clin Endocrinol Metab. 2014;28:481–94.
Surks MI, Ortiz E, Daniels GH, et al. Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. JAMA. 2004;291:228–38.
Swan KZ, Nielsen VE, Bibby BM et al. Is the reproducibility of shear wave elastography of thyroid nodules high enough for clinical use? A methodological study. Clin Endocrinol (Oxf). 2016. https://doi.org/10.1111/cen.13295. [Epub ahead of print]
Tan GH, Gharib H. Thyroid incidentalomas: management approaches to nonpalpable nodules discovered incidentally on thyroid imaging. Ann Intern Med. 1997;126:226–31.
Tan GH, Gharib H, Reading CC. Solitary thyroid nodule. Comparison between palpation and ultrasonography. Arch Intern Med. 1995;155:2418–23.
Thusoo TK, Gupta U, Kochhar K, et al. Upper airway obstruction in patients with goiter studies by flow volume loops and effect of thyroidectomy. World J Surg. 2000;24:1570–2.
Torchio R, Gulotta C, Perboni A, et al. Orthopnea and tidal expiratory flow limitation in patients with euthyroid goiter. Chest. 2003;124:133–40.
Vanderpump MP, Tunbridge WM, French JM, et al. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clin Endocrinol. 1995;43:55–68.
Vejbjerg P, Knudsen N, Perrild H, et al. Effect of a mandatory iodization program on thyroid gland volume based on individuals' age, gender, and preceding severity of dietary iodine deficiency: a prospective, population-based study. J Clin Endocrinol Metab. 2007;92:1397–401.
Verloop H, Louwerens M, Schoones JW, et al. Risk of hypothyroidism following hemithyroidectomy: systematic review and meta-analysis of prognostic studies. J Clin Endocrinol Metab. 2012;97:2243–55.
Wallace C, Siminoski K. The Pemberton sign. Ann Intern Med. 1996;125:568–9.
Watt T, Grønvold M, Rasmussen AK, et al. Quality of life in patients with benign thyroid disorders. A review. Eur J Endocrinol. 2006;154:501–10.
Watt T, Cramon P, Frendl DM, et al. Assessing health-related quality of life in patients with benign non-toxic goitre. Best Pract Res Clin Endocrinol Metab. 2014;28:559–75.
Wesche MF, Tiel-Van Buul MM, Lips P, et al. A randomized trial comparing levothyroxine with radioactive iodine in the treatment of sporadic nontoxic goiter. J Clin Endocrinol Metab. 2001;86:998–1005.

Thyroid Nodule

6

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Abstract

Benign thyroid nodules are highly prevalent in iodine-deficient areas. Histologically, benign thyroid nodules are characterized by morphologic criteria as encapsulated lesions (true adenomas) or adenomatous nodules, which lack a capsule. On functional grounds, nodules are classified as either "cold," "normal," or "hot," depending on whether they show decreased, normal, or increased uptake on scintiscan. Approximately 50–85% of all nodules are "cold," up to 40% are scintigraphically indifferent and about 10% are "hot," although the prevalence will vary geographically with the ambient iodine supply and with the clinical setting.

Hot thyroid nodules (autonomously functioning thyroid nodules, AFTNs) are mainly due to mutations which confer a constitutive activation of the cAMP cascade (e.g., TSHR and $G_s\alpha$ mutations) which results in a stimulation of growth and function. In contrast, the molecular etiology of cold thyroid nodules (CTNs) is still largely unknown.

Thyroid autonomy (i.e., AFTNs) is an almost exclusively benign disease, and there is little evidence in the literature to suggest the contrary. In contrast, the differential diagnosis of scintigraphically indifferent and CTNs includes benign follicular adenoma and adenomatous nodules, as well as papillary thyroid carcinoma and its variants and follicular thyroid carcinoma. While fine-needle aspiration cytology is currently the most sensitive and specific tool to select thyroid nodules for surgery after prioritization by assessment of ultrasound malignancy criteria, it is characterized by an inherent limitation, resulting in "indeterminate" cytologies. Molecular tests in the form of "rule out" and "rule in" malignancy tests have been proposed to fill this diagnostic gap.

The most important therapeutic options for hot nodules are radioiodine therapy or surgery and surgery for scintigraphically indifferent and CTNs if symptomatic or associated with increased malignancy risk.

Keywords

Autonomously functioning thyroid nodules · Cold thyroid nodules · Mutations · Ultrasonography · Scintigraphy · Fine-needle aspiration cytology · Molecular diagnostics · Antithyroid drug treatment · Surgery · Radioiodine therapy · Thyroid hormone treatment · Percutaneous interventional therapy

Definition and Clinical Manifestations

Thyroid nodules can be symptomatic or asymptomatic, visible on inspection, or palpable or identified by imaging techniques, such as ultrasound. Benign thyroid nodules are highly prevalent in iodine-deficient areas. Histologically, benign thyroid nodules are characterized by morphologic criteria – according to the World Health Organization (WHO) classification – as encapsulated lesions (true adenomas) or adenomatous nodules, which lack a capsule (Chan et al. 2004). On functional grounds, nodules are classified as either "cold," "normal," or "hot," depending on whether they are hypofunctional, normofunctional, or hyperfunctional, and show decreased, normal, or increased uptake on scintiscan (Fig. 1). Approximately 50–85% of all nodules are "cold," up to 40% are scintigraphically indifferent, and

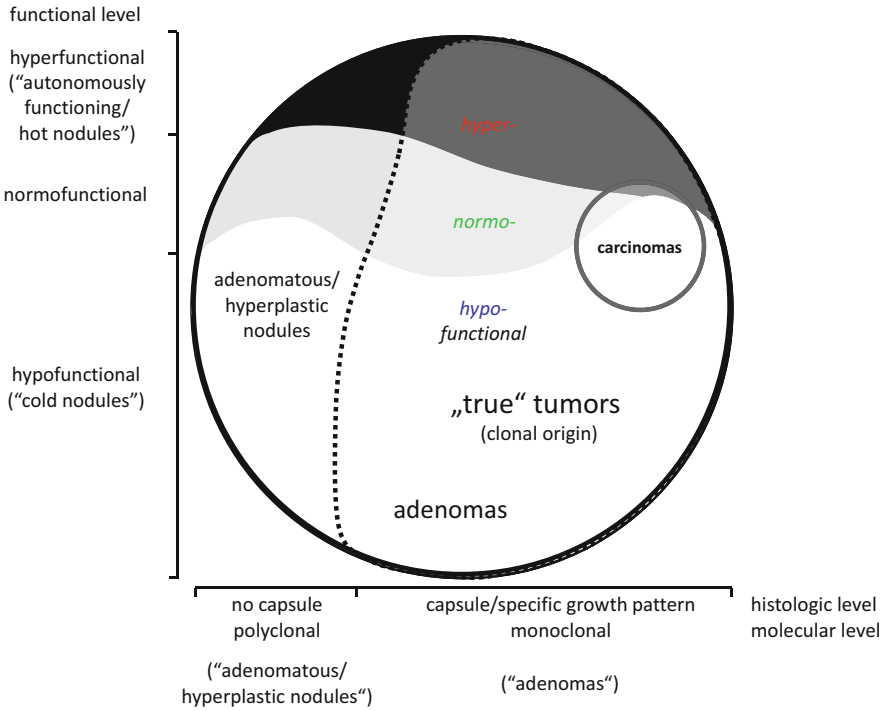

Fig. 1 The classification of thyroid nodules on the basis of functional, histologic, and molecular criteria. On the functional level, thyroid nodules are classified as either "cold," "normal," or "hot," depending on whether they are hypofunctional, normofunctional, or hyperfunctional. On the histological level, thyroid nodules are classified as adenomas in the presence of a capsule and a growth pattern that is different from the surrounding normal parenchyma or as adenomatous/ hyperplastic nodules when lacking a capsule. On the molecular level, monoclonal nodules are considered as true tumors (adenomas/carcinomas), while polyclonal nodules are considered as adenomatous/hyperplastic nodules. Clonality studies imply that the majority of thyroid nodules are "true" thyroid tumors compared to polyclonal hyperplastic nodules. However, there is no clear overlap between histologic and molecular classification

about 10% are "hot" (Belfiore et al. 1992; Knudsen et al. 2000), although the prevalence will vary geographically with the ambient iodine supply and with the clinical setting.

The classification of thyroid nodules on the molecular level, based on results from clonality studies, implies that the majority of thyroid nodules are "true" thyroid tumors (monoclonal) as opposed to polyclonal adenomatous/hyperplastic nodules. Traditionally, only thyroid adenomas are considered true tumors, on the basis of an exclusive histologic definition – the presence of a capsule and a growth pattern that is different from the surrounding normal parenchyma in an otherwise normal thyroid gland. Strict histologic criteria for an adenoma and its differentiation from adenomatous/hyperplastic thyroid nodules (without a capsule) are, however, difficult to obtain in the frequent presence of goiter or thyroiditis. The biological basis for separating adenomatous/hyperplastic thyroid nodules from true tumors should, therefore, also depend on their clonality (Chan et al. 2004). Since many thyroid nodules without a capsule (adenomatous/hyperplastic nodules) are monoclonal, a mixed histologic and molecular definition of true thyroid tumors, as outlined in Fig. 1**,** appears more objective and consistent.

Mutagenesis as the Cause of Thyroid Nodules

Mutations that confer a growth advantage (e.g., TSHR or $G_s\alpha$ protein mutations) very likely initiate focal growth. Hence, autonomously functioning thyroid nodules (AFTNs; "hot" thyroid nodules) are likely to develop from small cell clones, which contain advantageous mutations as shown for the TSHR in "hot" microscopic regions of euthyroid goiters (Krohn et al. 2000).

Based on the identification of somatic mutations and the predominant clonal origin of AFTNs and cold thyroid nodules (CTNs), the following sequence of events could lead to thyroid nodular transformation in three steps (Krohn et al. 2005). First, iodine deficiency, nutritional goitrogens, or autoimmunity cause diffuse thyroid hyperplasia. Then, at this stage of thyroid hyperplasia, increased proliferation together with a possible DNA damage due to H_2O_2 action causes a higher mutation load, i.e., a higher number of cells bearing mutations (Krohn et al. 2007). Some of these spontaneous mutations confer constitutive activation of the cAMP cascade (e.g., TSHR and $G_s\alpha$ mutations) which stimulates growth and function. Finally, in a proliferating thyroid, growth factor expression [e.g., insulin-like growth factor 1 (IGF-1), transforming growth factor-β (TGF-β), or epidermal growth factor (EGF)] is increased. As a result of growth factor co-stimulation, most cells divide and form small clones. After increased growth factor expression ceases, small clones with activating mutations will further proliferate if they can achieve self-stimulation. They could thus form small foci, which could develop into thyroid nodules. This mechanism could explain AFTNs by advantageous mutations that both initiate growth and function of the affected thyroid cells as well as CTNs by mutations that stimulate proliferation only (e.g., *RAS* mutations or other mutations in the RAS/RAF/MEK/ERK/MAP cascade) (Xing 2013).

Autonomously Functioning Thyroid Nodules (Hot Thyroid Nodules)

Somatic point mutations that constitutively activate the TSHR were first identified by Parma and co-workers in AFTNs (Parma et al. 1993). However, in different studies the prevalence of TSHR and $G_s\alpha$ mutations in AFTNs has been reported to vary from 8–82% to 8–75%, respectively (Parma et al. 1993; Fuhrer et al. 1997; Georgopoulos et al. 2003; Gozu et al. 2006). Available studies differ in the extent of mutation detection and the screening methods. A comparison with respect to the obvious differences between the studies has been done elsewhere (Vassart 2004; Krohn and Paschke 2001a). A comprehensive study using the more sensitive denaturing gradient gel electrophoresis (Garcia-Delgado et al. 1998; Trulzsch et al. 1999) revealed a frequency of 57% *TSHR* mutations and 3% $G_s\alpha$ mutations in 75 consecutive AFTNs (Trulzsch et al. 2001). More recently, a recurrent hot-spot mutation (c.1712A>G; p.Gln571Arg) in the enhancer of zeste homolog 1 (*EZH1*) gene, which codes for a catalytic subunit of the polycomb complex, has been identified by a whole-exome sequencing approach (Calebiro et al. 2016). Targeted screening in an independent cohort confirmed that this mutation occurs with high frequency (27%) in AFTNs, strongly associated with the occurrence of TSHR and $G_s\alpha$ protein mutations (Calebiro et al. 2016). Functional studies have revealed that this mutation increases proliferation of thyroid cells (Calebiro et al. 2016). The association between *EZH1* and *TSHR* mutations suggests a two-hit model for the pathogenesis of these tumors, whereby constitutive activation of the cAMP pathway and EZH1 mutations cooperate to induce the hyperproliferation of thyroid cells (Calebiro et al. 2016). These results raise the question of the molecular etiology of *TSHR* and $G_s\alpha$ mutation-negative nodules. A possible answer is given by clonal analysis of these AFTNs which demonstrates a predominant clonal origin of thyroid nodules and implies a neoplastic process driven by genetic alteration.

In addition to the intracellular signaling network that is connected to the TSHR, the extracellular action of different growth factors enhances the complexity of the signal flux into the thyroid cell. Growth factors like IGF-1, EGF, TGF-β, and fibroblast growth factor (FGF) stimulate growth and dedifferentiation of thyroid epithelial cells (Van Sande et al. 1995). Studies focused on insulin and IGF show a permissive effect of insulin and IGF-I on TSH signaling (Dugrillon et al. 1990; Roger et al. 1983) and a cooperative interaction of TSH and insulin/IGF-1 (Eggo et al. 1990). Other studies suggest inactivation of TGF-β signaling in AFTNs due to constitutively activated TSHR (e.g., resulting from TSHR mutations) (Eszlinger et al. 2004). This assumption is supported by the finding of a decreased expression of TGF-β 1 mRNA after TSH stimulation of thyrocytes (Gärtner et al. 1997). Because TGF-β 1 has been shown to inhibit iodine uptake, iodine organification, and thyroglobulin expression (Taton et al. 1993), as well as cell proliferation in different cell culture systems (Depoortere et al. 2000; Grubeck-Loebenstein et al. 1989), these findings suggest that inactivation of TGF-β signaling is a major prerequisite for increased proliferation in AFTNs (Krohn et al. 1999). Signal modulation of the TSHR that would define the etiology of AFTNs and the clinical

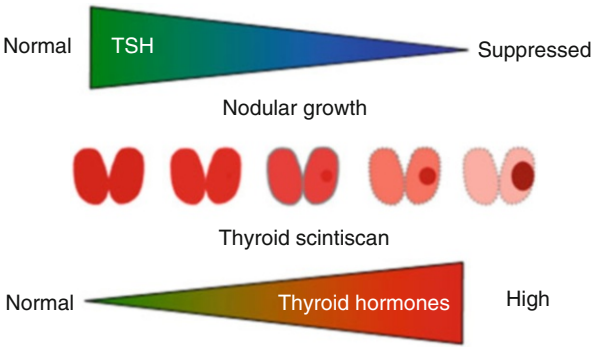

Fig. 2 The slow development of thyroid autonomy over years to decades

phenotype could therefore take part at a number of stages and very likely involves genetic/epigenetic, gender-related, and environmental factors.

In vivo characteristics of AFTNs include increased iodide uptake, thyroid peroxidase messenger RNA (mRNA), and protein content, as well as increased thyroid hormone secretion, which could be further stimulated by TSH (Deleu et al. 2000). Upregulation of the sodium/iodide (Na^+/I^-) symporter (NIS) and pendrin, contributing to the augmented iodine-trapping characteristics of AFTNs, has been reported by other investigators (Mian et al. 2001). Stimulation of growth has been investigated in AFTNs using the proliferation markers Ki67 and PCNA (Krohn et al. 1999; Deleu et al. 2000). However, the increase in the proliferation index in AFTNs was found to be relatively low (two- to threefold) compared to the surrounding normal thyroid tissue. These data are in agreement with the slow course for the evolution of nodule growth and overt hyperfunction in AFTNs (Fig. 2). However, the data is difficult to reconcile with the evolution of macroscopic AFTNs from a single mutated cell (estimated number of 30 cell divisions to yield a nodule volume of ~1 mL) (Krohn and Paschke 2001a; Kimura et al. 2001) and thus more likely reflects the end-stage rather than the initiation of disease.

Cold Thyroid Nodules

The term "cold" indicates reduced uptake on scintiscan. Since a histological diagnosis is typically employed to exclude thyroid malignancy, many investigations of thyroid nodules only refer to the histological diagnosis of thyroid adenoma. This histological entity should not be confounded with the scintigraphically characterized entity "cold nodule," which like AFTNs or "warm nodules" can histologically appear as thyroid adenomas or adenomatous nodules according to the WHO classification (Hedinger 1988). In contrast, focal hyperplasia is not very well explained at the molecular level and has been discussed in detail elsewhere as the cause of thyroid tumors (Derwahl and Studer 2001; Studer et al. 1989). A monoclonal origin has been detected for the majority of cold thyroid nodules, which implies nodular development from a single mutated thyroid cell (Krohn et al. 2001).

With reference to their functional status (i.e., reduced iodine uptake), failure in the iodide transport system or failure of the organic binding of iodide has been detected as functional aberrations of cold thyroid nodules long before the molecular components of the iodine metabolism were known. Subsequently, a decreased expression of the Na^+/I^- symporter (NIS) in thyroid carcinomas and benign cold thyroid nodules was suggested as the molecular mechanism underlying the failure of the iodide transport [reviewed in (Dohan et al. 2001, 2003)]. However, a defective cell membrane targeting of the NIS protein is a more likely molecular mechanism accounting for the failure of the iodine uptake in CTNs (Dohan et al. 2001; Tonacchera et al. 2002). The ultimate cause of this defect is currently unknown.

Compared to iodine transport, the organic binding of iodine is a multistep process with a number of protein components that still awaits final characterization (Dunn and Dunn 2001). mRNA expression of enzymatic components (e.g., thyroperoxidase (TPO) or flavoproteins) and the substrate of iodination [i.e., thyroglobulin (TG)] has been quantified in CTNs without significant differences compared with normal follicular thyroid tissue (Lazar et al. 1999; Caillou et al. 2001). TPO, TG, and thyroid-specific oxidases (THOX) have been successfully screened for molecular defects especially in congenital hypothyroidism (de Vijlder 2003).

Although CTNs could be considered as a form of focal hypothyroidism, somatic mutations in enzymes that catalyze organic binding of iodine would need to exert a growth advantage on the affected cell to cause the development of a thyroid nodule. At least in the case of inactivating mutations in the TPO or THOX genes, growth advantage could result from a lack of enzyme activity which would not only reduce thyroid hormone synthesis but also follicular iodide trapping in organic iodo-compounds. Because these compounds have been shown to inhibit thyroid epithelial cell proliferation (Pisarev et al. 1994), reduced synthesis could have a proliferative effect. Therefore, somatic TPO or THOX mutations could be a molecular cause of CTN. However, mutations in the TPO gene have not been detected (Krohn and Paschke 2001b). A study of 40 cold thyroid adenomas and adenomatous nodules detected *RAS* mutations in only a single case (Krohn et al. 2001). Moreover, in the same set of CTNs, no point mutations in the mutational hot spots of the BRAF gene were detected (Krohn and Paschke 2004). This is in line with the lack of BRAF mutations in benign follicular adenomas in other studies (Xing et al. 2004; Puxeddu et al. 2004). So far, only one study has detected a single BRAF mutation in a set of 51 follicular adenomas (Soares et al. 2003). Moreover, the gene expression for approximately 10,000 full-length genes was compared between CTNs and their corresponding normal surrounding tissue (Eszlinger et al. 2005). Increased expression of histone mRNAs and of cell cycle-associated genes like cyclin D1, cyclin H-/cyclin-dependent kinase (CDK) 7, and cyclin B most likely reflects a molecular setup for an increased proliferation in CTNs (Krohn et al. 2003). In line with the low prevalence of *RAS* mutations in CTNs (Krohn et al. 2001), a reduced expression of RAS-MAPK cascade-associated genes was found which might suggest a minor importance of this signaling cascade. Furthermore, gene rearrangements unique to thyroid adenomas have recently been in focus [reviewed in (Bol et al. 1999)]. These studies have led to the identification of the thyroid adenoma-associated gene (THADA) that encodes a death receptor-interacting protein (Rippe et al. 2003). Although also

reported for thyroid follicular carcinoma (Ward et al. 1998), the finding of loss of heterozygosity (LOH) at the TPO locus is characteristic for some CTNs (about 15%) but rather points to defects in a gene near TPO on the short arm of chromosome 2. Although the frequency of each of these DNA aberrations is rather low, together these chromosomal changes need to be considered in the further elucidation of the molecular etiology of CTNs.

Clinical Aspects

In a patient with thyroid autonomy, clinical features can be attributed to (a) symptoms of hyperthyroidism and (b) the growth of the nodule.

The clinical presentation of hyperthyroidism varies with age, and in the elderly it is often not symptomatic (Hegedus et al. 2003; Chiovato et al. 1997). In a series of 84 French patients with overt hyperthyroidism, atrial fibrillation and anorexia dominated in the older age group ($\geq$70 years), while classical signs of hyperthyroidism, e.g., nervousness, weight loss despite increased appetite, palpitations, tremor, and heat intolerance, were more frequently observed in younger patients ($\leq$50 years) (Trivalle et al. 1996). Alternatively, a patient may present with a lump or disfigurement of the neck, intolerance of a tight collar, or increase in collar size.

Subclinical hyperthyroidism, defined by low or suppressed serum TSH with normal free thyroxine (T_4) and free triiodothyronine (T_3) levels, is more commonly observed in older patients with toxic multinodular goiter (TMNG) (Toft 2001; Berghout et al. 1990). Moreover, the incidental finding of low or suppressed TSH levels on routine investigation for other indications is frequently the first evidence of the presence of thyroid autonomy, particularly in regions with iodine deficiency. Subclinical hyperthyroidism may be associated with atrial fibrillation and a reduced bone density (Toft 2001; Abrahamsen et al. 2014). In addition, an increased overall mortality as well as cardiovascular mortality rate in patients with low serum TSH levels is well documented (Parle et al. 2001; Brandt et al. 2012; Laulund et al. 2014).

Thyroid autonomy is widely viewed as an almost exclusively benign disease, and there is little evidence in the literature to suggest the contrary. Detailed pathological analyses of reported "toxic thyroid cancer" cases (0.7–6% in different series) have shown that, if classified correctly, the cancer (a) usually occurred coincidentally within the same or even in the contralateral thyroid lobe, (b) mostly represented a microcancer ($<$1 cm), and (c) was rarely located within the hot nodule itself (Hegedus et al. 2003; Schroder and Marthaler 1996). To date, seven cases of differentiated, scintigraphically hot, thyroid carcinomas with somatic constitutively activating TSH mutations have been reported (Jaeschke et al. 2010).

Patients with isocaptant or cold thyroid nodules may have no symptoms or may suffer from compressive symptoms, whereas dysphonia, hoarseness, and dysphagia are infrequent late symptoms of thyroid cancer as most thyroid cancers present without symptoms.

Diagnosis

Diagnosis of thyroid autonomy is based on three characteristics:

1. Confirmation of clinically suspected hyperthyroidism by an abnormal thyroid function test. Typically, there is overt thyrotoxicosis (low TSH, high free thyroid hormones). However, dependent on the autonomous cell mass, subclinical hyperthyroidism (TSH low, free T_3 and free T_4 normal) or even euthyroidism may be found.
2. Presence of palpable or ultrasonographic nodule(s).
3. Increased radionuclide uptake in the nodule(s) concomitant with a decreased uptake in the surrounding extranodular thyroid tissue. The most commonly used isotopes are ^{99m}Tc and ^{123}I, both of which are transported into the thyrocytes by the sodium-iodide symporter, but only ^{123}I is organified. Several studies have revealed a comparable diagnostic value of the two isotopes. However, discrepancies due to trapping-only nodules can be found in about 5% of AFTNs, most of which are associated with normal serum TSH (Reschini et al. 2006). The advantages of ^{99m}Tc, which is more frequently used in Europe, are its short half-life (6 h) and low cost (Nygaard et al. 1999; Ryo et al. 1983).

If thyroid autonomy is suspected in a euthyroid patient, a "suppression" scan can be performed after administration of thyroid hormone (i.e., 75 μg/day T_4 for 2 weeks followed by 150 μg/day for 2 weeks), after which uptake in all non-autonomous tissue will be suppressed and thyroid autonomy is unmasked (Bahre et al. 1988).

Long-term studies of patients with AFTNs have shown that the natural course evolves over years or even decades. Sandrock et al. (1993) reported an overall 4.1% annual incidence of hyperthyroidism in a group of 375 untreated euthyroid AFTN patients in Germany who were followed for a mean of 53 months. Hamburger (1980) highlighted the correlation between nodule size and development of hyperthyroidism in a group of 349 patients with AFTNs: 93.5% of patients with overt hyperthyroidism had AFTNs >3 cm in size, and patients with a euthyroid AFTN of >3 cm in size had a 20% risk of developing thyrotoxicosis during a 6-year follow-up period, as opposed to a 2–5% risk in patients with nodules <2.5 cm in size.

Diagnostic Imaging

Although simple and cheap, neck palpation is notoriously imprecise, both with regard to thyroid gland morphology and size determination (Jarlov et al. 1998). For this purpose, several imaging methods are available: ultrasonography, scintigraphy, computed tomography (CT) scan, magnetic resonance (MR) imaging, and, while infrequently indicated, also positron-emission tomography (PET). Of these, ultrasonography is the first priority (Bennedbaek et al. 1999; Bennedbaek and Hegedus 2000; Bonnema et al. 2000, 2002).

Ultrasonography

Ultrasonography, which is often used in Europe (Bennedbaek et al. 1999; Bonnema et al. 2000) and, at least until recently, less so in the United States (Bennedbaek and Hegedus 2000; Bonnema et al. 2002), allows determination of total thyroid volume and individual nodule evaluation for size, echogenicity, internal content, margin, shape, calcifications and regional lymph nodes, regional blood flow, nodule vascularity, and elasticity (Hegedus et al. 2003; Rago et al. 2007; Hegedus 2010). Classification of thyroid nodules according to a thyroid imaging reporting and data system (TI-RADS), including elastography, results in high sensitivity and negative predictive value (NPV) for the diagnosis of thyroid carcinoma. About 50% of the nodules can be classified as benign by ultrasound criteria with a risk of false negatives of only 3% (Russ et al. 2013). Ultrasonography aids in performing accurate biopsies (Hegedus 2001) and is of great help in therapeutic procedures such as cyst punctures and alcohol and laser sclerosis of solid or cystic nodules (Hegedus 2001; Bennedbaek et al. 1997; Dossing et al. 2005; Bennedbaek and Hegedus 2003).

In the differentiation between malignant and benign thyroid lesions, [18F]-2-deoxy-2-fluoro-D-glucose positron-emission tomography (FDG-PET) may be a potentially useful tool in the evaluation of thyroid nodules with indeterminate cytological findings. Since this method has a very low false-negative rate for the detection of malignant lesions, a number of unnecessary thyroidectomies may be avoided (Sebastianes et al. 2007). Noteworthy, focal thyroid lesions, detected incidentally, are seen in around 1.6% of patients undergoing FDG-PET for non-thyroid reasons, and 35% of these cases harbor thyroid cancer (Soelberg et al. 2012). Importantly, these numbers may vary considerably depending on the selection of patients examined.

Scintigraphy

Scintigraphy has little place in the anatomo-topographic evaluation of the nodule, but it aids in verification of the clinical diagnosis and allows determination of the relative mass of hyperfunctioning (hot) (Fig. 3) and nonfunctioning (cold) (Fig. 4) thyroid areas. Nodules with a high uptake by scintigraphy almost never harbor clinically significant malignancy, although exceptions have been reported. ^{99m}Tc used as tracer may result in false-positive uptake in 3–8% of thyroid nodules (Hegedus et al. 2003), while iodine isotopes are devoid of this problem. Nevertheless, comparative studies have been unable to demonstrate any clinically significant differences between the two tracers (Hegedus et al. 2003). Tracers like ^{201}thallium and ^{99m}Tc-methoxy-isobutyl-isonitrile (MIBI) have an increased uptake in differentiated malignant thyroid nodules, but the sensitivity and specificity do not support their general use (Okumura et al. 1999; Demirel et al. 2003). If the use of quantitative ^{99m}Tc-MIBI scintigraphy can better stratify patient, risk for a malignant lesion remains to be confirmed (Campenni et al. 2016). Many disregard thyroid scintigraphy in the initial evaluation of patients with nontoxic nodular goiter (Hegedus et al. 2003; Bonnema et al. 2000, 2002).

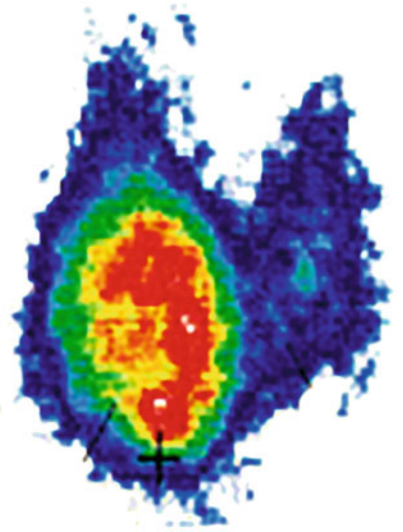

Fig. 3 Scintigraphy of a thyroid with an autonomously functioning thyroid nodule ("hot" nodule) in the right lobe

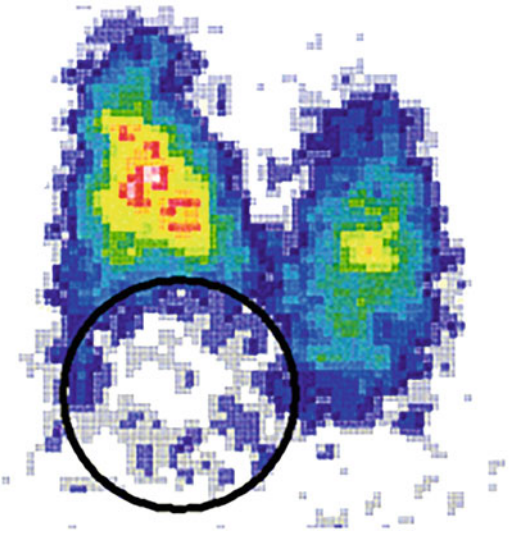

Fig. 4 Scintigraphy of a thyroid with a "cold" thyroid nodule in the right lobe

Nevertheless, more than two thirds of ETA members (Bennedbaek et al. 1999; Bonnema et al. 2000) routinely use scintigraphy, while less than 25% of ATA members prefer such a strategy (Bennedbaek and Hegedus 2000; Bonnema et al. 2002). Indisputable indications for scintigraphy, in the setting of a nodular goiter, are hyperthyroidism (to visualize hot nodules suitable for ^{131}I therapy) or when fine-needle aspiration (FNA) cytology has shown a follicular neoplasm, since hot nodules with great certainty are benign (Hegedus et al. 2003).

Fine-Needle Aspiration (FNA) Cytology

Current guidelines for the differential diagnosis and treatment of thyroid nodules recommend clinical assessment, thyroid-stimulating hormone (TSH), calcitonin (in Europe), and ultrasound for cancer risk stratification to select thyroid nodules with an increased malignancy risk to undergo fine-needle aspiration (FNA) biopsy (Gharib et al. 2016; Haugen et al. 2016). A thorough ultrasound malignancy risk stratification of thyroid nodules by standardized assessment of ultrasound characteristics can classify about 50% of thyroid nodules as definitely or very probably benign [thyroid imaging reporting and data system (TIRADS) 2 and 3)] with a false-negative risk of 0.3% (Russ et al. 2013). The remaining thyroid nodules with an increased malignancy risk (TIRADS 4A, 4B, 5) should undergo subsequent FNA cytology rated according to the Bethesda system for reporting thyroid cytopathology (Bethesda classification) (Cibas and Ali 2009).

The Bethesda classification is based on five cytological diagnostic categories (nondiagnostic, benign, indeterminate, suspicious for malignancy (SFM), and malignant) with a subdivision of the indeterminate category into atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) and follicular neoplasm/suspicious for a follicular neoplasm (FN/SFN). According to the National Cancer Institute Thyroid FNA State of the Science Conference, the cytological categories differ by their implied risk of malignancy (ROM) which has to be verified for each local setting (Cibas and Ali 2009). While nondiagnostic and benign cytologies are characterized by a low ROM (1–4% and 0–3%, respectively), the ROM for SFM and malignant cytologies is high (60–75% and 97–99%, respectively). The two indeterminate categories are characterized by an intermediate ROM of 5–15% (AUS/FLUS) and 15–30% (FN/SFN) (Gharib et al. 2016; Haugen et al. 2016). A recent meta-analysis reports the cytologically benign results for 39–74% of the nodules, and 2–16% of thyroid FNAs are cytologically malignant (Bongiovanni et al. 2012). Indeterminate (AUS/FLUS, FN/SFN) FNA cytology results are due to an intrinsic limitation of thyroid FNA cytology. Vascular or capsular invasion, which are the criteria distinguishing follicular adenomas (FAs) or adenomatous nodules from follicular carcinomas (FTCs) and follicular variant papillary thyroid carcinomas (fvPTC), cannot be detected in cytology samples. Thus, a high proportion of patients with FNAs classified as indeterminate will undergo diagnostic, potentially unnecessary, thyroid surgery with possible complications with the ultimate histology revealing only a 20% malignancy rate (Gharib et al. 2016; Haugen et al. 2016). AUS/FLUS results were observed in 1–27%, FN/SFN in 1–25%, SFM in 1–6%, and nondiagnostic in 2–24% of FNAs (Bongiovanni et al. 2012).

Because of known interobserver variations of AUS/FLUS and FN/SFN diagnoses (Lewis et al. 2009; Wang et al. 2011), consensus cytopathology is an effective way to reduce discrepant or indeterminate FNA results (Cibas et al. 2013). Moreover, current guidelines also recommend repeat FNA for AUS/FLUS FNA results to increase the chance for a definitive FNA cytology diagnosis (Gharib et al. 2016). Unfortunately, evidence-based ultrasound criteria and standardized FNA cytology, according to the Bethesda classification, are not always applied in everyday practice.

FNA was only used in 21% of patients undergoing surgery for a single thyroid nodule in Germany (Wienhold et al. 2013), and similar low preoperative FNA rates were reported in Belgium (van den Bruel et al. 2013) and France (L'assurance maladie – Caisse Nationale 2013). Therefore, the differential diagnosis and malignancy risk stratification of thyroid nodules require multidisciplinary expertise and a determination of both local ultrasound practices and local malignancy rates for a given FNA result. However, even in such a multidisciplinary setting, the inherent limitation of FNA cytology described above cannot be rectified.

However, accumulating evidence suggests that this limitation of AUS/FLUS and FN/SFN cytology can be compensated for by molecular diagnostic approaches which capitalize on the increasing knowledge about the molecular etiology of thyroid nodules by performing a comprehensive analysis of the transcriptional and mutational landscape of thyroid cancers (Cancer Genome Atlas Research Network 2014; Yoo et al. 2016). It was recently shown (Aragon et al. 2014) that any molecular diagnostic test, to compensate for the inherent limitation of AUS/FLUS and FN/SFN cytology, will require and cannot replace a multidisciplinary approach to the management of patients with thyroid nodules. In principle, the different molecular diagnostic methodologies can be broadly classified into two categories: "rule out" malignancy or "rule in" malignancy approaches (Fig. 5). While the former aims to

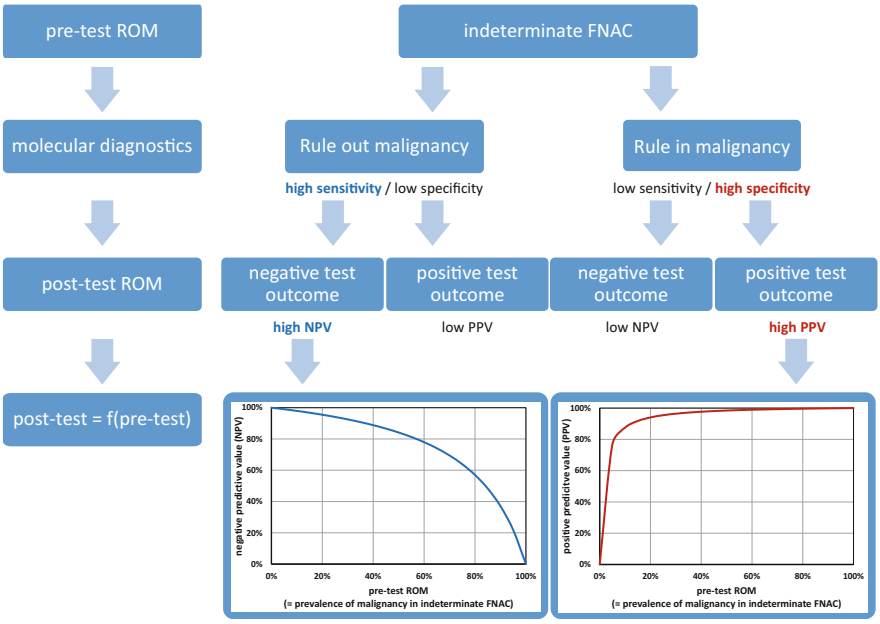

Fig. 5 Rule out and rule in approaches and their relation to diagnostic parameters such as sensitivity, specificity, PPV, and NPV. The post-test ROM (i.e., PPV and NPV) is also shown as a function of the pre-test ROM (prevalence of malignancy in the respective cytological category). Reproduced from Eszlinger et al. 2017a

reduce the overtreatment of benign nodules, the latter aims to optimize surgical planning (primary total thyroidectomy vs. a two-staged approach with "diagnostic" lobectomy and subsequent completion surgery). Interestingly, recent data suggest that the most recent "rule in" strategies may also have the potential to rule out malignancy due to a remarkably high sensitivity (Nikiforov et al. 2014, 2015).

"Rule Out" Malignancy

A "rule out" test aiming to identify benign nodules (i.e., to rule out malignancy for FNA cytology indeterminate nodules) and thereby decrease the number of diagnostic thyroid surgeries is the Afirma gene expression classifier (GEC).

In essence, the analysis of the GEC is done in two steps. While in the first step neoplasias of non-thyroidal origin are filtered out based on the expression patterns of 25 transcripts, in the second step the gene expression patterns of 142 genes are analyzed. Based on these expression patterns, the samples are classified as "benign" or "suspicious." The analytical performance including analytical sensitivity, analytical specificity, robustness, and quality control of the GEC has been verified (Walsh et al. 2012). It has been shown that RNA content and quality within the FNAs preserved in a RNA-stabilizing solution are stable for up to 6 days and that FNA storage and shipping do not significantly affect the GEC results. Furthermore, the analytical sensitivity tolerates variable RNA input and dilution of malignant FNA material down to 20%, while analytical specificity tolerates contamination with blood (up to 83%) and genomic DNA (up to 30%) with regard to false-negative results. However, high proportions of blood contamination demonstrated a potential for false-positive results (Walsh et al. 2012).

The GEC is based on an exploratory study in which the expression patterns of more than 247,186 transcripts were analyzed in 315 thyroid nodules (Chudova et al. 2010). Subsequently, the established GEC was validated in 265 indeterminate FNAs (Alexander et al. 2012). The majority of the malignant samples analyzed were classical papillary thyroid cancer (cPTC) and fvPTC (72%), and only 12% were FTCs. The reported negative predictive value (NPV) for AUS/FLUS, SFN/FN, and SFM was 95%, 94%, and 85%, respectively. Based on the high NPV, the authors recommend the GEC to "rule out" malignancy in AUS/FLUS and also SFN/FN cytologies. However, the initial data regarding the rate of benign GEC for indeterminate FNAs of 53% (Alexander et al. 2012) are at variance with data from subsequent independent studies with low patient numbers reporting benign GEC rates for indeterminate FNAs of only 26% and 27% (Marti et al. 2015; McIver et al. 2014). This would lower the positive predictive value (PPV) of the GEC for suspicious test outcomes from 44% to 47% (Alexander et al. 2012, 2014) to 16% (McIver et al. 2014). Such a low PPV, if accurate, calls into question the claimed reduction of diagnostic surgeries by 74% for the GEC for indeterminate FNAs (AUS/FLUS, SFN/FN, and SFM cytologies) (Li et al. 2011). Based on the above data, a reduction of diagnostic surgeries for indeterminate FNAs of only 25% appears more realistic. This also influences the interpretation of a previously

published cost-efficiency analysis (Li et al. 2011) for the GEC. A much less compelling cost reduction is also supported by an independent cost-efficiency analysis (Najafzadeh et al. 2012).

Most importantly, the pre-test risk of malignancy (ROM), i.e., the prevalence of malignancy in the local test setting, profoundly influences the test reliability (Fig. 5). For the GEC, a pre-test ROM of <23% is required to achieve an NPV of >95% and a post-test ROM of <5% (McIver 2013). Whereas the pre-test ROM in the initial study (Alexander et al. 2012) was <23% for AUS/FLUS and thus achieved an NPV of >95%, highly variable ROMs for AUS/FLUS have been reported from other institutions, ranging from 6% to 48% (Wang et al. 2011) or 13–62% (Vanderlaan et al. 2011). Thus, for an institution with a ROM of 48%, the NPV for the GEC would drop to 85% (McIver 2013). This calculation illustrates that the pre-test ROM as determined by the local center's cytopathologist strongly influences the local utility of the GEC. Despite this, many practitioners work with cytologists whose ROM is unknown.

Similar calculations could also be made for SFN/FN cytologies that also show poor results for the GEC, since higher ROMs have repeatedly been reported for SFN/FN as compared to AUS/FLUS (Cibas and Ali 2009). This would make the GEC unsuitable for SFN/FN cytologies. According to the National Comprehensive Cancer Network (NCCN) guideline, a post-test ROM of <5% is required to opt for watchful waiting instead of diagnostic surgery (Tuttle et al. 2010). Whether or not this goal can be achieved for the "rule out," GEC clearly depends on the respective pre-test ROM for the specific cytology category, which needs to be determined for all local settings.

Thyroid nodules with benign FNA cytology should be followed up, possibly with the option to do a second FNA to reduce false-negative results (Gharib et al. 2016). Several follow-up studies, including 1 of 11,000 thyroid nodules investigated by FNAs (Oertel et al. 2007), showed that the detection of thyroid carcinoma in a previously FNA benign nodule is only 1% (Oertel et al. 2007; Kuma et al. 1992; Lim et al. 2013; Nou et al. 2014). Since follow-up has also been suggested for GEC benign results obtained for indeterminate, AUS/FLUS, or SFN cytology, longitudinal follow-up studies with long-term outcomes, determination of false-negative GEC results, and quality of life data are clearly needed for these cases to provide the necessary evidence for this claim. Currently published prospective follow-up for GEC benign nodules is limited to 8.5 months (Alexander et al. 2014). Based on these results, the recently revised American Association of Clinical Endocrinologists (AACE) guideline does not recommend in favor of or against the use of the GEC for cytologically indeterminate thyroid nodules (Gharib et al. 2016). The American Thyroid Association (ATA) guidelines recommend that diagnostic surgical excision is the long-established standard of care for the management of FN/SFN cytology nodules without specifying a specific molecular test approach. However, after considering clinical and ultrasonographic features, molecular testing may be used to supplement malignancy risk assessment data in lieu of proceeding directly with surgery. Informed patient preference and feasibility should be considered in clinical decision-making (Haugen et al. 2016).

"Rule In" Malignancy

7-Gene Panel to "Rule In" Malignancy

In contrast to the GEC, which is used to exclude malignancy, the detection of cancer-specific mutations can be used to detect malignancy in a "rule in" approach. Early studies (Cantara et al. 2010; Moses et al. 2010; Nikiforov et al. 2009; Ohori et al. 2010), analyzing a 7-gene panel comprising *BRAF*, *NRAS*, *HRAS*, *KRAS*, *RET/PTC1*, *RET/PTC3*, and *PAX8/PPARG* mutations, showed that about 60% of the cytologically indeterminate carcinomas can be identified by this approach (Ferraz et al. 2011). Meanwhile, major differences with respect to diagnostic parameters of the "rule in" approach have been reported with sensitivities and specificities for the 7-gene panel ranging from 18–100% to 82–100%, respectively (Nishino 2016).

In their 2011 single-center study, Nikiforov and co-workers (2011) analyzed the 7-gene panel in 967 indeterminate FNAs, of which 53% had a histological diagnosis. They obtained sensitivities of 63–57% concomitant with PPVs of 88–87%, in the subcategories AUS/FLUS and FN/SFN, respectively (Nikiforov et al. 2011). Beaudenon-Huibregtse and co-workers (2014) analyzed 97 indeterminate FNAs with the 7-gene panel in a prospective multicenter study (55% with a histological diagnosis). In contradistinction to Nikiforov et al. (2011), they obtained a rather low sensitivity of 36% concomitant with a PPV of 67% in the AUS/FLUS category (Nishino 2016). Labourier et al. (2015) analyzed 282 indeterminate FNAs using a combination of the 7-gene panel and a miRNA classifier in a prospective multicenter study, 39% with a histological diagnosis. They reported a sensitivity of 69% and a PPV of 71% for the mutation panel alone for their indeterminate FNAs (Nishino 2016). Varying sensitivities and PPVs can be explained by high intra- and interobserver variabilities in the cytological and histological evaluation of thyroid nodules (Cibas et al. 2013; Fassina et al. 1993; Franc et al. 2003), different proportions of PTCs, FTCs, and fvPTCs with different mutation frequencies, a different prevalence of mutations in different populations, and variable local malignancy rates in identical cytological categories largely due to the referral practice pattern of the individual center (Ho et al. 2014).

All initial studies (Cantara et al. 2010; Moses et al. 2010; Nikiforov et al. 2009, 2011; Ohori et al. 2010) used fresh FNA material stored at −80 °C, which is difficult to implement in daily routine practice, as opposed to using routine air-dried FNA material (Ferraz et al. 2011). We have shown the feasibility of detecting *PAX8/PPARG* and *RET/PTC* rearrangements in air-dried FNAs smears (Ferraz et al. 2012). Molecular testing of routine FNA samples offers several advantages over obtaining additional fresh FNA material: the same indeterminate material analyzed by cytopathology is also analyzed for point mutations and rearrangements. This obviates the need to prepare additional FNA material for RNA preservation or to store FNA material until completion of cytological diagnosis. The latter would then reveal about 20% of samples with indeterminate cytology that would require molecular analysis. It also precludes the need for a second FNA for molecular diagnostics. Therefore, the burden to the patient and society is reduced. This approach was evaluated in two retrospective studies (Eszlinger et al. 2014, 2015). It is also feasible on residual biopsy material using ThinPrep technology (Krane et al. 2015) or the

residual needle aspirate after cytologic analysis (Nikiforov et al. 2011). Most importantly, it has the advantage of not requiring additional FNA procedures or dedicated passes or special preservation and storage.

After Nikiforov et al. (2011), the second largest prospective study for the 7-gene panel is the evaluation of a 2-year prospective application of the 7-gene panel for routine air-dried FNA material (Eszlinger et al. 2017b). With available histology/follow-up data for 63% of the analyzed FNAs, this study is comparable to the study by Nikiforov et al. (2011). In contrast, the studies of Beaudenon-Huibregtse et al. (2014) and Labourier et al. (2015) showed follow-up data for only 15% and 39% of the FNAs, respectively. While the sensitivity for molecular testing in the AUS/FLUS category is comparable between the studies of Nikiforov et al. (2011) and Eszlinger et al. (2017b) (63% vs. 58%, respectively), the specificity in the AUS/FLUS category of the Eszlinger study (Eszlinger et al. 2017b) is lower compared to the Nikiforov study (Nikiforov et al. 2011) (82% vs. 99%, respectively) but identical to the specificity described in Beaudenon-Huibregtse et al. (2014).

Interestingly, the sensitivity in the FN/SFN category in the Eszlinger et al. study (Eszlinger et al. 2017b) is lower than in the studies of Nikiforov et al. (2011) and Beaudenon-Huibregtse et al. (2014). This low sensitivity in the FN/SFN category is similar to a previous retrospective study (Eszlinger et al. 2014) and is mainly due to two findings. First is a very low mutation prevalence in FTCs. Second, while the prevalence of fvPTCs in the AUS/FLUS and FN/SFN categories is nearly identical, there was an exceptionally low mutation frequency in the fvPTCs in the FN/SFN category (Eszlinger et al. 2017b). While the specificities in the AUS/FLUS and FN/SFN category for the Eszlinger study (Eszlinger et al. 2017b) are comparable to the specificities observed in several previous studies (Beaudenon-Huibregtse et al. 2014; Labourier et al. 2015; Eszlinger et al. 2014, 2015), they are lower than those obtained by Nikiforov et al. (2011). As in previous studies (Eszlinger et al. 2014, 2015), this lower specificity is due to the prevalence of *RAS*/*PAX8*/*PPARG* mutations in histologically benign nodules. Therefore, the PPV of *RAS* mutations is particularly low (Eszlinger et al. 2017b).

Of note, histological reevaluation of nodules initially diagnosed as FAs in two studies resulted in a reclassification of three out of ten *RAS*-positive tumors as minimally invasive FTCs (Eszlinger et al. 2015) and 1 out of 17 benign nodules as FTCs, while five FAs/hyperplastic nodules were reclassified as follicular tumor of uncertain malignant potential (FT-UMP) (Eszlinger et al. 2017b). These findings highlight the dilemma of high intra- and interobserver variabilities not only in the cytological but also in the histological evaluation of thyroid nodules. This illustrates the difficulty in establishing histology as the gold standard for diagnosis (Cibas et al. 2013; Fassina et al. 1993; Franc et al. 2003).

Most importantly, due to different referral settings (primary vs. tertiary), the ROMs in these four prospective studies were quite different. The ROM based on cytology alone was 15% in the AUS/FLUS category in the Eszlinger study (Eszlinger et al. 2017b), which is comparable to both studies from the Nikiforov group (Nikiforov et al. 2009, 2011). However, the study of Beaudenon-Huibregtse et al. (2014) had a very high (50%) ROM in the AUS/FLUS category. In contrast to the AUS/FLUS category, the ROM for the FN/SFN category (17%) in the Eszlinger

study (Eszlinger et al. 2017b) is considerably lower in comparison to the studies by Nikiforov et al. (2011) and Beaudenon-Huibregtse et al. (2014) with a 27% and a 32% ROM, respectively. Since the PPV of a test increases with an increasing prevalence of malignancy (i.e., pre-test ROM) (Fig. 5), this low prevalence of malignant tumors in the primary referral setting, especially in the FN/SFN category, results in low PPVs in the indeterminate categories (Eszlinger et al. 2017b). The low diagnostic impact in the latter setting is also due to a low prevalence of *RAS* mutations in FTCs (resulting in a low sensitivity) and a rather high prevalence of *RAS* mutations in benign nodules (resulting in a low specificity). Therefore, whereas favorable results have been reported for tertiary referral settings with higher ROMs and higher ROM for *RAS* mutations, the application of the 7-gene panel as a "rule in" malignancy test in a primary referral setting with low ROM does not improve the presurgical diagnosis of thyroid FNAs.

Next-Generation Sequencing to "Rule In" Malignancy

Recently, the Thyroid Cancer Genome Atlas (TCGA) study comprehensively investigated the molecular etiology of 496 differentiated thyroid cancers with several molecular platforms and identified driver mutations in 96.5% of differentiated thyroid cancers (Cancer Genome Atlas Research Network 2014). These results clearly support the expansion of the 7-gene panel with newly identified mutations. A few months after this publication, a targeted next-generation sequencing (tNGS) panel was published that tests for point mutations in 12 cancer genes (Nikiforova et al. 2013) and 42 types of gene fusions (Nikiforova et al. 2013, 2014, 2015). In terms of diagnostic parameters, this should result in a higher sensitivity of the assay and finally in a higher NPV, since the higher the number of analyzed cancer-specific mutations, the higher the possibility of identifying malignant nodules in the group of cytologically indeterminate FNAs. Indeed, among 98 nodules with AUS/FLUS cytology and known outcome, this tNGS panel was able to classify 20 out of the 22 cancers correctly showing a sensitivity of 91%. Moreover, this tNGS approach was also characterized by a high specificity of 92% resulting in both a high PPV and NPV (77% and 97%, respectively) (Nikiforov et al. 2015). Similar results were reported for 143 consecutive FN/SFN FNA samples (Nikiforov et al. 2014). In both studies the most frequently mutated genes were *NRAS* and *HRAS*. If these results can be confirmed in a prospective multicenter study with a larger number of samples, the reported NPV of 96% could modify the current clinical management of mutation-negative indeterminate thyroid nodules by allocating these nodules with a cancer risk $<5\%$ to follow up instead of diagnostic lobectomy as suggested by the NCCN guideline (Tuttle et al. 2014). However, no prospective independent validation studies for the tNGS panels with histologic evaluation blinded to molecular testing have yet been published. Moreover, as point mutations were only detected in a few hot spots in the tNGS studies comprising *NRAS*, *HRAS*, *KRAS*, *PTEN*, *EIF1AX*, *BRAF*, *TERT*, and gene fusions were detected in only 5 of 42 analyzed gene fusions, more limited mutation panels analyzed with different technologies like mass spectrometry may be a more cost-efficient alternative to tNGS.

Finally, a recent study using RNA sequencing reported mutations in only 19 out of 32 histologically malignant tumors. Furthermore, in a limited number of FNAs, genetic alterations were detected in only 19 out of 44 malignant samples (43% sensitivity) (Pagan et al. 2016). These findings are at variance with the results of the TCGA study (Cancer Genome Atlas Research Network 2014) and also with two preliminary studies using tNGS for indeterminate FNAs (Nikiforov et al. 2014, 2015). Nonetheless, tNGS strategies for a mutation detection based on "rule in" and possibly also a "rule out" approach may well have a gap. This remains to be determined in multicenter studies with sufficient sample sizes and known FNA malignancy risks.

Diagnostic Relevance of *RAS* Mutations for "Rule In" Malignancy

While the presurgical detection of *BRAF* mutations and *RET/PTC* fusions has been associated with a 100% ROM (Cantara et al. 2010; Nikiforov et al. 2009, 2011; Beaudenon-Huibregtse et al. 2014; Eszlinger et al. 2014), the ROM for *RAS* mutations is uncertain since its association with thyroid cancer varies between 19% and 85% (Ohori et al. 2010; Nikiforov et al. 2011; Beaudenon-Huibregtse et al. 2014; Eszlinger et al. 2014, 2015; Krane et al. 2015).

To further elucidate these discrepancies, we reviewed reports of *RAS* mutations in malignant [FTCs, fvPTCs, and Hurthle cell carcinoma (HCC)] and benign [FAs, adenomatous nodules, benign goiters, Hurthle cell adenomas (HCA)] thyroid lesions from 1995 to present. We included studies of adults with a sample size over 50, and with analyses of FNA and/or surgical samples by a pathologist. Reviews and studies lacking methodological details were excluded. Among 51 studies, two independent investigators identified 20 studies that met the inclusion and exclusion criteria (Cantara et al. 2010; Nikiforov et al. 2009, 2011 Ohori et al. 2010; Beaudenon-Huibregtse et al. 2014; Eszlinger et al. 2014; 2015; Krane et al. 2015; An et al. 2015; Capella et al. 1996; Esapa et al. 1999; Fukahori et al. 2012; Jang et al. 2014; Lee et al. 2013; Liu et al. 2004; Medici et al. 2015; Schulten et al. 2013; Vasko et al. 2003; Yip 2015; Yoon et al. 2015). A variety of both FNA and surgical samples were analyzed, and preparations included fresh and air-dried FNAs, as well as frozen or formalin-fixed and paraffin-embedded tissue samples. The analysis of point mutations in codons 12 and 13 of *KRAS* and codon 61 of *HRAS* and *NRAS* predominated applying different methodologies for mutation detection.

Overall, the analysis revealed a substantial variability with regard to the *RAS* mutation prevalence within the different lesions and across the different studies. While for the malignant lesions a mean *RAS* mutation prevalence of 41% was determined, the mean *RAS* mutation prevalence in the benign entity was 10%. The highest prevalence in the malignant group has been shown for fvPTCs (52%) and in the benign group for FAs (19%). To extrapolate the diagnostic potential of *RAS* mutations from the *RAS* mutation prevalence in the different entities, specificity was calculated as 100% – total *RAS* mutation prevalence in benign lesions resulting in a mean specificity of 90%. The *RAS* mutation prevalence in malignant lesions is equivalent to the sensitivity resulting in a mean sensitivity of 41%.

Interpretation of these results is difficult given the large variability across the studies. Several factors could account for the variability including differences in sample preparation, mutation analysis technique, and variability across pathologists. Ultimately, the role of *RAS* mutations in a diagnostic ruling in approach would need to be investigated by a multicenter, blinded study. *RAS*-positive FAs may represent tumors for which capsular or vascular invasion was not detected by histologic examination (Lang et al. 1980). This assumption is supported by a recent prospective study defining the limitations of histologic thyroid nodule evaluation by Cibas et al. (2013). In 61% of FTCs/HCCs with capsular invasion and in 50% of FTCs/HCCs with vascular invasion, discordant diagnoses (benign vs. malignant) were established by expert histopathologists (Cibas et al. 2013). Similar results have previously been published by others (Fassina et al. 1993; Franc et al. 2003; Lang et al. 1980). This high rate of histopathologic discordance is a dilemma for translational studies of molecular markers aiming to improve the presurgical diagnosis because the histologic reference for these studies is obviously imprecise. Together with the well-known high interobserver variability for the diagnosis of FTC (Franc et al. 2003) and the increased likelihood for a FTC diagnosis with the examination of an increased number of paraffin blocks per nodule (Lang et al. 1980), this raises the question of whether histology can really be the diagnostic gold standard for thyroid nodules and FNA cytology. We will therefore most likely witness an evolution toward a diagnostic approach that combines histology with mutation analysis like in other areas of pathology.

Similarly, the *PAX8/PPARG* fusion oncogene has previously been reported in both FAs and FTCs (Cheung et al. 2003; Marques et al. 2002; Nikiforova et al. 2002). More recently it was reported that *PAX8/PPARG* rearrangements found in 22 thyroid nodules had a 100% predictive value for differentiated thyroid cancer (Armstrong et al. 2014). Therefore, for *PAX8/PPARG* gene fusions, the same diagnostic problem emerges as for *RAS* mutations. Nevertheless, *RAS*- and *PAX8/PPARG*-positive FAs might be preinvasive FTCs. Several studies suggest that *RAS* mutations are involved in malignant transformation and tumor dedifferentiation (Fukahori et al. 2012; Basolo et al. 2000; Garcia-Rostan et al. 2003; Zhu et al. 2003). In support, in vitro studies have shown that mutant *RAS* initiates cell proliferation, promotes chromosomal instability, and thereby may predispose to a more malignant phenotype (Fagin 2002; Saavedra et al. 2000). Moreover, transgenic mouse models carrying *RAS* mutations have been shown to develop thyroid carcinomas (Kim and Zhu 2009; Rochefort et al. 1996). Consequently, *RAS*- or *PAX8/PPARG*-positive (histologically benign) nodules may well be a subgroup of histologically benign nodules with faster growth (Sapio et al. 2011) and a higher likelihood of requiring therapy during follow-up. Unfortunately, this is a difficult subject to investigate because surgical removal of a noninvasive nodule impacts the natural history of a tumor. Thus, it is not possible to know whether a *RAS*- or *PAX8/PPARG*-positive FA is destined to become a bona fide invasive FTC but simply has not been given the opportunity.

MicroRNAs (miRNAs) in FNA Samples

In order to compensate for the possibility of a mutation panel NPV <95%, and to further characterize *RAS*-positive but histologically benign thyroid nodules, it may be necessary to complement the "rule in"/mutation detection with additional approaches, for example, the quantification of specific microRNAs (miRNAs) in thyroid FNA cytology specimen. miRNAs are less susceptible to degradation than mRNAs and have been shown to allow better diagnostic classifications than mRNA classifiers (Lu et al. 2005). This has also been demonstrated for the differential classification of benign thyroid nodules (Rossing 2013; Eszlinger and Paschke 2010).

Whereas most of the thyroid tumor samples investigated in these studies were PTCs, the diagnostic challenge for AUS/FLUS, SFN/FN, and SFM samples is trying to differentiate between FAs and FTCs. Only a few studies have addressed the identification of miRNAs differentially expressed in FTCs as compared to normal thyroid tissues or FAs/adenomatous nodules (Rossing 2013; Lodewijk et al. 2012). However, this would be a first step to identify miRNAs for the further discrimination of mutation-negative indeterminate cytologies. Moreover, the differentially expressed miRNAs identified in these studies show little overlap between the different studies analyzing FTCs, normal thyroid tissues, FAs, or adenomatous nodules (Stokowy et al. 2013). Possible reasons for these discrepant results are the limited numbers and the heterogeneity of samples included in the studies. Therefore, we recently applied microarray technology (Stokowy et al. 2015) and miRNA high-throughput sequencing (Stokowy et al. 2016) to compare the miRNA expression profiles of (mutation-negative) FAs and FTCs. The aim of these studies was to identify diagnostically promising miRNAs which might be used in an add-on approach subsequent to mutation testing. While no single miRNA had satisfactory predictive power, several classifiers comprising two miRNAs were generated. Finally, the best six classifiers could be validated in a set of 44 FNAs representing 24 FTCs and 20 FAs. Despite the preliminary nature of the small validation set, promising results were obtained. The three high-throughput sequencing-derived classifiers miR-484/miR-148b-3p, miR-484/miR-139-5p, and miR484/miR-145-5p were especially characterized by a high sensitivity and specificity. This makes the diagnostic application of these miRNA classifiers promising, particularly in combination with a "rule in" mutation panel (Stokowy et al. 2016).

Two-Step Approach Combining Mutation Testing and miRNA Classifiers

Labourier et al. (2015) published a multiplatform mutation and miRNA test combining the classic 7-gene panel with a miRNA gene expression classifier. This miRNA classifier is based on the expression levels of ten miRNAs (miR-29b-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-551b-3p), which were determined in an initial training cohort comprising 240 surgically resected, well-characterized benign and malignant thyroid lesions (Labourier et al. 2015). In addition, the miRNA thresholds were optimized using additional resected thyroid tissues and FNAs. Finally, the combination of the 7-gene panel and the 10-miRNA classifier was applied to 109 indeterminate FNAs. While the combination of both panels revealed a 20% sensitivity increase (up to 89%), the specificity did not change significantly (85% for the combined panel vs. 86% for the 7-gene panel alone). In their preliminary specific setting with a prevalence of malignancy of 32%, they obtained a NPV and PPV of 94% and 74%, respectively (Labourier et al. 2015). This approach requires further validation with sufficient sample numbers.

Such a two-step approach allows the identification in the first step of mutation-positive samples, which are characterized by an increased ROM and which should therefore undergo surgery (often a total thyroidectomy, especially for BRAF-positive samples). Furthermore, this approach would result in a decreased ROM for the mutation-negative indeterminate FNAs. This reduced ROM would then have to be low enough to obtain a NPV >94% with the miRNA classifiers in the second step to be able to support watchful waiting instead of surgery for the miRNA classifier benign FNAs. In contrast, the FNAs suspicious for malignancy according to the miRNA classifiers should undergo lobectomy for histologic verification. However, the identified miRNA classifiers (Labourier et al. 2015; Stokowy et al. 2016) require further validation in a large set of cytologically indeterminate FNAs preferably using an absolute method for miRNA quantification, e.g., digital PCR.

Implications of the Introduction of NIFTP on Molecular Diagnosis

Based on the observation that noninvasive encapsulated fvPTCs are characterized by a very low risk of recurrence or other adverse outcomes, a recent international multidisciplinary study suggested renaming this entity to “noninvasive follicular thyroid neoplasms with papillary-like nuclear features” (NIFTP) (Nikiforov et al. 2016). The authors of the study estimate that this reclassification might affect >45,000 patients worldwide per year by reducing the psychological burden, medical overtreatment, expense, and other consequences associated with a cancer diagnosis (Nikiforov et al. 2016). Since the ROM for each of the Bethesda classification categories (Cibas and Ali 2009) has been established considering noninvasive encapsulated fvPTC as malignant tumors, the reclassification of this tumor entity as NIFTP will also markedly impact the ROMs for FNA results. This is particularly relevant for the indeterminate categories and will also influence the PPVs and NPVs of molecular tests. Although data are still limited, a considerable reduction of the ROM of the indeterminate categories has been shown (Faquin et al. 2016; Strickland et al. 2015). Nevertheless, due to the limited data, the precise impact that a diagnosis of NIFTP will have on the ROM cannot currently be predicted. The effects are likely to vary depending on case demographics and the institutional frequency of diagnosing noninvasive encapsulated fvPTC/NIFTP (Baloch et al. 2016). It has to be

stressed that NIFTP can only be diagnosed on a surgical specimen allowing the pathologist an adequate sampling and evaluation of the tumor capsule interface to exclude capsular/vascular invasion (Nikiforov et al. 2016). With regard to molecular thyroid FNA diagnostics, one could anticipate that a reduction of the ROM in the indeterminate categories might be in favor of the "rule out" approach (since its NPV might increase) and against the "rule in" approach (i.e., the 7-gene panel) due to dropping PPVs. However, a "rule in" test with detection of a *RAS* mutation, which are prevalent in encapsulated fvPTC (Howitt et al. 2013; Rivera et al. 2010) in the situation whereby a subsequent histological diagnosis of a NIFTP is made, should not be considered a false positive. Instead, in terms of a personalized medicine approach, a mutation-specific ROM should tailor the most appropriate extent of surgery. For example, the detection of a *RAS* mutation or *PAX8/PPARG* fusion should lead to lobectomy followed by a careful histopathologic evaluation. If a NIFTP can then be diagnosed, a de-escalated management seems appropriate (Nikiforov et al. 2016). This would be in line with the view of *RAS*- and *PAX8/PPARG*-positive FAs as possible "preinvasive" FTCs, which require a careful initial histopathologic evaluation.

Nevertheless, all past investigations of the "rule in" and "rule out" approaches were unable to account for this recent pathology reclassification and new NIFTP diagnosis. Therefore, not only NPVs and PPVs but also sensitivities and specificities will now have to be reassessed for both approaches.

Treatment of Autonomously Functioning Thyroid Nodules

Therapy is only dealt with in brief. Further information on all the following treatment options are given in the chapters on Graves' disease as well as multinodular toxic and nontoxic goiter.

Antithyroid Drugs

Management of thyroid autonomy has been excellently reviewed by Hegedus et al. (2003), Hermus et al. (Hermus and Huysmans 1998), and others. Antithyroid drugs, usually in combination with beta-blocking drugs (preferably nonselective propranolol), are the first-line treatment in all patients with overt thyrotoxicosis. Depending on the type of antithyroid drug, an initial dosage of 30 mg/day of methimazole, 40–60 mg/day of carbimazole, or 300 mg/day of propylthiouracil is recommended. Higher dosages are associated with more frequent adverse effects (3–12%) and will only result in marginally faster resolution of thyrotoxicosis (Hegedus et al. 2003). Furthermore, a trial of low-dose drug therapy (5–10 mg methimazole per day) may be justified in selected patients with symptomatic subclinical thyrotoxicosis (Toft 2001); alternatively, beta-blocking drugs can be used.

Treatment of AFTNs with antithyroid drugs normalizes thyroid function, but remission is extremely rare and lifelong treatment would be necessary. While the

purpose of antithyroid drug therapy is to render the patient euthyroid, there is, in contrast to Graves' disease (Smith and Hegedus 2016), practically no spontaneous resolution of hyperthyroidism in AFTNs. This implies that once thyroid autonomy becomes clinically manifest, definitive treatment is indicated. Elderly patients with severe non-thyroidal illness may be an exception to this rule. However, benefits and risks of such "long-term" drug therapy have to be considered against the very low risk of definitive treatment (Hegedus et al. 2003; Cooper 2005; Gemsenjager 1992; Thomusch et al. 2000). Two ablative treatment options are available for TA: thyroid surgery or radioiodine treatment. Percutaneous ethanol injection (Hegedus et al. 2003; Hermus and Huysmans 1998; Ferrari et al. 1996) has not gained wide acceptance, mainly due to ethanol seepage along the needle tract causing fibrosis and pain.

Antithyroid drugs are indicated before thyroid surgery to lower the operative risk and can be stopped in the immediate postoperative period (Weetman 2007). To reduce the risk of exacerbation of hyperthyroidism, it has been recommended to render the patient euthyroid with antithyroid drugs prior to ^{131}I treatment. Usually, the antithyroid drug is discontinued at least 4 days before and resumed no sooner than 3 days afterward (Weetman 2007). A meta-analysis, based on studies in mainly Graves' disease, found that the use of methimazole as well as propylthiouracil in conjunction with ^{131}I therapy results in a decrease in the remission rate (Walter et al. 2007). This most likely also applies to toxic nodules (Ferrari et al. 1996).

Surgery

The purpose of thyroid surgery is to cure hyperthyroidism by removing all autonomously functioning thyroid tissue and other macroscopically visible nodular thyroid tissue. The extent of surgery is determined by preoperative ultrasound and importantly intraoperative morphological inspection (Gemsenjager 1992). For TA, hemithyroidectomy is usually adequate. The advantages of surgery (removal of all nodular tissue, rapid and permanent resolution of hyperthyroidism, and definite histological diagnosis) have to be weighed against general (risk of anesthesia, inpatient treatment) and thyroid-specific side effects, the latter of which are largely dependent on the surgeons' training (vocal cord paralysis ~1 to 2%, hypoparathyroidism is not an issue with lobectomy and 1–2% with bilateral surgery for an experienced endocrine surgeon) (Gemsenjager 1992; Thomusch et al. 2000). The incidence of postoperative hypothyroidism depends on the extent of thyroid resection and is very low following hemithyroidectomy.

While thyroid surgery is usually performed after euthyroidism has been achieved with antithyroid drug treatment, surgery is also advocated in patients with overt hyperthyroidism who, prior to surgery, have had adverse effects of a drug or in patients with thyrotoxic storm who are treatment resistant. Rapid control of severe hyperthyroidism can be achieved by the administration of glucocorticoids, beta-blockers, and iodide or iopanoic acid in patients allergic to the antithyroid drugs (Panzer et al. 2004).

Radioiodine Therapy

^{131}I treatment is considered safe and appropriate in nearly all types of hyperthyroidism, especially in elderly patients (Weetman 2007; Bonnema and Hegedus 2012). Generally, ^{131}I is thought to carry a lower rate of complications and a lower cost than surgery (Weetman 2007; Bonnema and Hegedus 2012). This fact has led a number of centers to offer ^{131}I as the first choice of therapy in the majority of patients. ^{131}I therapy is widely used for treatment of thyroid autonomy and is highly effective in terms of eradicating hyperthyroidism and reducing thyroid gland volume. The success rate for ^{131}I therapy has been reported to range between 85% and 100% in TA (Nygaard et al. 1999).

Different protocols have been suggested for ^{131}I therapy in benign thyroid disease. Some investigators prefer to administer a standard activity, e.g., 10 or 20 miC (370–740 MBq), while others apply a certain ^{131}I activity per gram of thyroid tissue (Hegedus et al. 2003; Hermus and Huysmans 1998; Ferrari et al. 1996). Different algorithms exist for dose calculation, e.g., in Germany ^{131}I dosage is mostly calculated according to the Marinelli formula, which takes into account the maximum ^{131}I uptake as well as the effective half-life determined after administration of an ^{131}I test dose (Marinelli et al. 1948; Reiners and Schneider 2002). However, most centers have given up this cumbersome and costly procedure, since it offers very little, if any advantage, in the long run (Gemsenjager 1992). Advantages mostly relate to ease of radioiodine administration and, in most countries, the outpatient-based therapy. Disadvantages are the "time to euthyroidism" (rarely more than 4–12 weeks) during which drug therapy may have to be continued and thyroid function monitored at 3- to 6-week intervals (Hegedus et al. 2003; Nygaard et al. 1999). Radioiodine treatment is contraindicated in pregnancy and during lactation, and contraception is advocated for at least 4 months after receiving ^{131}I therapy according to EU regulations.

Side effects of radioiodine treatment may rarely include transient local pain and tenderness. Exacerbation of thyrotoxicosis due to destructive thyroiditis is very rare and mostly mild and easy to control (Hegedus et al. 2003; Ferrari et al. 1996). Population-based studies comprising more than 35,000 patients treated with ^{131}I have not shown an increased risk of thyroid cancer, leukemia or other malignancies, reproductive abnormalities, or congenital defects in the offspring. Thus, ^{131}I therapy is considered a very safe treatment in adults (Franklyn et al. 1999; Hall et al. 1992; Holm et al. 1991). However, caution has been raised concerning ^{131}I safety in children on the basis of the Chernobyl accident epidemiological data (Farahati et al. 2000), and there is no consensus on a lowest age limit.

Postradioiodine hypothyroidism in AFTNs usually develops insidiously. The prevalence of hypothyroidism depends on the extent of TSH suppression prior to ^{131}I therapy and the protocol applied (Nygaard et al. 1999; Reiners and Schneider 2002) and, importantly, increases with the duration of follow-up. In a retrospective study of 346 patients with AFTNs, the occurrence of hypothyroidism was 7.6% at 1 year, 28% at 5 years, 46% at 10 years, and 60% at 20 years of follow-up (Ceccarelli et al. 2005). Although most have found much lower prevalences of hypothyroidism

Table 1 Advantages and disadvantages of different treatments for AFTNs

Treatment option	Advantages	Disadvantages
Surgery	Rapid control of thyrotoxicosis	Inpatient treatment
	Rapid relief of pressure symptoms	Side effects of anesthesia and thyroid surgery
	Removal of all nodular tissue ~100% cure rate	
	Definite histology	
Radioiodine	Outpatient therapy	Slow induction of euthyroidism
	Easy applicability	Variable reduction in volume
		Long-term risk of hypothyroidism
Antithyroid drug	Outpatient therapy effective for short term	No remission/cure of thyrotoxicosis
		Side effects 1–5%
	Easy applicability	Frequent follow-up and compliance required

(Gemsenjager 1992), these data emphasize the necessity of long-term monitoring of thyroid function in all patients who have received ^{131}I therapy. A summary of the advantages and disadvantages of the different treatments for AFTNs is shown in Table 1.

Cost-Effective Management of Thyroid Autonomy

Vidal-Trecan et al. (2002) have described an analytical decision model, based on the cost accounting system of 50 nonprofitable Parisian hospitals (inpatient cost) and the national reimbursement schedule of the French Social Security (outpatient costs), to examine the cost-effectiveness of different therapeutic options in TA (hemithyroidectomy, radioactive iodine, and lifelong antithyroid drug therapy). In their model, surgery was the most effective and least costly strategy in a 40-year-old woman with TA (EUR 1391 for surgery vs. EUR 2825 for ^{131}I and EUR 5760 for long-term drug therapy, followed by ^{131}I in cases of adverse drug reactions). However, radioiodine therapy is more favorable if surgical mortality exceeded 0.6% (e.g., in the elderly with an increased likelihood of multimorbidity). Lifelong drug treatment was the preferred and cost-effective treatment in women >85 years of age. Thus, in choosing the appropriate treatment strategy for TA, several factors (e.g., age, comorbidity, locally available treatment facilities and expertise, and their costs) which likewise may vary locally, must be considered. Importantly, such data do not take into consideration patient preference and patient-related outcomes (Watt et al. 2015).

Follow-Up

The long-term management of patients with thyroid autonomy is aimed at the detection and adequate treatment of thyroid dysfunction, prevention and detection

of novel nodular thyroid disease, and, in the case of surgery, detection and treatment of postsurgical hypoparathyroidism. With ^{131}I therapy, long-term follow-up for the development of hypothyroidism is mandatory (Holm et al. 1991). Furthermore, the solitary radioiodine-treated nodule often remains palpable and firm (although smaller), and FNA biopsy may reveal suspicious cells secondary to the radiation. In iodine-deficient areas, iodine supplementation may be appropriate to prevent recurrent nodular thyroid disease (Gharib et al. 2016).

Treatment of Cold and Isocaptant Thyroid Nodules

Thyroid Hormone Treatment

In a German study, L-T4 in combination with elementary iodine supplementation resulted in a nodule volume reduction of 17.3% within 1 year, as compared with placebo (Grussendorf et al. 2011).

L-T4 dose has often been targeted toward a partly suppressed serum TSH level (Bonnema et al. 2000, 2002; Grussendorf et al. 2011). The consequence is subclinical hyperthyroidism affecting adversely the skeleton and the cardiovascular system (Abrahamsen et al. 2014; Surks et al. 2004). Since lifelong therapy is probably needed to avoid nodule regrowth, L-T4 treatment is in fact not feasible in the majority of the patients (Fast et al. 2008). Based on the aforementioned, L-T4 treatment should be abandoned for this indication (Hegedus et al. 2003; Hegedus 2004; Cooper et al. 2006), in line with the recommendations of major specialist societies (Gharib et al. 2016; Haugen et al. 2016).

Surgery

The goal of surgery is removal of all thyroid tissue with a nodular appearance, usually by a hemithyroidectomy and, if indicated, subtotal resection of the contralateral lobe. Only extremely rarely is a thoracic approach necessary. Further resection is not usually recommended if final pathologic evaluation incidentally reveals a unilateral cancer less than 1 cm in size. This not uncommon finding accounts for most cancers found in surgical series, the majority of which are of little if any clinical significance (Ito et al. 2003). Macroscopically normal perinodular tissue often harbors microscopic growth foci, which explains the relatively high risk of recurrence in these patients (Hegedus et al. 1999).

Not all patients are surgical candidates, but among those undergoing surgery, the surgical mortality rate is less than 1% in experienced hands and high-volume centers. Disadvantages include the general risks and side effects of a surgical procedure. Specific risks for bilateral surgery include transient (6%) or permanent (2%) vocal cord paralysis, transient (6%) or permanent (5%) hypoparathyroidism, and postoperative bleeding (1%) (Erickson et al. 1998). Others have found lower figures (al-Suliman et al. 1997).

Percutaneous Interventional Therapy

Percutaneous ethanol injection therapy (PEIT) has been used for more than two decades in solitary hot, toxic, and even cold thyroid nodules (Hegedus et al. 2003; Bennedbaek and Hegedus 2003). The most convincing effect is seen in solitary thyroid cysts (Bennedbaek and Hegedus 2003). Drawbacks are related to pain, risk of recurrent laryngeal nerve damage, and the possibility of extrathyroidal fibrosis complicating subsequent surgery. Laser ablation, used for more than a decade, seems to have the same, or better, efficacy than PEIT with much fewer side effects (Dossing et al. 2005, 2011, 2013). A balanced account of indications, efficacy, side effects, and cost related to the major available techniques for nonsurgical thyroid nodule ablation (PEIT, laser ablation, and radiofrequency ablation) can be obtained from recent reviews (Gharib et al. 2013; Papini et al. 2014a, b).

References

Abrahamsen B, Jorgensen HL, Laulund AS, Nybo M, Brix TH, Hegedus L. Low serum thyrotropin level and duration of suppression as a predictor of major osteoporotic fractures-the OPENTHYRO register cohort. J Bone Miner Res. 2014;29(9):2040–50.

Alexander EK, Kennedy GC, Baloch ZW, et al. Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. N Engl J Med. 2012;367(8):705–15.

Alexander EK, Schorr M, Klopper J, et al. Multicenter clinical experience with the afirma gene expression classifier. J Clin Endocrinol Metab. 2014;99(1):119–25.

al-Suliman NN, Ryttov NF, Qvist N, Blichert-Toft M, Graversen HP. Experience in a specialist thyroid surgery unit: a demographic study, surgical complications, and outcome. Eur J Surg. 1997;163(1):13–20.

An JH, Song KH, Kim SK, et al. RAS mutations in indeterminate thyroid nodules are predictive of the follicular variant of papillary thyroid carcinoma. Clin Endocrinol. 2015;82(5):760–6.

Aragon HP, Olson MT, Fazeli R, et al. The impact of molecular testing on the surgical management of patients with thyroid nodules. Ann Surg Oncol. 2014;21(6):1862–9.

Armstrong MJ, Yang H, Yip L, et al. PAX8/PPARgamma rearrangement in thyroid nodules predicts follicular-pattern carcinomas, in particular the encapsulated follicular variant of papillary carcinoma. Thyroid. 2014;24(9):1369–74.

Bahre M, Hilgers R, Lindemann C, Emrich D. Thyroid autonomy: sensitive detection in vivo and estimation of its functional relevance using quantified high-resolution scintigraphy. Acta Endocrinol. 1988;117(2):145–53.

Baloch ZW, Seethala RR, Faquin WC et al. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP): a changing paradigm in thyroid surgical pathology and implications for thyroid cytopathology. Cancer Cytopathol. 2016.

Basolo F, Pisaturo F, Pollina LE, et al. N-ras mutation in poorly differentiated thyroid carcinomas: correlation with bone metastases and inverse correlation to thyroglobulin expression. Thyroid. 2000;10(1):19–23.

Beaudenon-Huibregtse S, Alexander EK, Guttler RB, et al. Centralized molecular testing for oncogenic gene mutations complements the local cytopathologic diagnosis of thyroid nodules. Thyroid. 2014;24(10):1479–87.

Belfiore A, La Rosa GL, La Porta GA, et al. Cancer risk in patients with cold thyroid nodules: relevance of iodine intake, sex, age, and multinodularity. Am J Med. 1992;93:363–9.

Bennedbaek FN, Hegedus L. Management of the solitary thyroid nodule: results of a North American survey. J Clin Endocrinol Metab. 2000;85(7):2493–8.

Bennedbaek FN, Hegedus L. Treatment of recurrent thyroid cysts with ethanol: a randomized double-blind controlled trial. J Clin Endocrinol Metab. 2003;88(12):5773–7.
Bennedbaek FN, Karstrup S, Hegedus L. Percutaneous ethanol injection therapy in the treatment of thyroid and parathyroid diseases. Eur J Endocrinol. 1997;136(3):240–50.
Bennedbaek FN, Perrild H, Hegedus L. Diagnosis and treatment of the solitary thyroid nodule. Results of a European survey. Clin Endocrinol. 1999;50(3):357–63.
Berghout A, Wiersinga WM, Smits NJ, Touber JL. Interrelationships between age, thyroid volume, thyroid nodularity, and thyroid function in patients with sporadic nontoxic goiter. Am J Med. 1990;89(5):602–8.
Bol S, Belge G, Thode B, Bartnitzke S, Bullerdiek J. Structural abnormalities of chromosome 2 in benign thyroid tumors. Three new cases and review of the literature. Cancer Genet Cytogenet. 1999;114(1):75–7.
Bongiovanni M, Spitale A, Faquin WC, Mazzucchelli L, Baloch ZW. The Bethesda System for Reporting Thyroid Cytopathology: a meta-analysis. Acta Cytol. 2012;56(4):333–9.
Bonnema SJ, Hegedus L. Radioiodine therapy in benign thyroid diseases: effects, side effects, and factors affecting therapeutic outcome. Endocr Rev. 2012;33(6):920–80.
Bonnema SJ, Bennedbaek FN, Wiersinga WM, Hegedus L. Management of the nontoxic multinodular goitre: a European questionnaire study. Clin Endocrinol. 2000;53(1):5–12.
Bonnema SJ, Bennedbaek FN, Ladenson PW, Hegedus L. Management of the nontoxic multinodular goiter: a North American survey. J Clin Endocrinol Metab. 2002;87(1):112–7.
Brandt F, Almind D, Christensen K, Green A, Brix TH, Hegedus L. Excess mortality in hyperthyroidism: the influence of preexisting comorbidity and genetic confounding: a danish nationwide register-based cohort study of twins and singletons. J Clin Endocrinol Metab. 2012;97(11):4123–9.
Caillou B, Dupuy C, Lacroix L, et al. Expression of reduced nicotinamide adenine dinucleotide phosphate oxidase (ThoX, LNOX, Duox) genes and proteins in human thyroid tissues. J Clin Endocrinol Metab. 2001;86(7):3351–8.
Calebiro D, Grassi ES, Eszlinger M, et al. Recurrent EZH1 mutations are a second hit in autonomous thyroid adenomas. J Clin Invest. 2016;126(9):3383–8.
Campenni A, Giovanella L, Siracusa M, et al. (99m)Tc-methoxy-isobutyl-isonitrile scintigraphy is a useful tool for assessing the risk of malignancy in thyroid nodules with indeterminate fine-needle cytology. Thyroid. 2016;26(8):1101–9.
Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. Cell. 2014;159(3):676–90.
Cantara S, Capezzone M, Marchisotta S, et al. Impact of proto-oncogene mutation detection in cytological specimens from thyroid nodules improves the diagnostic accuracy of cytology. J Clin Endocrinol Metab. 2010;95(3):1365–9.
Capella G, Matias-Guiu X, Ampudia X, de Leiva A, Perucho M, Prat J. Ras oncogene mutations in thyroid tumors: polymerase chain reaction-restriction-fragment-length polymorphism analysis from paraffin-embedded tissues. Diagn Mol Pathol. 1996;5(1):45–52.
Ceccarelli C, Bencivelli W, Vitti P, Grasso L, Pinchera A. Outcome of radioiodine-131 therapy in hyperfunctioning thyroid nodules: a 20 years' retrospective study. Clin Endocrinol. 2005;62(3):331–5.
Chan JKC, Hirokawa M, et al. Tumours of the thyroid and parathyroid: follicular adenoma. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C, editors. WHO classification of tumours: pathology & genetics of tumours of endocrine organs. Lyon: IARC Press; 2004. p. 98–103.
Cheung L, Messina M, Gill A, et al. Detection of the PAX8-PPAR gamma fusion oncogene in both follicular thyroid carcinomas and adenomas. J Clin Endocrinol Metab. 2003;88(1):354–7.
Chiovato L, Mariotti S, Pinchera A. Thyroid diseases in the elderly. Bailliere Clin Endocrinol Metab. 1997;11(2):251–70.
Chudova D, Wilde JI, Wang ET, et al. Molecular classification of thyroid nodules using high-dimensionality genomic data. J Clin Endocrinol Metab. 2010;95(12):5296–304.
Cibas ES, Ali SZ. The Bethesda System for Reporting Thyroid Cytopathology. Am J Clin Pathol. 2009;132(5):658–65.

Cibas ES, Baloch ZW, Fellegara G, et al. A prospective assessment defining the limitations of thyroid nodule pathologic evaluation. Ann Intern Med. 2013;159(5):325–32.
Cooper DS. Antithyroid drugs. N Engl J Med. 2005;352(9):905–17.
Cooper DS, Doherty GM, Haugen BR, et al. Management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid. 2006;16(2):109–42.
de Vijlder JJ. Primary congenital hypothyroidism: defects in iodine pathways. Eur J Endocrinol. 2003;149(4):247–56.
Deleu S, Allory Y, Radulescu A, et al. Characterization of autonomous thyroid adenoma: metabolism, gene expression, and pathology. Thyroid. 2000;10(2):131–40. 2000; 10(2):131-140.
Demirel K, Kapucu O, Yucel C, Ozdemir H, Ayvaz G, Taneri F. A comparison of radionuclide thyroid angiography, (99m)Tc-MIBI scintigraphy and power Doppler ultrasonography in the differential diagnosis of solitary cold thyroid nodules. Eur J Nucl Med Mol Imaging. 2003;30(5):642–50.
Depoortere F, Pirson I, Bartek J, Dumont JE, Roger PP. Transforming growth factor beta(1) selectively inhibits the cyclic AMP-dependent proliferation of primary thyroid epithelial cells by preventing the association of cyclin D3-cdk4 with nuclear p27(kip1). Mol Biol Cell. 2000;11(3):1061–76.
Derwahl M, Studer H. Nodular goiter and goiter nodules: Where iodine deficiency falls short of explaining the facts. Exp Clin Endocrinol Diabetes. 2001;109(5):250–60.
Dohan O, Baloch Z, Banrevi Z, LiVolsi V, Carrasco N. Rapid communication: predominant intracellular overexpression of the Na(+)/I(−) symporter (NIS) in a large sampling of thyroid cancer cases. J Clin Endocrinol Metab. 2001;86(6):2697–700.
Dohan O, De la Vieja, Paroder V, et al. The sodium/iodide symporter (NIS): characterization, regulation, and medical significance. Endocr Rev. 2003;24(1):48–77.
Dossing H, Bennedbaek FN, Hegedus L. Effect of ultrasound-guided interstitial laser photocoagulation on benign solitary solid cold thyroid nodules – a randomised study. Eur J Endocrinol. 2005;152(3):341–5.
Dossing H, Bennedbaek FN, Hegedus L. Long-term outcome following interstitial laser photocoagulation of benign cold thyroid nodules. Eur J Endocrinol. 2011;165(1):123–8.
Dossing H, Bennedbaek FN, Hegedus L. Interstitial laser photocoagulation (ILP) of benign cystic thyroid nodules – a prospective randomized trial. J Clin Endocrinol Metab. 2013;98(7):E1213–7.
Dugrillon A, Bechtner G, Uedelhoven WM, Weber PC, Gärtner R. Evidence that an iodolactone mediates the inhibitory effect of iodide on thyroid cell proliferation but not on adenosine 3′,5′-monophosphate formation. Endocrinology. 1990;127(1):337–43.
Dunn JT, Dunn AD. Update on intrathyroidal iodine metabolism. Thyroid. 2001;11(5):407–14.
Eggo MC, Bachrach LK, Burrow GN. Interaction of TSH, insulin and insulin-like growth factors in regulating thyroid growth and function. Growth Factors. 1990;2(2–3):99–109.
Erickson D, Gharib H, Li H, van Heerden JA. Treatment of patients with toxic multinodular goiter. Thyroid. 1998;8(4):277–82.
Esapa CT, Johnson SJ, Kendall-Taylor P, Lennard TW, Harris PE. Prevalence of Ras mutations in thyroid neoplasia. Clin Endocrinol. 1999;50(4):529–35.
Eszlinger M, Paschke R. Molecular fine-needle aspiration biopsy diagnosis of thyroid nodules by tumor specific mutations and gene expression patterns. Mol Cell Endocrinol. 2010;322(1–2):29–37.
Eszlinger M, Krohn K, Frenzel R, Kropf S, Tonjes A, Paschke R. Gene expression analysis reveals evidence for inactivation of the TGF-beta signaling cascade in autonomously functioning thyroid nodules. Oncogene. 2004;23(3):795–804.
Eszlinger M, Krohn K, Berger K, et al. Gene expression analysis reveals evidence for increased expression of cell cycle-associated genes and Gq-protein-protein kinase C signaling in cold thyroid nodules. J Clin Endocrinol Metab. 2005;90(2):1163–70.
Eszlinger M, Krogdahl A, Munz S, et al. Impact of molecular screening for point mutations and rearrangements in routine air-dried fine-needle aspiration samples of thyroid nodules. Thyroid. 2014;24(2):305–13.
Eszlinger M, Piana S, Moll A, et al. Molecular testing of thyroid fine needle aspirations (FNA) improves pre-surgical diagnosis and supports the histological identification of minimally invasive follicular thyroid carcinomas. Thyroid. 2015;25(4):401–9.

Eszlinger M, Lau L, Ghaznavi S, Symonds C, Chandarana SP, Khalil M, Paschke R. Molecular profiling of thyroid nodule fine-needle aspiration cytology. Nat Rev Endocrinol. 2017a. https://doi.org/10.1038/nrendo.2017.24.

Eszlinger M, Böhme K, Ullmann M, et al. Evaluation of a two-year routine application of molecular testing of thyroid fine needle aspirations (FNA) using a 7-gene panel in a primary refferral setting in Germany. Thyroid. 2017b. https://doi.org/10.1089/thy.2016.0445.

Fagin JA. Minireview: branded from the start-distinct oncogenic initiating events may determine tumor fate in the thyroid. Mol Endocrinol. 2002;16(5):903–11.

Faquin WC, Wong LQ, Afrogheh AH, et al. Impact of reclassifying noninvasive follicular variant of papillary thyroid carcinoma on the risk of malignancy in The Bethesda System for Reporting Thyroid Cytopathology. Cancer Cytopathol. 2016;124(3):181–7.

Farahati J, Demidchik EP, Biko J, Reiners C. Inverse association between age at the time of radiation exposure and extent of disease in cases of radiation-induced childhood thyroid carcinoma in Belarus. Cancer. 2000;88(6):1470–6.

Fassina AS, Montesco MC, Ninfo V, Denti P, Masarotto G. Histological evaluation of thyroid carcinomas: reproducibility of the "WHO" classification. Tumori. 1993;79(5):314–20.

Fast S, Bonnema SJ, Hegedus L. The majority of Danish nontoxic goitre patients are ineligible for Levothyroxine suppressive therapy. Clin Endocrinol. 2008;69(4):653–8.

Ferrari C, Reschini E, Paracchi A. Treatment of the autonomous thyroid nodule: a review. Eur J Endocrinol. 1996;135(4):383–90.

Ferraz C, Eszlinger M, Paschke R. Current state and future perspective of molecular diagnosis of fine-needle aspiration biopsy of thyroid nodules. J Clin Endocrinol Metab. 2011;96(7):2016–26.

Ferraz C, Rehfeld C, Krogdahl A, et al. Detection of PAX8/PPARG and RET/PTC rearrangements is feasible in routine air-dried fine needle aspiration smears. Thyroid. 2012;22(10):1025–30.

Franc B, de la Salmoniere P, Lange F, et al. Interobserver and intraobserver reproducibility in the histopathology of follicular thyroid carcinoma. Hum Pathol. 2003;34(11):1092–100.

Franklyn JA, Maisonneuve P, Sheppard M, Betteridge J, Boyle P. Cancer incidence and mortality after radioiodine treatment for hyperthyroidism: a population-based cohort study. Lancet. 1999;353(9170):2111–5.

Fuhrer D, Holzapfel HP, Wonerow P, Scherbaum WA, Paschke R. Somatic mutations in the thyrotropin receptor gene and not in the Gs alpha protein gene in 31 toxic thyroid nodules. J Clin Endocrinol Metab. 1997;82(11):3885–91.

Fukahori M, Yoshida A, Hayashi H, et al. The associations between RAS mutations and clinical characteristics in follicular thyroid tumors: new insights from a single center and a large patient cohort. Thyroid. 2012;22(7):683–9.

Garcia-Delgado M, Gonzalez-Navarro CJ, Napal MC, Baldonado C, Vizmanos JL, Gullon A. Higher sensitivity of denaturing gradient gel electrophoresis than sequencing in the detection of mutations in DNA from tumor samples. Biotechniques. 1998;24(1):72. 74, 76.

Garcia-Rostan G, Zhao H, Camp RL, et al. ras mutations are associated with aggressive tumor phenotypes and poor prognosis in thyroid cancer. J Clin Oncol. 2003;21(17):3226–35.

Gärtner R, Schopohl D, Schaefer S, et al. Regulation of transforming growth factor beta 1 messenger ribonucleic acid expression in porcine thyroid follicles in vitro by growth factors, iodine, or delta-iodolactone. Thyroid. 1997;7(4):633–40.

Gemsenjager E. The surgical treatment of autonomous nodular goiter. Prospective long-term study. Schweiz Med Wochenschr. 1992;122(18):687–92.

Georgopoulos NA, Sykiotis GP, Sgourou A, et al. Autonomously functioning thyroid nodules in a former iodine-deficient area commonly harbor gain-of-function mutations in the thyrotropin signaling pathway. Eur J Endocrinol. 2003;149(4):287–92.

Gharib H, Hegedus L, Pacella CM, Baek JH, Papini E. Clinical review: nonsurgical, image-guided, minimally invasive therapy for thyroid nodules. J Clin Endocrinol Metab. 2013;98(10):3949–57.

Gharib H, Papini E, Garber JR, et al. American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi medical guidelines for clinical practice for the diagnosis and management of thyroid nodules. Endocr Pract. 2016;22(5):622–39.

Gozu HI, Bircan R, Krohn K, et al. Similar prevalence of somatic TSH receptor and Gsalpha mutations in toxic thyroid nodules in geographical regions with different iodine supply in Turkey. Eur J Endocrinol. 2006;155(4):535–45.
Grubeck-Loebenstein B, Buchan G, Sadeghi R, et al. Transforming growth factor beta regulates thyroid growth. Role in the pathogenesis of nontoxic goiter. J Clin Invest. 1989;83(3):764–70.
Grussendorf M, Reiners C, Paschke R, Wegscheider K. Reduction of thyroid nodule volume by levothyroxine and iodine alone and in combination: a randomized, placebo-controlled trial. J Clin Endocrinol Metab. 2011;96(9):2786–95.
Hall P, Boice Jr JD, Berg G, et al. Leukaemia incidence after iodine-131 exposure. Lancet. 1992;340(8810):1–4.
Hamburger JI. Evolution of toxicity in solitary nontoxic autonomously functioning thyroid nodules. J Clin Endocrinol Metab. 1980;50(6):1089–93.
Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association Guidelines Task Force on thyroid nodules and differentiated thyroid cancer. Thyroid. 2016;26(1):1–133.
Hedinger C. Histological typing of thyroid tumors. Berlin: Springer Verlag; 1988.
Hegedus L. Thyroid ultrasound. Endocrinol Metab Clin N Am. 2001;30(2):339–ix.
Hegedus L. Clinical practice. The thyroid nodule. N Engl J Med. 2004;351(17):1764–71.
Hegedus L. Can elastography stretch our understanding of thyroid histomorphology? J Clin Endocrinol Metab. 2010;95(12):5213–5.
Hegedus L, Nygaard B, Hansen JM. Is routine thyroxine treatment to hinder postoperative recurrence of nontoxic goiter justified? J Clin Endocrinol Metab. 1999;84(2):756–60.
Hegedus L, Bonnema SJ, Bennedbaek FN. Management of simple nodular goiter: current status and future perspectives. Endocr Rev. 2003;24(1):102–32.
Hermus AR, Huysmans DA. Treatment of benign nodular thyroid disease. N Engl J Med. 1998;338(20):1438–47.
Ho AS, Sarti EE, Jain KS, et al. Malignancy rate in thyroid nodules classified as Bethesda category III (AUS/FLUS). Thyroid: Off J Am Thyroid Assoc. 2014;24(5):832–9.
Holm LE, Hall P, Wiklund K, et al. Cancer risk after iodine-131 therapy for hyperthyroidism. J Natl Cancer Inst. 1991;83(15):1072–7.
Howitt BE, Jia Y, Sholl LM, Barletta JA. Molecular alterations in partially-encapsulated or well-circumscribed follicular variant of papillary thyroid carcinoma. Thyroid. 2013;23(10):1256–62.
Ito Y, Uruno T, Nakano K, et al. An observation trial without surgical treatment in patients with papillary microcarcinoma of the thyroid. Thyroid. 2003;13(4):381–7.
Jaeschke H, Mueller S, Eszlinger M, Paschke R. Lack of in vitro constitutive activity for four previously reported TSH receptor mutations identified in patients with nonautoimmune hyperthyroidism and hot thyroid carcinomas. Clin Endocrinol. 2010;73(6):815–20.
Jang EK, Song DE, Sim SY, et al. NRAS codon 61 mutation is associated with distant metastasis in patients with follicular thyroid carcinoma. Thyroid. 2014;24(8):1275–81.
Jarlov AE, Nygaard B, Hegedus L, Hartling SG, Hansen JM. Observer variation in the clinical and laboratory evaluation of patients with thyroid dysfunction and goiter. Thyroid. 1998;8(5):393–8.
Kim CS, Zhu X. Lessons from mouse models of thyroid cancer. Thyroid. 2009;19(12):1317–31.
Kimura T, Van Keymeulen A, Golstein J, Fusco A, Dumont JE, Roger PP. Regulation of thyroid cell proliferation by tsh and other factors: a critical evaluation of in vitro models. Endocr Rev. 2001;22(5):631–56.
Knudsen N, Perrild H, Christiansen E, Rasmussen S, Dige-Petersen H, Jorgensen T. Thyroid structure and size and two-year follow-up of solitary cold thyroid nodules in an unselected population with borderline iodine deficiency. Eur J Endocrinol. 2000;142(3):224–30.
Krane JF, Cibas ES, Alexander EK, Paschke R, Eszlinger M. Molecular analysis of residual ThinPrep material from thyroid FNAs increases diagnostic sensitivity. Cancer Cytopathol. 2015;123(6):356–61.

Krohn K, Paschke R. Progress in understanding the etiology of thyroid autonomy. J Clin Endocrinol Metab. 2001a;86(7):3336–45.
Krohn K, Paschke R. Loss of heterozygocity at the thyroid peroxidase gene locus in solitary cold thyroid nodules. Thyroid. 2001b;11(8):741–7.
Krohn K, Paschke R. BRAF mutations are not an alternative explanation for the molecular etiology of ras-mutation negative cold thyroid nodules. Thyroid. 2004;14(5):359–61.
Krohn K, Emmrich P, Ott N, Paschke R. Increased thyroid epithelial cell proliferation in toxic thyroid nodules. Thyroid. 1999;9(3):241–6.
Krohn K, Wohlgemuth S, Gerber H, Paschke R. Hot microscopic areas of iodine-deficient euthyroid goitres contain constitutively activating TSH receptor mutations [In Process Citation]. J Pathol. 2000;192(1):37–42.
Krohn K, Reske A, Ackermann F, Muller A, Paschke R. Ras mutations are rare in solitary cold and toxic thyroid nodules. Clin Endocrinol. 2001;55(2):241–8.
Krohn K, Stricker I, Emmrich P, Paschke R. Cold thyroid nodules show a marked increase in proliferation markers. Thyroid. 2003;13(6):569–75.
Krohn K, Fuhrer D, Bayer Y, et al. Molecular pathogenesis of euthyroid and toxic multinodular goiter. Endocr Rev. 2005;26(4):504–24.
Krohn K, Maier J, Paschke R. Mechanisms of disease: hydrogen peroxide, DNA damage and mutagenesis in the development of thyroid tumors. Nat Clin Pract Endocrinol Metab. 2007;3(10):713–20.
Kuma K, Matsuzuka F, Kobayashi A, et al. Outcome of long standing solitary thyroid nodules. World J Surg. 1992;16(4):583–7.
L'assurance maladie – Caisse Nationale. Pertinence des soins: une analyse de la pratique des thyroidectomies chez l'adulte. http://www.ameli.fr/fileadmin/user_upload/documents/23102013_DP_thyroidectomies.pdf. 2013. Ref Type: Online Source.
Labourier E, Shifrin A, Busseniers AE, et al. Molecular testing for miRNA, mRNA and DNA on fine needle aspiration improves the preoperative diagnosis of thyroid nodules with indeterminate cytology. J Clin Endocrinol Metab. 2015;100(7):2743–50.
Lang W, Georgii A, Stauch G, Kienzle E. The differentiation of atypical adenomas and encapsulated follicular carcinomas in the thyroid gland. Virchows Arch A Pathol Anat Histol. 1980;385(2):125–41.
Laulund AS, Nybo M, Brix TH, Abrahamsen B, Jorgensen HL, Hegedus L. Duration of thyroid dysfunction correlates with all-cause mortality. the OPENTHYRO Register Cohort. PLoS ONE. 2014;9(10):e110437.
Lazar V, Bidart JM, Caillou B, et al. Expression of the Na+/I− symporter gene in human thyroid tumors: a comparison study with other thyroid-specific genes. J Clin Endocrinol Metab. 1999;84(9):3228–34.
Lee SR, Jung CK, Kim TE, et al. Molecular genotyping of follicular variant of papillary thyroid carcinoma correlates with diagnostic category of fine-needle aspiration cytology: values of RAS mutation testing. Thyroid. 2013;23(11):1416–22.
Lewis CM, Chang KP, Pitman M, Faquin WC, Randolph GW. Thyroid fine-needle aspiration biopsy: variability in reporting. Thyroid. 2009;19(7):717–23.
Li H, Robinson KA, Anton B, Saldanha IJ, Ladenson PW. Cost-effectiveness of a novel molecular test for cytologically indeterminate thyroid nodules. J Clin Endocrinol Metab. 2011;96(11):E1719–26.
Lim DJ, Kim JY, Baek KH, et al. Natural course of cytologically benign thyroid nodules: observation of ultrasonographic changes. Endocrinol Metab (Seoul). 2013;28(2):110–8.
Liu RT, Hou CY, You HL, et al. Selective occurrence of ras mutations in benign and malignant thyroid follicular neoplasms in Taiwan. Thyroid. 2004;14(8):616–21.
Lodewijk L, Prins AM, Kist JW, et al. The value of miRNA in diagnosing thyroid cancer: a systematic review. Cancer Biomark. 2012;11(6):229–38.
Lu J, Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. Nature. 2005;435(7043):834–8.
Marinelli LD, Quimby EH, Hine GJ. Dosage determination with radioactive isotopes; practical considerations in therapy and protection. Am J Roentgenol Radium Ther. 1948;59(2):260–81.

Marques AR, Espadinha C, Catarino AL, et al. Expression of PAX8-PPAR gamma 1 rearrangements in both follicular thyroid carcinomas and adenomas. J Clin Endocrinol Metab. 2002;87(8):3947–52.
Marti JL, Avadhani V, Donatelli LA, et al. Wide inter-institutional variation in performance of a molecular classifier for indeterminate thyroid nodules. Ann Surg Oncol. 2015;22(12):3996–4001.
McIver B. Evaluation of the thyroid nodule. Oral Oncol. 2013;49(7):645–53.
McIver B, Castro MR, Morris JC, et al. An independent study of a gene expression classifier (Afirma) in the evaluation of cytologically indeterminate thyroid nodules. J Clin Endocrinol Metab. 2014;99(11):4069–77.
Medici M, Kwong N, Angell TE, et al. The variable phenotype and low-risk nature of RAS-positive thyroid nodules. BMC Med. 2015;13:184.
Mian C, Lacroix L, Alzieu L, et al. Sodium iodide symporter and pendrin expression in human thyroid tissues. Thyroid. 2001;11(9):825–30.
Moses W, Weng J, Sansano I, et al. Molecular testing for somatic mutations improves the accuracy of thyroid fine-needle aspiration biopsy. World J Surg. 2010;34(11):2589–94.
Najafzadeh M, Marra CA, Lynd LD, Wiseman SM. Cost-effectiveness of using a molecular diagnostic test to improve preoperative diagnosis of thyroid cancer. Value Health. 2012;15(8):1005–13.
Nikiforov YE, Steward DL, Robinson-Smith TM, et al. Molecular testing for mutations in improving the fine needle aspiration diagnosis of thyroid nodules. J Clin Endocrinol Metab. 2009;94(6):2092–8.
Nikiforov YE, Ohori NP, Hodak SP, et al. Impact of mutational testing on the diagnosis and management of patients with cytologically indeterminate thyroid nodules: a prospective analysis of 1056 FNA samples. J Clin Endocrinol Metab. 2011;96(11):3390–7.
Nikiforov YE, Carty SE, Chiosea SI, et al. Highly accurate diagnosis of cancer in thyroid nodules with follicular neoplasm/suspicious for a follicular neoplasm cytology by ThyroSeq v2 next-generation sequencing assay. Cancer. 2014;120(23):3627–34.
Nikiforov YE, Carty SE, Chiosea SI, et al. Impact of the multi-gene ThyroSeq next-generation sequencing assay on cancer diagnosis in thyroid nodules with atypia of undetermined significance/follicular lesion of undetermined significance cytology. Thyroid. 2015;25(11):1217–23.
Nikiforov YE, Seethala RR, Tallini G et al. Nomenclature revision for encapsulated follicular variant of papillary thyroid carcinoma: a paradigm shift to reduce overtreatment of indolent tumors. JAMA Oncol. 2016.
Nikiforova MN, Biddinger PW, Caudill CM, Kroll TG, Nikiforov YE. PAX8-PPARgamma rearrangement in thyroid tumors: RT-PCR and immunohistochemical analyses. Am J Surg Pathol. 2002;26(8):1016–23.
Nikiforova MN, Wald AI, Roy S, Durso MB, Nikiforov YE. Targeted next-generation sequencing panel (ThyroSeq) for detection of mutations in thyroid cancer. J Clin Endocrinol Metab. 2013;98(11):E1852–60.
Nishino M. Molecular cytopathology for thyroid nodules: A review of methodology and test performance. Cancer Cytopathol. 2016;124(1):14–27.
Nou E, Kwong N, Alexander LK, Cibas ES, Marqusee E, Alexander EK. Determination of the optimal time interval for repeat evaluation after a benign thyroid nodule aspiration. J Clin Endocrinol Metab. 2014;99(2):510–6.
Nygaard B, Hegedus L, Nielsen KG, Ulriksen P, Hansen JM. Long-term effect of radioactive iodine on thyroid function and size in patients with solitary autonomously functioning toxic thyroid nodules. Clin Endocrinol. 1999;50(2):197–202.
Oertel YC, Miyahara-Felipe L, Mendoza MG, Yu K. Value of repeated fine needle aspirations of the thyroid: an analysis of over ten thousand FNAs. Thyroid. 2007;17(11):1061–6.
Ohori NP, Nikiforova MN, Schoedel KE, et al. Contribution of molecular testing to thyroid fine-needle aspiration cytology of "follicular lesion of undetermined significance/atypia of undetermined significance". Cancer Cytopathol. 2010;118(1):17–23.
Okumura Y, Takeda Y, Sato S, et al. Comparison of differential diagnostic capabilities of 201Tl scintigraphy and fine-needle aspiration of thyroid nodules. J Nucl Med. 1999;40(12):1971–7.

Pagan M, Kloos RT, Lin CF, et al. The diagnostic application of RNA sequencing in patients with thyroid cancer: an analysis of 851 variants and 133 fusions in 524 genes. BMC Bioinformatics. 2016;17(Suppl 1):6.

Panzer C, Beazley R, Braverman L. Rapid preoperative preparation for severe hyperthyroid Graves' disease. J Clin Endocrinol Metab. 2004;89(5):2142–4.

Papini E, Pacella CM, Misischi I, et al. The advent of ultrasound-guided ablation techniques in nodular thyroid disease: towards a patient-tailored approach. Best Pract Res Clin Endocrinol Metab. 2014a;28(4):601–18.

Papini E, Pacella CM, Hegedus L. Diagnosis of endocrine disease: thyroid ultrasound (US) and US-assisted procedures: from the shadows into an array of applications. Eur J Endocrinol. 2014b;170(4):R133–46.

Parle JV, Maisonneuve P, Sheppard MC, Boyle P, Franklyn JA. Prediction of all-cause and cardiovascular mortality in elderly people from one low serum thyrotropin result: a 10-year cohort study. Lancet. 2001;358(9285):861–5.

Parma J, Duprez L, Van Sande J, et al. Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. Nature. 1993;365(6447):649–51.

Pisarev MA, Krawiec L, Juvenal GJ, et al. Studies on the goiter inhibiting action of iodolactones. Eur J Pharmacol. 1994;258(1–2):33–7.

Puxeddu E, Moretti S, Elisei R, et al. BRAF(V599E) mutation is the leading genetic event in adult sporadic papillary thyroid carcinomas. J Clin Endocrinol Metab. 2004;89(5):2414–20.

Rago T, Santini F, Scutari M, Pinchera A, Vitti P. Elastography: new developments in ultrasound for predicting malignancy in thyroid nodules. J Clin Endocrinol Metab. 2007;92(8):2917–22.

Reiners C, Schneider P. Radioiodine therapy of thyroid autonomy. Eur J Nucl Med Mol Imaging. 2002;29(Suppl 2):S471–8.

Reschini E, Ferrari C, Castellani M, et al. The trapping-only nodules of the thyroid gland: prevalence study. Thyroid. 2006;16(8):757–62.

Rippe V, Drieschner N, Meiboom M, et al. Identification of a gene rearranged by 2p21 aberrations in thyroid adenomas. Oncogene. 2003;22(38):6111–4.

Rivera M, Ricarte-Filho J, Knauf J, et al. Molecular genotyping of papillary thyroid carcinoma follicular variant according to its histological subtypes (encapsulated vs infiltrative) reveals distinct BRAF and RAS mutation patterns. Mod Pathol. 2010;23(9):1191–200.

Rochefort P, Caillou B, Michiels FM, et al. Thyroid pathologies in transgenic mice expressing a human activated Ras gene driven by a thyroglobulin promoter. Oncogene. 1996;12(1):111–8.

Roger PP, Servais P, Dumont JE. Stimulation by thyrotropin and cyclic AMP of the proliferation of quiescent canine thyroid cells cultured in a defined medium containing insulin. FEBS Lett. 1983;157(2):323–9.

Rossing M. Classification of follicular cell-derived thyroid cancer by global RNA profiling. J Mol Endocrinol. 2013;50(2):R39–51.

Russ G, Royer B, Bigorgne C, Rouxel A, Bienvenu-Perrard M, Leenhardt L. Prospective evaluation of thyroid imaging reporting and data system on 4550 nodules with and without elastography. Eur J Endocrinol. 2013;168(5):649–55.

Ryo UY, Vaidya PV, Schneider AB, Bekerman C, Pinsky SM. Thyroid imaging agents: a comparison of I-123 and Tc-99m pertechnetate. Radiology. 1983;148(3):819–22.

Saavedra HI, Knauf JA, Shirokawa JM, et al. The RAS oncogene induces genomic instability in thyroid PCCL3 cells via the MAPK pathway. Oncogene. 2000;19(34):3948–54.

Sandrock D, Olbricht T, Emrich D, Benker G, Reinwein D. Long-term follow-up in patients with autonomous thyroid adenoma. Acta Endocrinol. 1993;128(1):51–5.

Sapio MR, Guerra A, Marotta V, et al. High growth rate of benign thyroid nodules bearing RET/PTC rearrangements. J Clin Endocrinol Metab. 2011;96(6):E916–9.

Schroder S, Marthaler B. Autonomy and malignancy of thyroid glad tumors. A critical analysis of the literature on the existence of hyperfunctioning follicular and papillary thyroid gland carcinomas. Pathologe. 1996;17(5):349–57.

Schulten HJ, Salama S, Al-Ahmadi A, et al. Comprehensive survey of HRAS, KRAS, and NRAS mutations in proliferative thyroid lesions from an ethnically diverse population. Anticancer Res. 2013;33(11):4779–84.

Sebastianes FM, Cerci JJ, Zanoni PH, et al. Role of 18F-fluorodeoxyglucose positron emission tomography in preoperative assessment of cytologically indeterminate thyroid nodules. J Clin Endocrinol Metab. 2007;92(11):4485–8.

Smith TJ, Hegedus L. Graves' disease. N Engl J Med. 2016;375(16):1552–65.

Soares P, Trovisco V, Rocha AS, et al. BRAF mutations and RET/PTC rearrangements are alternative events in the etiopathogenesis of PTC. Oncogene. 2003;22(29):4578–80.

Soelberg KK, Bonnema SJ, Brix TH, Hegedus L. Risk of malignancy in thyroid incidentalomas detected by 18F-fluorodeoxyglucose positron emission tomography: a systematic review. Thyroid. 2012;22(9):918–25.

Stokowy T, Wojtas B, Fujarewicz K, Jarzab B, Eszlinger M, Paschke R. miRNAs with the potential to distinguish follicular thyroid carcinomas from benign follicular thyroid tumors – results of a meta-analysis. Horm Metab Res. 2013;46(3):171–80.

Stokowy T, Wojtas B, Krajewska J, et al. A two miRNA classifier differentiates follicular thyroid carcinomas from follicular thyroid adenomas. Mol Cell Endocrinol. 2015;399:43–9.

Stokowy T, Wojtas B, Jarzab B, et al. Two-miRNA-classifiers differentiate mutation-negative follicular thyroid carcinomas and follicular thyroid adenomas in Fine Needle Aspirations with high specificity. Endocrine. 2016;54(2):440–7.

Strickland KC, Howitt BE, Marqusee E, et al. The impact of noninvasive follicular variant of papillary thyroid carcinoma on rates of malignancy for fine-needle aspiration diagnostic categories. Thyroid. 2015;25(9):987–92.

Studer H, Peter HJ, Gerber H. Natural heterogeneity of thyroid cells: the basis for understanding thyroid function and nodular goiter growth. Endocr Rev. 1989;10(2):125–35.

Surks MI, Ortiz E, Daniels GH, et al. Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. JAMA. 2004;291(2):228–38.

Taton M, Lamy F, Roger PP, Dumont JE. General inhibition by transforming growth factor beta 1 of thyrotropin and cAMP responses in human thyroid cells in primary culture. Mol Cell Endocrinol. 1993;95(1–2):13–21.

Thomusch O, Machens A, Sekulla C, et al. Multivariate analysis of risk factors for postoperative complications in benign goiter surgery: prospective multicenter study in Germany. World J Surg. 2000;24(11):1335–41.

Toft AD. Clinical practice. Subclinical hyperthyroidism. N Engl J Med. 2001;345(7):512–6.

Tonacchera M, Viacava P, Agretti P, et al. Benign nonfunctioning thyroid adenomas are characterized by a defective targeting to cell membrane or a reduced expression of the sodium iodide symporter protein. J Clin Endocrinol Metab. 2002;87(1):352–7.

Trivalle C, Doucet J, Chassagne P, et al. Differences in the signs and symptoms of hyperthyroidism in older and younger patients. J Am Geriatr Soc. 1996;44(1):50–3.

Trulzsch B, Krohn K, Wonerow P, Paschke R. DGGE is more sensitive for the detection of somatic point mutations than direct sequencing. Biotechniques. 1999;27(2):266–8.

Trulzsch B, Krohn K, Wonerow P, et al. Detection of thyroid-stimulating hormone receptor and Gsalpha mutations: in 75 toxic thyroid nodules by denaturing gradient gel electrophoresis. J Mol Med. 2001;78(12):684–91.

Tuttle RM, Ball DW, Byrd D, et al. Thyroid carcinoma. J Natl Compr Cancer Netw. 2010;8(11):1228–74.

Tuttle RM, Haddad RI, Ball DW, et al. Thyroid carcinoma, version 2.2014. J Natl Compr Cancer Netw. 2014;12(12):1671–80.

van den Bruel A, Francart J, Dubois C, et al. Regional variation in thyroid cancer incidence in Belgium is associated with variation in thyroid imaging and thyroid disease management. J Clin Endocrinol Metab. 2013;98(10):4063–71.

Van Sande J, Parma J, Tonacchera M, Swillens S, Dumont J, Vassart G. Somatic and germline mutations of the TSH receptor gene in thyroid diseases. J Clin Endocrinol Metab. 1995;80(9):2577–85.

Vanderlaan PA, Krane JF, Cibas ES. The frequency of 'atypia of undetermined significance' interpretations for thyroid fine-needle aspirations is negatively correlated with histologically proven malignant outcomes. Acta Cytol. 2011;55(6):512–7.
Vasko V, Ferrand M, Di Cristofaro J, Carayon P, Henry JF, de Micco C. Specific pattern of RAS oncogene mutations in follicular thyroid tumors. J Clin Endocrinol Metab. 2003;88(6):2745–52.
Vassart G. Activating mutations of the TSH receptor. Thyroid. 2004;14(1):86–7.
Vidal-Trecan GM, Stahl JE, Durand-Zaleski I. Managing toxic thyroid adenoma: a cost-effectiveness analysis. Eur J Endocrinol. 2002;146(3):283–94.
Walsh PS, Wilde JI, Tom EY, et al. Analytical performance verification of a molecular diagnostic for cytology-indeterminate thyroid nodules. J Clin Endocrinol Metab. 2012;97(12):E2297–306.
Walter MA, Briel M, Christ-Crain M, et al. Effects of antithyroid drugs on radioiodine treatment: systematic review and meta-analysis of randomised controlled trials. BMJ. 2007;334(7592):514.
Wang CC, Friedman L, Kennedy GC, et al. A large multicenter correlation study of thyroid nodule cytopathology and histopathology. Thyroid. 2011;21(3):243–51.
Ward LS, Brenta G, Medvedovic M, Fagin JA. Studies of allelic loss in thyroid tumors reveal major differences in chromosomal instability between papillary and follicular carcinomas. J Clin Endocrinol Metab. 1998;83(2):525–30.
Watt T, Bjorner JB, Groenvold M, et al. Development of a short version of the thyroid-related patient-reported outcome ThyPRO. Thyroid. 2015;25(10):1069–79.
Weetman AP. Radioiodine treatment for benign thyroid diseases. Clin Endocrinol. 2007; 66(6):757–64.
Wienhold R, Scholz M, Adler JR, Nster G, Paschke R. The management of thyroid nodules: a retrospective analysis of health insurance data. Dtsch Arztebl Int. 2013;110(49):827–34.
Xing M. Molecular pathogenesis and mechanisms of thyroid cancer. Nat Rev Cancer. 2013; 13(3):184–99.
Xing M, Vasko V, Tallini G, et al. BRAF T1796A transversion mutation in various thyroid neoplasms. J Clin Endocrinol Metab. 2004;89(3):1365–8.
Yip L. Molecular markers for thyroid cancer diagnosis, prognosis, and targeted therapy. J Surg Oncol. 2015;111(1):43–50.
Yoo SK, Lee S, Kim SJ, et al. Comprehensive analysis of the transcriptional and mutational landscape of follicular and papillary thyroid cancers. PLoS Genet. 2016;12(8):e1006239.
Yoon JH, Kwon HJ, Lee HS, Kim EK, Moon HJ, Kwak JY. RAS mutations in AUS/FLUS cytology: does it have an additional role in BRAFV600E mutation-negative nodules? Medicine (Baltimore). 2015;94(27):e1084.
Zhu Z, Gandhi M, Nikiforova MN, Fischer AH, Nikiforov YE. Molecular profile and clinical-pathologic features of the follicular variant of papillary thyroid carcinoma. An unusually high prevalence of ras mutations. Am J Clin Pathol. 2003;120(1):71–7.

Part III

Thyroiditis

Hashimoto's Thyroiditis

7

Wilmar M. Wiersinga

Contents

Abstract

Hashimoto's thyroiditis is best defined as an organ-specific autoimmune disease, characterized by autoimmune-mediated destruction of the thyroid gland. Diagnostic criteria have changed dramatically since the first description in 1912; they now include the presence of antibodies against thyroid peroxidase (TPOAb) and thyroglobulin, hypoechogenicity on thyroid ultrasound, and often but not always hypothyroidism. Distinct pathologic phenotypes are recognized: goitrous and atrophic variants but also an IgG4-related variant, hashitoxicosis, juvenile thyroiditis, and

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silent or painless thyroiditis. With a prevalence of 10–12% in the general population, it is the most common autoimmune disease. The prevalence is higher in females than in males, increases with advancing age, and is highest in Whites and lowest in Blacks. The incidence of autoimmune hypothyroidism is about 350 cases/100,000/year for women and 60 cases/100,000/year for men in iodine-sufficient regions and 44 (females) and 12 (males) per 100,000 per year in iodine-deficient areas. Breakdown of self-tolerance against thyroid antigens may lead to thyroid autoimmunity. Loss of T_{reg} inhibitory actions and gain of Th17 proinflammatory actions (reflected by a shift to higher values of Th17/Th10 ratio in peripheral blood) play a crucial role in the loss of tolerance against thyroid antigens. Cytotoxic CD8+ T cells directed against TPO and Tg mediate thyroid gland destruction, either by the granule exocytosis pathway or apoptosis (programmed cell death). TPOAb and TgAb may cause antibody-dependent cell-mediated cytotoxicity (ADCC) via complement-mediated lysis of thyrocytes. Hashimoto's thyroiditis often runs in families as evident from a high sibling risk ratio of 28. Twin studies suggest genes contribute about 73% of the liability to the development of TPOAb and TgAb; environmental factors would thus contribute about 20–30%. Polymorphisms in *TSHR*, *Tg*, *HLA*, *CTLA-4*, *IL2RA*, and *FOXP3* have all been associated with Hashimoto's thyroiditis but account for only a small proportion of the heritability. Genome-wide association studies continue to detect novel genetic loci linked to TPOAb. Smoking and moderate alcohol consumption to a certain extent protect against Hashimoto's thyroiditis. Low selenium or vitamin D intake are presumably related to a higher prevalence of TPOAb, but presently there is no convincing evidence that selenium or vitamin D supplementation may lower TPOAb concentration. Infections may provoke Hashimoto's thyroiditis, but available epidemiological studies do not support a causative role.

Keywords
Hashimoto's thyroiditis · History · Diagnosis · Epidemiology · Immunopathogenesis · IgG4 · Genetic polymorphisms · Environment

Introduction

Autoimmune thyroid disease (AITD) can be defined as a complex disease characterized by an autoimmune response against thyroid antigens, which may develop against a certain genetic background and facilitated by exposure to particular environmental factors. Hashimoto's disease and Graves' disease are well-known examples of AITD: Hashimoto's disease is associated with antibodies against thyroid peroxidase (TPOAb) and hypothyroidism, whereas Graves' disease is associated with antibodies against thyroid-stimulating hormone receptor (stimulating TSHRAb) and hyperthyroidism (Aijan and Weetman 2015; Smith and Hegedus 2016). Thus, at first sight, there is a clear distinction between Hashimoto's and Graves' disease, TPOAb being the hallmark of Hashimoto's disease and TSHRAb being the hallmark of Graves' disease. The neat

dichotomy, however, is deceptive. TPOAb are also present in 70% of patients with Graves' disease, and hypothyroidism occurs in the long run in up to 20% of patients with Graves' hyperthyroidism who have entered remission after a course of antithyroid drugs; blocking TSHRAb contributed to the late development of hypothyroidism in one third of cases and chronic autoimmune thyroiditis in the remaining two thirds (Wood and Ingbar 1979; Hedley et al. 1989; Tamai et al. 1989). Conversely, some patients with Hashimoto's disease may have either stimulating or blocking TSHRAb (Konishi et al. 1983), and cases have been described in which Hashimoto's hypothyroidism converts into overt Graves' hyperthyroidism likely explained by a change from blocking to stimulating TSHRAb (Bell et al. 1985; McLachlan and Rapoport 2013). One may consider Hashimoto's hypothyroidism and Graves' hyperthyroidism as the extreme ends of a continuous spectrum of thyroid autoimmunity (Prummel and Wiersinga 2002; Mariotti 2012). It is tempting to speculate that the natural history of autoimmune thyroid disease is in principle that of Hashimoto's thyroiditis characterized by the occurrence of TPOAb and TgAb, with the slow development over years of subclinical and finally overt autoimmune hypothyroidism in some but not all patients (Effraimidis et al. 2011a). Development of Graves' hyperthyroidism in contrast is rather fast, happening in a few months; its occurrence might be considered the default of AITD, which usually follows the course of Hashimoto's thyroiditis. Hashimoto's thyroiditis can probably best be defined as an organ-specific autoimmune disease, characterized by autoimmune-mediated destruction of the thyroid gland. The diagnosis is nowadays suggested by a typical ultrasound pattern and the presence of TPOAb and/or TgAb (Radetti 2014). Indeed thyroid hypoechogenicity, among patients with goiter and circulating thyroid antibodies, identifies those with Hashimoto's thyroiditis who are prone to develop hypothyroidism (Fig. 1; Marcocci et al. 1991). Such characteristics are

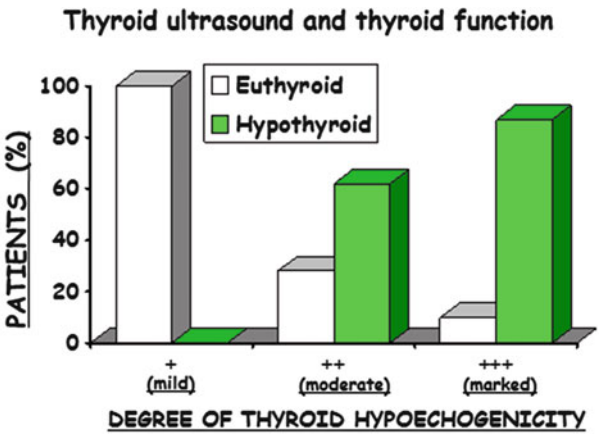

Fig. 1 Prevalence of hypothyroidism according to the degree of thyroid hypoechogenicity in patients with Hashimoto's thyroiditis and diffuse low thyroid echogenicity (Modified from Marcocci et al. 1991)

a world apart from the original description by Hashimoto himself, which mentions goitrous enlargement of the thyroid gland with lymphocytic infiltration ("struma lymphomatosa") (Hashimoto 1912). At the time of Hashimoto's paper, the very existence of thyroid antibodies was unknown (they were detected only in 1956 by Roitt et al.) (Roitt et al. 1956), and thyroid ultrasonography was gradually introduced in clinical practice not earlier than in the 1980s. The concept of Hashimoto's thyroiditis as a particular disease entity thus has evolved enormously over the past century. We will investigate the accuracy of the various diagnostic criteria for Hashimoto's thyroiditis. Applied criteria are from the disciplines of pathology (lymphocytic infiltrate?), clinical medicine (goiter?), immunology (thyroid antibodies?), radiology (thyroid hypoechogenicity?), and biochemistry (abnormal thyroid function?).

Diagnostic Criteria

Histopathology

Hashimoto's thyroiditis is typically a diffuse inflammation of the thyroid gland consisting of a combination of epithelial cell destruction, lymphoid cellular infiltration, and fibrosis. The thyroid cells are packed with mitochondria and have an acidophilic character; they are called Hűrthle or Askenazy cells. Clusters of macrophage-like cells can be seen within the follicles. The lymphoid infiltration in the interstitial tissue is accompanied by actual lymphoid follicles and germinal centers (Ben-Skowronek et al. 2011).

In the early 1960s, the consistency of the histopathological diagnosis of Hashimoto's disease was studied among pathologists (Masi et al. 1965). At that time uncertainty existed about the proper use of the terms Hashimoto's disease versus chronic thyroiditis: were the lesions designated by each term distinguishable from each other and were there specific criteria for each diagnosis? Randomly selected surgical specimens which had been indexed in the pathology department of the Johns Hopkins Hospital as either Hashimoto's disease, chronic thyroiditis, or other fibro-lymphocytic disorders were investigated in a blinded fashion by eight experts. They had to mark as absent, minimal, moderate, or severe each of the following histopathologic characteristics present in the original description of Hashimoto: lymphoid infiltration, lymphoid follicle formation, plasma cell infiltration, Hűrthle cell dysplasia, epithelial hyperplasia, epithelial desquamation, follicular atrophy, colloid nodules, and scarring and then make a diagnosis: Hashimoto's disease, chronic thyroiditis, or other disease. There was considerable difference between observers in their use of the terms Hashimoto's disease and chronic thyroiditis: both diagnoses were frequently made on the same slides by different observers. If, however, the diagnoses Hashimoto's disease and chronic thyroiditis were combined, the interobserver agreement increased appreciably to 85%. Hashimoto's disease was diagnosed preferentially on the more fully developed lesions showing marked alterations in all fields, whereas slides diagnosed as chronic thyroiditis tended to show focal alterations. The alterations were indistinguishable from each other under high-power magnification. The authors concluded that both terms indicated the same

disease entity, which they preferred to call Hashimoto's disease (Masi et al. 1965). But the label chronic lymphocytic thyroiditis seemed equally acceptable. The issue of focal thyroiditis has been given much consideration. Early studies recognized its frequent occurrence in macroscopically normal thyroids at necropsy, chiefly in middle-aged and elderly women; it was regarded as a precursor or a nonprogressive form of the diffuse chronic thyroiditis associated with primary myxedema (Williams and Doniach 1962). A quantitative study on the postmortem incidence of focal thyroiditis was done in all thyroids sectioned over 5 years (1948–1953) at the Hammersmith Hospital in London, UK. Thyroid sections were available in 40% of all necropsies; availability depended more on the person carrying out the examination than on the nature of the disease, and therefore it was thought the observed trends in sex and age distribution were generally reliable. The sole criterion applied for the existence of focal thyroiditis was the presence of a collection of lymphocytes or plasma cells easily visible with a low-power objective in 6-μ-thick sections. The presence of follicle breakdown or oxyphil cell change was not considered a prerequisite for the diagnosis of focal thyroiditis, although follicle breakdown was almost invariably and oxyphil change frequently present in those cases with more than 10 foci per square cm. Sections from 724 thyroids were examined. Focal thyroiditis (>10 foci/cm^2) was present in 6% of adult males and 22% of adult females, with a higher incidence in older women (Williams and Doniach 1962). An increase in the incidence and severity of focal thyroiditis was found in the presence of the autoimmune disease pernicious anemia, suggesting that focal thyroiditis points to weakening of immune tolerance. Whether all cases of focal thyroiditis belong to the disease entity of Hashimoto's disease is uncertain. On the one hand, the presence of focal thyroiditis in thyroid tissue obtained by biopsy and at autopsy from patients with no evidence of hypothyroidism during life is correlated to raised serum concentrations of TPOAb and TgAb (Vanderpump 2011). On the other hand, focal thyroiditis is not uncommon, and practically all thyrotoxic glands removed at operation show small lymphocytic foci. Does this mean that the thyroid is limited in its pattern of reaction and that several distinct processes lead to the same morphological end result? Such questions are still not answered in a satisfactory manner.

To celebrate the centennial of Hashimoto's original paper in 2012, the surgical pathology archives of the Johns Hopkins Hospital were searched for cases of Hashimoto's thyroiditis in the period 1889–2012 (Caturegli et al. 2013). Cases with lymphocytic infiltration of the thyroid gland with germinal center formation and Hűrthle cell metaplasia were accepted as Hashimoto's thyroiditis. However, the presence of a simple lymphocytic infiltration was not considered indicative of Hashimoto's thyroiditis; such lesions of focal nature without germinal centers and lack of Hűrthle cells are often referred to as chronic nonspecific thyroiditis. The first case labeled as Hashimoto appeared in 1942, 30 years after the original description. Prior to this date, there were three cases satisfying the diagnostic criteria (in 1928, 1935, and 1939). Between 1942 and 2012, a total of 867 cases of Hashimoto's thyroiditis were identified, 6% of all thyroid surgical specimens. Approximately half of the cases ($n = 462$) were isolated, meaning Hashimoto's thyroiditis was the sole pathologic finding. The remaining half ($n = 405$) were those in which Hashimoto's thyroiditis was found incidentally in association with other pathologies, mainly with

papillary thyroid carcinoma (but also with Hűrthle cell carcinoma and medullary thyroid carcinoma). Papillary thyroid carcinoma was found in 231 of the 867 cases (26.6%) of Hashimoto's thyroiditis (Caturegli et al. 2013). This prevalence is similar to that reported from Krakow (106 papillary cancers out of 452 cases of Hashimoto's thyroiditis, 23.5%); interestingly, this prevalence of 23.5% is threefold higher than the 7.5% prevalence of papillary thyroid carcinoma in non-Hashimoto's thyroiditis patients (Konturek et al. 2013). The number of annual Hashimoto cases at Johns Hopkins increased significantly over the period 1943–1967 and then remained constant between 1968 and 1992, before increasing again between 1993 and 2012 (Caturegli et al. 2013). The main contributor to the recent increase in incidence was Hashimoto's thyroiditis associated with papillary thyroid carcinoma. The annual increase in papillary thyroid carcinoma was accompanied not only by increases in cases associated with Hashimoto's thyroiditis but also by increases in cases associated with less prominent lymphocytic infiltration sometimes referred to as chronic nonspecific thyroiditis. The overall conclusion is that modern-day pathologic features of Hashimoto's thyroiditis are nearly identical to the ones originally reported by Hashimoto in 1912 (Caturegli et al. 2013).

Thyroid Antibodies

After the 1956 discovery of thyroid antibodies in the serum of patients with Hashimoto's disease (Roitt et al. 1956), measurement of TPOAb (originally known as thyroid microsomal antibodies) and TgAb gradually entered daily clinical practice. Consequently, as of the 1970s the presence of TPOAb and/or TgAb in serum was considered as positive proof for the existence of Hashimoto's thyroiditis. The original assays (e.g., applied in the Whickham Survey in 1972) used a semi-quantitative particle agglutination technique. But improved technology employing purified antigens and monoclonal antibodies led to quantitative immunoassays with higher sensitivity and specificity in the next decades. It resulted in assays capable to detect TPOAb and TgAb in the serum of every single healthy person (Zophel et al. 2003). This generated the quest for reliable reference intervals, as only elevated concentrations of thyroid antibodies in serum would permit the diagnosis of thyroid autoimmunity. The upper normal limit can be determined in various ways:

1. By a traditional nonparametric scale
2. By NACB (National Academy of Clinical Biochemistry) criteria, i.e., restricting blood sampling to males younger than 30 years without risk factors for thyroid autoimmunity and with TSH values between 0.5 and 2.5 mU/L (Demers and Spencer 2003)
3. By a model of "composite logarithmic Gaussian distributions" (Jensen et al. 2006)

The 97.5% upper limits (using an immunometric assay with detection limits of $<$1.0 kU/L for both TPOAb and TgAb) according to these three models were

284, 24, and 9.8 kU/L for TPOAb, respectively, and 84, 22, and 19 lU/L for TgAb. The decision value (defined as the concentration corresponding to 0.1% false positives) was 15 kU/L for TPOAb and 31 kU/L for TgAb (Jensen et al. 2006). For meaningful interpretation of results, the assay methodology and especially its upper normal limit should be known. Patients with hypothyroidism caused by either atrophic or goitrous autoimmune thyroiditis have rather high serum concentrations of TPOAb and TgAb, but these antibodies are also present – albeit usually at lower concentrations – in 70% of patients with Graves' hyperthyroidism. Serum TPOAb may not be detected in about 10% of individuals with ultrasound evidence of Hashimoto's thyroiditis (Biondi and Cooper 2008). Cases of seronegative Hashimoto's thyroiditis have been described in which thyroid autoantibody production was localized to the thyroid (Baker et al. 1988). Nevertheless, serum TPOAb and TgAb remain sensitive and specific markers for thyroid autoimmunity. Interestingly, antibodies against the TSHR have also been described in Hashimoto's thyroiditis. Blocking TSHRAb were detected already in the 1980s (Konishi et al. 1983; Endo et al. 1978), but their role in various conditions remained controversial. With the advent of more accurate assays for blocking and stimulating TSHRAb, it has become clear that patients can exhibit a mixture of both blocking and stimulating TSHRAb, the ratio of which may vary over time and influence the clinical presentation (Evans et al. 2010; Li et al. 2013a; Diana et al. 2016). Stimulating TSHRAb are present in all patients with Graves' hyperthyroidism with or without Graves' orbitopathy but also in 5.5% of patients with Hashimoto's thyroiditis (defined as euthyroid or hypothyroid patients with at least fivefold increased levels of TPOAb and heterogeneous hypoechoic pattern on thyroid ultrasound) and in 68.2% of patients with Hashimoto's thyroiditis and coexistent Graves' orbitopathy (Kahaly et al. 2016). Autoimmunity against the TSH receptor consequently is not limited to Graves' disease but is involved in Hashimoto's thyroiditis as well.

Goiter

Following wider application of TPOAb and TgAb assays, it became obvious that these antibodies were also present in subjects without goiter. Actually, the vast majority of patients with Hashimoto's thyroiditis have no goiter, and at the time of diagnosis only a minority of patients with overt autoimmune hypothyroidism have goiter (Laurberg et al. 1999). The prevalence of goiter in the setting of Hashimoto's thyroiditis is in the order of 5–10% according to a rough estimate.

Thyroid Function

In a study published in 1992, a correlation was sought between thyroid function and histology in 601 patients with chronic thyroiditis, as characterized by mononuclear cell infiltration (Mizukami et al. 1992). There were 137 patients (23%) with oxyphilic chronic thyroiditis (moderate to severe diffuse cell infiltration, follicular

epithelial changes almost in all cells, mild to severe fibrosis); their median age was 44 years and 85% were hypothyroid (latent in 38% and overt in 47%). In the group of 161 patients (27% of total) with mixed chronic thyroiditis (moderate diffuse cell infiltration, various follicular epithelial changes, and minimal to mild fibrosis), median age was 38 years; 39% had hypothyroidism (latent in 33.5% and overt in 5.5%), 38% were euthyroid and 23% hyperthyroid. Focal thyroiditis was observed in 149 patients (25%) with a median age of 37 years; hypothyroidism was present in 15% (all except one had latent hypothyroidism), euthyroidism in 83%, and hyperthyroidism in 2%. The greater the extent of cell infiltration in focal thyroiditis, the smaller the proportion of patients who were euthyroid and the greater the proportion of latent hypothyroid patients. Hyperplastic chronic thyroiditis (mild to moderate focal cell infiltration, mild to severe diffuse hyperplastic epithelial cell changes) occurred in 154 patients (26%) with a median age of 33 years; hypothyroidism was present in 10% (latent in 3% and overt in 7%), euthyroidism in 5%, and hyperthyroidism in 85%. Taken together, patients with the classic oxyphilic variant were the oldest and those with hyperplastic thyroiditis the youngest (44 vs. 33 years), whereas hypothyroidism was most prevalent in the oxyphilic variant (85%) and hyperthyroidism most in the hyperplastic variant (85%). Patients with focal thyroiditis had the highest proportion of euthyroidism (83%), and their mean age of 37 years lay in between those of the two other groups. Hashimoto's thyroiditis may thus occur in the presence of hypothyroidism, euthyroidism, or hyperthyroidism, and thyroid function by itself lacks sufficient sensitivity and specificity for the diagnosis of Hashimoto's thyroiditis.

Thyroid Ultrasonography

Marked diffuse or inhomogeneous hypoechogenicity or patchy echo pattern are typical sonographic signs of Hashimoto's thyroiditis (Bennedbaek and Hegedus 2007). Hypoechogenicity is a consequence of lymphocytic aggregations, which appear as very homogeneous tissue without reflecting surfaces (Radetti 2014). Thus, abnormal echogenicity is observed in all patients with Hashimoto's thyroiditis (Fig. 2). The high sensitivity of thyroid ultrasonography is further illustrated by reports that a hypoechoic ultrasound pattern or an irregular echo pattern may precede the occurrence of TPOAb in serum (Biondi and Cooper 2008). Specificity of hypoechogenicity is however more limited: e.g., a homogeneous and diffusely hypoechoic echo pattern is observed in Graves' disease.

Taken together, none of the abovementioned diagnostic criteria has 100% sensitivity and specificity for the diagnosis of Hashimoto's thyroiditis. Histopathology comes close, although the issue of focal thyroiditis has not been resolved satisfactorily. TPOAb and TgAb, if elevated, do indicate thyroid autoimmunity in general; they are more associated with Hashimoto's thyroiditis and hypothyroidism than with Graves' hyperthyroidism. The absence of goiter and hypothyroidism does not exclude the diagnosis of Hashimoto's thyroiditis. The finding of thyroid hypoechogenicity can be of great help. It follows that the combination of thyroid

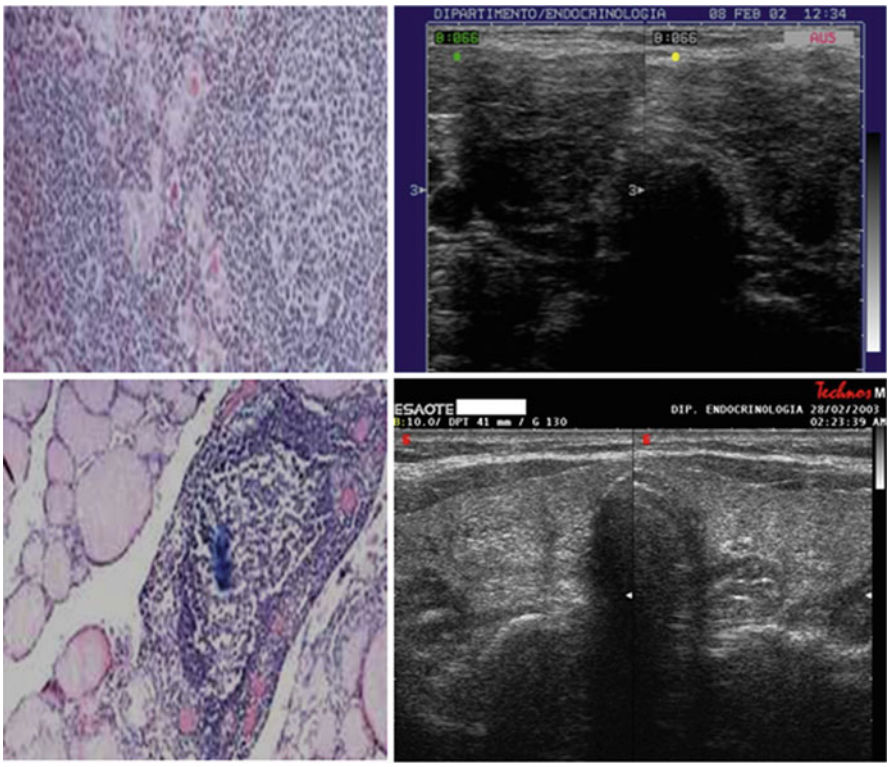

Fig. 2 Thyroid ultrasonography and cytology in two patients with Hashimoto's thyroiditis: note hypoechogenicity and lymphocytic infiltration in patient A with diffusely enlarged thyroid gland (*top panel*: TSH 1.8 mU/L, TPOAb >1,000 kU/L, TgAb 157 kU/L) and patient B with nodular goiter (*bottom panel*: TSH 0.5 mU/L, TPOAb 1,000 kU/L, TgAb 89 kU/L) (Courtesy of Prof. Paolo Vitti, Pisa, Italy)

ultrasound, thyroid function tests, and thyroid antibodies usually allows to make a reliable diagnosis of Hashimoto's thyroiditis. Cytology and histopathology are nowadays rarely required to ascertain the diagnosis.

Classification of Hashimoto's Thyroiditis and Its Variants

A number of variants of Hashimoto's thyroiditis have been described (Doniach et al. 1979; Livolsi 1994; Caturegli et al. 2014) and listed in Table 1:

Fibrous variant. The fibrosis is dominant (in contrast to what is seen in the classic variant and the IgG4-related variant), still remaining within the thyroid capsule (thus distinguishing it from Riedel's thyroiditis with its adhesion to the surrounding structures) (Caturegli et al. 2014). Pathologically, the thyroid architecture is destroyed; there is marked follicular atrophy, dense keloid-like fibrosis, and

Table 1 Characteristics of the various forms of Hashimoto's thyroiditis

	Juvenile	Hashitoxicosis	IgG4 related	Classic (goitrous)	Fibrous (atrophic)
Peak age at onset	10–18 year	40–60 year	40–50 year	40–60 year	60–70 year
F:M ratio	6:1	5:1	3:1	12:1	10:1
Thyroid function at presentation	Normal/subclinical hypo	Hyperthyroid	Hypothyroid	Mostly normal	Hypothyroid
Ultrasonography	Hypoechogenicity	Hypoechogenicity	Strong hypoechogenicity	Hypoechogenicity	Hypoechogenicity + nodularity
24 h RAI uptake	Variable	Increased	Unknown	Variable	Decreased
Fibrosis	No	No	Yes	Yes	Severe

Modified from Caturegli et al. 2014

prominent squamous metaplasia (Livolsi 1994). It occurs in about 10% of cases, often affecting elderly people with symptomatic goiters and hypothyroidism (Katz and Vickery 1974). The fibrous variant coincides mostly with the atrophic subtype. Both names, however, do not always indicate the same condition. For example, there are patients belonging to the fibrous variant, who present with a goiter and consequently cannot be regarded as having atrophic thyroiditis.

Goitrous and atrophic variants. Chronic autoimmune thyroiditis has a goitrous (classic) form often referred to as Hashimoto's disease (in agreement with the presence of a goiter in the patients described by Hashimoto himself in 1912) and an atrophic form sometimes referred to as Ord's disease (as first reported by W.M. Ord in 1888 in the famous Report on Myxedema from the Clinical Society of London, mentioning the thyroid as "in every case reduced in size, and described variously as durated, fibrous and structureless") (Dayan and Daniels 1996; Davies 2003). Goitrous autoimmune thyroiditis is characterized by diffuse lymphocytic infiltration with occasional germinal centers, thyroid follicles of reduced size containing sparse colloid, and fibrosis. Although the follicles are small, individual thyroid cells often appear enlarged and contain cytoplasma that is granular and pink (oxyphil change), known as Hűrthle or Askenazy cells (Dayan and Daniels 1996). In atrophic autoimmune thyroiditis, the thyroid gland is small, with lymphocytic infiltration and fibrous tissue replacing the thyroid parenchyma. It has been proposed that goitrous autoimmune thyroiditis and atrophic autoimmune thyroiditis (also known as primary or idiopathic myxedema) are two separate disease entities, the former associated with HLA-DR5 and the latter with HLA-DR3/B8 (Irvine et al. 1978; Doniach 1981). Atrophic thyroiditis, as compared to goitrous thyroiditis, is further associated with greater antibody-dependent cell-mediated cytotoxicity (Bogner et al. 1995) and a higher prevalence of TSH receptor-blocking antibodies (Takasu et al. 1987; Chiovato et al. 1990; Cho et al. 1995). It has been hypothesized that atrophic thyroiditis represents the end stage of goitrous thyroiditis. Actually, a number of studies argue against this hypothesis. The age at presentation is not different between either subtypes (Bogner et al. 1995; Carlé et al. 2009). Little histologic progression has been observed in patients with Hashimoto's thyroiditis undergoing second biopsies up to 20 years after the first (Vickery and Hamblin 1961; Hayashi et al. 1985). Goitrous lymphocytic thyroiditis changed little as a function of time in many patients regardless of whether thyroid hormone was administered (Hayashi et al. 1985). In a population-based study in Denmark, the prevalence of subclinical goitrous Hashimoto's disease was 0.62% (thyroid volume $>$14.9 ml) and of subclinical autoimmune atrophic thyroiditis was 0.24% (thyroid volume $<$6.6 ml); there was a strong association between large volume and TPOAb/TgAb but only in subjects with elevated TSH ($>$3.6 mU/L) (Bulow Pedersen et al. 2005). In another Danish population-based study, all patients with incident overt autoimmune hypothyroidism were prospectively identified (Carlé et al. 2009). Thyroid volume showed a Gaussian distribution in both males and females with no bimodal pattern. Thyroid volume was positively associated with TPOAb and TgAb concentrations and negatively with echogenicity and serum TSH. Thyroid volume was not related to the prevalence of

TSH receptor antibodies nor to duration of symptoms before diagnosis. The authors conclude that goitrous thyroiditis and atrophic autoimmune thyroiditis are only extremes of a continuous Gaussian distribution and do not represent separate disorders. However, patients with low or high thyroid volume differ with respect to several characteristics.

IgG4-related variant. IgG4-related disease is a newly recognized fibro-inflammatory condition characterized by a tendency to form tumefactive lesions at multiple sites, a peculiar histopathologic appearance, and often but not always elevated serum IgG4 concentrations (Deshpande et al. 2012a). Critical histopathological features are a dense lymphoplasmacytic infiltrate (predominantly T cells with scattered aggregates of B cells; plasma cells are essential and may predominate), a storiform pattern of fibrosis (irregularly whorled pattern), and obliterative phlebitis. The diagnosis of IgG4-related diseases requires tissue IgG4 immunostaining: $IgG4^+$ plasma cells that number >50 per high-power field might be specific but appropriate cutoffs vary per organ. The $IgG4^+/IgG^+$ plasma cell ratio might be a more accurate diagnostic tool than $IgG4^+$ plasma cell count, with a ratio of >40% as a comprehensive cutoff value in any organ. The serum IgG4 concentration is elevated in many patients with IgG4-related disease but is normal in up to 40% of patients with biopsy-proven IgG4-related disease. Neither elevated serum IgG4 nor elevated numbers of $IgG4^+$ plasma cells in tissue are specific for IgG4-related disease. Morphological features in the appropriate clinical context form the basis for the diagnosis of IgG4-related disease (Deshpande et al. 2012a). Riedel's thyroiditis is the prime example of an IgG4-related thyroid disease (Dahlgren et al. 2010). IgG4-related variants of Hashimoto's thyroiditis were first described in 2009 (Li et al. 2009). Among 70 Japanese patients, who had undergone thyroidectomy because of goitrous Hashimoto's thyroiditis, 19 (27%) had IgG4 thyroiditis (Li et al. 2010). Patients with IgG4 thyroiditis showed a higher grade of stromal fibrosis, lymphoplasmacytic infiltration, and follicular cell degeneration than non-IgG4 thyroiditis patients. IgG4 thyroiditis was further associated with a higher proportion of male gender, a shorter disease duration, more prevalent subclinical hypothyroidism, higher serum IgG4, higher concentration of TPOAb and TgAb, and more frequently low echogenicity at ultrasound examination. The prevalence of IgG4-related Hashimoto's thyroiditis was 36%, 23%, and 13% among all cases of Hashimoto's thyroiditis in subsequent studies (Tasli et al. 2014; Zhang et al. 2014; Jokisch et al. 2016). The IgG4-related variant in these studies was associated with younger age, relatively more males, more fibrosis, and greater tissue expression of TGF-β1. The fibrous variant of Hashimoto's thyroiditis was proposed to be a IgG4-related thyroid disease (Deshpande et al. 2012b). A recent German study observed a 96% prevalence of the fibrous variant among IgG4-related Hashimoto's thyroiditis, whereas the prevalence was 18% in non-IgG4-related Hashimoto's thyroiditis (Jokisch et al. 2016). So the majority of cases with the fibrous variant are not IgG4 related. The clinical relevance of identifying the IgG4-related variant is unclear, especially because the pathogenetic role of IgG4 – if any – has not been resolved. IgG4 (in contrast to IgG1) is

not causing damage to thyroid cells (Guo et al. 1997). However, it could be speculated that IgG4-related thyroiditis might benefit from steroids which could diminish progression of fibrosis (Minamino et al. 2016).

Hashitoxicosis. Thyroidal lymphocytic infiltration is similar to that in the classic (goitrous) form, but germinal center formation is rare or absent, follicular atrophy absent or actually replaced by hyperplasia, Hűrthle cell metaplasia less extensive, and fibrosis milder (Caturegli et al. 2014). Hashitoxicosis, described first by Fatourechi in 1971 (Fatourechi et al. 1971), has the clinical features of Graves' hyperthyroidism (elevated thyroidal radioiodine uptake and presence of stimulating TSHRAb) but the histopathology of Hashimoto's thyroiditis. The hyperthyroidism is transient and evolves into permanent hypothyroidism after 3–24 months (Wasniewska et al. 2012a). This sequence of events is frequently related to a change from stimulating to blocking TSHRAb (Nabhan et al. 2005).

Juvenile thyroiditis has the same pathology as hashitoxicosis. Most children have goiter but are usually asymptomatic. At time of diagnosis, 43% are euthyroid, and 24% have subclinical hypothyroidism, 21% overt hypothyroidism, 9% overt hyperthyroidism, and 3% subclinical hyperthyroidism (Demirbilek et al. 2007; Wasniewska et al. 2012b). The natural history is variable, with remissions and recurrences or evolution to permanent hypothyroidism.

Silent or painless thyroiditis and *postpartum thyroiditis* can be considered as still other variants of Hashimoto's thyroiditis. Thyroid biopsy shows follicular disruption and lymphocytic thyroiditis, while stromal fibrous and oxyphilic changes are rare. Dissimilarities with the classic form include the relative lack of oncocytic metaplasia, minimal to absent follicular atrophy, and mild or no fibrosis (Mizukami et al. 1988, 1993). Typically, these conditions have a triphasic pattern, starting with thyrotoxicosis, followed by hypothyroidism, and then recovery. The initial thyrotoxicosis is not caused by excessive production of thyroid hormones by the thyroid gland but rather by the release of preformed hormones from the thyroid follicles caused by destructive thyroiditis. Whereas most patients recover from the subsequent hypothyroid phase with restoration of the normal thyroid architecture, permanent hypothyroidism may occur later. The natural history of these conditions fits with the definition of Hashimoto's thyroiditis as an autoimmune-mediated destruction of the thyroid gland.

Classification system. There is no satisfactory or internationally accepted classification of Hashimoto's thyroiditis and its variants. Hashimoto's thyroiditis, chronic lymphocytic thyroiditis, and chronic autoimmune thyroiditis are used in the literature almost as synonyms, often without mentioning clear diagnostic criteria. Pathologists continue to employ terms in their histologic diagnoses of AITD that clinicians rarely use because they have little clinical relevance (Table 1; Davies and Amino 1993). One attempt for a clinically and scientifically useful and meaningful classification is presented in Table 2 (Davies 2003; Davies and Amino 1993). The scheme has not attracted much attention, and it is not being used. Nevertheless, a classification system which is agreed upon by the scientific community would be very helpful. It could form the basis for further exploration of differences in immunopathogenesis between the various variants, which up to now remain largely unknown.

Table 2 Proposed classification of human autoimmune thyroiditis

Type	Thyroid function	Thyroid antibodies
Type 1. Euthyroid autoimmune thyroiditis (Hashimoto's thyroiditis)		
1A. Goitrous	TSH normal	TPOAb and TgAb
1B. Non-goitrous		
Type 2. Hypothyroid autoimmune thyroiditis (Hashimoto's thyroiditis)		
2A. Goitrous	TSH increased FT4 decreased	TPOAb and TgAb TSHRAb (blocking) in non-goitrous subset
2B. Non-goitrous		
2C. Transient aggravation[a]	Initial TSH decreased later TSH increased	TPOAb and TgAb
Type 3. Autoimmune thyroiditis (Graves' disease)		
3A. Hyperthyroid	TSH decreased FT4 increased	TSHRAb (stimulating) usually TPOAb and TgAb
3B. Euthyroid	TSH normal FT4 normal	TSHRAb (stimulating) usually TPOAb and TgAb
3C. Hypothyroid[b]	TSH increased FT4 decreased	TSHRAb (stimulating) usually TPOAb and TgAb

Modified from Davies and Amino (1993) and Davies (2003)
[a]May start as transient destructive thyrotoxicosis, often followed by transient hypothyroidism (postpartum thyroiditis, painless or silent thyroiditis)
[b]Hypothyroidism with Graves' orbitopathy

Epidemiology

Hashimoto's thyroiditis was considered a rare condition in the first half of the twentieth century (Caturegli et al. 2013). This changed after the detection of thyroid antibodies in serum in 1956 and the introduction of simple immunoassays for these antibodies as of the 1970s. Nowadays, Hashimoto's thyroiditis appears to be very common and actually is the most prevalent autoimmune disease among all autoimmune diseases. Comparison of incidence and prevalence figures between studies is hampered by a number of factors (McLeod and Cooper 2012). First, disease definition may be different: diagnostic methods vary between eras, and disease definition is not standardized. Second, laboratory techniques vary among eras and may not be standardized even when similar techniques are used, and positive cutoff points are not standardized. Third, study populations are rarely comparable. Thus, the combination of genetic background and environmental exposures is certainly different, and there is no standardization for age, gender, and race among studies. Despite these inherent difficulties, available data allow a number of general conclusions about the epidemiology of Hashimoto's thyroiditis. Data of the most relevant studies have been summarized in a number of recent papers (Vanderpump 2011; McLeod and Cooper 2012; McGrogan et al. 2008; Endocrine Society 2015). Hashimoto's thyroiditis in the majority of these studies was diagnosed by the presence of TPOAb and/or TgAb, with or without autoimmune hypothyroidism. However, postpartum thyroiditis and painless/silent

thyroiditis were not included in most studies, although these conditions could be considered as still other variants of Hashimoto's thyroiditis in view of their autoimmune-mediated destructive type of thyroiditis.

Prevalence

The epidemiological studies allow firm conclusions with respect to the prevalence of Hashimoto's thyroiditis: (a) the prevalence is very high in the general population; (b) the prevalence is higher in females than in males; (c) the prevalence increases with advancing age; and (d) the prevalence is highest in Whites and lowest in Blacks, whereas the prevalence in Hispanics lies in between those of Whites and Blacks (Tables 3, 4, and 5). The reported prevalence of 10–12% for Hashimoto's thyroiditis in the general population is higher than for any other autoimmune disease. Prevalences vary between populations. One explanation for such differences is ambient iodine intake: prevalence of Hashimoto's thyroiditis is lower in iodine-deficient and higher in iodine-replete regions (Bulow Pedersen et al. 2002). In line with other autoimmune diseases, there is a strong *female preponderance* (Table 3). For instance, in the NHANES III study, the prevalence of TPOAb was 17.0% in women and 8.7% in men, and the prevalence of TgAb was 15.2% in women and 7.6% in men (Hollowell et al. 2002). The female to male ratio varies between 5:1 and 2:1. An equal sex ratio of 1:1 was only observed in APS1 (autoimmune polyglandular syndrome type 1), caused by a genomic mutation in *AIRE* (autoimmune regulator gene) (Nithiyananthan et al. 2000; Meyer et al. 2001). In a large collective of 686 children and adolescents with Hashimoto's disease, the female to male ratio was 2.8:1 (Demirbilek et al. 2007; Wasniewska et al. 2012b; Radetti et al. 2006). Available data suggest that the female preponderance becomes more marked in adulthood. The question why thyroid autoimmunity preferentially occurs in women remains incompletely resolved. Sex steroids might be involved as well as pregnancy. Recovering from the immune effects of pregnancy, a rebound reaction may activate the Th1-mediated pathway leading to cellular immunity and destruction of thyroid follicular cells (Weetman 2010). In susceptible subjects it could result in postpartum thyroiditis, a prevalent condition associated with the development of permanent autoimmune hypothyroidism (Azizi 2005; Stagnaro-Green et al. 2011). However, conflicting results are reported in the literature with regard to a possible association between parity, thyroid antibodies, and autoimmune thyroid disease (Walsh et al. 2005; Bulow Pedersen et al. 2006; Friedriech et al. 2008). A recent paper reports a dose-dependent association between development of autoimmune overt hypothyroidism and the number of live births and induced abortions but only in premenopausal women: odds ratios for hypothyroidism after 1, 2, or $\geq$3 live births were 1.72, 3.12, and 4.51, respectively, and after 1 or $\geq$2 induced abortions 1.02 and 2.70, respectively (Carlé et al. 2014). The prevalence increased with *advancing age* (Table 4). In the NHANES III study, the prevalence of TPOAb increased fivefold (from 4.8% to 23.9%) and of TgAb threefold (from 6.3% to 21.6%) between the second and ninth decades of life. The effect of age was even more prominent when

Table 3 Incidence and prevalence figures of Hashimoto's thyroiditis

Author and year	*Population*	*Method*	*Incidence per 100,000/year*[a]	*Prevalence*[b]
Furszyfer et al. (1970)	Olmsted Co, USA $n = 240$ cases, all F, Age 0–70^{+} Year 1935–1967	Tissue sample Clinical signs	F only 6.5 (1935–1944) 21.4 (1945–1954) 67.0 (1955–1964) 69.0 (1965–1967)	F, 10.5% M, 0.26%
Tunbridge et al. (1977)	Whickham, UK $n = 2779$ Age ≥20 Year 1972	Hemagglutination TgAb		F, 16.2% M, 4.3%
Sundbeck et al. (1991)	Gothenburg, Sweden $n = 514$ Age 70–79 Year 1971–1988	TSH	F, 243	
Galofre et al. 1994 (1994)	Vigo, Spain $n = 278{,}370$ Year 1990–1992	TSH, FT4 TPOAb and TgAb ultrasonography	F, 45.4 M, 2.2	
Vanderpump et al. (1995)	Whickham, UK $n = 1877$ 1972–1992	Serum TSH, FT4	F, 350 M, 60	F, 10.3% M, 2.7%
Hollowell et al. (2002)	USA population $n = 17{,}353$ Age ≥12 Year 1988–2004	Serum TSH, FT4 TPOAb and TgAb		F, 17.0% M, 8.7%
Leese et al. (2008)	Tayside, Scotland $n = 390{,}000$ 1994–2001	Serum TSH TPOAb and TgAb	F, 448 M, 92	
Zaletel et al. (2011)	Ljubljana, Slovenia $n = 1{,}000{,}000$ Adults 1999 and 2009	Serum TSH, FT4 TPOAb and TgAb ultrasonography	36.9 (1999) 68.8 (2009)[c]	
Carlé et al. (2006a)	Denmark $n = 538{,}734$ 1997–2000	Serum TSH, FT4 TPOAb and TgAb	F, 44.4 M, 11.9	
Lombardi et al. (2013)	Pescopagano, Italy 1995, $n = 1{,}411$ 2010, $n = 1{,}148$[c] All ages	Serum TSH, FT4 TPOAb and TgAb ultrasonography		1995 F,17.2%, M, 5.8% 2010[c] F,25.6%, M, 10.7%
McLeod et al. (2014)	US military 20,270,688 year Age 20–54 year 1997–2011	ICD-9-CM code 245.2	F, 26.3 M, 3.2	

F females, *M* males

[a]Limited to autoimmune hypothyroidism, see also text

[b]Based on frequency of TPOAb and TgAb

[c]After salt iodization as of 1999

Table 4 Gender-specific prevalence of TPOAb and TgAb as a function of age in the total NHANES III population (Hollowell et al. 2002)

	TPO antibodies %		Tg antibodies %	
Age group	Males	Females	Males	Females
12–19 year	2.9	6.7	5.2	7.3
20–29 year	5.7	11.3	5.2	9.2
30–39 year	9.5	14.2	7.8	14.5
40–49 year	11.2	18.0	7.4	16.4
50–59 year	11.0	20.7	8.8	18.6
60–69 year	11.7	27.3	10.3	22.4
70–79 year	13.2	29.0	14.1	22.3
80–89 year	12.3	30.2	11.3	27.0

studied in the first two decades. The prevalence of TPOAb and/or TgAb in an iodine-sufficient region of Spain was 0.6, 4.6, and 6.2% in the age groups 1–6, 6–12, and 12–16 years, respectively (Garcia-Garcia et al. 2012). The prevalence was associated with age (odds ratio 1.30) and female sex (odds ratio 2.78). The prevalence of thyroid antibodies in girls was 5.0% and in boys 2.3%; at prepubertal age these figures were 3.3% in girls and 1.5% in boys and at postpubertal age 8.1% and 3.9%, respectively. Another study reported a higher frequency of TPOAb in girls in Tanner stage II–IV than in girls in Tanner stage I (8.2% vs. 2.2%) (Kaloumenou et al. 2008). So puberty is one of the factors promoting thyroid autoimmunity. If the disease manifests itself already during childhood, one may hypothesize this happens preferentially in subjects harboring a rather high genetic susceptibility for autoimmune thyroid disease. Conversely, if one contracts the disease later in life, it is more likely that exposure to environmental insults play a more dominant role in the pathogenesis. Naturally, the environment will play a less prominent role in children because their young age prevents a long exposure time to environmental stressors. Lastly, the prevalence of Hashimoto's thyroiditis has been found to differ between *ethnic groups* (Table 5). Interestingly, the prevalence (and incidence) of Hashimoto's thyroiditis is highest in Whites and lowest in Blacks and Asian/Pacific Islanders, whereas the opposite is true for Graves' disease (highest incidence in Blacks and Asian/Pacific Islanders and lowest in Whites (McLeod et al. 2014). Little is known about the presumably genetic factors responsible for these ethnic differences.

Incidence

The medical-indexing and record-retrieval system at the Mayo Clinics in Rochester, MN, assured the identification of practically all Olmsted County residents in whom the diagnosis of significant illness had been made. Using this register, the incidence of Hashimoto's thyroiditis among female residents of Olmsted County increased significantly over a 33-year period, especially between 1935 and 1954, but not any longer between 1955 and 1967 (Table 2; Furszyfer et al. 1970). In the same period, the incidence of Graves' disease did not change (Furszyfer et al.

Table 5 Ethnic differences in the incidence and prevalence of Hashimoto's thyroiditis and incidence of Graves' disease

Ethnic group[a,b]	*Prevalence TPOAb*	*Prevalence TgAb*
White, non-Hispanic	14.3%	12.9%
Mexican American	10.9%	8.8%
Black, non-Hispanic	5.3%	3.0%
Ethnic group[c,d]	*Incidence rate ratio Hashimoto's thyroiditis*	*Incidence rate ratio Graves' disease*
White	1.00	1.00
Black	F, 0.33 (95% CI 0.21–0.51) M, 0.22 (95% CI 0.11–0.47)	F, 1.92 (95% CI 1.56–2.37) M, 2.53 (95% CI 2.01–3.18)
Asian/Pacific Islander	F, 0.31 (95% CI 0.17–0.56) M, 0.23 (95% CI 0.07–0.72)	F, 1.78 (95% CI 1.20–2.66) M, 3.36 (95% CI 2.57–4.40)

F females, *M* males
[a]Hollowell et al. (2002)
[b]Total NHANES III population
[c]McLeod et al. (2014)
[d]Incidence rate ratio of Hispanic group lies in between those of Whites and Blacks

1972). Between 1935 and 1944, the diagnosis of Hashimoto's thyroiditis was exclusively made by thyroidectomy, but between 1965 and 1967, the diagnosis was established in 21% by thyroidectomy, in 28% by needle biopsy, and in 51% by clinical features (like diffusely enlarged sometimes lobulated thyroid gland with rubbery feeling, low basal metabolic rate, normal or increased protein-bound iodine, low butanol-extractable iodine, high tanned red cell agglutination titer). Changes in applied diagnostic methods may explain to a large extent the observed rising incidence of Hashimoto's thyroiditis, especially between 1935 and 1955, but the authors speculate that increased ingestion of iodide triggers the disease and might be partly responsible for the increasing incidence up to 70/100,000/year in women in the 1960s (Furszyfer et al. 1970). The incidence rate of autoimmune hypothyroidism for women in the iodine-sufficient Spanish city of Vigo in the early 1990s was 45.4/100,000/year (Galofre et al. 1994). Much higher incidence rates of spontaneous overt hypothyroidism (presumably of autoimmune origin) were reported in the 1990s in a 20-year follow-up of the Whickham Survey in Northern England, the first (and rightly famous) population-based survey on thyroid autoimmunity (Vanderpump et al. 1995): 350 cases/100,000/year in women and 60 cases/100,000 per year in men. Odds ratios for development of spontaneous hypothyroidism in women were 14 [95% CI 9–24] for raised TSH at baseline, 13 [95% CI 8–19] for positive TPOAb and/or TgAb at baseline, and 38 [95% CI 22–65] for raised TSH and positive thyroid antibodies combined; corresponding values in men were consistently higher, with odds ratios of 44 [95% CI 19–104], 25 [95% CI 10–63], and 173 [95% CI 81–370], respectively. Various subsequent longitudinal studies have confirmed that a serum TSH >2.5 mU/L and the presence of TPOAb and/or TgAb increase the risk of developing overt hypothyroidism (Strieder et al. 2008; Walsh et al. 2010). Incidence rates of hypothyroidism in the

same order of magnitude (448/100,000/year for women and 92/100,000/year for men) are published for the population of Tayside in Scotland from 1994 to 2001 (Leese et al. 2008). The data are based on the Thyroid Epidemiology, Audit, and Research Study (TEARS), in which hypothyroidism is defined as continuous long-term replacement therapy because of an underactive thyroid, excluding past thyroid surgery or hyperthyroidism from the register; consequently it is assumed that almost all cases of incident hypothyroidism are due to autoimmune thyroid disease. An interesting study from Slovenia demonstrated a clear increase in the incidence of hypothyroid Hashimoto's thyroiditis after mandatory salt iodization in 1999 (Zaletel et al. 2011).The incidence of hypothyroid Hashimoto's thyroiditis increased from 36.9 before to 68.8 per 100,000 per year after the rise in iodine intake (RR 1.86, 95% CI 1.64–2.12). The increase of Hashimoto's thyroiditis (hypothyroid and euthyroid cases combined) was even more dramatic, from 73.2/100,000 in 1999 to 166.4/ 100,000 in 2009. Similar results were obtained in the small rural community of Pescopagano in Italy. Fifteen years after voluntary iodine prophylaxis, the prevalence of TPOAb and TgAb had increased significantly, and Hashimoto's thyroiditis (defined as hypo- or euthyroidism with raised thyroid antibodies or euthyroidism without detectable antibodies but hypoechogenicity at thyroid ultrasound) had become more frequent in 2010 than in 1995 (14.5% and 3.5%, respectively), both in females and in males (Lombardi et al. 2013). Much relevant information has been generated by the DanThyr program, the Danish joint iodine fortification program (Laurberg 2015). The sale of iodized salt was prohibited in Denmark until 1998, when a voluntary program was started of adding KI to all salt for human consumption. Due to the ineffectiveness of this voluntary program, it was changed after 2 years into a mandatory program: the level of iodization of household and bread salt was set to 13 ppm, that is, 13 μg iodine per g salt, which would provide a daily iodine intake of about 50 μg at an average salt intake of 4 g per day. The mandatory program was implemented during the period July 2000 to April 2001, and 4–5 years later the median urinary iodine concentration had increased toward 101 μg/L (Rasmussen et al. 2008). This value is just above the lower limit of 100 μg/L compatible with an adequate iodine intake as recommended by WHO/UNICEF/ICCIDD. Before the mandatory program, the unadjusted incidence rate of spontaneous hypothyroidism was 44.4 (females) and 11.9 (males) per 100,000 per year (Carlé et al. 2006a). Hypothyroidism in this study had most likely an autoimmune origin, as serum thyroid antibodies were measurable in practically all patients (Carlé et al. 2006b). The incidence of spontaneous hypothyroidism increased nearly exponentially with age, with a sharp increase above 50 years of age; half of the patients were 67.6 years or older. Of much interest is the higher standardized incidence rate in the mildly iodine-deficient region of Copenhagen than in the moderately iodine-deficient region of Aalborg: 35.0 and 23.1 per 100,000 per year, respectively, and standardized incidence rate ratio of 1.53 (95% CI 1.29–1.80). Thus spontaneous hypothyroidism was 53% more common in Copenhagen with higher – but still deficient – iodine intake. Median urinary iodine excretion values in this study were 61 μg/L (93 μg/24 h) in Copenhagen and 45 μg/L (62 μg/24 h) in Aalborg. After

implementation of the mandatory iodization program, the median urinary iodine concentrations increased to 108 μg/L in Copenhagen and 93 μg/L in Aalborg (Rasmussen et al. 2002). This was associated with an increased prevalence of mild (but not overt) hypothyroidism in both regions: figures before/after for the Copenhagen region were 4.45%/5.60% and for the Aalborg region 3.24%/5.40% (Vejbjerg et al. 2009). In line with the increased prevalence of hypothyroidism, a doubling in the use of thyroid hormone replacement was documented: in Copenhagen the incidence rate increased by 75% (incident users/100,000 person-years were 72.2 in 1997 and 126.6 in 2008) and in Aalborg by 87% (from 86.9 to 162.9, respectively) (Cerqueira et al. 2011). A subsequent longitudinal study within the DanThyr cohort reported a significant increase in serum TSH during an 11-year follow-up: from 1.3 mU/L in 1997–1998 to 1.5 mU/L in 2008–2010 (Bjergved et al. 2012). Urinary iodine excretion in this cohort had increased from 52 to 75 μg/L. The prevalence of TPOAb had increased from 16.1% to 23.9% and of TgAb from 12.1% to 21.5%. The DanThyr studies provide compelling evidence that increasing iodine intake is associated with higher incidence of Hashimoto's thyroiditis and autoimmune hypothyroidism.

The incidence of spontaneous hypothyroidism in iodine-deficient Denmark is about 50% lower than in iodine-sufficient Sweden. The incidence rate of spontaneous hypothyroidism in Sweden in women aged 38–66 years is 156/100,000 person-years and in Denmark in women aged 40–69 years 68.6/100,000 person-years (Carlé et al. 2006b; Nystrom et al. 1981); in women aged 70–79 years, the incidence rates were 243 in Sweden and 121 in Denmark (Sundbeck et al. 1991; Carlé et al. 2006b). The relevance of ambient iodine intake in a population for the frequency of thyroid diseases in that population has again been demonstrated nicely in large studies from China (Teng et al. 2006). Three regions with mildly deficient iodine intake, more than adequate iodine intake, and excessive iodine intake were compared, in 1999 at baseline and during a 5-year follow-up between 1999 and 2004. Both the prevalence and incidence of TPOAb, TgAb, and autoimmune thyroiditis (defined as TPOAb >100 kU/L with overt or subclinical hypothyroidism, called Hashimoto's thyroiditis in the presence of goiter but atrophic thyroiditis in the absence of goiter) were in general lowest in regions with mild iodine deficiency and higher in the regions with adequate or excessive iodine intake (Table 6; Teng et al. 2006). Other studies from China confirm that higher iodine intake increases the prevalence of hypothyroidism and autoimmune thyroiditis (Teng et al. 2011; Shan et al. 2016).

In conclusion, the incidence of Hashimoto's thyroiditis (like the prevalence) is higher in females, in old age, and in regions with high iodine intake. Although raising the iodine intake (by salt iodization) increases the incidence rate of Hashimoto's thyroiditis and autoimmune hypothyroidism, differences in ambient iodine intake are unlikely to be the sole explanation for differences in frequency of Hashimoto's thyroiditis between populations. Differences in genetic makeup (see Table 4 for ethnic differences) and environmental exposures, apart from iodine, must be involved as well. The present data cannot answer whether there is a secular trend toward a worldwide increasing incidence of Hashimoto's thyroiditis.

Table 6 Prevalence and cumulative incidence[a] of thyroid autoimmunity in three regions of China with different iodine intake (Modified from Teng et al. 2006)

	Mildly deficient iodine intake	*More than adequate iodine intake*	*Excessive iodine intake*
N in 1999 and 2004	1,103 and 884	1,584 and 1270	1,074 and 864
Urinary iodine[b] in 1999 and 2004	84–88 μg/L	243–214 μg/L	651–634 μg/L
TPOAb ≥50 kU/L	Prevalence 9.2% Incidence 2.8%	Prevalence 9.8% Incidence 4.1%	Prevalence10.5% Incidence 3.7%
TgAb ≥40 kU/L	Prevalence 9.0% Incidence 3.3%	Prevalence 9.0% Incidence 3.9%	Prevalence 9.4% Incidence 5.1%
Autoimmune thyroiditis[c]	Prevalence 0.5% Incidence 0.2%	Prevalence 1.7% Incidence 1.0%	Prevalence 2.8% Incidence 1.3%
Goitrous autoimmune hypothyroidism	Prevalence 0.4% Incidence 0%	Prevalence 1.0% Incidence 0.3%	Prevalence 1.5% Incidence 0.5%
Atrophic autoimmune hypothyroidism	Prevalence 0.1% Incidence 0.2%	Prevalence 0.7% Incidence 0.7%	Prevalence 1.3% Incidence 0.8%

[a]As % of persons followed-up for 5 years
[b]Median values
[c]Defined as TPOAb >100 kU/L with overt or subclinical hypothyroidism, subdivided in Hashimoto's thyroiditis (with goiter) and atrophic thyroiditis (without goiter)

Pathogenesis

Immunological synapse. The immunological synapse refers to the interaction between antigen-presenting cells (APCs) and T lymphocytes (T cells) (Weetman 2016). Macrophages (Mϕ), dendritic cells (DC), and also B lymphocytes (B cells) act as professional APCs. APCs take up antigens and process them into peptides of 12–18 amino acids (the epitopes of the antigen) which bind to major histocompatibility complex (MHC) class II molecules on the cell surface (MHC is termed human leukocyte antigen, HLA, in humans). The complex of HLA and antigenic epitope may be recognized by T-cell receptors (TCR) on helper T cells (identified by expression of the surface molecule CD4; CD stands for cluster of differentiation). Formation of the trimolecular complex HLA class II, antigenic epitope, and TCR activates CD4+ T cells, which involves expression of the interleukin-2 receptor (IL-2R) and autocrine stimulation by IL-2 release. Activation of T cells, however, requires, next to recognition of the antigen, costimulation. Formation of the trimolecular complex induces CD40 ligand on T cells, which binds to constitutively expressed CD40 on APCs; it results in induction of B7-1 (CD80) or B7-2 (CD81) molecules on APCs, which then binds to constitutively expressed CD28 on T cells. This induces CTLA-4 (cytotoxic T-lymphocyte-associated protein-4, now named CD152) on T cells, which reduces the interaction between APCs and T cells and terminates the immune response (Weetman 2016; McLachlan and Rapoport 2014). Activated CD4+ T cells may develop into type 1 helper T cells (Th1) or type 2 helper T cells (Th2). Th1 cells (typically producing INF-γ) promote cell destruction; Th2

cells (typically producing IL-4) promote antibody production. Whereas CD4 is the receptor for MHC class II molecules on APCs, CD8+ is the coreceptor for HLA class I molecules. CD8+ T cells are typically cytotoxic but were presumed to have suppressor functions in the past. Cytotoxicity in CD8+ T cells is directed against antigenic epitopes that are synthesized in the target cell (e.g., products of viral infection or malignant transformation) and presented by MHC class I molecules. B cells may turn into plasma cells secreting antibodies. Some B cells act as memory B cells or as APCs by their cell surface immunoglobulins that function as specific antigen receptor. These specific antigens are then internalized, processed, and presented to T cells (McLachlan and Rapoport 2014; Wiersinga 2014).

Tolerance to self-antigens. Mounting an immune response against exogenous antigens (e.g., from invading microorganisms) has obvious advantages, but presentation of endogenous "self"-antigens to T cells may result in autoimmunity. A number of complex regulatory mechanisms serve to prevent an immune response directed against self-antigens. Self-tolerance is enacted in the thymus (central tolerance) and in peripheral tissues (peripheral tolerance). Immature T cells from the bone marrow enter the thymus, where they undergo a process of selection and finally exit as CD4+ or CD8+ T cells depleted of high-affinity binding sites of self-peptides (McLachlan and Rapoport 2014). This central tolerance is accomplished by negative selection of autoreactive T cells in the thymic medulla. Self-reactive T cells emerge during the random recombination of gene segments that encode variable parts of the TCR for the antigen (Geenen et al. 2013). Thymic medullary epithelial cells express peptides from self-proteins, which in cooperation with dendritic cells are presented to immature T cells. T cells that recognize these self-peptides with high affinity are deleted. T cells that have moderate affinities for self-peptides are positively selected and leave the thymus to become mature T cells. In general, the higher the concentration of autoantigen in the thymus is, the greater the degree of self-tolerance will be (Wiersinga 2014).

Central tolerance may not eliminate all self-reactive T cells. Interaction of the TCR on naïve T cells with an MHC class II molecule plus epitope, in the absence of CD28 ligation with CD80, induces anergy, that is, the T cell is paralyzed and unable to respond. Similarly, engagement of CTLA4 results in T-cell anergy (Weetman 2016). Furthermore, self-antigen presentation in the thymus generates regulatory T cells (T_{reg}) that can inhibit in peripheral tissues those self-reactive T cells that escaped negative selection in the thymus (Geenen et al. 2013). T_{reg} can be classified as either natural regulatory T cells (nT_{reg}, constitutive, developing in the thymus) or inducible regulatory T cells (iT_{reg}, involved in the adaptive immune response) which are generated from naïve T cells in the periphery after antigenic stimulation (Marazuela et al. 2006). T_{reg} are characterized by the expression of CD4, CD25 (the interleukin-2 receptor α-chain), and the transcription factor FoxP3 (forkhead box P3 protein); they produce interleukin-10 (IL-10) and transforming growth factor-β (TGF-β). T_{reg} might act directly on effector CD4+ and CD8+ lymphocytes by cell-to-cell contact, hampering their activation and proliferation, or indirectly via secretion of IL-10 and TGF-β (Bossowski et al. 2016). Control of autoreactive T cells in the periphery may be considered as a secondary or "fail-safe" mechanism in

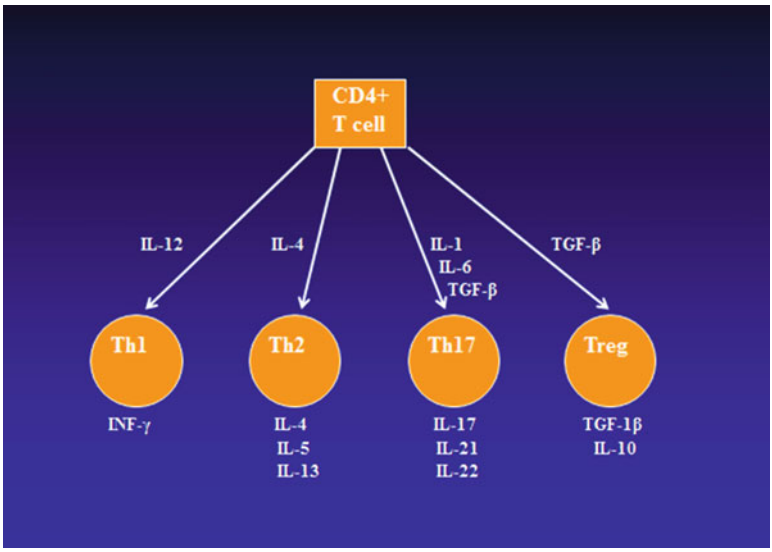

Fig. 3 Differentiation of CD4+ T cells. Indicated are cytokines that promote differentiation into one of the four T-cell subsets and the cytokines that are predominantly produced by each T-cell subset (Modified from Pyzik et al. 2015)

the prevention of autoimmune reactions. Recently, next to Th1, Th2, and T_{reg}, a fourth subset of T helper cells has been identified, designated Th17 cells generating interleukin-17 (IL-17) (Fig. 3; Pyzik et al. 2015). These cells are involved in the clearance of extracellular pathogens but have also been associated with several autoimmune diseases. IL-6 together with TGF-β promotes differentiation into Th17 cells, which are highly proinflammatory and may lead to severe autoimmune responses. T_{reg} are natural suppressors that control overactive cells. Thus a balance between Th17 and T_{reg} might be crucial for immune homeostasis (Bossowski et al. 2016).

Breakdown of self-tolerance. Breakdown of self-tolerance against thyroid antigens may lead to thyroid autoimmunity (Fig. 4; Weetman 2003). The three major thyroid antigens are thyroid peroxidase (TPO), thyroglobulin (Tg), and TSH receptor (TSHR). In general, immunogenicity of antigens is higher in case of (a) genetic polymorphisms, (b) a higher number of peptides available for binding to MHC molecules on APCs, (c) membrane-bound antigens, and (d) a high degree of glycosylation which facilitates antigen binding to cell surface mannose receptors on APCs. According to these features, the immunogenicity of Tg is higher than that of TPO and TSHR (McLachlan and Rapoport 2014; Wiersinga 2014). The interplay between thyroid antigens and immunocompetent cells determines the outcome of the immune response. In Hashimoto's thyroiditis, the massive lymphocytic infiltration in the thyroid gland is composed mostly (up to 50%) of B lymphocytes. There is also an abundance of CD8+ cytotoxic/suppressor T cells but less CD4+ T cells (Ben-Skowronek et al. 2011; Pyzik et al. 2015; Zha et al. 2014). Th1 cells activate

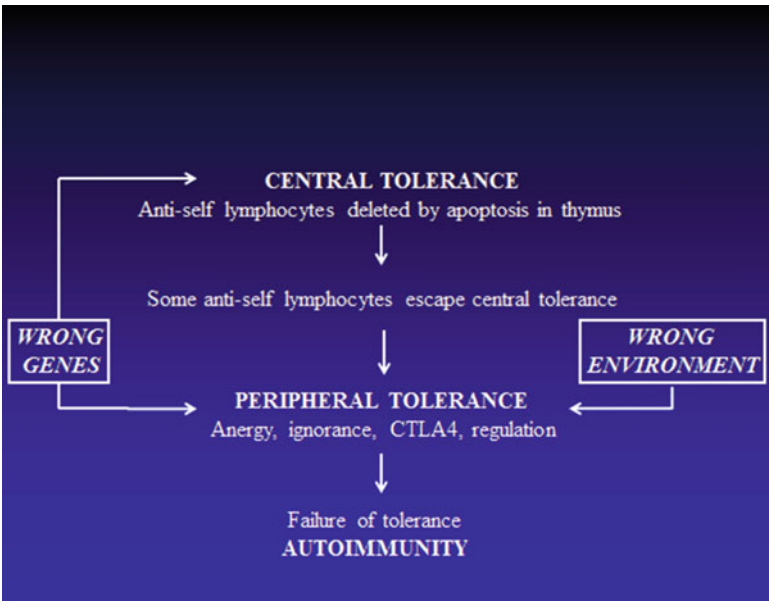

Fig. 4 Interaction between genes and environment in the development of autoimmune thyroid disease (Modified from Weetman 2003)

cytotoxic lymphocytes and macrophages, directly destroying thyroid follicular cells. Activation of Th2 cells results in stimulation of B cells and plasma cells, which produce antibodies against thyroid antigens. The number of T_{reg} in peripheral blood in patients with Hashimoto's thyroiditis, as compared to healthy controls, has been reported as increased, normal, or decreased; however, T_{reg} of Hashimoto patients are less capable of inhibiting proliferation of T cells (Pellegrini et al. 2012; Bossowski et al. 2013; Glick et al. 2013; Liu et al. 2014). Patients with Hashimoto's thyroiditis have enhanced levels of T cells synthesizing IL-17 and IL-22 in their peripheral blood; in addition, a stronger expression of IL-17 and IL-22 and of IL-23R+ cells is observed in their thyroid glands. In vitro differentiation of T lymphocytes into Th17 cells (induced by IL-23/IL-6) is also enhanced (Figueroa-Vega et al. 2010). It indicates increased differentiation of Th17 cells and enhanced synthesis of IL-17 in Hashimoto's thyroiditis. This was confirmed in another study demonstrating an increase of thyroid-infiltrating Th17 cells and IL-17 mRNA, which correlated with local fibrosis; serum IL-17 was also increased, inversely related to the degree of hypothyroidism (Li et al. 2013b). Whereas Th17 by their proinflammatory nature would be pathogenic, Th10 would play a protective role (IL-10 being produced by T_{reg}). Several studies describe a shift to higher values of the Th17/Th10 ratio in peripheral blood of patients with Hashimoto's thyroiditis. A skew to higher ratios would promote autoimmunity, and indeed a direct relationship has been found between Th17/Th10 ratios and TPOAb, TgAb, and TSH (Liu et al. 2014; Kristensen et al. 2014; Xue et al. 2015a, b). These findings strengthen the hypothesis that loss of

T_{reg} inhibitory actions and gain of Th17 proinflammatory actions play a crucial role in the loss of tolerance to thyroid antigens in AITD.

Further support for this proposition comes from a recent study on microvesicles. Circulating microvesicles are emerging as important contributors to the development of inflammatory and autoimmune diseases. Microvesicles are involved in intercellular communication; they can modulate immune reactions by transferring membrane and cytoplasmic components and genetic information (including microRNAs) between cells. In plasma samples from AITD patients, the proportion of platelet-derived microvesicles was increased, and that of leukocyte- and endothelial cell-derived microvesicles decreased compared with healthy controls (Rodriguez-Munoz et al. 2015). Functional assays showed that microvesicles from Hashimoto patients inhibit the in vitro differentiation of FoxP3+ T_{reg} and induce expression of Th17 pathogenic (IL-17+IFNγ$^-$) cells.

A reduced frequency of IL-10-producing regulatory B cells in peripheral blood of patients with Hashimoto's thyroiditis has not been observed (Kristensen et al. 2015). It has further been investigated whether defects in immunoregulatory mechanisms exist in circulating and thyroid dendritic cells (DCs) (Leskela et al. 2013). Tolerogenic DCs have an important role in the intrathymic deletion of autoreactive lymphocytes as well as in the maintenance of peripheral tolerance to self-antigens. This immunoregulatory function is exerted through different mechanisms, including the induction of anergy or programmed cell death of effector lymphocytes and the generation of regulatory T cells. The tolerogenic activity of DCs seems to be closely associated with the expression and function of different cell surface receptors, including the P-selectin glycosylated ligand-1 (PSGL-1), the inhibitory isoforms of immunoglobulin-like transcripts (ILTs or CD85), the ligands of the programmed death 1 receptor (PD-L1,2), and CD69. DCs synthesize various cytokines that activate and proliferate T_{reg} and effector lymphocytes. DCs also express indoleamine 2,3-dioxygenase (IDO) which through tryptophan (Trp) starvation and generation of Trp metabolites seems to participate in differentiation of T_{reg}. AITD patients (either with Graves' disease or Hashimoto's thyroiditis) have a diminished number of peripheral blood plasmacytoid DCs (but not of conventional immunogenic DCs), a defective expression of several immunoregulatory molecules (including ILT3, PSGL-1, CD69, and IDO), and a diminished generation of Trp metabolites, mainly in those with severe disease. In thyroid tissue of AITD patients, there are more plasmacytoid DCs, and expression of regulatory molecules like ILT3 and PSGL-1 is diminished (Leskela et al. 2013).

Effector mechanisms. Autoimmune-mediated destruction of the thyroid gland (the hallmark of Hashimoto's thyroiditis) is mediated by both cellular and humoral immune responses. Cytotoxic CD8+ T cells directed against both TPO and Tg mediate thyroid gland destruction (Ehlers et al. 2012). Cytotoxicity is directed against antigenic epitopes presented by MHC class I molecules on thyrocytes. Expression of MHC class I and adhesion molecules on thyrocytes in Hashimoto's thyroiditis enhance cytotoxicity by the binding of CD8+ cytotoxic T cells to thyrocytes. Destruction of target cells is by the granule exocytosis pathway (utilizing perforin and granzyme A and B) and by apoptosis or programmed cell death. Perforin-containing T cells have been detected in Hashimoto's thyroiditis and may contribute to thyroid cell destruction (Popko et al. 2015). There is increased

expression of the apoptotic molecule Fas on thyroid follicular cells from patients with Hashimoto's thyroiditis, which can be mediated by cytokines like IL-1 (Kotani et al. 1995; Baker 1999); expression of Fas renders thyrocytes liable to apoptosis by cytotoxic T cells expressing Fas ligand (Fas expression is generalized, whereas Fas ligand is restricted to cells of the immune system) (Weetman 2016). Indeed, upregulation of caspase-3 and downregulation of bcl-2 in the thyroid of patients with Hashimoto's disease support a pathogenetic role of apoptosis (Kaczmarek et al. 2011). Disruption of the thyroxisome (caveolin-1, thyroid peroxidase, and dual oxidase) at the apical membrane of the thyrocyte induced by Th1 cytokines (IFN-γ, IL-1α) may lead to uncontrolled oxidative stress and cell apoptosis (Marique et al. 2014). Apoptosis may also occur via TRAIL (TNF-related apoptosis-inducing ligand), which can be induced on thyrocytes by cytokines like TNF and IFN-γ (Pyzik et al. 2015). IL-1β may directly reduce expression of tight junction proteins, thus disturbing thyroid epithelium integrity and mediating thyroid follicular cell destruction in Hashimoto's thyroiditis (Rebuffat et al. 2013). Peripheral blood CD4+ and CD8+ T cells from children with Hashimoto's thyroiditis show lower surface expression of the cytotoxic lymphocyte antigen-4 (CTLA-4) than controls, which may enhance the immune response (Kucharska et al. 2013).

TPOAb and TgAb may cause antibody-dependent cell-mediated cytotoxicity (ADCC) via complement-mediated lysis of thyrocytes. The ability to fix complement depends on the IgG subclass. ADCC would cause more damage to the thyroid gland in comparison to T cells and cytokine-mediated apoptosis (Chiovato et al. 1993). TSHR blocking antibodies may also contribute to thyroid atrophy, albeit only in a small minority of Hashimoto's thyroiditis cases. Thyroid-infiltrating immunocompetent cells may release a wide variety of cytokines which could enhance the autoimmune response and further modulate thyroid hormone production and thyroid growth.

Genes and Environment

Breaking tolerance to self-antigens can happen in subjects who have the wrong genes and who are exposed to the wrong environment (Fig. 4; Weetman 2003). AITD often runs in families, which can be quantified by the sibling risk ratio (Brix et al. 1998). The sibling risk ratio (λs) is defined as the ratio of the risk for developing AITD in siblings of AITD patients to the frequency of AITD in the general population. The λs value for AITD is 16.9, for Graves' disease 11.6, and for Hashimoto's thyroiditis 28.0 (Villanueva et al. 2003). Family members may share the same genes but also the same environment. Twin studies are uniquely suitable to assess the relative contribution of genes in the development of AITD. A series of elegant twin studies in Denmark have concluded that genes contribute 79% (95% CI 38–90%) of the liability to developing Graves' hyperthyroidism and 73% (95% CI 46–89%) of the liability to developing TPOAb and/or TgAb (Brix et al. 2001; Hansen et al. 2006; Brix and Hegedus 2012). The implication is that the contribution of the environment to AITD would be more limited, in the order of 20–30%.

Genetic Factors

Polymorphisms in thyroid-specific genes (*TSHR, Tg*) and immunoregulatory genes (*HLA*, *CTLA-4*, *CD40*, *CD25*, *FOXP3)* have all been associated with Graves' disease but in general less so with Hashimoto's thyroiditis (Smith and Hegedus 2016; Effraimidis and Wiersinga 2014). Genetic variants may influence whether patients with AITD develop Graves' disease or Hashimoto's disease, sometimes in a gender-specific manner (Walsh et al. 2011; Campbell et al. 2015).

1. *Thyroid-specific genes TSHR and Tg.* Single-nucleotide polymorphisms (SNPs) in *TSHR* have been specifically associated with Graves' disease, but not with autoimmune hypothyroidism in a Caucasian population; however, more recently an association has been described between *TSHR* intron SNPs and Hashimoto's thyroiditis in a Chinese Han population (Liu et al. 2012). Functional analyses of *TSHR* intron 1 polymorphisms provide direct evidence of a link between central tolerance and these SNPs. The disease-predisposing genotype (TT) of SNP rs12101261 was associated with decreased thymic expression levels of TSHR mRNA (Stefan et al. 2014; Lee et al. 2015). Multiple SNPs in *Tg* have been associated with both Graves' and Hashimoto's disease; SNPs were located in exons in Caucasians and Indians and in introns in the Japanese (Jacobson and Tomer 2007; Ban et al. 2012; Patel et al. 2016).
2. *Immunoregulatory genes HLA*, *CTLA-4*, *and CD40* (involved in antigen presentation and T-cell activation) (Lee et al. 2015). Associations with *HLA class I and II* molecules have been recognized for a long time. *HLA-A*02:07* and *HLA-DRB4* confer susceptibility to Hashimoto's thyroiditis in Japanese subjects, whereas the haplotype *HLA-A*33:03-C*14:03-B*44:03-DRB1*13:02-DQB1*06:04-DPB1*04:01* conferred protection (Ueda et al. 2014). *HLA-B* appears to be a risk factor for Hashimoto's thyroiditis in Han Chinese (Huang et al. 2012). Only Tg peptides were found to be bound to HLA-DR within thyroid glands of Graves' patients, suggesting that presentation of Tg peptides by HLA-DR to T cells may be the initial trigger of AITD (Lee et al. 2015; Muixi et al. 2008). Two polymorphisms in *CTLA-4* (+49 A/G and CT60) have been linked to Hashimoto's thyroiditis in Taiwanese and Indian people (Patel et al. 2016; Ting et al. 2016). Polymorphisms in *CD40* are associated with Graves' disease, but not with Hashimoto's thyroiditis (Li et al. 2012).
3. *Immunoregulatory genes CD25 and FOXP3* (involved in the establishment of peripheral tolerance) (Lee et al. 2015). *IL2RA* and *FOXP3* encode markers for T_{reg}. *CD25* is marker for the interleukin-2 receptor-α chain present predominantly on CD25+ T cells, a susceptibility locus for Graves' disease (Brand et al. 2007). *FOXP3* encodes a forkhead/winged helix transcription factor expressed in naturally arising T_{reg}, committing naïve T cells to become T_{reg}. Mutations in *FOXP3* result in the fatal IPEX syndrome (immunodysregulation, polyendocrinopathy, enteropathy, X-linked). Polymorphisms in *FOXP3* have been associated with AITD in Caucasians (especially with Graves' disease developing below the age of 30 years), but not in Japanese (Owen et al. 2006; Ban et al. 2007). The location of *FOXP3* on the X-chromosome might contribute to the female preponderance of AITD.

Mutations in *AIRE* (autoimmune regulator gene), expressed in thymic medullary epithelial cells, result in failure to present self-antigens correctly in the thymus leading to a loss of self-tolerance and thereby to autoimmune polyglandular syndrome type 1. However, *AIRE* mutations are rarely present in adult AITD patients (in about 0.3–0.6% of patients with Graves' disease and autoimmune hypothyroidism). Therefore *AIRE* is not considered as a susceptibility gene contributing to the more common autoimmune endocrinopathies (Nithiyananthan et al. 2000; Meyer et al. 2001).

4. *Genome-wide association studies (GWAS).* GWAS offer a hypothesis-free approach to understanding disease susceptibility, which allows the discovery of novel pathways (Simmonds 2013). A genome-wide direct comparison between Hashimoto's thyroiditis and Graves' disease revealed an SNP at the VAV3 locus, associated with Hashimoto's thyroiditis (Oryoji et al. 2015). A large GWAS investigated 18,297 individuals for TPOAb positivity (1,769 TPOAb positives and 16,528 TPOAb negatives) and 12,353 individuals for TPOAb serum levels, with replications in 8,990 individuals (Medici et al. 2014). Significant associations ($p < 5 \times 10^{-8}$) were detected at TPO-rs11675434, ATXN2-rs653178, and BACH2-rs10944479 for TPOAb positivity and at TPO-rs11675434, MAGI3-rs1230666, and KALRN-rs20110099 for TPOAb levels. Individuals with a high genetic risk score (based on the effects of these variants) had an increased risk of TPOAb positivity and an increased level of TSH and a decreased risk of goiter; the MAGI3 variant was also associated with an increased risk of hypothyroidism. The results provide insight into why individuals with thyroid autoimmunity do or do not eventually develop thyroid disease, and these markers may therefore predict which TPOAb-positive subjects are particularly at risk of developing clinical thyroid dysfunction.
5. *X-chromosome inactivation (XCI).* In female mammalian cells, one of the two X-chromosomes is inactivated in early embryonic life. Female tissues are thus mosaics of two cell lines, one with the paternal X-chromosome and the other with the maternal X-chromosome as the active X. Usually, there is a random 50:50 ratio of the two cell lines. A skewed X-chromosome inactivation (XCI) is defined as inactivation of the same X-chromosome in $\geq$80% of cells. The consequence could be that self-antigens on one X-chromosome are not expressed at sufficiently high levels in the thymus or at peripheral sites, thereby failing to induce tolerance to these self-antigens (Effraimidis and Wiersinga 2014; Brix et al. 2005). Skewed XCI could be an explanation for the female preponderance in AITD. A meta-analysis of one UK and four non-UK Caucasian studies reports significant skewing of XCI with Graves' disease (OR 2.54, 95% CI 1.58–4.10] and Hashimoto's thyroiditis (OR 2.40, 95% CI 1.10–5.26) (Brix et al. 2005; Simmonds et al. 2014).

Polymorphisms in immunoregulatory genes may promote AITD, but they are not specific for AITD as they are associated with other autoimmune diseases as well. It explains why various autoimmune diseases may occur in the same patient. The mechanism of action of many susceptibility loci is incompletely understood. Why

are so many SNPs located in noncoding parts of the gene? Gene-gene or gene-environment interactions have hardly been studied. GWAS continue to detect additional genes and loci conferring risk for AITD. The odds ratio of each locus for AITD is rather low in the order of 1.5–2.0, with slightly higher odds for *HLA*. Taking together the effects of known susceptibility loci, it accounts for only a small proportion of the heritability of AITD. It follows that there must be many undetected susceptibility genes, each locus contributing just a little to the development of AITD, and that our current understanding of the etiology of AITD is gravely underestimated (Effraimidis and Wiersinga 2014; Brix and Hegedus 2011).

Environmental Factors

1. *Iodine intake*. As outlined in the above section on epidemiology, an increase in the ambient iodine intake in a population is followed by an increase in the prevalence and incidence of Hashimoto's thyroiditis: the frequencies of TPOAb, TgAb, and autoimmune hypothyroidism all rise. The mechanisms linking thyroid autoimmunity and iodine use in humans are incompletely understood. Using monoclonal TgAb-Fab directed to various epitopes on Tg, it has been suggested that the unmasking of a cryptic epitope on Tg contributes to iodine-induced thyroid autoimmunity in humans (Latrofa et al. 2013; Fiore et al. 2015). Excess iodine contributes further to autophagy suppression and apoptosis of thyroid follicular epithelial cells, which could be predisposing to increased risk of developing Hashimoto's thyroiditis (Xu et al. 2016).
2. *Smoking.* Whereas smoking is a clear risk factor for Graves' hyperthyroidism and even more so for Graves' ophthalmopathy, it has been recognized only in the last few years that smoking to a certain extent has a protective effect against the development of Hashimoto's thyroiditis (Wiersinga 2013). The prevalence of TPOAb is lower in smokers than in non-smokers, both in the Amsterdam AITD cohort (odds ratio 0.69, 95% CI 0.48–0.99) and in the third NHANES survey (odds ratio 0.57, 95% CI 0.48–0.67) (Strieder et al. 2003a; Belin et al. 2004). In the population-based HUNT study in Norway, the prevalence of subclinical hypothyroidism (OR 0.54, 95% CI 0.45–0.66) and overt hypothyroidism (OR 0.60, 95% CI 0.38–0.95) was lower in smokers compared to never smokers (Asvold et al. 2007). In a prospective study among healthy female relatives of AITD patients, discontinuation of smoking increased the risk of developing de novo TPOAb and/or TgAb (Effraimidis et al. 2009). In the prospective DanThyr study, patients diagnosed with autoimmune hypothyroidism had more often stopped smoking in the last 2 years before diagnosis than matched controls (16.4% vs 3.4%) (Carle et al. 2012a). The increased risk of autoimmune hypothyroidism after quitting smoking was transient: odds ratios <1 year, 1–2 years, and 3–10 years after cessation of smoking were 7.36 (95% CI 2.27–23.90), 6.34 (95% CI 2.59–15.3), and 0.75 (95% CI 0.30–1.87), respectively.
   Danish nationwide registration of maternal smoking during pregnancy adds further evidence that smoking reduces the risk of hypothyroidism (adjusted

hazard ratio 0.75, 95% CI 0.70–0.81) and increases the risk of hyperthyroidism (adjusted hazard ratio 1.38, 95% CI 1.27–1.49) (Andersen et al. 2014). The contrasting effects of smoking on the risks for Graves' hyperthyroidism and Hashimoto's hypothyroidism remain unexplained. One may hypothesize involvement of nicotine, which reduces experimental autoimmune encephalomyelitis. Anatabine – a tobacco alkaloid with a structure similar to nicotine – reduces the incidence and severity of experimental autoimmune thyroiditis (Caturegli et al. 2012).

3. *Alcohol.* Early studies suggested a direct toxic effect of alcohol on the thyroid gland, since thyroid volume was smaller in patients with alcoholic liver cirrhosis than in matched controls (Hegedus 1984). A nested case-control study in the Amsterdam AITD cohort did not find a relationship between alcohol consumption and de novo development of TPOAb, but participants who developed overt autoimmune hypothyroidism consumed less alcohol than those who remained euthyroid (Effraimidis et al. 2012a). A population-based case-control study in Denmark likewise observed that moderate alcohol consumption reduced the risk of overt autoimmune hypothyroidism: odds ratios were 1.98 (95% CI 1.21–3.33) for 0 units of alcohol per week, 1.00 for 1–10 units/week (reference), 0.41 (95% CI 0.20–0.83) for 11–20 units/week, and 0.90 (95% CI 0.41–2.00) for ≥21 units/week (Carle et al. 2012b). The observed associations were independent of gender, smoking, type of alcohol (beer or wine), and iodine intake. Interestingly, alcohol consumption also protects against Graves' hyperthyroidism (Carle et al. 2013). How alcohol exerts this protective effect is unknown; suffice it to say that the same protective effect of alcohol has been recorded in other autoimmune diseases like rheumatoid arthritis and type 1 diabetes mellitus.
4. *Selenium.* Recent epidemiological studies from China provide strong circumstantial evidence that low selenium intake is associated with Hashimoto's thyroiditis (Wu et al. 2015). Comparing prevalences between counties with low Se intake (serum Se 57 μg/L, IQR 39–82) and counties with adequate Se intake (serum Se 104 μg/L, IQR 80–136), prevalences were higher in counties with low Se intake for hypothyroidism (4.2% vs 2.0%, $p < 0.001$), subclinical hypothyroidism (21.4 vs. 11.7%, $p < 0.001$), and autoimmune thyroiditis (3.4% vs. 2.2%, $p = 0.007$). Upon dividing all participants in quintiles according to their serum Se concentration, those with serum Se in quintile 1 (<47 μg/L) and quintile 2 (47–69 μg/L) had higher prevalences of autoimmune thyroiditis, subclinical hypothyroidism, and hypothyroidism than those with serum Se in quintiles 3, 4, and 5 (69 −≥120 μg/L) (in whom prevalences were similar). Glutathione peroxidases are selenoproteins, protecting thyrocytes from oxidative stress generated by the action of H_2O_2. Low selenium levels have been associated with poor immune function. Thus, mild nutritional selenium deficiency may promote thyroid autoimmunity, and selenium supplementation might have a beneficial effect on thyroid autoimmunity. This has been investigated in eight randomized clinical trials, comparing the effect of selenium supplementation with placebo on the concentration of serum TPOAb in patients with Hashimoto's thyroiditis and TPOAb. Baseline TSH was either normal or slightly elevated, and exogenous

levothyroxine was used in some of the trials. TPOAb concentrations decreased in four trials, and did not change in the other four trials (Wiersinga 2016). The contrasting outcomes could not be explained from baseline serum Se or TSH concentrations, type of selenium preparation (sodium selenite or selenomethionine), concomitant use of levothyroxine, sample size, or glutathione peroxidase genotypes. A systematic review and meta-analysis of the controlled trials investigating the effect of selenium supplementation concluded that selenium supplementation reduced serum TPOAb levels; whether this effect was clinically relevant remained doubtful (Wichman et al. 2016; Winther et al. 2016). Consequently, it is difficult to make confident decisions about the use of selenium supplementation for Hashimoto's thyroiditis. The outcome of a large randomized controlled trial is therefore eagerly awaited (Winther et al. 2014). Of interest is a polymorphism in the promotor region of the selenoprotein S gene (*SEPS1*), which contributes to genetic susceptibility for Hashimoto's thyroiditis (odds ratio 2.24) but requires replication (Santos et al. 2014).

Two more placebo-controlled studies on selenium supplementation have been done, both in pregnant women. Selenium in a dose of 200 μg daily given as of gestational week 12 up to 1 year after delivery lowered the postpartum surge of TPOAb and reduced the incidence of postpartum thyroid dysfunction (Negro et al. 2007). Selenium supplementation with 60 μg/day as of 12–14 gestational weeks did not change the prevalence of TPOAb, TgAb, or subclinical hypothyroidism in the second and third trimesters (Mao et al. 2016). Adequate nutritional supply of selenium that saturates expression of circulating selenoprotein P, together with optimal iodine and iron intake, is required for a healthy thyroid development (Kohrle 2015), but the utility of selenium supplementation to combat thyroid autoimmunity has not yet been established (Hegedus et al. 2016).

5. *Vitamin D.* Immunocompetent cells express the vitamin D-activating enzyme CYP27B1 and the vitamin D receptor (VDR). The hormone $1,25(OH)_2D$ (converted locally from 25(OH)D or derived from the blood) binds to VDR modulating innate and adaptive immunity (van Belle et al. 2011). Low vitamin D levels have been identified as risk factors for various autoimmune diseases like rheumatoid arthritis, type 1 diabetes mellitus, and multiple sclerosis. Whether this is true also for AITD is presently uncertain. AITD patients (either with Graves' disease or Hashimoto's thyroiditis) had lower 25(OH)D levels than controls in a meta-analysis of 20 case-control studies: standardized mean difference was −0.99 ng/ml with 95% CI −1.31 to −0.66 ng/ml (Wang et al. 2015). This meta-analysis, however, did not adjust for the many confounders of vitamin D measurements (such as age, sex, body mass index, smoking, estrogen use, and seasonal variation). Avoiding these confounders, a Korean study in subjects undergoing routine health checkups observed lower 25(OH)D levels in women with TPOAb than in women without TPOAb (22.0 ng/ml vs. 23.5 ng/ml, $p = 0.03$) (Choi et al. 2014). The difference was observed in premenopausal women, but not in postmenopausal women and men. The prevalence of TPOAb was 21.2%, 15.5%, and 12.6% in women with vitamin D deficiency (<10 ng/ml), vitamin D insufficiency (10–30 ng/ml), and vitamin D sufficiency (>30 ng/ml),

respectively. Corresponding odds ratios adjusted for age, BMI, serum calcium, smoking, menopause, and season were 1.95, 1.31, and 1.00, respectively. A prospective study embedded in the Amsterdam AITD cohort, in contrast, did not find differences in serum 25(OH)D or $1,25(OH)_2D3$ between cases (women who developed de novo TPOAb) and controls (women who remained TPOAb negative), neither at baseline nor at the time of the occurrence of thyroid antibodies; in this study controls were matched to cases for age, BMI, smoking, estrogen use, season, and duration of follow-up (Effraimidis et al. 2012b). Matters become even more complicated by reported associations between polymorphisms in *VDR* and AITD (Feng et al. 2013; Inoue et al. 2014; Meng et al. 2015). Recent studies either confirm or refute an association between vitamin D deficiency and Hashimoto's thyroiditis (Mazokopakis et al. 2015; Yasmeh et al. 2016). Caucasian patients with Hashimoto's thyroiditis living on the island of Crete had low serum 25(OH)D, inversely correlated with serum TPOAb; serum TPOAb concentration decreased by 20% after 4 months of treatment with oral cholecalciferol in a daily dose of 1,200–4,000 IU (Mazokopakis et al. 2015).

6. *Infections.* MHC class II molecules are present on thyroid follicular cells in patients with Hashimoto's thyroiditis, but not in normal subjects. Expression of these molecules can be induced by IFN-γ and indirectly by viruses, enabling thyrocytes to present antigens (either foreign or self) to T cells, thereby activating T cells and initiating a thyroid autoimmune response. High endogenous IFN-α levels are seen in patients infected with certain viruses. Infections thus may provoke thyroid autoimmunity (Weetman 2016; Prummel and Laurberg 2003). No association was found between *Helicobacter pylori* infection and Hashimoto's thyroiditis in women (Shmuely et al. 2016). The relationship between *Yersinia enterocolitica* infections and AITD has been studied extensively. In the Amsterdam AITD cohort study, the prevalence of antibodies against YOP (*Y. enterocolitica* outer membrane protein) in healthy female relatives of AITD patients was higher than in controls (Strieder et al. 2003b). During follow-up, the proportion of subjects with YOP antibodies did not differ between cases (those who developed TPOAb) and controls (those who remained TPOAb negative), neither at baseline, at 1 year before seroconversion, nor at the time of seroconversion; the same negative results were obtained when analyzing hypothyroid cases and their respective controls (Effraimidis et al. 2011b). The data argue against a role of *Yersinia enterocolitica* in the pathogenesis of Hashimoto's thyroiditis. The higher prevalence of YOP antibodies in AITD relatives and in twins affected with Graves' disease (Brix et al. 2008) might be explained by assuming that susceptibility genes for AITD may also confer risk to *Y. enterocolitica* infection.
7. *Stress.* Whereas it is widely believed that stress exposure may provoke Graves' hyperthyroidism, there is a paucity of data about the role of stress in Hashimoto's thyroiditis (Wiersinga 2016). Stress exposure was assessed annually by questionnaires on recent life events (both pleasant and unpleasant) and daily hassles during the 5-year follow-up of the Amsterdam AITD cohort (Effraimidis et al. 2012c). No association was found between stress exposure and de novo occurrence of TPOAb or the development of overt autoimmune hypothyroidism.

Modulation of exposure to environmental factors in order to decrease the risk of developing Hashimoto's thyroiditis is maneuvering between Scylla and Charybdis (Laurberg et al. 2011). One should use iodized salt in order to prevent iodine deficiency disorders, but too high iodized salt intake has an unfavorable effect on blood pressure and increases the risk on Hashimoto's thyroiditis. To continue smoking may decrease the likelihood of developing Hashimoto's thyroiditis but increases the risk of Graves' disease, cardiovascular diseases, and cancer. To consume alcohol protects to a certain extent against Hashimoto's thyroiditis, but excessive amounts of alcohol are detrimental for health. There is no good evidence that supplementation with selenium or vitamin D will prevent Hashimoto's thyroiditis. To avoid pregnancy will indeed decrease the risk of developing Hashimoto's thyroiditis, but that kind of recommendation is not very realistic. Taken together, preventive interventions to diminish the risk of Hashimoto's thyroiditis are nowadays few, not always feasible, and probably of limited efficacy (Wiersinga 2016). Obtaining better data, and being able to predict the consequences of the interaction between susceptibility genes and the environmental triggers, at the level of the individual, is needed to implement better preventive measures.

Modified from Caturegli et al. (2014)

References

Aijan RA, Weetman AP. The pathogenesis of Hashimoto's thyroiditis: further developments in our understanding. Horm Metab Res. 2015;47:702–10.

Andersen SL, Olsen J, Wu CS, Laurberg P. Smoking reduces the risk of hypothyroidism and increases the risk of hyperthyroidism: evidence from 450,842 mothers giving birth in Denmark. Clin Endocrinol. 2014;80:307–14.

Asvold BO, Bjoro T, Nilsen TIL, Vatten LJ. Tobacco smoking and thyroid function: a population-based study. Arch Intern Med. 2007;167:1428–32.

Azizi F. The occurrence of permanent thyroid failure in patients with subclinical postpartum thyroiditis. Eur J Endocrinol. 2005;153:367–71.

Baker Jr JR. Dying (apoptosing?) for a consensus on the Fas pathway in the thyroid. J Clin Endocrinol Metab. 1999;84:2593–5.

Baker JR, Saunders NB, Wartofsky L, Tseng Y-C, Burman KD. Seronegative Hashimoto thyroiditis with thyroid autoantibody localized to the thyroid. Ann Intern Med. 1988;108:26–30.

Ban Y, Tozaki T, Tobe T, Ban Y, Jacobson EM, Concepcion ES, Tomer Y. The regulatory T cell gene FOXP3 and genetic susceptibility to thyroid autoimmunity: an association analysis in Caucasian and Japanese cohorts. J Autoimmun. 2007;28:201–7.

Ban Y, Tozaki T, Taniyama M, Skrabanek L, Nakano Y, Ban Y, Hirano T. Multiple SNPs in intron 41 of thyroglobulin gene are associated with autoimmune thyroid disease in the Japanese population. PLoS ONE. 2012;7:e37501. https://doi.org/10.1371/journal.pone.0037501.

Belin RM, Astor BC, Powe NR, Ladenson PW. Smoke exposure is associated with a lower prevalence of serum thyroid autoantibodies and thyrotropin concentration elevation and a higher prevalence of mild thyrotropin concentration suppression in the third National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab. 2004;89:6077–86.

Bell PM, Sinnamon DG, Smyth PPA, Drexhage HA, Haire M, Botazzo GF, Atkinson AB. Hyperthyroidism following primary hypothyroidism in association with polyendocrine autoimmunity. Acta Endocrinol. 1985;108:491–7.

Bennedbaek FN, Hegedus L. Imaging of the normal and affected thyroid in childhood. Paediatr Adolesc Med. 2007;11:225–69.

Ben-Skowronek I, Szewczyk L, Ciechanek R, Korobowicz E. Interactions of lymphocytes, thyrocytes and fibroblasts in Hashimoto's thyroiditis: an immunohistochemical and ultrastructural study. Horm Res Paediatr. 2011;76:335–42.

Biondi B, Cooper DS. The clinical significance of subclinical thyroid dysfunction. Endocr Rev. 2008;29:76–131.

Bjergved L, Jorgensen T, Perrild H, Carle A, Cerqueira C, Krejbjerg A, Laurberg P, Ovesen L, Bulow Pedersen I, Banke RL, Knudsen N. Predictors of change in serum TSH after iodine fortification: an 11-year follow-up to the DanThyr study. J Clin Endocrinol Metab. 2012;97:4022–9.

Bogner U, Hegedus L, Hansen JM, Finke R, Schleusener H. Thyroid cytotoxic antibodies in atrophic and goitrous autoimmune thyroiditis. Eur J Endocrinol. 1995;132:69–74.

Bossowski A, Moniuszko M, Dabrowska M, Sawicka B, Rusak M, Jeznach M, et al. Lower proportions of CD4+CD25(high) and CD4+FoxP3, but not CD4+CD25+CD127(low)FoxP3+ T cell levels in children with autoimmune thyroid diseases. Autoimmunity. 2013;46:222–30.

Bossowski A, Moniuszko M, Idzkowska E, Grubczak K, Snight P, Bossoska A, Diana T, Kahaly GJ. Decreased proportions of CD4+ IL17+/CD4+CD25+CD127- and CD4+IL17+/CD4+CD25+CD127-FoxP3+ T cells in children with autoimmune thyroid diseases. Autoimmunity. 2016;49:320–8.

Brand OJ, Lowe CE, Heward JM, Franklyn JA, Cooper JD, Todd JA, Gough SC. Association of the interleukin-2 receptor α (IL2Rα)/CD25 gene region with Graves' disease using a multilocus test and tag SNPs. Clin Endocrinol. 2007;66:508–12.

Brix TH, Hegedus L. The complexity of the etiology of autoimmune thyroid disease is gravely underestimated. Thyroid. 2011;12:1289–92.

Brix TH, Hegedus L. Twin studies as a model for exploring the aetiology of autoimmune thyroid disease. Clin Endocrinol. 2012;76:457–64.

Brix TH, Kyvik KO, Hegedus L. What is the evidence of genetic factors in the etiology of Graves' disease? A brief review. Thyroid. 1998;8:627–34.

Brix TH, Kyvik KO, Christensen K, Hegedus L. Evidence for a major role of heredity in Graves' disease: a population-based study of two Danish twin cohorts. J Clin Endocrinol Metab. 2001;86:930–4.

Brix TH, Knudsen GPS, Kristiansen M, Kyvik KO, Orstavik KH, Hegedus L. High frequency of skewed X-chromosome inactivation in females with autoimmune thyroid disease: a possible explanation for the female predisposition to thyroid autoimmunity. J Clin Endocrinol Metab. 2005;90:5949–53.

Brix TH, Hansen PS, Hegedus L, Wenzel BE. Too early to dismiss Yersinia enterocolitica infection in the aetiology of Graves' disease: evidence from a twin case-control study. Clin Endocrinol. 2008;69:491–6.

Bulow Pedersen I, Knudsen N, Jorgensen T, Perrild H, Ovesen L, Laurberg P. Large differences in incidences of overt hyper- and hypothyroidism associated with a small difference in iodine intake: a prospective comparative register-based population survey. J Clin Endocrinol Metab. 2002;87:4462–9.

Bulow Pedersen I, Laurberg P, Knudsen N, Jorgensen T, Perrild H, Ovesen L, Rasmussen LB. A population study of the association between thyroid autoantibodies in serum and abnormalities in thyroid function and structure. Clin Endocrinol. 2005;62:713–20.

Bulow Pedersen I, Laurberg P, Knudsen N, Jorgensen T, Perrild H, Ovesen L, Rasmussen LB. Lack of association between thyroid antibodies and parity in a population study argues against microchemerism as a trigger of thyroid autoimmunity. Eur J Endocrinol. 2006;154:39–45.

Campbell P, Brix TH, Wilson SG, Ward LC, Hui J, Beilby JP, Hegedus L, Walsh JP. Common genetic variants associated with thyroid function may be risk alleles for Hashimoto'disease and Graves' disease. Clin Endocrinol. 2015;84:278–83.

Carlé A, Laurberg P, Knudsen N, Perrild H, Ovesen L, Rasmussen LB, Jorgensen T, Pedersen IB. Thyroid peroxidase and thyroglobulin auto-antibodies in patients with newly diagnosed overt hypothyroidism. Autoimmunity. 2006a;39:497–503.

Carlé A, Laurberg P, Pedersen IB, Knudsen N, Perrild H, Ovesen L, Rasmussen LB, Jorgensen T. Epidemiology of subtypes of hypothyroidism in Denmark. Eur J Endocrinol. 2006b;154:21–8.

Carlé A, Pedersen IB, Knudsen N, Perrild H, Ovesen L, Jorgensen T, Laurberg P. Thyroid volume in hypothyroidism due to autoimmune disease follows a unimodal distribution: evidence against primary thyroid atrophy and autoimmune thyroiditis being distinct diseases. J Clin Endocrinol Metab. 2009;94:833–9.

Carle A, Bulow Pedersen I, Knudsen N, Perrild H, Ovesen L, Banke Rasmussen L, et al. Smoking cessation is followed by a sharp but transient rise in the incidence of overt autoimmune hypothyroidism – a population-based, case-control study. Clin Endocrinol. 2012a;77:764–72.

Carle A, Bulow Pedersen I, Knudsen N, Perrild H, Ovesen L, Rasmussen LB, et al. Moderate alcohol consumption may protect against overt autoimmune hypothyroidism: a population-based case-control study. Eur J Endocrinol. 2012b;167:483–90.

Carle A, Bulow Pedersen I, Knudsen N, Perrild H, Ovesen L, Rasmussen LB, et al. Graves' hyperthyroidism and moderate alcohol consumption: evidence for disease prevention. Clin Endocrinol. 2013;79:111–9.

Carlé A, Pedersen IB, Knudsen N, Perrild H, Ovesen L, Rasmussen LB, Laurberg P. Development of autoimmune overt hypothyroidism is highly associated with live births and induced abortions but only in premenopausal women. J Clin Endocrinol Metab. 2014;99:2241–9.

Caturegli P, De Remigis A, Ferlito M, Landek-Salgado MA, Iwama S, Tzou S-C, Ladenson PW. Anatabine ameliorates experimental autoimmune thyroiditis. Endocrinology. 2012;153:4580–7.

Caturegli P, De Remigis A, Chuang K, Dembele M, Iwama A, Iwama S. Hashimoto thyroiditis: celebrating the centennial through the lens of the Johns Hopkins hospital surgical pathology records. Thyroid. 2013;23:142–50.

Caturegli P, De Remigis A, Rose NR. Hashimoto thyroiditis: clinical and diagnostic criteria. Autoimmun Rev. 2014;13:391–7.

Cerqueira C, Knudsen N, Ovesen L, Laurberg P, Perrild H, Rasmussen LB, Jorgensen T. Doubling in the use of thyroid hormone replacement therapy in Denmark: association to iodization of salt? Eur J Epidemiol. 2011;26:629–35.

Chiovato L, Vitti P, Santini F, Lopez G, Mammoli C, Bassi P, et al. Incidence of antibodies blocking thyrotropin effect in vitro in patients with euthyroid or hypothyroid autoimmune thyroiditis. J Clin Endocrinol Metab. 1990;71:40–5.

Chiovato L, Bassi P, Santini F, et al. Antibodies producing complement-mediated thyroid cytotoxicity in patients with atrophic or goitrous autoimmune thyroiditis. J Clin Endocrinol Metab. 1993;77:1700–5.

Cho BY, Kim WB, Chung JH, Yi KH, Shong YK, Lee HK, Koh CS. High prevalence and little change in TSH receptor blocking antibody titres with thyroxine and antithyroid drug therapy in patients with non-goitrous autoimmune thyroiditis. Clin Endocrinol. 1995;43:465–71.

Choi YM, Kim WG, Kim TY, Bae SJ, Kim HK, Jang EK, et al. Low levels of serum vitamin D3 are associated with autoimmune thyroid disease in pre-menopausal women. Thyroid. 2014;24:655–61.

Dahlgren M, Khosroshahi A, Nielsen GP, Deshpande V, Stone JH. Riedel's thyroiditis and multifocal fibrosclerosis are part of the IgG4-related systemic disease spectrum. Arthritis Care Res. 2010;62:1312–8.

Davies TF. Ord-Hashimoto's disease: renaming a common disorder – again. Thyroid. 2003;13:317.

Davies TF, Amino N. A new classification for human autoimmune thyroid disease. Thyroid. 1993;3:331–3.

Dayan CM, Daniels G. Chronic autoimmune thyroiditis. N Engl J Med. 1996;335:99–107.

Demers LM, Spencer CA. Laboratory medicine practice guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid. 2003;13:3–126.

Demirbilek H, Kandemir N, Gonc EN, Ozon A, Alikasifoglu A, Yordam N. Hashimoto's thyroiditis in children and adolescents: a retrospective study on clinical, epidemiological and laboratory properties of the disease. J Pediatr Endocrinol Metab. 2007;20:1199–205.
Deshpande V, Huck A, Ooi E, Stone JH, Faquin WC, Nielsen GP. Fibrosing variant of Hashimoto thyroiditis is an IgG4-related disease. J Clin Pathol. 2012a;65:725–8.
Deshpande V, Zen Y, Chan JK, Yi EE, Sato Y, Yoshino T, et al. Consensus statement on the pathology of IgG4-related disease. Mod Pathol. 2012b;25:1181–92.
Diana T, Li Y, Olivo PD, Lackner KJ, Kim H, Kanitz M, Kahaly GJ. Analytical performance and validation of a bioassay for thyroid-blocking antibodies. Thyroid. 2016;26:734–40.
Doniach D. Hashimoto's thyroiditis and primary myxoedema viewed as separate entities. Eur J Clin Investig. 1981;11:245–7.
Doniach D, Bottazzo GF, Russell RCG. Goitrous autoimmune thyroiditis (Hashimoto's disease). Clin Endocrinol Metab. 1979;8:63–80.
Effraimidis G, Wiersinga WM. Autoimmune thyroid disease: old and new players. Eur J Endocrinol. 2014;170:R241–52.
Effraimidis G, Tijssen JGP, Wiersinga WM. Discontinuation of smoking increases the risk for developing thyroid peroxidase antibodies and/or thyroglobulin antibodies: a prospective study. J Clin Endocrinol Metab. 2009;94:1324–8.
Effraimidis G, Strieder TGA, Tijssen JGP, Wiersinga WM. Natural history of the transition from euthyroidism to overt autoimmune hypo- or hyperthyroidism: a prospective study. Eur J Endocrinol. 2011a;164:107–13.
Effraimidis G, Tijssen JGP, Strieder TGA, Wiersinga WM. No causal relationship between Yersinia enterocolitica infection and autoimmune thyroid disease: evidence from a propsective study. Clin Exp Immunol. 2011b;165:38–43.
Effraimidis G, Badenhoop K, Tijssen JG, Wiersinga WM. Vitamin D deficiency is not associated with early stages of thyroid autoimmunity. Eur J Endocrinol. 2012a;167:43–8.
Effraimidis G, Tijssen JG, Brosschot JF, Wiersinga WM. Involvement of stress in the pathogenesis of autoimmune thyroid disease: a prospective study. Psychoneuroendocrinology. 2012b;37:1191–8.
Effraimidis G, Tijssen JG, Wiersinga WM. Alcohol consumption as a risk factor for autoimmune thyroid disease: a prospective study. Eur Thyroid J. 2012c;1:99–104.
Ehlers M, Thiel A, Bernecker C, Porwol D, Papewalis C, Willenberg HS, et al. Evidence of a combined cytotoxic thyroglobulin and thyroperoxidase epitope-specific cellular immunity in Hashimoto's thyroiditis. J Clin Endocrinol Metab. 2012;97:1347–54.
Endo K, Kasagi K, Konishi J, Ikekubo K, Okuno T, Takeda Y, Mori T, Torizuka K. Detection and properties of TSH-binding inhibitor immunoglobulins in patients with Graves' disease and Hashimoto's thyroiditis. J Clin Endocrinol Metab. 1978;46:734–9.
Endocrine Society. Endocrine facts and figures: thyroid. 1st ed. 2015. Accessed 2 Aug 2016 http://endocrinefacts.org/health-conditions/thyroid/5-thyroiditis
Evans M, Sanders J, Tagami T, Sanders P, Young S, Roberts E, et al. Monoclonal antibodies to the TSH receptor, one with stimulating activity and one with blocking activity, obtained from the same blood sample. Clin Endocrinol. 2010;73:404–12.
Fatourechi V, McConahey WM, Woolner LB. Hyperthyroidism associated with histologic Hashimoto's thyroiditis. Mayo Clin Proc. 1971;46:682–9.
Feng M, Li H, Chen S-F, Li W-F, Zhang F-B. Polymorphisms in the vitamin D receptor gene and risk of autoimmune thyroid diseases: a meta-analysis. Endocrine. 2013;43:318–26.
Figueroa-Vega N, Alfonso-Perez M, Benedicto I, Sanchez-Madrid F, Gonzalez-Amaro R, Marazuela M. Increased circulating pro-inflammatory cytokines and Th17 lymphocytes in Hashimoto's thyroiditis. J Clin Endocrinol Metab. 2010;95:953–62.
Fiore E, Latrofa F, Vitti P. Iodine, thyroid autoimmunity and cancer. Eur Thyroid J. 2015;4:26–35.
Friedriech N, Schwarz S, Thonack J, John U, Wallaschofski H, Volzke H. Association between parity and autoimmune thyroiditis in a general female population. Autoimmunity. 2008;41:174–80.

Furszyfer J, Kurland LT, Woolner LB, Elveback LR, McConahey WM. Hashimoto's thyroiditis in Olmsted County, Minnesota, 1935 through 1967. Mayo Clin Proc. 1970;45:586–96.

Furszyfer J, Kurland LT, McConahey WM, Woolner LB, Elveback LR. Epidemiologic aspects of Hashimoto's thyroiditis and Graves' disease in Rochester, Minnesota (1935–1967), with special reference to temporal trends. Metabolism. 1972;21:197–204.

Galofre JC, Garcia-Mayor RV, Fluiters E, Fernandez-Calvet L, Rego A, Paramo C, Andrade MA. Incidence of different forms of thyroid dysfunction and its degree in an iodine sufficient area. Thyroidology. 1994;6:49–54.

Garcia-Garcia E, Vazquez-Lopez MA, Garcia-Fuentes E, Rodriquez-Sanchez FI, Munoz FJ, Bonillo-Perales A, Soriquer F. Iodine intake and prevalence of thyroid autoimmunity and autoimmune thyroiditis in children and adolescents aged between 1 and 16 years. Eur J Endocrinol. 2012;167:387–92.

Geenen V, Bodart G, Henry S, Michaux H, Dardenne O, Chatlet-Renard C, Martens H, Hober D. Programming of neuroendocrine self in the thymus and its defect in the development of neuroendocrine autoimmunity. Front Neurosci. 2013;7:187. https://doi.org/10.3389/fnins.2013.00187.

Glick AB, Wodzinski A, Fu P, Levine AD, Wald DN. Impairment of regulatory T-cell function in autoimmune thyroid disease. Thyroid. 2013;23:871–7.

Guo J, Jaume JC, Rapoport B, McLachlan SM. Recombinant thyroid peroxidase-specific Fab converted to immunoglobulin G (IgG) molecules: evidence for thyroid cell damage by IgG1, but not IgG4, autoantibodies. J Clin Endocrinol Metab. 1997;82:925–31.

Hansen PS, Brix TH, Iachine I, Kyvik KO, Hegedus L. The relative importance of genetic and environmental effects for the early stages of thyroid autoimmunity: a study of healthy Danish twins. Eur J Endocrinol. 2006;154:29–38.

Hashimoto H. Zur Kentniss der Lymphomatosen Veranderung der Schilddruse (Struma Lymphomatosa). Arch Klin Chir. 1912;97:219–48.

Hayashi Y, Tamai H, Fukuta S, Hirota Y, Katayama S, Kuma K, et al. A long term clinical, immunological, and histological follow-up study of patients with goitrous lymphocytic thyroiditis. J Clin Endocrinol Metab. 1985;61:1172–8.

Hedley AJ, Young RE, Jones SJ, Alexander WD, Bewsher PD. Antithyroid drugs in the treatment of hyperthyroidism of Graves' disease: long-term follow-up of 434 patients. Clin Endocrinol. 1989;31:209–18.

Hegedus L. Decreased thyroid gland volume in alcoholic cirrhosis of the liver. J Clin Endocrinol Metab. 1984;58:930–3.

Hegedus L, Bonnema SJ, Winther KH. Selenium in the treatment of thyroid diseases: an element in search of the relevant indication? Eur Thyroid J. 2016;5:149–51.

Hollowell J, Staehling N, Flanders W, Hannon W, Gunter E, Spencer C, Braverman L. Serum TSH, T4 and thyroid antibodies in the United States population (1988–1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab. 2002;87:489–99.

Huang CY, Chang TY, Chu CC, Lo FS, Ting WH, Lin CH, Wu YL, Chu SY, Chang SC, Chen WF, et al. The HLA-B gene and Hashimoto disease in Han children: a case-control and family-based study. Tissue Antigens. 2012;80:431–6.

Inoue N, Watanabe M, Ishido N, Katsumata Y, Kagawa T, Hidaka Y, Iwatani Y. The functional polymorphisms of VDR, GC and CYP2R1 are involved in the pathogenesis of autoimmune thyroid diseases. Clin Exp Immunol. 2014;178:262–9.

Irvine WJ, Gray RS, Morris PJ, Ting A. HLA in primary atrophic hypothyroidism and Hashimoto goitre. J Clin Lab Immunol. 1978;1:193–5.

Jacobson EM, Tomer Y. The *CD40, CTLA-4, thyroglobulin, TSH receptor,* and *PTPN22* gene quintet and its contribution to thyroid autoimmunity: back to the future. J Autoimmun. 2007;28:85–98.

Jensen EA, Petersen PH, Blaabjerg O, Hansen PS, Brix TH, Hegedus L. Establishment of reference distributions and decision values for thyroid antibodies against thyroid peroxidase (TPOAb), thyroglobulin (TgAb) and the thyrotropin receptor (TRAb). Clin Chem Lab Med. 2006;44:991–8.

Jokisch F, Kleinlein I, Haller B, Seehaus T, Fuerst H, Kremer M. A small subgroup of Hashimoto's thyroiditis is associated with IgG4-related disease. Virchows Arch. 2016;468:321–7.
Kaczmarek E, Lacka A, Jarmalowska-Jurczyszyn D, Sidor A, Majewski P. Changes of B and T lymphocytes and selected apoptosis markers in Hashimoto's thyroiditis. J Clin Pathol. 2011;64:626–30.
Kahaly GJ, Diana T, Glang J, Kanitz M, Pitz S, Konig J. Thyroid stimulating antibodies are highly prevalent in Hashimoto's thyroiditis and associated orbitopathy. J Clin Endocrinol Metab. 2016;101:1998–2004.
Kaloumenou I, Mastorakos G, Alevizaki M, Duntas LH, Mantzou E, Ladopoulos C, Antoniou A, Chiotis D, Papassotiriou I, Chrousos GP, Dacou-Voutetakis C. Thyroid autoimmunity in schoolchildren in an area with long-standing iodine sufficiency: correlation with gender, pubertal stage, and maternal thyroid autoimmunity. Thyroid. 2008;18:747–54.
Katz SM, Vickery AL. The fibrous variant of Hashimoto's thyroiditis. Hum Pathol. 1974;5:161–70.
Kohrle J. Selenium and thyroid. Curr Opin Endocrinol Diabetes Obes. 2015;5:392–401.
Konishi J, Iida Y, Endo K, Misaki T, Nohara Y, Matsuura N, et al. Inhibition of thyrotropin-induced adenosine 3′5′-monophosphate increase by immunoglobulins from patients with primary myxedema. J Clin Endocrinol Metab. 1983;57:544–9.
Konturek A, Barczynski M, Wierzchowski W, Stopa M, Nowak W. Coexistence of papillary thyroid cancer with Hashimoto's thyroiditis. Langenbeck's Arch Surg. 2013;398:389–94.
Kotani T, Aratake Y, Hirai K, Fukazawa Y, Sato H, Ohtaki S. Apoptosis in thyroid tissue from patients with Hashimoto's thyroiditis. Autoimmunity. 1995;20:231–6.
Kristensen B, Hegedus L, Madsen HO, Smith TJ, Nielsen CH. Altered balance between self-reactive T helper (Th)17 cells and Th10 cells and between full-length forkhead box protein 3 (FoxP3) and FoxP3 splice variants in Hashimoto's thyroiditis. Clin Exp Immunol. 2014;180:58–69.
Kristensen B, Hegedus L, Lundy SK, Brimnes MK, Smith TJ, Nielsen CH. Characterization of regulatory B cells in Graves' disease and Hashimoto's thyroiditis. PLoS ONE. 2015;10: e0127949. https://doi.org/10.1371/journal.pone.0127949.
Kucharska AM, Gorska E, Wasik M, Demkow U. Expression of cytotoxic T lymphocyte antigen-4 in T cells from children with Hashimoto's thyroiditis. Adv Exp Med Biol. 2013;756:163–8.
Latrofa F, Fiore E, Rago T, Antonangeli L, Montanelli L, Ricci D, et al. Iodine contributes to thyroid autoimmunity in humans by unmasking a cryptic epitope on thyroglobulin. J Clin Endocrinol Metab. 2013;98:E1768–74.
Laurberg P. The Danish investigation of iodine intake and thyroid disease: DanThyr – 20 years of iodine monitoring and research. Thyroid Int. 2015; issue 2, pp. 3–26.
Laurberg P, Bulow Pedersen I, Pedersen KM, Vestergaard H. Low incidence rate of overt hypothyroidism compared with hyperthyroidism in an area with moderately low iodine intake. Thyroid. 1999;9:33–8.
Laurberg P, Andersen S, Bulow Pedersen I, Knudsen N, Carlé A. Prevention of autoimmune hypothyroidism by modifying iodine intake and the use of tobacco and alcohol is manoeuvring between Scylla and Charibdis. Hormones. 2011;12:30–8.
Lee HJ, Li CW, Hammerstad SS, Stefan M, Tomer Y. Immunogenetics of autoimmune thyroid disease: a comprehensive review. J Autoimmun. 2015;64:82–90.
Leese GP, Flynn RV, Jung RT, Macdonald TM, Murphy MJ, Morris AD. Increasing prevalence and incidence of thyroid disease in Tayside, Scotland: the Thyroid Epidemiology Audit and Research Study (TEARS). Clin Endocrinol. 2008;68:311–6.
Leskela S, Rodriguez-Munoz A, de la Fuente H, Figueroa-Vega N, Bonay P, Martin P, et al. Plasmacytoid dendritic cells in patients with autoimmune thyroid disease. J Clin Endocrinol Metab. 2013;98:2822–33.
Li Y, Bai Y, Liu Z, Ozaki T, Taniguchi E, Mori I, et al. Immunohistochemistry of IgG4 can help subclassify Hashimoto's autoimmune thyroiditis. Pathol Int. 2009;59:636–41.
Li Y, Nishihara E, Hirokawa M, Taniguchi E, Miyauchi A, Kakudo K. Distinct clinical, serological, and sonographic characteristics of Hashimoto's thyroiditis based with and without IgG4-positive plasma cells. J Clin Endocrinol Metab. 2010;95:1309–17.

Li M, Sun H, Liu S, Yu J, Li Q, Liu P, Shen H, Sun D. CD40 C/T-1 polymorphism plays different roles in Graves' disease and Hashimoto's thyroiditis: a meta-analysis. Endocr J. 2012;59:1041–50.
Li D, Cai W, Gu R, Zhang Y, Zhang H, Tang K, et al. Th17 cells play a role in the pathogenesis of Hashimoto's thyroiditis in patients. Clin Immunol. 2013a;149:411–20.
Li Y, Kim J, Diana T, Klasen R, Olivo PD, Kahaly GJ. A novel bioassay for anti-thyrotropin receptor antibodies detects both thyroid-blocking and stimulating activity. Clin Exp Immunol. 2013b;173:390–7.
Liu L, Wu HQ, Wang Q, Zhu YF, Zhang W, Guan LJ, Zhang JA. Association between thyroid stimulating hormone receptor intron polymorphisms and autoimmune thyroid disease in a Chinese Han population. Endocr J. 2012;59:717–23.
Liu Y, Tang X, Tian J, Zhu C, Peng H, Rui K, et al. Th17/Treg cells imbalance and GITRL profile in patients with Hashimoto's thyroiditis. Int J Mol Sci. 2014;15:21674–86.
Livolsi VA. The pathology of autoimmune thyroid disease: a review. Thyroid. 1994;4:333–9.
Lombardi AF, Fiore E, Tonacchera M, Antonangeli L, Rago T, Frigeri M, et al. The effect of voluntary iodine prophylaxis in a small rural community: the Pescopagano survey 15 years later. J Clin Endocrinol Metab. 2013;98:1031–9.
Mao J, Pop VJ, Bath SC, Vader HL, Redman CWG, Rayman MP. Effect of low-dose selenium on thyroid autoimmunity and thyroid function in UK pregnant women with mild-to-moderate iodine deficiency. Eur J Nutr. 2016;55:55–61.
Marazuela M, Garcia-Lopez MA, Figueroa-Vega N, et al. Regulatory T cells in human autoimmune thyroid disease. J Clin Endocrinol Metab. 2006;91:3639–46.
Marcocci C, Vitti P, Cetani F, Catalano F, Concetti R, Pinchera A. Thyroid ultrasonography helps to identify patients with diffuse lymphocytic thyroiditis who are prone to develop hypothyroidism. J Clin Endocrinol Metab. 1991;72:209–13.
Mariotti S. Chronic autoimmune thyroiditis. In: Monaco F, editor. Thyroid diseases. Boca Raton: CRC Press; 2012. p. 77–96.
Marique L, Van RV, Gerard AC, Craps J, Senou M, Marbaix E, et al. The expression of dual oxidase, thyroid peroxidase, and caveolin-1 differs according to the type of immune response (Th1/Th2) involved in thyroid autoimmune disorders. J Clin Endocrinol Metab. 2014;99:1722–32.
Masi AT, Sartwell PE, Hartmann WH, Shulman LE. Hashimoto's disease. A study of the consistency of its histopathological diagnosis. J Chronic Dis. 1965;18:23–33.
Mazokopakis EE, Papadomanolaki MG, Tsekouras KC, Evangelopoulos AD, Kotsiris DA, Tzortzinis AA. Is vitamin D related to pathogenesis and treatment of Hashimoto's thyroiditis? Hell J Nucl Med. 2015;18:222–7.
McGrogan A, Seaman HE, Wright JW, de Vries CS. The incidence of autoimmune thyroid disease: a systematic review. Clin Endocrinol. 2008;69:687–96.
McLachlan SM, Rapoport B. Thyrotropin-blocking autoantibodies and thyroid-stimulating antibodies: potential mechanisms involved in the pendulum swinging from hypothyroidism to hyperthyroidism or vice versa. Thyroid. 2013;23:14–24.
McLachlan SM, Rapoport B. Breaking tolerance to thyroid antigens: changing concepts in thyroid autoimmunity. Endocr Rev. 2014;35:59–105.
McLeod DS, Cooper DS. The incidence and prevalence of thyroid autoimmunity. Endocrine. 2012;42:252–65.
McLeod DS, Caturegli P, Cooper DS, Matos PG, Hutfless S. Variation in rates of autoimmune thyroid disease by race/ethnicity in US military personnel. JAMA. 2014;311:1563–5.
Medici M, Porcu E, Pistis G, Teumer A, Brown SJ, Jensen RA, et al. Identification of novel genetic loci associated with thyroid peroxidase antibodies and clinical thyroid disease. PLoS Genet. 2014;10:e1004123. https://doi.org/10.1371/journal.pgen.1004123.
Meng S, He ST, Jiang WJ, Xiao L, Li DF, Xu J, et al. Genetic susceptibility to autoimmune thyroid diseases in a Chinese Han population: role of vitamin D receptor gene polymorphisms. Ann Endocrinol (Paris). 2015;76:684–9.

Meyer G, Donner H, Herwig J, Bohles H, Usadel KH, Badenhoop K. Screening for an AIRE-1 mutation in patients with Addison's disease, type 1 diabetes, Graves' disease and Hashimoto's thyroiditis as well as APECED syndrome. Clin Endocrinol. 2001;54:335–8.
Minamino H, Inaba H, Ariyasu H, Furuta H, Nishi M, Yoshimasu T, et al. A novel immunopathological association of IgG4-RD and vasculitis with Hashimoto's thyroiditis. Endocrinol Diabetes Metab Case Rep. 2016. https://doi.org/10.1530/EDM-16-0004.
Mizukami Y, Michigishi T, Hashimoto T, et al. Silent thyroiditis: a histological and immunohistochemical study. Hum Pathol. 1988;19:423–31.
Mizukami Y, Michigishi T, Kawato M, Sato T, Nonomura A, Hashimoto T, Matsubara F. Thyroid function and histologic correlations in 601 cases. Hum Pathol. 1992;23:980–8.
Mizukami Y, Michigishi T, Nonomura A, et al. Postpartum thyroiditis: a clinical, histological and immunopathological study of 15 cases. Am J Clin Pathol. 1993;100:200–5.
Muixi L, Carrascal M, Alvarez I, Daura X, Marti M, Armengol MP, et al. Thyroglobulin peptides associate in vivo to HLA-DR in autoimmune thyroid glands. J Immunol. 2008;181:795–807.
Nabhan ZM, Kreher NC, Eugster EA. Hashitoxicosis in children: clinical features and natural history. J Pediatr. 2005;146:533–6.
Negro R, Greco G, Mangieri T, Pezzarossa A, Dazzi D, Hassan H. The influence of selenium supplementation on postpartum thyroid status in pregnant women with thyroid peroxidase autoantibodies. J Clin Endocrinol Metab. 2007;92:1263–8.
Nithiyananthan R, Howard JM, Allahabadia A, Barnett AH, Franklyn JA, Cough SCL. A heterozygous deletion of the autoimmune regulator *(AIRE1)* gen, autoimmune thyroid disease, and type 1 diabetes: no evidence for association. J Clin Endocrinol Metab. 2000;85:1320–2.
Nystrom E, Bengtsson C, Lindquist O, Noppa H, Lindstedt G, Lundberg PA. Thyroid disease and high concentration of serum thyrotrophin in a population sample of women. A 4-year follow-up. Acta Med Scand. 1981;210:39–46.
Oryoji D, Ueda S, Yamamoto K, Yoshimura Noh J, Okamura K, Noda M, et al. Identification of a Hashimoto thyroiditis susceptibility locus via a genome wide comparison with Graves' disease. J Clin Endocrinol Metab. 2015;100:E319–24.
Owen CJ, Eden JA, Jennings CE, Wilson V, Cheetham TD, Pearce SH. Genetic association studies of the FOXP3 gene in Graves' disease and autoimmune Addison's disease in the United Kingdom population. J Mol Endocrinol. 2006;37:97–104.
Patel H, Mansuri MS, Singh M, Begum R, Shastri M, Misra A. Association of cytotoxic T-lymphocyte antigen 4 (CTLA4) and thyroglobulin (TG) genetic variants with autoimmune hypothyroidism. PLoS ONE. 2016;11:e0149441. https://doi.org/10.1371/journal.pone.0149441.
Pellegrini FP, Marinoni M, Frangione V, Tedeschi A, Gandini V, Ciglia F, et al. Down syndrome, autoimmunity and T regulatory cells. Clin Exp Immunol. 2012;169:238–43.
Popko K, Osinska I, Kucharska A, Demkow U. Cytometric analysis of perforin expression in NK cells, CD8+, and CD4+ lymphocytes in children with autoimmune Hashimoto's thyroiditis – a preliminary study. J Pediatr Endocrinol Metab. 2015;28:789–92.
Prummel MF, Laurberg P. Interferon-alpha and autoimmune thyroid disease. Thyroid. 2003;13:547–51.
Prummel MF, Wiersinga WM. Autoimmune thyroid diseases. In: Gill RG, Harmon JT, Maclaren NK, editors. Immunologically mediated endocrine diseases. Philadelphia: Lippincott Williams & Wilkins; 2002. p. 373–96.
Pyzik A, Grywalska E, Matyjaszek-Matuszek B, Rolinski J. Immune disorders in Hashimoto's thyroiditis: what do we know so far? J Immunol Res. 2015. https://doi.org/10.1155/2015/979167.
Radetti G. Clinical aspects of Hashimoto's thyroiditis. Endocr Dev. 2014;26:158–70.
Radetti G, Gottardi E, Bona G, Corrias A, Salardi S, Loche S, et al. The natural history of euthyroid Hashimoto's thyroiditis in children. J Pediatr. 2006;149:827–32.
Rasmussen LB, Ovesen L, Bulow I, Jorgensen A, Knudsen N, Laurberg P, Perrild H. Dietary iodine intake and urinary iodine excretion in a Danish population: effect of geography, supplements and food choice. Br J Nutr. 2002;87:61–9.
Rasmussen LB, Carlé A, Jorgensen T, Knudsen N, Laurberg P, Pedersen IB, Perrild H, Vejbjerg P, Ovesen L. Iodine intake before and after mandatory iodization in Denmark: results from the

Danish investigation of iodine intake and thyroid diseases (DanThyr) study. Br J Nutr. 2008;100:166–73.
Rebuffat SA, Kammoun-Krichen M, Charfeddine I, Ayadi H, Bougacha-Elleuch N, Peraldi-Roux S. Il-1β and TSH disturb thyroid epithelium integrity in autoimmune thyroid disease. Immunobiology. 2013;218:285–91.
Rodriguez-Munoz A, Martinez-Hernandez R, Ramos-Levi AM, Serrano-Somavilla A, Gonzalez-Amaro R, Sanchez-Madrid F, de la Fuente H, Marazuela M. Circulating microvesicles regulate Treg and Th17 differentiation in human autoimmune thyroid disorders. J Clin Endocrinol Metab. 2015;100:E1531–9.
Roitt IM, Doniach D, Campbell PN, Hudson RV. Autoantibodies in Hashimoto's diseases (lymphadenoid goiter). Lancet. 1956;ii:820–2.
Santos LR, Duraes C, Mendes A, Prazeres H, Alvelos MI, Moreira CS, et al. A polymorphism in the promotor region of the selenoprotein S gene (SEPS1) contributes to Hashimoto's thyroiditis susceptibility. J Clin Endocrinol Metab. 2014;99:E719–23.
Shan Z, Chen L, Lian X, Liu C, Shi B, Shi L, et al. Iodine status and prevalence of thyroid disorders after introduction of mandatory universal salt iodization for 16 years in China: a cross-sectional study in 10 cities. Thyroid. 2016;26:1125–30.
Shmuely H, Shimon I, Gitter LA. Helicobacter pylori infection in women with Hashimoto thyroiditis: a case-control study. Medicine. 2016;95:e4074. https://doi.org/10.1097/MD.000000004074.
Simmonds MJ. GWAS in autoimmune thyroid disease: redefining our understanding of pathogenesis. Nat Rev Endocrinol. 2013;9:277–87.
Simmonds MJ, Kavvoura FK, Brand OJ, Newby PR, Jackson LE, Hargreaves CE, Franklyn JA, Gough SC. Skewed X chromosome inactivation and female preponderance in autoimmune thyroid disease: an association study and meta-analysis. J Clin Endocrinol Metab. 2014;99:E127–31.
Smith TJ, Hegedus L. Graves' disease. N Engl J Med. 2016;375:1552–65.
Stagnaro-Green A, Schwartz A, Gismondi R, Tinelli A, Mangieri T, Negro R. High rate of persistent hypothyroidism in a large-scale prospective study of postpartum thyroiditis in southern Italy. J Clin Endocrinol Metab. 2011;96:652–7.
Stefan M, Wei C, Lombardi A, Li CW, Concepcion ES. Inabnet 3rd WB et al. Genetic-epigenetic dysregulation of thymic TSH receptor gene expression triggers thyroid autoimmunity. Proc Natl Acad Sci U S A. 2014;111:12562–7.
Strieder TGA, Prummel MF, JGP T, Endert E, Wiersinga WM. Risk factors for and prevalence of thyroid disorders in a cross-sectional study among healthy female relatives of patient with autoimmune thyroid disease. Clin Endocrinol. 2003a;59:396–401.
Strieder TGA, Wenzel BE, Prummel MF, Tijssen JGP, Wiersinga WM. Increased prevalence of antibodies to enteropathogenic Yersinia enterocolitica virulence proteins in relatives of patients with autoimmune thyroid disease. Clin Exp Immunol. 2003b;132:278–82.
Strieder TGA, Tijssen JGP, Wenzel BE, Endert E, Wiersinga WM. Prediction of progression to overt hypothyroidism or hyperthyroidism in female relatives of patients with autoimmune thyroid disease using the Thyroid Events Amsterdam (THEA) score. Arch Intern Med. 2008;168:1657–63.
Sundbeck G, Lundberg PA, Lindstedt G, Ingenburg R, Eden S. Incidence and prevalence of thyroid disease in elderly women: results from the longitudinal population study of elderly people in Gothenburg, Sweden. Age Ageing. 1991;20:291–8.
Takasu N, Yamada T, Katakura M, Yamauchi K, Shimizu Y, Ishizuki Y. Evidence for thyrotropin (TSH)-blocking activity in goitrous Hashimoto's thyroiditis with assays measuring inhibition of TSH receptor binding and TSH-stimulated thyroid adenosine 3′,5′-monophosphate responses/cell growth by immunoglobulins. J Clin Endocrinol Metab. 1987;64:239–45.
Tamai H, Kasagi K, Takaichi Y, Takamatsu J, Komaki G, Matsubayashi S, et al. Development of spontaneous hypothyroidism in patients with Graves' disease treated with antithyroid drugs: clinical, immunological, and histological findings in 26 patients. J Clin Endocrinol Metab. 1989;69:49–53.

Tasli F, Ozkok G, Argon A, Ersoz D, Yagci A, Uslu A, et al. The role of IgG4(+) plasma cells in the association of Hashimoto's thyroiditis with papillary carcinoma. APMIS. 2014;122:1259–65.
Teng W, Shan Z, Teng X, Guan H, Li Y, Teng D, et al. Effect of iodine intake on thyroid diseases in China. N Engl J Med. 2006;354:2783–93.
Teng X, Shan Z, Chen Y, Lai Y, Yu J, Shan L, et al. More than adequate iodine intake may increase subclinical hypothyroidism and autoimmune thyroiditis: a cross-sectional study based on two Chinese communities with different iodine intake levels. Eur J Endocrinol. 2011;164:943–50.
Ting WH, Chien MN, Lo FS, Wang CH, Huang CY, Lin CL, et al. Association of cytotoxic T-lymphocyte-associated protein 4 (CTLA4) gene polymorphisms with autoimmune thyroid disease in children and adults: case-control study. PLoS ONE. 2016;11:e0154394. https://doi.org/10.1371/journal.pone.0154394.
Tunbridge WM, Evered DC, Hall R, Appleton D, Brewis M, Clark F, et al. The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol. 1977;7:481–93.
Ueda S, Oryoji D, Yamamoto K, Noh JY, Okamura K, Noda M, et al. Identification of independent susceptible and protective HLA alleles in Japanese autoimmune thyroid disease and their epistasis. J Clin Endocrinol Metab. 2014;99:E379–83.
van Belle TL, Gysemans C, Mathieu C. Vitamin D in autoimmune, infectious and allergic diseases: a vital player? Best Pract Res Clin Endocrinol Metab. 2011;25:617–32.
Vanderpump MPJ. The epidemiology of thyroid disease. Br Med Bull. 2011;99:39–51.
Vanderpump MP, Tunbridge WM, French JM, Appleton D, Bates D, Clark F, Grimley Evans J, Hasan DM, Rodgers H, Tunbridge F. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clin Endocrinol. 1995;43:55–68.
Vejbjerg P, Knudsen N, Perrild H, Laurberg P, Carlé A, Pedersen IB, Rasmussen LB, Ovesen L, Jorgensen T. Lower prevalence of mild hypothyroidism related to a higher iodine intake in the population: prospective study of a mandatory iodization programme. Clin Endocrinol. 2009;71:440–5.
Vickery AL, Hamblin Jr E. Struma lymphomatosa (Hashimoto's thyroiditis): observations on repeated biopsies in sixteen patients. N Engl J Med. 1961;264:226–9.
Villanueva R, Greenberg DA, Davies TF, Tomer Y. Sibling recurrence risk in autoimmune thyroid disease. Thyroid. 2003;13:761–4.
Walsh JP, Bremner AP, Bulsara MK, O'Leary P, Leedman PJ, Feddema P, Michelangeli V. Parity and the risk of autoimmune thyroid disease: a community-based study. J Clin Endocrinol Metab. 2005;90:5309–12.
Walsh JP, Bremmer AP, Feddema P, Leedman PJ, Brown SJ, O'Leary P. Thyrotropin and thyroid antibodies as predictors of hypothyroidism: a 13-year longitudinal study of a community-based cohort using current immunoassay techniques. J Clin Endocrinol Metab. 2010;85:1095–104.
Walsh JP, Berry J, Liu S, Panicker V, Dayan CM, Brix TH, Hegedus L, Hou P, Shi B, Morahan G. The clinical presentation of autoimmune thyroid disease in men is associated with *IL12B* genotype. Clin Endocrinol. 2011;74:508–12.
Wang J, Lv S, Chen G, Gao C, He J, Zhong H, et al. Meta-analysis of the association between vitamin D and autoimmune thyroid disease. Nutrition. 2015;7:2485–98.
Wasniewska M, Corrias A, Salerno M, Lombardo F, Aversa T, Mussa A, et al. Outcomes of children with Hashitoxicosis. Horm Res Paediatr. 2012a;77:36–40.
Wasniewska M, Corrias A, Salerno M, Mussa A, Capalbo D, Messina MF, Aversa T, Bombaci S, De Luca F, Valenzise M. Thyroid function patterns at Hashimoto's thyroiditis presentation in childhood and adolescence are mainly conditioned by patient's age. Horm Res Paediatr. 2012b;78:232–6.
Weetman AP. Autoimmune thyroid disease: propagation and progression. Eur J Endocrinol. 2003;148:1–9.
Weetman AP. Immunity, thyroid function and pregnancy: molecular mechanisms. Nat Rev Endocrinol. 2010;6:311–8.
Weetman AP. Autoimmune thyroid disease. Chapter 18. In: Jameson LJ, et al., editors. Endocrinology: adult and pediatric. 7th ed. Philadelphia: Elsevier/Saunders; 2016. p. 1423–36.

Wichman J, Winther KH, Bonnema SJ, Hegedus L. Selenium supplementation significantly reduces thyroid autoantibody levels in patients with chronic autoimmune thyroiditis: a systematic review and meta-analysis. Thyroid. 2016. https://doi.org/10.1089/thy.2016.0256. [Epub ahead of print].
Wiersinga WM. Smoking and thyroid. Clin Endocrinol. 2013;79:145–51.
Wiersinga WM. Thyroid autoimmunity. Endocr Dev. 2014;26:139–57.
Wiersinga WM. Clinical relevance of environmental factors in the pathogenesis of autoimmune thyroid disease. Endocrinol Metab. 2016;31:213–22.
Williams ED, Doniach I. The post-mortem incidence of focal thyroiditis. J Pathol Bacteriol. 1962;83:255–64.
Winther KH, Watt T, Bjorner JB, Cramon P, Feldt-Rasmussen U, Gluud C, et al. The chronic autoimmune thyroiditis quality of life selenium trial (CATALYST): study protocol for a randomized controlled trial. Trials. 2014;15:115.
Winther KH, Wichman JE, Bonnema SJ, Hegedus L, Insufficient documentation for clinical efficacy of selenium supplementation in chronic autoimmune thyroiditis, based on a systematic review and meta-analysis. Endocrine. 2016. https://doi.org/10.1007/s12020-016-1098z. [Epub ahead of print].
Wood LC, Ingbar SH. Hypothyroidism as a late sequela in patients with Graves' disease treated with antithyroid agents. J Clin Invest. 1979;64:1429–36.
Wu Q, Rayman MP, Lv H, Schomburg L, Cui B, Gao C, et al. Low population selenium status is associated with increased prevalence of thyroid disease. J Clin Endocrinol Metab. 2015;100:4037–47.
Xu C, Wu F, Mao C, Wang X, Zheng T, Bu L et al. Excess iodine promotes apoptosis of thyroid follicular epithelial cells by inducing autophagy suppression and is associated with Hashimoto thyroiditis disease. J Autoimmun 2016; https://doi.org/10.1016/j.jaut2016.07.008. [E-pub ahead of print].
Xue H, Yang Y, Zhang Y, Song S, Zhang L, Ma L, et al. Macrophage migration inhibitory factor interacting with Th17 cells may be involved in the pathogenesis of autoimmune damage in Hashimoto's thyroiditis. Mediat Inflamm. 2015a. https://doi.org/10.1155/2015/621072.
Xue H, Yu X, Ma L, Song S, Li Y, Zhang L, et al. The possible role of CD4+CD25^{high}Foxp3+/CD4+IL-17A+ cell imbalance in the autoimmunity of patients with Hashimoto thyroiditis. Endocrine. 2015b;50:665–73.
Yasmeh J, Farpour F, Rizzo V, Kheradnam S, Sachmechi I. Hashimoto's thyroiditis not associated with vitamin D deficiency. Endocr Pract. 2016. https://doi.org/10.4158/EP15934.OR. [E-pub ahead of print].
Zaletel K, Gaberscek S, Pirnat E, Krhin B, Hojker S. Ten-year follow-up of thyroid epidemiology in Slovenia after increase in salt iodization. Croat Med J. 2011;52:615–21.
Zha B, Huang X, Lin J, Liu J, Hou Y, Wu G. Distribution of lymphocyte subpopulations in thyroid glands of human autoimmune thyroid disease. J Clin Lab Anal. 2014;28:249–54.
Zhang J, Zhao L, Gao Y, Liu M, Li T, Huang Y, et al. A classification of Hashimoto's thyroiditis based on immunohistochemistry for IgG4 and IgG. Thyroid. 2014;24:364–70.
Zophel K, Saller B, Wunderlich G, Gruning T, Koch R, Wilde J, et al. Autoantibodies to thyroperoxidase (TPOAb) in a large population of euthyroid subjects: implications for the definition of TPOAb reference intervals. Clin Lab. 2003;49:591–600.

Postpartum Thyroiditis and Silent Thyroiditis

8

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Abstract

Postpartum thyroiditis (PPT) is an autoimmune "destructive" thyroiditis that occurs in the first year following delivery and, rarely, after a miscarriage. The autoimmune nature of the disease is confirmed by the presence of thyroid peroxidase antibodies (TPOAb) in most affected women (and occasionally thyroglobulin antibodies) and immune cell infiltration of the thyroid gland. These are usually combined with the imaging characteristics of a destructive thyroiditis (low radioactive iodine or technetium uptake and ultrasound hypoechogenicity).

PPT may present in several ways – a mild and short thyrotoxic phase followed by a more symptomatic and longer hypothyroid phase occur in the biphasic form of PPT, in about 24–28% subjects. However, the thyrotoxic and hypothyroid phases may occur independently of each other, with hypothyroidism occurring alone in about 50% of cases. The thyrotoxic phase usually requires symptomatic treatment only, but the hypothyroid phase produces more severe and longer-lasting symptoms and requires thyroxine replacement in the majority. Early permanent hypothyroidism occurs in a minority of women, and a high percentage of those who are antibody positive and develop PPT develop long-term thyroid dysfunction. There is approximately a 70% chance of PPT recurring in subsequent pregnancies. The presence of TPOAb, gestational and type 1 diabetes mellitus, autoimmune thyroid disease, and previous PPT are risk factors for the development of PPT. The strategies for screening and prevention are not clear, and there is no consensus currently.

Sporadic silent thyroiditis has identical pathological and clinical features to postpartum thyroiditis but occurs sporadically, outside the postpartum setting. It is characterized by a similar destructive thyroiditis with thyrotoxic, hypothyroid, and euthyroid phases. The absence of thyroid pain or raised inflammatory markers distinguishes this form of thyroiditis from acute and subacute thyroiditis. Although silent thyroiditis is typically self-limiting, some patients have severe and recurrent episodes, and permanent hypothyroidism is seen in about 10–20% of patients.

Key words

Sporadic thyroiditis · Postpartum thyroiditis · Pregnancy · Thyrotoxicosis · Hypothyroidism

Introduction

The term thyroiditis refers to a heterogeneous group of thyroid disorders that arise from a variety of causes including autoimmunity, infections, drugs, and infiltrative conditions (Table 1). The clinical manifestations of these conditions range from thyrotoxicosis to hypothyroidism and goiter and may vary in severity from mild disease to severe complicated illnesses. Postpartum thyroiditis (PPT) and silent thyroiditis are forms of "destructive" thyroiditis in which there is destruction of thyroid follicular cells resulting in release of pre-formed thyroid hormones into the circulation. These conditions often arise de novo but may also develop on the background of preexisting thyroid disease such as Graves' disease and Hashimoto's thyroiditis. The typical clinical course is that of an initial thyrotoxic phase, followed by a hypothyroid phase, and finally a return to euthyroid state. The two conditions are now believed to be essentially the same illness with PPT developing within the first postpartum year and silent thyroiditis occurring sporadically in the patient who has not been pregnant. Patients with silent and postpartum thyroiditis can be distinguished from those with subacute or acute infectious thyroiditis by the absence of pain, fever, or laboratory markers of acute inflammation. Because these conditions are typically self-limiting, it is essential that they are promptly recognized so as to prevent unwarranted therapy with antithyroid drugs and to allow discontinuation of levothyroxine therapy at the end of the illness.

Postpartum Thyroiditis

Postpartum thyroiditis (PPT) is defined as the occurrence of thyrotoxicosis or hypothyroidism alone or thyrotoxicosis followed by hypothyroidism in the first postpartum year in women without preexisting thyroid disease. However, there is evidence to suggest that the biochemical changes of PPT can occur in a high percentage of women on thyroxine therapy given for hypothyroidism predating

Table 1 Causes of thyroiditis

Autoimmune
Chronic autoimmune (Hashimoto's) thyroiditis
Postpartum thyroiditis
Silent thyroiditis
Infections
Acute viral, bacterial, fungal, and parasitic infections
Drugs
Lithium, interferon, amiodarone
Miscellaneous
Riedel's thyroiditis, radiation-induced thyroiditis

Table 2 A concise "history" of postpartum thyroiditis

Time	Contributors	Remarks
98–138 AD	Soranus	Noted "swelling of neck after pregnancy"
1260–1473	Various artists: Duccio, van der Weyden, Fra Filippo Lippi, Da Vinci	Painted women with goiters, holding infants in their laps
Nineteenth century	Caleb Hillier Parry, Armand Trousseau	Case reports of thyroid dysfunction after delivery
1888	Sir Horatio Bryan Donkin	Possible early report of postpartum hypothyroidism
1891	George Murray	Used thyroid extract in a patient with possible PPT. Noted multiparity as a risk factor for hypothyroidism
1948	Roberton	Successful treatment of hypothyroid phase of PPT with thyroid extracts
1961	Parker and Beierwaltes	Described thyroid antibody modulation during pregnancy and postpartum
1976–1982	Amino	Case series characterizing PPT
1977	Ginsburg and Walfish	First description of biphasic PPT
1990 onwards	Hall, Stagnaro-Green, Davies, Lazarus	Produced evidence of epidemiology, immunology, clinical features, long-term outcome, and screening for PPT

Adapted from Smallridge and Clark (2014)

pregnancy (Sergi et al. 2015). PPT may also occur less commonly after spontaneous miscarriages (Stagnaro-Green 2012).

Historical Aspects

The earliest descriptions of PPT were attributed to Soranus in the first and second centuries AD, when he noted "swelling in the neck" in women who had recently delivered. Many centuries later, Leonardo Da Vinci and other artists painted women with visible goiters, holding infants in their laps, indicating the postpartum state (Smallridge and Clark 2014). But it was not until the 1940s that physicians began to associate the postpartum period with thyroid dysfunction. Roberton in his report in the British Medical Journal first described the successful treatment of the hypothyroid phase of PPT with thyroid extract (Roberton 1948). Since then there have been many case reports, research studies, and reviews of the risk factors for PPT, its immunology, epidemiology, clinical features, long-term outcome, and the clinical utility of screening (Table 2).

The Incidence of PPT

PPT is one of the most common endocrine disorders, but precise incidence rates are difficult to estimate due to significant variability of studies in terms of PPT

Table 3 Clinical, immunological, and ultrasound evidence that PPT is an autoimmune disease

Characteristics of PPT	Remarks
Presence of antithyroid antibodies	Thyroid peroxidase antibodies (TPOAb) in the majority
	Thyroglobulin antibodies (TgAb) in the rest
Postpartum increase in complement activating subclasses of TPOAb	Only IgG1-IgG3 increase. No increase in IgG4 which cannot activate complement
Histological features of thyroiditis	Lymphocyte infiltration and follicle formation similar to autoimmune thyroiditis
Association with specific HLA haplotypes: DR3, DR4, DR5	Similar pattern to autoimmune thyroiditis
Characteristic hypoechogenic ultrasound features	Similar pattern to autoimmune thyroiditis

definition, study design, timing of antibody screening, frequency and length of follow-up, and thyroid antibody testing methodology. In one study, the pooled prevalence of PPT, defined as an abnormal thyroid-stimulating hormone (TSH) level, for the general population was 8.1% (95% confidence interval 6.6–10.0) although there was marked geographic and population genetic heterogeneity in the studies analyzed (Nicholson et al. 2006). For example, incidence estimates of PPT for the USA is 5.7%, Asia 4.4%, Spain 9.3%, Sweden 7.3%, and the Netherlands 6.3% (Nicholson et al. 2006). An incidence rate of 4–8% is perhaps an accurate estimate, i.e., almost 1 in every 20 women or more, who delivers, is likely to get PPT.

Pathogenesis of PPT

Evidence that PPT Is an Autoimmune Disease

There are many features of PPT, which point to an autoimmune origin (Table 3; Stagnaro-Green 2012; Feldt-Rasmussen et al. 1990). Thyroid-related antibodies, which are markers for thyroid autoimmunity, are present in the majority of women who develop PPT. It is estimated that 33–52% of those who are antibody positive in early pregnancy will develop PPT. The majority of these antibody-positive women have thyroid peroxidase antibodies (TPOAb), and the rest have thyroglobulin antibodies (TgAb). The nature of thyroid dysfunction in those who are antibody negative (a small minority) but develop PPT is speculative. There is also good evidence that the TPOAb titer in the first trimester is predictive of PPT (Premawardhana et al. 2004). Furthermore, the typical histological features of PPT closely resemble those of autoimmune thyroid disease – there is lymphocyte and immune cell infiltration and follicle formation reminiscent of Hashimoto's thyroiditis. Although complement subclasses capable of activating the complement cascade (i.e., IgG1-IgG3) are increased during the postpartum period (and IgG4, the complement component which cannot activate this cascade, remains unchanged), there is no conclusive immunological proof of complement activation (Okosieme et al. 2002).

The Immune Tolerance of Pregnancy

To understand the immunological basis of PPT, it is important to appreciate the maternal immune changes that take place during pregnancy and the "rebound" that occurs when these changes are reversed in the postpartum period. These changes to maternal immunity in pregnancy are designed to immunologically "tolerate" and prevent rejection of the fetus with its paternal antigens. The fetus is semi-allogeneic (i.e., it contains antigens from both mother and father), and therefore the unmodulated maternal immune system has the potential to reject it. However, maternal "immunomodulation" results in tolerance and preservation of the fetus. The fetal trophoblast, maternal T cells, and maternal antibody production are all modified in a manner that enhances tolerance and reduces rejection. This immune tolerance seems to be localized and specific to paternal antigens in humans, and the important role of regulatory T cells (Tregs) in this process is now being elucidated (La Rocca et al. 2014).

T Regulatory Cells

Tregs (previously called T suppressor cells) form a small subset of the T cell population in humans and comprise approximately 5–15% of the CD4+ T cell load in humans. These cells are either thymic or peripheral and bear the surface markers CD25 and Foxp3. Tregs suppress the activity of immunocompetent cells (natural killer [NK] cells, CD4+ and CD8+ T cells, dendritic cells, and B cells) when in close proximity to them and in the presence of interleukin-2. This suppressive action is exerted by means of several mechanisms including cell-to-cell contact, the use of soluble factors (IL-10, TGF-beta, CD 35), competitive inhibition, cytolysis, and metabolic disruption (Galustian and Dasgupta 2001). Tregs specific to paternal antigen-bearing cells appear in uterine lymph nodes very early – usually the day after implantation. Their number increases in the peripheral blood in the first trimester, peaks in the second trimester in parallel with increasing immune tolerance, and rapidly decreases in the postpartum period. Their immunosuppressor function is of major importance in the immunomodulation of pregnancy, and the immune rebound that occurs during the postpartum period is thought to be due to their reduction soon after delivery.

Other Cellular Mechanisms

A number of additional mechanisms other than Tregs are likely to be involved in the process of pregnancy-induced immunomodulation. Briefly, these are: (a) inhibition of maternal T cell proliferation by depriving trophoblasts and macrophages of tryptophan, through suppression of the enzyme indoleamine 2,3-dioxygenase (Durr and Kindler 2013); (b) apoptosis of activated maternal T cells by the expression of Fas ligand by fetal trophoblast cells (Hunt et al. 1997); (c) suppression of NK cells, mainly decidual NK cells, by HLA-G expression (Rouas-Freiss et al. 1997); (d) limited expression of MHC class I and class II molecules on trophoblasts (Erlebacher et al. 2007); and (e) reduction of complement activation by CD46, CD55, and CD59 expression (Xu et al. 2000). Lastly, the T helper cell 1 (Th1) and T helper cell 2 (Th2) paradigms have been proposed as a further model of immune

tolerance in pregnancy. During pregnancy, there appears to be a Th2 predominance (resulting in a cytokine profile inhibiting humoral immunity) with suppression of Th1 (which usually produces a cytokine profile inhibiting cellular immunity) (La Rocca et al. 2014). However, the Th2 predominant model cannot explain all the features of maternal immune tolerance, and there are clearly more complex features that need to be accounted for, e.g., the involvement of Th17 cells. However, a Treg-induced conversion from a Th1 to a Th2 paradigm is plausible though unproven.

Hormonal Influences

The main drivers for these cellular and immune changes are thought to be the changes in the maternal hormonal milieu that occur during pregnancy. Placental production of progesterone and estradiol is increased in pregnancy and is of critical importance in the survival and maintenance of the fetal allograft. During pregnancy, progesterone and estradiol influence a variety of immune cells including macrophages, monocytes, natural killer cells, and dendritic cells. These effects are achieved through hormone binding to specific cell surface receptors or indirectly via modulation of cytokine and growth factor activity (Schumacher et al. 2014). Pregnancy-related hormones also influence development of B cell subsets that promote fetal survival thus creating a complex network of hormonal and cellular interactions (Schumacher et al. 2014). In addition, high concentrations of immunomodulatory hormones, namely, cortisol, norepinephrine, and 1,25-dihydroxyvitamin-D, have been observed in conjunction with low levels of pro-inflammatory cytokines in the third trimester of pregnancy with reciprocal changes in the postpartum (Elenkov et al. 2001). In another study, patients who subsequently developed PPT had lower cortisol levels and higher concentrations of IFN-gamma at 36 weeks of gestation than women who remained euthyroid suggesting that the immunological determinants of PPT occur to some extent in the antenatal period (Kokandi et al. 2003).

The Postpartum Immunological Rebound

These immunomodulatory mechanisms are highly efficient during pregnancy, ensuring fetal survival, but they reverse in the immediate postpartum period when all the above constraints on maternal immune function are removed. The "heightened" state of immunity in the immediate postpartum period may be the result of several mechanisms. Following delivery of the fetus, there is a decline in pregnancy-related hormones as well as a relative reduction in the concentration of various immunomodulatory hormones thus allowing reversal to the pro-inflammatory cellular and cytokine milieu (Elenkov et al. 2001). Another possible mechanism relates to Tregs as indicated above. A rapid fall of Tregs occurs soon after, delivery and their controlling effects on immunity are rapidly lost. It is plausible that having been released from Treg-mediated suppression, preexisting thyroid-reactive T cells may cause a "destructive" thyroiditis. Microchimerism where fetal cells cross over through the placenta to reside in maternal tissues for long periods of time is another plausible mechanism (Brix et al. 2009).

Risk Factors for PPT

Thyroid Peroxidase Antibodies (TPOAb)

The presence of TPOAb in pregnancy identifies a subset of women who are at a higher risk of developing PPT. Between 33% and 52% of subjects who have TPOAb in the first trimester develop PPT. However, TPOAb alone is a weak predictor of PPT, and its positive predictive value (PPV) is only 0.31–0.55 (Lazarus et al. 1999). In a recent Australian study, the presence of TPOAb in the first trimester predicted PPT with a PPV of only 0.44 (0.2–0.71) (Ekinci et al. 2015).

Previous PPT

PPT in a previous pregnancy in TPOAb-positive women increases the risk of PPT in subsequent pregnancies. One study reported that 69% of such women developed PPT in a subsequent pregnancy compared to only 25% who were TPOAb positive but did not have PPT (Lazarus et al. 1997).

Type 1 Diabetes Mellitus (T1DM) and Gestational Diabetes Mellitus (GDM)

Several studies from geographically disparate areas have examined the incidence of PPT in T1DM and have shown an increase in incidence of between three and four times when those with T1DM were compared to those without T1DM (Table 4; Stagnaro-Green 2012; Bech et al. 1991; Feldt-Rasmussen et al. 1990; Gerstein 1993; Walfish et al. 1992; Alvarez-Marfany et al. 1994; Stagnaro-Green et al. 1992; Gallas et al. 2002; Kuijpens et al. 1998). There is recent evidence that women who have GDM are also at increased risk of PPT. In a study from Iran, 334 women with GDM were compared to 313 women who did not have GDM, during follow-up for 1 year postpartum. There was a significant difference in the incidence of PPT between the two groups (16.6 vs. 6.1%; $p < 0.001$) although there was no difference in the prevalence of thyroid antibodies between them (Maleki and Tavosi 2015).

Previous Autoimmune Thyroid Disease (AITD)

Recent studies of women on thyroxine replacement therapy for preexisting autoimmune thyroid disease have shown that in 68% of them biochemical thyroid function

Table 4 The incidence of PPT in women with T1DM

Country	Incidence of PPT	
	Women without T1DM	Women with T1DM
Denmark (Bech et al. 1991; Feldt-Rasmussen et al. 1990)	3.3	10.5
Canada (Gerstein 1993; Walfish et al. 1992)	6	25
USA (Alvarez-Marfany et al. 1994; Stagnaro-Green et al. 1992)	8.8	25
Netherlands (Gallas et al. 2002; Kuijpens et al. 1998)	5.2	15.9

Reviewed in Stagnaro-Green (2012)

fluctuated postpartum in a manner suggestive of PPT (Sergi et al. 2015). This is a significantly higher proportion compared to those who did not have preexisting AITD.

Other Autoimmune Disorders

An increased incidence of PPT has been shown in women with SLE and autoimmune hepatitis in a few studies (Stagnaro-Green 2012). However, it is not clear from these studies if this increased risk is related to the presence of thyroid autoantibodies or to other disease-specific antibodies.

Environmental and Other Factors

The role of environmental factors and factors such as smoking, the presence of a goiter, and a family history of thyroid disease is unclear and needs to be further elucidated. Early studies of smoking and PPT demonstrated an increased risk of developing the disease (Kuijpens et al. 1998; Fung et al. 1988), but further studies are needed to define this relationship more precisely. Selenium given to pregnant TPOAb-positive women reduced TPOAb titers and the incidence of PPT (Negro et al. 2007). This needs clarification in further studies too.

Clinical Features of PPT

Clinical Subtypes

The typical biphasic form of PPT occurs in only about a quarter to a third of patients developing PPT. In these patients, an early thyrotoxic phase (with a median time to onset of about 12–13 weeks) gives way to a hypothyroid phase (median time to onset of about 19 weeks) usually after a period of biochemical euthyroidism. However, isolated thyrotoxicosis may occur in a quarter to a third of women and isolated hypothyroidism in about 50% of those who develop PPT (Lazarus and Premawardhana 2011) (Fig. 1).

The symptoms associated with the thyrotoxic phase of PPT are mild, lasting only a few weeks, and self-limiting. Fatigue, palpitations, and nervousness occurred more frequently in women with PPT than in euthyroid controls (Stagnaro-Green 2012). The hypothyroid phase was associated with more severe symptoms lasting several months and required thyroxine replacement therapy in the majority. Tiredness and memory impairment also occur more commonly in women with PPT than in euthyroid women, and in one study fatigue, cold intolerance, hoarseness, dry hair, and paresthesia were more common in TPOAb-positive compared to TPOAb-negative women with PPT (Kuijpens et al. 2001).

Differential Diagnosis of the Thyrotoxic Phase

It is important to differentiate thyrotoxic PPT (which is about 20 times more common in the postpartum period) from postpartum Graves' disease (GD) as their management differs. It is important to remember that about 40–45% of women who

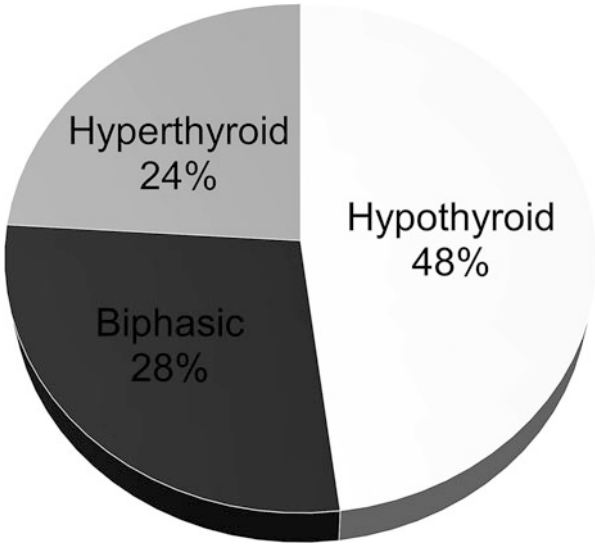

Fig. 1 Incidence of clinical subtypes of postpartum thyroiditis

Table 5 Differentiating between thyrotoxic PPT and postpartum Graves' disease

Feature	Thyrotoxic PPT	Postpartum GD
Onset of symptoms	Early (12–13 weeks)	Late (6–12 months)
Severity of symptoms	Usually mild	Often moderate or severe
Clinical signs	Small goiter (30–40%)	Smooth goiter (90%) with bruit, rarely the presence of orbitopathy, acropachy and pretibial myxedema
	No extra-thyroidal signs	
T4/T3 ratio	T4 predominant	T3 predominant
Thyrotropin receptor antibody (TRAb)	Negative	Positive
Thyroid blood flow	Low	High
Thyroid scintigraphy	Low to absent	High uptake

develop GD in Denmark, Japan, and the USA do so in the postpartum period (Andersen et al. 2015). The following clinical features and investigations may help differentiate thyrotoxic PPT from GD (Table 5).

Onset of Symptoms and Clinical Features

The onset of symptoms of GD is usually between 6 and 12 months postpartum, compared to a median time of 12–13 weeks for thyrotoxic PPT (Ide et al. 2014). However, there may be an overlap in a minority, and in one study, 22% of postpartum GD occurred earlier (Ide et al. 2014). Although no symptom or symptom

complex is specific for either of these conditions, the presence of a goiter, often with a bruit over it, and the presence of extra-thyroidal manifestations (e.g., orbitopathy, pretibial myxedema) would favor GD.

Free T4 and Free T3 Ratio

Thyrotoxic PPT is an autoimmune "destructive" thyroiditis, and the clinical features of thyrotoxicosis are caused by the release of pre-formed thyroid hormones stored intra-thyroidally, i.e., there will be T4 predominance as T4 is preferentially stored within the thyroid gland. However, in postpartum GD, there is T3 predominance due to thyroidal secretion of T3 and peripheral conversion from T4. Although the T4/T3 ratio was used in the past to differentiate between the two conditions, this ratio is not in common use now.

Thyrotropin Receptor Antibodies (TRAb)

Third-generation TRAb assays are very sensitive and specific in the diagnosis of GD – both estimated to be between 97% and 99% (Tozzoli et al. 2012). The use of third-generation assays has eliminated the overlap that used to exist between the two conditions with early assays. TRAb testing is extremely helpful in differentiating between thyrotoxic PPT and early onset of postpartum GD (particularly in the first 3–6 months) (Ide et al. 2014).

Thyroid Blood Flow (TBF) and Ultrasound Scans (TUS)

Thyroid volume increases during pregnancy (Rasmussen et al. 1989a), and ultrasound thyroid hypoechogenicity may predict the onset of PPT (Adams et al. 1992). However, thyroid ultrasound and thyroid blood flow measurements may not be easily available to clinicians at short notice. But where available, they may help in differentiating between the highly vascular thyroid of GD from the relatively avascular thyroid of PPT (Ide et al. 2014).

Thyroid Scintigraphy

Thyroid scintigraphy is rarely required or desirable in the immediate postpartum period, particularly if the mother is breastfeeding. Guidelines recommend stopping breastfeeding for 4–12 h (discarding milk from one to three breastfeeds) for technetium-labeled compounds and cessation of breast feeding for 12 h (discarding milk for three feeds) to 3 weeks for most iodine-labeled compounds (Leide-Svegborn et al. 2016; Mattsson et al. 2015). Although most forms of technetium have a short half-life (measured in several hours) and may theoretically be used, the above clinical features and investigations are sufficient to make a differential diagnosis in the majority of women.

Differential Diagnosis of the Hypothyroid Phase of PPT

The differentiation of hypothyroid PPT from Hashimoto's thyroiditis is difficult. Both conditions present late, and the majority of affected women are TPOAb positive. However, the majority of those with hypothyroid PPT will not require thyroxine beyond the first postpartum year, and drug withdrawal is safe in them –

they will remain symptom-free with TSH levels within the reference range. However, it is prudent to remember that at the end of the first postpartum year, between 4% and 54% of these women may require long-term thyroxine therapy as they may have become permanently hypothyroid (Stagnaro-Green 2012). Patients with Hashimoto's thyroiditis on the other hand will become symptomatically and biochemically hypothyroid when thyroxine is withdrawn and will require long-term thyroxine replacement therapy.

Long-Term Outcome of PPT

Although the majority of women with PPT recover at the end of the first postpartum year, there is a significant proportion with persistent thyroid dysfunction requiring long-term thyroxine therapy. These women often have higher TPOAb titers and higher TSH levels during the hypothyroid phase of PPT and are more likely to be multiparous and older with a greater degree of ultrasound hypoechogenicity during the index illness (Premawardhana et al. 2000). However, prediction of long-term hypothyroidism in individual subjects is difficult.

The mechanism of persistent thyroid dysfunction is currently unclear. An organification defect was demonstrated by means of a perchlorate discharge test, in 41% of Italian and 64% of Welsh women 4–7 years after the index pregnancy (Creagh et al. 1994). Continuing thyroid damage was also demonstrated by a higher prevalence of thyroid ultrasound hypoechogenicity in those women who had TPOAb and had hypothyroid PPT, compared to those who did not and those who were TPOAb negative during 66–144 months of follow-up (Premawardhana et al. 2000). It appears therefore that low-grade thyroid damage, possibly autoimmune in nature, continues after pregnancy although the maximum damage would have occurred at the time of the initial illness.

Permanent hypothyroidism with a need for long-term thyroxine was present in 30% of subjects with TPOAb and PPT at the end of the first postpartum year in a study from South Wales (Premawardhana et al. 2000). However, there has been a variable prevalence of 12–61% of permanent hypothyroidism reported from other parts of the world (Lazarus 2011). This variability was likely due to a lack of uniformity in the definition of PPT, length and frequency of follow-up, and possible environmental and genetic heterogeneity.

A variable prevalence of hypothyroidism has also been reported in longer-term studies of PPT from geographically disparate regions (Table 6; Premawardhana et al. 2000; Nikolai et al. 1987; Tachi et al. 1988; Othman et al. 1990; Barca et al. 2000; Azizi 2004, 2005). In the Welsh study (Premawardhana et al. 2000), 98 TPOAb-positive women (of whom 48 developed PPT) and 70 TPOAb-negative controls were followed up for 66–140 months. Forty-six percent of women who developed PPT were hypothyroid (some subclinically) compared to only 4% of women who were TPOAb positive but did not develop PPT and 1.4% of women who were TPOAb negative. The rate of conversion to hypothyroidism in women who were TPOAb positive and developed PPT was 7.1% per year, higher than that reported for

Table 6 Long-term follow-up of PPT

Author	Follow up years	Number of patients	% hypothyroid[a]
Nikolai et al. (1987)	3	27	12
Tachi et al. (1988)	8.7	44	29
Othman et al. (1990)	3.5	43	23
Premawardhana et al. (2000)	6.6	98	24.5
Barca et al. (2000)	2	49	61
Azizi (2004)	2	148	63
Azizi (2005)	2	172[a]	63

[a]Includes overt and subclinical hypothyroidism

women in community-based follow-up studies. In a study from Japan with a mean follow-up after PPT of 8.7 years, Tachi found a 29% prevalence of permanent hypothyroidism (Tachi et al. 1988). Similarly, Jansson reported a 30% prevalence of hypothyroidism at 5 years in Sweden (Jansson et al. 1984). The reason for a high prevalence of 61% with hypothyroidism at the end of 2 years after PPT in Brazil is unclear (Barca et al. 2000).

Management of PPT

The management of PPT is empirical as there have been no randomized studies of intervention. The thyrotoxic phase of PPT is mild, short lasting, and self-limiting as described above and may only require symptom control with beta-adrenergic agents, e.g., bisoprolol or propranolol in suitable doses in the absence of contraindications. The medication may safely be withdrawn in a few weeks. Thionamides (carbimazole, methimazole, and propylthiouracil) have no place in the treatment of this destructive phase of PPT.

However, the hypothyroid phase may require thyroxine therapy. Treatment should be considered for all symptomatic women with a raised TSH and women who are planning further pregnancies or are breastfeeding even if subclinical hypothyroidism is present. Thyroxine may be started in full replacement doses, e.g., 100–125 mcg/day, as these women are young and otherwise healthy. Treatment may be withdrawn at the end of the first postpartum year on a trial basis unless a further pregnancy is planned or the woman is actually pregnant or breastfeeding at this stage. In view of the substantial risk of developing permanent hypothyroidism, we recommend long-term monitoring of thyroid function after an episode of postpartum thyroiditis and in subsequent pregnancies.

Screening for PPT

There is no consensus about screening for PPT. Specialist organizations and learned societies such as the US Preventive Services Task Force, the American Thyroid Association, the Endocrine Society, and the American College of Obstetricians and

Table 7 Recommendations for thyroid screening in pregnancy

Society[a] (year)	Screening strategy
ACOG (2015)	High-risk targeted
ATA (2011) (Stagnaro-Green et al. 2011)	High-risk targeted
ES (USA) (2012) (De Groot et al. 2012)	High-risk targeted
AACE (2012) (Garber et al. 2012)	Universal screening not recommended
	High-risk targeted screening not addressed
SEEN (2012) (Vila et al. 2012)	Universal screening
ETA (2014) (Lazarus et al. 2014)	High-risk targeted

[a]*ACOG* American College of Obstetricians and Gynecologists, *ATA* American Thyroid Association, *ES* Endocrine Society (USA), *AACE* American Association of Clinical Endocrinologists, *SEEN* Spanish Society of Endocrinology and Nutrition, *ETA* European Thyroid Association

Gynecologists counsel against universal screening for PPT because of the paucity of evidence from RCTs (Table 7; ACOG 2015; Stagnaro-Green et al. 2011; De Groot et al. 2012; Garber et al. 2012; Vila et al. 2012; Lazarus et al. 2014). The proponents of screening would point to the following to support their views – (a) PPT is a common disease occurring in almost 1 in every 20 women who give birth; (b) PPT causes significant morbidity in the first postpartum year, particularly during the hypothyroid phase (which is present in nearly three fourths of those with PPT); (c) about 70% of those who have PPT will have a recurrence in subsequent pregnancies; (d) a significant percentage of those who had PPT will develop early (end of the first postpartum year) and late long-term hypothyroidism (Table 6); (e) effective treatment is freely and cheaply available; and (f) screening is cost-effective (Bonds and Freedberg 2001).

However, those against screening for PPT would disagree because of the lack of evidence for a suitable screening tool, among other factors. Although TPOAb is sensitive and specific, it remains a poor predictor of PPT. The ten studies that investigated its utility for prediction were very variable in their design, timing of TPOAb testing, specific antibody tested (microsomal vs. TPOAb) and did not provide clear answers (Adlan and Premawardhana 2011). Also, the occurrence of PPT in some thyroid antibody-negative women would detract from the use of TPOAb as a screening tool. It is also unclear whether combining TPOAb with thyroglobulin estimation, ultrasound scans of the thyroid, or complement activation would improve prediction.

Preventing PPT

Although there is little evidence to support measures to prevent the occurrence of PPT, the following interventions are worth noting.

Selenium

Negro and colleagues in a study from Italy recruited 151 TPOAb-positive women and a cohort of TPOAb-negative women at 10 weeks of gestation (Negro et al. 2007). This group of subjects had blood Se levels at the lower end of the population

reference range. Selenium (Se) was given to 77 of the TPOAb-positive women. They showed a significant reduction in TPOAb compared to untreated women (62.4 vs. 43.9%; $p < 0.01$). The mean TPOAb levels and postpartum peak TPOAb titers 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 TPOAb-positive group. Further evidence is needed to corroborate these findings although selenium is increasingly prescribed in practice (Hegedüs et al. 2016; Negro et al. 2016).

Radioiodine Treatment of GD

The effects of radioactive iodine (RAI) for the treatment of GD, on the incidence of PPT, were examined in a Japanese study (Yoshihara et al. 2014). These investigators retrospectively reviewed 118 women who had RAI before pregnancy for GD and found a reduced incidence of PPT (2.1%), compared to the group who had subtotal thyroidectomy (23.6%) or antithyroid drugs (55.1%). It is unclear and premature to comment on a possible effect of RAI compared to the other modalities of GD treatment. We need more evidence before firm conclusions can be drawn.

Sporadic Painless Thyroiditis

Definition

Sporadic painless thyroiditis is a destructive thyroiditis occurring in the nonpregnant population. The condition is essentially identical to postpartum thyroiditis and runs an identical clinical course characterized by a hyperthyroid phase, followed by a hypothyroid phase, and, finally, a return to a euthyroid state (Fig. 2). Several synonyms have been used to describe the syndrome including painless thyroiditis, silent thyroiditis, transient thyrotoxicosis, atypical subacute thyroiditis, and lymphocytic thyroiditis with spontaneously resolving hyperthyroidism. Silent thyroiditis should be distinguished from subacute or acute thyroiditis which is commonly due to viral or bacterial infections of the thyroid gland. Unlike silent thyroiditis, the clinical course of acute or subacute thyroiditis is marked by features of thyroid gland inflammation such as neck pain, dysphagia, fever, and raised inflammatory markers in serum.

Epidemiology

There is significant variation in the reported incidence of silent thyroiditis. An initial retrospective clinic study from the USA suggested that silent thyroiditis was present inasmuch as 10–20% of patients with hyperthyroidism although it is possible that some of these cases could have been induced by iodine ingestion (Nikolai et al. 1980). A subsequent review in another US hospital showed only one definite and three possible cases of silent thyroiditis among 86 thyrotoxic patients (1–5%) (Vitug and Goldman 1985). More recently a study from Denmark based on reviews of Tc-99 pertechnetate

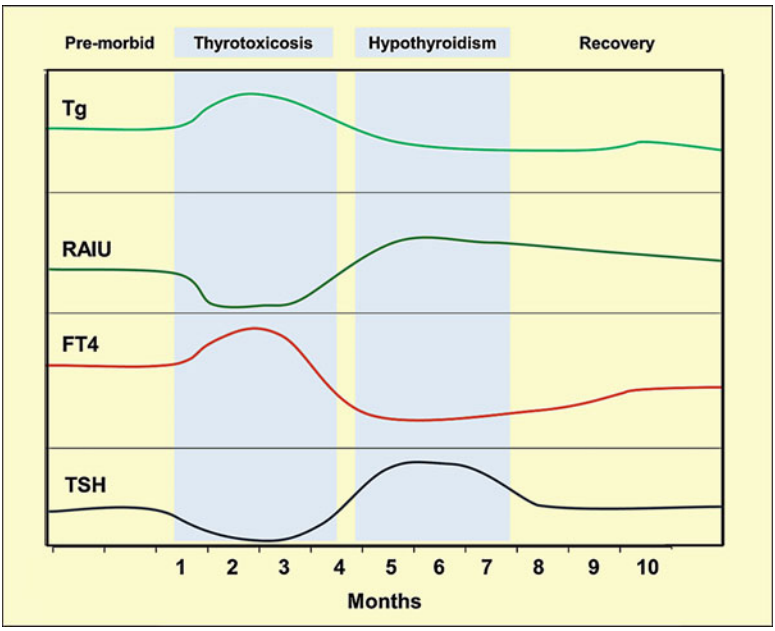

Fig. 2 Clinical course of painless thyroiditis

scans over a 10-year period estimated an incidence rate of 0.49 per 100,000 person-years with a prevalence of 0.51% among patients with thyrotoxicosis (Schwartz et al. 2013). Another study in the same population revealed that 0.8% of newly diagnosed patients with hyperthyroidism had uptake-proven silent thyroiditis (Rasmussen et al. 1989b). These latter figures would suggest a reasonably rare disease but are probably underestimates since many patients with thyroiditis may not have undergone thyroid uptake scans due to the fleeting nature of the thyrotoxic disease phase.

Most series report a slight female preponderance (2–3:1) with the majority of patients aged between 30 and 50 years (Nikolai et al. 1980; Tajiri et al. 1986). Some authors have suggested a geographic variation in the occurrence of silent thyroiditis with the largest series reported in coastal regions of Japan and in the areas around the great lakes of North America (Vitug and Goldman 1985). It has been suggested that this trend may be due to increased iodine intake in previously iodine-deficient areas (Vitug and Goldman 1985). This is supported by reportedly high rates of silent thyroiditis (10%) in coastal areas of Japan with iodine-rich diets (Tajiri et al. 1986). However, it is possible that other local factors are contributory.

Pathogenesis

Silent thyroiditis is due to destructive inflammation of the thyroid gland. Thyrotoxicosis results from leakage of pre-formed thyroid hormones into the

circulation following damage to the thyroid epithelial follicular cells. Hyperthyroidism is absent in the true sense, and thus radioactive iodine uptake is low in the face of clinical and biochemical thyrotoxicosis. Thyroid histology shows a diffuse or focal lymphocytic thyroiditis with lymphocytic cellular infiltration, moderate to severe follicular disruption, and the presence of giant cells in some cases. The extensive follicular disruption together with the absence of marked fibrosis, Hürthle cells, or lymphoid germinal center formation further serves to distinguish silent thyroiditis from Hashimoto's thyroiditis (Nikolai et al. 1980; Mizukami et al. 1988). Samples taken in the late recovery phase may show normal thyroid follicular architecture suggesting that the histological features recover to some extent (Mizukami et al. 1988). Immunohistochemical studies reveal that the majority of infiltrating lymphocytes express a T cell phenotype mostly aggregated around disrupted thyroid follicles with some cells expressing B cell phenotypes (Mizukami et al. 1988).

Precipitating Factors

Hormonal Factors

The initiating event in silent thyroiditis is unknown although several reports have implicated a mix of factors making it likely that there are multifactorial triggers. As discussed above, in pregnancy, it is believed that thyroiditis is initiated by the immunological rebound that follows the immunosuppressed state of pregnancy. Following delivery, the immunological milieu reverts towards the pro-inflammatory state that favors the expression of thyroid autoimmunity including the reemergence of the Th1 cytokine phenotype, suppression of T regulatory cell activity, and activation of components of the complement cascade. Similar triggers might play a role in the initiation of sporadic thyroiditis in the nonpregnant population. Silent thyroiditis has been described after adrenalectomy in a patient with Cushing's syndrome (Takasu et al. 1990), following cessation of steroid therapy in patients with rheumatoid arthritis (Maruyama et al. 1982) and allergic rhinitis (Morita et al. 2001) or in patients with isolated adrenocorticotropin deficiency (Mizokami et al. 2012), Addison's disease (Whitfield et al. 2009), and hypopituitarism (Sasaki et al. 1991). The patients described in these reports mostly had evidence of preexisting autoimmune thyroid disease with positive thyroid antibodies. Steroid cessation or a hypo-adrenal state could have therefore led to loss of the protective effect of glucocorticoids on thyroid autoimmunity thereby aggravating thyroiditis via a mechanism similar to the rebound phenomenon postulated to account for postpartum thyroiditis.

Thyroid Autoimmunity

It is likely that thyroid autoimmunity plays a role in the development of silent thyroiditis. Most patients have positive thyroid autoantibodies and have histological features of lymphocytic thyroiditis that are indistinguishable from those seen in patients with Hashimoto's thyroiditis and postpartum thyroiditis. In pregnancy, 30–50% of patients with positive thyroid peroxidase antibodies ultimately develop

postpartum thyroiditis (Lazarus et al. 1996) and the antibody titer may predict the occurrence and severity of hypothyroidism (Jansson et al. 1984). Although thyroid antibodies occur more frequently in patients with postpartum thyroiditis and Hashimoto's thyroiditis, many authorities would consider silent thyroiditis and postpartum thyroiditis as subacute variants of Hashimoto's thyroiditis (Dayan and Daniels 1996). Furthermore, these conditions may occur sequentially in the same patient.

Miscellaneous Factors

Cases of thyroiditis have been reported following trauma to the thyroid gland and even after manipulation of the gland at surgery. Painless thyroiditis has also been reported in the aftermath of a drug-induced hypersensitivity state associated with reactivation of multiple herpes viruses although the mechanism of induction of thyroiditis in this case is unclear (Ito et al. 2013). A case of silent thyroiditis was reported in the first trimester of pregnancy although the patient had had a spontaneous abortion 9 months earlier which would make this a case of postpartum thyroiditis (Sato et al. 2012).

Drug-Induced Painless Thyroiditis

Although many drugs are known to cause thyroid dysfunction, only a few of these have been specifically linked with the occurrence of silent thyroiditis. These include amiodarone, lithium, and α-interferon. However the exact mechanisms by which these drugs bring about thyroid dysfunction is difficult to ascertain since they exert effects on the thyroid gland via multiple pathogenic pathways. This difficulty is compounded by the fact that many cases of thyroiditis pass unnoticed or are diagnosed retrospectively casting doubts as to the diagnosis. Furthermore patients with drug-induced painless thyroiditis may evolve into other forms of thyroid dysfunction further blurring the clinical picture.

Interferon-α

The interferons are glycoproteins that possess immunomodulatory properties and induce the production of various antibodies (Liang and Ghany 2013). Recombinant human interferon-α is used in the treatment of hepatitis C infection and is associated with a spectrum of thyroid dysfunction that includes Graves' disease, Hashimoto's thyroiditis, and silent thyroiditis (Tomer and Menconi 2009). Interferon-α-treated patients who develop silent thyroiditis typically have preexisting autoimmune thyroid disease. Thyrotoxicosis in patients receiving interferon-α may be caused by Graves' disease or silent thyroiditis, and both conditions may coexist or evolve from one condition to the other in the same patient. Some patients show a triphasic disease pattern evolving from silent thyroiditis to hypothyroidism and, finally, Graves' hyperthyroidism (Bohbot et al. 2006). The mechanism of this syndrome is speculative, but Graves' disease could arise from induction of TSH receptor antibodies as a consequence of the thyroid follicular destruction that occurs in the course of silent thyroiditis. One example of this mechanism is the development of GD following radioiodine therapy of nodular nontoxic goiter (Nygaard et al. 1997).

Lithium

Lithium has been used effectively for the treatment of bipolar disorders for several decades. Its effects on the thyroid hormone axis are well known, and in practice it has been associated with hyperthyroidism, hypothyroidism, and goiter. Clinically, lithium exerts multiple effects on thyroid physiology through mechanisms involving hypothalamo-pituitary-thyroid axis inhibition, aggravation of thyroid autoimmunity, inhibition of peripheral T4 deiodination, and inhibition of cyclic AMP-mediated cellular pathways (Lazarus 2009). Lithium is avidly concentrated by the thyroid follicular cell and effectively inhibits thyroid hormone synthesis and release by interfering with iodine uptake and iodination and coupling of iodotyrosines (Lazarus 2009). Accordingly it is has been used as an adjunctive therapy for hyperthyroidism in patients that are intolerant to thionamides (Lazarus 2009). Somewhat paradoxically however lithium also induces a silent thyroiditis. In a retrospective study of 400 patients with hyperthyroidism who underwent thyroid uptake scanning, patients with silent thyroiditis were more likely to have been exposed to lithium compared to those with Graves' disease (OR 4.7 95% CI: 1.3, 17) (Miller and Daniels 2001).

The mechanism of lithium-induced thyroiditis is uncertain but may be due to induction of thyroid autoimmunity. Up to a fifth of patients on lithium therapy have positive thyroid antibodies, and lithium-treated patients have been observed to exhibit increased B cell activity, reduced suppressor T cell activity, and in vitro augmentation of human lymphocyte immunoglobulin production. It is also possible that lithium induces thyroiditis through direct toxic effects on the thyroid gland. Thyroid antibodies are not always present in affected patients and toxic levels of intra-thyroidal lithium, and follicular cell disruption in the absence of lymphocytic infiltration has been seen in some thyroid biopsy samples of patients with lithium-induced thyroiditis (Miller and Daniels 2001).

Amiodarone

Amiodarone is an iodine-rich benzofuranic acid derivative with structural similarities to thyroxine (Martino et al. 2001). It is extensively used in clinical practice to treat a variety of atrial and ventricular arrhythmias (Basaria and Cooper 2005). The effects of amiodarone on thyroid function are well recognized and are mediated through toxic effects of the excess iodine load. Approximately 37.5% of amiodarone is made up of iodine, and each 200 mg tablet contains 75 mg of iodine (Martino et al. 2001). The incidence of amiodarone-induced thyroid dysfunction is highly variable but ranges from 2% to 24% and depends on population iodine nutrition status among other factors (Martino et al. 2001). Hypothyroidism occurs from iodide blocking of organification of iodine and thyroid hormone production, the so-called Wolff-Chaikoff effect. Hyperthyroidism may also occur as a result of accelerated thyroid hormone synthesis induced by excess iodine, and this form of hyperthyroidism has been called type 1 amiodarone-induced thyrotoxicosis or AIT 1 (Tsang and Houlden 2009). In addition amiodarone-induced thyroid hyperthyroidism may also occur in the form of a silent destructive thyroiditis or AIT 2 (Tsang and Houlden 2009).

Amiodarone-induced thyroiditis is difficult to predict, and its occurrence is neither dependent on the dose nor duration of amiodarone therapy. Thyroiditis may develop at various times in the course of therapy and may even be seen in individuals who have been on treatment for many years. Thyroiditis usually occurs in individuals with normal thyroid glands but can also be seen in patients with preexisting thyroid autoimmunity or in conjunction with other forms of amiodarone-induced thyroid dysfunction. The distinction between amiodarone-induced thyrotoxicosis (AIT 1) and silent thyroiditis is crucial if clinicians are to avoid subjecting patients with self-limiting thyroiditis to high doses of antithyroid therapy with thionamides or perchlorate. Clinically this distinction may not always be possible, but patients with thyroiditis have low radioactive iodine uptake scan, typically lack goiters or thyroid antibodies, and are more commonly resident in iodine-replete areas, in contrast to AIT 1 which is seen in iodine-deficient individuals with long-standing thyroid nodules or in patients with Graves' disease (Tsang and Houlden 2009). Furthermore, thyroid color flow Doppler in patients with silent thyroiditis does not show the typical vascularity patterns of hyperthyroidism (Bogazzi et al. 1997). Interleukin-6 has also been shown to be significantly more elevated in patients with thyroiditis compared to AIT 1 (Bartalena et al. 1994).

The mechanism of thyroiditis is uncertain but may be due to a direct toxic effect of amiodarone on thyroid follicular cells. The management of amiodarone-induced silent thyroiditis is debated. Antithyroid drugs are not indicated, and some authorities advocate a short course of steroid therapy, e.g., 40–60 mg of prednisolone, tapered over 2–3 months. However, there is no consensus on whether amiodarone should be discontinued, and the drug may be continued in cases where discontinuation proves difficult for the management of arrhythmias. The hypothyroid phase may require transient levothyroxine for symptom control. Other measures have been tried for difficult cases of prolonged thyrotoxicosis including lithium therapy, thyroidectomy, and plasmapheresis.

Other Drugs

A number of other drugs have been linked to the development of a destructive thyroiditis including interleukin-2 therapy (Krouse et al. 1995) and the tyrosine kinase inhibitors such as sunitinib and imatinib (de Groot et al. 2005). However, the exact mechanisms and nature of the thyroid destructive effects of these drugs are still being unraveled, and destructive thyroiditis might be only one of a number of proposed pathways by which thyroid dysfunction develops in individuals taking these medications (Hamnvik et al. 2011).

Clinical Features

Patients with silent thyroiditis typically but not invariably show a triphasic disease pattern characterized by an initial phase of hyperthyroidism, a hypothyroid phase, and a return to euthyroidism (Fig. 2).

Thyrotoxic Phase

The thyrotoxic phase is seen in 5–20% of patients and is characterized by typical symptoms such as heat intolerance, sweating, palpitations, anxiety, and tremors. The ratio of T3:T4 is less than in the hyperfunctioning thyroid gland, and so symptoms are generally less severe than in Graves' disease or toxic nodules. Unlike in patients with acute and subacute thyroiditis, thyroid pain is absent, and there are no preceding features of fever and constitutional symptoms. Furthermore, patients with silent thyroiditis may not have preexisting thyroid disease. There is no goiter in about 50% of cases, and signs of thyroid orbitopathy or pretibial myxedema as seen in patients with Graves' disease are lacking. This disease phase usually lasts for about 3 months although shorter or much longer periods are seen. The vast majority of cases have no discernible trigger, but a proportion of cases occur following drug exposure or on the background of preexisting autoimmune thyroid disease. At this stage, thyroid hormones will show a suppressed TSH together with elevated concentrations of FT4 and/or FT3 (overt hyperthyroidism) or with normal FT4 and FT3 (subclinical hyperthyroidism). In addition, thyroid tests done at the end of this phase may be normal if the patient is already in the transitory phase from hyperthyroidism to hypothyroidism. Radioactive iodine or technetium uptake is low. Occasionally, thyrotoxicosis may be severe, and a variety of complications such as acute myocardial infarction, periodic paralysis (Oh et al. 2012), and severe hypercalcemia (Thewjitcharoen and Lumlertgul 2012) have all been reported in the literature.

Hypothyroid Phase

During the hypothyroid phase, patients may experience typical hypothyroid symptoms including lethargy, cold intolerance, constipation, weight gain, and low mood. Thyroid tests will show an elevated TSH concentration with low FT4 or F3 (overt hypothyroidism) or with normal FT4 and FT3 (subclinical hypothyroidism). This phase may be misdiagnosed as primary autoimmune hypothyroidism or may be missed altogether if thyroid hormones are checked during the transitory euthyroid window. Hypothyroidism typically lasts 3–6 months and is then followed by a recovery phase with the entire duration of the illness lasting less than 1 year. Permanent hypothyroidism occurs in about 20% of patients, but this is probably overestimated since many patients start levothyroxine during the hypothyroid phase and subsequently continue treatment thereby losing the opportunity to establish thyroid function recovery. Features which predict permanent hypothyroidism include high TPO antibody titers, severe hypothyroidism, persistent hypoechogenicity on ultrasound, and a brief or undiagnosed thyrotoxic phase (Premawardhana et al. 2000; Pearce et al. 2003; Kamijo 2010).

Differential Diagnosis

A number of important differential diagnoses should be considered at each phase of the disease. This is essential to ensure appropriate therapy and avoid undue treatment

Table 8 Differential diagnosis of silent thyroiditis

	Silent thyroiditis	Acute thyroiditis	Subacute thyroiditis	Graves' disease
Goiter	Some patients	Yes	Yes	Yes
Neck pain	No	Yes	Yes	No
Pain and fever	No	Yes	Yes	No
T3:T4 ratio	T4 predominant	T4 predominant	T4 predominant	T3 predominant
Thyroid antibodies	Yes	Negative	Negative	Positive
Thyroid ultrasound	Hypoechogenic	Hypoechogenic, abscess	Hypoechogenic	Diffuse, hypoechogenic
Uptake scan	Low	Normal	Low	High
CRP, ESR	Normal	High	High	Normal

CRP C-reactive protein, *ESR* erythrocyte sedimentation rate

with antithyroid drugs in patients with self-limiting disease. These conditions and the relevant investigations are summarized in Table 8. The hyperthyroid phase should be distinguished from other causes of hyperthyroidism such as Graves' disease, toxic nodules, and subacute and acute thyroiditis. Patients with silent thyroiditis will typically have no history of thyroid pain, and physical signs such as orbitopathy and thyroid vascular bruit are absent. Goiter is present in 50% of patients and is typically small in size and diffuse. Patients with thyroiditis have relatively lower levels of T3 and T3:T4 ratios than those with Graves' disease. In silent thyroiditis, TSH receptor antibodies are negative or elevated to a lesser extent than seen in Graves' disease although a reliably discriminatory cutoff point is lacking (Kamijo 2010).

Inflammatory markers such as C-reactive protein and erythrocyte sedimentation rate are elevated in patients with acute and subacute thyroiditis but are typically normal in silent thyroiditis. A thyroid ultrasound will confirm the absence of thyroid nodules, but the thyroid texture in thyroiditis is hypoechoic and heterogeneous and may be difficult to differentiate from the sonographic features of Graves' disease. Color flow Doppler ultrasound will show typical flow patterns of thyroid vascularity in patients with Graves' hyperthyroidism. A radioiodine uptake is low in thyroiditis unlike in other causes of hyperthyroidism where this is elevated. Other causes of low uptake should be borne in mind including factitious thyrotoxicosis (Table 9). Serum thyroglobulin is useful in distinguishing patients with factitious thyroiditis as thyroglobulin is elevated in thyroiditis but is low following ingestion of thyroxine (Mariotti et al. 1982). The uptake scan should be done without delay during the thyrotoxic phase as a radioiodine scan in the hypothyroid stage may show a normal or even increased uptake leading to diagnostic confusion. The key differential diagnosis in patients at the hypothyroid disease phase is primary autoimmune or Hashimoto's thyroiditis. This may be difficult to ascertain since some patients with silent thyroiditis have goiters and positive thyroid peroxidase antibodies. Where there is uncertainty, a withdrawal of levothyroxine may be attempted to monitor for recovery thus allowing a retrospective diagnosis.

Table 9 Causes of thyrotoxicosis and low radioactive iodine uptake

Thyroiditis
Silent thyroiditis
Postpartum thyroiditis
Subacute thyroiditis
Drug-induced thyroiditis
Iodine intake
Iodine-induced thyrotoxicosis
Iodine-rich diet before test
Miscellaneous
Factitious thyrotoxicosis
Struma ovarii

Management

Thyrotoxic Phase

The thyrotoxic phase is due to the leakage of pre-formed hormones from the thyroid gland and typically does not require specific treatment with antithyroid drugs. Patients with mild symptoms can be observed without treatment, but beta-adrenergic blockers may be used for controlling adrenergic symptoms such as tremors, anxiety, sweating, and heat intolerance. The nonspecific beta-adrenergic blocking agent propranolol is more effective than cardio-specific beta-blockers and may be given at dose ranges of 40–160 mg daily. Calcium channel antagonists like verapamil or diltiazem may be used as an alternative to beta-blockers in patients with asthma and chronic obstructive airway disease. Although most patients can be safely managed conservatively, occasionally severe and recurrent cases may in exceptional circumstances require definitive therapy with thyroidectomy (Ishii et al. 2013).

Hypothyroid Phase

Hypothyroidism should be anticipated, and thyroid function tests should be carefully monitored so that the evolution to hypothyroidism is recognized. The expected course and symptoms of the disease should be explained to the patient. Mild symptoms of hypothyroidism can be simply monitored, and a decision to start levothyroxine will depend on the clinical and biochemical severity. For severe cases, levothyroxine should be started and the dose adjusted according to serial thyroid hormones. Most patients will require treatment for 6–12 months after which levothyroxine should be stopped and the patient monitored for recovery (Samuels 2012). About 20% of patients have persistent hypothyroidism beyond 12 months, and these individuals should then continue on lifelong levothyroxine (Pearce et al. 2003; Nikolai et al. 1981). Levothyroxine should also be continued in patients with persistent goiters or in women who are being evaluated for infertility or trying to conceive in order to minimize the risk of fetal harm from gestational hypothyroidism.

Recurrent Silent Thyroiditis

It is estimated that about 5–10% of patients develop recurrent episodes of thyroiditis although rates as high as 60% have been reported in Japan (Samuels 2012). Some patients suffer multiple repeated episodes within several years, and up to nine episodes have been reported in the same patient (Mittra and McDougall 2007). There does not appear to be any established predisposing factors for recurrence, and prolonged treatment of the hypothyroid phase with levothyroxine does not prevent recurrence. In some cases, patients have received ablative thyroid gland treatment with radioactive iodine and subsequently treated with lifelong levothyroxine therapy (Mittra and McDougall 2007).

References

ACOG. Practice Bulletin No. 148: thyroid disease in pregnancy. Obstet Gynecol. 2015;125 (4):996–1005.

Adams H, Jones MC, Othman S, Lazarus JH, Parkes AB, Hall R, et al. The sonographic appearances in postpartum thyroiditis. Clin Radiol. 1992;45(5):311–5.

Adlan MA, Premawardhana LD. Thyroid peroxidase antibody and screening for postpartum thyroid dysfunction. J Thyroid Res. 2011;2011:745135.

Alvarez-Marfany M, Roman SH, Drexler AJ, Robertson C, Stagnaro-Green A. Long-term prospective study of postpartum thyroid dysfunction in women with insulin dependent diabetes mellitus. J Clin Endocrinol Metab. 1994;79(1):10–6.

Andersen SL, Olsen J, Carle A, Laurberg P. Hyperthyroidism incidence fluctuates widely in and around pregnancy and is at variance with some other autoimmune diseases: a danish population-based study. J Clin Endocrinol Metab. 2015;100(3):1164–71.

Azizi F. Age as a predictor of recurrent hypothyroidism in patients with post-partum thyroid dysfunction. J Endocrinol Invest. 2004;27(11):996–1002.

Azizi F. The occurrence of permanent thyroid failure in patients with subclinical postpartum thyroiditis. Eur J Endocrinol. 2005;153(3):367–71.

Barca MF, Knobel M, Tomimori E, Cardia MS, Medeiros-Neto G. Prevalence and characteristics of postpartum thyroid dysfunction in Sao Paulo, Brazil. Clin Endocrinol (Oxf). 2000;53(1):21–31.

Bartalena L, Grasso L, Brogioni S, Aghini-Lombardi F, Braverman LE, Martino E. Serum interleukin-6 in amiodarone-induced thyrotoxicosis. J Clin Endocrinol Metab. 1994;78 (2):423–7.

Basaria S, Cooper DS. Amiodarone and the thyroid. Am J Med. 2005;118(7):706–14.

Bech K, Hoier-Madsen M, Feldt-Rasmussen U, Jensen BM, Molsted-Pedersen L, Kuhl C. Thyroid function and autoimmune manifestations in insulin-dependent diabetes mellitus during and after pregnancy. Acta Endocrinol. 1991;124(5):534–9.

Bogazzi F, Bartalena L, Brogioni S, Mazzeo S, Vitti P, Burelli A, et al. Color flow Doppler sonography rapidly differentiates type I and type II amiodarone-induced thyrotoxicosis. Thyroid. 1997;7(4):541–5.

Bohbot NL, Young J, Orgiazzi J, Buffet C, Francois M, Bernard-Chabert B, et al. Interferon-alpha-induced hyperthyroidism: a three-stage evolution from silent thyroiditis towards Graves' disease. Eur J Endocrinol. 2006;154(3):367–72.

Bonds DE, Freedberg KA. Cost-effectiveness of prenatal screening for postpartum thyroiditis. J Womens Health Gend Based Med. 2001;10(7):649–58.

Brix TH, Hansen PS, Kyvik KO, Hegedus L. Aggregation of thyroid autoantibodies in twins from opposite-sex pairs suggests that microchimerism may play a role in the early stages of thyroid autoimmunity. J Clin Endocrinol Metab. 2009;94(11):4439–43.

Creagh FM, Parkes AB, Lee A, Adams H, Hall R, Richards CJ, et al. The iodide perchlorate discharge test in women with previous post-partum thyroiditis: relationship to sonographic appearance and thyroid function. Clin Endocrinol (Oxf). 1994;40(6):765–8.

Dayan CM, Daniels GH. Chronic autoimmune thyroiditis. N Engl J Med. 1996;335(2):99–107.

de Groot JW, Zonnenberg BA, Plukker JT, van Der Graaf WT, Links TP. Imatinib induces hypothyroidism in patients receiving levothyroxine. Clin Pharmacol Ther. 2005;78(4):433–8.

De Groot L, Abalovich M, Alexander EK, Amino N, Barbour L, Cobin RH, et al. Management of thyroid dysfunction during pregnancy and postpartum: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2012;97(8):2543–65.

Durr S, Kindler V. Implication of indolamine 2,3 dioxygenase in the tolerance toward fetuses, tumors, and allografts. J Leukoc Biol. 2013;93(5):681–7.

Ekinci EI, Chiu WL, Lu ZX, Sikaris K, Churilov L, Bittar I, et al. A longitudinal study of thyroid autoantibodies in pregnancy: the importance of test timing. Clin Endocrinol (Oxf). 2015;82 (4):604–10.

Elenkov IJ, Wilder RL, Bakalov VK, Link AA, Dimitrov MA, Fisher S, et al. IL-12, TNF-alpha, and hormonal changes during late pregnancy and early postpartum: implications for autoimmune disease activity during these times. J Clin Endocrinol Metab. 2001;86(10):4933–8.

Erlebacher A, Vencato D, Price KA, Zhang D, Glimcher LH. Constraints in antigen presentation severely restrict T cell recognition of the allogeneic fetus. J Clin Invest. 2007;117(5):1399–411.

Feldt-Rasmussen U, Høier-Madsen M, Rasmussen NG, Hegedüs L, Hornnes P. Anti-thyroid peroxidase antibodies during pregnancy and postpartum. Relation to postpartum thyroiditis. Autoimmunity. 1990;6(3):211–4.

Fung HY, Kologlu M, Collison K, John R, Richards CJ, Hall R, et al. Postpartum thyroid dysfunction in Mid Glamorgan. Br Med J (Clin Res Ed). 1988;296(6617):241–4.

Gallas PR, Stolk RP, Bakker K, Endert E, Wiersinga WM. Thyroid dysfunction during pregnancy and in the first postpartum year in women with diabetes mellitus type 1. Eur J Endocrinol. 2002;147(4):443–51.

Galustian C, Dasgupta P. Regulatory T cells. eLS: Chichester: Wiley; 2001.

Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, et al. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. Endocr Pract. 2012;18(6):988–1028.

Gerstein HC. Incidence of postpartum thyroid dysfunction in patients with type I diabetes mellitus. Ann Intern Med. 1993;118(6):419–23.

Hamnvik OP, Larsen PR, Marqusee E. Thyroid dysfunction from antineoplastic agents. J Natl Cancer Inst. 2011;103(21):1572–87.

Hegedüs L, Bonnema SJ, Winther KH. Selenium in the treatment of thyroid diseases: an element in search of the relevant indications? Eur Thyroid J. 2016;5(3):149–51.

Hunt JS, Vassmer D, Ferguson TA, Miller L. Fas ligand is positioned in mouse uterus and placenta to prevent trafficking of activated leukocytes between the mother and the conceptus. J Immunol. 1997;158(9):4122–8.

Ide A, Amino N, Kang S, Yoshioka W, Kudo T, Nishihara E, et al. Differentiation of postpartum Graves' thyrotoxicosis from postpartum destructive thyrotoxicosis using antithyrotropin receptor antibodies and thyroid blood flow. Thyroid. 2014;24(6):1027–31.

Ishii H, Takei M, Sato Y, Ito T, Ito K, Sakai Y, et al. A case of severe and recurrent painless thyroiditis requiring thyroidectomy. Med Princ Pract. 2013;22(4):408–10.

Ito K, Akita Y, Ishida N, Kasugai C, Tamada Y, Watanabe D. Painless thyroiditis in drug-induced hypersensitivity syndrome with prolonged reactivation of herpesviruses. Int J Dermatol. 2013;52(4):475–7.

Jansson R, Bernander S, Karlsson A, Levin K, Nilsson G. Autoimmune thyroid dysfunction in the postpartum period. J Clin Endocrinol Metab. 1984;58(4):681–7.

Kamijo K. Study on cutoff value setting for differential diagnosis between Graves' disease and painless thyroiditis using the TRAb (Elecsys TRAb) measurement via the fully automated electrochemiluminescence immunoassay system. Endocr J. 2010;57(10):895–902.

Kokandi AA, Parkes AB, Premawardhana LD, John R, Lazarus JH. Association of postpartum thyroid dysfunction with antepartum hormonal and immunological changes. J Clin Endocrinol Metab. 2003;88(3):1126–32.

Krouse RS, Royal RE, Heywood G, Weintraub BD, White DE, Steinberg SM, et al. Thyroid dysfunction in 281 patients with metastatic melanoma or renal carcinoma treated with interleukin-2 alone. J Immunother Emphasis Tumor Immunol. 1995;18(4):272–8.

Kuijpens JL, Pop VJ, Vader HL, Drexhage HA, Wiersinga WM. Prediction of post partum thyroid dysfunction: can it be improved? Eur J Endocrinol. 1998;139(1):36–43.

Kuijpens JL, Vader HL, Drexhage HA, Wiersinga WM, van Son MJ, Pop VJ. Thyroid peroxidase antibodies during gestation are a marker for subsequent depression postpartum. Eur J Endocrinol. 2001;145(5):579–84.

La Rocca C, Carbone F, Longobardi S, Matarese G. The immunology of pregnancy: regulatory T cells control maternal immune tolerance toward the fetus. Immunol Lett. 2014;162(1 Pt A):41–8.

Lazarus JH. Lithium and thyroid. Best Pract Res Clin Endocrinol Metab. 2009;23(6):723–33.

Lazarus JH. The continuing saga of postpartum thyroiditis. J Clin Endocrinol Metab. 2011;96(3):614–6.

Lazarus J, Premawardhana L. Postpartum thyroiditis. In: Weetman A, editor. Contemporary endocrinology – autoimmune diseases in endocrinology. Totowa: Humana Press; 2011.

Lazarus JH, Hall R, Othman S, Parkes AB, Richards CJ, McCulloch B, et al. The clinical spectrum of postpartum thyroid disease. QJM. 1996;89(6):429–35.

Lazarus JH, Ammari F, Oretti R, Parkes AB, Richards CJ, Harris B. Clinical aspects of recurrent postpartum thyroiditis. Br J Gen Pract. 1997;47(418):305–8.

Lazarus JH, Parkes AB, Premawardhana LD, Harris B. Screening for postpartum thyroiditis. J Clin Endocrinol Metab. 1999;84(11):4295–6.

Lazarus J, Brown RS, Daumerie C, Hubalewska-Dydejczyk A, Negro R, Vaidya B. 2014 European thyroid association guidelines for the management of subclinical hypothyroidism in pregnancy and in children. Eur Thyroid J. 2014;3(2):76–94.

Leide-Svegborn S, Ahlgren L, Johansson L, Mattsson S. Excretion of radionuclides in human breast milk after nuclear medicine examinations. Biokinetic and dosimetric data and recommendations on breastfeeding interruption. Eur J Nucl Med Mol Imaging. 2016;43(5):808–21.

Liang TJ, Ghany MG. Current and future therapies for hepatitis C virus infection. N Engl J Med. 2013;368(20):1907–17.

Maleki N, Tavosi Z. Evaluation of thyroid dysfunction and autoimmunity in gestational diabetes mellitus and its relationship with postpartum thyroiditis. Diabet Med. 2015;32(2):206–12.

Mariotti S, Martino E, Cupini C, Lari R, Giani C, Baschieri L, et al. Low serum thyroglobulin as a clue to the diagnosis of thyrotoxicosis factitia. N Engl J Med. 1982;307(7):410–2.

Martino E, Bartalena L, Bogazzi F, Braverman LE. The effects of amiodarone on the thyroid. Endocr Rev. 2001;22(2):240–54.

Maruyama H, Kato M, Mizuno O, Kataoka K, Matsuki S. Transient thyrotoxicosis occurred after cessation of steroid therapy in a patient with autoimmune thyroiditis and rheumatoid arthritis. Endocrinol Jpn. 1982;29(5):583–8.

Mattsson S, Johansson L, Leide Svegborn S, Liniecki J, Nosske D, Riklund KA, et al. Radiation dose to patients from radiopharmaceuticals: a compendium of current information related to frequently used substances. Ann ICRP. 2015;44(2 Suppl):7–321.

Miller KK, Daniels GH. Association between lithium use and thyrotoxicosis caused by silent thyroiditis. Clin Endocrinol (Oxf). 2001;55(4):501–8.

Mittra ES, McDougall IR. Recurrent silent thyroiditis: a report of four patients and review of the literature. Thyroid. 2007;17(7):671–5.

Mizokami T, Itoh Y, Sato Y, Nunoi K, Okamura K. Isolated adrenocorticotropin deficiency associated with painless thyroiditis: a case report and review of the literature. Kurume Med J. 2012;59(3–4):71–7.

Mizukami Y, Michigishi T, Hashimoto T, Tonami N, Hisada K, Matsubara F, et al. Silent thyroiditis: a histologic and immunohistochemical study. Hum Pathol. 1988;19(4):423–31.

Morita S, Ueda Y, Yokoyama N. Painless thyroiditis induced by the cessation of betamethasone. Intern Med (Tokyo, Japan). 2001;40(8):744–6.
Negro R, Greco G, Mangieri T, Pezzarossa A, Dazzi D, Hassan H. The influence of selenium supplementation on postpartum thyroid status in pregnant women with thyroid peroxidase autoantibodies. J Clin Endocrinol Metab. 2007;92(4):1263–8.
Negro R, Attanasio R, Grimaldi F, Guglielmi R, Papini E. A 2015 Italian Survey of Clinical Practice Patterns in the Management of Graves' Disease: comparison with European and North American Surveys. Eur Thyroid J. 2016;5(2):112–9.
Nicholson WK, Robinson KA, Smallridge RC, Ladenson PW, Powe NR. Prevalence of postpartum thyroid dysfunction: a quantitative review. Thyroid. 2006;16(6):573–82.
Nikolai TF, Brosseau J, Kettrick MA, Roberts R, Beltaos E. Lymphocytic thyroiditis with spontaneously resolving hyperthyroidism (silent thyroiditis). Arch Intern Med. 1980;140(4):478–82.
Nikolai TF, Coombs GJ, McKenzie AK. Lymphocytic thyroiditis with spontaneously resolving hyperthyroidism and subacute thyroiditis. Long-term follow-up. Arch Intern Med. 1981;141(11):1455–8.
Nikolai TF, Turney SL, Roberts RC. Postpartum lymphocytic thyroiditis. Prevalence, clinical course, and long-term follow-up. Arch Intern Med. 1987;147(2):221–4.
Nygaard B, Knudsen JH, Hegedus L, Scient AV, Hansen JE. Thyrotropin receptor antibodies and Graves' disease, a side-effect of 131I treatment in patients with nontoxic goiter. J Clin Endocrinol Metab. 1997;82(9):2926–30.
Oh SB, Ahn J, Oh MY, Choi BG, Kang JH, Jeon YK, et al. Thyrotoxic periodic paralysis associated with transient thyrotoxicosis due to painless thyroiditis. J Korean Med Sci. 2012;27(7):822–6.
Okosieme OE, Parkes AB, McCullough B, Doukidis D, Morgan BP, Richards CJ, et al. Complement activation in postpartum thyroiditis. QJM. 2002;95(3):173–9.
Othman S, Phillips DI, Parkes AB, Richards CJ, Harris B, Fung H, et al. A long-term follow-up of postpartum thyroiditis. Clin Endocrinol (Oxf). 1990;32(5):559–64.
Pearce EN, Farwell AP, Braverman LE. Thyroiditis. N Engl J Med. 2003;348(26):2646–55.
Premawardhana LD, Parkes AB, Ammari F, John R, Darke C, Adams H, et al. Postpartum thyroiditis and long-term thyroid status: prognostic influence of thyroid peroxidase antibodies and ultrasound echogenicity. J Clin Endocrinol Metab. 2000;85(1):71–5.
Premawardhana LD, Parkes AB, John R, Harris B, Lazarus JH. Thyroid peroxidase antibodies in early pregnancy: utility for prediction of postpartum thyroid dysfunction and implications for screening. Thyroid. 2004;14(8):610–5.
Rasmussen NG, Hornnes PJ, Hegedus L. Ultrasonographically determined thyroid size in pregnancy and post partum: the goitrogenic effect of pregnancy. Am J Obstet Gynecol. 1989a;160 (5 Pt 1):1216–20.
Rasmussen NG, Hansen JM, Hegedus L. Frequency of thyroiditis and postpartum thyroiditis in a 10-year consecutive hyperthyroid Danish population. Thyroidology/APRIM. 1989b;1(3):143–7.
Roberton H. Lassitude, coldness, and hair changes following pregnancy, and their response to treatment with thyroid extract. Br Med J. 1948;2:93–4.
Rouas-Freiss N, Goncalves RM, Menier C, Dausset J, Carosella ED. Direct evidence to support the role of HLA-G in protecting the fetus from maternal uterine natural killer cytolysis. Proc Natl Acad Sci U S A. 1997;94(21):11520–5.
Samuels MH. Subacute, silent, and postpartum thyroiditis. Med Clin North Am. 2012;96 (2):223–33.
Sasaki H, Ohnishi O, Okudera T, Okumura M. Simultaneous occurrence of transient resolving thyrotoxicosis due to painless thyroiditis, hypopituitarism and diabetes insipidus following pituitary apoplexy. Postgrad Med J. 1991;67(783):75–7.
Sato S, Endo K, Iizaka T, Saiki R, Iwaku K, Sato S, et al. A case of painless thyroiditis in a very early stage of pregnancy. Intern Med (Tokyo, Japan). 2012;51(5):475–7.
Schumacher A, Costa SD, Zenclussen AC. Endocrine factors modulating immune responses in pregnancy. Front Immunol. 2014;5:196.

Schwartz F, Bergmann N, Zerahn B, Faber J. Incidence rate of symptomatic painless thyroiditis presenting with thyrotoxicosis in Denmark as evaluated by consecutive thyroid scintigraphies. Scand J Clin Lab Invest. 2013;73(3):240–4.

Sergi M, Tomlinson G, Feig DS. Changes suggestive of post-partum thyroiditis in women with established hypothyroidism: incidence and predictors. Clin Endocrinol (Oxf). 2015;83(3):389–93.

Smallridge RC, Clark T. Sawin historical vignette: what do criminology, Harry Houdini, and King George V have in common with postpartum thyroid dysfunction? Thyroid. 2014;24(12):1752–8.

Stagnaro-Green A. Approach to the patient with postpartum thyroiditis. J Clin Endocrinol Metab. 2012;97(2):334–42.

Stagnaro-Green A, Roman SH, Cobin RH, el-Harazy E, Wallenstein S, Davies TF. A prospective study of lymphocyte-initiated immunosuppression in normal pregnancy: evidence of a T-cell etiology for postpartum thyroid dysfunction. J Clin Endocrinol Metab. 1992;74(3):645–53.

Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, et al. Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. Thyroid. 2011;21(10):1081–125.

Tachi J, Amino N, Tamaki H, Aozasa M, Iwatani Y, Miyai K. Long term follow-up and HLA association in patients with postpartum hypothyroidism. J Clin Endocrinol Metab. 1988;66(3):480–4.

Tajiri J, Nakashima M, Higashi K, Morita M, Umeda T, Sato T. Silent thyroiditis. Arch Intern Med. 1986;146(8):1644.

Takasu N, Komiya I, Nagasawa Y, Asawa T, Yamada T. Exacerbation of autoimmune thyroid dysfunction after unilateral adrenalectomy in patients with Cushing's syndrome due to an adrenocortical adenoma. N Engl J Med. 1990;322(24):1708–12.

Thewjitcharoen Y, Lumlertgul N. Painless thyroiditis complicating with hypercalcemic encephalopathy. J Med Assoc Thai. 2012;95(1):119–23.

Tomer Y, Menconi F. Interferon induced thyroiditis. Best Pract Res Clin Endocrinol Metab. 2009;23(6):703–12.

Tozzoli R, Bagnasco M, Giavarina D, Bizzaro N. TSH receptor autoantibody immunoassay in patients with Graves' disease: improvement of diagnostic accuracy over different generations of methods. Systematic review and meta-analysis. Autoimmun Rev. 2012;12(2):107–13.

Tsang W, Houlden RL. Amiodarone-induced thyrotoxicosis: a review. Can J Cardiol. 2009;25(7):421–4.

Vila L, Velasco I, Gonzalez S, Morales F, Sanchez E, Lailla JM, et al. Detection of thyroid dysfunction in pregnant women: universal screening is justified. Endocrinol Nutr. 2012;59(9):547–60.

Vitug AC, Goldman JM. Silent (painless) thyroiditis. Evidence of a geographic variation in frequency. Arch Intern Med. 1985;145(3):473–5.

Walfish PG, Meyerson J, Provias JP, Vargas MT, Papsin FR. Prevalence and characteristics of postpartum thyroid dysfunction: results of a survey from Toronto, Canada. J Endocrinol Invest. 1992;15(4):265–72.

Whitfield P, Bagg W, Holdaway I. Concurrent Addison's disease and silent thyroiditis. Intern Med J. 2009;39(12):e1–2.

Xu C, Mao D, Holers VM, Palanca B, Cheng AM, Molina H. A critical role for murine complement regulator crry in fetomaternal tolerance. Science (New York, NY). 2000;287(5452):498–501.

Yoshihara A, Noh JY, Watanabe N, Iwaku K, Kobayashi S, Suzuki M, et al. Lower incidence of postpartum thyrotoxicosis in women with Graves disease treated by radioiodine therapy than by subtotal thyroidectomy or with antithyroid drugs. Clin Nucl Med. 2014;39(4):326–9.

Acute and Subacute Thyroiditis

9

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Abstract

The term thyroiditis refers to inflammation of the thyroid gland, which has many causes. The tempo of clinical presentation is used to classify thyroiditis into acute, subacute, and chronic types, but in practice there is considerable overlap. Acute forms of thyroiditis include acute infectious thyroiditis caused by bacterial or fungal infection (most commonly arising from a piriform sinus tract fistula or hematogenous spread) and radiation-induced and traumatic thyroiditis. Many types of thyroiditis may present in a subacute fashion, but the term subacute thyroiditis is generally reserved for a specific type of thyroiditis characterized pathologically by granulomatous inflammation and the presence of giant cells, which is thought to be precipitated by viral infection in genetically predisposed individuals. Acute infectious thyroiditis and subacute thyroiditis each present with painful anterior neck swelling and constitutional symptoms; careful clinical assessment, supported by judicious use of imaging and fine-needle aspiration biopsy, allows diagnostic differentiation. Thyroid dysfunction in subacute thyroiditis classically follows a triphasic course of hyperthyroidism, followed by hypothyroidism and eventual resolution to euthyroidism, but these phases are not all observed in all patients. In hyperthyroid patients, it can be difficult to distinguish clinically between subacute thyroiditis, lymphocytic thyroiditis, and Graves' disease, and inflammatory markers, thyroid antibody measurement, and radionuclide imaging can be diagnostically helpful. The treatment of acute infectious thyroiditis is with systemic antimicrobial treatment and surgical drainage. For subacute thyroiditis, treatment is not always required; when it is warranted, nonsteroidal anti-inflammatory drugs or glucocorticoid treatment can be offered for the painful thyroiditis and beta-blockade for symptoms of thyrotoxicosis. Follow-up of thyroid function is recommended to ensure resolution.

Keywords

Thyroiditis · Acute thyroiditis · Infectious thyroiditis · Piriform sinus tract fistula · Radiation thyroiditis · Traumatic thyroiditis · Subacute thyroiditis · De Quervain's thyroiditis · Subacute granulomatous thyroiditis

Introduction

The term thyroiditis describes a heterogeneous group of disorders characterized by thyroid gland inflammation. Thyroiditis may present as an acutely tender gland associated with fever or with symptoms of thyroid dysfunction or may be asymptomatic, with the diagnosis made after the detection of thyroid dysfunction on routine blood test. There is neither a standardized classification system nor terminology for the causes of thyroiditis. The tempo of clinical presentation is commonly used to classify thyroiditis into acute, subacute, and chronic types, but these terms are not formally defined, and in practice considerable overlap in presentation exists. Types of thyroiditis, with alternate nomenclature, classified according to their causes and tempo of presentation are listed in Table 1.

Table 1 Classification of thyroiditis

Tempo of presentation	Disease	Alternative terms	Cause
Acute	Infectious thyroiditis	Acute thyroiditis Suppurative thyroiditis May also present as chronic thyroiditis	Bacterial or fungal infection
	Radiation thyroiditis		Radioactive iodine External beam radiotherapy Other forms of ionizing radiation
	Traumatic thyroiditis	Palpation thyroiditis	Trauma to thyroid
Subacute	Subacute thyroiditis	De Quervain's thyroiditis Subacute granulomatous thyroiditis Subacute nonsuppurative thyroiditis Giant cell thyroiditis Viral thyroiditis	Viral infection in genetically predisposed individuals
	Silent thyroiditis	Lymphocytic thyroiditis Painless thyroiditis Lymphocytic thyroiditis with spontaneously resolving hyperthyroidism Hashitoxicosis Autoimmune thyroiditis Includes postpartum thyroiditis	Destructive autoimmune thyroiditis
	Thyroid manifestations of generalized disease		Sarcoid Amyloid IgG4-related
	Drug induced		Amiodarone Interferon Monoclonal antibodies (e.g., ipilimumab, alemtuzumab) Tyrosine kinase inhibitors (e.g., sunitinib)
Chronic	Hashimoto's thyroiditis		Autoimmune destructive thyroiditis
	Fibrosing thyroiditis	Riedel's thyroiditis Multifocal fibrosing thyroiditis	Unknown

This chapter discusses in further detail the acute causes of thyroiditis and subacute thyroiditis. For the purpose of this chapter, the term subacute thyroiditis refers to the specific disease entity thought to be precipitated by viral infection and

associated with distinct pathological findings of granulomatous changes with giant cell formation; of course other types of thyroiditis may also present in a subacute fashion. Other causes of thyroiditis are discussed elsewhere (see ▶ Chaps. 7, "Hashimoto's Thyroiditis", ▶ 8, "Postpartum Thyroiditis and Silent Thyroiditis", and ▶ 25, "Drugs and Other Substances Interfering with Thyroid Function").

Infectious Thyroiditis

Presentation

Acute infectious thyroiditis is a rare condition, with its precise incidence uncertain. Of 1309 consecutive thyroid operations performed by a single surgeon between 1933 and 1955, 6 cases (0.5%) of acute suppurative infection were reported (Hendrick 1957). The rarity of thyroid infection has been attributed to protective trophic factors and anatomic characteristics inherent to the thyroid gland. This includes its high iodine content, hydrogen peroxide production, capsular encasement, rich blood supply with anastomotic arterial network, and abundant lymphatic drainage (Paes et al. 2010).

Infectious thyroiditis typically presents acutely with neck pain, fever, and constitutional symptoms. Sore throat, dysphagia, and dysphonia may also occur. Most patients have a unilateral neck mass which moves on swallowing and may be fluctuant (indicating abscess formation); overlying skin may be erythematous and edematous. Patients may avoid neck extension and keep their neck flexed to avoid pressure on the thyroid gland. Infectious thyroiditis can be potentially life-threatening due to airway compromise or complications resulting from sepsis. There is a risk of infection spreading to the chest causing necrotizing mediastinitis and pericarditis (Pereira et al. 2010).

Patients with acute infectious thyroiditis are usually euthyroid, although hyperthyroidism can occur due to destruction of thyroid follicles causing release of thyroxine and triiodothyronine. More chronic, destructive infection can result in hypothyroidism (Luiz et al. 2013).

Etiology

The most common cause for acute infectious thyroiditis, especially in children, is piriform sinus tract fistula. Other mechanisms for thyroid infection include spread from adjacent infected tissue, hematogenous spread, direct thyroid inoculation (e.g., fine-needle aspiration (FNA) biopsy, central venous line placement, or intravenous drug use), or esophageal perforation or rupture (Paes et al. 2010).

Piriform sinus fistula is a congenital fistula arising from the apex of the piriform recess and often ending in (or in the vicinity of) the thyroid gland. The embryologic origin of the fistula suggests that it is a remnant that arises in the most caudal embryonic pharyngeal pouch as a result of the migration of C cells of the thyroid

from their origin in the ultimobranchial body (Miyauchi 2010). The right ultimobranchial body is often atrophic and does not develop in humans, explaining the predilection for acute thyroiditis to affect the left lobe of the thyroid (Al-Dajani and Wootton 2007). In the case of piriform sinus tract fistula, recurrent infectious thyroiditis is common unless the fistula is definitively treated.

In the absence of piriform sinus tract fistula, predisposing factors for thyroid infection include existing thyroid disease (goiter, adenoma, or carcinoma), preceding infection in a distant site, trauma, postpartum or postabortal status, advanced age, diabetes mellitus, smoking, immunocompromised state, and chemotherapy (Al-Dajani and Wootton 2007).

The etiological agents responsible for infectious thyroiditis are typically bacterial although fungal infections also occur. Virtually any bacterium can infect the thyroid gland with gram-positive aerobic bacteria (*Staphylococcus aureus* and *Streptococcus* species) being the most frequent cause (Paes et al. 2010). Gram-negative aerobes and anaerobic organisms are also reported with anaerobic infections commonly polymicrobial. Unusual pathogens such as mycobacteria (*Mycobacterium avium-intracellulare* and *Mycobacterium tuberculosis*) and the fungi *Pneumocystis jiroveci* and *Nocardia* species are also described (Goldani et al. 2006; Luiz et al. 2013; Paes et al. 2010). In the case of *Mycobacterium tuberculosis*, symptoms of thyroiditis are more likely to be subacute or even chronic and can mimic carcinoma or multinodular goiter. The diagnosis is made on fine-needle aspiration findings of caseous necrosis, with or without visualization of acid-fast bacilli, and culture (Baidya et al. 2015; Majid and Islam 2011). Unusual pathogens are more often described in immunocompromised patients such as those with human immunodeficiency virus (HIV) infection, transplant recipients, or those receiving chemotherapy.

Diagnostic Evaluation

The initial laboratory evaluation of acute infectious thyroiditis typically includes a complete blood count, C-reactive protein (CRP), and thyroid function testing. There is usually leukocytosis and elevated CRP. Thyroid function tests are useful to determine the presence of thyroid dysfunction, which if present should be monitored with serial testing. Screening for human immunodeficiency virus (HIV) should be considered.

Laboratory markers may not be particularly useful in differentiating acute thyroiditis from aggressive thyroid cancer, subacute thyroiditis, or abscess formation in nearby anatomical structures as these conditions may each give rise to elevated white cell count and CRP. Imaging and FNA targeting any mass and/or fluid collection are therefore required.

Imaging characteristics in acute infectious thyroiditis vary depending on the stage of inflammation. Imaging features in the early inflammatory stage, when obvious abscess formation has not yet occurred, are subtle (Masuoka et al. 2011). During the early inflammatory stage, ultrasound performed by a skilled operator may be helpful; characteristic features include a hypoechoic area in the thyroid gland with a

perithyroidal hypoechoic space and effacement of the plane between the thyroid and perithyroidal tissues. Imaging findings on CT scan obtained shortly after inflammation onset are less specific and include a low-density area in the thyroid lobe and slight lobular swelling; it is not until the acute stage that abscess formation is evident on CT.

While ultrasound may be the most direct imaging method to differentiate acute infectious thyroiditis from its differentials, CT imaging with intravenous contrast of the neck and chest offers improved anatomic assessment and is recommended for the evaluation of acute infectious thyroiditis. CT with intravenous contrast can define soft tissue enhancement and whether there is abscess extension into the neck or mediastinum. CT scans may also identify a piriform sinus tract fistula as the cause for the thyroiditis, particularly if a trumpet maneuver is performed at the time of CT. A trumpet maneuver involves the patient blowing into a blunt needle attached to a syringe as if inflating a balloon or playing a trumpet. Air is thus used as a contrast medium to detect piriform sinus fistula and clarify the anatomical path of the fistula. Imaging with barium swallow is reported to have a higher sensitivity for piriform sinus fistula detection than CT with trumpet maneuver (Masuoka et al. 2011; Mou et al. 2014).

It may be difficult to identify fistula in the acute inflammatory period with either CT and/or barium contrast study, and it may be necessary to repeat imaging after inflammation subsides (Mou et al. 2014). Direct inspection by endoscopic hypopharyngoscopy may have the highest diagnostic yield for piriform sinus fistula (Kim et al. 2000; Paes et al. 2010), but this requires large-bore direct laryngoscopy under general anesthesia; fistula opening is infrequently observed with a thin flexible fiberscope, and therefore the utility of this technique as a diagnostic test has been questioned (Miyauchi 2010).

Having characterized acute infectious thyroiditis with CT scan, ultrasound allows diagnostic and therapeutic intervention with FNA. FNA can be useful when a differential diagnosis is still being considered (as it can help differentiate infection from subacute thyroiditis and malignancy) and is also essential for establishing infectious etiology and antimicrobial sensitivity.

The relative diagnostic utility of magnetic resonance imaging in infectious thyroiditis is unknown as no studies have directly compared MRI, CT, or other imaging modalities in this condition in a comprehensive fashion. Radionuclide thyroid scanning is not indicated, since infection, inflammation, and malignancy are each associated with focal or generalized reduction in tracer uptake, leading to a low discriminatory value.

Differential Diagnosis

Acute infectious thyroiditis needs to be differentiated from other conditions causing painful anterior neck swelling and fever, as presented in Table 2, adapted from Paes et al. (Paes et al. 2010). Examination findings, imaging studies, and FNA targeting any mass and/or fluid collection are the most helpful methods for differentiating between conditions.

Table 2 Differential diagnosis of painful anterior neck swelling and fever

Thyroid disorders	Subacute thyroiditis
	Thyroid nodule hemorrhage
	Thyroid cyst rupture
	Aggressive thyroid cancer
	Thyroid lymphoma
Conditions involving nearby anatomical structures	Abscess formation in a lymph node, the parapharyngeal/retropharyngeal space, or sternocleidomastoid muscle
	Parathyroid hemorrhage
	Branchial arches 1–4 anomalies (cysts, fistulas, sinuses)
	Thyroglossal duct cyst infection or perforation

Treatment

Treatment of acute infectious thyroiditis is with broad-spectrum, empiric intravenous antimicrobial therapy and percutaneous drainage with subsequent targeted antimicrobial therapy guided by microbiology results. In the setting of airway compromise, urgent transcutaneous or open surgical drainage is recommended, whereas in a stable patient, therapeutic ultrasound-guided FNA drainage may be appropriate. Conservative management with only intravenous antibiotics has been described (Segni et al. 2011). In the event of clinical deterioration despite percutaneous drainage, surgery may be required. Surgery may consist of open surgical drainage, or if extensive disease is present, total, near-total, or hemithyroidectomy.

If a piriform sinus fistula is present, surgical resection or nonsurgical obliteration of the fistula is recommended due to the risk of recurrent infection. Open surgery to excise a piriform sinus tract may be effective but can be complicated by injury to the recurrent laryngeal nerve and other structures. Delaying open surgery until resolution of inflammation improves identification of vital structures in the neck, thereby reducing potential surgical complications. Endoscopic cauterization of the piriform sinus internal opening either via chemocauterization or electrical cauterization is an alternative to open surgery with lower risk of complication (Kim et al. 2000; Miyauchi et al. 2009). Cauterization may be performed during acute infection if incision and drainage of the abscess are concomitantly performed. Often endoscopic cauterization is performed after resolution of the acute infection (Kim et al. 2000; Miyauchi et al. 2009).

Long-term Outcomes and Recurrence Rates

Without intervention to a piriform sinus tract fistula, further episodes of acute infectious thyroiditis generally occur within months to several years.

With effective antibiotic therapy and elimination of formed abscesses, patients with acute infectious thyroiditis typically have an excellent prognosis if they survive the acute episode. Thyroid function usually remains normal, although transient thyrotoxicosis or transient hypothyroidism or even permanent hypothyroidism may occur as a result of the disease or its treatment (Goldani et al. 2006; Luiz et al. 2013).

Radiation Thyroiditis

Clinically apparent thyroiditis, manifested by anterior neck pain and/or swelling, can occur following radioactive iodine (RAI) therapy for hyperthyroidism or thyroid cancer or from incidental irradiation of the thyroid in the course of external beam radiotherapy to the neck.

Following RAI for hyperthyroidism (toxic nodular thyroid disease or Graves' disease), approximately 1–5% of patients develop symptomatic thyroiditis (Ross 2011; Shah et al. 2015). There is a higher incidence of thyroiditis following RAI for thyroid remnant ablation in the setting of thyroid cancer with one series reporting mild thyroiditis (requiring simple analgesia only) in 16% of patients, whereas severe thyroiditis (requiring glucocorticoid treatment) occurred in 5% of patients (Cherk et al. 2008). The incidence and severity of thyroiditis were higher in patients with higher remnant RAI uptake (Cherk et al. 2008).

The onset of radiation thyroiditis is generally a few days after RAI therapy but may occur up to 2 weeks after treatment. Symptoms range from mild thyroidal discomfort to an exquisitely tender thyroid with dysphagia, and an enlarging goiter may be apparent (Bonnema and Hegedus 2012; Shah et al. 2015). Simple analgesia including nonsteroidal anti-inflammatory drugs or, in more severe cases, glucocorticoid therapy may be used to manage symptoms (Cherk et al. 2008). Exacerbation of thyrotoxicosis may occur, caused by the release of stored thyroid hormones from the gland. Thyroid storm occurring post-RAI therapy has been reported (Bonnema and Hegedus 2012; McDermott et al. 1983).

External beam radiotherapy causing thyroiditis is limited to case reports (Aizawa et al. 1998). The interval between radiotherapy and thyroiditis onset is generally months to years although shorter intervals are described (Aizawa et al. 1998). In contrast to RAI-induced thyroiditis, external beam radiotherapy-associated thyroiditis usually causes minimal or no symptoms but may be identified on thyroid function testing (Nishiyama et al. 1996). Later development of hypothyroidism is common (Aizawa et al. 1998).

Traumatic Thyroiditis

Direct anterior neck pressure or manipulation at the time of surgery or accident has been reported to be associated with thyroiditis. It was first described in 1975 as vigorous palpation of the thyroid gland inducing an inflammatory reaction and histological changes characteristic of multifocal granulomatous folliculitis (Carney et al. 1975). The described pathological findings were not thought to be clinically relevant nor pose a risk of thyrotoxicosis, but subsequent literature suggests otherwise. The incidence of traumatic thyroiditis is difficult to estimate because of under-recognition, under-reporting, and the lack of well-defined diagnostic criteria. In a parathyroidectomy surgical series, a 31% incidence rate of postoperative hyperthyroidism was reported with 4% of patients overtly thyrotoxic with symptoms requiring medical management (Stang et al. 2005).

Thyroiditis associated with trauma is usually non-tender although pain and tenderness have been observed. Clinical presentation is usually with features of thyrotoxicosis; the development of atrial fibrillation associated with traumatic thyroiditis is described (Mai et al. 2008). Specific imaging features have not been reported. Radionuclide scanning usually shows reduced tracer, as with other types of destructive thyroiditis (Mai et al. 2008).

Traumatic thyroiditis is generally self-limiting with complete biochemical remission occurring from 12 days to 3 months postoperatively in surgical series (Stang et al. 2005). Symptomatic treatment and in some cases glucocorticoid therapy, as used in other forms of inflammatory thyroiditis, have been employed (Espiritu and Dean 2010; Mai et al. 2008).

Subacute Thyroiditis

Subacute thyroiditis was first described in 1895 by Mygind as a thyroiditis affecting a previously normal thyroid gland without abscess formation (Mygind 1895). In 1905, Fritz de Quervain, a Swiss surgeon who specialized in thyroid disease, differentiated subacute thyroiditis from other forms of thyroiditis by pathological findings (Engkakul et al. 2011). He described granulomatous changes with giant cells as the unique pathological findings in the thyroid of affected individuals. Subsequent to this pathological description, subacute thyroiditis has commonly been referred to as de Quervain's thyroiditis.

Pathogenesis

Subacute thyroiditis is a self-limiting, inflammatory thyroid disorder characterized by neck pain and systemic symptoms, generally accompanied by thyroid dysfunction. The pathogenesis of the condition is not well understood, but it is thought to be caused by a viral infection or a post-viral inflammatory process in a genetically predisposed individual. The inflammatory process causes thyroid follicle damage with proteolysis of stored thyroglobulin leading to unregulated release of thyroid hormones into the circulation and resultant hyperthyroidism. The hyperthyroid state lasts until thyroglobulin stores are exhausted. Due to thyroid follicular cell damage and TSH inhibition from the raised serum T4 and T3 levels, new thyroid hormone synthesis ceases; this can result in progression from hyperthyroidism through to euthyroidism and then hypothyroidism. As inflammation subsides, thyroid follicles can regenerate, and normal, regulated, thyroid hormone synthesis and secretion resume.

The initial hypothesis of a viral or post-viral etiology in subacute thyroiditis was formulated on the tendency for the disease to have an antecedent history of an upper respiratory tract infection and/or to be associated with a typical viral prodrome of myalgia and malaise. A viral precipitant is also supported by clusters of the disease occurring during outbreaks of viral infection. Some studies have reported an

increased incidence during summer (Kitchener and Chapman 1989; Martino et al. 1987; Nishihara et al. 2008), but this has not been confirmed in all literature (Benbassat et al. 2007; Bennedbaek and Hegedus 1997; Fatourechi et al. 2003).

Numerous viruses have been reported to be associated with subacute thyroiditis, including mumps, coxsackievirus, enterovirus, echovirus, adenovirus, influenza, Epstein-Barr virus, hepatitis E, HIV, cytomegalovirus, dengue fever, and rubella (Assir et al. 2012; Bouillet et al. 2009; Desailloud and Hober 2009; Engkakul et al. 2011; Martinez-Artola et al. 2015). Virological evidence to support these viruses as causative agents includes either positive viral culture from thyroid tissue or high titers of virus-specific antibody (Desailloud and Hober 2009). While virological evidence of infection exists in some cases, other subacute thyroiditis patient series have not reliably identified a causal virus (Luotola et al. 1998; Mori et al. 1998). Luotola et al., focusing on the association of subacute thyroiditis with enterovirus but also considering other common viral pathogens, only identified one patient with acute cytomegalovirus among 27 cases of subacute thyroiditis. All other patients were negative in antibody tests, virus isolation, polymerase chain reaction, and antigen detection (Luotola et al. 1998). In addition to the implicated viral precipitants, there has also been a report of subacute thyroiditis associated with bacterial infection due to *Chlamydophila psittaci* (formerly *Chlamydia psittaci*) (Schofield and Keal 1986).

While subacute thyroiditis may be precipitated by viral infection, genetic predisposition also appears to be important, particularly class I major histocompatibility antigen status. A strong association with HLA-B35 across many ethnic groups has been reported (Nyulassy et al. 1977; Ohsako et al. 1995), and familial clustering of subacute thyroiditis is also associated with HLA-B35 status (Hamaguchi et al. 2005; Kramer et al. 2004; Zein et al. 2007). HLA-B67 antigen has also been associated with subacute thyroiditis in a Japanese population, although in a smaller percentage of patients and with a different phenotype from HLA-B35 (Ohsako et al. 1995). HLA-B67-associated subacute thyroiditis occurred mostly in summer or autumn and showed a higher incidence of a hypothyroid phase, whereas HLA-B35-subacute thyroiditis disease occurred year-round and was more likely to progress from hyperthyroidism to euthyroidism without a hypothyroid phase. The authors concluded that these differences suggested different viruses associating with the different HLA antigens (Ohsako et al. 1995). It is proposed that a viral infection provides an antigen, either directly or from virus-induced host tissue damage that binds to HLA molecules on macrophages. The resulting antigen-HLA complex is recognized by cytolytic T cells that then damage thyroid follicular cells because of molecular mimicry. Unlike autoimmune thyroid disease, however, the immune reaction is not self-perpetuating, so the process is self-limiting.

Epidemiology

Subacute thyroiditis is an uncommon condition and an uncommon cause of hyperthyroidism; it is however the most common cause of painful thyroid disease in adults (Engkakul et al. 2011). In a Danish population-based cohort from 1997 to 2000,

subacute thyroiditis accounted for 2.3% of newly diagnosed overt hyperthyroidism cases and had an age-adjusted standardized incident rate of 2.0 per 100,000 person-years (Carle et al. 2011). Data from the Rochester Epidemiology Project showed an overall age and sex-adjusted incidence rate from 1960 through 1997 of 4.9 cases per 100,000/year. The incidence rate decreased from the 1960s onwards although was stable in the 1980s and 1990s with 3.6 cases per 100,000/year in the 1990s (Fatourechi et al. 2003). Subacute thyroiditis occurs more commonly in women than men, with a sex ratio between 3:1 to 7:1 (Carle et al. 2011; Fatourechi et al. 2003; Nishihara et al. 2008). The peak incidence of the condition is in the fifth decade of life; it is very rare in children (Fatourechi et al. 2003; Nishihara et al. 2008).

Presentation/Clinical Features

Neck pain is a cardinal presenting feature of subacute thyroiditis and may be of sudden or gradual onset. The pain may initially be unilateral or bilateral with unilateral pain at onset reported in approximately two-thirds of patients (Bennedbaek and Hegedus 1997; Nishihara et al. 2008); unilateral pain may become bilateral over days or weeks. The pain may be limited to the thyroid gland or radiate to the upper neck, jaw, throat, or ears. Dysphagia may occur and cause the disorder to be mistaken for pharyngitis. On palpation the thyroid gland is tender and is slightly or moderately enlarged; this may be diffuse or asymmetric and nodules may be palpable (Fatourechi et al. 2003).

Systemic symptoms are common in subacute thyroiditis. Patients may describe features of an upper respiratory tract infection in the preceding weeks as well as fever, fatigue, arthralgia/myalgia, and/or anorexia. At symptom onset, a temperature of greater than 38 °C is described in more than a quarter of patients (Nishihara et al. 2008). Rarely, subacute thyroiditis may present as a fever of unknown origin. The pain and systemic symptoms of subacute thyroiditis may reach their peak within days but more typically gradually progress over 1–2 weeks and continue with a fluctuating intensity for weeks up to months.

Thyroid dysfunction in subacute thyroiditis typically has a triphasic course of hyperthyroidism followed by hypothyroidism and then resolving to euthyroidism. However, not all patients demonstrate all phases. The entire clinical course typically lasts about 3 months although may be longer, particularly in those who develop a hypothyroid phase (Benbassat et al. 2007).

Hyperthyroidism, evidenced biochemically by a suppressed TSH, has been reported to occur in all cases of subacute thyroiditis at initial presentation (Benbassat et al. 2007). Symptomatic hyperthyroidism occurred in more than 60% of patients presenting with subacute thyroiditis in a large, Japanese series of 852 affected individuals (Nishihara et al. 2008). In this series, elevations in free T4, free T3, and thyroglobulin reached their peaks within 7 days of symptom onset, whereas full suppression of TSH occurred somewhat later. Hyperthyroid symptoms generally resolve over weeks to months without treatment; a median duration of 43 days (range 15–90) has been reported (Benbassat et al. 2007). Hyperthyroidism is usually

mild both clinically and biochemically but can precipitate thyrotoxic complications such as cardiac arrhythmia, psychosis, and very rarely thyroid storm.

A hypothyroid phase is reported to occur in 30–60% of patients with subacute thyroiditis (Benbassat et al. 2007; Fatourechi et al. 2003). In most cases, this is mild, transient and often asymptomatic. These patients mostly have subclinical or mild overt hypothyroidism with median peak TSH concentrations being 9.6 mU/L and 15.8 mU/L in two cohorts studied (Benbassat et al. 2007; Fatourechi et al. 2003). Peak TSH concentrations above 10 mU/L and 50 mU/L are reported to occur in 39% and 13% of hypothyroid individuals, respectively (Benbassat et al. 2007). Ultimately, around 90% of patients recover normal thyroid function (Benbassat et al. 2007; Fatourechi et al. 2003).

Diagnostic Evaluation

The clinical presentation of subacute thyroiditis is often characteristic, allowing a clinical diagnosis to be made. Routine investigations include a full blood count, CRP, and thyroid function testing. A radionuclide thyroid scan is also frequently requested (during the hyperthyroid phase) and can help confirm the diagnosis. Other investigations would generally be reserved for those cases with atypical features or where the diagnosis is uncertain.

Laboratory

Subacute thyroiditis is typically associated with an elevation in CRP which can be marked. The blood leukocyte count is usually normal but may be mildly elevated (Engkakul et al. 2011). A markedly elevated white blood cell count should raise suspicion for suppurative thyroiditis. Normochromic normocytic anemia may be noted. Liver function test abnormalities may be seen related possibly to the precipitating infection or the hyperthyroidism.

In the acute phase of subacute thyroiditis, most patients have biochemical evidence of hyperthyroidism with elevated serum T4 and T3 and suppressed TSH. The T3 to T4 ratio is often relatively low, reflecting the predominance of T4 over T3 within thyroid follicles (Amino et al. 1981). Impairment of peripheral deiodination of T4 to T3 from acute illness can also result in a relatively low T3 level. Serum thyroglobulin is elevated in almost all patients with subacute thyroiditis, consistent with follicular destruction, but is not part of routine evaluation.

Thyroid antibodies such as anti-thyroglobulin, antithyroid peroxidase, and TSH receptor antibodies are usually absent in subacute thyroiditis, although transient low-titer antibodies may be found (Benbassat et al. 2007; Bennedbaek and Hegedus 1997; Engkakul et al. 2011; Erdem et al. 2007; Mariotti et al. 1990). Positive antithyroid antibodies (anti-thyroglobulin and antithyroid peroxidase) are associated with the development of a hypothyroid phase (Benbassat et al. 2007). These antithyroid antibodies disappear during the recovery phase in most.

Imaging Studies

Radionuclide thyroid scanning typically shows markedly reduced or absent tracer uptake during the acute phase of subacute thyroiditis (see Fig. 1). In the recovery phase, the thyroid gland has an increased iodine-trapping ability, which can result in irregular or diffusely increased tracer uptake on scanning before eventually normalizing.

Thyroid ultrasound is not required as part of the diagnostic workup of subacute thyroiditis. If it is performed, subacute thyroiditis is characterized by a hypoechogenic area at the site of thyroid gland pain (Bennedbaek and Hegedus 1997; Nishihara et al. 2008) (see Fig. 2). Ultrasonographic thyroid gland enlargement is also common and improves with recovery (Bennedbaek and Hegedus 1997). During the hyperthyroid phase, color Doppler sonography shows low flow (see Fig. 2), whereas in Graves' hyperthyroidism there is usually enhanced flow. Subacute thyroiditis is also associated with thyroid nodules on ultrasound with one study reporting nodules of >4 mm in more than 70% of patients (Benbassat et al. 2007). Most of these patients had multiple nodules with the median size of the largest nodule being 17 mm. On follow-up, the majority of these nodules spontaneously resolve. Therefore, if clinical suspicion of malignancy is low, imaging can be repeated when subacute thyroiditis has resolved, and FNA biopsy considered only if nodules persist (Benbassat et al. 2007).

Pathology

Tissue diagnosis is rarely needed for the diagnosis of subacute thyroiditis. In doubtful cases such as the area of tenderness being limited to a solitary nodule or localized area, FNA biopsy may be useful to distinguish unilateral involvement of subacute thyroiditis from infection, bleeding into a cyst, or neoplasm.

Cytopathological findings are often characteristic and include the presence of multinucleated giant cells and granulomatous changes (see Fig. 3). Other

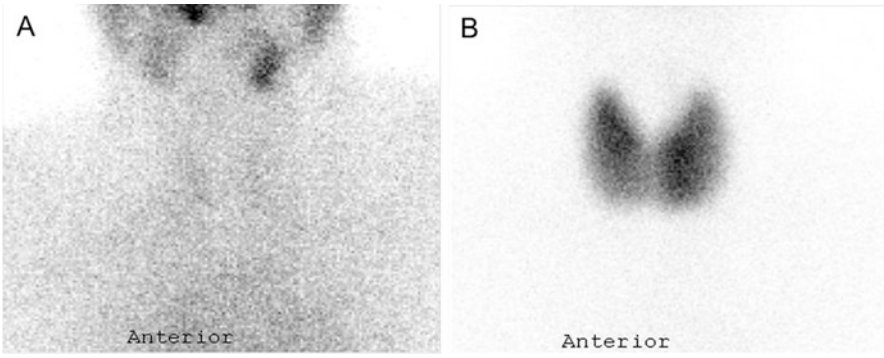

Fig. 1 Technetium scintigraphic appearance of subacute thyroiditis (**a**) during the hyperthyroid phase of the illness, demonstrating absent tracer uptake within the thyroid gland. By comparison, in Graves' disease (**b**) there is diffuse uptake of tracer, usually above the reference range

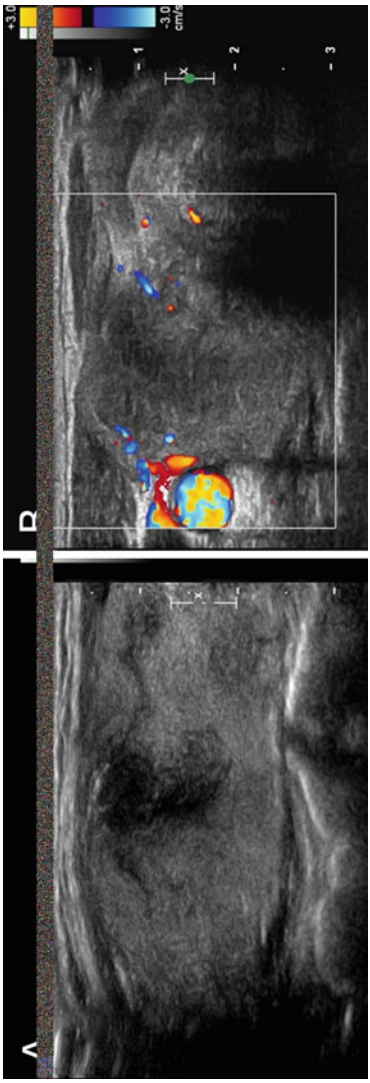

Fig. 2 Ultrasound appearance of subacute thyroiditis demonstrating thyroid enlargement with hypoechogenicity and pseudonodular appearance (**a**). Using color flow Doppler, there is low vascularity (**b**)

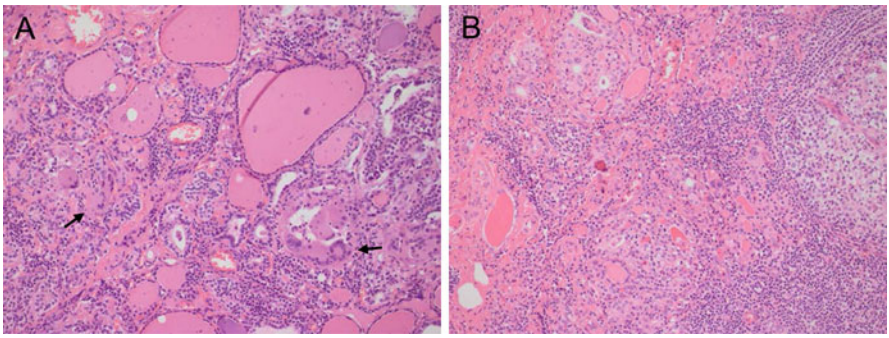

Fig. 3 (**a**) Pathological changes of subacute thyroiditis, showing characteristic features of granulomatous change with multinucleated giant cells (*arrows*) and interfollicular infiltration by mononuclear cells and lymphocytes. For comparison, pathological appearances of Hashimoto's thyroiditis are shown (**b**), with follicular atrophy and extensive lymphocytic infiltration

pathological findings include, in the early stage, infiltration of thyroid follicles with mononuclear cells and lymphocytes and ultimately patchy disruption of the thyroid follicle epithelial lining, and loss of follicular integrity is seen. Interfollicular fibrosis may occur later, but eventually pathological changes return to normal with minimal residual thyroid fibrosis seen after resolution of the disease (Engkakul et al. 2011).

Differential Diagnosis

The primary differential diagnosis of a tender, inflamed thyroid with systemic symptoms is acute infectious thyroiditis, which is a more common condition in children than subacute thyroiditis. Other causes of painful anterior neck swelling and fever should also be considered (see Table 2). Occasionally, subacute thyroiditis may mimic temporal arteritis, with fever, nonspecific constitutional symptoms, and pain at the angle of the jaw (Cunha et al. 2010; Rosenstein and Kramer 1994).

During the thyrotoxic phase, differential diagnoses of Graves' disease and silent thyroiditis need to be considered (see ▶ Chaps. 8, "Postpartum Thyroiditis and Silent Thyroiditis" and ▶ 14, "Graves' Disease"). A history of a preceding viral illness is common in subacute thyroiditis but not in Graves' disease and silent thyroiditis, and inflammatory markers such as CRP are rarely elevated in autoimmune thyroid disease. Neck pain is unusual in Graves' disease or silent thyroiditis, but some patients with these conditions do have tenderness of the thyroid on palpation. Characteristic physical signs of Graves' disease such as thyroid bruit and proptosis are not a feature of subacute thyroiditis, although ocular manifestations of hyperthyroidism such as lid lag and retraction may be present (Samuels 2012). Serum T3 concentrations and the T3 to T4 ratio tend to be lower in subacute thyroiditis than in Graves' disease (in which increased T4 to T3 conversion occurs within the thyroid), but there is overlap (Amino et al. 1981; Izumi et al. 2002). High concentrations of TSH receptor antibodies (TRAb) indicate a diagnosis of Graves' disease, but positive TRAb levels have been

reported (albeit infrequently) in subacute thyroiditis (Takasu et al. 2004). Radionuclide scanning reliably distinguishes between Graves' disease and thyroiditis in the thyrotoxic phase, with diffuse tracer uptake in Graves' disease and reduced or absent uptake in subacute or silent thyroiditis (see Fig. 1). Care must be taken to perform radionuclide scanning while the patient is thyrotoxic, as tracer uptake may increase during the hypothyroid phase of subacute thyroiditis, with the potential for diagnostic confusion (Engkakul et al. 2011; Samuels 2012).

During the hypothyroid phase of subacute thyroiditis, the main differential diagnosis is Hashimoto's disease (see ▶ Chap. 7, "Hashimoto's Thyroiditis"). Measurement of antithyroid antibodies may be useful, because they tend to be absent or in low titer in subacute thyroiditis (Engkakul et al. 2011; Mariotti et al. 1990). A history of neck pain can be helpful as, unlike subacute thyroiditis, Hashimoto's disease is typically painless. If no obvious intervening thyrotoxic phase is brought to medical attention, however, patients may forget a history of neck pain (Samuels 2012). Hashimoto's thyroiditis may also rarely cause chronic neck pain (Kon and DeGroot 2003). If the diagnosis is uncertain, then prolonged monitoring of thyroid function or a period of levothyroxine replacement with attempted withdrawal of treatment some months later may be required to see if hypothyroidism has resolved.

Treatment

Treatment for subacute thyroiditis aims at relieving pain and ameliorating hyperthyroid symptoms. In many patients, no treatment or simple analgesia is all that is required. Mild to moderately painful thyroiditis responds to nonsteroidal anti-inflammatory drugs (NSAIDs). In cases where NSAIDs are ineffective or where symptoms are more severe at disease onset, glucocorticoid treatment such as oral prednisolone is employed. Glucocorticoid treatment should only be commenced once acute suppurative thyroiditis has been excluded either clinically or on investigation. There is no evidence that NSAIDs or glucocorticoid therapy alters the disease process (Benbassat et al. 2007). Beta-adrenergic blockers may be used for control of hyperthyroid symptoms, but antithyroid drugs (which block thyroid hormone synthesis) are not indicated since the thyrotoxicosis arises from leakage of stored thyroid hormone rather than increased synthesis and secretion (Engkakul et al. 2011).

Oral glucocorticoid treatment is administered in subacute thyroiditis in moderate and severe cases and can improve symptoms within hours (Bennedbaek and Hegedus 1997). Prednisolone is the most commonly used glucocorticoid with a starting dose of 40 mg daily for 1–2 weeks recommended; this is then tapered over 2–4 weeks or longer (Bahn et al. 2011). A median prednisolone treatment duration of 1–2 months is reported (Bennedbaek and Hegedus 1997; Fatourechi et al. 2003). One study of 23 consecutive patients with subacute thyroiditis reported that of the 22 patients treated with prednisolone at a starting dose of 37.5 mg daily, all had complete resolution of pain within 2 days (Bennedbaek and Hegedus 1997). In another study,

patients treated with prednisolone had a median time to resolution of pain of 4 days, compared with 21 days for those treated with NSAIDs and acetylsalicylic acid (Fatourechi et al. 2003). Low-dose glucocorticoid therapy may also be effective. In an uncontrolled Japanese study, an initial prednisolone dose of 15 mg daily was employed, with tapering of the daily dose by 5 mg every 2 weeks as permitted by clinical response. In this study, 80% of patients improved within 8 weeks, allowing prednisolone cessation. The authors concluded that the proportion of patients requiring prolonged prednisolone treatment was similar to that in previous studies where higher prednisolone doses were used (Kubota et al. 2013).

Symptoms of subacute thyroiditis may recur with tapering or cessation of glucocorticoids; a relapse rate of 10–35% is described (Arao et al. 2015; Bennedbaek and Hegedus 1997; Fatourechi et al. 2003; Mizukoshi et al. 2001). Relapse does not appear to correlate with sex, age, clinical features, laboratory findings, or initial prednisolone dose (Arao et al. 2015; Mizukoshi et al. 2001). In one study of 26 patients (including 4 who relapsed), rapid dose tapering appeared to be a predictor of relapse with non-recurrence and recurrence groups having 44.3±15.3 days and 19.0±15.3 days, respectively, until a prednisolone dose of 5 mg/day was reached (Arao et al. 2015). Based on their findings, these authors recommended a period greater than 6 weeks before tapering prednisolone to 5 mg daily. Other authors have speculated that the period of time of prednisolone 10 mg daily may be the breakpoint for recurrence, suggesting that extending the period of treatment with prednisolone 10 mg daily may help reduce the risk of relapse (Mizukoshi et al. 2001). Taken together these recommendations suggest that once prednisolone dose has been tapered to 10 mg daily, more gradual dose tapering with at least a 6-week course of therapy before dropping to 5 mg daily of prednisolone may reduce the risk of recurrence.

Recently, a novel therapeutic strategy of intrathyroidal injection of lidocaine and dexamethasone has been described for the treatment of subacute thyroiditis. This treatment achieved more rapid pain relief and required a shorter duration of treatment than a tapered regimen of oral prednisolone treatment (Ma et al. 2014).

Glucocorticoid treatment does not prevent late (or early) hypothyroidism and in fact may be associated with a higher rate of hypothyroidism on long-term follow-up (Fatourechi et al. 2003). In the pathogenesis of late-onset hypothyroidism, autoimmunity and development of antithyroid and blocking antibodies have been speculated. However, the association between glucocorticoid therapy and hypothyroidism may reflect the more severe underlying disease process in the patient requiring such treatment rather than being a treatment effect.glucocorticoid

Levothyroxine is rarely needed in the early hypothyroid phase of subacute thyroiditis because the hypothyroidism is usually transient in nature. If symptoms of hypothyroidism are more pronounced, however, levothyroxine treatment can be instituted. The dose should be adjusted to achieve a serum TSH concentration in the reference range. It should not be assumed that this treatment is required indefinitely and the ongoing need for treatment should be evaluated after several months, with a view to attempted withdrawal.

Long-Term Outcomes and Recurrence Rates

Late-onset, permanent hypothyroidism is reported to occur between 2 and 24 years after the initial episode of subacute thyroiditis, but it is uncertain whether this is directly related to the subacute thyroiditis or is from the subsequent development of autoimmune thyroid disease (Fatourechi et al. 2003). Graves' disease in the months to years following an episode of subacute thyroiditis has also been reported, perhaps caused by subacute thyroiditis triggering autoreactive B cells to produce TSH receptor antibodies (Bennedbaek et al. 1996; Iitaka et al. 1998).

A recurrence rate for subacute thyroiditis of 1.4–4% has been reported with recurrences occurring 6–23 years after the initial episode (Fatourechi et al. 2003; Iitaka et al. 1996; Yamamoto et al. 1988). Repeated episodes of recurrence have been reported in some patients, with recurrences generally milder than the original episode (Iitaka et al. 1996; Yamamoto et al. 1988).

Summary

The term thyroiditis refers to inflammatory conditions of the thyroid with a wide range of etiologies, which may present in an acute, subacute, or chronic fashion. Careful clinical assessment supported by judicious use of investigations allows the correct diagnosis to be made. Acute infectious thyroiditis is managed with systemic antimicrobial treatment, surgical drainage, and identification of the underlying cause. Subacute thyroiditis of presumed viral etiology may present primarily with local neck symptoms or with thyrotoxic symptoms. It is managed expectantly and symptomatically with nonsteroidal anti-inflammatory or glucocorticoid treatment if required for neck pain, and beta-blockade if necessary for thyrotoxic symptoms.

Cross-References

- ▶ Drugs and Other Substances Interfering with Thyroid Function
- ▶ Graves' Disease
- ▶ Hashimoto's Thyroiditis
- ▶ Postpartum Thyroiditis and Silent Thyroiditis

References

Aizawa T, Watanabe T, Suzuki N, Suzuki S, Miyamoto T, Ichikawa K, Oguchi M, Hashizume K. Radiation-induced painless thyrotoxic thyroiditis followed by hypothyroidism: a case report and literature review. Thyroid. 1998;8(3):273–5.

Al-Dajani N, Wootton SH. Cervical lymphadenitis, suppurative parotitis, thyroiditis, and infected cysts. Infect Dis Clin North Am. 2007;21(2):523–41, viii.

Amino N, Yabu Y, Miki T, Morimoto S, Kumahara Y, Mori H, Iwatani Y, Nishi K, Nakatani K, Miyai K. Serum ratio of triiodothyronine to thyroxine, and thyroxine-binding globulin and

calcitonin concentrations in Graves' disease and destruction-induced thyrotoxicosis. J Clin Endocrinol Metab. 1981;53(1):113–6.
Arao T, Okada Y, Torimoto K, Kurozumi A, Narisawa M, Yamamoto S, Tanaka Y. Prednisolone dosing regimen for treatment of subacute thyroiditis. J UOEH. 2015;37(2):103–10.
Assir MZ, Jawa A, Ahmed HI. Expanded dengue syndrome: subacute thyroiditis and intracerebral hemorrhage. BMC Infect Dis. 2012;12:240.
Bahn RS, Burch HB, Cooper DS, Garber JR, Greenlee MC, Klein I, Laurberg P, McDougall IR, Montori VM, Rivkees SA, Ross DS, Sosa JA, Stan MN. Hyperthyroidism and other causes of thyrotoxicosis: management guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists. Endocr Pract. 2011;17(3):456–520.
Baidya A, Singha A, Bhattacharjee R, Dalal BS. Tuberculosis of the thyroid gland: two case reports. Oxf Med Case Rep. 2015;2015(4):262–4.
Benbassat CA, Olchovsky D, Tsvetov G, Shimon I. Subacute thyroiditis: clinical characteristics and treatment outcome in fifty-six consecutive patients diagnosed between 1999 and 2005. J Endocrinol Invest. 2007;30(8):631–5.
Bennedbaek FN, Gram J, Hegedus L. The transition of subacute thyroiditis to Graves' disease as evidenced by diagnostic imaging. Thyroid. 1996;6(5):457–9.
Bennedbaek FN, Hegedus L. The value of ultrasonography in the diagnosis and follow-up of subacute thyroiditis. Thyroid. 1997;7(1):45–50.
Bonnema SJ, Hegedus L. Radioiodine therapy in benign thyroid diseases: effects, side effects, and factors affecting therapeutic outcome. Endocr Rev. 2012;33(6):920–80.
Bouillet B, Petit JM, Piroth L, Duong M, Bourg JB. A case of subacute thyroiditis associated with primary HIV infection. Am J Med. 2009;122(4):e5–6.
Carle A, Pedersen IB, Knudsen N, Perrild H, Ovesen L, Rasmussen LB, Laurberg P. Epidemiology of subtypes of hyperthyroidism in Denmark: a population-based study. Eur J Endocrinol. 2011;164(5):801–9.
Carney JA, Moore SB, Northcutt RC, Woolner LB, Stillwell GK. Palpation thyroiditis (multifocal granulomatour folliculitis). Am J Clin Pathol. 1975;64(5):639–47.
Cherk MH, Kalff V, Yap KS, Bailey M, Topliss D, Kelly MJ. Incidence of radiation thyroiditis and thyroid remnant ablation success rates following 1110 MBq (30 mCi) and 3700 MBq (100 mCi) post-surgical 131I ablation therapy for differentiated thyroid carcinoma. Clin Endocrinol (Oxf). 2008;69(6):957–62.
Cunha BA, Chak A, Strollo S. Fever of unknown origin (FUO): de Quervain's subacute thyroiditis with highly elevated ferritin levels mimicking temporal arteritis (TA). Heart Lung. 2010;39(1):73–7.
Desailloud R, Hober D. Viruses and thyroiditis: an update. Virol J. 2009;6:5.
Engkakul P, Mahachoklertwattana P, Poomthavorn P. Eponym: de Quervain thyroiditis. Eur J Pediatr. 2011;170(4):427–31.
Erdem N, Erdogan M, Ozbek M, Karadeniz M, Cetinkalp S, Ozgen AG, Saygili F, Yilmaz C, Tuzun M, Kabalak T. Demographic and clinical features of patients with subacute thyroiditis: results of 169 patients from a single university center in Turkey. J Endocrinol Invest. 2007;30(7):546–50.
Espiritu RP, Dean DS. Parathyroidectomy-induced thyroiditis. Endocr Pract. 2010;16(4):656–9.
Fatourechi V, Aniszewski JP, Fatourechi GZ, Atkinson EJ, Jacobsen SJ. Clinical features and outcome of subacute thyroiditis in an incidence cohort: Olmsted County, Minnesota, study. J Clin Endocrinol Metab. 2003;88(5):2100–5.
Goldani LZ, Zavascki AP, Maia AL. Fungal thyroiditis: an overview. Mycopathologia. 2006;161 (3):129–39.
Hamaguchi E, Nishimura Y, Kaneko S, Takamura T. Subacute thyroiditis developed in identical twins two years apart. Endocr J. 2005;52(5):559–62.
Hendrick JW. Diagnosis and management of thyroiditis. J Am Med Assoc. 1957;164(2):127–33.
Iitaka M, Momotani N, Ishii J, Ito K. Incidence of subacute thyroiditis recurrences after a prolonged latency: 24-year survey. J Clin Endocrinol Metab. 1996;81(2):466–9.
Iitaka M, Momotani N, Hisaoka T, Noh JY, Ishikawa N, Ishii J, Katayama S, Ito K. TSH receptor antibody-associated thyroid dysfunction following subacute thyroiditis. Clin Endocrinol (Oxf). 1998;48(4):445–53.

Izumi Y, Hidaka Y, Tada H, Takano T, Kashiwai T, Tatsumi KI, Ichihara K, Amino N. Simple and practical parameters for differentiation between destruction-induced thyrotoxicosis and Graves' thyrotoxicosis. Clin Endocrinol (Oxf). 2002;57(1):51–8.

Kim KH, Sung MW, Koh TY, Oh SH, Kim IS. Pyriform sinus fistula: management with chemocauterization of the internal opening. Ann Otol Rhinol Laryngol. 2000;109(5):452–6.

Kitchener MI, Chapman IM. Subacute thyroiditis: a review of 105 cases. Clin Nucl Med. 1989;14 (6):439–42.

Kon YC, DeGroot LJ. Painful Hashimoto's thyroiditis as an indication for thyroidectomy: clinical characteristics and outcome in seven patients. J Clin Endocrinol Metab. 2003;88(6):2667–72.

Kramer AB, Roozendaal C, Dullaart RP. Familial occurrence of subacute thyroiditis associated with human leukocyte antigen-B35. Thyroid. 2004;14(7):544–7.

Kubota S, Nishihara E, Kudo T, Ito M, Amino N, Miyauchi A. Initial treatment with 15 mg of prednisolone daily is sufficient for most patients with subacute thyroiditis in Japan. Thyroid. 2013;23(3):269–72.

Luiz HV, Pereira BD, Silva TN, Veloza A, Matos C, Manita I, Cordeiro MC, Raimundo L, Portugal J. Thyroid tuberculosis with abnormal thyroid function – case report and review of the literature. Endocr Pract. 2013;19(2):e44–9.

Luotola K, Hyoty H, Salmi J, Miettinen A, Helin H, Pasternack A. Evaluation of infectious etiology in subacute thyroiditis – lack of association with coxsackievirus infection. APMIS. 1998;106(4):500–4.

Ma SG, Bai F, Cheng L. A novel treatment for subacute thyroiditis: administration of a mixture of lidocaine and dexamethasone using an insulin pen. Mayo Clin Proc. 2014;89(6):861–2.

Mai VQ, Glister BC, Clyde PW, Shakir KM. Palpation thyroiditis causing new-onset atrial fibrillation. Thyroid. 2008;18(5):571–3.

Majid U, Islam N. Thyroid tuberculosis: a case series and a review of the literature. J Thyroid Res. 2011;2011:359864.

Mariotti S, Caturegli P, Piccolo P, Barbesino G, Pinchera A. Antithyroid peroxidase autoantibodies in thyroid diseases. J Clin Endocrinol Metab. 1990;71(3):661–9.

Martinez-Artola Y, Poncino D, Garcia ML, Munne MS, Gonzalez J, Garcia DS. Acute hepatitis E virus infection and association with a subacute thyroiditis. Ann Hepatol. 2015;14(1):141–2.

Martino E, Buratti L, Bartalena L, Mariotti S, Cupini C, Aghini-Lombardi F, Pinchera A. High prevalence of subacute thyroiditis during summer season in Italy. J Endocrinol Invest. 1987; 10(3):321–3.

Masuoka H, Miyauchi A, Tomoda C, Inoue H, Takamura Y, Ito Y, Kobayashi K, Miya A. Imaging studies in sixty patients with acute suppurative thyroiditis. Thyroid. 2011;21(10):1075–80.

McDermott MT, Kidd GS, Dodson Jr LE, Hofeldt FD. Radioiodine-induced thyroid storm. Case report and literature review. Am J Med. 1983;75(2):353–9.

Miyauchi A. Thyroid gland: a new management algorithm for acute suppurative thyroiditis? Nat Rev Endocrinol. 2010;6(8):424–6.

Miyauchi A, Inoue H, Tomoda C, Amino N. Evaluation of chemocauterization treatment for obliteration of pyriform sinus fistula as a route of infection causing acute suppurative thyroiditis. Thyroid. 2009;19(7):789–93.

Mizukoshi T, Noguchi S, Murakami T, Futata T, Yamashita H. Evaluation of recurrence in 36 subacute thyroiditis patients managed with prednisolone. Intern Med. 2001;40(4):292–5.

Mori K, Yoshida K, Funato T, Ishii T, Nomura T, Fukuzawa H, Sayama N, Hori H, Ito S, Sasaki T. Failure in detection of Epstein-Barr virus and cytomegalovirus in specimen obtained by fine needle aspiration biopsy of thyroid in patients with subacute thyroiditis. Tohoku J Exp Med. 1998;186(1):13–7.

Mou JW, Chan KW, Wong YS, Houben CH, Tam YH, Lee KH. Recurrent deep neck abscess and piriform sinus tract: a 15-year review on the diagnosis and management. J Pediatr Surg. 2014;49 (8):1264–7.

Mygind H. Thyroiditis akuta simplex. J Laryngol Rhinol Otol. 1895;9:181–93.

Nishihara E, Ohye H, Amino N, Takata K, Arishima T, Kudo T, Ito M, Kubota S, Fukata S, Miyauchi A. Clinical characteristics of 852 patients with subacute thyroiditis before treatment. Intern Med. 2008;47(8):725–9.

Nishiyama K, Kozuka T, Higashihara T, Miyauchi K, Okagawa K. Acute radiation thyroiditis. Int J Radiat Oncol Biol Phys. 1996;36(5):1221–4.

Nyulassy S, Hnilica P, Buc M, Guman M, Hirschova V, Stefanovic J. Subacute (de Quervain's) thyroiditis: association with HLA-Bw35 antigen and abnormalities of the complement system, immunoglobulins and other serum proteins. J Clin Endocrinol Metab. 1977;45(2):270–4.

Ohsako N, Tamai H, Sudo T, Mukuta T, Tanaka H, Kuma K, Kimura A, Sasazuki T. Clinical characteristics of subacute thyroiditis classified according to human leukocyte antigen typing. J Clin Endocrinol Metab. 1995;80(12):3653–6.

Paes JE, Burman KD, Cohen J, Franklyn J, McHenry CR, Shoham S, Kloos RT. Acute bacterial suppurative thyroiditis: a clinical review and expert opinion. Thyroid. 2010;20(3):247–55.

Pereira O, Prasad DS, Bal AM, McAteer D, Abraham P. Fatal descending necrotizing mediastinitis secondary to acute suppurative thyroiditis developing in an apparently healthy woman. Thyroid. 2010;20(5):571–2.

Rosenstein ED, Kramer N. Occult subacute thyroiditis mimicking classic giant cell arteritis. Arthritis Rheum. 1994;37(11):1618–20.

Ross DS. Radioiodine therapy for hyperthyroidism. N Engl J Med. 2011;364(6):542–50.

Samuels MH. Subacute, silent, and postpartum thyroiditis. Med Clin North Am. 2012;96 (2):223–33.

Schofield PM, Keal EE. Subacute thyroiditis associated with *Chlamydia psittaci* infection. Postgrad Med J. 1986;62(723):33–4.

Segni M, Turriziani I, di Nardo R, Pucarelli I, Serafinelli C. Acute suppurative thyroiditis treated avoiding invasive procedures in a child. J Clin Endocrinol Metab. 2011;96(10):2980–1.

Shah KK, Tarasova V, Davidian M, Anderson RJ. Painful acute radiation thyroiditis induced by 131I treatment of Graves' disease. BMJ Case Rep. 2015. doi:10.1136/bcr-2014-207670.

Stang MT, Yim JH, Challinor SM, Bahl S, Carty SE. Hyperthyroidism after parathyroid exploration. Surgery. 2005;138(6):1058–64.

Takasu N, Kamijo K, Sato Y, Yoshimura H, Nagata A, Ochi Y. Sensitive thyroid-stimulating antibody assay with high concentrations of polyethylene glycol for the diagnosis of Graves' disease. Clin Exp Pharmacol Physiol. 2004;31(5–6):314–9.

Yamamoto M, Saito S, Sakurada T, Tamura M, Kudo Y, Yoshida K, Kaise K, Kaise N, Fukazawa H, Itagaki Y, et al. Recurrence of subacute thyroiditis over 10 years after the first attack in three cases. Endocrinol Jpn. 1988;35(6):833–9.

Zein EF, Karaa SE, Megarbane A. Familial occurrence of painful subacute thyroiditis associated with human leukocyte antigen-B35. Presse Med. 2007;36(5 Pt 1):808–9.

Part IV

Hypothyroidism

Classification and Etiopathogenesis of Hypothyroidism

10

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Abstract

Hypothyroidism is a graded phenomenon characterized by a deficient thyroidal production of thyroid hormone. This condition comprises multiple functional or structural disorders, both congenital and acquired. The clinical expression of hypothyroidism varies between individuals, depending on the cause, duration, and severity of the hypothyroid state, but almost all organs and systems may be affected by a deficiency of thyroid hormone production. This chapter describes the epidemiology, multiple etiologies, and clinical manifestations of hypothyroidism.

Keywords

Hypothyroidism · Subclinical hypothyroidism · Classification · Epidemiology · Etiopathogenesis · Clinical features

Hypothyroidism

Definition and Classification of Hypothyroidism

Hypothyroidism is a clinical condition characterized by reduced synthesis and secretion of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). As detailed in Table 1, the most common cause of hypothyroidism is represented by loss of functional thyroid cell mass (primary hypothyroidism), which in turn may be the consequence of an autoimmune attack (such as Hashimoto's thyroiditis), surgery, irradiation, drugs, and other more rare thyroid injuries. The distinctive features of primary hypothyroidism are represented by an increase in serum thyrotropin (TSH) concentration, which may stimulate thyroid growth and goiter formation. Primary hypothyroidism is a chronic condition which almost unavoidably leads to permanent thyroid failure, but transient hypothyroidism may be typically observed as a phase of subacute or painless thyroiditis (see ▶ Chap. 8, "Postpartum Thyroiditis and Silent Thyroiditis"). Less frequently hypothyroidism is the consequence of reduced TSH and/or thyrotropin-releasing hormone (TRH) production, as observed in several pituitary and hypothalamic diseases, leading to reduced stimulation of an otherwise normal thyroid gland (secondary or central hypothyroidism). Serum TSH concentration is low or even undetectable in most cases of central hypothyroidism, but may be normal or even slightly high, although the absolute levels appear to be inadequately low as compared to the low circulating thyroid hormone levels. For a complete discussion of central hypothyroidism, the reader is referred to ▶ Chap. 12, "Central Hypothyroidism".

Clinical signs and symptoms of hypothyroidism may rarely be observed in conditions associated with reduced thyroid hormone activity at the tissue level, even in the presence of normal or increased thyroid hormone production. These conditions include abnormal peripheral metabolism of thyroid hormone and target tissue resistance to thyroid hormones. Abnormalities of deiodinating thyroid hormone metabolism associated with decreased central TSH secretion with low circulating T3, normal,

Table 1 Classification of hypothyroidism

Primary hypothyroidism
Congenital
Agenesia, dyskinesia, ectopia
Dyshormonogenetic goiter
Iodide transport or utilization defect (NIS or pendrin mutations)
Iodotyrosine dehalogenase deficiency
Organification disorders (TPO deficiency or dysfunction)
Defects in thyroglobulin synthesis or processing
TSH receptor defects and other forms of idiopathic TSH unresponsiveness
Thyroidal Gs protein abnormalities (pseudohypoparathyroidism type 1a)
Acquired
Thyroiditis
Autoimmune
Hashimoto's thyroiditis (goitrous and atrophic forms)
Painless and postpartum thyroiditis (transient, may result in permanent thyroid failure)
Other thyroiditis
Subacute (transient, rarely result in permanent thyroid failure)
Riedel's thyroiditis
Thyroid infiltration
Amyloidosis
Hemochromatosis
Sarcoidosis
Cystinosis
Scleroderma
Iodine deficiency, goitrogens in foodstuffs, pollutants
Iodine excess
Iatrogenic
^{131}I
Surgery
External irradiation for nonthyroidal malignancy
Drugs blocking synthesis or release of thyroxine
Antithyroid drugs (methimazole, carbimazole, propylthiouracil)
Other drugs: lithium, ethionamide, sulfonamides, iodide
Tyrosine kinase inhibitors (TKIs): sunitinib, regorafenib, others
Cytokines (interferon-α, interleukin-2, others)
Consumptive hypothyroidism
Type 3 deiodinase (D3) expression in large tumors
Central hypothyroidism
Congenital
TSH deficiency or structural abnormality
TRH and TRH receptor defects
Acquired
Pituitary (secondary) or hypothalamic (tertiary) disorders
Bexarotene (retinoid X receptor agonist)
Dopamine or severe illness

or low T4 and normal/low TSH are typically observed in patients with nonthyroidal illnesses (NTI; see ▶ Chap. 24, "Non-thyroidal Illness"), but NTI is rarely associated with significant signs and symptoms of hypothyroidism (Boelen et al. 2011; Fliers et al. 2015). Other forms of recently identified defective thyroid hormone metabolism, potentially leading to hypothyroidism, are consumptive hypothyroidism and congenital defects of inactive T4 conversion into active T3 (Refetoff and Dumitrescu 2007). Consumptive hypothyroidism is caused by increased inactivation of T4 and T3 by type 3 iodothyronine deiodinase (D3). Generalized resistance to thyroid hormone (RTH), due to mutations in nuclear thyroid hormone receptor (TR) or cofactors (see ▶ Chap. 19, "Differentiated Thyroid Carcinoma of Follicular Origin"), is associated with increased circulating TSH and thyroid hormone concentrations (Refetoff et al. 1993) and no evident clinical hypothyroid features, although there is evidence of decreased thyroid hormone action in some tissues with higher expression of the mutant genes (Ortiga-Carvalho et al. 2014).

As detailed later in this chapter, hypothyroidism is a systemic condition affecting multiple organs, and the severity of the corresponding symptoms is related to the degree of thyroid hormone deficiency, independently from the etiology of the thyroid failure. The degree of hypothyroidism is variable from severe overt hypothyroidism to milder and subtle forms currently called subclinical hypothyroidism. Although several poorly defined clinical manifestations have been associated with subclinical hypothyroidism, this term should be employed only to describe the condition characterized by increased serum TSH concentration in the presence of normal serum FT3 and FT4 concentrations. Long-term severe hypothyroidism is also called "myxedema": this term derives from one of the hallmarks of long-standing thyroid failure, i.e., the nonpitting edema caused by accumulation of glycosaminoglycans in derma and other tissues. Myxedema is rarely observed today, due to very effective laboratory tools which allow early diagnosis and treatment of any form of hypothyroidism (Devdhar et al. 2007).

Epidemiology of Hypothyroidism

Prevalence of Hypothyroidism

Primary hypothyroidism accounts for about 99% of all forms of thyroid failure. As reviewed by Vanderpump (2011), the prevalence of previously undiagnosed hypothyroidism is highly variable in the general population, ranging from 0.6% to 12% in women and 1.3% to 4.0% in men, with the highest values observed in elderly subjects (Parle et al. 1991; Hollowell et al. 2002). Lower prevalence of hypothyroidism is reported in areas of mild to moderate iodine deficiency (Aghini-Lombardi et al. 1999; Knudsen et al. 1999). The prevalence of subclinical hypothyroidism (increased serum TSH with normal serum FT4 concentration (Biondi and Cooper 2008)) has been evaluated in several epidemiological surveys. In the first survey carried out in Wickham, UK (Tunbridge et al. 1977), subclinical thyroid failure was detected in 3% of men and 8% of women of all ages (10% in women over 55 years).

In the USA 9.4% of the population had primary thyroid failure, mostly (9.0%) subclinical (Colorado study (Canaris et al. 2000), while in the National Health and Nutrition Examination Survey (NHANES III), increased TSH was detected in 4–21% of women and in 3–16% of men (Hollowell et al. 2002). In the same survey, serum TSH progressively increased with age in both genders with higher values observed in Caucasians as compared to blacks. Similar findings have been reported by Kanaya et al. (2002) in another US cohort. Iodine intake may affect the prevalence of hypothyroidism, subclinical thyroid failure being more frequent in areas of high iodine intake (Vanderpump 2011). This phenomenon is possibly related to the effects of iodine on triggering/exacerbating thyroid autoimmunity (Laurberg et al. 2010).

The high prevalence of mild forms of hypothyroidism in elderly populations reported in the above, and several other studies (reviewed by Vanderpump (2011)) deserves further comment, since the precise interpretation of these epidemiological data in terms of thyroid pathology is still unclear. According to a further analysis of NHANES III data (Surks and Hollowell 2007), a substantial proportion of subjects aged >80 years had serum TSH concentrations above the upper limit (97.5 centile) of the reference range calculated for the entire population (4.5 mIU/L). However, only 30% of these elderly subjects with serum TSH >4.5 mIU/L had serum TSH above the 97.5 centile reference range calculated in the subjects of the same age (7.49 mIU/L). This, together with the observation that only 40% of >80-year-old subjects with increased serum TSH had positive serum antithyroid antibodies, suggests the need of defining different serum TSH reference ranges (Surks and Hollowell 2007) in the elderly subjects. Although there is a general consensus that mild TSH elevation in older individuals may not reflect thyroid dysfunction, but rather represent a consequence of normal aging, the use of different age-specific reference ranges for TSH is not yet advocated by current guidelines for diagnosis and treatment of hypothyroidism (Garber et al. 2012; Jonklaas et al. 2014). A further difficulty in interpreting epidemiology of hypothyroidism in the elderly is represented by the frequent spontaneous recovery of subclinical thyroid failure observed in about one third of old subjects in the subsequent follow-up (Vanderpump 2011).

Incidence of Hypothyroidism The Wickham cohort study, with its 20-year follow-up (Tunbridge et al. 1977; Vanderpump et al. 1995; Vanderpump 2011), represents the first study providing the incidence of overt spontaneous hypothyroidism, which was estimated to be between 0.6 new cases/1000/year in men and 3.5 new cases/1000/year in women. This study also identified risk factors for developing overt thyroid failure. These were a serum TSH concentration > 2.0 mIU/L and high serum levels of antithyroid peroxidase (at that time microsomal) antibodies (TPOAb). Other studies have provided similar values of standardized incidence rates of primary hypothyroidism, comprising between 3.90 and 4.89 new cases/1000/year in women and between 0.65 and 1.01 new cases/1000/year in men (Vanderpump 2011). Interestingly, from 1994 to 2001, the mean age at diagnosis of hypothyroidism significantly decreased in women (Vanderpump 2011).

Etiopathogenesis of Primary Hypothyroidism

Congenital and acquired conditions known to cause primary hypothyroidism are listed in Table 1: in all cases the two factors responsible for the thyroid failure are loss of functional thyroid tissue or defects in thyroid hormone biosynthesis and/or secretion.

Congenital primary hypothyroidism may be the consequence of several inborn defects involving key steps needed for development and function of the thyroid gland and associated with different clinical features. These conditions include thyroid agenesis or dysplasia, several variants of dyshormonogenetic goiters, TSH receptor defects or idiopathic TSH unresponsiveness, and thyroidal Gs protein abnormalities (such as pseudohypoparathyroidism type 1a). A detailed description of the genetic and epigenetic mechanisms leading to the various forms of congenital hypothyroidism is beyond the scope of this chapter and the reader is referred to Chap. 11 ▶ "Congenital Hypothyroidism" for a complete coverage of this field.

Several conditions are responsible for acquired primary hypothyroidism and these are briefly summarized in the following paragraphs.

Autoimmune Thyroiditis

Autoimmune (Hashimoto's) thyroiditis (HT) is the most frequent cause of acquired primary hypothyroidism in adults (Mariotti 2012a). HT fulfills all the classic and revised criteria proposed by Milgrom, Witebsky, and Rose (and Bona 1993) for organ-specific autoimmune diseases, such as the presence of cellular and humoral immune response to thyroid-specific antigens identified at the molecular level, the frequent association with other autoimmune diseases, and the availability of several experimental and spontaneous models of the disease. The precise cause of thyroid autoimmunity remains unknown, but it most probably results from the combination of a peculiar genetic background (Brix et al. 2000; Taylor et al. 2006) with several exogenous and endogenous environmental factors leading to the loss of immunological tolerance (Ajjan and Weetman 2015; Effraimidis and Wiersinga 2014). Current understanding of thyroid autoimmune mechanisms and the clinical spectrum of HT are extensively reviewed elsewhere in this book (see ▶ Chaps. 7, "Hashimoto's Thyroiditis" and ▶ 8, "Postpartum Thyroiditis and Silent Thyroiditis"). In this paragraph attention will be focused only on the cellular and humoral immune mechanism leading to thyroid damage and hypothyroidism (See Fig. 1). The major cause of autoimmune destruction of thyroid follicular cells is represented by cell-mediated cytotoxicity exerted by thyroid antigen-specific perforin-containing cytotoxic CD8+ T cells, present within the lymphocytic infiltrate in Hashimoto's thyroiditis. Interaction between Fas (CD95) and Fas ligand (FasL) abnormally expressed on the cell surface provides an additional pathway for thyroid follicular cell destruction. The autoimmune thyroid infiltrate also contains increased number of the pro-inflammatory subsets of T-helper (TH) cells (TH1 and TH17) that locally produce several cytokines (mostly IL-2, IFN-γ, and TNF-α), which concur in thyroid destruction and self-perpetuation of the autoimmune reaction. In particular, cytokines stimulate the expression of HLA, co-stimulatory molecules, and chemokines

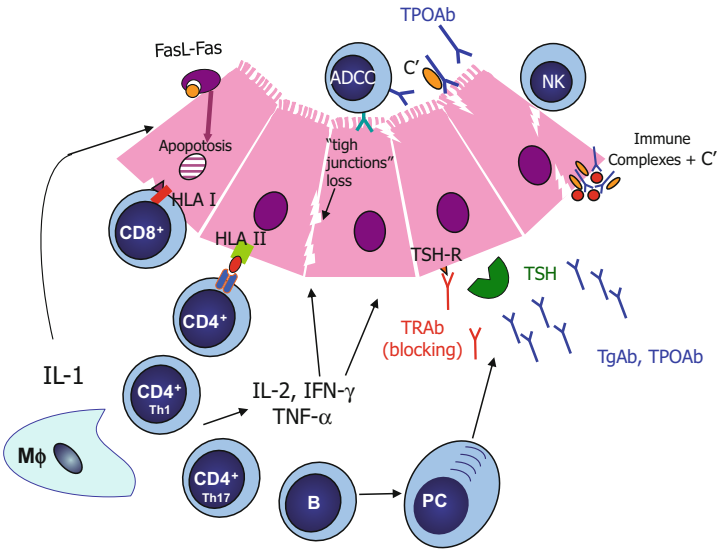

Fig. 1 Cellular and humoral immune mechanisms leading to thyroid damage in autoimmune hypothyroidism. *ACDD* Antibody-dependent cellular cytotoxicity, *AbTPO* anti-thyroid peroxidase antibody, *TgAb* anti-thyroglobulin antibody, *TRAb* TSH-receptor antibody, *C′* complement

on the surface of thyroid cells, able to increase T-cell cytotoxic potential. Other cellular and humoral immune mechanisms potentially involved in thyroid destruction/hypothyroidism are antibody-dependent cell-mediated cytotoxicity (ADCC), natural killer (NK) cells, complement (C′)-fixing autoantibodies, and blocking type TSH receptor antibodies (TRBAb). With the exception of TRBAb, which has ability to induce transient neonatal hypothyroidism, if transferred across placenta, the actual in vivo relevance of other thyroid antibodies has never been proven, since placental transfer of anti-Tg e and anti-TPO antibodies is devoid of any effect on fetal thyroid function.

Thyroid autoimmunity is also involved in the transient hypothyroidism observed in painless postpartum and painless sporadic thyroiditis (see ▶ Chap. 8, "Postpartum Thyroiditis and Silent Thyroiditis"), following the initial destructive thyrotoxic phase due to acute exacerbation of autoimmune effector mechanisms (Amino et al. 1999). Although initially the hypothyroidism appears self-limiting, up to 60% of postpartum and 20% of sporadic painless thyroiditis patients with time develop permanent hypothyroidism (Stagnaro-Green et al. 2011; Mariotti 2012b).

Other Non-Autoimmune Thyroiditis

With the exception of Riedel's thyroiditis (Hennessey 2011), where thyroid failure is a frequent consequence of extensive fibrosis, other forms of non-autoimmune thyroiditis are rarely responsible of permanent hypothyroidism (5–10% in subacute De Quervain's thyroiditis and only exceptionally in acute bacterial thyroiditis) (Lio 2012).

Thyroid Infiltration

Thyroid infiltration in the course of amyloidosis, hemochromatosis, sarcoidosis, cystinosis, scleroderma, and leukemia may rarely produce hypothyroidism and goiter (Singer 2013). Sarcoid infiltration of the thyroid is also associated with thyroid autoimmunity, and HT may concur with granulomatous infiltration to thyroid insufficiency (Nakamura et al. 1997)

Iodine Intake

Hypothyroidism may result from both severe iodine deficiency and excess.

Patients living in iodine-deficient areas develop goiter which is generally associated with euthyroidism due to adaptive mechanisms including increased T3 and decreased T4 thyroidal synthesis and increased activity of thyroidal deiodinases D1 and D2 (Gereben et al. 2008). However, in severe iodine deficiency (<25 mcg/day), large goiters and variable degrees of hypothyroidism ranging from subclinical to overt thyroid failure may be observed at any age (Zimmermann and Boelaert 2015).

Dietary substances interfering with thyroid hormone synthesis (naturally occurring goitrogens and polluted water) (Pearce and Braverman 2009) may concur with iodine deficiency to exacerbate hypothyroidism. Natural goitrogens are present in many vegetables; cruciferous vegetables (cabbage, cauliflower, broccoli, turnip, and others) contain glucosinolates whose metabolites compete with iodine for thyroidal uptake. Other vegetables (cassava, lima beans, sweet potatoes, and others) contain cyanogenic glucosides, which are metabolized to thiocyanates competing with iodine for thyroidal uptake (Zimmermann 2009). Flavonoids contained in soy and millet may impair thyroid peroxidase (TPO) activity and could affect thyroid function in infants, while in healthy adults, soy-based products appear to have negligible effects on thyroid function (Zimmermann 2009). Unclean drinking water containing humic substances and/or industrial pollutants (resorcinol, phthalic acid, perchlorate) may also impair thyroid hormone iodination, but most of these goitrogenic substances are not able to induce hypothyroidism in conditions of coexisting iodine deficiency (Zimmermann 2009).

Iodine excess (intake >1–2 mg/day) acutely inhibits iodine organification and thyroid hormone synthesis (Wolff–Chaikoff effect). Exposure to iodine excess does not cause hypothyroidism in most euthyroid subjects, due to an adaptive mechanism (escape) of the normal thyroid gland (Leung and Braverman 2014). However, patients with preexisting thyroid abnormalities (autoimmune thyroiditis; previous episode of painless, postpartum, or subacute thyroiditis or other forms of destructive thyrotoxicosis; previous partial thyroidectomy or radioiodine treatment or concomitant use of goitrogens) may fail to escape the Wolff–Chaikoff effect and develop hypothyroidism (iodine-induced hypothyroidism) (Leung and Braverman 2014). High iodine concentration is also able to reduce the release of thyroid hormone from the thyroid (Leung and Braverman 2014).

Many substances may be responsible for excess iodine intake: these include drugs (presently almost exclusively represented by amiodarone, an iodine-rich molecule employed in the treatment of ventricular and supraventricular tachyarrhythmias), kelp

tablets, dietary supplements, topical iodinated antiseptic solutions (e.g., Betadine), and iodinated radiologic contrast agents. Among these substances, amiodarone is presently the most common source of drug-induced thyroid dysfunction. The use of this drug is associated with a high frequency of both hypo- and hyperthyroidism, hypothyroidism being more frequently observed in subjects living in iodine-sufficient areas (Martino et al. 2001).

Iatrogenic Hypothyroidism

Hypothyroidism may be frequently observed during treatment of hyperthyroid conditions with antithyroid drugs (methimazole, carbimazole, and propylthiouracil), and this effect is easily avoided by tapering the dose or withdrawing the drug.

Administration of ^{131}I for treating hyperthyroidism is often followed by hypothyroidism, but the incidence of thyroid failure is different according to the dose administered, the etiology of hyperthyroidism, and the follow-up time. In particular, hypothyroidism develops much more frequently in patients with Graves' disease (24% after 1 year and up to 90% after 25 years) than in those with toxic multinodular goiter (4% after 1 year and 24–60% after 25 years) (Metso et al. 2004; Ross 2011; Bonnema and Hegedüs 2012).

Total thyroidectomy is followed by severe permanent hypothyroidism, while the risk of hypothyroidism after hemithyroidectomy is variable. According to a recent meta-analysis (Verloop et al. 2012), the overall risk of hypothyroidism after hemithyroidectomy was 22%, mostly represented by subclinical hypothyroidism, while overt thyroid failure was found in about one in 25 operated patients. Interestingly, positive serum TPOAb is highly predictive of hypothyroidism after surgery. The same has also been reported for radioiodine treatment (Mariotti et al. 1986).

Primary hypothyroidism (both overt and subclinical) is a frequent complication of external irradiation of the head and neck region for lymphomas and other neck or central nervous system malignancies exceeding 25–30 Gy (Hancock et al. 1995). The incidence of radiation-induced hypothyroidism depends on multiple factors, but it is generally reported between 20% and 30%, half of the events appearing within the first 5 years after treatment, with a peak after 2–3 years (Jereczek-Fossa et al. 2004). Significant risk factors for hypothyroidism are female gender, surgery involving the thyroid gland, other neck surgeries, and Caucasian ethnicity (Vogelius et al. 2011). There is a radiation dose–response relation with a 50% risk of HT at a dose of 45 Gy but with considerable variation between studies (Vogelius et al. 2011). More recently, a model to predict radiation-induced hypothyroidism has been developed and validated for patients with squamous cell carcinomas of the head and neck (Rønjom et al. 2013; Rønjom et al. 2015). Significant prediction factors were thyroid volume, mean radiation dose to the thyroid, and time elapsed after radiation. In this model precise estimation of thyroid volume was particularly relevant in planning optimized treatment (Rønjom et al. 2015).

Several other drugs used for treatment of disorders unrelated to the thyroid gland may cause primary hypothyroidism and/or goiter. For a detailed description of the complex interference of drugs on thyroid function, see ▶ Chap. 25, "Drugs and Other Substances Interfering with Thyroid Function". Lithium, when employed for bipolar manic-

depressive psychosis, is associated with goiter in 40% and hypothyroidism in 20% of cases, by blocking both release and synthesis of thyroid hormones by mechanisms still poorly understood, which may include a promoting effect on thyroid autoimmunity in predisposed individuals (Lazarus 2009). Goiter and hypothyroidism have been occasionally observed with the use of para-aminosalicylic acid, phenylbutazone, aminoglutethimide, and ethionamide which interfere with iodine organification and thyroid hormone synthesis, as well as after drugs inducing the microsomal enzymes in the liver such as rifampicin and carbamazepine (Curran and DeGroot 1991). Excess iodine and iodine-containing compounds may induce hypothyroidism, as described above.

Administration of cytokines (e.g., interferons and interleukins) may precipitate hypothyroidism, in general by exacerbation of preexisting autoimmune thyroiditis and by a direct cytotoxic effect on thyroid follicular cells (Tomer and Menconi 2009). In these patients, hypothyroidism is often heralded by a transient phase of destructive thyrotoxicosis (Menconi et al. 2011). The administration of the lymphocyte depleting anti-DC52 monoclonal antibody (alemtuzumab, formerly called Campath H1) in multiple sclerosis is also often followed by development of thyroid autoimmune dysfunction, but this is almost exclusively represented by TSH receptor antibody (TRAb)-mediated hyperthyroidism indistinguishable from Graves' disease (Coles et al. 1999; Daniels et al. 2014).

A continuously growing number of novel anticancer drugs, including tyrosine kinase inhibitors (TKIs) and monoclonal antibodies, have recently been shown to induce severe endocrine disruption involving the thyroid, the pituitary, and other endocrine glands (Torino et al. 2013). Anti-CTLA4 monoclonal antibodies (ipilimumab), currently employed in patients with metastatic melanoma, may be occasionally associated with autoimmune thyroiditis but more often induce central hypothyroidism resulting from lymphocytic hypophysitis (Iwama et al. 2014). Among TKIs, severe thyroid toxicity with overt primary hypothyroidism and thyroid atrophy is observed in cancer patients treated with sunitinib and other TKIs targeting key kinase receptors in angiogenic pathways, but not other kinase receptors such epidermal growth factor receptor family or c-KIT (Torino et al. 2013). Transient destructive thyrotoxicosis, mostly subclinical, may be observed in up to 40% of patients developing sunitinib-induced hypothyroidism (Torino et al. 2013). The mechanism(s) involved in the thyroid damage is(are) still not fully understood, but de novo triggering of thyroid autoimmunity may be an important cofactor in sunitinib-induced hypothyroidism (Pani et al. 2015).

Consumptive Hypothyroidism

A paraneoplastic syndrome called "consumptive hypothyroidism" has recently been identified in newborns with very rare vascular tumors in the liver (hemangioendotheliomas) (Luongo et al. 2013). This syndrome results from the aberrant uncontrolled expression of type 3 deiodinase (D3) that is responsible for a severe form of hypothyroidism by inactivation of thyroid hormones T4 and T3 in tumor tissue, in spite of a normally functioning thyroid gland. This rare form of hypothyroidism affects patients in the first years of life, and has distinct features in terms of diagnosis, treatment, and prognosis, with respect to other forms of hypothyroidism. Interestingly, there is circumstantial evidence that D3 activation

may contribute to the development of some cases of TKI-induced thyroid failure (Kappers et al. 2011)

Clinical Picture of (Overt) Hypothyroidism

Described by Gull in 1874 (Fig. 2), the full expression of acquired hypothyroidism appeared to be similar to myxedematous endemic cretinism, except for the time of onset and the absence of permanent mental retardation. The condition was also known as cachexia strumipriva, because it was mainly observed after surgical removal of the thyroid gland. Myxedema is typically characterized by skin changes, increased body weight, loss of hair and teeth, and neurological symptoms such as variable degrees of impairment of speech, movement, and intellect.

Nowadays, myxedema is rarely observed, and the clinical expression of thyroid hormone deficiency usually varies between individuals depending on age, cause, duration, and severity of the hypothyroid state. The common physiopathologic feature involves a slowing of physical and mental activity and the impairment of many organ functions.

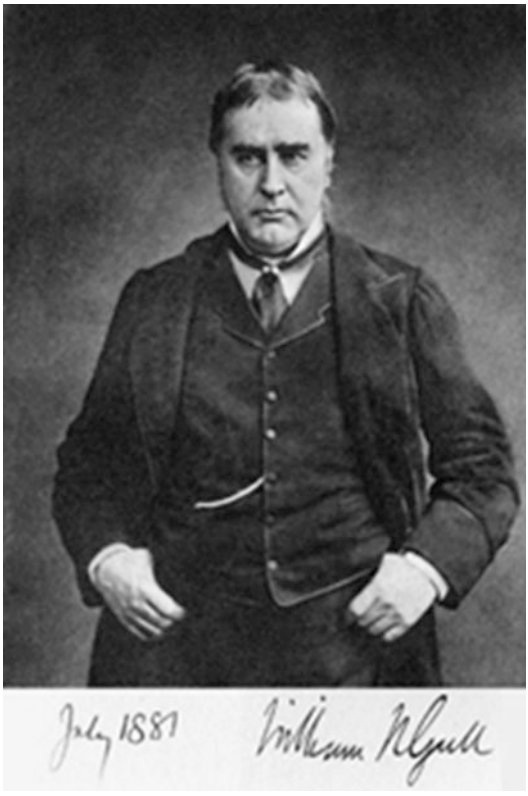

Fig. 2 Gull WW, author of the description of a cretinoid state supervening in adult life in women in 1874

Metabolic Changes

Energy Balance

Thyroid hormones are involved in the regulation of energy balance in mammals, acting not only in the periphery but also in brain areas involved in the regulation of food intake and energy expenditure (Herwig et al. 2014). Triiodothyronine (T3) is responsible for about 30% of the resting energy expenditure (REE) due to its significant role in temperature homeostasis. This role is mediated by thyroid hormone receptor alpha (TRa), while TR beta is a key regulator of cholesterol metabolism (Santini et al. 2014). In hypothyroid rats, mRNA expression for the thyroid hormone transporter MCT8 was found to be influenced by T3, and the expression of agouti-related protein (Agrp), a key component in the hypothalamic energy balance homeostatic mechanism, can also be regulated by T3 (Herwig et al. 2014).

In patients with complete lack of thyroid hormones, due to athyreosis, REE falls between 35 and 45 percent below normal, leading to a slight net gain in energy stores. As a consequence, body weight increases (on average by 10%) due to an increase in body fat and a retention of water and salt (Fig. 3). The effect of hypothyroidism on appetite regulation is still debated, but in adult rats, methimazole-induced hypothyroidism impairs hypothalamic leptin signaling and the anorectic response to the peripheral administration of leptin. These findings suggest that thyroid hormones are essential for the effect of leptin on food intake. An increase of adipose tissue mass results in an increase in serum leptin, which mediates a decrease in energy intake while energy disposal increases, eventually leading to a reduction in adipose tissue mass. A large number of studies have investigated the relationship between thyroid dysfunction and circulating levels of leptin, but the results have been conflicting. As a matter of fact, the effect of hypothyroidism on

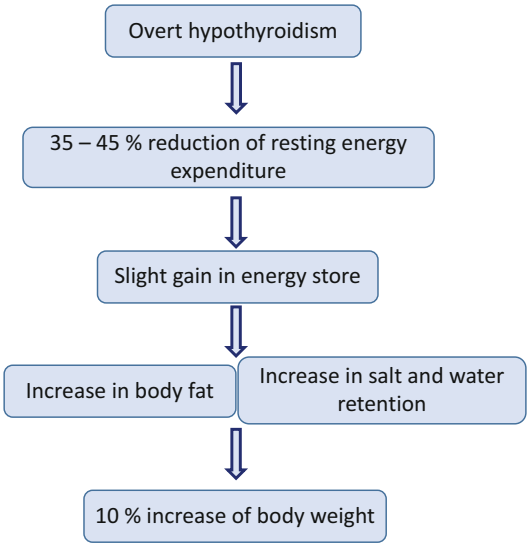

Fig. 3 Effects of overt hypothyroidism on body weight

energy balance might be largely independent of leptin. Indeed, in animal models, hypothyroidism induces a negative energy balance by reducing food intake due to the modulation of hypothalamic mediators in the arcuate nucleus, namely, an increase of proopiomelanocortin (POMC) and a decrease of neuropeptide Y (NPY) with consequent predominance of the anorexigenic pathway (Calvino et al. 2016).

Protein Metabolism

Hypothyroidism is a condition of positive nitrogen balance, despite a reduction of both synthesis and degradation of protein. In contrast, treatment of myxedema is accompanied by a marked but temporary negative nitrogen balance, due to mobilization of extracellular protein.

Carbohydrate Metabolism

Low thyroid hormone levels impair the ability of insulin-mediated translocation of glucose by the glucose transporter 4 (GLUT-4), thus reducing intestinal glucose absorption and promoting insulin resistance. Hypothyroidism is also associated with a reduced hepatic glucose production. The reduction of hepatic glucose production and its intestinal absorption are balanced by a slower glucose metabolism and by a diminished utilization (Guastamacchia et al. 2015). Indeed, in hypothyroid patients, the intravenously glucose tolerance test is characterized by a prolonged glucose disappearance rate, despite a normal peak value in magnitude and in time of occurrence. The insulin response is blunted and slightly delayed. The occurrence of hypoglycemia in hypothyroid patients should alert the physician to concomitant diseases (e.g., hypopituitarism or Addison's disease). The development of hypothyroidism in patients with insulin-dependent diabetes mellitus may require lowering of the insulin dose to counteract the decreased rate of insulin degradation. Similarly, in diabetic patients treated with glucose-lowering drugs, the development of hypothyroidism may increase the risk of hypoglycemia due to reduced endogenous production of glucose.

Lipid Metabolism

Thyroid hormones have multiple effects on the regulation of lipid synthesis, absorption, and metabolism. Therefore, overt and subclinical hypothyroidism significantly affects the lipid profile and promotes cardiovascular disease. A recent study evaluating lipid profile and TSH levels in a large Spanish population including euthyroid and hypothyroid individuals identified a serum TSH level cutoff of 2.57 mU/l for detecting significant differences in circulating lipid levels (Santos-Palacios et al. 2013).

The main changes in lipid profile (Table 2) are characterized by increased total and low-density lipoprotein (LDL) cholesterol levels with increased or normal high-

Table 2 Changes in lipid profile in overt hypothyroidism

↑	Total and LDL cholesterol
↑ =	HDL cholesterol
↑	HDL2 subfraction
↑ =	Triglycerides

density lipoprotein (HDL) levels. Usually, the HDL2 subfraction, and not the HDL3, is elevated. Triglyceride levels are normal or slightly elevated due to a decreased lipoprotein lipase activity, although the levels of lipoprotein (a) may be increased (Duntas and Brenta 2012). Hypothyroidism-related lipid abnormalities are secondary to a decreased biosynthesis and degradation of lipoproteins, resulting in the reduction of catabolic pathways. The reduction in the number of LDL receptors causes an excess of circulating LDL particles, which are also susceptible to increased oxidation. A reduced activity of the enzymes lipoprotein lipase and hepatic lipase and of the cholesteryl ester transfer protein (CETP) is found in hypothyroidism. Treatment of overt hypothyroidism with LT4 reverses lipid abnormalities.

Skin and Mucosae

The skin manifestations of overt, long-standing hypothyroidism have been described since the 1800s. Myxedema, the original name for hypothyroidism, referred to the edema-like skin condition caused by increased glycosaminoglycan and protein deposition in the interstitial spaces of the skin. The clinical picture is a nonpitting swelling, most marked around the eyes and hands (Safer 2012). The skin in hypothyroidism is rough, dry, and cool and covered with fine scales, particularly on the extensor extremities (35–37). These changes are frequent (80% of hypothyroid patients) and are related to a decreased cutaneous metabolism, reduced secretion of sweat and sebaceous glands, vasoconstriction, thinning of the epidermis, and hyperkeratosis of the stratum corneum. The skin is usually pale because of the excess fluid and mucopolysaccharide content in the dermis, vasoconstriction, and anemia. Increased dermal carotene may contribute to yellow hue on the palms, soles, and nasolabial folds. The palpebral fissure may be narrowed due to diminished tone of the sympathetic nervous fibers to Müller's levator palpebra superior muscle. Loss of the lateral third of the eyebrow may be observed (Queen Anne's sign) (Fig. 4). The hair is dry, coarse, and slow growing. A similar picture may be observed for nails which present as thickened, brittle, and slow growing. Candida folliculitis may sometimes affect hypothyroid patients.

The enlarged tongue may result in a husky, low-pitched, and coarse voice, as well as in a low and deliberate speech. Nowadays, this picture is very rare and the vast majority of patients have very vague symptoms and subclinical disease.

Nervous System

The central nervous system is an important target of thyroid hormones as also shown in animal models where deprivation of thyroid hormone during critical periods of brain development affects processes such as neuronal migration, outgrowth and differentiation, synaptogenesis, myelination, and glial cell proliferation. In humans, fetal and neonatal hypothyroidism leads to severe intellectual deficits, abnormal balance, impaired fine motor skills, spasticity, and deafness according to the timing

Fig. 4 Anne of Denmark (1612) portraiture showing the thinning or loss of the outer third of the eyebrows

Table 3 Neurologic, cognitive, and psychiatric effects of overt hypothyroidism

Neurological symptoms and signs
Somnolence, lethargy
Slow speech
Delayed relaxation of deep tendon reflexes
Ataxia
Sensory–motor polyneuropathy
EEG changes
Dizziness, vertigo, and tinnitus
Deafness
Mood and cognitive changes
Anxiety and depression
Memory deficits
Calculation difficulties
Myxedema madness

of the onset of thyroid hormone action in the developing brain (Heuer 2007). The onset of hypothyroidism in adult life causes less severe neurological manifestations that usually regress after substitution treatment (Table 3).

Early studies found that regional cerebral blood flow and measures of regional brain metabolic activity, such as cerebral glucose metabolism, were decreased in

hypothyroidism, thus suggesting a decreased brain activity and/or a reduction of central blood flow as a consequence of increased vascular resistance. More recently, ^{32}P nuclear magnetic resonance spectroscopy of the frontal lobe of the adult hypothyroid patients reported reversible alterations in phosphate metabolism, suggesting impairment of mitochondrial metabolism (Constant et al. 2001). Positron emission tomography (PET) studies have reported decreased glucose metabolism in hypothyroid patients in many regions of the brain including the hippocampus. Voxel-based morphometry (VBM) studies have demonstrated gray and white matter volume reduction in different brain regions (Singh et al. 2016). Furthermore, a significant decrease of glutamate and myoinositol levels in the hippocampus of hypothyroid patients, persisting after the restoring of euthyroidism, has been demonstrated by in vivo proton magnetic resonance spectroscopy. Collectively, these studies indicate that even in adults, the thyroid hormones influence metabolism and structure and function of the brain.

Neurological Signs and Symptoms

In long-standing and severe hypothyroidism, ataxia, intention tremor, nystagmus, and dysdiadochokinesis have been described, possibly caused by deposition of mucinous material in the cerebellar tissue. A delayed relaxation phase of the deep tendon reflexes is more frequently observed. These signs promptly revert after thyroid hormone replacement therapy. The absence of alpha waves and the presence of low-amplitude theta and delta waves may sometimes be present on EEG.

In the past, neurologic complaints were reported by up to 80% of patients with hypothyroidism. Most of these patients, having a severe long-standing hypothyroidism, complained of major neurological symptoms, such as burning and lancinating extremity pain due to a sensory–motor polyneuropathy with a distal–proximal progression, first involving the lower limbs and then the upper ones. These major neurologic complaints are nowadays rarely observed, probably due to earlier diagnosis and adequate correction of hypothyroidism with levothyroxine (LT4). A metachromatic infiltrate was also described in the lateral femoral cutaneous and sural nerves, together with axon cylinder degeneration. More recently, a reduction in the intraepidermal nerve fiber density, associated with a small-fiber sensory neuropathy, was observed (Magri et al. 2010).

Vestibular abnormalities and both nerve and conduction deafness are reported. More frequent symptoms are dizziness, vertigo, and tinnitus, suggesting damage of the eighth cranial nerve, the labyrinth, or possibly the cerebellum. Thyroid hormone replacement therapy significantly improves hearing defects associated with hypothyroidism, unlike with the sensorineural deafness of Pendred's syndrome. A deficiency in the pigment retinene may be responsible for rare cases of night blindness.

Hashimoto encephalopathy is a steroid-responsive encephalopathy, associated with elevated TPOAbs without brain tumor, stroke, or infection of the central nervous system. The clinical presentation includes behavioral changes, confusion, cognitive decline, stroke-like episodes, amnestic syndrome, ataxia, seizures, and myoclonus. The condition is thought to be of autoimmune rather than metabolic origin (Zhou et al. 2017).

Mental Symptoms

In adults with hypothyroidism, memory, and in particular the verbal component, is impaired. Changes in overall intelligence, attention, concentration, perceptual function, language, and psychomotor function are reported. A prospective, open-label interventional study found a specific deficit in memory rather than a general cognitive slowing in overtly hypothyroid patients (Correia et al. 2009). Usually, these mental symptoms are reverted by treatment with LT4, although not completely in all cases. Functional imaging studies have demonstrated decrease in cerebral blood flow and function, both globally and in regions that mediate attention, visuospatial processing, working memory, and motor speed (Correia et al. 2009; Nagamachi et al. 2004).

Psychiatric Symptoms

Slowing of thought and speech is frequently found in hypothyroid patients, together with decreased attentiveness and apathy. Agitation and psychosis, the so-called myxedema madness, is nowadays rare if ever observed. These symptoms usually revert with thyroid hormone replacement therapy.

The most frequent psychiatric condition associated with hypothyroidism is depression, probably due to a reduced activity of 5-hydroxytryptamine in the brain. A recent Danish observational cohort study demonstrated an increased risk of being diagnosed with a psychiatric disorder and an increased risk of being treated with antipsychotics, both before and after the diagnosis of hypothyroidism (Thvilum et al. 2014). As a matter of fact, administration of high-dose T4 or T3 might potentiate the effect of tricyclic antidepressant drugs.

Cardiovascular System

The actions of thyroid hormones (particularly T3) on the heart are mediated by genomic and non-genomic mechanisms targeted to membrane proteins, cytoskeletal components, and organelles. The genomic actions are mediated by nuclear receptors (TR) alpha and beta, the former being predominantly represented in the heart (Table 4). T3-activated TR regulates myosin heavy chain (MHC) genes (encoding for the two contractile protein isoforms of the thick filament of the cardiomyocyte). T3 regulates the expression of the sarcoplasmic reticulum Ca2þ (SERCa2þ) whose function is to sequester calcium in the sarcoplasmic reticulum during the relaxation phase of myocyte contraction. T3 also inhibits the expression and the degree of phosphorylation of the membrane protein phospholamban (PLB), a key regulator of SERCa2beta. In hypothyroidism, changes of SERCa2beta and PLB are responsible for an impaired diastolic function. Other T3-responsive cardiac genes are the ones encoding for Na1/K1-ATPase, beta1-adrenergic receptor, atrial natriuretic hormone, voltage-gated potassium channels (positively regulated) and adenylyl cyclase catalytic subunits, thyroid hormone receptor alpha-1, Na/Ca exchanger, thyroid hormone transporters (MCT8), and adenine nucleotide translocase-1 (ANT1 (negatively regulated)) (Danzi and Klein 2014).

Table 4 Thyroid hormone genomic actions on the heart

Positive effects on expression	Negative effects on expression
Sarcoplasmic reticulum Ca2þ	Phospholamban
Alpha myosin heavy chain	Beta myosin heavy chain
Beta1-adrenergic receptor	Thyroid hormone receptor alpha-1
Na1/K1-ATPase	Na1/Ca21 exchanger
Atrial natriuretic hormone	Adenylyl cyclase catalytic subunits
Voltage-gated potassium channels	Thyroid hormone transporters (MCT8)

Table 5 Cardiovascular and electrocardiographic features of hypothyroidism

Cardiovascular features	Electrocardiographic features
Reduction of	Low-voltage complexes
Pulse rate	
Stroke volume	
Cardiac output	
Cardiac preload	
Increase of	Flat or negative T waves
Systemic vascular resistance	
Systolic and diastolic dysfunction	Sinus bradycardia
Cardiomegaly	Prolonged QTc (possible cause of cause torsade de pointes ventricular tachycardia)

The original description of the myxedema heart, as reported by Zondek in 1918, included an enlarged cardiac silhouette associated with diminished heart sounds, caused by pericardial effusion. Interstitial edema and swelling of muscle fibers with loss of striations were reported as the main histologic features of the myxedema heart. Although this typical picture is nowadays rarely observed, cardiovascular changes are frequently reported in hypothyroid patients. Systemic manifestations typically include a reduction of pulse rate, of stroke volume, and, as a consequence, of cardiac output. Other commonly observed cardiovascular features of hypothyroidism are increased systemic vascular resistance, systolic and diastolic dysfunction, decreased cardiac preload, and cardiomegaly (Table 5). As a consequence, hypothyroid patients carry an excess risk of being diagnosed with cardiovascular diseases before the diagnosis of hypothyroidism, excess risk persisting also thereafter (Thvilum et al. 2013). Characteristic electrocardiographic features of overt hypothyroidism are low-voltage complexes, flat or negative T waves, sinus bradycardia, and prolonged QTc, which might cause torsade de pointes ventricular tachycardia. ECG abnormalities, which are related to histologic changes of the myocardium and to the presence pericardial effusion, can resolve upon thyroid hormone replacement therapy. Bradycardia, due to sinus node dysfunction, may be responsible for failure of the heart rate to accelerate in stress conditions, such as

fever, infection, or cardiovascular failure. The sinus dysfunction is usually proportional to the decrease in the body's metabolic rate. A prolonged circulation time is frequently observed in overt hypothyroidism.

In most tissues the decrease in blood flow results in a proportional reduction in oxygen consumption. As a consequence, the arteriovenous oxygen difference remains normal or slightly increased.

Venous pressure is normal while peripheral arterial resistance is increased. Indeed, the mean arterial pressure rises, and 20% of hypothyroid patients have diastolic hypertension. Endothelial dysfunction contributes to hypertension due to its effects on atherosclerosis and increased arterial stiffness. In nearly 50% of patients with hypothyroidism and hypertension, a severe impairment of the elastic properties of the aorta may be responsible for the incomplete normalization of blood pressure after replacement therapy. Congestive heart failure may occur in severe or untreated hypothyroidism particularly when underlying heart disease is present. In this regard it is worth noting that conventional cardiac drugs, such as glycosides or digoxin, might be less effective or poorly tolerated in hypothyroid patients.

Autopsy studies first reported an increased risk of atherosclerosis in hypothyroid patients. Pathogenic links include increased arterial stiffness, systemic vascular resistance, and hypothyroidism- induced dyslipidemia, which is characterized by high levels of LDL cholesterol and of the atherogenic LDL variant and apolipoprotein B. Other independent risk factors for coronary atherosclerosis such as hyperhomocysteinemia, high levels of C-reactive protein, and coagulation abnormalities may be observed in hypothyroid patients. Angina pectoris may occur before treatment of hypothyroidism, usually reflecting a poor myocardial oxygenation. More frequently, angina pectoris worsens or appears for the first time after starting thyroid hormone replacement therapy, suggesting a coronary flow impairment.

Gastrointestinal Changes

Gastrointestinal manifestations of hypothyroidism are not rare and involve different digestive organs (Table 6). Modest weight gain, mainly due to fluid accumulation, in spite of reduced appetite, can occur in hypothyroidism.

Oropharyngeal dysphagia, esophagitis, and hiatus hernia are described. Both the mean esophageal transit time and the gastric emptying time are reported as markedly increased in hypothyroid patients, when assessed by endoscopy and scintigraphy (Yaylali et al. 2009). This delay is mainly in the emptying phase. The prolonged gastric emptying time tends to resolve with hypothyroidism treatment. The reduction of peristalsis is the main cause of constipation, which remains the most frequent gastrointestinal complaint. An impairment of the Cl−/HCO3- anion exchange also contributes to the reduced intestinal motility. However, the syndrome of ileus, megacolon, or colonic pseudo-obstruction is a rare event, usually associated with severe hypothyroidism or myxedema coma. More than one-half of hypothyroid patients show a small intestinal bacterial overgrowth frequently persisting after the

Table 6 Gastrointestinal manifestations of hypothyroidism

Weight gain (modest)	Increased liver function tests
Oropharyngeal dysphagia, esophagitis, and hiatus hernia	Increased esophageal transit time and gastric emptying time
Constipation	Increased risk for nonalcoholic fatty liver disease
Small intestinal bacterial overgrowth	Increased prevalence of[a]: Pernicious anemia Celiac disease Inflammatory bowel disease Primary biliary cirrhosis

[a]When hypothyroidism is dependent on an autoimmune thyroid disease

establishment of euthyroidism. In these cases, antibiotic therapy may be necessary (Ebert 2010).

Changes in liver function tests are present in about half of patients with hypothyroidism, despite normal hepatic histology. Hypothyroidism increases the risk of gallstones due to a reduced gallbladder motility and bilirubin excretion and increased serum cholesterol. Nonalcoholic fatty liver disease (NAFLD) is also frequently observed in hypothyroidism. In a case–control study, hypothyroidism was found to be significantly associated to an increased risk of hepatocellular carcinoma, even after adjustment for other known risk factors (Hassan et al. 2009).

When hypothyroidism is due to autoimmunity, autoimmune disorders of the digestive system, such as pernicious anemia, celiac disease, inflammatory bowel disease, and primary biliary cirrhosis, may be present. The coexistence of pernicious anemia causes vitamin B12 deficiency and bone marrow megaloblastosis. The prevalence of celiac disease in hypothyroid patients ranges between 3% and 5%, while the prevalence in the general populations is about 1%. In contrast, autoimmune thyroid diseases may be present in 6–20% of individuals with celiac disease. In fact, celiac disease and other conditions with malabsorption are the commonest cause of treatment refractory hypothyroidism.

Respiratory System

Patients with overt, long-standing hypothyroidism may complain about shortness of breath, sputum production, cough, wheezing, and airway hyperresponsiveness. Pleural effusion was described as a cause of respiratory symptoms, but depression of the respiratory center in the brain, neuropathy, and myopathy changes due to hypothyroidism may also have a pathogenic role (Fig. 5). However, these data must be interpreted with caution because a recent systematic review on the respiratory manifestations of hypothyroidism found substantial biases in all studies addressing the impact of hypothyroidism on respiratory symptoms and ventilation. The curative effect of LT4 substitution on ventilation is also controversial, because the two studies

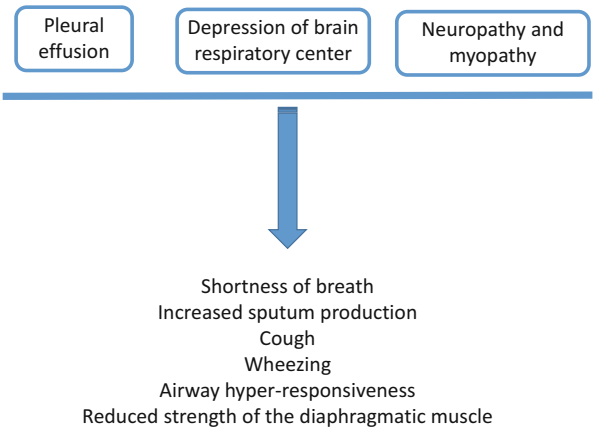

Fig. 5 Respiratory changes during overt hypothyroidism

included in the review gave opposing results (Sorensen et al. 2016). The strength of the diaphragmatic muscle is reduced in hypothyroid patients. The few available studies report an improvement by either LT4 or LT3 treatment. The forced vital capacity (FVC) and the forced expiratory volume in 1 second (FEV1) may be reduced in overt hypothyroidism.

Nocturnal breathing abnormalities are frequently reported in overt hypothyroid patients. In particular obstructive sleep apnea syndrome (OSAS) may be found in up to 30% of affected patients (Jha et al. 2006). Several factors may predispose hypothyroid patients to the development of OSAS, such as the increased size of the tongue and of pharyngeal skeletal muscles, a slow and sustained pharyngeal muscle contraction pattern, and diminished neural output by the respiratory center. After LT4 replacement therapy, apnea periods, oxygen desaturation events, and snoring usually improve (Sorensen et al. 2016).

Musculoskeletal System

Muscles

Thyroid hormone signaling is required for skeletal muscle development, contractile function, and muscle regeneration (Salvatore et al. 2014). Thus, hypothyroid patients frequently report pain, cramps, stiffness, easy fatigability, and weakness, symptoms which are aggravated by exposure to cold. Physical examination shows muscle hypertrophy, proximal muscle weakness, and delayed relaxation phase of deep tendon reflexes (Table 7). Hoffman's syndrome is a rare form of hypothyroid myopathy first described in 1897 and characterized by proximal weakness and pseudohypertrophy of muscles. The same clinical picture in children with cretinism is referred to as Kocher–Debré–Sémélaigne syndrome.

The serum levels of muscle enzymes, such as CK, myoglobin, and lactate dehydrogenase, are frequently elevated, but electromyography is often normal. At histology the hypothyroid muscle shows atrophy of type II muscle fibers, relative

Table 7 Musculoskeletal symptoms, signs, and laboratory findings in hypothyroidism

Symptoms	Sign	Lab findings
Pain, cramps	Muscle hypertrophy	Increased CK, myoglobin, and lactate dehydrogenase levels
Stiffness	Proximal muscle weakness	Electromyography is often normal
Easy fatigability and weakness	Delayed relaxation phase of deep tendon reflexes	Atrophy of type II muscle fibers and relative hypertrophy in type I muscle fibers

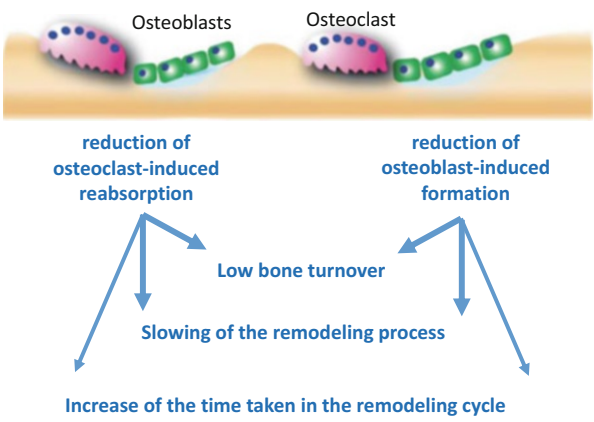

Fig. 6 Effects of thyroid hormone deficiency on the bone remodelling cycle

hypertrophy in type I muscle fibers, increase in the percentage of type I fibers, presence of internalized nuclei, and core-like structures. In most cases symptoms resolve after replacement therapy (Anwar and Gibofsky 2010).

Reflex contraction and relaxation time is prolonged and it is also known as Woltman's sign. The pathophysiology of delayed reflex relaxation is related to a severe reduction of Ca 2+ − ATPase activity of the fast-twitch variety (SERCA1) in hypothyroidism, with impairment of calcium reuptake as a consequence, and to a reduction of muscle levels of myosin ATPase. Carpal tunnel syndrome may be associated with hypothyroidism.

Bone and Joints

In the adult skeleton, thyroid hormone deficiency leads to a reduction of osteoclast-induced reabsorption and of osteoblast-induced formation resulting in a low bone turnover, a slowing of the remodeling process, and an increase of the time taken in the remodeling cycle. Bone mass may be normal or slightly increased (Fig. 6). However, hypothyroid patients show a two- to threefold increased risk of fracture. In some patients fractures may occur before bone mass reaches levels compatible with osteoporosis, reflecting an impairment of bone quality (Bassett et al. 2010). Interestingly, recent data suggest that high TSH levels may be linked to the risk of osteoporotic fractures only in young and middle-aged men, while in

postmenopausal women, the long-term risk of hip and other osteoporotic fractures is strongly related to the cumulative duration of LT4 overtreatment (Abrahamsen et al. 2015).

Increased serum levels of PTH are frequently observed in hypothyroidism, probably due to a resistance to the action of PTH. Serum 1,25-dihydroxycholecalciferol levels are consequently increased. The concentrations of calcium and phosphorus in serum are usually normal, but calcium may be slightly elevated due to the effects of the increase in PTH and vitamin D. Serum alkaline phosphatase and osteocalcin levels are often decreased.

Hypothyroidism may share some symptoms with rheumatic disease, but the coexistence of rheumatoid arthritis, seronegative spondyloarthropathies, psoriatic arthritis, and fibromyalgia should be considered.

Renal Function

Thyroid hormones play an important role in growth and development of the kidney and in water and electrolyte homeostasis. However, hypothyroidism is normally not associated with significant impairment of renal function, with the exception of those patients with coexisting advanced renal disease. The glomerular filtration rate and the renal plasma flow are usually reduced in overt hypothyroidism and normalize after replacement therapy. The mechanisms involved are increased peripheral resistance and reduction of myocardial contractility and stroke volume, hyperlipidemia, and paracrine or endocrine mechanisms mediated by insulin-like growth factor type 1 and vascular endothelial growth factor (Iglesias and Diez 2009).

In more than half of hypothyroid patients, serum creatinine levels are elevated. In these patients the occurrence of hyponatremia is frequent. Hyponatremia is mainly related to a reduction of the glomerular filtration rate causing a reduced water delivery to the distal diluting segment of the nephron. Hyponatremia may be also due to an inappropriate ADH secretion syndrome-like disorder.

In spite of reduced renal blood flow and blood volume, the total body sodium content is increased, probably resulting from binding to extracellular mucopolysaccharides. No significant changes in serum potassium are reported, while magnesium as well as homocysteine concentration may be elevated.

Hematopoietic System

Studies on the influence of thyroid hormones, through their receptors (TRa-1 and TRb-1), on hematopoiesis in patients with hypothyroidism demonstrate a modulation of cell production in the bone marrow. The prevalence of anemia in overt hypothyroidism is high, ranging from 30% in adults and 60% in children and adolescents.

Table 8 Types and causes of anemia in overt hypothyroidism

Types	Causes
Normochromic normocytic anemia	Bone marrow depression and decreased production of erythropoietin
Macrocytic anemia	Reduced absorption of vitamin B12
Microcytic anemia	Reduced iron absorption, menorrhagia

Usually anemia is normochromic normocytic and caused by bone marrow depression and decreased production of erythropoietin due to a reduced need of oxygen. A hypocellular structure of the bone marrow with fat tissue infiltration may be observed. Anemia may also be macrocytic, as a consequence of reduced absorption of vitamin B12, as observed in pernicious anemia due to intrinsic factor deficiency. Reduced iron absorption resulting from autoimmune gastritis and achlorhydria may lead to microcytic anemia. In hypothyroid women menorrhagia is frequent and causes iron deficiency contributing to microcytic hypochromic anemia (Table 8) (M'Rabet-Bensalah et al. 2016).

In overt hypothyroidism clotting abnormalities may be observed. In most cases only abnormal laboratory findings are present but sometimes clinically significant episodes of bleeding are described.

The main coagulopathy described in hypothyroidism is the acquired von Willebrand syndrome type 1. This coagulopathy should be taken into account when planning invasive procedures in hypothyroid patients. The mild or moderate acquired von Willebrand syndrome is reversible after restoration of euthyroidism. Laboratory findings indicating prolongation of activated partial thromboplastin time (aPTT), prothrombin time (PT) and clotting time, reduction in factor VIII activity, and vWF and platelet levels indicating a hypocoagulation state have been described. On the other hand, there are also data suggesting existence of a hypercoagulation and hypofibrinolytic state, particularly in subclinical or moderate hypothyroidism (Kyriakakis et al. 2016).

Reproductive System

Men

The main laboratory findings in male hypothyroid patients are a decrease in sex hormone binding globulin (SHBG) and total testosterone concentrations, while free testosterone may be either normal or reduced. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) serum levels are normal but may exhibit a blunted response to GnRH stimulation. Dehydroepiandrosterone (DHEA), DHEA sulfate, and androstenediol and its sulfate and pregnenolone sulfate are decreased in men with hypothyroidism, compared with normal controls (Table 9) (Krassas and Pontikides 2004). The most frequent complaints are decreased libido or impotence. Several studies have shown abnormalities in sperm morphology in hypothyroid men. These abnormalities improve or normalize when euthyroidism is restored. Little is

Table 9 Pituitary–gonadal changes in male hypothyroid patients

SHBG	↓
Total testosterone	↓
Free testosterone	= ↓
LH and FSH	= blunted responses to GnRH stimulation
DHEA, DHEA sulfate, androstenediol and its sulfate and pregnenolone sulfate	↓

Table 10 Pituitary–gonadal changes in female hypothyroid patients

SHBG	↓
Estradiol	↓
Free estradiol	= ↑
Total testosterone	↓
Free testosterone	= ↑
LH and FSH	= blunted responses to GnRH stimulation
Prolactin	↑

known about the effect of hypothyroidism on male fertility. According to Poppe et al. (2006), the prevalence of thyroid dysfunction and autoimmunity are similar in men with normal and abnormal semen characteristics. For this reason, screening for thyroid disorders is not recommended in infertile men. The testicles are histologically immature if hypothyroidism precedes puberty and show tubular involution if onset is after puberty. In children, precocious sexual development has been reported (Anasti et al. 1995).

Women

The serum levels of SHBG and both total testosterone and E2 are decreased, but their unbound fractions are increased. FSH and LH are usually normal, but sometimes their response to GnRH stimulation is delayed or blunted, probably due to the increased prolactin levels (Table 10). Anovulation and inadequate secretion of progesterone are frequently associated with proliferative endometrium. The frequency of menstrual disturbances, and particularly of oligomenorrhea, is high in hypothyroid women. Studies focusing on the role of overt hypothyroidism as a cause of infertility are scarce and limited in the number of subjects examined. In contrast, many studies have evaluated the association between hypothyroidism and obstetrical/neonatal morbidity. When compared to euthyroid women, pregnant women with overt hypothyroidism have a higher risk of spontaneous abortion during early gestation, gestation-induced hypertension (and/or preeclampsia), placental abruption, preterm delivery, low birth weight, stillbirth, and/or perinatal death (Krassas et al. 2010). Euthyroid neonates born to mothers who were hypothyroid during pregnancy have been reported to achieve a lower IQ later in life (Haddow et al. 1999).

Other Endocrine Glands

Pituitary

Long-standing primary hypothyroidism may cause enlargement of the pituitary sella. The prevalence of hyperprolactinemia in overt hypothyroidism is 40% and usually normalizes with L-thyroxine treatment. Elevated prolactin levels may be due to the influence of increased TRH levels as well as to a decreased prolactin clearance. Hypothyroidism is a well-known cause of growth retardation, because growth hormone (GH) secretion and GH action are deficient in hypothyroid children. GH response to insulin-induced hypoglycemia may be also impaired. GH secretion is decreased as a consequence of an increase in hypothalamic somatostatinergic tone and results in low serum levels of IGF-1, IGF-2, IGFBP-1, and IGFBP-3 concentrations. These changes are reversible after thyroid hormone replacement therapy.

Adrenal Glands

Idiopathic isolated ACTH deficiency is a rare condition of unclear etiology. When present, it may be associated with thyroid disease and particularly with primary hypothyroidism (Hannon and O'Halloran 2011). Adrenal function may be impaired in patients with primary hypothyroidism. In long-standing hypothyroidism, a significant reduction in cortisol secretion after ACTH has been documented in primary adrenal cell culture and it was recently confirmed also in humans (Rodriguez-Gutierrez et al. 2014).

Similarly, the turnover rate of aldosterone is decreased in hypothyroidism but balanced by a lower secretion rate. Angiotensinogen and renin production is reduced. These abnormalities usually are not responsible for changes in sodium and potassium homeostasis.

Conclusion

Hypothyroidism is a clinical condition characterized by reduced synthesis and secretion of the thyroid hormones due to loss of functional thyroid cell mass which in turn may be the consequence of an autoimmune disease, surgery, irradiation, drugs, and other more rare thyroid injuries.

Hypothyroidism is a systemic condition affecting multiple organs, and the severity of symptoms may range from life threatening to mild signs or symptoms.

Cross-References

- ► Acute and Subacute Thyroiditis
- ► Central Hypothyroidism
- ► Congenital Hypothyroidism
- ► Diagnosis and Treatment of Hypothyroidism
- ► Drugs and Other Substances Interfering with Thyroid Function

▶ Hashimoto's Thyroiditis
▶ Non-thyroidal Illness
▶ Postpartum Thyroiditis and Silent Thyroiditis
▶ Tests of Thyroid Function
▶ Thyroid Autoantibodies
▶ Thyroid Physiology and Thyroid Diseases in Pregnancy

References

Abrahamsen B, Jørgensen HL, Laulund AS, Nybo M, Bauer DC, Brix TH, Hegedüs L. The excess risk of major osteoporotic fractures in hypothyroidism is driven by cumulative hyperthyroid as opposed to hypothyroid time: an observational register-based time-resolved cohort analysis. J Bone Miner Res. 2015;30:898.

Aghini-Lombardi F, Antonangeli L, Martino E, Vitti P, Maccherini D, Leoli F, Rago T, Grasso L, Valeriano R, Balestrieri A, Pinchera A. The spectrum of thyroid disorders in an iodine-deficient community: the Pescopagano survey. J Clin Endocrinol Metab. 1999;84:561.

Ajjan RA, Weetman AP. The Pathogenesis of Hashimoto's Thyroiditis: Further Developments in our Understanding. Horm Metab Res. 2015;47:702.

Amino N, Tada H, Hidaka Y. Postpartum autoimmune thyroid syndrome: a model of aggravation of autoimmune disease. Thyroid. 1999;9:705.

Anasti JN, Flack MR, Froehlich J, Nelson LM, Nisula BC. A potential novel mechanism for precocious puberty in juvenile hypothyroidism. J Clin Endocrinol Metab. 1995;80:276.

Anwar S, Gibofsky A. Musculoskeletal manifestations of thyroid disease. Rheum Dis Clin N Am. 2010;36:637.

Bassett JH, Boyde A, Howell PG, Bassett RH, Galliford TM, Archanco M, Evans H, Lawson MA, Croucher P, St Germain DL, Galton VA, Williams GR. Optimal bone strength and mineralization requires the type 2 iodothyronine deiodinase in osteoblasts. Proc Natl Acad Sci USA. 2010; 107:7604.

Biondi B, Cooper DS. The clinical significance of subclinical thyroid dysfunction. Endocr Rev. 2008;29:76.

Boelen A, Kwakkel J, Fliers E. Beyond low plasma T3: local thyroid hormone metabolism during inflammation and infection. Endocr Rev. 2011;32:670.

Bonnema SJ, Hegedüs L. Endocr Rev. 2012;33:920.

Brix TH, Kyvik KO, Hegedüs L. A population-based study of chronic autoimmune hypothyroidism in Danish twins. J Clin Endocrinol Metab. 2000;85:536.

Calvino C, Imperio GE, Wilieman M, Costa ESRH, Souza LL, Trevenzoli IH, Pazos-Moura CC. Hypothyroidism Induces Hypophagia Associated with Alterations in Protein Expression of Neuropeptide Y and Proopiomelanocortin in the Arcuate Nucleus, Independently of Hypothalamic Nuclei-Specific Changes in Leptin Signaling. Thyroid. 2016;26:134.

Canaris GJ, Manowitz NR, Mayor G, Ridgway EC. The Colorado thyroid disease prevalence study. Arch Intern Med. 2000;160:526.

Coles AJ, Wing M, Smith S, Coraddu F, Greer S, Taylor C, Weetman A, Hale G, Chatterjee VK, Waldmann H, Compston A. Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. Lancet. 1999;354:1691.

Constant EL, de Volder AG, Ivanoiu A, Bol A, Labar D, Seghers A, Cosnard G, Melin J, Daumerie C, Clin J. Cerebral blood flow and glucose metabolism in hypothyroidism: a positron emission tomography study. Endocrinol Metab. 2001;86:3864.

Correia N, Mullally S, Cooke G, Tun TK, Phelan N, Feeney J, Fitzgibbon M, Boran G, O'Mara S, Gibney J, Clin J. Evidence for a specific defect in hippocampal memory in overt and subclinical hypothyroidism. Endocrinol Metab. 2009;94:3789.

Curran PG, DeGroot LJ. The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. Endocr Rev. 1991;12:135.
Daniels GH, Vladic A, Brinar V, Zavalishin I, Valente W, Oyuela P, Palmer J, Margolin DH, Hollenstein J. Alemtuzumab-related thyroid dysfunction in a phase 2 trial of patients with relapsing-remitting multiple sclerosis. J Clin Endocrinol Metab. 2014;99:80.
Danzi S, Klein I. Thyroid disease and the cardiovascular system. Endocrinol Metab Clin N Am. 2014;43:517.
Devdhar M, Ousman YH YH, Burman KD. Hypothyroidism. Endocrinol Metab Clin N Am. 2007;36:595.
Duntas LH, Brenta G. The effect of thyroid disorders on lipid levels and metabolism. Med Clin North Am. 2012;96:269.
Ebert EC. The thyroid and the gut. J Clin Gastroenterol. 2010;44:402.
Effraimidis G, Wiersinga WM. Mechanisms in endocrinology: autoimmune thyroid disease: old and new players. Eur J Endocrinol. 2014;170:R241.
Fliers E, Bianco AC, Langouche L, Boelen A. Thyroid function in critically ill patients. Lancet Diabetes Endocrinol. 2015;3:816.
Garber JR, Cobin HR, Gharib H, Hennessey JV, Klein I, Mechanick J, Pessah-Pollack R, Singer PA, Woeber KA. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. Thyroid. 2012;22:1220.
Gereben B, Zavacki AM, Ribich S, Kim BW, Huang SA, Simonides WS, Zeöld A, Bianco AC. Cellular and molecular basis of deiodinase-regulated thyroid hormone signaling. J Endocr Rev. 2008;29:898.
Guastamacchia E, Triggiani V, Aglialoro A, Aiello A, Ianni L, Maccario M, Zini M, Giorda C, Guglielmi R, Betterle C, Attanasio R, Borretta G, Garofalo P, Papini E, Castello R, Ceriello A. Italian Association of Clinical Endocrinologists (AME) & Italian Association of Clinical Diabetologists (AMD) Position Statement : Diabetes mellitus and thyroid disorders: recommendations for clinical practice. Endocrine. 2015;49:339.
Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, Mitchell ML, Hermos RJ, Waisbren SE, Faix JD, Klein RZ, Engl N. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. J Med. 1999;341:549.
Hancock SL, McDougall IR, Constine L-S. Thyroid abnormalities after therapeutic external radiation. Int J Radiat Oncol Biol Phys. 1995;31:1165.
Hannon MJ, O'Halloran DJ. Isolated acquired ACTH deficiency and primary hypothyroidism: a short series and review. Pituitary. 2011;14:358.
Hassan MM, Kaseb A, Li D, Patt YZ, Vauthey JN, Thomas MB, Curley SA, Spitz MR, Sherman SI, Abdalla EK, Davila M, Lozano RD, Hassan DM, Chan W, Brown TD, Abbruzzese JL. Association between hypothyroidism and hepatocellular carcinoma: a case-control study in the United States. Hepatology. 2009;49:1563.
Hennessey JV. Clinical review: Riedel's thyroiditis: a clinical review. J Clin Endocrinol Metab. 2011;96:3031.
Herwig A, Campbell G, Mayer CD, Boelen A, Anderson RA, Ross AW, Mercer JG, Barrett P. A thyroid hormone challenge in hypothyroid rats identifies T3 regulated genes in the hypothalamus and in models with altered energy balance and glucose homeostasis. Thyroid. 2014;24:1575.
Heuer H. The importance of thyroid hormone transporters for brain development and function. Best Pract Res Clin Endocrinol Metab. 2007;21:265.
Hollowell NW, Staehling WD, Flanders WH, Hannon EW, Gunter CA, Spencer LE. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab. 2002;87:489.
Iglesias P, Diez JJ. Thyroid dysfunction and kidney disease. Eur J Endocrinol. 2009;160:503.
Iwama S, De Remigis A, Callahan MK, Slovin SF, Wolchok JD, Caturegli P. Pituitary expression of CTLA-4 mediates hypophysitis secondary to administration of CTLA-4 blocking antibody. Sci Transl Med. 2014;6:230ra45.

Jereczek-Fossa BA, Alterio D, Jassem J, Gibelli B, Tradati N, Orecchia R. Radiotherapy-induced thyroid disorders. Cancer Treat Rev. 2004;30:369.

Jha A, Sharma SK, Tandon N, Lakshmy R, Kadhiravan T, Handa KK, Gupta R, Pandey RM, Chaturvedi PK. Thyroxine replacement therapy reverses sleep-disordered breathing in patients with primary hypothyroidism. Sleep Med. 2006;7:55.

Jonklaas J, Bianco AC, Bauer AJ, Burman KD, Cappola AR, Celi FS, Cooper DS, Kim BW, Peeters RP, Rosenthal MS, Sawka AM. Guidelines for the treatment of hypothyroidism: prepared by the american thyroid association task force on thyroid hormone replacement. Thyroid. 2014;24:1670.

Kanaya AM, Harris F, Volpato S, Pérez-Stable EJ, Harris T, Bauer DC. Association between thyroid dysfunction and total cholesterol level in an older biracial population: the health, aging and body composition study. Arch Intern Med. 2002;162:773.

Kappers MHW, van Esch JHM, Smedts FMM, de Krijger RR, Eechoute K, Mathijssen RHJ, Sleijfer S, Leijten F, Danser AHJ, van den Meiracker AH, Visser TJ. Sunitinib-induced hypothyroidism is due to induction of type 3 deiodinase activity and thyroidal capillary regression. J Clin Endocrinol Metab. 2011;96:3087.

Knudsen N, Jùrgensen T, Rasmussen S, Christiansen E, Perrild H. The prevalence of thyroid dysfunction in a population with borderline iodine deficiency. Clin Endocrinol. 1999;51:361.

Krassas GE, Pontikides N. Male reproductive function in relation with thyroid alterations. Best Pract Res Clin Endocrinol Metab. 2004;18:183.

Krassas GE, Poppe K, Glinoer D. Thyroid function and human reproductive health. Endocr Rev. 2010;31:702.

Kyriakakis N, Lynch J, Ajjan R, Murray RD. The effects of pituitary and thyroid disorders on haemostasis: potential clinical implications. Clin Endocrinol. 2016;84:473.

Laurberg P, Cerqueira C, Ovesen L, Rasmussen LB, Perrild H, Andersen S, Pedersen IB, Carlé A. Iodine intake as a determinant of thyroid disorders in populations. Best Pract Res Clin Endocrinol Metab. 2010;24:13.

Lazarus JH. Lithium and thyroid. Best Pract Res Clin Endocrinol Metab. 2009;23:723.

Leung AM, Braverman LE. Consequences of excess iodine. Nat Rev Endocrinol. 2014;10:136.

Lio S. In: Monaco F, editor. Thyroid diseases. Boca Raton: CRC Press; 2012. p. 49.

Luongo C, Trivisano L, Alfano F, Salvatore D. Type 3 deiodinase and consumptive hypothyroidism: a common mechanism for a rare disease. Front Endocrinol (Lausanne). 2013;4:115.

Magri F, Buonocore M, Oliviero A, Rotondi M, Gatti A, Accornero S, Camera A, Chiovato L. Intraepidermal nerve fiber density reduction as a marker of preclinical asymptomatic small-fiber sensory neuropathy in hypothyroid patients. Eur J Endocrinol. 2010;163:279.

Mariotti S. In: Monaco F, editor. Thyroid diseases. Boca Raton: CRC Press; 2012a. p. 77.

Mariotti S. In: Monaco F, editor. Thyroid diseases. Boca Raton: CRC Press; 2012b. p. 97.

Mariotti S, Martino E, Francesconi M, Ceccarelli C, Grasso L, Lippi F, Baschieri L, Pinchera A. Serum thyroid autoantibodies as a risk factor for development of hypothyroidism after radioactive iodine therapy for single thyroid 'hot' nodule. Acta Endocrinol. 1986;113:500.

Martino E, Bartalena L, Bogazzi F, Braverman LE. The effects of amiodarone on the thyroid. Endocr Rev. 2001;22:240.

Menconi F, Hasham A, Tomer Y. Environmental triggers of thyroiditis: hepatitis C and interferon-α. J Endocrinol Investig. 2011;34:78.

Metso S, Jaatinen P, Huhtala H, Luukkaala T, Oksala H, Salmi J. Long-term follow-up study of radioiodine treatment of hyperthyroidism. Clin Endocrinol. 2004;61:641.

M'Rabet-Bensalah K, Aubert CE, Coslovsky M, Collet TH, Baumgartner C, den Elzen WP, Luben R, Angelillo-Scherrer A, Aujesky D, Khaw KT, Rodondi N. Thyroid dysfunction and anaemia in a large population-based study. Clin Endocrinol. 2016; 84:627.

Nagamachi S, Jinnouchi S, Nishii R, Ishida Y, Fujita S, Futami S, Kodama T, Tamura S, Kawai K. Cerebral blood flow abnormalities induced by transient hypothyroidism after thyroidectomy–analysis by tc-99m-HMPAO and SPM96. Ann Nucl Med. 2004;18:469.

Nakamura H, Yamashita S, Ashizawa K, Kitahara A, Natsume H, Andoh S, Nagasawa S, Nishiyama K, Chida K, Sato A, Yoshimi T. High incidence of positive autoantibodies against thyroid peroxidase and thyroglobulin in patients with sarcoidosis. Clin Endocrinol. 1997;46:467.

Ortiga-Carvalho TM, Sidhaye AR, Wondisford FE. Thyroid hormone receptors and resistance to thyroid hormone disorders. Nat Rev Endocrinol. 2014;10:582.

Pani F, Atzori F, Baghino G, Boi F, Tanca L, Ionta MT, Mariotti S. Thyroid dysfunction in patients with metastatic carcinoma treated with sunitinib: Is thyroid autoimmunity involved?. Thyroid. 2015;25:1255.

Parle JV, Franklyn JA JA, Cross KW, Jones SC, Sheppard MC. Prevalence and follow-up of abnormal thyrotrophin (TSH) concentrations in the elderly in the United Kingdom. Clin Endocrinol. 1991;34:77.

Pearce EN, Braverman LE. Environmental pollutants and the thyroid. Best Pract Res Clin Endocrinol Metab. 2009;23(6):801–13.

Poppe K, Glinoer D, Tournaye H, Maniewski U, Haentjens P, Velkeniers B. Is systematic screening for thyroid disorders indicated in subfertile men?. Eur J Endocrinol. 2006;154:363.

Refetoff S, Dumitrescu AM. Syndromes of reduced sensitivity to thyroid hormone: genetic defects in hormone receptors, cell transporters and deiodination. Best Pract Res Clin Endocrinol Metab. 2007;21:277.

Refetoff S, Weiss RE, Usala SJ. The syndromes of resistance to thyroid hormone. Endocr Rev. 1993;14:348.

Rodriguez-Gutierrez R, Gonzalez-Velazquez C, Gonzalez-Saldivar G, Villarreal-Perez JZ, Gonzalez-Gonzalez JG. Glucocorticoid functional reserve in full-spectrum intensity of primary hypothyroidism. Int J Endocrinol. 2014;2014:313519.

Rønjom MF, Brink C, Bentzen SM, Hegedüs L, Overgaard J, Johansen J. Hypothyroidism after primary radiotherapy for head and neck squamous cell carcinoma: normal tissue complication probability modeling with latent time correction. Radiother Oncol. 2013;109:317.

Rønjom MF, Brink C, Lorenzen FL, Hegedüs L, Johansen J. Variation of normal tissue complication probability (NTCP) estimates of radiation-induced hypothyroidism in relation to changes in delineation of the thyroid gland. Acta Oncol. 2015;54:1188.

Rose NR, Bona C. Defining criteria for autoimmune diseases (Witebsky's postulates revisited). Immunol Today. 1993;14:426.

Ross DS. Radioiodine therapy for hyperthyroidism. N Engl J Med. 2011;364:542.

Safer JD. Thyroid hormone action on skin. Curr Opin Endocrinol Diabetes Obes. 2012;19:388.

Salvatore D, Simonides WS, Dentice M, Zavacki AM, Larsen PR. Thyroid hormones and skeletal muscle–new insights and potential implications. Nat Rev Endocrinol. 2014;10:206.

Santini F, Marzullo P, Rotondi M, Ceccarini G, Pagano L, Ippolito S, Chiovato L, Biondi B. Eur J Endocrinol. 2014;171:R137.

Santos-Palacios S, Brugos-Larumbe A, Guillen-Grima F, Galofre JC. Clin Endocrinol. 2013; 79:874.

Singer PA. Werner & Ingbar's thyroid. A fundamental and clinical text. 10th ed. Philadelphia: Wolters Kluver/Lippincot Williams & Wikins; 2013. p. 552.

Singh S, Rana P, Kumar P, Shankar LR, Khushu S. J Neuroendocrinol. 2016;28:1.

Sorensen JR, Winther KH, Bonnema SJ, Godballe C, Hegedus L. Respiratory Manifestations of Hypothyroidism: A Systematic Review. Thyroid. 2016;26:1519.

Stagnaro-Green A, Schwartz A, Gismondi R, Tinelli A, Mangieri T, Negro R. High rate of persistent hypothyroidism in a large-scale prospective study of postpartum thyroiditis in southern Italy. J Clin Endocrinol Metab. 2011;96:652.

Surks MI, Hollowell JG. Age-specific distribution of serum thyrotropin and antithyroid antibodies in the US population: implications for the prevalence of subclinical hypothyroidism. J Clin Endocrinol Metab. 2007;92:4575.

Taylor JC, Gough SC, Hunt PJ, Brix TH, Chatterjee K, Connell JM, Franklyn JA, Hegedüs L, Robinson BG, Wiersinga WM, Wass JA, Zabaneh D, Mackay I, Weetman AP. A genome-wide

screen in 1119 relative pairs with autoimmune thyroid disease. J Clin Endocrinol Metab. 2006;91:646.
Thvilum M, Brandt F, Almind D, Christensen K, Brix TH, Hegedüs L. Type and extent of somatic morbidity before and after the diagnosis of hypothyroidism. a nationwide register study. PLoS One. 2013;16:e75789.
Thvilum M, Brandt F, Almind D, Christensen K, Brix TH, Hegedüs L. Increased psychiatric morbidity before and after the diagnosis of hypothyroidism: a nationwide register study. Thyroid. 2014;24:802.
Tomer Y, Menconi F. Interferon induced thyroiditis. Best Pract Res Clin Endocrinol Metab. 2009;23:703.
Torino F, Barnabei A, Paragliola R, Baldelli R, Appetecchia M, Corsello SM. Thyroid dysfunction as an unintended side effect of anticancer drugs. Thyroid. 2013; 23:1345.
Tunbridge WM, Evered DC, Hall R, Appleton D, Brewis M, Clark F, Evans JG, Young E, Bird T, Smith PA. The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol. 1977;7:481.
Vanderpump MPJ. The epidemiology of thyroid disease. Br Med Bull. 2011;99:39.
Vanderpump MPJ, Tunbridge WMG, French JM, Appleton D, Bates D, Clark F, Grlmley Evans J, Hasan DM, Rodgers H, Tunbridge F, Young ET. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clin Endocrinol. 1995;43:55.
Verloop H, Louwerens M, Schoones JW, Kievit J, Smit JWA, Dekkers OM. Risk of hypothyroidism following hemithyroidectomy: systematic review and meta-analysis of prognostic studies. J Clin Endocrinol Metab. 2012;97:2243.
Vogelius IR, Bentzen SM, Maraldo MV, Petersen PM, Specht L. Risk factors for radiation-induced hypothyroidism: a literature-based meta-analysis. Cancer. 2011;117:5250.
Yaylali O, Kirac S, Yilmaz M, Akin F, Yuksel D, Demirkan N, Akdag B. Does hypothyroidism affect gastrointestinal motility?. Gastroenterol Res Pract. 2009;2009:529802.
Zhou JY, Xu B, Lopes J, Blamoun J, Li L. Hashimoto encephalopathy: literature review. Acta Neurol Scand. 2017;135:285.
Zimmermann MB. Iodine deficiency. Endocr Rev. 2009;30:376.
Zimmermann MB, Boelaert K. Iodine deficiency and thyroid disorders. Lancet Diabetes Endocrinol. 2015;3:286.

Congenital Hypothyroidism

11

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Abstract

Congenital hypothyroidism (CH) is the most frequent congenital endocrine disorder with a reported incidence more than doubled in recent years. Delayed diagnosis and treatment of CH lead to growth retardation and neurological and psychiatric impairment, which can be largely prevented if thyroid hormone replacement therapy (L-T_4 therapy) is commenced within the first 2 weeks of life. Development of neonatal screening programs in most industrialized countries, measuring thyroid-stimulating hormone (TSH) and total thyroxine (T_4) in dried blood spots, has allowed early diagnosis of CH. As soon as the diagnosis is confirmed by measuring serum TSH and thyroid hormone levels, L-T_4 therapy should be started. Other diagnostic studies (i.e., scintigraphy and ultrasound) to determine the underlying cause of CH should not delay initiation of treatment. Controversies still exist regarding the optimal screening program and management of this disorder.

This chapter provides a summary of significant recent advances in the epidemiology, etiopathogenesis, and treatment of congenital hypothyroidism.

Keywords

Congenital hypothyroidism · Hyperthyrotropinemia · Newborn screening · Thyroid dysgenesis · Thyroid dyshormonogenesis · Clinical features · Management

Definition and Epidemiology

Congenital hypothyroidism (CH) defines a condition of insufficient thyroid hormone supply to the organism starting in utero. Owing to the essential role played by thyroid hormone in growth and brain development (Bernal et al. 2003; Williams 2008), if not immediately treated, CH can lead to growth retardation and neurological and psychiatric impairment, including intellectual disability, spasticity, disturbances of gait and coordination, and speech and language deficits (Zoeller and Rovet 2004; Porterfield and Hendrich 1993). All these alterations can be largely prevented by immediate thyroid hormone replacement therapy.

As diagnosis of CH can be difficult at birth, in developed countries neonatal screening programs have been instituted to allow a prompt diagnosis and treatment of the condition. The first newborn screening program for CH was started in Quebec in the mid-1970s and then rapidly developed in other countries (Dussault 1999; Rastogi and LaFranchi 2010; Rose et al. 2006; Van Vliet and Czernichow 2004). Before the screening era, the incidence of CH was estimated to be 1:6700 live births (Grosse and Van Vliet 2011). The use of more sensitive tests and different newborn screening strategies (see the "Newborn Thyroid Screening Tests" section) has

allowed the early detection of a larger number of CH cases, with a reported initial incidence of about 1:3000–4000 live births, which made CH the most frequent congenital endocrine disease (Dussault 1999; Rastogi and LaFranchi 2010; Rose et al. 2006; Van Vliet and Czernichow 2004). Recent reports have indicated that the incidence of primary CH may be increasing in some countries, particularly for cases with a normally located (eutopic) thyroid gland and milder dysfunction (Corbetta et al. 2009). An increased incidence has been reported in New York (1:1415) (Harris and Pass 2007), in Massachusetts (1:1660) (Mitchell et al. 2011), in the United Kingdom (1:1077) (Pearce et al. 2010), in Italy (1:2200) (Italian National Registry of Infants with Congenital Hypothyroidism 2013), and in Greece (1:1749) (Mengreli et al. 2010). The reasons for the increased incidence remain largely unclear. However, several factors have been proposed to explain this increase, among them are changes in screening thresholds and strategies, in particular the use of lower TSH cutoff levels (Deladoëy et al. 2011; Olivieri et al. 2013), altered ethnicities of the screened population with a higher proportion of neonates with an Asian and Hispanic background at higher susceptibility to CH (Hinton et al. 2010), increased multiple and premature births which can present a transient rise in TSH levels (Olivieri et al. 2007; Radetti et al. 2007), and iodine status (Zimmermann et al. 2005).

Classification

According to site, CH can be classified in primary (thyroid) or secondary/central (pituitary and/or hypothalamic). The most common form is primary CH, characterized by high thyroid-stimulating hormone (TSH) levels reflecting various types of abnormal thyroid gland development (dysgenesis) or thyroid hormone biosynthesis defects (dyshormonogenesis). Secondary CH is much less frequent. It results from a deficiency of TSH, isolated, or most commonly associated with other pituitary hormone deficiencies.

Peripheral CH is a distinct category resulting from defects of thyroid hormone cell membrane transport, metabolism, or nuclear action.

According to severity, CH can be severe (elevated TSH levels associated with subnormal levels of free thyroxine, FT_4), mild, or subclinical (elevated TSH without decreased FT_4 concentrations). The term "hyperthyrotropinemia" refers to a form of subclinical CH in which there is a mild increase in TSH concentration (e.g., 6–20 mU/L) with normal thyroid hormone concentrations.

Furthermore, CH can be classified into permanent and transient. Permanent CH refers to a deficiency of thyroid hormone requiring lifelong treatment, whereas transient CH refers to a temporary deficiency of thyroid hormone reverting to euthyroidism in the first few months or years of postnatal life.

Table 1 summarizes all forms of CH.

Table 1 Classification and etiology of congenital hypothyroidism (CH)

Primary CH
Thyroid dysgenesis
Aplasia
Hypoplasia
Ectopic gland
Thyroid dyshormonogenesis
Sodium-iodide symporter defect
Thyroid peroxidase defect
Hydrogen peroxidase generation defects (DUOX2, DUOXA2 gene mutations)
Pendrin defect (Pendred's syndrome)
Thyroglobulin defect
Iodotyrosine deiodinase defect
Resistance to TSH binding or signaling
TSH receptor defect
G protein defect (pseudohypoparathyroidism type 1A and 1B)
Secondary (central) CH
Isolated TSH deficiency
Congenital hypopituitarism (combined pituitary hormone deficiencies)
Peripheral CH
Thyroid hormone cell membrane transport defect (*MCT8* gene mutations)
Thyroid hormone metabolism defect (*SECISBP2* gene mutations)
Thyroid hormone resistance (*thyroid hormone receptor α and β* gene mutations)
Transient CH
Subclinical or mild CH/Hyperthyrotropinemia
Transient or permanent (genetic and/or environmental factors)

Permanent CH

Primary Congenital Hypothyroidism

Thyroid Dysgenesis

Thyroid dysgenesis (TD) represents 80–85% of all cases of primary CH. It constitutes a heterogeneous group of affections deriving from the disruption of one of the steps of thyroid development going from the generation of functional thyrocytes from endodermal pluripotent stem cells to the migration of thyroid from the foramen caecum, where the gland originates, to its definitive pretracheal position.

TD includes thyroid agenesis or athyreosis (~35–40% of the cases), hypoplastic thyroid or hypoplasia (~5% of the cases), and thyroid ectopy (~30–45% of the cases) (De Felice and Di Lauro 2004; Park and Chatterjee 2005; Szinnai 2014a). Thyroid agenesis is the most severe form of TD. It is characterized by the highest serum TSH levels and undetectable serum thyroglobulin (TG) levels. It is still unknown whether athyreosis derives from arrest of differentiation of pluripotent endodermal cells or

from apoptosis of thyrocyte precursor pool during early steps of organogenesis. Patients with thyroid hypoplasia can present with mild isolated increase in TSH (hyperthyrotropinemia) or severe thyroid insufficiency depending on the size of functional thyroid tissues. Severe hypoplasia can at scintigraphy mimic athyreosis; however, measurable serum TG levels and thyroid tissue detectable at ultrasound help to differentiate between the two conditions.

Thyroid ectopy is the most frequent form of TD; it is associated with variable thyroid dysfunction. Affected newborns can present with severe hypothyroidism at birth or can develop hypothyroidism later in life, thus escaping diagnosis by neonatal screening (Szinnai 2014a).

The pathogenesis of TD is still largely unknown. Although most cases of CH with TD occur as sporadic disease, accumulating evidence indicates that genetic factors are involved in the pathogenesis of the disorder. This includes the observation that female prevalence is significant for ectopy but not for athyreosis (Devos et al. 1999) and that different incidence of TD occurs in different ethnic groups (i.e., higher susceptibility in Asian Indian and Hispanic and lower in Black infants) (Grant and Smith 1988; Knobel and Medeiros-Neto 2003; Lorey and Cunningham 1992). Moreover, CH incidence is higher in those populations with high blood-related marriages (Ordookhani et al. 2004), and approximately 2% of patients with TD have one affected relative with a dysgenetic gland (Castanet et al. 2001). The association in 5–6% of TD cases of other major birth defects supports the genetic origin of TD as well (Devos et al. 1999; Olivieri et al. 2002). Lastly, mutations in genes involved in thyroid development cause TD in animal models (De Felice and Di Lauro 2004). On the other hand, TD has been found discordant in monozygotic twins (Perry et al. 2002), and a linkage and mutational analysis of familial cases has demonstrated genetic heterogeneity (Castanet et al. 2005). Furthermore, a mouse model has demonstrated multigenic origin of TD in mice (Amendola et al. 2005). All together these data suggest that there is a spectrum of TD ranging from monogenic to multifactorial genetic etiologies and environmental as well as epigenetic modifiers are likely to be contributing factors.

TD has been associated to mutations of genes encoding for transcription factors involved in thyroid development (*PAX-8*, *NKX2.1*, *FOXE1*, and *NKX2.5*) and for the TSH receptor (*TSHR*) and the $G_S\alpha$ gene. Monogenetic forms of TD can be divided into nonsyndromic and syndromic. Mutations in *PAX8*, *NKX2.5*, and *TSHR* account for nonsyndromic TD, whereas mutations in *FOXE1* and *NKX2.1* lead to the Bamforth-Lazarus syndrome and the brain-lung-thyroid syndrome, respectively. Mutations in the $G_S\alpha$ gene represent the molecular cause of pseudohypoparathyroidism.

PAX 8 gene mutations. PAX8 (paired box gene 8) is a transcription factor of the mammalian Pax protein family, which plays an important role in thyroid cell development when thyroid bud evaginates from the floor of the pharynx (Plachov et al. 1990); its expression is maintained in thyroid follicular cells during all stages of development and in adulthood (De Felice and Di Lauro 2004) and has a fundamental role not only in initiating thyroid cell differentiation but also in maintaining the differentiated state essential for thyroid cell proliferation

(Pasca di Magliano et al. 2000). In the adult thyroid, PAX8 acts as transcription factor of *TG*, thyroperoxidase (*TPO*), and sodium/iodide symporter *(NIS/SLC5A5*) genes. Since their first description in 1998 (Macchia et al. 1998), 15 different *PAX8* mutations have so far been reported (Szinnai 2014b). Mutations occur either de novo or are transmitted in an autosomal dominant fashion. The clinical and biochemical phenotype associated with the *PAX8* mutations is variable even within the same family, ranging from severe CH to mild hyperthyrotropinemia and from athyreosis over hypoplasia to eutopic thyroid gland. Thyroid ectopy is a rare finding in PAX8 defects (Ramos et al. 2013). Besides TD, patients harboring *PAX8* mutations can present with kidney agenesis because PAX8 also plays a role during kidney development.

NKX2.1 gene mutations: brain-lung-thyroid syndrome. NK2 Homeobox 1, formerly called TTF1 or TITF1 (thyroid transcription factor 1), is a homeodomain-containing transcription factor which controls the expression of several important thyroid-specific and lung-specific genes, namely, *TG*, *TPO*, *TSHR*, and *NIS* in the thyroid and surfactant genes in the lung. Studies in mice have shown that Nkx2.1, homologous to human NKX2.1, is expressed at early and late stages of thyroid development as well as during adulthood (Lazzaro et al. 1991; Kimura et al. 1996). During development, Nkx2.1 is essential for survival of the precursors of the thyroid follicular cells, for folliculogenesis, and in differentiated thyroid follicular cells for regulating the expression of the thyroid-specific genes. In the adult murine thyroid, Nkx2.1 is required for the maintenance of an ordered follicular architecture and function of the differentiated thyroid. Besides the thyroid and lung, in the human NKX2.1 is also expressed in restricted regions of the central nervous system including the hypothalamus and basal ganglia.

The phenotype associated to *NKX2.1* mutations is characterized by a triad of CH, severe surfactant deficiency syndrome (with recurrent pulmonary infections, asthma, bronchodysplasia), and generalized hypotonia at birth evolving to benign hereditary chorea (BHC), a nonprogressive and hyperkinetic movement disorder, usually around 1 year of age (Devriendt et al. 1998; Krude et al. 2002; Pohlenz et al. 2002). However, the clinical phenotype is variable with only 50% of affected patients showing the classical triad, 30% showing thyroid and neurologic disorders, 13% presenting with isolated BHC, and 7% with absence of BHC (Carrè et al. 2009; Thorwarth et al. 2014). With respect to morphological and functional thyroidal abnormalities reported, most patients show hypoplasia or a eutopic thyroid and moderate hypothyroidism or hyperthyrotropinemia. Athyreosis is a less frequent finding, and only one case with thyroid ectopy has so far been reported (de Filippis et al. 2014). Patients can be missed at neonatal screening due to mild hyperthyrotropinemia and be diagnosed later because of the lung and/or neurologic manifestations. Motor delay and hypotonia have been reported as the first clinical manifestations of BHC in early infancy, even before the onset of movement disorder (Gras et al. 2012).

FOXE1 gene mutations: Bamforth-Lazarus syndrome. FOXE1 (Forkhead Box E1), formerly called TTF2 (thyroid transcription factor 2), is a forkhead/winged-helix transcription factor expressed during human thyroid development at a later

stage than PAX8 and NKX2.1, in palate, Rathke's pouch, pharyngeal structures, and hair follicles (Trueba et al. 2005; Szinnai et al. 2007).

FOXE1 mutations cause the Bamforth-Lazarus syndrome, a syndrome characterized by the combination of TD (mainly athyreosis or severe hypoplasia), cleft palate, choanal atresia, bifid epiglottis, and spiky hair (Bamforth et al. 1989; Clifton-Bligh et al. 1998). Mutations in *FOXE1* are the rarest monogenetic form of TD, with only eight patients harboring six different mutations described (Carrè et al. 2014). While *FOXE1* mutations have not been found in patients with isolated TD in large cohorts, they have been identified in patients with isolated cleft palate (Moreno et al. 2009).

NKX2.5 gene mutations. NK2 Homeobox 5 is a homeodomain-containing transcription factor expressed during heart and thyroid development (Reamon-Buettner and Borlak 2010). Mutations in *NKX2.5* have been identified in about 3% of patients suffering from isolated congenital heart disease. As congenital defects of heart development are the most frequently associated malformations in TD patients, *NKX2.5* appears to be a good candidate gene for TD. Furthermore, in *Nkx2.5* null mouse embryos in spite of a normal budding of the thyroid gland, evidenced by the expression of the thyroid-specific transcription factors *Nkx2.1*, *Foxe1*, and *Pax8*, a smaller sized bud was detected on embryonic day 9.5 (E9.5), confirming a role of Nkx2.5 during the organogenesis of the thyroid (Dentice et al. 2006). In 2006, four patients with three different heterozygous *NKX2.5* mutations and ectopy or athyreosis were reported among a cohort of 241 individuals affected by TD (Dentice et al. 2006). However, the role of *NKX2.5* mutations in the pathogenesis of thyroid dysgenesis is still not clear since the parents and one sibling of probands, harboring the same mutations, had no morphological or biochemical evidence of CH (Dentice et al. 2006). Moreover, since 2006, *NKX2.5* mutations were screened for in large cohorts of CH patients, without identifying any further mutations (Szinnai 2014a). Lastly, a recent study showed that patients with congenital heart disease harboring the *NKX2.5* mutation, reported by Dentice et al. (2006) (p.A119S), had normal thyroid function and morphology (Van Engelen et al. 2012). In the same study, in contrast to what was previously reported (Dentice et al. 2006), functional analysis revealed no transactivation differences between the mutant protein and its WT counterpart. Given all these observations, it is now accepted that *NKX2.5* mutations are not a major contributor in the pathogenesis of TD, although their role as a genetic modifier cannot be excluded.

Inactivating thyroid-stimulating hormone receptor mutations (TSHR): thyroid-stimulating hormone resistance. The TSHR is a member of the glycoprotein hormone receptor subfamily of the G protein-coupled receptors, which acts as the main regulator of thyroid activity in the mature thyroid gland (Kleinau et al. 2013). The TSHR is expressed at a later stage of human and mouse thyroid development than *PAX8*, *NKX2.1*, and *FOXE1* but before activation of the fetal hypothalamic-pituitary-thyroid axis and TSH secretion (Szinnai 2014a). Based on these observations and studies in mice, it was assumed that the TSHR without its ligand does not play a central role for thyroid organogenesis. Indeed, *Tshr* knockout mice show a normal thyroid morphogenesis and only reduction in *Nis* and *Tpo* expression compared to

wild-type mice (Postiglione et al. 2002). In men, mutations in the *TSHR* gene are associated with TSH resistance (RTSH).

RTSH is a congenital syndrome of variable hyposensitivity to a biologically active TSH molecule, characterized by elevated serum TSH and normal to very low serum levels of thyroid hormones in the presence of a hypoplastic or a normally sized gland in situ (Grasberger and Refetoff 2017). The degree of hyposensitivity to TSH depends on the type and location of the *TSHR* mutations and whether the patient is homozygous or heterozygous. More severe inactivating mutations manifest as CH, whereas mild or heterozygous mutations present as hyperthyrotropinemia in childhood or adulthood (Tonacchera et al. 2004). The main feature of *TSHR* gene mutations is the high level of phenotypic variability of the same mutation, either within families or between unrelated subjects. Genetic rearrangements or mutations in yet unanalyzed regions or elements of the *TSHR* gene, other genes in addition to *TSHR* (digenic inheritance), or environmental factors might account for this phenotypic variability. Mutations in the *TSHR* gene are the most frequent cause of monogenetic forms of TD, with a reported frequency in ten large cohorts of CH patients of 4.3% (Szinnai 2014a). The highest frequencies have been observed in Japan, Korea, and Taiwan. A high prevalence (11–29%) of *TSHR* mutations has been recently reported also in children with isolated hyperthyrotropinemia and no history of CH or of autoimmune thyroid disease (Calebiro et al. 2012; Rapa et al. 2009; Nicoletti et al. 2009).

Mild forms are considered stable compensated conditions not requiring replacement therapy, whereas incompletely compensated forms showing reduced FT_4 levels over time may require treatment (Tenenbaum-Rakover et al. 2015).

CH associated with parathyroid disorders:pseudohypoparathyroidismtype-1A and 1-B. Pseudohypoparathyroidism (PHP) is an uncommon disorder resulting from molecular alterations within or upstream of the GNAS locus (Mantovani et al. 2016). The disorder presents either as a sporadic or as a familial defect, being inherited in an autosomal dominant fashion in the familial cases (Farfel et al. 1981; Patten et al. 1990).

Affected patients may have the classical Albright's hereditary osteodystrophy (AHO) characterized by resistance to the action of parathyroid hormone (PTH) with hypocalcemia, hyperphosphatemia and elevated serum PTH levels, and additional physical features, such as brachydactyly (specifically a shortening of the third through fifth metacarpals and distal phalanx of the thumb), ectopic ossifications, short stature, obesity, and mental retardation (Albright et al. 1942). A blunted response of urinary cyclic AMP to exogenous PTH administration with a failure of the expected phosphaturic response confirms the diagnosis.

Patients can present with resistance to the action of PTH associated with resistance to other hormones that rely on the $G_s\alpha$ protein for signal transduction. The most frequent variants of PHP include PHP type-1A (PHP1A) and PHP type-1B (PHP1B). PHP1A presents with hypocalcemia and hyperphosphatemia and features of AHO with most of affected individuals showing resistance to TSH and a minority to gonadotropins, growth hormone-releasing hormone (GHRH), and calcitonin (Mantovani et al. 2016; Weinstein et al. 2001).

PHP1B is characterized by renal resistance to PTH in the absence of physical abnormalities. Resistance to TSH, often mild, is observed in at least half of PHP1B patients (Liu et al. 2003; Mantovani et al. 2007; Molinaro et al. 2015).

Regarding TSH resistance, patients with PHP1A and PHP1B develop resistance to this hormone during childhood or adolescence, but hypothyroidism may be detected at neonatal screening as well (Levine et al. 1985; Pohlenz et al. 2003; Pinsker et al. 2006). TSH resistance is usually mild, with normal or slightly low thyroid hormone levels and absence of goiter.

Further Genes Involved in TD

GLIS3. GLIS3 (transcription factor GLI similar*)* is an ubiquitous nuclear protein which in humans plays a role in transcriptional activation and repression during embryogenesis (Kim et al. 2003). *GLIS3* mutations present with multisystem involvement. In addition to CH and neonatal diabetes, renal cystic dysplasia, progressive liver fibrosis, and osteopenia can be present (Senee et al. 2006; Dimitri et al. 2011, 2015). All reported patients but one suffer from CH (Dimitri 2017). The pathology leading to hypothyroidism ranges from thyroid agenesis to normal thyroid morphology with suspected dyshormonogenesis and possible temporary thyroid hormone resistance to treatment. So far, no patients with ectopy have been described. Affected patients treated with L-T_4 may not normalize their serum TSH levels despite T_4 levels increasing to the upper end of the normal range. Dividing the daily dose of L-T_4 into three or four doses may help to normalize TSH in this condition, although the mechanism by which this occurs is unclear (Dimitri 2017).

JAG1. The jagged 1 protein (JAG1) is a member of the jagged protein family, which includes ligands expressed on the cell surface interacting with Notch receptors on adjacent cells. The Notch pathway plays an essential role in thyroid development since disruption of the Notch signal leads to thyroid dysgenesis in zebra fish (Porazzi et al. 2012). Heterozygous *JAG1* mutations have been reported in the Alagille syndrome type 1 (ALGS1), characterized by variable involvement of the liver, heart, skeleton, eye, and facial defects (Jurkiewicz et al. 2014). In a recent study, thyroid function was assessed in 21 ALGS1 patients with *JAG1* mutations, and *JAG1* mutations were searched in 100 patients with CH and TD. Non-autoimmune hypothyroidism was observed in six AGLS1 patients, two of them having thyroid hypoplasia. Four CH patients showed heterozygous *JAG1* mutations: two had thyroid hypoplasia, one thyroid ectopy, and one a normal gland in situ (de Filippis et al. 2016). Functional studies of the mutations in zebra fish showed variable defects of thyroid development which were rescued by WT *jag1* transcripts (de Filippis et al. 2016). These findings suggest that JAG1 can contribute to the pathogenesis of thyroid dysgenesis (mainly thyroid hypoplasia).

BOREALIN. Borealin is a major component of the chromosomal passenger complex (CPC), expressed among other tissues in thyrocytes (Gassmann et al. 2004). The CPC has well-known functions in mitosis. Whole-exome sequencing of familial cases of TD has showed biallelic (homozygous) mutations of the *BOREALIN* gene in two cases from one consanguineous family and monoallelic

mutations in two other sporadic cases of CH with TD (out of 134 cases sequenced) (Carrè et al. 2017) highlighting the role of this gene in TD.

NTN1. Netrin1 is a protein involved in the regulation of various developmental processes including angiogenesis, non-neuronal cell migration, and epithelial morphogenesis (Cirulli and Yebra 2007; Levy-Strumpf and Culotti 2014). Deletion in the Netrin-1 gene (*NTN1*) has been reported in one patient with congenital heart and thyroid disorder (ectopy) (Opitz et al. 2015). Studies in zebra fish have shown that *ntn1a* and *ntn1b* are not expressed in thyroid tissue, but *ntn1a* is expressed in pharyngeal arch mesenchyme (Opitz et al. 2015); *ntn1a*-deficient zebra fish embryos display defective aortic arch artery formation and abnormal thyroid morphogenesis. The abnormal thyroid morphogenesis results from a lack of proper guidance exerted by the dysplastic vasculature of *ntn1a*-deficient embryos. These findings suggest that, as for other congenital disorders such as Kallmann's syndrome (Van Vliet and Deladoëy 2015), extrinsic signal during thyroid morphogenesis might contribute to the pathogenesis of TD.

Thyroid Dyshormonogenesis

Inborn errors of thyroid hormone biosynthesis (dyshormonogenesis) account for 15–20% of cases of CH (Grasberger and Refetoff 2011; Targovnik et al. 2017). Thyroid dyshormonogenesis is caused by mutations in genes involved in one of the steps of thyroid hormone synthesis. Mutations of distinct genes are responsible for iodine transport defect (*NIS/SLC5A5*), iodine organification defects (*TPO*, *DUOX2*, *DUOXA2*, *SLC26A4/PDS*), thyroglobulin (TG) synthesis or transport defects, and intrathyroidal iodine recycling (IYD/DEHAL1) impairment (Grasberger and Refetoff 2011; Targovnik et al. 2017). With the exception of disorders related to *DUOX2* and *IYD* mutations, all forms of dyshormonogenesis are inherited in an autosomal recessive fashion.

Goiter is a frequent finding in dyshormonogenesis. It can be present already in utero or can develop postnatally, especially when diagnosis and treatment are delayed, but is a rare finding in newborns detected by neonatal screening. Except for deafness, no other malformations are associated with dyshormonogenesis (see below).

Radioiodine uptake and perchlorate discharge test (PDT) can help with the differential diagnosis of dyshormonogenetic defects (Fig. 1). The PDT allows detection of partial iodide organification defects (PIOD, iodide discharge between 10% and 90%) and total iodide organification defects (TIOD, iodide discharge >90%). Measurement of serum TG levels represents a further diagnostic tool.

Mutations in the SLC5A5 (NIS) gene and iodine transport defect (ITD). The sodium-iodide symporter (NIS) is a transmembrane glycoprotein mediating the active uptake of iodide through the basolateral membrane of thyrocytes (Dai et al. 1996). Mutations in *SLC5A5* cause congenital ITD (Spitzweg and Morris, 2010), an uncommon form of dyshormonogenetic CH (the incidence is unknown). Affected patients suffer from hypothyroidism and goiter. The severity and onset of hypothyroidism correlate with the residual function of the mutated NIS and range from a severe hypothyroidism diagnosed at birth to a mild hypothyroidism diagnosed during

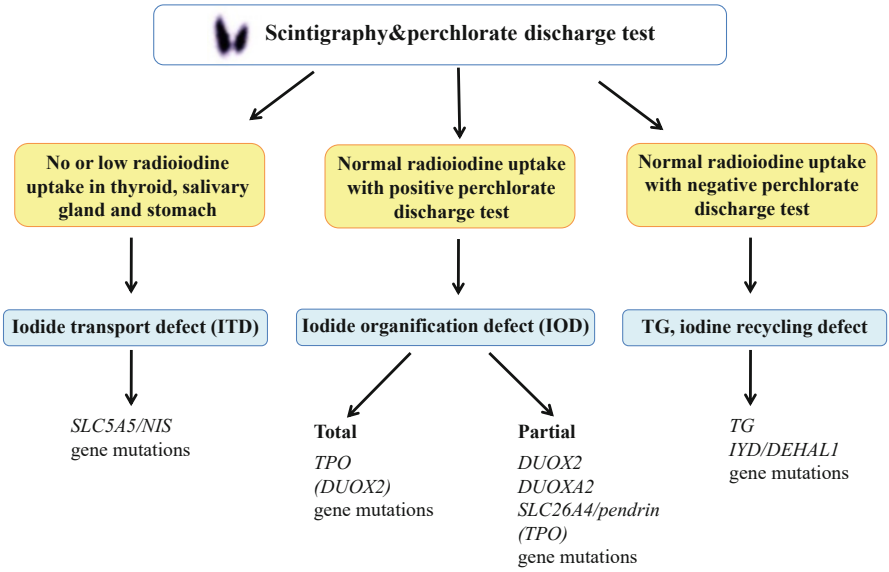

Fig. 1 Differential diagnosis of dyshormonogenetic defects based on radioiodine uptake and the perchlorate discharge test with the possible genetic defects involved (Modified from Szinnai 2014a)

infancy or childhood. In ITD, thyroid scintigraphy shows blunted or absent radioiodide uptake leading to a misdiagnosis of athyreosis, especially when goiter is absent or missed or serum TG levels are not measured. Reduced uptake in salivary glands and gastric mucosa (where NIS is also expressed), a low iodide saliva to plasma ratio, and detection of goiter by ultrasound allow diagnosing defects of *SLC5A5*.

Iodide supplementation at a supraphysiological dose has been proven to improve thyroid function in patients with residual NIS activity (Wolff 1983; Tonacchera et al. 2003) and should be considered when treating defects of NIS.

Mutations in the SLC26A4 gene and Pendred's syndrome. The *SLC26A4* gene encodes for pendrin (PDS), a multifunctional anion exchanger expressed at the apical membrane of thyrocytes in the inner ear and kidneys. In the thyrocytes PDS facilitates the passive efflux of iodide across the apical membrane into the follicular lumen. Inactivating mutations in the *SLC26A4* are linked to Pendred's syndrome (Everett et al. 1997). Pendred's syndrome is a rare cause of thyroid dyshormonogenesis characterized by the combination of congenital bilateral sensorineural hearing loss (associated with malformation of the cochlea), diffuse or multinodular goiter, hypothyroidism of different degrees, and usually PIOD. Pendred's syndrome is rarely detected by neonatal screening for CH, and patients are rather identified because of severe congenital deafness (Banghova et al. 2008). Goiter usually develops in late childhood or early adolescence. As for other dyshormonogenetic disorders, thyroid phenotype is influenced by the nutritional iodine intake, so that in condition of iodine sufficiency, about 90% of patients are euthyroid (Reardon et al. 1997).

Disorders caused by mutations in the thyroid peroxidase (TPO) gene. TPO is a membrane-bound thyroid-specific glycoprotein located at the apical membrane of thyrocytes. Using hydrogen peroxide as cofactor, TPO catalyzes two crucial steps of thyroid hormone synthesis: iodination of thyrosyl residues in TG to generate mono- and diiodotyrosines (MIT and DIT) and coupling of iodotyrosines to generate iodothyronines (T_4, T_3, and rT_3).

Biallelic inactivating mutations in the *TPO* gene are the most frequent cause of thyroid dyshormonogenesis with permanent CH (Ris-Stalpers and Bikker 2010). Characteristics of these disorders are severe CH associated to an intact iodide uptake, normal TG protein expression, and TIOD. Recently, patients with PIOD and TPO defects have also been reported (Szinnai 2014a). Goiter is a frequent finding, sometimes detected by ultrasound in utero. The latter finding may prompt to perform an in utero treatment aiming at preventing dystocia related to large goiters and improving growth and neurological development. The treatment consists of intra-amniotic injection of L-T_4 (Agrawal et al. 2002). However, experience with this procedure is limited and at risk of provoking premature labor or infections.

Disorders due to DUOX2 and DUOXA2 mutations. The Dual oxidase 2 (*DUOX2*) gene encodes for a protein located at the apical membrane of thyrocytes involved in the Ca2-reduced nicotinamide adenine dinucleotide phosphate-dependent generation of H_2O_2. Hydrogen peroxide is an essential electron acceptor for the iodination and coupling reactions mediated by TPO. Mutations in the *DUOX2* gene cause transient or permanent CH associated with PIOD (Grasberger 2010; Muzza and Fugazzola 2017); TIOD has been reported only in single cases (Muzza and Fugazzola 2017). Characteristic of these disorders is the detection of low FT_4 and high TSH serum levels at the first postnatal serum sampling, despite borderline blood spot TSH (Muzza et al. 2014). In contrast with most of the dyshormonogenetic defects, a single *DUOX2* allele mutation suffices to cause CH. Specifically, monoallelic *DUOX2* mutations cause mild CH, while biallelic mutations can lead to mild to severe CH. Transient CH has been linked to monoallelic as well as biallelic mutations (De Marco et al. 2011; Tonacchera et al. 2009). The reason for the variable expressivity of *DUOX2* defects is not known, but the influence of genetic background and the different iodine intake in the affected subjects are possible explanations. Evidence for a role of iodine intake in the expressivity of *DUOX2* mutations is provided by the case of two siblings harboring the same compound heterozygous mutations. One boy was affected by CH, whereas the other, exposed to iodine overload in the perinatal period, had normal thyroid function (Vigone et al. 2005). The recent finding of *DUOX2* mutations in children with CH and thyroid ectopy suggests that DUOX2, and specifically its N-terminal domain where mutations localize, might play a role in thyroid development (Kizys et al. 2017).

DUOXA2 mutations. The dual oxidase maturation factor 2 (DUOXA2) is the factor required to express DUOX2 enzymatic activity (Grasberger and

Refetoff 2006). The first mutation of *DUOXA2* gene was reported in 2008 in a Chinese girl with mild permanent CH and PIOD (Zamproni et al. 2008). The patient was homozygous for a nonsense mutation, while heterozygous carriers of the same mutation were euthyroid. Following the first report, *DUOXA2* mutations have been identified in four other CH patients (Sugisawa et al. 2017). The phenotype of DUOXA2 mutations is similar in all cases, being characterized by overt hypothyroidism with goiter at first presentation and subsequent improvement of the thyroid hormone-producing capacity with age. Of note, in the last reported case, the proband's brother harboring the same homozygous mutation was unaffected, suggesting that the phenotype of *DUOXA2* gene mutations could be broader than currently recognized (Sugisawa et al. 2017).

Disorders caused by TG gene mutations. Thyroglobulin (TG) is a large glycoprotein functioning as a matrix for thyroid hormone synthesis and storage of the inactive form of thyroid hormones and iodine. TG is synthesized by thyrocytes and excreted mostly into the follicular lumen and in small amounts into the bloodstream. Biallelic *TG* gene mutations lead to CH, usually severe, associated with high iodide uptake, normal organification of iodide, and very low/undetectable serum TG levels (Targovnik et al. 2010, 2011). Goiter is present at birth or appears shortly after birth. Following the first report of a TG mutation in 1991, 117 deleterious mutations in the human *TG* gene have so far been reported (Targovnik et al. 2017). *TG* mutations cause CH by reducing TG synthesis or altering the protein structure with consequent alteration of protein folding or assembly and biosynthesis of thyroid hormone. TG defects are a relatively common cause of thyroid dyshormonogenesis with an estimated incidence of approximately 1 in 100,000 newborns (Targovnik et al. 2010, 2011).

Defects in recycling of intrathyroidal iodine caused by mutation in IYD gene. The iodotyrosine deiodinase (IYD, also known as DEHAL1) is the enzyme deiodinating MIT and DIT to recycle iodine within the thyrocytes. Besides the thyroid it is also expressed in the liver and kidney.

The first patients with IYD defect were described in the 1950s–1960s and presented with hypothyroidism, goiter, and mental retardation. They showed a rapid and high initial radioiodine uptake followed by a relatively rapid decline of the accumulated iodine and a negative perchlorate discharge test (Hutchison and McGirr 1954; Murray et al. 1965). Subsequently IYD mutations have been reported in subjects from consanguineous families of Turkish, Scottish, and Moroccan descent suffering from goitrous hypothyroidism negative at neonatal screening for CH (Moreno et al. 2008; Afink et al. 2008). In contrast to other dyshormonogenetic disorders, some heterozygous family members developed goiter and hypothyroidism during follow-up (Afink et al. 2008). High urinary MIT and DIT detected by high-performance liquid chromatography and tandem mass spectrometry is diagnostic for IYD defects (Moreno et al. 2008).

Regarding treatment, supplementation with high dose of iodine may be used to correct the thyroid function alterations related to IYD deficiency (Hirsch et al. 1986).

Secondary or Central Congenital Hypothyroidism (CCH)

CCH is a rare form of permanent CH due to hypothalamic or pituitary defects, characterized by low serum FT_4 levels and inappropriately low or normal serum TSH levels. Few screening programs measure total or free T_4 and TSH (simultaneously or stepwise) enabling detection of CCH (Zwaveling-Soonawala et al. 2015). Due to the diverse screening strategies used in the different countries, the incidence of CCH is variable worldwide being about 1:100,000 in the United States, 1:30,000 in Japan, and 1:16,000 in the Netherlands (Vigone et al. 2015).

Congenital forms of central hypothyroidism can be isolated, if the defect is limited to thyrotroph function (isolated TSH deficiency), or associated with combined pituitary hormone deficiency (CPHD).

The molecular mechanisms underlying CCH are still largely unknown. At present, isolated TSH deficiency has been associated with inactivating mutations in four genes: *TSH β-subunit (TSHβ)*, *thyrotropin-releasing hormone receptor* (*TRH-R*), *immunoglobulin superfamily member 1* (*IGSF1)*, and *transducin β-like protein 1X (TBL1X)* genes (Beck-Peccoz et al. 2017). Most patients described with isolated CCH harbor defects in *TSHβ*and *IGSF1* genes. *TRH-R* mutations have so far been reported in only four families (Beck-Peccoz et al. 2017; García et al. 2017). The inheritance is recessive in all cases with *TSH β* and *TRH-R* mutations. Patients with *TSHβ* mutations present with severe isolated central hypothyroidism of neonatal onset and pituitary hyperplasia. Serum levels of TSH are low or normal, while levels of α-glycoprotein hormone subunit (α-GSU) are high.

TRH receptor mutations manifest with isolated central hypothyroidism with apparently uneventful infantile development and with childhood to adulthood onset (growth retardation). Levels of TSH are normal and a TRH test shows blunted TSH and prolactin response.

In 2012, loss-of-function mutations of *IGSF1* were identified as an X-linked cause of central hypothyroidism associated with macroorchidism in 50% of cases (Joustra et al. 2016). *IGSF1* encodes for the plasma membrane glycoprotein IGSF1, which is highly expressed in the pituitary and testis. IGSF1 might be involved in pituitary paracrine regulation, although its specific function remains largely unknown.

Missense mutations in the *TBL1X* gene have recently been reported in eight patients (six men and two women) with CCH and hearing loss (Heinen et al. 2016). *TBL1X* encodes for transducin β-like protein 1X. TBL1X is an essential subunit in the complex formed by NCOR (also known as N-CoR1) and SMRT (also known as N-CoR2), which is the major thyroid hormone receptor corepressor involved in T_3-regulated gene expression. The NCOR-SMRT complex activates the transcription of negatively regulated genes in the absence of T_3. As a consequence, defects in this complex result in decreased *TRH* and *TSHβ* transcription.

CPHD is due to mutations in genes encoding for transcription factors involved in pituitary development and differentiation such as *POU1F*, *PROP1*, *HESX1*, *LHX3*, *LHX4*, *SOX3*, *OTX2*, and *LEPR* (Beck-Peccoz et al. 2017). In addition to the features related to multiple hormone deficiency (i.e., hypoglycemia due to GH and/or ACTH/

cortisol deficiency and micropenis and undescended testes due to LH/FSH deficiencies), features of midline defects such as decreased vision or nystagmus (syndrome of septooptic dysplasia) can be present in the affected patients.

Peripheral CH Due to Reduced Sensitivity to Thyroid Hormone

Uncommon causes of CH include defects in thyroid hormone cell membrane transport (mutations in *the monocarboxylate transporter 8*, *MCT8*), metabolism (mutations in *the selenocysteine insertion sequence-binding protein 2*, *SBP2*), and nuclear action (mutations in *the thyroid hormone receptor α and β genes*, *THRA and THRB*) (Dumitrescu and Refetoff 2015; Visser et al. 2013). The clinical, genetic, and laboratory characteristics of these defects are shown in Table 2. As most routine neonatal screening programs are based on the determination of TSH, MCT8 defects are rarely identified at birth. In neonatal screening programs based on T_4

Table 2 Clinical, genetic and laboratory characteristics of the different forms of peripheral CH due to reduced sensitivity to thyroid hormone (modified from Dumitrescu and Refetoff 2015)

		Phenotype	
Level of defect	**Gene involved**	**Laboratory**	**Clinical**
Monocarboxylate transporter 8 (MCT8) defect	*MCT8* (*SLC16A2*) gene X-chromosome linked	High T_3, low rT_3 and T_4, normal or slightly elevated TSH	Hypotonia with poor head control; spastic quadriplegia; no speech, or dysarthria; mental retardation not walking, or rarely ataxic gate; paroxysmal dyskinesia, seizures; low BMI; hypermetabolism
Selenocysteine insertion sequence binding protein 2 (SBP2) defect	*SBP2* (*SECISBP2*) gene Recessive	High T_4 and rT_3 low T_3, normal or slightly elevated TSH	Growth retardation azoospermia; delayed bone maturation; hearing impairment; photosensitivity immunodeficiency; myopathy; delayed developmental milestones
Resistance to thyroid hormone (RTH)	*THRB* gene Dominant negative Rarely recessive	High serum FT_4, FT_3 and rT_3 non suppressed TSH, high TG	Goiter, tachycardia attention deficit hyperactivity disorder (ADHD)
RTH alpha1	*THRA* gene	Low serum T4/T3 ratio; low rT3	Cognitive impairment; constipation; anemia; short lower limbs; delayed closure of skull sutures; delayed bone and dental development; skeletal dysplasia; macrocephaly; seizures; placid behavior

measurements, a low concentration could potentially identify new cases. The same holds true for the other forms of reduced sensitivity to thyroid hormone. A limited neonatal survey by measuring blood T_4 concentration suggested the occurrence of RTHβ of one case per 40,000 live births (LaFranchi et al. 2003; Tajima et al. 2009). The incidence of all the other forms is not known.

Transient Congenital Hypothyroidism

The prevalence of transient CH varies depending on the iodine status being higher in the areas of more severe iodine deficiency. Indeed, it is more common in Europe (1:100) than in the United States (1:50,000) (Gaudino et al. 2005). Studies conducted in the last years in Europe have shown that one-third of patients with CH and normally located glands may have transient thyroid dysfunction (Eugster et al. 2004; Gaudino et al. 2005; Castanet et al. 2015).

Transient CH has been associated with transplacental transfer of antithyroid drugs used to treat maternal hyperthyroidism (Rastogi and LaFranchi 2010; Gaudino et al. 2005); in this case hypothyroidism lasts for a few days to 2 weeks after birth. Prematurity and perinatal iodine deficiency or overload are other causes of transient CH. Iodine overload can be due to maternally administered drugs rich in iodine, such as amiodarone, or maternal or neonatal iodine exposure through skin disinfection. Exposure to high amounts of iodine leads to hypothyroidism because in the neonatal thyroid gland, the escape from the Wolff-Chaikoff effect can be delayed (Connely et al. 2012; Theodoropoulos et al. 1979). Iodine contamination is nowadays generally avoided during obstetric procedures, and iodine has been removed from antiseptics used in most intensive care nurseries.

Transplacental passage of thyrotropin receptor-blocking antibodies (TRB-Abs) is another well known and relatively rare cause of transient CH. The incidence of CH due to TRB-Abs is approximately 1:100,00 neonates (Brown et al. 1993). Maternal antithyroid antibodies can cross the placenta and block the TSH receptor in the neonatal thyroid. This condition is characterized by the coexistence of maternal autoimmune thyroid disease (i.e., atrophic autoimmune thyroiditis), the finding of more than one affected infant born to the mother, presence of goiter in the infant, and hypothyroidism resolving over 3 to 6 months as the maternal antibody is cleared (LaFranchi 1999).

Congenital liver hemangiomas producing large amounts of the enzyme type 3 iodothyronine deiodinase have been reported to cause a consumptive type of congenital hypothyroidism. Serum T_4 levels are low, whereas TSH and reverse T_3 levels are increased. Hypothyroidism resolves after tumor treatment (Huang et al. 2000).

Mutations of the *DUOX2* gene can account for some cases of transient CH (Muzza and Fugazzola 2017).

As shown by recent studies (Gaudino et al. 2005; Castanet et al. 2015; Mitchell et al. 2011), however, all the above-listed risk factors (see also Table 3) can explain only a small fraction of cases of transient CH, while most remain unexplained.

Table 3 Etiology of transient CH

Maternal intake of antithyroid drugs
Maternal or neonatal excess iodine exposure
Maternal or neonatal iodine deficiency
Transplacental passage of maternal TSH receptor blocking antibodies (TRB-abs) Prematurity, IUGR, UTI
DUOX2 gene mutations
Congenital hepatic hemangioma/hemangioendothelioma

Since it can be difficult during the neonatal period to differentiate permanent from transient hypothyroidism, replacement therapy with levothyroxine ($L\text{-}T_4$) should be started in all neonates with confirmed hypothyroidism (see section "Treatment and Monitoring of CH"). Diagnostic reevalution should be performed at 3 years of age to distinguish between permanent and transient CH. Reevaluation can be anticipated at the end of the first or second year of life in patients with a high suspicion of transient CH (exposure to excess iodine, maternal antithyroid antibodies) and/or in those not requiring L-T4 dose adjustment during the first months of life.

Diagnosis

Symptoms and Signs

The clinical symptoms and signs of CH include prolonged neonatal jaundice, lethargy, hypotonia, macroglossia, umbilical hernia, not waking for feeds, poor and slow feeding, cold extremities, dry skin with or without a coarse/puffy face, hoarse cry, and constipation (Table 4). Persistence of the posterior fontanelle, a large anterior fontanelle, and a wide sagittal suture can be other signs of the disease reflecting delayed bone maturation. A few infants with thyroid dyshormonogenesis may also present with a palpable goiter and with goiter and deafness in the case of Pendred's syndrome. However, most of these manifestations are not present at birth, and clinical features are often initially subtle and nonspecific. This is in part due to the protective effect of maternal thyroid hormone, which crosses the placenta until the end of pregnancy, and in part to some functioning thyroid tissue being present in the most common form of CH.

If CH remains untreated, the clinical manifestations become more evident in the second half of the first year of life, with growth retardation and delay in motor development. In addition to the delay in the development of motor skills, intellectual disability is the most relevant and devastating clinical feature of CH, as it is not reversible.

A rare manifestation of long-standing CH is the Kocher-Debre-Semelaigne (DKS) syndrome consisting of generalized muscular hypertrophy, involving particularly the calf, giving the child a "Herculean" appearance (Tashko et al. 1999).

Table 4 Symptoms of CH present at the time of diagnosis by neonatal screening

Prolonged jaundice
Feeding difficulties
Lethargy
Umbilical hernia
Macroglossia
Constipation
Cold or mottled skin
Hypothermia
Abnormal cry (hoarse cry)
Periorbital edema
Enlarged anterior and posterior fontanelle
Hypotonia

Cramps, muscle pain, and stiffness can also be parts of the syndrome. This form of myopathy resolves with thyroid hormone treatment (Tashko et al. 1999). The KDS syndrome has become a rare finding in CH, restricted to countries where hypothyroidism remains endemic.

Almost 10% of neonates affected by CH present with other congenital malformations. Of these, congenital heart defects are the most common, occurring in up to 50% of patients (Olivieri et al. 2002). Other associated malformations include genitourinary malformations, neurologic abnormalities, cleft palate, and spiky hair (Olivieri et al. 2002; Law et al. 1998; Kumar et al. 2009). CH can be part of rare genetic syndromes, such as the Bamforth-Lazarus syndrome, the brain-lung-thyroid syndrome, and the pseudohypoparathyroidism (see also the "Thyroid Dysgenesis" section). Clinical symptoms of an underlying syndrome usually precede diagnosis of CH.

CH is also more frequent in children with Down's and Williams-Beuren's syndromes (Cutler et al. 1986; Stagi et al. 2008).

Newborn Thyroid Screening Tests

To allow an early diagnosis of CH, in the mid-1970s, tests for hypothyroidism have been added to existing neonatal screening programs for congenital disorders in most industrialized countries (Dussault 1999; Rastogi and LaFranchi 2010; Rose et al. 2006; Van Vliet and Czernichow 2004). Screening programs for CH have been developed in Canada, the United States, parts of Mexico, Western Europe, Japan, Australia, New Zealand, and Israel, while they are still under development in parts of Eastern Europe, Asia, South America, and Africa.

The screening for CH is performed on whole blood samples collected in the first days after birth. The best "window" for testing is 48 to 72 hours after birth because earlier measurement of TSH has a high frequency of false-positive results due to the physiological neonatal TSH surge that occurs at birth.

Blood is spotted onto filter paper, allowed to dry, and eluted into a buffer for TSH and/or T_4 analysis.

Three screening strategies have been followed (Mitchell and Larson 2003): (1) a primary T_4 method with confirmatory TSH testing in infants with a T_4 below a selected cutoff, more common in North America; (2) a primary TSH method, more common in Europe, Japan, and Oceania; and (3) a combination of a primary T_4 and a primary TSH method. Each approach has advantages and disadvantages.

Primary T_4 screening has a low specificity because hypothyroxinemia is common in preterm infants, in ill neonates, and in congenital defects of the thyroxine binding globulin (TBG, 1/4.000 live births), but it can detect some infants with secondary or central hypothyroidism and infants with delayed TSH rise.

Primary TSH screening is the most sensitive test for detecting primary CH, even mild or "subclinical." Serum TSH levels as well as log TSH are in fact inversely proportional to the FT_4 concentration; therefore, small changes in FT_4 are reflected in large changes in serum TSH.

Neither TSH nor FT_4 methods will detect all infants with defects of thyroid transport, metabolism, or action (see the "Peripheral CH Due to Reduced Sensitivity to Thyroid Homone" section). In order to detect all of these disorders, pilot programs measuring both T_4 and TSH have been undertaken in some countries (van Tijn et al. 2005).

Each newborn screening program sets cutoffs for test results. Generally, if the screening T_4 is below the 10th percentile and/or TSH is greater than the specific cutoff of the screening center, an infant is recalled for serum testing. In cases with "intermediate results," e.g., low T_4 but TSH below cutoff, a second heel prick screening specimen is recommended.

Cutoff values for TSH have changed over the years passing from 20–40 mU/l followed at introduction of neonatal screening to 10–7 mU/l in the last years (Lain et al. 2017). Predictably, an increase in the incidence of primary CH occurs when cutoff levels for TSH are lowered (Corbetta et al. 2009; Mengreli et al. 2010), with an increased detection of milder forms. These new screening strategies pose some still unsolved issues regarding the increase in false-positive tests and the benefits and costs related to the early detection and treatment of mild forms of CH (LaFranchi 2011). On the other hand, lowering TSH cutoff may enable the detection of additional cases of permanent CH, some of which have defects of thyroid development and severe hypothyroidism at confirmation of the diagnosis (Olivieri et al. 2013; Rabbiosi et al. 2013).

In a subgroup of neonates, primary CH may be masked by reduced levels of TSH at screening due to hypothalamic-pituitary immaturity, fetal blood mixing in multiple births, serious neonatal illnesses, and administration of some medications such as dopamine and glucocorticoids. Therefore, a multiple sampling strategy consisting of repetition of spot TSH test at 2 and/or 4 weeks of life has been recommended for preterm newborns, babies with low or very low birth weight, neonates from multiple births, and sick newborns admitted to a neonatal intensive care unit (Léger et al. 2014).

Confirmatory Serum Thyroid Testing

Infants with abnormal screening test results should undergo confirmation of the diagnosis by measurement of TSH, T_4, either total or free T_4, and T_3 on a serum sample (LaFranchi 1999). The obtained results have to be compared with age-normal reference ranges. In the first few days of life, because of the TSH surge occurring shortly after birth, serum TSH can be as high as 39 mU/ml (Rastogi and LaFranchi 2010). At 1 to 2 weeks of life, when confirmatory serum tests are usually obtained, the upper TSH range falls to approximately 10 mUL (Elmlinger et al. 2001). As for TSH, levels of thyroid hormones are higher in the first days of life, falling closer to the levels typically seen in infancy by 2–4 weeks of age (Elmlinger et al. 2001).

Elevated serum TSH levels and a low free or total T_4 at recall test confirm the diagnosis of primary hypothyroidism. The finding of an elevated serum TSH associated with a normal free or total T_4 is consistent with subclinical primary hypothyroidism or hyperthyrotropinemia.

The screening programs that undertake a primary T_4 test and recall infants with persistently low T_4 levels detect some infants with central hypothyroidism. Confirmatory serum testing will show a low free or total T_4, with either a low TSH or an "inappropriately normal" TSH level.

Low levels of T_4 in the presence of a non-elevated TSH can also be found in TBG deficiency. In this X-liked disorder, serum testing will show a low total T_4 but normal free T_4 (Pappa et al. 2015). The diagnosis of the disorder can be confirmed by finding a low serum TBG levels. Affected infants are euthyroid and do not require replacement therapy.

Diagnosis of the Underlying Etiology and Severity of CH

Once the diagnosis of congenital hypothyroidism is confirmed, a clinical evaluation, imaging studies, and some additional laboratory tests are required to determine the underlying etiology.

It is recommended to collect information about family history, maternal history (maternal thyroid diseases, drugs and medications taken, iodine supply), pregnancy and labor features, and personal clinical history (birth weight, gestational age, perinatal features, chromosome disorders, exposure to drugs/substances interfering with thyroid function).

Imaging studies used for the diagnosis of CH include thyroid scintigraphy and ultrasound. Thyroid scintigraphy, using either 10–20 MBq of technetium-99 m (^{99m}Tc) or 1–2 MBq of iodine-123 (^{123}I) (I-131 delivers a higher dose to the thyroid and total body and should not be used in newborns/children), is the most informative diagnostic test for thyroid dysgenesis (Fig. 2). Scintigraphy should not be considered when it can delay the start of therapy or when a CH due to excess iodine intake through exposure or to transplacental passage of TRB-Ab is suspected. In fact, in the latter conditions, scintigraphy may show no uptake despite the presence of a eutopic

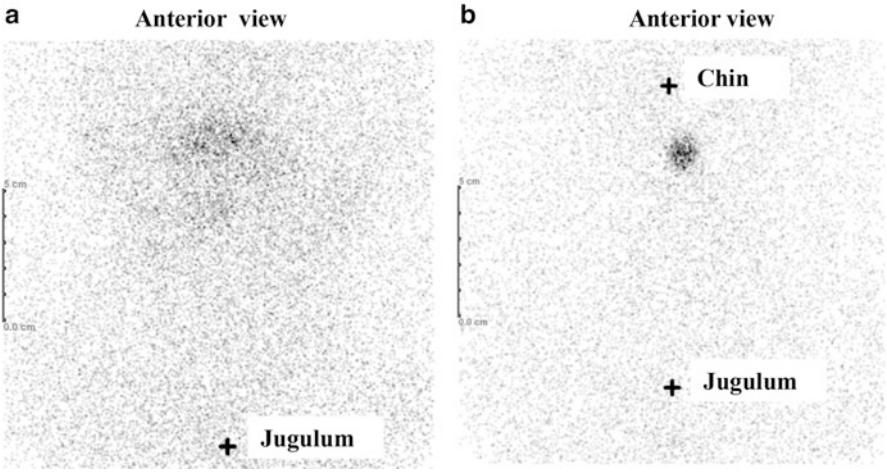

Fig. 2 Scintigraphic images (tracer iodine-123) of thyroid agensis (**a**) and ectopy (**b**)

thyroid gland. The same holds true for inactivating mutations in the *TSHr* and the *NIS* genes.

When a defect in thyroid hormone biosynthesis is suspected, scintigraphy can be combined with a perchlorate discharge test to demonstrate a defective oxidation and organification of iodide. However, this investigation can be postponed until reevaluation of the initial diagnosis, which is usually performed at 3 years of age.

Thyroid ultrasound is a noninvasive method enabling to detect the presence or absence of thyroid tissue in the neck. However, it cannot always detect lingual and sublingual thyroid ectopy (Karakoc-Aydiner et al. 2012), although the use of color Doppler facilitates the identification of thyroid tissue by demonstrating marked increases in blood flow (Ohnishi et al. 2003). An experienced investigator is strongly recommended as the interpretation of ultrasound features may be difficult at the neonatal age; often, nonthyroidal hyperechoic tissue in the thyroid fossa can be misdiagnosed as a dysplastic thyroid gland in situ (Jones et al. 2010).

Detection of serum TG may be useful to confirm the presence of thyroid tissue and to distinguish between TG defects, with undetectable/low serum TG, and other causes of hypothyroidism with in situ gland. Serum TG levels reflect the amount of thyroid tissue and generally are elevated when TSH is elevated.

Maternal autoimmune thyroid disease raises the possibility of an antibody-mediated congenital hypothyroidism. This can be confirmed by determination of TRB-Ab in mother and newborn.

Measurement of urinary iodine excretion in a single urine sample can be useful to detect iodine excess in case of infants with a history of iodine overload.

Finally, an X-ray or ultrasound examination of the knee can be performed at birth to evaluate bone maturation as indicators of prenatal CH severity (Leshem et al. 2002).

Treatment and Monitoring of CH

The main goal of the treatment of CH is to achieve, in all affected children, a normal growth and psychomotor and cognitive development (Mitchell and Larson 2003; LaFranchi 1999). To accomplish this goal, the thyroid defect has to be diagnosed and treated as soon as possible after birth. Recent studies suggest that a treatment within 7–14 days of life with appropriate doses of L-T_4 can rapidly normalize TSH and FT_4 and restore a normal intellectual outcome even in individuals with severe CH (Tillotson et al. 1994; Salerno et al. 2002; Bongers-Schokking et al. 2000).

L-T4 is the treatment of choice for CH; T_4 is converted in peripheral tissues and in the central nervous system (SNC) into the biologically active T_3; in particular, most brain T_3 derives from local deiodination of circulating T_4 rather than from circulating T_3. Therefore, combined treatment with T_4 plus T_3 has been shown not to significantly improve the outcome of CH (Cassio et al. 2003; Grozinsky-Glasberg et al. 2006).

To properly treat CH, some peculiarities of replacement therapy with L-T_4 in newborns have to be considered: (1) thyroid hormone requirement in newborns is much higher than in adults; (2) the primary goal of L-T_4 therapy is to rapidly normalize serum TSH and FT_4; and (3) children with CH show a relative resistance of TSH to the negative feedback exerted by thyroid hormone (Fisher et al. 2000; Bagattini et al. 2014). This resistance is as more severe as more delayed is the start of treatment with L-T_4 in the neonatal period. As a consequence, to obtain normal serum TSH levels in children with CH, serum FT_4 levels have to be maintained at the upper limit of the normal range.

Initial Treatment: Doses and Methods of Administration of L-T_4

The recent Consensus Guidelines of the European Society for Paediatric Endocrinology suggest that if TSH spot concentrations are $\geq$40 mU/L, treatment should be started as soon as a good venous sample can be obtained, without waiting for the venous blood test result, unless thyroid function test (TFT) results are available on the same day (Léger et al. 2014). If TSH spot concentrations are $<$40 mU/L, the clinician may wait for the results of TFT, provided that these results are available on the following day.

An initial daily L-T4 dose of 10–15 μg/kg (according to the prenatal severity of CH, i.e., by using the highest end of the dosage range in more severe forms) is recommended (Léger et al. 2014). The optimal starting dose of L-T_4 for children with CH has been controversial for many years. A meta-analysis published in 2001 (Hrytsiuk et al. 2002) showed that high starting doses of L-T_4 did not improve neurocognitive outcome but rather might increase the risk of behavioral problems in school-age children (Hrytsiuk et al. 2002; Rovet 2004). However, subsequent studies have shown a significant improvement of neurocognitive outcome in children with severe CH (or treated after the second week of life) treated with higher starting doses ($>$9.5–13.5 μg/kg/day) compared with those treated with lower doses

(Salerno et al. 2002; Bongers-schokking et al. 2000). Moreover, other studies considering neuropsychological follow-up in CH children treated with high doses of L-T_4 seem to exclude behavioral problems (Kooistra et al. 2004; Bongers-Schokking 2005).

L-T_4 should be administered orally or, if oral administration is not possible, it can be administered *iv* at a dose of no more than 80% of the oral dose. L-T_4 is usually administered in tablet form. The tablets can be crushed and administered via a small spoon, with suspension in a few ml of water or milk. The intake of substances that can interfere with L-T_4 absorption (i.e., soy, fiber, calcium, and iron) at the time of L-T_4 administration should be avoided (Rastogi and LaFranchi 2010). There is also a liquid form of L-T_4. The liquid form allows an easier administration; however, the bioequivalence between the two formulations and the possible side effects related to the use of ethanol as excipient of liquid form have not been extensively studied yet, and studies are still discordant (Cassio et al. 2013; Peroni et al. 2014).

Treatment Monitoring

Hormonal Evaluation

L-T_4 dose should be adjusted according to periodic TSH and FT_4 determinations, maintaining TSH in the age-specific reference range and serum FT_4 concentrations in the upper half of the age-specific reference range. In the presence of a prolonged TSH suppression and an increase in FT_4, suggestive of overtreatment, dose of L-T_4 should be rapidly decreased to avoid acceleration of growth and skeletal maturation, premature cranial suture fusion, and behavioral problems.

The first follow-up examination should be 1 to 2 weeks after the start of L-T_4 treatment (especially in children treated with high doses and/or in those with mild forms, to avoid phases of overtreatment); subsequently, every 2 weeks, until TSH levels are completely normalized; and thereafter every 1 to 3 months, until the age of 12 months (Léger et al. 2014). Between the ages of 1 and 3 years, children should undergo clinical and laboratory evaluations every 2 to 4 months, with regular evaluations every 3 to 12 months thereafter until growth is completed (Table 5). Measurements should be performed more frequently in case of low adherence to treatment and 4 to 6 weeks after any change in L-T_4 dose or formulation (Léger et al. 2014).

Growth Evaluation

Clinical and auxological parameters should be routinely assessed in CH children during follow-up.

Neuropsychological Development Evaluation

A normal neuropsychological development should be assessed at least once between 3 and 5 years of age by testing balance, limb coordination, fine motor development, and head movement control (Bargagna et al. 2000). At the same age, language development should also be tested. If neuropsychological or language deficits are observed, a rehabilitative program in preschool age is recommended (Bargagna et al. 1999).

Table 5 Treatment monitoring: FT_4 and TSH evaluation according to *"the European Society for Paediatric Endocrinology Consensus Guidelines 2014"* (Léger et al. 2014)

1 to 2 wks after the beginning of L-T4 therapy
Every 1–3 months in the first 12 months
Every 2–4 months between 1 and 3 yrs. of age
Every 3–12 months to end of growth
4–6 weeks after any change of therapy

Reevaluation of the Diagnosis

Diagnostic reevaluation of the thyroid axis is generally performed at 2–3 years of age to distinguish between permanent and transient CH in patients with in situ thyroid in whom no permanent cause of CH was found at diagnosis and in cases in which no etiological diagnostic assessment was carried out at the start of therapy. Reevaluation can be performed at the end of the first or the second year of life in neonates with positive TRB-Ab and/or in children who have required no increase in L-T_4 dose since infancy.

Reevaluation is not indicated when thyroid dysgenesis has been conclusively shown on imaging or (with the exception of DUOX2 mutations) when dyshormonogenesis has been confirmed by molecular genetic testing.

If reevaluation aims at identifying the precise etiology, L-T_4 therapy should be phased out over a 4- to 6-week period and a full reevaluation carried out at the end of this period, with biochemical testing and thyroid imaging. Complete evaluation includes assessment of TSH, FT_4, and TG and thyroid ultrasound evaluation. Scintigraphy should be done during reevaluation if not performed in the neonatal period, whereas ^{123}I scintiscan with perchlorate discharge test and/or analysis of genes related to CH (see above) should be reserved for selected cases (in situ normal-sized or hyperplastic thyroid gland, positive family history of thyroid disorders, elevated TG at diagnosis).

If it is important to establish the presence or absence of primary hypothyroidism rather than to obtain an exact diagnosis, L-T_4 dose may be decreased by 30% for 2 to 3 weeks. An increase in TSH concentration to $\geq$10 mU/L during this period confirms that CH is permanent (Léger et al. 2014).

Outcomes of Treated CH

Neuropsychological and Cognitive Outcomes

After implementation of the neonatal screening program and early treatment of CH, severe neuropsychological, cognitive, and growth deficits related to CH have disappeared. The mean global IQ in large cohorts of children with CH is now 10 to 30 points higher than in the prescreening era (Grosse and Van Vliet 2011).

However, some affected patients still have neurocognitive and behavioral sequelae of CH persisting into adolescence and adulthood. These have been related to disease severity and therefore associated to athyreosis, absence of knee epiphyses at birth, very low T4, and very high TSH concentrations at diagnosis (Dimitropoulos et al. 2009; Kempers et al. 2007; Rovet and Ehrlich 2000, Rovet 2005). Studies have also showed that cognitive outcome is related to age at treatment and L-T_4 dose (Selva et al. 2005) and to the parents' socioeducational status (Tillotson et al. 1994). No increase in the risk of attention deficit hyperactivity disorder is found in patients with CH, but they may suffer from sustained attention problems related to episodes of overtreatment (Alvarez et al. 2010) and, in more severe cases, can present with slower information processing (Oerbeck et al. 2007).

Lastly, subtle and specific memory deficits and reduced hippocampal volumes (Wheeler et al. 2011) as well as fine motor impairment (Hauri-Hohl et al. 2011) may be observed in some patients.

Hearing, Visual, and Verbal Development

Hearing defects may result from lack of thyroid hormone which plays a role in cochlear development and auditory function during development (François et al. 1994; Rovet et al. 1996). Hearing loss is reported in about 30–50% of patients with CH born before the introduction of neonatal screening, whose L-T_4 replacement treatment began late. However, a higher prevalence of hearing impairment in childhood and adult life has also been observed in patients with CH identified at neonatal screening and treated early, although reports are conflicting (Léger 2015). A French nationwide study of 1202 young adults with CH has recently shown that a significantly higher proportion of the CH population than the general population have hearing impairment (9.5% vs. 2.5%) at a median age of 23.4 years (Lichtenberger-Geslin et al. 2013). The hearing loss was mostly detected at a median age of 7 years and was bilateral, mild to moderate, and of the sensorineural type. In the study, hearing impairment was associated with severity and type of CH, patients with athyreosis and gland in situ being more frequently affected than those with thyroid ectopy.

An undiagnosed hearing impairment may alter speech development, school performance, and social interactions (Bess et al. 1998). Therefore, early and regular evaluations of hearing acuity, starting in childhood before school age and continuing throughout childhood and early adulthood, are advisable in the CH population (Léger et al. 2014).

Visual processing problems have also been described in patients affected by CH and born after implementation of neonatal screening (Zoeller and Rovet 2004).

Growth, Puberty, and Fertility

Thyroid hormone is essential for growth and bone maturation during postnatal life and for bone maturation during fetal development as well. Hypothyroidism retards

bone maturation, decreases growth velocity, and impairs height growth. Early and appropriate replacement treatment can prevent all these detrimental effects (Léger and Czernichow 1989). Indeed, linear growth and final height are reported as normal in children with CH adequately treated from the first weeks of life (Salerno et al. 2001). No relationship has been found between growth pattern and severity of CH at diagnosis or L-T_4 starting dose. Age at starting therapy, adequacy of treatment, and familial genetic growth potential are the major factors determining final height in the CH patients (Salerno et al. 2001).

Hypothyroidism can impair puberty and fecundity through different pathogenetic mechanisms (Anasti et al. 1995; Nelson et al. 2011). It can cause precocious puberty with macroorchidism in boys and bilateral ovary enlargement with multicystic ovaries in girls. Replacement therapy improves or normalizes these alterations (Krassas et al. 2010). Provided treatment is adequate, onset of puberty, age at menarche, and menstrual cycles are reported normal in CH patients (Salerno et al. 2001).

Fecundity is generally normal and only slightly decreased in the most severely affected female patients because of a possible detrimental effect of severe hypothyroidism on the reproductive tract during fetal development (Hassani et al. 2012).

Bone Health

Thyroid hormone is an important regulator of skeletal development and adult bone mass and strength maintenance (Bassett and Williams 2016). Overtreatment with L-T_4 increases bone resorption, leading to bone loss. Only a few studies have evaluated the impact of long-term L-T_4 treatment on bone mineral density. These studies report a normal bone mineral density in children as well as in young adults with CH treated early in life with L-T_4 (Leger et al. 1997; Salerno et al. 2004). As in a healthy population, weight and current intake of calcium seem to be major determinants of bone density in CH children. However, there are no data on patients treated with the currently used doses of L-T_4.

BMI and Metabolic and Cardiovascular Health

In healthy subjects, body mass index (BMI) increases rapidly in infancy and peaks at around the first year of life. After the first BMI peak, BMI decreases gradually and reaches a nadir on average at around 5 years of age (Chen and Chang 2010; Whitaker et al. 1998). The subsequent rise is defined as the adiposity rebound, which if it occurs earlier is associated with higher BMI in later life (Whitaker et al. 1998).

An earlier adiposity rebound and a higher risk of being overweight or obese have been reported in children and adolescents affected by CH (Wong et al. 2004; Livadas et al. 2007; Chen et al. 2013). As a consequence, CH patients are at risk of developing metabolic alterations. Therefore, in affected subjects, weight should be monitored and lifestyle interventions, including diet and exercise, encouraged.

Besides the higher risk of congenital heart malformations associated with congenital thyroid defects (Olivieri et al. 2002), young adults affected by CH show a slight increase in cardiovascular abnormalities related to treatment inadequacy (Oliviero et al. 2010; Salerno et al. 2008).

The cardiovascular system is sensitive to thyroid hormone action (Klein and Ojamaa 2001), and overt as well as subclinical thyroid dysfunctions have been associated with a wide spectrum of cardiovascular alterations, leading to atherosclerosis, myocardial infarction, and cardiovascular death (Biondi et al. 2002; Fatourechi 2001; Lillevang-Johansen et al. 2017; Thvilum et al. 2013).

During long-term L-T_4 replacement therapy, CH patients often present with subclinical thyroid dysfunction, namely, serum TSH concentration above or below the normality range. This finding is commonly attributed to both the need to maintain serum TSH levels within the normal range at the expense of increased FT_4 levels (see also the "Treatment" section) and, particularly during adolescence, to inadequate compliance with treatment.

Impairment of diastolic function, reduction of exercise capacity and cardiopulmonary performance, and increased intima-media thickness are the cardiovascular abnormalities found in a group of young adult with CH (mean age 18.1 $\pm$ 0.2 year) treated with L-T_4 within the first month of life (Salerno et al. 2008). These abnormalities appear related to nonphysiological fluctuations of TSH levels during treatment, with episodes of subclinical hyperthyroidism and, more frequently, subclinical hypothyroidism (Salerno et al. 2008).

High TSH levels, inadequately corrected by L-T_4 therapy especially during puberty, have been shown to impair the elastic and functional vessel properties of adult CH patients as well (Oliviero et al. 2010).

Based on the aforementioned, an optimal treatment of CH is essential for cardiovascular health.

References

Afink G, Kulik W, Overmars H, de Randamie J, Veenboer T, van Cruchten A, Craen M, Ris-Stalpers C. Molecular characterization of iodotyrosine dehalogenase deficiency in patients with hypothyroidism. J Clin Endocrinol Metab. 2008;93:4894–901.

Agrawal P, Ogilvy-Stuart A, Lees C. Intrauterine diagnosis and management of congenital goitrous hypothyroidism. Ultrasound Obstet Gynecol. 2002;19(5):501–5.

Albright F, Burnett CH, Smith CH, et al. Pseudohypoparathyroidism: an example of "Seabright-Bantam syndrome". Endocrinology. 1942;30:922e32.

Alvarez M, Iglesias Fernández C, Rodríguez Sánchez A, Dulín Lñi- guez E, Rodríguez Arnao MD. Episodes of overtreatment during the first six months in children with congenital hypothyroidism and their relationships with sustained attention and inhibitory control at school age. Horm Res Paediatr. 2010;74:114–20.

Amendola E, De Luca P, Macchia PE, Terracciano D, Rosica A, Chiappetta G, Kimura S, Mansouri A, Affuso A, Arra C, Macchia V, Di Lauro R, De Felice M. A mouse model demonstrates a multigenic origin of congenital hypothyroidism. Endocrinology. 2005;146 (12):5038–47.

Anasti JN, Flack MR, Froehlich J, Nelson LM, Nisula BCA. potential novel mechanism for precocious puberty in juvenile hypothyroidism. J Clin Endocrinol Metab. 1995;80(1): 276–9.

Bagattini B, Cosmo CD, Montanelli L, Piaggi P, Ciampi M, Agretti P, Marco GD, Vitti P, Tonacchera M. The different requirement of L-T4 therapy in congenital athyreosis compared with adult-acquired hypothyroidism suggests a persisting thyroid hormone resistance at the hypothalamic-pituitary level. Eur J Endocrinol. 2014;171(5):615–21. https://doi.org/10.1530/EJE-14-0621.

Bamforth JS, Hughes IA, Lazarus JH, Weaver CM, Harper PS. Congenital hypothyroidism, spiky hair, and cleft palate. J Med Genet. 1989;26:49–51.

Banghova K, Al Taji E, Cinek O, Novotna D, Pourova R, Zapletalova J, Hnikova O, Lebl J. Pendred syndrome among patients with congenital hypothyroidism detected by neonatal screening: identification of two novel PDS/SLC26A4 mutations. Eur J Pediatr. 2008;167(7):777–83.

Bargagna S, Canepa G, Costagli C, Dinetti D, Marcheschi M, Millipiedi S, Montanelli L, Pinchera A, Chiovato L. Neuropsychological follow-up in early treated congenital hypothyroidism: a problem-oriented approach. Thyroid. 2000;10:243–9.

Bargagna S, Dinetti D, Pinchera A, Marcheschi M, Montanelli L, Presciuttini S, Chiovato L. School attainments in children with congenital hypothyroidism detected by neonatal screening and treated early in life. Eur J Endocrinol. 1999;140(5):407–13.

Bassett JH, Williams GR. Role of Thyroid Hormones in Skeletal Development and Bone Maintenance. Endocr Rev. 2016;37(2):135–87. https://doi.org/10.1210/er.2015-1106.

Beck-Peccoz P, Rodari G, Giavoli C, Lania A. Central hypothyroidism - a neglected thyroid disorder. Nat Rev Endocrinol. 2017;13(10):588–98. https://doi.org/10.1038/nrendo.2017.47.

Bernal J, Guadano-Ferraz A, Morte B. Perspectives in the study of thyroid hormone action on brain development and function. Thyroid. 2003;13:1005–12.

Bess FH, Dodd-Murphy J, Parker RA. Children with minimal sensorineural hearing loss: prevalence, educational performance, and functional status. Ear Hear. 1998;19:339–54.

Biondi B, Palmieri EA, Lombardi G, Fazio S. Effects of subclinical thyroid dysfunction on the heart. Ann Intern Med. 2002;137(11):904–14.

Bongers-Schokking JJ. de Muinck Keizer-Schrama SMPF.Influence and timing of the dose of thyroid hormone replacement on mental, psychomotor, and behavioral development in children with congenital hypothyroidism. J Pediatr. 2005;147:768–74.

Bongers-Schokking JJ, Koot HM, Wiersma D, Verkerk PH. de Muinck Keizer-Schrama SM. Influence of timing and dose of thyroid hormone replacement on development in infants with congenital hypothyroidism. J Pediatr. 2000;136(3):292–7.

Brown RS, Bellisario RL, Mitchell E, Keating P, Botero D. Detection of thyrotropin binding inhibitory activity in neonatal blood spots. J Clin Endocrinol Metab. 1993;77(4):1005–8.

Calebiro D, Gelmini G, Cordella D, Bonomi M, Winkler F, Biebermann H, de Marco A, Marelli F, Libri DV, Antonica F, Vigone MC, Cappa M, Mian C, Sartorio A, Beck-Peccoz P, Radetti G, Weber G, Persani L. Frequent TSH receptor genetic alterations with variable signaling impairment in a large series of children with nonautoimmune isolated hyperthyrotropinemia. J Clin Endocrinol Metab. 2012;97(1):E156–60.

Carré A, Stoupa A, Kariyawasam D, Gueriouz M, Ramond C, Monus T, Léger J, Gaujoux S, Sebag F, Glaser N, Zenaty D, Nitschke P, Bole-Feysot C, Hubert L, Lyonnet S, Scharfmann R, Munnich A, Besmond C, Taylor W, Polak M. Mutations in BOREALIN cause thyroid dysgenesis. Hum Mol Genet. 2017;26(3):599–610. https://doi.org/10.1093/hmg/ddw419.

Carré A, Hamza RT, Kariyawasam D, Guillot L, Teissier R, Tron E, Castanet M, Dupuy C, El Kholy M, Polak M. A novel FOXE1 mutation (R73S) in Bamforth-Lazarus syndrome causing increased thyroidal gene expression. Thyroid. 2014;24(4):649–54. https://doi.org/10.1089/thy.2013.0417.

Carré A, Szinnai G, Castanet M, Sura-Trueba S, Tron E, Broutin-L'Hermite I, Barat P, Goizet C, Lacombe D, Moutard ML, Raybaud C, Raynaud-Ravni C, Romana S. YthierH, Léger J, Polak M. Five new TTF1/NKX2.1 mutations in brain-lung-thyroid syndrome: rescue by

PAX8 synergism in one case. Hum Mol Genet. 2009;18(12):2266–76. https://doi.org/10.1093/hmg/ddp162.

Cassio A, Monti S, Rizzello A, Bettocchi I, Baronio F, D'Addabbo G, Bal MO, Balsamo A. Comparison between liquid and tablet formulations of levothyroxine in the initial treatment of congenital hypothyroidism. J Pediatr. 2013;162(6):1264–9, 1269.e1–2. https://doi.org/10.1016/j.jpeds.2012.11.070.

Cassio A, Cacciari E, Cicognani A, Damiani G, Missiroli G, Corbelli E, Balsamo A, Bal M, Gualandi S. Treatment for congenital hypothyroidism: thyroxine alone or thyroxine plus triiodothyronine? Pediatrics. 2003;111:1055–60.

Castanet M, Goischke A, Léger J, Thalassinos C, Rodrigue D, Cabrol S, Zenaty D, al-Harbi M, Polak M, Czernichow P, Fédération Parisienne pour le Dépistage et la Prévention des Handicaps de l"Enfant (FDPHE). Natural history and management of congenital hypothyroidism with in situ thyroid gland. Horm Res Paediatr. 2015;83(2):102–10. https://doi.org/10.1159/000362234.

Castanet M, Sura-Trueba S, Chauty A, Carré A, de Roux N, Heath S, Léger J, Lyonnet S, Czernichow P, Polak M. Linkage and mutational analysis of familial thyroid dysgenesis demonstrate genetic heterogeneity implicating novel genes. Eur J Hum Genet. 2005;13(2):232–9.

Castanet M, Polak M, Bonaïti-Pellié C, Lyonnet S, Czernichow P, Léger J, AFDPHE (Association Française pour le Dépistage et la Prévention des Handicaps de l'Enfant). Nineteen years of national screening for congenital hypothyroidism: familial cases with thyroid dysgenesis suggest the involvement of genetic factors. J Clin Endocrinol Metab. 2001;86(5):2009–14.

Chen SY, Lin SJ, Lin SH, Chou YY. Early adiposity rebound and obesity in children with congenital hypothyroidism. Pediatr Neonatol. 2013;54(2):107–12. https://doi.org/10.1016/j.pedneo.2012.10.008.

Chen W, Chang MH. New growth charts for Taiwanese children and adolescents based on World Health Organization standards and health-related physical fitness. Pediatr Neonatol. 2010;51:69–79.

Cirulli V, Yebra M. Netrins: beyond the brain. Nat Rev Mol Cell Biol. 2007;8(4):296–306.

Clifton-Bligh RJ, Wentworth JM, Heinz P, Crisp MS, John RLJ, Ludgate M, Chatterjee VK. Mutation of the gene encoding human TTF-2 associated with thyroid agenesis, cleft palate and choanal atresia. Nat Genet. 1998;1:399–401.

Connelly KJ, Boston BA, Pearce EN, Sesser D, Snyder D, Braverman LE, Pino S, LaFranchi SH. Congenital hypothyroidism caused by excess prenatal maternal iodine ingestion. J Pediatr. 2012;161(4):760–2. https://doi.org/10.1016/j.jpeds.2012.05.057.

Corbetta C, Weber G, Cortinovis F, Calebiro D, Passoni A, Vigone MC, Beck-Peccoz P, Chiumello G, Persani L. A 7-year experience with low blood TSH cutoff levels for neonatal screening reveals an unsuspected frequency of congenital hypothyroidism (CH). Clin Endocrinol. 2009;71(5):739–45. https://doi.org/10.1111/j.1365-2265.2009.03568.x.

Cutler AT, Benezra-Obeiter R, Brink SJ. Thyroid function in young children with Down syndrome. Am J Dis Child. 1986;140(5):479–83.

Dai G, Levy O, Carrasco N. Cloning and characterization of the thyroid iodide transporter. Nature. 1996;379(6564):458–60.

De Felice M, Di Lauro R. Thyroid development and its disorders: genetics and molecular mechanisms. Endocr Rev. 2004;25(5):722–46.

de Filippis T, Marelli F, Nebbia G, et al. JAG1 Loss-Of-Function Variations as a Novel Predisposing Event in the Pathogenesis of Congenital Thyroid Defects. J Clin Endocrinol Metab. 2016;101(3):861–70.

de Filippis T, Marelli F, Vigone MC, Di Frenna M, Weber G, Persani L. Novel NKX2-1 Frameshift Mutations in Patients with Atypical Phenotypes of the Brain-Lung- Thyroid Syndrome. Eur Thyroid J. 2014;3(4):227–33.

De Marco G, Agretti P, Montanelli L, Di Cosmo C, Bagattini B, De Servi M, Ferrarini E, Dimida A, Freitas Ferreira AC, Molinaro A, Ceccarelli C, Brozzi F, Pinchera A, Vitti P, Tonacchera M.

Identification and functional analysis of novel dual oxidase 2 (DUOX2) mutations in children with congenital or subclinical hypothyroidism. J Clin Endocrinol Metab. 2011;96(8):E1335–9. https://doi.org/10.1210/jc.2010-2467.

Deladoëy J, Ruel J, Giguère Y, Van Vliet G. Is the incidence of congenital hypothyroidism really increasing? A 20-year retrospective population based study in Québec. J Clin Endocrinol Metab. 2011;96:2422–9.

Dentice M, Cordeddu V, Rosica A, Ferrare M, Santarpia L, Salvatore D, Chiovato L, Perri A, Moschini L, Fazzini C, Olivieri A, Costa P, Stoppioni V, Baserga M, De Felice M, Sorcini M, Fenzi G, Di Lauro R, Tartaglia M, Macchia PE. Missense mutation in the transcription factor NKX2–5: a novel molecular event in the pathogenesis of thyroid dysgenesis. J Clin Endocrinol Metab. 2006;91:1428–33.

Devos H, Rodd C, Gagné N, Laframboise R, Van Vliet G. A search for the possible molecular mechanisms of thyroid dysgenesis: sex ratios and associated malformations. J Clin Endocrinol Metab. 1999;84(7):2502–6.

Devriendt K, Vanhole C, Matthijs G, de Zegher F. Deletion of thyroid transcription factor-1 gene in an infant with neonatal thyroid dysfunction and respiratory failure. N Engl J Med. 1998;338:1317–8.

Dimitri P. The role of GLIS3 in thyroid disease as part of a multisystem disorder. Best Pract Res Clin Endocrinol Metab. 2017;31(2):175–82. https://doi.org/10.1016/j.beem.2017.04.007.

Dimitri P, Habeb AM, Garbuz F, Millward A, Wallis S, Moussa K, et al. Expanding the Clinical Spectrum Associated With GLIS3 Mutations. J Clin Endocrinol Metab. 2015;100(10):E1362–9. https://doi.org/10.1210/jc.2015-1827.

Dimitri P, Warner JT, Minton JA, Patch AM, Ellard S, Hattersley AT, Barr S, Hawkes D, Wales JK, Gregory JW. Novel GLIS3 mutations demonstrate an extended multisystem phenotype. Eur J Endocrinol. 2011;164(3):437–43. https://doi.org/10.1530/EJE-10-0893.

Dimitropoulos A, Molinari L, Etter K, Torresani T, Lang-Muritano M, Jenni OG, Largo RH, Latal B. Children with congenital hypothyroidism: long-term intellectual outcome after early high-dose treatment. Pediatr Res. 2009;65:242–8. https://doi.org/10.1203/PDR.0b013e31818d2030.

Dumitrescu AM, Refetoff S. Impaired sensitivity to thyroid hormone: defects of transport, metabolism and action. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, Koch C, Korbonits M, McLachlan R, New M, Purnell J, Rebar R, Singer F, Vinik A, editors. Endotext [Internet]. South Dartmouth: MDText.com, Inc.; 2015. 20 Aug 2015.

Dussault JH. The anecdotal history of screening for congenital hypothyroidism. J Clin Endocrinol Metab. 1999;84(12):4332–4.

Elmlinger MW, Kühnel W, Lambrecht HG, Ranke MB. Reference intervals from birth to adulthood for serum thyroxine (T4), triiodothyronine (T3), free T3, free T4, thyroxine binding globulin (TBG) and thyrotropin (TSH). Clin Chem Lab Med. 2001;39(10):973–9.

Eugster EA, LeMay D, Zerin JM, Pescovitz OH. Definitive diagnosis in children with congenital hypothyroidism. J Pediatr. 2004;144:643–7.

Everett LA, Glaser B, Beck JC, Idol JR, Buchs A, Heyman M, Adawi F, Hazani E, Nassir E, Baxevanis AD, Sheffield VC, Green ED. Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). Nat Genet. 1997;17(4):411–22.

Fatourechi V. Adverse effects of subclinical hyperthyroidism. Lancet. 2001;358(9285):856–7.

François M, Bonfils P, Leger J, Czernichow P, Narcy P. Role of congenital hypothyroidism in hearing loss in children. J Pediatr. 1994;124:444–6.

Farfel Z, Brothers VM, Brickman AS, Conte F, Neer R, Bourne HR. Pseudohypoparathyroidism: inheritance of deficient receptor-cyclase coupling activity. Proc Natl Acad Sci U S A. 1981;78(5):3098–102.

Fisher DA, Schoen EJ, La Franchi S, Mandel SH, Nelson JC, Carlton EI, Goshi JH. The hypothalamic-pituitary-thyroid negative feedback control axis in children with treated congenital hypothyroidism. J Clin Endocrinol Metab. 2000;85:2722–7.

García M, González de Buitrago J, Jiménez-Rosés M, Pardo L, Hinkle PM, Moreno JC. Central Hypothyroidism Due to a TRHR Mutation Causing Impaired Ligand Affinity and Transactivation of Gq. J Clin Endocrinol Metab. 2017;102(7):2433–42. https://doi.org/10.1210/jc.2016-3977.

Gaudino R, Garel C, Czernichow P, Léger J. Proportion of various types of thyroid disorders among newborns with congenital hypothyroidism and normally located gland: a regional cohort study. Clin Endocrinol. 2005;62(4):444–8.

Grant DB, Smith I. Survey of neonatal screening for primary hypothyroidism in England, Wales, and Northern Ireland 1982-4. Br Med J (Clin Res Ed). 1988;296(6633):1355–8.

Gras D, Jonard L, Roze E, Chantot-Bastaraud S, Koht J, Motte J, Rodriguez D, Louha M, Caubel I, Kemlin I, Lion-François L, Goizet C, Guillot L, Moutard ML, Epaud R, Héron B, Charles P, Tallot M, Camuzat A, Durr A, Polak M, Devos D, Sanlaville D, Vuillaume I, Billette de Villemeur T, Vidailhet M, Doummar D. Benign hereditary chorea: phenotype, prognosis, therapeutic outcome and long term follow-up in a large series with new mutations in the TITF1/NKX2-1 gene. J Neurol Neurosurg Psychiat. 2012;83(10):956–62. https://doi.org/10.1136/jnnp-2012-302505.

Grasberger H, Refetoff S. Resistance to thyrotropin. Best Pract Res Clin Endocrinol Metab. 2017;31(2):183–94. https://doi.org/10.1016/j.beem.2017.03.004.

Grasberger H, Refetoff S. Genetic causes of congenital hypothyroidism due to dyshormonogenesis. Curr Opin Pediatr. 2011;23(4):421–8. https://doi.org/10.1097/MOP.0b013e32834726a4.

Grasberger H. Defects of thyroidal hydrogen peroxide generation in congenital hypothyroidism. Mol Cell Endocrinol. 2010;322(1–2):99–106. https://doi.org/10.1016/j.mce.2010.01.029.

Grasberger H, Refetoff S. Identification of the maturation factor for dual oxidase. Evolution of an eukaryotic operon equivalent. J Biol Chem. 2006;281(27):18269–72.

Gassmann R, Carvalho A, Henzing AJ, Ruchaud S, Hudson DF, Honda R, Nigg EA, Gerloff DL, Earnshaw WC. Borealin: a novel chromosomal passenger required for stability of the bipolar mitotic spindle. J Cell Biol. 2004;166(2):179–91.

Grosse SD, Van Vliet G. Prevention of intellectual disability through screening for congenital hypothyroidism: how much and at what level? Arch Dis Child. 2011;96:374–9.

Grozinsky-Glasberg S, Fraser A, Nahshoni E, Weizman A, Leibovici L. Thyroxine-triiodothyronine combination therapy versus thyroxine monotherapy for clinical hypothyroidism: meta-analysis of randomized controlled trials. J Clin Endocrinol Metab. 2006;91:2592–9.

Harris KB, Pass KA. Increase in congenital hypothyroidism in New York State and in the United States. Mol Genet Metab. 2007;91(3):268–77. Epub 2007 May 23. Erratum in: Mol Genet Metab. 2008 May;94(1):140.

Hassani Y, Larroque B, Dos Santos S, Ecosse E, Bouyer J, Léger J. Fecundity in young adults treated early for congenital hypothyroidismis related to the initial severity of the disease: a longitudinal population-based cohort study. J Clin Endocrinol Metab. 2012;97:1897–904.

Hauri-Hohl A, Dusoczky N, Dimitropoulos A, Leuchter RH, Molinari L, Caflisch J, Jenni OG, Latal B. Impaired neuromotor outcome in school-age children with congenital hypothyroidism receiving early high-dose substitution treatment. Pediatr Res. 2011;70:614–8. https://doi.org/10.1203/PDR.0b013e3182321128.

Heinen CA, Losekoot M, Sun Y, Watson PJ, Fairall L, Joustra SD, Zwaveling-Soonawala N, Oostdijk W, van den Akker EL, Alders M, Santen GW, van Rijn RR, Dreschler WA, Surovtseva OV, Biermasz NR, Hennekam RC, Wit JM, Schwabe JW, Boelen A, Fliers E, van Trotsenburg AS. Mutations in TBL1X Are Associated With Central Hypothyroidism. J Clin Endocrinol Metab. 2016;101(12):4564–73.

Hinton CF, Harris KB, Borgfeld L, Drummond-Borg M, Eaton R, Lorey F, Therrell BL, Wallace J, Pass KA. Trends in incidence rates of congenital hypothyroidism related to select demographic factors: data from the United States, California, Massachusetts, New York, and Texas. Pediatrics. 2010;125(Suppl 2):S37–47. https://doi.org/10.1542/peds.2009-1975D.

Hirsch HJ, Shilo S, Spitz IM. Evolution of hypothyroidism in familial goitre due to deiodinase deficiency: report of a family and review of the literature. Postgrad Med J. 1986;62(728):477–80.
Hrytsiuk I, Gilbert R, Logan S, Pindoria S, Brook CG. Starting dose of levothyroxine for the treatment of congenital hypothyroidism: a systematic review. Arch Pediatr Adolesc Med. 2002;146:485–91.
Huang SA, Tu HM, Harney JW, Venihaki M, Butte AJ, Kozakewich HP, Fishman SJ, Larsen PR. Severe hypothyroidism caused by type 3 iodothyronine deiodinase in infantile hemangiomas. N Engl J Med. 2000;343(3):185–9.
Hutchison JH, McGirr EM. Hypothyroidism as an inborn error of metabolism. J Clin Endocrinol. 1954;14:869–86.
Italian National Registry of Infants with Congenital Hypothyroidism (INRICH). http://www.iss.it/rnic/. Accessed 8 Feb 2013.
Jones JH, Attaie M, Maroo S, Neumann D, Perry R, Donaldson MD. Heterogeneous tissue in the thyroid fossa on ultrasound in infants with proven thyroid ectopia on isotope scan—a diagnostic trap. Pediatr Radiol. 2010;40:725–31.
Joustra SD, Heinen CA, Schoenmakers N, Bonomi M, Ballieux BE, Turgeon MO, Bernard DJ, Fliers E, van Trotsenburg AS, Losekoot M, Persani L, Wit JM, Biermasz NR, Pereira AM, Oostdijk W. IGSF1 Clinical Care Group. IGSF1 Deficiency: Lessons From an Extensive Case Series and Recommendations for Clinical Management. J Clin Endocrinol Metab. 2016;101(4):1627–36. https://doi.org/10.1210/jc.2015-3880.
Jurkiewicz D, Gliwicz D, Ciara E, Gerfen J, Pelc M, Piekutowska-Abramczuk D, Kugaudo M, Chrzanowska K, Spinner NB, Krajewska-Walasek M. Spectrum of JAG1 gene mutations in Polish patients with Alagille syndrome. J Appl Genet. 2014;55(3):329–36. https://doi.org/10.1007/s13353-014-0212-2.
Karakoc-Aydiner E, Turan S, Akpinar I, Dede F, Isguven P, Adal E, Guran T, Akcay T, Bereket A. Pitfalls in the diagnosis of thyroid dysgenesis by thyroid ultrasonography and scintigraphy. Eur J Endocrinol. 2012;166:43–8. https://doi.org/10.1530/EJE-11-0140.
Kempers MJ, van der Sluijs Veer L, Nijhuis-van der Sanden RW, Lanting CI, Kooistra L, Wiedijk BM, Last BF, de Vijlder JJ, Grootenhuis MA, Vulsma T. Neonatal screening for congenital hypothyroidism in The Netherlands: cognitive and motor outcome at 10 years of age. J Clin Endocrinol Metab. 2007;92:919–24.
Kim YS, Nakanishi G, Lewandoski M, Jetten AM. GLIS3, a novel member of the GLIS subfamily of Kruppel-like zinc finger proteins with repressor and activation functions. Nucleic Acids Res. 2003;31(19):5513–25.
Kimura S, Hara Y, Pineau T, Fernandez-Salguero P, Fox CH, Ward JM, Gonzalez FJ. The T/ebp null mouse: thyroid-specific enhancer-binding protein is essential for the organogenesis of the thyroid, lung, ventral forebrain, and pituitary. Genes Dev. 1996;10:60–9.
Kizys MML, Louzada RA, Mitne-Neto M, Jara JR, Furuzawa GK, de Carvalho DP, Dias-da-Silva MR, Nesi-França S, Dupuy C, Maciel RMB. DUOX2Mutations Are Associated With Congenital Hypothyroidism With Ectopic Thyroid Gland. J Clin Endocrinol Metab. 2017;102(11):4060–71. https://doi.org/10.1210/jc.2017-00832.
Klein I, Ojamaa K. Thyroid hormone and the cardiovascular system. N Engl J Med. 2001;344(7):501–9.
Kleinau G, Neumann S, Grüters A, Krude H, Biebermann H. Novel insights on thyroid-stimulating hormone receptor signal transduction. Endocr Rev. 2013;34:691–724.
Knobel M, Medeiros-Neto G. An outline of inherited disorders of the thyroid hormone generating system. Thyroid. 2003;13(8):771–801.
Kooistra L, Vulsma T, van der Meere J. An investigation of impulsivity in children with early-treated congenital hypothyroidism. Dev Neuropsychol. 2004;26:595–610.
Krassas GE, Poppe K, Glinoer D. Thyroid function and human reproductive health. Endocr Rev. 2010;31(5):702–55. https://doi.org/10.1210/er.2009-0041. Epub 2010 Jun 23.
Krude H, Schutz B, Bibermann H, von Moers A, Schnabel D, Neitzel H, Tonnies H, Weise D, Lafferty A, Schwarz S, De Felice M, von Deimling A, van Landeghem F, Di Lauro R,

Grueters A. Choreoathetosis, hypothyroidism, and pulmonary alterations due to human NKX2-1 haploinsufficiency. J Clin Invest. 2002;109:475–80.
Kumar J, Gordillo R, Kaskel FJ, Druschel CM, Woroniecki RP. Increased prevalence of renal and urinary tract anomalies in children with congenital hypothyroidism. J Pediatr. 2009;154 (2):263–6. https://doi.org/10.1016/j.jpeds.2008.08.023. Epub 2008 Sep 27.
LaFranchi SH. Increasing incidence of congenital hypothyroidism: some answers, more questions. J Clin Endocrinol Metab. 2011;96(8):2395–7. https://doi.org/10.1210/jc.2011-1850.
LaFranchi SH, Snyder DB, Sesser DE, Skeels MR, Singh N, Brent GA, Nelson JC. Follow-up of newborns with elevated screening T4 concentrations. J Pediatr. 2003;143:296–301.
LaFranchi S. Congenital hypothyroidism: etiologies, diagnosis, and management. Thyroid. 1999;9 (7):735–40.
Lain S, Trumpff C, Grosse SD, Olivieri A, Van Vliet G. Are lower TSH cutoffs in neonatal screening for congenital hypothyroidism warranted? Eur J Endocrinol. 2017;177(5):D1–D12. https://doi.org/10.1530/EJE-17-0107.
Lazzaro D, Price M, De Felice M, Di Lauro R. The transcription factor TTF-1 is expressed at the onset of thyroid and lung morphogenesis and in restricted regions of the foetal brain. Development. 1991;113:1093–104.
Law WY, Bradley DM, Lazarus JH, John R, Gregory JW. Congenital hypothyroidism in Wales (1982-1993): demographic features, clinical presentation and effects on early neurodevelopment. Clin Endocrinol. 1998;48(2):201–7.
Léger J. Congenital hypothyroidism: a clinical update of long-term outcome in young adults. Eur J Endocrinol. 2015;172(2):R67–77. https://doi.org/10.1530/EJE-14-0777.
Léger J, Olivieri A, Donaldson M, Torresani T, Krude H, van Vliet G, Polak M, Butler G. ESPE-PES-SLEP-JSPE-APEG-APPES-ISPAE; Congenital Hypothyroidism Consensus Conference Group. European Society for Paediatric Endocrinology consensus guidelines on screening, diagnosis, and management of congenital hypothyroidism. J Clin Endocrinol Metab. 2014; 99(2):363–84. https://doi.org/10.1210/jc.2013-1891.
Léger J, Ruiz JC, Guibourdenche J, Kindermans C, Garabedian M, Czernichow P. Bone mineral density and metabolism in children with congenital hypothyroidism after prolonged L-thyroxine therapy. Acta Paediatr. 1997;86:704–10.
Léger J, Czernichow P. Congenital hypothyroidism: decreased growth velocity in the first weeks of life. Biol Neonate. 1989;55(4–5):218–23.
Leshem E, Bialik V, Hochberg Z. Ultrasonographic assessment of bone maturity in newborns. Horm Res. 2002;57(5–6):180–6.
Levine MA, Jap TS, Hung W. Infantile hypothyroidism in two sibs: an unusual presentation of pseudohypoparathyroidism type Ia. J Pediatr. 1985;107:919e22.
Levy-Strumpf N, Culotti JG. Netrins and Wnts function redundantly to regulate antero -posterior and dorso-ventral guidance in C. elegans. PLoS Genet. 2014;10(6):e1004381. https://doi.org/10.1371/journal.pgen.1004381. eCollection 2014 Jun.
Lichtenberger-Geslin L, Dos Santos S, Hassani Y, Ecosse E, Van Den Abbeele T, Leger J. Factors associated with hearing impairment in patients with congenital hypothyroidism treated since the neonatal period: a national population-based study. J Clin Endocrinol Metab. 2013;98:3644–52. https://doi.org/10.1210/jc.2013-1645.
Lillevang-Johansen M, Abrahamsen B, Jørgensen HL, Brix TH, Hegedüs L. Excess Mortality in Treated and Untreated Hyperthyroidism Is Related to Cumulative Periods of Low Serum TSH. J Clin Endocrinol Metab. 2017;102(7):2301–2309. https://doi.org/10.1210/jc.2017-00166.
Liu J, Erlichman B, Weinstein LS. The stimulatory G protein a subunit is imprinted in human thyroid glands: implications for thyroid function in pseudohypoparathyroidism type 1A and 1B. J Clin Endocrinol Metab. 2003;88:4336e41.
Livadas S, Magiakou MA, Mengreli C, Girginoudis P, Galani C, Smyrnaki P, Kanaka-Gantenbein C, Xekouki P, Chrousos GP, Dacou-Voutetakis C. Obesity and attenuated adiposity rebound in children with congenital hypothyroidism. Normalization of BMI values in adolescents. Horm Metab Res. 2007;39(7):524–8.

Lorey FW, Cunningham GC. Birth prevalence of primary congenital hypothyroidism by sex and ethnicity. Hum Biol. 1992;64(4):531–8.

Macchia PE, Lapi P, Krude H, Pirro MT, Missero C, Chiovato L, Souabni A, Baserga M, Tassi V, Pinchera A, Fenzi G, Gruters A, Busslinger M, Di Lauro R. PAX8 mutations associated with congenital hypothyroidism caused by thyroid dysgenesis. Nat Genet. 1998;19:83–6.

Mantovani G, Spada A, Elli FM. Pseudohypoparathyroidism and Gsα-cAMP-linked disorders: current view and open issues. Nat Rev Endocrinol. 2016;12(6):347–56. https://doi.org/10.1038/nrendo.2016.52.

Mantovani G, Bondioni S, Linglart A, Maghnie M, Cisternino M, Corbetta S, Lania AG, Beck-Peccoz P, Spada A. Genetic analysis and evaluation of resistance to thyrotropin and growth hormone-releasing hormone in pseudohypoparathyroidism type Ib. J Clin Endocrinol Metab. 2007;92:3738e42.

Mengreli C, Kanaka-Gantenbein C, Girginoudis P, Magiakou MA, Christakopoulou I, Giannoulia-Karantana A, Chrousos GP, Dacou-Voutetakis C. Screening for congenital hypothyroidism: the significance of threshold limit in false-negative results. J Clin Endocrinol Metab. 2010;95(9):4283–90. https://doi.org/10.1210/jc.2010-0057.

Mitchell ML, Hsu HW, Sahai I. Massachusetts Pediatric Endocrine Work Group.The increased incidence of congenital hypothyroidism: fact or fancy? Clin Endocrinol. 2011;75(6):806–10. https://doi.org/10.1111/j.1365-2265.2011.04128.x.

Mitchell ML, Larson CA. Neonatal screening for thyroid disease. In: Braverman LE, editor. Diseases of the thyroid. Humana Press, Totowa; 2003. p. 53–62.

Molinaro A, Tiosano D, Takatani R, Chrysis D, Russell W, Koscielniak N, Kottler ML, Agretti P, De Marco G, Ahtiainen P, Christov M, Mäkitie O, Tonacchera M, Jüppner H. TSH elevations as the first laboratory evidence for pseudohypoparathyroidism type Ib (PHP-Ib). J Bone Miner Res. 2015;30(5):906–12. https://doi.org/10.1002/jbmr.2408.

Moreno JC, Klootwijk W, van Toor H, et al. Mutations in the iodotyrosine deiodinase gene and hypothyroidism. N Engl J Med. 2008;358:1811–8.

Moreno LM, Mansilla MA, Bullard SA, Cooper ME, Busch TD, Machida J, Johnson MK, Brauer D, Krahn K, Daack-Hirsch S, L'heureux J, Valencia-Ramirez C, Rivera D, López AM, Moreno MA, Hing A, Lammer EJ, Jones M, Christensen K, Lie RT, Jugessur A, Wilcox AJ, Chines P, Pugh E, Doheny K, Arcos-Burgos M, Marazita ML, Murray JC, Lidral AC. FOXE1 association with both isolated cleft lip with or with- out cleft palate, and isolated cleft palate. Hum Mol Genet. 2009;18:4879–96.

Murray P, Thomson JA, McGirr EM, Wallace TJ, Macdonald EM, Maccabe HM. Absent and defective iodotyrosine deiodination in a family some of whose members are goitrous cretins. Lancet. 1965;1:183–5.

Muzza M, Fugazzola L. Disorders of H2O2 generation. Best Pract Res Clin Endocrinol Metab. 2017;31(2):225–40. https://doi.org/10.1016/j.beem.2017.04.006.

Muzza M, Rabbiosi S, Vigone M, Zamproni I, Cirello V, Maffini M, Maruca K, Schoenmakers N, Beccaria L, Gallo F, Park SM, Beck-Peccoz P, Persani L, Weber G, Fugazzola L. The clinical and molecular characterization of patients with dyshormonogeneic congenital hypothyroidism reveals specific diagnostic clues for DUOX2 defects. J Clin Endocrinol Metab. 2014;99:E544–53.

Nelson ER, Allan ER, Pang FY, Habibi HR. Auto-regulation of thyroid hormone receptors in the goldfish ovary and testis. Gen Comp Endocrinol. 2011;172(1):50–5. https://doi.org/10.1016/j.ygcen.2010.12.017.

Nicoletti A, Bal M, De Marco G, Baldazzi L, Agretti P, Menabò S, Ballarini E, Cicognani A, Tonacchera M, Cassio A. Thyrotropin-stimulating hormone receptor gene analysis in pediatric patients with non-autoimmune subclinical hypothyroidism. J Clin Endocrinol Metab. 2009;94(11):4187–94. https://doi.org/10.1210/jc.2009-0618.

Oerbeck B, Reinvang I, Sundet K, Heyerdahl S. Young adults with severe congenital hypothyroidism: cognitive event related potentials (ERPs) and the significance of an early start of thyroxine treatment. Scand J Psychol. 2007;48:61–7.

Ohnishi H, Sato H, Noda H, Inomata H, Sasaki N. Color Doppler ultrasonography: diagnosis of ectopic thyroid gland in patients with congenital hypothyroidism caused by thyroid dysgenesis. J Clin Endocrinol Metab. 2003;88:5145–9.

Olivieri A, Corbetta C, Weber G, Vigone MC, Fazzini C, Medda E, Italian Study Group for Congenital Hypothyroidism. Congenital hypothyroidism due to defects of thyroid development and mild increase of TSH at screening: data from the Italian National Registry of infants with congenital hypothyroidism. J Clin Endocrinol Metab. 2013;98(4):1403–8. https://doi.org/10.1210/jc.2012-3273. Epub 2013 Feb 26.

Olivieri A, Medda E, De Angelis S, Valensise H, De Felice M, Fazzini C, Cascino I, Cordeddu V, Sorcini M, Stazi MA, Study Group for Congenital Hypothyroidism. High risk of congenital hypothyroidism in multiple pregnancies. J Clin Endocrinol Metab. 2007;92(8):3141–7.

Olivieri A, Stazi MA, Mastroiacovo P, Fazzini C, Medda E, Spagnolo A, De Angelis S, Grandolfo ME, Taruscio D, Cordeddu V, Sorcini M, Study Group for Congenital Hypothyroidism. A population-based study on the frequency of additional congenital malformations in infants with congenital hypothyroidism: data from the Italian Registry for Congenital Hypothyroidism (1991-1998). J Clin Endocrinol Metab. 2002;87(2):557–62.

Oliviero U, Cittadini A, Bosso G, Cerbone M, Valvano A, Capalbo D, Apuzzi V, Calabrese F, Lettiero T, Salerno M. Effects of long-term L-thyroxine treatment on endothelial function and arterial distensibility in young adults with congenital hypothyroidism. Eur J Endocrinol. 2010;162:289–94. https://doi.org/10.1530/EJE-09-0674.

Opitz R, Hitz MP, Vandernoot I, Trubiroha A, Abu-Khudir R, Samuels M, Désilets V, Costagliola S, Andelfinger G, Deladoëy J. Functional zebrafish studies based on human genotyping point to netrin-1 as a link between aberrant cardiovascular development and thyroid dysgenesis. Endocrinology. 2015;156(1):377–88. https://doi.org/10.1210/en.2014-1628.

Ordookhani A, Mirmiran P, Moharamzadeh M, Hedayati M, Azizi FA. high prevalence of consanguineous and severe congenital hypothyroidism in an Iranian population. J Pediatr Endocrinol Metab. 2004;17(9):1201–9.

Pappa T, Ferrara AM, Refetoff S. Inherited defects of thyroxine-binding proteins. Best Pract Res Clin Endocrinol Metab. 2015;29(5):735–47. https://doi.org/10.1016/j.beem.2015.09.002.

Park SM, Chatterjee VK. Genetics of congenital hypothyroidism. J Med Genet. 2005;42(5):379–89.

Pasca di Magliano M, Di Lauro R, Zannini M. Pax8 has a key role in thyroid cell differentiation. Proc Natl Acad Sci U S A. 2000;97(24):13144–9.

Patten JL, Johns DR, Valle D, Eil C, Gruppuso PA, Steele G, Smallwood PM, Levine MA. Mutation in the gene encoding the stimulatory G protein of adenylate cyclase in Albright's hereditary osteodystrophy. N Engl J Med. 1990;322(20):1412–9.

Plachov D, Chowdhury K, Walther C, Simon D, Guenet JL, Gruss P. Pax8, a murine paired box gene expressed in the developing excretory system and thyroid gland. Development. 1990; 110(2):643–51.

Pearce MS, Korada M, Day J, Turner S, Allison D, Kibirige M, Cheetham TD. Increasing Incidence, but Lack of Seasonality, of Elevated TSH Levels, on Newborn Screening, in the North of England. J Thyroid Res. 2010;2010:101948. https://doi.org/10.4061/2010/101948.

Peroni E, Vigone MC, Mora S, Bassi LA, Pozzi C, Passoni A, Weber G. Congenital hypothyroidism treatment in infants: a comparative study between liquid and tabletformulations of levothyroxine. Horm Res Paediatr. 2014;81(1):50–4. https://doi.org/10.1159/000356047.

Perry R, Heinrichs C, Bourdoux P, Khoury K, Szöts F, Dussault JH, Vassart G, Van Vliet G. Discordance of monozygotic twins for thyroid dysgenesis: implications for screening and for molecular pathophysiology. J Clin Endocrinol Metab. 2002;87(9):4072–7.

Pinsker JE, Rogers W, McLean S, Schaefer FV, Fenton C. Pseudohypoparathyroidism type 1a with congenital hypothyroidism. J Pediatr Endocrinol Metab. 2006;19:1049–52.

Pohlenz J, Ahrens W, Hiort O. A new heterozygous mutation (L338N) in the human Gsa (GNAS1) gene as a cause for congenital hypothyroidism in Albright's hereditary osteodystrophy. Eur J Endocrinol. 2003;148:463e8.

Pohlenz J, Dimitrescu A, Zundel D, Martine U, Schonberger W, Koo E, Weiss RE, Cohen RN, Kimura S, Refetoff S. Partial deficiency of thyroid transcription factor 1 produces predominantly neurological defects in humans and mice. J Clin Invest. 2002;109:469–73.

Porazzi P, Marelli F, Benato F, de Filippis T, Calebiro D, Argenton F, Tiso N, Persani L. Disruptions of global and JAGGED1-mediatedNotch signaling affect thyroid morphogenesis in the zebrafish. Endocrinology. 2012;153(11):5645–58. https://doi.org/10.1210/en.2011-1888.

Porterfield SP, Hendrich CE. The role of thyroid hormones in prenatal and neonatal neurological development – current perspectives. Endocr Rev. 1993;14:94–106.

Postiglione MP, Parlato R, Rodriguez-Mallon A, Rosica A, Mithbaokar P, Maresca M, Marians RC, Davies TF, Zannini MS, De Felice M, Di Lauro R. Role of the thyroid-stimulating hormone receptor signalling in development and differentiation of the thyroid gland. Proc Natl Acad Sci U S A. 2002;99:15462–7.

Rabbiosi S, Vigone MC, Cortinovis F, Zamproni I, Fugazzola L, Persani L, Corbetta C, Chiumello G, Weber G. Congenital hypothyroidism with eutopic thyroid gland: analysis of clinical and biochemical features at diagnosis and after re-evaluation. J Clin Endocrinol Metab. 2013;98(4):1395–402. https://doi.org/10.1210/jc.2012-3174.

Radetti G, Fanolla A, Pappalardo L, Gottardi E. Prematurity may be a risk factor for thyroid dysfunction in childhood. J Clin Endocrinol Metab. 2007;92(1):155–9. Epub 2006 Oct 10.

Ramos HE, Carre A, Szinnai G, Tron E, Cerqueira TLO, Leger J, Cabrol S, Puel O, de Roux N, Polak M, Chevrier L, Castanet M. PAX8 mutations in congenital hypothyroidism: new evidence for phenotypic variability from normal to ectopic thyroid gland. 2013. https://endo.confex.com/endo/2013endo/webprogram/Paper5804.html.

Rapa A, Monzani A, Moia S, et al. Subclinical hypothyroidism in children and adolescents: a wide range of clinical, biochemical, and genetic factors involved. J Clin Endocrinol Metab. 2009;94(7):2414–20.

Rastogi MV, LaFranchi SH. Congenital hypothyroidism. Orphanet J Rare Dis. 2010;5:17. https://doi.org/10.1186/1750-1172-5-17.

Reamon-Buettner SM, Borlak J. NKX2-5: an update on this hypermutable homeodomain protein and its role in human congenital heart disease (CHD). Hum Mutat. 2010;31:1185–94.

Reardon W, Coffey R, Phelps PD, Luxon LM, Stephens D, Kendall-Taylor P, Britton KE, Grossman A, Trembath R. Pendred syndrome–100 years of underascertainment? QJM. 1997;90(7):443–7.

Ris-Stalpers C, Bikker H. Genetics and phenomics of hypothyroidism and goiter due to TPO mutations. Mol Cell Endocrinol. 2010;322(1–2):38–43. https://doi.org/10.1016/j.mce.2010.02.008.

Rose SR, Brown RS, Foley T, Kaplowitz PB, Kaye CI, Sundararajan S, Varma SK. Update of newborn screening and therapy for congenital hypothyroidism. Pediatrics. 2006;117(6):2290–303.

Rovet JF. Children with congenital hypothyroidism and their sib- lings: do they really differ? Pediatrics. 2005;115:e52–7.

Rovet JF. In search for the optimal therapy for congenital hypothyroidism. J Pediatr. 2004;144:698–700.

Rovet JF, Ehrlich R. Psychoeducational outcome in children with early-treated congenital hypothyroidism. Pediatrics. 2000;105:515–22.

Rovet J, Walker W, Bliss B, Buchanan L, Ehrlich R. Long-term sequelae of hearing impairment in congenital hypothyroidism. J Pediatr. 1996;128:776–83.

Salerno M, Oliviero U, Lettiero T, Guardasole V, Mattiacci DM, Saldamarco L, Capalbo D, Lucariello A, Saccà L, Cittadini A. Long-term cardiovascular effects of levothyroxine therapy in young adults with congenital hypothyroidism. J Clin Endocrinol Metab. 2008;93(7):2486–91. https://doi.org/10.1210/jc.2007-2315.

Salerno M, Lettiero T, Esposito-del Puente A, et al. Effect of long-term L-thyroxine treatment on bone mineral density in young adults with congenital hypothyroidism. Eur J Endocrinol. 2004;151:689–94.

Salerno M, Militerni R, Bravaccio C, Micillo M, Capalbo D, Di MS, Tenore A. Effect of different starting doses of levothyroxine on growth and intellectual outcome at four years of age in congenital hypothyroidism. Thyroid. 2002;12(1):45–52.

Salerno M, Micillo M, Di Maio S, Capalbo D, Ferri P, Lettiero T, Tenore A. Longitudinal growth, sexual maturation and final height in patients with congenital hypothyroidism detected by neonatal screening. Eur J Endocrinol. 2001;145(4):377–83.

Selva KA, Harper A, Downs A, Blasco PA, Lafranchi SH. Neurodevelopmental outcomes in congenital hypothyroidism: comparison of initial T4 dose and time to reach target T4 and TSH. J Pediatr. 2005;147:775–80.

Senée V, Chelala C, Duchatelet S, Feng D, Blanc H, Cossec JC, Charon C, Nicolino M, Boileau P, Cavener DR, Bougnères P, Taha D, Julier C. Mutations in GLIS3 are responsible for a rare syndrome with neonatal diabetes mellitus and congenital hypothyroidism. Nat Genet. 2006;38(6):682–7.

Spitzweg C, Morris JC. Genetics and phenomics of hypothyroidism and goiter due to NIS mutations. Mol Cell Endocrinol. 2010;322(1–2):56–63. https://doi.org/10.1016/j.mce.2010.02.007.

Stagi S, Manoni C, Salti R, Cecchi C, Chiarelli F. Thyroid hypoplasia as a cause of congenital hypothyroidism in Williams syndrome. Horm Res. 2008;70(5):316–8. https://doi.org/10.1159/000157879.

Sugisawa C, Higuchi S, Takagi M, Hasegawa Y, Taniyama M, Abe K, Hasegawa T, Narumi S. Homozygous DUOXA2mutation (p.Tyr138*) in a girl with congenital hypothyroidism and her apparently unaffected brother: Case report and review of the literature. Endocr J. 2017;64(8):807–12. https://doi.org/10.1507/endocrj.EJ16-0564.

Szinnai G. Clinical genetics of congenital hypothyroidism. Endocr Dev. 2014a;26:60–78. https://doi.org/10.1159/000363156.

Szinnai G. Genetics of normal and abnormal thyroid development in humans. Best Pract Res Clin Endocrinol Metab. 2014b;28:133–50.

Szinnai G, Lacroix L, Carré A, Guimiot F, Talbot M, Martinovic J, Delezoide AL, Vekemans M, Michiels S, Caillou B, Schlumberger M, Bidart JM, Polak M. Sodium/iodide symporter (NIS) gene expression is the limiting step for the onset of thyroid function in the human fetus. J Clin Endocrinol Metab. 2007;92:70–6.

Tajima T, Jo W, Fujikura K, Fukushi M, Fujieda K. Elevated free thyroxine levels detected by a neonatal screening system. Pediatr Res. 2009;66:312–6.

Targovnik HM, Citterio CE, Rivolta CM. Iodide handling disorders (NIS, TPO, TG, IYD). Best Pract Res Clin Endocrinol Metab. 2017;31(2):195–212. https://doi.org/10.1016/j.beem.2017.03.006.

Targovnik HM, Citterio CE, Rivolta CM. Thyroglobulin gene mutations in congenital hypothyroidism. Horm Res Paediatr. 2011;75:311–21.

Targovnik HM, Esperante SA, Rivolta CM. Genetics and phenomics of hypothyroidism and goiter due to thyroglobulin mutations. Mol Cell Endocrinol. 2010;322:44–55.

Tashko V, Davachi F, Baboci R, Drishti G, Hoxha P. Kocher-Debré Sémélaigne syndrome. Clin Pediatr (Phila). 1999;38(2):113–5.

Tenenbaum-Rakover Y, Almashanu S, Hess O, Admoni O, Hag-Dahood Mahameed A, Schwartz N, Allon-Shalev S, Bercovich D, Refetoff S. Long-term outcome of loss-of-function mutations in thyrotropin receptor gene. Thyroid. 2015;25(3):292–9. https://doi.org/10.1089/thy.2014.0311.

Theodoropoulos T, Braverman LE, Vagenakis AG. Iodide-induced hypothyroidism: a potential hazard during perinatal life. Science. 1979;205(4405):502–3.

Thorwarth A, Schnittert-Hübener S, Schrumpf P, Müller I, Jyrch S, Dame C, Biebermann H, Kleinau G, Katchanov J, Schuelke M, Ebert G, Steininger A, Bönnemann C, Brockmann K, Christen HJ, Crock P, deZegher F, Griese M, Hewitt J, Ivarsson S, Hübner C, Kapelari K, Plecko B, Rating D, Stoeva I, Ropers HH, Grüters A, Ullmann R, Krude H. Comprehensive

genotyping and clinical characterisation reveal 27 novel NKX2-1 mutations and expand the phenotypic spectrum. J Med Genet. 2014;51(6):375–87. https://doi.org/10.1136/jmedgenet-2013-102248.

Thvilum M, Brandt F, Almind D, Christensen K, Hegedüs L, Brix TH. Excess mortality in patients diagnosed with hypothyroidism: a nationwide cohort study of singletons and twins. J Clin Endocrinol Metab. 2013;98(3):1069–75. https://doi.org/10.1210/jc.2012-3375.

Tillotson SL, Fuggle PW, Smith I, Ades AE, Grant DB. Relation between biochemical severity and intelligence in early treated congenital hypothyroidism: a threshold effect. BMJ. 1994;309 (6952):440–5.

Tonacchera M, De Marco G, Agretti P, Montanelli L, Di Cosmo C, Freitas Ferreira AC, Dimida A, Ferrarini E, Ramos HE, Ceccarelli C, Brozzi F, Pinchera A, Vitti P. Identification and functional studies of two new dual-oxidase 2 (DUOX2) mutations in a child with congenital hypothyroidism and a eutopic normal-size thyroid gland. J Clin Endocrinol Metab. 2009;94(11):4309–14. https://doi.org/10.1210/jc.2009-0426.

Tonacchera M, Perri A, De Marco G, Agretti P, Banco ME, Di Cosmo C, Grasso L, Vitti P, Chiovato L, Pinchera A. Low prevalence of thyrotropin receptor mutations in a large series of subjects with sporadic and familial nonautoimmune subclinical hypothyroidism. J Clin Endocrinol Metab. 2004;89(11):5787–93.

Tonacchera M, Agretti P, de Marco G, Elisei R, Perri A, Ambrogini E, De Servi M, Ceccarelli C, Viacava P, Refetoff S, Panunzi C, Bitti ML, Vitti P, Chiovato L, Pinchera A. Congenital hypothyroidism due to a new deletion in the sodium/iodide symporter protein. Clin Endocrinol. 2003;59(4):500–6.

Trueba SS, Augé J, Mattei G, Etchevers H, Marti- novic J, Czernichow P, Vekemans M, Polak M, Attié- Bitach T. PAX8, TITF1, and FOXE1 gene expression patterns during human development: new insights into human thyroid development and thyroid dys- genesis-associated malformations. J Clin Endocrinol Metab. 2005;90:445–62.

Van Engelen K, Mommersteeg MTM, Baars MJH, Lam J, Ilgun A, van Trotsenburg AS, Smets AM, Christoffels VM, Mulder BJ, Postma AV. The ambiguous role of NKX2-5 mutations in thyroid dysgenesis. PLoS One. 2012;7:e52685.

van Tijn DA, de Vijlder JJ, Jr VB, Verkerk PH, Vulsma T. Neonatal detection of congenital hypothyroidism of central origin. J Clin Endocrinol Metab. 2005;90(6):3350–9.

Van Vliet G, Deladoey J. Sublingual thyroid ectopy: similarities and differences with Kallmann syndrome. F1000Prime Rep. 2015;7:20.

Van Vliet G, Czernichow P. Screening for neonatal endocrinopathies: rationale, methods and results. Semin Neonatol. 2004;9(1):75–85.

Vigone MC, Di Frenna M, Weber G. Heterogeneous phenotype in children affected by non-autoimmune hypothyroidism: an update. J Endocrinol Investig. 2015;38(8):835–40. https://doi.org/10.1007/s40618-015-0288-5.

Vigone MC, Fugazzola L, Zamproni I, Passoni A, Di Candia S, Chiumello G, Persani L, Weber G. Persistent mild hypothyroidism associated with novel sequence variants of the DUOX2 gene in two siblings. Hum Mutat. 2005;26(4):395.

Visser WE, van Mullem AA, Visser TJ, Peeters RP. Different causes of reduced sensitivity to thyroid hormone: diagnosis and clinical management. Clin Endocrinol. 2013;79(5):595–605. https://doi.org/10.1111/cen.12281.

Weinstein LS, Yu S, Warner DR, Liu J. Endocrine manifestations of stimulatory G protein a-subunit mutations and the role of genomic imprinting. Endocr Rev. 2001;22:675–705.

Wheeler SM, Willoughby KA, McAndrews MP, Rovet JF. Hippocampal size and memory functioning in children and adolescents with congenital hypothyroidism. J Clin Endocrinol Metab. 2011;96:E1427–34.

Whitaker RC, Pepe MS, Wright JA, Seidel KD, Dietz WH. Early adiposity rebound and the risk of adult obesity. Pediatrics. 1998;101:E5.

Williams GR. Neurodevelopmental and neurophysiological actions of thyroid hormone. J Neuroendocrinol. 2008;20(6):784–94. https://doi.org/10.1111/j.13652826.2008.01733.x.

Wolff J. Congenital goiter with defective iodide transport. Endocr Rev. 1983;4(3):240–54.
Wong SC, Ng SM, Didi M. Children with congenital hypothyroidism are at risk of adult obesity due to early adiposity rebound. Clin Endocrinol. 2004;61(4):441–6.
Zamproni I, Grasberger H, Cortinovis F, Vigone MC, Chiumello G, Mora S, Onigata K, Fugazzola L, Refetoff S, Persani L, Weber G. Biallelic inactivation of the dual oxidase maturation factor 2 (DUOXA2) gene as a novel cause of congenital hypothyroidism. J Clin Endocrinol Metab. 2008;93(2):605–10. Epub 2007 Nov 27.
Zimmermann MB, Aeberli I, Torresani T, Bürgi H. Increasing the iodine concentration in the Swiss iodized salt program markedly improved iodine status in pregnant women and children: a 5-y prospective national study. Am J Clin Nutr. 2005;82(2):388–92.
Zoeller RT, Rovet J. Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. J Neuroendocrinol. 2004;16:809–18.
Zwaveling-Soonawala N, van Trotsenburg AS, Verkerk PH. The severity of congenital hypothyroidism of central origin should not be underestimated. J Clin Endocrinol Metab. 2015;100(2): E297–300. https://doi.org/10.1210/jc.2014-2871.

Central Hypothyroidism

12

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Contents

Abstract

Central hypothyroidism (CH) is a clinical condition characterized by a defect in thyroid hormone secretion due to an insufficient stimulation by thyrotropin (TSH) of an otherwise normal thyroid gland. CH is a rare and heterogeneous disorder that is caused by abnormalities of either the pituitary gland or the hypothalamus, and it may be congenital or acquired. The clinical manifestations are usually milder than those observed in primary hypothyroidism, and the CH diagnosis is

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based on low circulating levels of free thyroid hormone and low/normal TSH. CH treatment is based on L-thyroxine (L-T4) supplementation, the adequacy of which is evaluated by measuring circulating free thyroxine (FT4). This chapter analyzes our current understanding of the causes of CH and highlights possible pitfalls in its diagnosis and treatment.

Keywords
Central hypothyroidism · L-thyroxine · Hypopituitarism

Epidemiology

Central hypothyroidism (CH) is a clinical condition characterized by a defect in thyroid hormone secretion due to an insufficient stimulation by thyrotropin (TSH) of an otherwise normal thyroid gland. CH is a rare and heterogeneous disease caused by abnormalities of either the pituitary gland (i.e., secondary hypothyroidism) or the hypothalamus (i.e., tertiary hypothyroidism).

The prevalence of CH ranges from 1:20,000 to 1:80,000 individuals in the general population (Price and Weetman 2001) and represents an uncommon cause of hypothyroidism (one of 1,000 hypothyroid patients). As far as congenital CH is concerned, its prevalence depends on the screening protocols adopted. In fact, when TSH-only-based protocols are used, CH is often unrecognized since it is usually associated with inappropriately normal/low TSH in the presence of low circulating FT4 levels. When screening programs for neonatal CH include both TSH and FT4 measurements, its prevalence increases to 1:160,000 (Asakura et al. 2002; Nebesio et al. 2010). Interestingly, CH prevalence further increases to 1 in 16,000 newborns if the screening algorithm is based on the combined measurement of TSH, T4, and thyroxine-binding globulin, which could be effective in diagnosing the milder forms of the disease (Kempers et al. 2006).

While primary hypothyroidism is mainly diagnosed in females (incidence 3.5:1000 in females vs 0.6:1000 in males), CH affects patients of all ages and equally in both sexes.

Etiopathogenesis

CH may be congenital or acquired (Table 1) and is caused by anatomical and/or functional abnormalities affecting either the pituitary (secondary hypothyroidism) or the hypothalamus (tertiary hypothyroidism). It is worth noting that in many instances, both the pituitary and the hypothalamus may be affected simultaneously. A quantitative defect in the amount of functional pituitary thyrotroph cells (i.e., thyrotropin reserve) is probably the pathogenic mechanism underlying most acquired CH cases. This quantitative defect in thyrotrophs is associated with a qualitative defect in the secreted TSH isoforms, which display an impaired

Table 1 Causes of central hypothyroidism

Acquired	Causes
Invasive	
	Pituitary macroadenomas, craniopharyngiomas, meningiomas, gliomas, metastases, carotid aneurysms
Iatrogenic	
	Cranial surgery or irradiation, drugs (e.g., bexarotene)
Injury	
	Head trauma, 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	
	TSH beta, TRHR, IGSF1
Combined	
	HESX1, LHX3, LHX4, SOX3, OTX2, PROP1, POU1F1

TRHR, TRH receptor; IGSF1, immunoglobulin superfamily member 1; HESX1, homeobox gene expressed in ES cells; LIM domain transcription factors 3 and 4, LHX3 and LHX4; SOX3, SRY-related HMG-box gene 3; OTX2, orthodenticle homeobox 2; PROP1, prophet of PIT1; POU1F1, POU domain class 1 transcription factor 1

biological activity and ability to bind thyroid TSH receptors despite a preserved immunoreactivity (Persani et al. 2000). In this setting, circulating levels of immunoreactive TSH may be normal or even slightly increased (Beck-Peccoz et al. 1985). Importantly, the secretion of bioinactive TSH is often related to CH forms associated with an impaired hypothalamic function (i.e., tertiary hypothyroidism). In this respect, studies have shown that the impaired biological activity of TSH observed in these patients is caused by changes in TSH carbohydrate structure leading to an impaired glycosylation (Papandreou et al. 1993; Persani et al. 1998).

In congenital CH, defects in TSH secretion may be quantitative and/or qualitative according to the cause of the disease. In this respect, in patients with loss of function TSH beta gene mutations, CH is caused by "abnormal" TSH molecules lacking 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 (Beck-Peccoz et al. 2006). Other mutations may form an incomplete heterodimer with preserved immunoreactivity in some of the methods for TSH measurement but completely devoid of bioactivity (Bonomi et al. 2001).

Congenital CH

Congenital CH may be classified as isolated or combined (Table 2). Isolated congenital CH is caused by mutations affecting genes coding for TSH beta, TRH receptor (TRHR), or immunoglobulin superfamily member 1 (IGSF1) (Shoenmakers et al. 2015). In the majority of patients, congenital CH is associated with different pituitary hormone deficiencies (combined CH), and some additional syndromic features may be present depending on the genes involved (Schoenmakers et al. 2015).

Isolated CH

TSH beta gene mutations cause severe CH of neonatal onset leading to impaired neurodevelopment. Neurological alterations associated with TSH beta mutations are related to treatment delay because affected subjects are not recognized by TSH-based CH screening programs and remain undiagnosed until the neurological consequences of the severe hypothyroidism are clinically manifest. Biallelic TRHR gene mutations represent an uncommon cause of isolated congenital CH. These

Table 2 Congenital forms of CH: clinical presentation

Gene	Pituitary function	Other clinical features	Neuroradiological findings
TSH beta	CH		Enlarged/normal pituitary
TRHR	CH, HYP		
IGSF1	CH, GHD (transient), HYP	Macroorchidism	
POUF1	CH, HYP, GHD		Variable pituitary hypoplasia
PROP1	CH, GHD, CHY, CHA (late)		Enlarged/normal/hypoplastic pituitary
HESX1	CH, GHD, CHY, CHA (late)	Septo-optic dysplasia	Pituitary hypoplasia
LHX3	CH, GHD, CHY, HYP	Limited neck rotation, short cervical spine, sensorineural deafness	Enlarged/normal/hypoplastic pituitary
LHX4	CH, GHD, CHY (variable), CHA	Cerebellar abnormalities	Pituitary hypoplasia
SOX3	CH, GHD, CHY, CHA	Mental retardation	Pituitary hypoplasia
OTX2	CH, GHD, CHY, CHA	Anopthalmy Retinal abnormalities	Variable pituitary hypoplasia

GHD, growth hormone deficiency; CH, central hypothyroidism; CHY, central hypogonadism; HYP, hypoprolactinemia; CHA, central hypoadrenalism; TRHR, TRH receptor; IGSF1, immunoglobulin superfamily member 1; PROP1, prophet of PIT1; POU1F1, POU domain class 1 transcription factor 1; HESX1, homeobox gene expressed in ES cells; LIM domain transcription factors 3 and 4, LHX3 and LHX4; SOX3, SRY-related HMG-box gene 3; OTX2, orthodenticle homeobox 2

mutations have so far been described in just three cases from two unrelated kindred. Affected males present subnormal T4 concentrations, growth retardation, and delayed bone age. Conversely, no neurological deficits (i.e., mental retardation) have been described in these patients. IGSF1 deficiency has recently been identified as an X-linked cause of CH and macroorchidism (Sun et al. 2012). A multicentric study has recently analyzed all clinical and biochemical characteristics associated with IGSF1 deficiency in a series of 42 patients (Joustra et al. 2013). In particular, the authors observed that in male patients CH is associated with hyperprolactinemia (67% of cases) and transient GH deficiency (13% of cases). Though puberty is delayed (including the growth spurt and pubic hair development), testicular growth starts at a normal age, and macroorchidism is described in all evaluable adults. Notably, body mass index, percent fat, and waist circumference are increased, with presence of the metabolic syndrome in the majority of patients above 55 years of age. Heterozygous female carriers have CH in 33% of cases, and, as observed in affected males, body mass index, percent fat, and waist circumference are relatively high.

Combined CH

LHX3 and LHX4 are LIM domain transcription factors involved in the early steps of pituitary development. Patients bearing LHX3 mutations present GH, TSH, and LH/FSH deficiencies, while central hypoadrenalism is inconsistently reported (Schoenmakers et al. 2015). Brain imaging studies reveal pituitary aplasia or hypoplasia in 60% of cases and hyperplasia in 30% of cases (Schoenmakers et al. 2015). Patients with LHX3 mutations may present extrapituitary disorders such as vertebral abnormalities, variable hearing alterations, and limited head and neck rotation (Netchine et al. 2000). LHX4 mutations lead to GH and variable LH/FSH, TSH, and ACTH deficiencies, anterior pituitary hypoplasia, hypoplastic sella turcica, cerebellar alterations, or Chiari malformation (Rochette et al. 2015).

Septo-optic dysplasia (SOD) is characterized by the combination of optic nerve hypoplasia and/or midline forebrain defects (i.e., agenesis of the corpus callosum, absent septum pellucidum) and/or hypopituitarism associated with pituitary hypoplasia (McCabe et al. 2011). Mutations affecting homeobox gene expressed in ES cells (HESX1), SRY-related HMG-box gene 3 (SOX3), and orthodenticle homeobox 2 (OTX2) genes have been found in patients with CH and SOD. HESX1 expression occurs early in the pituitary placode, and its reduction is necessary for prophet of PIT1 (PROP1) and POU domain class 1 transcription factor 1 (POU1F1) expression, leading to differentiation of GH-, TSH-, and PRL-secreting cells. Patients with homozygous mutations are usually characterized by a more severe phenotype. While GH deficiency is diagnosed in all patients, other pituitary deficiencies, including CH, are found in 50% of cases. Optic nerve anomalies are observed in 30% of cases, and MRI imaging reveals pituitary hypoplasia in 80% of cases, ectopic posterior pituitary in 50–60%, and corpus callosum agenesis or hypoplasia in 25% of cases. OTX2 is a paired homeodomain transcription factor involved in the early steps of brain development. OTX2 mutations are responsible for 2–3% of anophthalmia/microphthalmia syndromes in humans. Pituitary deficiencies range from isolated GH deficiency to panhypopituitarism. Brain MRI may reveal normal or hypoplastic

pituitary. Moreover, ectopic posterior pituitary or Chiari syndrome may be identified in these patients. Mutations affecting the SOX3 gene lead to X-linked hypopituitarism, ranging from isolated growth hormone deficiency to combined pituitary hormone deficiency, including evolving TSH deficiency (Stagi et al. 2014).

PROP1 is a pituitary-specific paired-like homeodomain transcription factor. Its expression is required for the development of GH-, PRL-, and TSH-secreting pituitary cells (i.e., POU1F1 lineage). PROP1 mutations are the most common cause of combined pituitary hormone deficiency and are associated with GH, TSH, LH/FSH, ACTH, and PRL deficiencies that may be diagnosed from childhood to adulthood (Fluck et al. 1998). Neuroradiological imaging studies can show transient pituitary hyperplasia or a normal or hypoplastic pituitary. Pituitary hyperplasia sometimes precedes spontaneous hypoplasia.

POU1F1 is expressed relatively late during pituitary development and its expression persists in adulthood. POU1F1 is required for the production of GH, PRL, and TSH beta as well as for the expression of GHRH receptor. Patients with autosomal recessive and dominant POU1F1 mutations are characterized by GH and PRL deficiency, which is normally present from early life. In contrast, TSH deficiency may be highly variable and hypothyroidism may occur later in childhood. In these patients, MRI shows a normal or a hypoplastic anterior pituitary.

Acquired

Neoplasias, affecting the hypothalamus-pituitary region as well as therapeutic interventions on sellar and extrasellar tumor masses (i.e., surgery and radiotherapy), represent the most frequent causes of acquired CH. In particular, pituitary macroadenomas may induce hypopituitarism by affecting either pituitary cells or the pituitary stalk. In this respect, nonfunctioning pituitary adenomas are the tumors most frequently involved. At presentation, isolated or multiple pituitary deficits are diagnosed in 62% of patients with pituitary nonfunctioning macroadenomas, with CH found in 27% of them (Ferrante et al. 2008; Dekkers et al. 2008). The risk and extent of postsurgical hypopituitarism depend on tumor size, tumor extension, and the experience of the surgeon. In particular, new pituitary hormone deficiency is described in 10% of patients who have undergone pituitary surgery in referral centers, with CH occurring in less than 3% of such cases (Losa et al. 2013).

Craniopharyngiomas are typically slowly growing extrasellar tumors, and visual field defects and hypopituitarism are the most common presenting clinical manifestations. In children, GH deficiency is the most common pituitary deficit diagnosed at presentation (up to 100% of patients), followed by TSH deficiency (up to 25% of patients). In adults, CH has been described in 40% of cases, growth hormone deficiency in 80–90% of cases, gonadotropin deficiency in 70% of patients, and ACTH in 40% of patients (Karavitaki et al. 2005, 2006; Muller 2014). Surgical intervention is associated with hypopituitarism in the majority of patients with craniopharyngiomas, with CH reported in 40 to 95% of cases (Karavitaki et al. 2006; Muller 2014).

Importantly, hypopituitarism may occur in patients who undergo neurosurgical intracranial procedures for conditions other than pituitary tumors. In this setting, the main pituitary hormone deficiencies are related to ACTH, GH, and LH/FSH insufficiency and only rarely to TSH insufficiency (Fleck et al. 2013).

Direct and indirect irradiation of the hypothalamic-pituitary axis may cause hypopituitarism. The risk of developing CH is related to both the effective dose given to the area and the total radiation dose delivered (Kanumakala et al. 2003; Schmiegelow et al. 2003). Radiation-induced CH occurs in patients who undergo radiotherapy, not only for pituitary tumors and craniopharyngiomas but also in 10–50% of patients irradiated for nasopharyngeal or paranasal sinus tumors (Samaan et al. 1987; Ratnasingam et al. 2015) and in 12–65% of patients irradiated for any site brain tumors (Constine et al. 1993; Kyriakakis et al. 2016). Unfortunately, data on the long-term effects on hypothalamic-pituitary function of proton beam therapy – whether by Leksell Gamma Knife or stereotactic linear accelerator – are still scarce and inconclusive. However, recent findings suggest that hypopituitarism (including CH) occurs even after these new irradiation methods (Xu et al. 2013). Analyses of the effects of Leksell Gamma Knife on pituitary function in a series of patients affected with Cushing's disease have demonstrated that new pituitary deficiency occurs in 58% of patients, with a latency of up to 160 months after radiation delivery. The most commonly deficient endocrine axis was the GH (33%) followed by the gonadotroph axis (28 %). Of interest, TSH deficiency was observed in 27% of cases, while CH occurred in 5%, 10%, and 27% of patients at 3, 5, and 10 years of follow-up, respectively (Cohen-Inbar et al. 2016).

Hypopituitarism may represent the consequences of traumatic brain injury (TBI), the prevalence of anterior pituitary dysfunction ranging from 15% to 68% (Fernandez-Rodriguez et al. 2015). In TBI patients CH frequency varies between series (from 5% up to 29%) (Fernandez-Rodriguez et al. 2015; Krewer et al. 2016), this discrepancy being possibly explained by either the timing of testing or the diagnostic procedure used to identify pituitary hormone deficiencies. Cerebrovascular accidents (i.e., subarachnoid hemorrhage or infarcts) can but rarely induce hypopituitarism, with CH diagnosed in less than 2% of cases (Klose et al. 2010).

Granulomatous diseases (i.e., sarcoidosis, tuberculosis, and histiocytosis X), as well as all iron overload states (i.e., hemochromatosis, patients with β-thalassemia who need several blood transfusions), can induce hypopituitarism and CH by directly acting on the pituitary stalk (Gamberini et al. 2008; Lewis et al. 2009).

Hypophysitis is a condition characterized by lymphocytic infiltration of the pituitary gland. On the basis of the histopathological picture, it can be classified as lymphocytic or granulomatous (Fukuoka 2015). Hypopituitarism is the most prevalent feature of lymphocytic hypophysitis, with CH as the pituitary hormone deficiency most frequently diagnosed after central hypoadrenalism and hypogonadotropic hypogonadism. In contrast, GH deficiency seems to be the least frequent (Fukuoka 2015; Honegger et al. 2015). Xanthogranulomatous hypophysitis is a very rare form of pituitary hypophysitis. It may either be primary (with an autoimmune etiology), secondary (as a reactive degenerative response to an epithelial lesion such as craniopharyngiomas, Rathke's cleft cyst, germinoma, and pituitary

adenomas), or part of a multiorgan systemic disease (e.g., tuberculosis, sarcoidosis, or granulomatosis). Recently, IgG4-related hypophysitis has been frequently diagnosed as a part of IgG4-related disease. This is a clinical entity characterized by IgG4 + plasma cell and lymphocyte infiltration and elevated serum IgG4 concentrations (Bando et al. 2013). The growing use of anti-CTLA-4 antibody treatment (i.e., ipilimumab and tremelimumab) for several cancer types has resulted in the appearance of hypophysitis in up to 10% of treated patients (Lam et al. 2015). In particular, most patients with ipilimumab-induced hypophysitis have multiple anterior pituitary hormone deficiencies. CH is the most frequent (up to 90% of cases), followed by central adrenal insufficiency and hypogonadotropic hypogonadism (Faje 2016).

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 (Yamamoto et al. 2011).

Clinical and Biochemical Presentation

Clinical features of CH depend on etiology, severity of the thyroid impairment, extent and severity of associated hormone deficiencies, and age of the patient at the time of disease onset. Congenital CH is clinically more severe than the acquired forms. Symptoms and signs are usually the same but milder than those of primary hypothyroidism and goiter is always absent. It has been proposed that residual thyrotroph function, as well as the physiological constitutive activity of the TSH receptor, may explain this discrepancy (Neumann et al. 2010; Barbesino et al. 2012). In the presence of combined pituitary deficiencies, other endocrine manifestations (i.e., growth failure, delayed puberty, adrenal insufficiency, and diabetes insipidus) lead the patients to seek medical attention before their hypothyroidism becomes severe.

In congenital CH, various syndromic and complex clinical features may be present depending on the genes involved (Table 2) (Schoenmakers et al. 2015). In patients with TSH beta mutations, CH is clinically undetectable at birth, biochemically associated with elevated glycoprotein hormone alpha subunit and an impaired TSH response to TRH stimulation, and characterized by severe signs and symptoms. Prolactin secretion is normal and fully responsive to TRH stimulation (Bonomi et al. 2001). CH characterized by the complete absence of TSH and PRL responses to TRH is caused by inactivating TRH receptor mutations (Collu et al. 1997; Bonomi et al. 2009; Koulouri et al. 2016). In the first reported cases, clinical manifestations were mild (growth retardation, delayed bone age) despite biochemical evidence of severe CH, with T4 levels ranging from 40% to 88% of the lower limit of normal. Surprisingly, despite the late treatment, no attributable neurological deficits were found, thus suggesting sufficient childhood thyroid hormone production. Importantly, T4 replacement was found effective in improving growth and quality of life in these individuals (Collu et al. 1997; Bonomi et al. 2009). Although the TRH receptor is expressed on lactotrophs and mediates prolactin secretion in response to exogenous TRH, a female homozygous for p.R17* *TRHR* underwent two pregnancies and

lactated normally (Bonomi et al. 2009). Immunoglobulin superfamily member 1 (IGSF1) is an X-linked cause of CH deficiency syndrome. Males with IGSF1 mutations present CH, increased body weight (in some cases metabolic syndrome has been described at adult age), macroorchidism, and sometimes hypoprolactinemia and/or transient growth hormone (GH) deficiency (Joustra et al. 2013; Hulle et al. 2016). A subset of female carriers (about 18%) also exhibit CH. A delayed adrenarche, as a consequence of PRL deficiency, seems to be part of the clinical phenotype of patients with IGSF1 deficiency (Hughes et al. 2016). Finally, mild deficits in attentional control, on formal testing, have been described in some adult male patients with IGSF1 deficiency (Joustra et al. 2016).

Due to the difficulties in recognizing CH clinically, the diagnosis is usually made biochemically by measuring circulating free thyroxine with direct "two-step" methods, provided that factors interfering in the assays have been ruled out (i.e., thyroid autoantibodies or abnormal binding proteins) (Gurnell et al. 2011). In CH, serum TSH levels are usually low/normal or even slightly increased in patients with tertiary (hypothalamic) hypothyroidism. The latter condition may be misdiagnosed as a condition of primary subclinical hypothyroidism (Koulouri et al. 2013).

CH is characterized by the presence of abnormalities in circadian TSH secretion leading to lack of the physiological nocturnal TSH rise, which normally demands inpatient evaluation (Darzy and Shalet 2005). A TRH stimulation test (TRH 200 mcg i.v.) has been proposed to differentiate pituitary from hypothalamic CH, the former characterized by an exaggerated/delayed and/or prolonged TSH response, which is impaired in the latter (Lania et al. 2008; Fig. 1). However, the practical utility of the TRH test is limited since the pituitary and the hypothalamus may be simultaneously involved in acquired CH. Importantly, absent or impaired FT4 and FT3 responses, as measured at 120 and 180 min after TRH injection, indirectly indicate the secretion of bioinactive TSH.

A 10% variation in FT4 may be considered as normal in euthyroid patients. Therefore, in patients followed for pituitary diseases, a decrease in circulating FT4

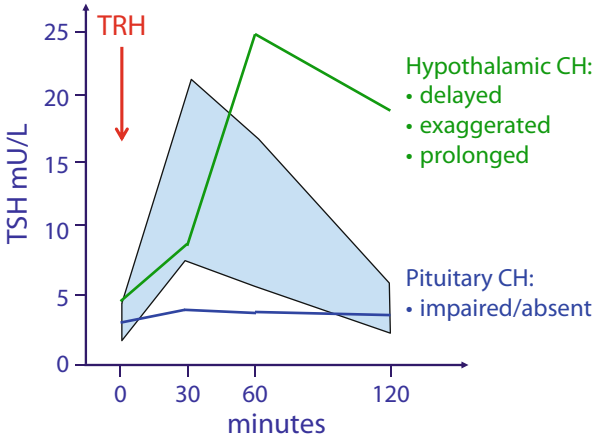

Fig. 1 TRH stimulation test (TRH 200 μg i.v. as a bolus) in CH diagnosis. Blood for TSH measurement is withdrawn at −30, 0, 20, 60, 120, and 180 min, while FT4 and FT3 were measured at the time 0, 120, and 180 min. TRH test may be helpful in differentiating hypothalamic from pituitary CH, the first being characterized by an exaggerated, delayed, and/or prolonged TSH response and the second by an impaired TSH response

Table 3 Clinical and biochemical features indicating possible CH

Clinical features
Presence of diseases affecting the hypothalamic-pituitary region
Neuroradiological imaging demonstrating alterations in the hypothalamic-pituitary region
Normal thyroid structure, at ultrasound scan
Biochemical features
Low FT4 and normal/low TSH levels
Absence of antithyroid autoantibodies
Presence of other pituitary hormone deficiencies

above 20% may suggest CH, even if FT4 concentrations are still in the normal range (Alexopoulou et al. 2004).

Patients with nonthyroidal illnesses (NTI), a relatively common finding following any acute or chronic illness (e.g., poor nutrition/starvation, sepsis, burns, malignancy, myocardial infarction, postsurgery, chronic liver, and renal disease), display thyroid function values that considerably overlap those of CH patients (Koulouri et al. 2013). It has been suggested that NTI may be due to factors such as down-regulation of TRH neurons in the paraventricular nucleus, reduced TSH secretion, and modifications in thyroid hormone metabolism. It is crucial to be aware of this transient phenomenon and to consider biochemical data in the context of clinical status in order to avoid inappropriate treatment. In this respect, a clue for distinguishing CH from NTI is the evaluation of serum FT3, which is reduced in NTI and normal in mild to moderate forms of CH.

Once the biochemical diagnosis has been confirmed, a family history of CH, a suggestive clinical history (e.g., head trauma, subarachnoidal hemorrhage, previous brain irradiation, or surgery), or specific symptoms (e.g., headaches or visual field defects) should lead to a pituitary MRI and evaluation of the other hypothalamic-pituitary axes.

In Table 3, clinical and biochemical features indicating possible CH are summarized.

Treatment and Follow-Up

CH treatment should lead to the restoration and maintenance of euthyroidism in analogy to that intended for patients with primary hypothyroidism. In this respect, L-thyroxine (L-T4) therapy is recommended since no evidence supports the superiority of combined treatment with L-T4 and triiodothyronine in either adults or children (Cassio et al. 2003; Grozinsky-Glasberg et al. 2006; Slawik et al. 2007, and Wiersinga 2014).

No consensus has been reached concerning the evaluation of the adequacy of L-T4 replacement dose in CH, as, unlike in primary hypothyroidism, serum TSH levels cannot be used for monitoring L-T4 therapy. In fact, TSH secretion is suppressed even during low-dose L-T4 treatment, a finding possibly related to the

negative feedback of circulating hormones on residual thyrotrophs (Ferretti et al. 1999; Shimon et al. 2002). It has been demonstrated that in the majority of CH patients, TSH is suppressed during L-T4 treatment even though serum FT4 levels were still in the hypothyroid range (Ferretti et al. 1999). These data suggest that the finding of normal serum TSH levels during L-T4 treatment reflects a possible CH undertreatment. In particular, it has been demonstrated that TSH levels above 1.0 mU/l should be considered as a sign of insufficient replacement in CH patients (Shimon et al. 2002). Nonetheless, several recent papers dealing with L-T4 substitution therapy in patients with CH have underlined the pitfalls in achieving optimal replacement (Beck-Peccoz 2011). In particular, by comparing FT4 values in these groups of patients with those found in patients with primary hypothyroidism and adequately treated with L-T4, it has been demonstrated that CH patients are generally undertreated (Koulouri et al. 2011). The same authors suggest that levels of FT4 around 16 pmol/l (reference range 9–25 pmol/l) might represent an appropriate target for considering CH patients adequately treated.

In CH, free thyroid hormones should be measured to evaluate the adequacy of L-T4 treatment. In this respect, low FT4 values may indicate undertreatment, while high FT3 levels possibly indicate overtreatment. During follow-up blood for FT4/FT3, measurement should be drawn before ingestion of the L-T4 tablets. Serum FT4 levels in the middle/upper part of the normal range is considered representing an appropriate target in L-T4 treated CH patients (Ferretti et al. 1999; Slawik et al. 2007; Iverson and Mariash 2008; Koulouri et al. 2011). In this respect, it has been demonstrated that the majority of CH patients reach normal circulating FT4 levels with a mean daily L-T4 dose ranging from 1.5 $\pm$ 0.3 to 1.6 $\pm$ 0.5 μg/kg body weight. These doses are similar to those commonly used for primary hypothyroidism (Alexopoulou et al. 2004; Ferretti et al. 1999). Finally, biochemical indices of thyroid hormone action at the tissue level (e.g., SHBG, cholesterol, Gla protein, BGP, and carboxyterminal telopeptide of type 1 collagen, ICTP) are of little help in monitoring L-T4 treatment in CH, since these parameters may be affected by the coexistence of alterations in adrenal, somatotroph, or gonadal function (Alexopoulou et al. 2004).

L-T4 treatment should be started at a low daily dosage and then gradually increased by 25 mcg every 2–3 weeks in order to reach full replacement dose. The majority of patients reach normal FT4 and FT3 levels with a daily L-T4 dose ranging from 1.5 to 1.6 μg/kg bw (Lania et al. 2008). Among CH patients, significant differences in L-T4 dose depend on concomitant treatment (e.g., estrogens, rhGH) or the individuals' age, with higher doses required in the young. Of crucial importance, in children L-T4 treatment should be started as early as possible, and with full replacement doses, in order to prevent serious damage of the brain.

In CH patients, concomitant estrogen or GH replacement therapy may require a significant increase in L-T4 dose to normalize circulating FT4 levels (Lania et al. 2008). The increase in L-T4 requirement, observed during estrogen therapy (Arafah et al. 2001), is possibly related to the transient increase of thyroxine-binding globulin levels that induce a reduction in FT4 bioavailability (Ain et al. 1987). During follow-up, it is recommended to evaluate circulating FT4 and FT3 levels 6–8 weeks after initiation of estrogen replacement therapy (Arafah 2001).

GH deficiency per se may mask subclinical forms of CH that can be diagnosed once rhGH has been initiated (Portes et al. 2000; Porretti et al. 2002; Agha et al. 2007; Giavoli et al. 2003; Losa et al. 2008). GH administration has been found to enhance peripheral deiodination of T4 to T3 (Jorgensen et al. 1994). This effect on T4 metabolism is biologically relevant only in patients with combined pituitary hormone deficiencies and a partial impairment of thyrotroph function (Portes et al. 2000; Giavoli et al. 2003; Losa et al. 2008). In fact, contrary to that observed in patients with multiple pituitary hormone deficiencies, rhGH replacement therapy does not induce central hypothyroidism in children with idiopathic isolated GHD. In this setting, slow growth (in spite of adequate rhGH substitution and normal IGF-I levels) is an important clinical marker of central hypothyroidism. Therefore, a strict monitoring of thyroid function is mandatory in treated children with multiple pituitary hormone deficiencies (Giavoli et al. 2003).

On a final note, it is mandatory to exclude concomitant central adrenal insufficiency prior to L-T4 therapy initiation, since restoration of euthyroidism might precipitate an adrenal crisis in unrecognized central hypoadrenalism. In fact, normalization of thyroid function increases cortisol metabolism, thereby leading to a greater glucocorticoid requirement. If adrenal function cannot be evaluated prior to start of L-thyroxine, prophylactic treatment with steroids (i.e., hydrocortisone or Cortone Acetate) should be considered.

Summary

CH is a rare and heterogeneous condition caused by anatomical and/or functional abnormalities of either the pituitary gland or the hypothalamus, and it may be congenital or acquired. Although the increasing knowledge on causes of CH, several CH cases classified as idiopathic remain unexplained. This is true for some familial CH forms as well as for acquired CH cases possibly related to specific anti-thyrotroph antibodies.

The clinical presentation is usually mild, and diagnosis is made on the basis of the coexistence of low circulating thyroid hormone levels and low/normal/slightly elevated TSH levels. CH treatment is based on L-T4 supplementation. Free thyroxine levels should be measured before drawing blood, in order to evaluate adequacy of the treatment. In this respect, we recommend reaching FT4 levels 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. Such information should provide more specific markers for a more precise tailoring of replacement therapy.

When managing CH patients, the possible interplay between CH treatment and potential coexistent pituitary hormone deficiencies should be taken into consideration. In particular, excluding concomitant central adrenal insufficiency, prior to initiation of L-T4 therapy, is crucial.

Cross-References

- ▶ Congenital Hypothyroidism
- ▶ Hashimoto's Thyroiditis
- ▶ Tests of Thyroid Function
- ▶ Thyroid Autoantibodies

References

Agha A, Walker D, Perry L, Drake WM, Chew SL, Jenkins PJ, Grossman AB, Monson JP. Unmasking of central hypothyroidism following growth hormone replacement in adult hypopituitaric patients. Clin Endocrinol (Oxf). 2007;66:72–7.

Ain KB, Mori Y, Refetoff S. Reduced clearance rate of thyroxine-binding globulin (TBG) with increased sialylation: a mechanism for estrogen-induced elevation of serum TBG concentration. J Clin Endocrinol Metab. 1987;65:689–96.

Alexopoulou O, Beguin C, De Nayer P, Maiter D. Clinical and hormonal characteristics of central hypothyroidism at diagnosis and during follow-up in adult patients. Eur J Endocrinol. 2004;150:1–8.

Arafah BM. Increased need for thyroxine in women with hypothyroidism during estrogen therapy. N Engl J Med. 2001;344:1743–9.

Asakura Y, Tachibana K, Adachi M, Suwa S, Yamagami Y. Hypothalamo-pituitary hypothyroidism detected by neonatal screening for congenital hypothyroidism using measurement of thyroid-stimulating hormone and thyroxine. Acta Paediatr. 2002;91:172–7.

Bando H, Iguchi G, Fukuoka H, Taniguchi M, Yamamoto M, Matsumoto R, Suda K, Nishizawa H, Takahashi M, Kohmura E, Takahashi Y. The prevalence of IgG4-related hypophysitis in 170 consecutive patients with hypopituitarism and/or central diabetes insipidus and review of the literature. Eur J Endocrinol. 2013;170:161–72.

Barbesino G, Sluss PM, Caturegli P. Central hypothyroidism in a patient with pituitary autoimmunity: evidence for TSH-independent thyroid hormone synthesis. J Clin Endocrinol Metab. 2012;97:345–50.

Beck-Peccoz P. Treatment of central hypothyroidism. Clin Endocrinol (Oxf). 2011;74:671–2.

Beck-Peccoz P, Amr S, Menezes-Ferreira MM, Faglia G, Weintraub BD. Decreased receptor binding of biologically inactive thyrotropin central hypothyroidism: effect of treatment with thyrotropin- releasing hormone. N Engl J Med. 1985;312:1085–90.

Beck-Peccoz P, Persani L, Calebiro D, Bonomi M, Mannavola D, Campi I. Syndromes of hormone resistance in the hypothalamic-pituitary-thyroid axis. Best Pract Res Clin Endocrinol Metab. 2006;20:529–46.

Bonomi M, Proverbio MC, Weber G, Chiumello G, Beck-Peccoz P, Persani L. Hyperplastic pituitary gland, high serum glycoprotein hormone α-subunit, and variable circulating thyrotropin (TSH) levels as hallmark of central hypothyroidism due to mutations of the TSHβ gene. J Clin Endocrinol Metab. 2001;86:1600–4.

Bonomi M, Busnelli M, Beck-Peccoz P, Costanzo D, Antonica F, Dolci C, Pilotta A, Buzi F, Persani L. A family with complete resistance to thyrotropin-releasing hormone. N Engl J Med. 2009;360:731–4.

Cassio A, Cacciari E, Cicognani A, Damiani G, Missiroli G, Corbelli E, Balsamo A, Bal M, Gualandi S. Treatment for congenital hypothyroidism: thyroxine alone or thyroxine plus triiodothyronine? Pediatrics. 2003;111:1055–60.

Cohen-Inbar O, Ramesh A, Xu Z, Vance ML, Schlesinger D, Sheehan JP. Gamma knife radiosurgery in patients with persistent acromegaly or Cushing's disease: long-term risk of hypopituitarism. Clin Endocrinol (Oxf). 2016;84(4):524–31.

Collu R, Tang J, Castagné J, Lagacé G, Masson N, Huot C, Deal C, Delvin E, Faccenda E, Eidne KA, Van Vliet G. A novel mechanism for isolated central hypothyroidism: inactivating mutations in the thyrotropin-releasing hormone receptor gene. J Clin Endocrinol Metab. 1997;82:1561–5.

Constine LS, Woolf PD, Cann D, Mick G, McCormick K, Raubertas RF, Rubin P. Hypothalamic-pituitary dysfunction after radiation for brain tumors. N Engl J Med. 1993;328:87–94.

Darzy KH, Shalet SM. Circadian and stimulated thyrotropin secretion in cranially irradiated adult cancer survivors. J Clin Endocrinol Metab. 2005;90:6490–7.

Dekkers OM, Pereira AM, Romijn JA. Treatment and follow-up of clinically nonfunctioning pituitary macroadenomas. J Clin Endocrinol Metab. 2008;93:3717–26.

Faje A. Immunotherapy and hypophysitis: clinical presentation, treatment, and biologic insights. Pituitary. 2016;19:82–92.

Fernandez-Rodriguez E, Bernabeu I, Castro AI, Casanueva FF. Hypopituitarism after traumatic brain injury. Endocrinol Metab Clin North Am. 2015;44:151–9.

Ferrante E, Ferraroni M, Castrignanò T, Menicatti L, Anagni M, Reimondo G, Del Monte P, Bernasconi D, Loli P, Faustini-Fustini M, Terzolo M, Borretta G, Losa M, Morabito A, Spada A, Beck-Peccoz P, Lania A. Non-functioning pituitary adenoma database: a useful resource to improve the clinical management of pituitary tumors. Eur J Endocrinol. 2008;155:823–9.

Ferretti E, Persani L, Jaffrain-Rea ML, Giambona S, Tamburrano G, Beck-Peccoz P. Evaluation of the adequacy of L-T4 replacement therapy in patients with central hypothyroidism. J Clin Endocrinol Metab. 1999;84:924–9.

Fleck SK, Wallaschofski H, Rosenstengel C, Matthes M, Kohlmann T, Nauck M, Schroeder HW, Spielhagen C. Prevalence of hypopituitarism after intracranial operations not directly associated with the pituitary gland. BMC Endocr Disord. 2013;13:51.

Fluck C, Deladoey J, Rutishauser K, Eble A, Marti U, Wu W, Mullis PE. Phenotypic variability in familial combined pituitary hormone deficiency caused by a PROP1 gene mutation resulting in the substitution of Arg– > Cys at codon 120 (R120C). J Clin Endocrinol Metab. 1998;83:3727–34.

Fukuoka H. Hypophysitis. Endocrinol Metab Clin North Am. 2015;44:143–9.

Gamberini MR, De Sanctis V, Gilli G. Hypogonadism, diabetes mellitus, hypothyroidism, hypoparathyroidism: incidence and prevalence related to iron overload and chelation therapy in patients with thalassaemia major followed from 1980 to 2007 in the Ferrara Centre. Pediatr Endocrinol Rev. 2008;6 Suppl 1:158–69.

Giavoli C, Porretti S, Ferrante E, Cappiello V, Ronchi CL, Travaglini P, Epaminonda P, Arosio M, Beck-Peccoz P. Recombinant hGH replacement therapy and the hypothalamus-pituitary-thyroid axis in children with GH deficiency: when should we be concerned about the occurrence of central hypothyroidism? Clin Endocrinol (Oxf). 2003;59:806–10.

Grozinsky-Glasberg S, Fraser A, Nahshoni E, Weizman A, Leibovici L. Thyroxine–triiodothyronine combination therapy versus thyroxine monotherapy for clinical hypothyroidism: meta-analysis of randomized controlled trials. J Clin Endocrinol Metab. 2006;91:2592–9.

Gurnell M, Halsall DJ, Chatterjee VK. What should be done when thyroid function tests do not make sense? Clin Endocrinol (Oxf). 2011;74:673–8.

Honegger J, Schlaffer S, Menzel C, Droste M, Werner S, Elbelt U, Strasburger C, Störmann S, Küppers A, Streetz-van der Werf C, Deutschbein T, Stieg M, Rotermund R, Milian M, Petersenn S, Pituitary Working Group of the German Society of Endocrinology. Diagnosis of primary hypophysitis in Germany. J Clin Endocrinol Metab. 2015;100:3841–9.

Hughes JN, Aubert M, Heatlie J, Gardner A, Gecz J, Morgan T, Belsky J, Thomas PQ. Identification of an IGSF1-specific deletion in a five generation pedigree with X-linked Central Hypothyroidism without macroorchidism. Clin Endocrinol (Oxf). 2016;85:609–15.

Hulle SV, Craen M, Callewaert B, Joustra S, Oostdijk W, Losekoot M, Wit JM, Turgeon MO, Bernard DJ, Schepper JD. Delayed adrenarche may be an additional feature of immunoglobulin super family member 1 deficiency syndrome. J Clin Res Pediatr Endocrinol. 2016;8:86–91.

Iverson JF, Mariash CN. Optimal free thyroxine levels for thyroid hormone replacement in hypothyroidism. Endocr Pract. 2008;14:550–5.

Jorgensen JO, Moller J, Laursen T, Orskov H, Christiansen JS, Weeke J. Growth hormone administration stimulates energy expenditure and extrathyroidal conversion of thyroxine to triiodothyronine in a dose-dependent manner and suppresses circadian thyrotrophin levels: studies in GH-deficient adults. Clin Endocrinol (Oxf). 1994;41:609–14.

Joustra SD, Schoenmakers N, Persani L, Campi I, Bonomi M, Radetti G, Beck-Peccoz P, Zhu H, Davis TM, Sun Y, Corssmit EP, Appelman-Dijkstra NM, Heinen CA, Pereira AM, Varewijck AJ, Janssen JA, Endert E, Hennekam RC, Lombardi MP, Mannens MM, Bak B, Bernard DJ, Breuning MH, Chatterjee K, Dattani MT, Oostdijk W, Biermasz NR, Wit JM, van Trotsenburg AS. The IGSF1 deficiency syndrome: characteristics of male and female patients. J Clin Endocrinol Metab. 2013;98:4942–52.

Joustra SD, Andela CD, Oostdijk W, van Trotsenburg AS, Fliers E, Wit JM, Pereira AM, Middelkoop HA, Biermasz NR. Mild deficits in attentional control in patients with the IGSF1 deficiency syndrome. Clin Endocrinol (Oxf). 2016;84:896–903.

Kanumakala S, Warne GL, Zacharin MR. Evolving hypopituitarism following cranial irradiation. J Paediatr Child Health. 2003;39:232–5.

Karavitaki N, Brufani C, Warner JT, Adams CB, Richards P, Ansorge O, Shine B, Turner HE, Wass JA. Craniopharyngiomas in children and adults: systematic analysis of 121 cases with long-term follow-up. Clin Endocrinol (Oxf). 2005;62:397–409.

Karavitaki N, Cudlip S, Adams CB, Wass JA. Craniopharyngiomas. Endocr Rev. 2006;27:371–97.

Kempers MJ, Lanting CI, van Heijst AF, van Trotsenburg AS, Wiedijk BM, de Vijlder JJ, Vulsma T. Neonatal screening for congenital hypothyroidism based on thyroxine, thyrotropin, and thyroxine-binding globulin measurement: potentials and pitfalls. J Clin Endocrinol Metab. 2006;91:3370–6.

Klose M, Brennum J, Poulsgaard L, Kosteljanetz M, Wagner A, Feldt-Rasmussen U. Hypopituitarism is uncommon after aneurysmal subarachnoid haemorrhage. Clin Endocrinol (Oxf). 2010;73:95–101.

Koulouri O, Auldin MA, Agarwal R, Kieffer V, Robertson C, Falconer Smith J, Levy MJ, Howlett TA. Diagnosis and treatment of hypothyroidism in TSH deficiency compared to primary thyroid disease: pituitary patients are at risk of underreplacement with levothyroxine. Clin Endocrinol (Oxf). 2011;74:744–9.

Koulouri O, Moran C, Halsall D, Chatterjee K, Gurnell M. Pitfalls in the measurement and interpretation of thyroid function tests. Best Pract Res Clin Endocrinol Metab. 2013;27:745–62.

Koulouri O, Nicholas AK, Schoenmakers E, Mokrosinski J, Lane F, Cole T, Kirk J, Farooqi IS, Chatterjee VK, Gurnell M, Schoenmakers N. A novel thyrotropin-releasing hormone receptor missense mutation (p81r) in central congenital hypothyroidism. J Clin Endocrinol Metab. 2016;101:847–51.

Krewer C, Schneider M, Schneider HJ, Kreitschmann-Andermahr I, Buchfelder M, Faust M, Berg C, Wallaschofski H, Renner C, Uhl E, Koenig E, Jordan M, Stalla GK, Kopczak A. Neuroendocrine disturbances one to five or more years after traumatic brain injury and aneurysmal subarachnoid hemorrhage: data from the german database on hypopituitarism. J Neurotrauma. 2016;33:1544–53.

Kyriakakis N, Lynch J, Orme SM, Gerrard G, Hatfield P, Loughrey C, Short SC, Murray RD. Pituitary dysfunction following cranial radiotherapy for adult-onset nonpituitary brain tumours. Clin Endocrinol (Oxf). 2016;84:372–9.

Lam T, Chan MM, Sweeting AN, De Sousa SM, Clements A, Carlino MS, Long GV, Tonks K, Chua E, Kefford RF, Chipps DR. Ipilimumab-induced hypophysitis in melanoma patients: an Australian case series. Intern Med J. 2015;45:1066–73.

Lania A, Persani L, Beck-Peccoz P. Central hypothyroidism. Pituitary. 2008;11:181–6.

Lewis AS, Courtney CH, Atkinson AB. All patients with “idiopathic” hypopituitarism should be screened for hemochromatosis. Pituitary. 2009;12:273–5.

Losa M, Scavini M, Gatti E, Rossini A, Madaschi S, Formenti I, Caumo A, Stidley CA, Lanzi R. Long-term effects of growth hormone replacement therapy on thyroid function in adults with growth hormone deficiency. Thyroid. 2008;18:1249–54.

Losa M, Donofrio CA, Barzaghi R, Mortini P. Presentation and surgical results of incidentally discovered nonfunctioning pituitary adenomas: evidence for a better outcome independently of other patients' characteristics. Eur J Endocrinol. 2013;169:735–42.

McCabe MJ, Alatzoglou KS, Dattani MT. Septo-optic dysplasia and other midline defects: the role of transcription factors: HESX1 and beyond. Best Pract Res Clin Endocrinol Metab. 2011;25:115–24.

Muller HL. Craniopharyngioma. Endocr Rev. 2014;35:513–43.

Nebesio TD, McKenna MP, Nabhan ZM, Eugster EA. Newborn screening results in children with central hypothyroidism. J Pediatr. 2010;156:990–3.

Netchine I, Sobrier ML, Krude H, Schnabel D, Maghnie M, Marcos E, Duriez B, Cacheux V, Av M, Goossens M, Grüters A, Amselem S. Mutations in LHX3 result in a new syndrome revealed by combined pituitary hormone deficiency. Nat Genet. 2000;25:182–6.

Neumann S, Raaka BM, Gershengorn MC. Constitutively active thyrotropin and thyrotropin-releasing hormone receptors and their inverse agonists. Methods Enzymol. 2010;485:147–60.

Papandreou MJ, Persani L, Asteria C, Ronin C, Beck-Peccoz P. Variable carbohydrate structures of circulating thyrotropin as studied by lectin affinity chromatography in different clinical conditions. J Clin Endocrinol Metab. 1993;77:393–8.

Persani L, Borgato S, Romoli R, Asteria C, Pizzocaro A, Beck-Peccoz P. Changes in the degree of sialylation of carbohydrate chains modify the biological properties of circulating thyrotropin isoforms in various physiological and pathological states. J Clin Endocrinol Metab. 1998;83:2486–92.

Persani L, Ferretti E, Borgato S, Faglia G, Beck-Peccoz P. Circulating thyrotropin bioactivity in sporadic central hypothyroidism. J Clin Endocrinol Metab. 2000;85:3631–5.

Porretti S, Giavoli C, Ronchi C, Lombardi G, Zaccaria M, Valle D, Arosio M, Beck-Peccoz P. Recombinant human GH replacement therapy and thyroid function in a large group of adult GH-deficient patients: when does L-T4 therapy become mandatory? J Clin Endocrinol Metab. 2002;87:2042–5.

Portes ES, Oliveira JH, MacCagnan P, Abucham J. Changes in serum thyroid hormones levels and their mechanisms during long-term growth hormone (GH) replacement therapy in GH deficient children. Clin Endocrinol (Oxf). 2000;53:183–9.

Price A, Weetman AP. Screening for central hypothyroidism is unjustified. BMJ. 2001;322:798.

Ratnasingam J, Karim N, Paramasivam SS, Ibrahim L, Lim LL, Tan AT, Vethakkan SR, Jalaludin A, Chan SP. Hypothalamic pituitary dysfunction amongst nasopharyngeal cancer survivors. Pituitary. 2015;18(4):448–55.

Rochette C, Jullien N, Saveanu A, Caldagues E, Bergada I, Braslavsky D, Pfeifer M, Reynaud R, Herman JP, Barlier A, Brue T, Enjalbert A, Castinetti F. Identifying the deleterious effect of rare LHX4 allelic variants, a challenging issue. PLoS One. 2015;10:e0126648.

Samaan NA, Schultz PN, Yang KP, Vassilopoulou-Sellin R, Maor MH, Cangir A, Goepfert H. Endocrine complications after radiotherapy for tumors of the head and neck. J Lab Clin Med. 1987;109:364–72.

Schmiegelow M, Feldt-Rasmussen U, Rasmussen AK, Poulsen HS, Muller J. A population-based study of thyroid function after radiotherapy and chemotherapy for a childhood brain tumor. J Clin Endocrinol Metab. 2003;88:136–40.

Schoenmakers N, Alatzoglou KS, Chatterjee VK, Dattani MT. Recent advances in central congenital hypothyroidism. J Endocrinol. 2015;227:R51–71.

Shimon I, Cohen O, Lubetsky A, Olchovsky D. Thyrotropin suppression by thyroid hormone replacement is correlated with thyroxine level normalization in central hypothyroidism. Thyroid. 2002;12:823–7.

Slawik M, Klawitter B, Meiser E, Schories M, Zwermann O, Borm K, Peper M, Lubrich B, Hug MJ, Nauck M, Olschewski M, Beuschlein F, Reincke M. Thyroid hormone replacement for

central hypothyroidism: a randomized controlled trial comparing two doses of thyroxine (T4) with a combination of T4 and triiodothyronine. J Clin Endocrinol Metab. 2007;92:4115–22.

Stagi S, Lapi E, Pantaleo M, Traficante G, Giglio S, Seminara S, de Martino M. A SOX3 (Xq26.3-27.3) duplication in a boy with growth hormone deficiency, ocular dyspraxia, and intellectual disability: a long-term follow-up and literature review. Hormones (Athens). 2014;13:552–60.

Sun Y, Bak B, Schoenmakers N, van Trotsenburg AS, Oostdijk W, Voshol P, Cambridge E, White JK, le Tissier P, Gharavy SN, Martinez-Barbera JP, Stokvis-Brantsma WH, Vulsma T, Kempers MJ, Persani L, Campi I, Bonomi M, Beck-Peccoz P, Zhu H, Davis TM, Hokken-Koelega AC, Del Blanco DG, Rangasami JJ, Ruivenkamp CA, Laros JF, Kriek M, Kant SG, Bosch CA, Biermasz NR, Appelman-Dijkstra NM, Corssmit EP, Hovens GC, Pereira AM, den Dunnen JT, Wade MG, Breuning MH, Hennekam RC, Chatterjee K, Dattani MT, Wit JM, Bernard DJ. Loss-of-function mutations in IGSF1 cause an X-linked syndrome of central hypothyroidism and testicular enlargement. Nat Genet. 2012;44:1375–81.

Wiersinga WM. Paradigm shifts in thyroid hormone replacement therapies for hypothyroidism. Nat Rev Endocrinol. 2014;10:164–74.

Xu Z, Lee Vance M, Schlesinger D, Sheehan JP. Hypopituitarism after stereotactic radiosurgery for pituitary adenomas. Neurosurgery. 2013;72:630–7.

Yamamoto M, Iguchi G, Takeno R, Okimura Y, Sano T, Takahashi M, Nishizawa H, Handayaningshi AE, Fukuoka H, Tobita M, Saitoh T, Tojo K, Mokubo A, Morinobu A, Iida K, Kaji H, Seino S, Chihara K, Takahashi Y. Adult combined GH, prolactin, and TSH deficiency associated with circulating PIT-1 antibody in humans. J Clin Invest. 2011;121:113–9.

Diagnosis and Treatment of Hypothyroidism

13

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Abstract

Hypothyroidism is a common medical condition which affects predominantly women and the elderly. Symptoms of hypothyroidism are nonspecific and frequently encountered in the general population; therefore the diagnosis is suspected in many but confirmed in only a few. Hence thyroid function tests are some of the most requested laboratory tests. The diagnosis of hypothyroidism is confirmed when serum thyrotropin (TSH) levels are above the reference range in the presence of low circulating thyroid hormones. However, the reference range for both serum TSH and thyroid hormones in the population is wide, and there is continuous debate as to what constitutes the "normal" range for a given individual.

Treatment of hypothyroidism with thyroid hormone replacement therapy is thought to be relatively straightforward and usually lifelong. Treatment of choice is levothyroxine (LT4), a synthetic thyroid hormone that is chemically identical to thyroxine (T4). The body is able to generate the active thyroid hormone triiodothyronine (T3) from LT4 peripherally by the action of deiodinasing enzymes. Therapy with appropriate doses of LT4 restores biochemical euthyroidism by increasing serum T4 and reducing serum TSH levels to within the reference range. However, some patients with hypothyroidism who are treated with LT4 therapy and whose serum TSH level is within the reference range complain of residual hypothyroid symptoms. In addition, circulating levels of T3 tend to be lower with LT4 monotherapy compared to euthyroid controls, even when serum TSH levels are similar. It is therefore argued that other forms of thyroid hormone replacement (such as T4 and T3 combinations or with desiccated thyroid extract) may be a more physiological form of replacement. The majority of interventional trials of these alternative therapies have so far been unable to show any benefit. It is possible that these alternative therapies may be useful in certain subgroups of patients but this is yet to be proven.

Keywords
Hypothyroidism · Management · L-Thyroxine · Diagnosis · Treatment

Diagnosis of Hypothyroidism

Introduction

The main function of the human thyroid gland is to produce the thyroid hormones (TH) thyroxine (T4) and triiodothyronine (T3). TH have an important role in development and growth and regulate metabolism of virtually all organs by both genomic and non-genomic action (Brent 2012). At the tissue level, modulation of TH is achieved by complex and tightly regulated processes involving TH secretion, plasma and cellular membrane transport, activation and deactivation, and interaction with intracellular TH receptors and their co-regulators. The production of TH by the thyroid gland is controlled by the pituitary and the hypothalamus by thyrotropin (TSH) and thyrotropin-regulating hormone (TRH), respectively. Both TSH and TRH secretion, in turn, are influenced by TH levels by a negative feedback mechanism. TSH regulates iodide uptake and the subsequent synthesis and secretion of TH by thyroid follicular cell via the TSH receptor. Deficiency of TH (or hypothyroidism) is classed as primary if it is due to reduced TH production and secretion by the thyroid gland itself or secondary if the TH deficiency is as a consequence of TSH or TRH deficiency (also referred to as central hypothyroidism). The differences between primary and secondary (or central) hypothyroidism are outlined in Table 1. A diagnosis of primary

Table 1 Biochemical and clinical differences between primary and secondary hypothyroidism

	Primary hypothyroidism	Secondary hypothyroidism
Serum TSH levels	High	Inappropriately low or normal
Serum thyroid hormone levels	Low or low-normal	Low or low-normal
Antithyroid antibodies	Usually positive[a]	Usually negative
TRH stimulation test[b]	Exaggerated TSH response	Flat or delayed TSH response
Clinical features	Symptoms are variable; goiter usually present; other pituitary hormones normal[c]	Symptoms are variable and can range from none to those of hypopituitarism; goiter absent; other pituitary hormones may be abnormal

[a]If cause is due to autoimmune thyroid destruction of the thyroid gland
[b]Details of this test are beyond scope of this chapter but have been provided in reference (Moncayo et al. 2007)
[c]Prolactin levels may be mildly elevated

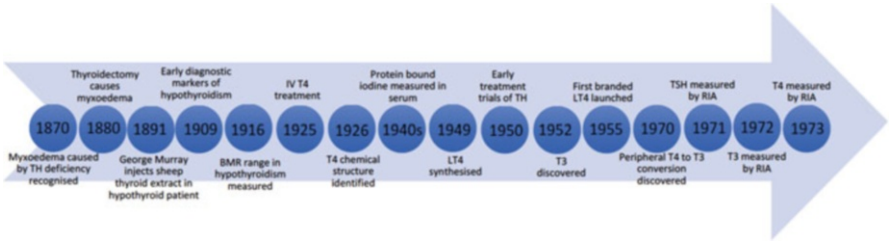

Fig. 1 Timeline chart of developments in thyroid function and treatment

hypothyroidism is confirmed by biochemical evidence of TH deficiency and a raised serum TSH level. The diagnosis is aided by the presence of relevant clinical symptoms and signs, but clinical examination on its own is not reliable (Cooper and Biondi 2012). Moreover, clinical assessment is even more unreliable in milder forms of the condition – which is the presenting feature in the vast majority of patients. Mild (or subclinical) hypothyroidism is characterized by raised serum TSH levels with TH concentrations within the reference range. Prior to the availability of biochemical thyroid function tests, a variety of methods were utilized, and only more severe forms of hypothyroidism were detected. This chapter describes various aspects of making a diagnosis as well as treatment of hypothyroidism.

Historical Aspects of Making a Diagnosis of Hypothyroidism

In the past, before biochemical testing for thyroid function was available, diagnosis of hypothyroidism relied largely on the presence of clinical symptoms and signs (Fig. 1). In 1874, Sir William Withey Gull presented before the Clinical Society of London two of the five cases he had seen of what he called "A Cretinoid State supervening in Adult Life in Women." In these cases, he described their cretin-like appearance, including a broad and thick tongue and the guttural voice and its pronunciation "as if the tongue were too large for the mouth" (Gull 1874). In 1922, Boothby and Sandiford showed that patients with hypothyroidism have a 10% lower basal metabolic rate compared to normal individuals and hence could be used as a diagnostic tool (Boothby and Sandiford 1922). The advent of assays for detecting TH was a game changer in diagnosing hypothyroidism. In the early 1950s, only one thyroid test was available: an indirect estimate of the serum total (free plus protein-bound) thyroxine (T4) concentration, using the protein-bound iodine (PBI) technique (Benotti and Benotti 1963). In 1952, Pitt-Rivers documented the presence of T3 in plasma using chromatography, studied its physiological function, and noted that it seemed to be three to four times more potent than T4 in preventing goiters (Gross and Pitt-Rivers 1952). Thyroid-stimulating hormone (TSH) was measured for the first time in 1963 using a first-generation radioimmunoassay (RIA) (Yiger et al. 1963). A few years later, RIA for measurement of serum T_3 (Surks et al. 1972) and T_4 (Larsen et al. 1973) were soon developed by Surks and Reed-Larsen, respectively. Measurement of TSH using RIA became the mainline test for assessing thyroid

status, but there was considerable overlap between values in hyperthyroid and euthyroid subjects. Therefore, its use declined as new immunometric assay techniques became available in the middle of the 1980s (Dunlap 1990). The new technique, which measured the presence or concentration of the molecule using antibodies, was more accurate, leading to the second, third, and even fourth generations of TSH immunoassays, with each generation possessing ten times greater functional sensitivity than the last. For the first time, automated methods were used in the third-generation immunoassay. Currently, fourth-generation TSH immunoassays are in use worldwide. Since 1970, technological advances in radioimmunoassays (Nicoloff and Spencer 1990), immunometric assays (Spencer and Nicoloff 1990), and more recently liquid chromatography-tandem mass spectrometry methodologies (Thienpont et al. 2010) have progressively improved the specificity and the sensitivity of thyroid testing methods (Dufour 2007).

Are Symptoms and Signs Not Sufficient to Diagnose Hypothyroidism?

Prevalence of Symptoms and Signs

Primary hypothyroidism is a graded condition, ranging from very mild cases, in which the individual is virtually asymptomatic but has biochemical abnormalities, to very severe cases which present with life-threatening myxedema coma. Since the onset of the disease is insidious, the obvious symptoms and signs can present late in the disease process and may be nonspecific (Wiersinga 2004). The commonest symptom in hypothyroidism, almost always present, is generalized weakness and/or tiredness. Other common symptoms include dry and coarse skin, weight gain, lethargy, and constipation (Fig. 2).

The symptoms reported by hypothyroid patients are nonspecific and have a high prevalence in the wider euthyroid adult population (Carlé et al. 2014). For instance, in a population-based survey of participants unaware of their thyroid status at the time of sampling, the symptom of tiredness was reported by more than 40 and 80% of respondents with euthyroidism and hypothyroidism, respectively. The presence of hypothyroid symptoms has low sensitivity and positive predictive value. However, there is a graded prevalence of the number of symptoms reported based on severity of TH deficiency: with more symptoms being present in overt hypothyroidism than subclinical hypothyroidism (Canaris et al. 2000a). Therefore, clinical suspicion of hypothyroidism needs to be confirmed by blood testing.

In the era prior to thyroid function testing, questionnaires to quantify symptoms and signs were developed with the aim to improve the accuracy of diagnosis of hypothyroidism. One of these is the Billewicz score, composed of points given in a weighted manner for the presence or absence of 17 symptoms and signs. Billewicz designed this in 1969 as a diagnostic index for hypothyroidism prior to the availability of biochemical thyroid function tests. He along with colleagues evaluated the clinical features of hypothyroidism in 152 patients with suspected hypothyroidism (Billewicz et al. 1969). The final diagnosis of hypothyroidism was made by 48-h radioactive iodine uptake, serum protein-bound iodine, thyroid autoantibodies, electrocardiogram, serum

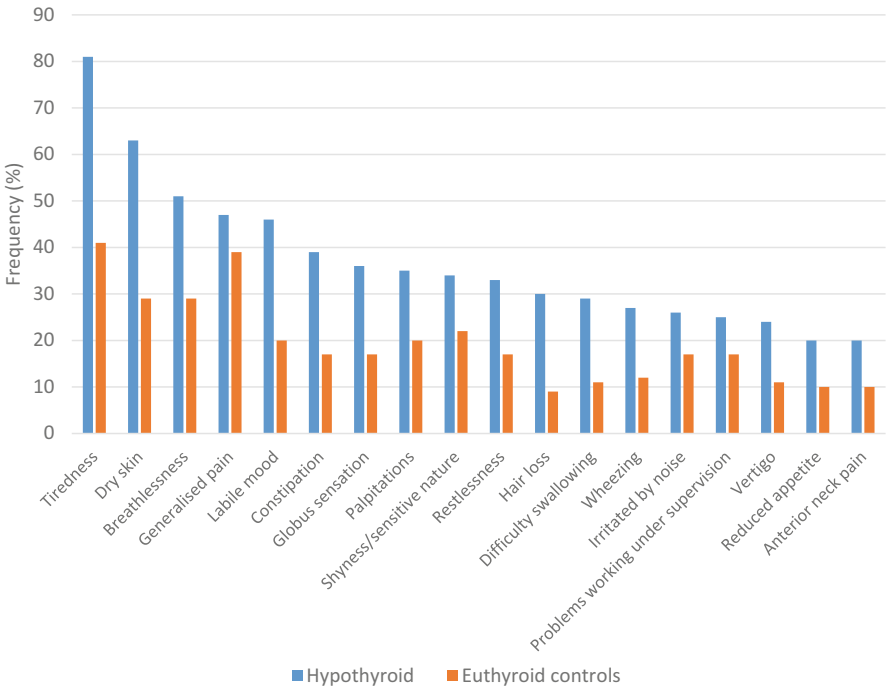

Fig. 2 Prevalence of common symptoms and signs of hypothyroidism in adults (Adapted from reference Carlé et al. 2014)

cholesterol, and therapeutic response to L-thyroxine replacement therapy. A higher positive score indicated a greater degree of clinical hypothyroidism. The usefulness of this score was confirmed by a larger follow-up study (Nyström et al. 1988).

More recently, Zulewski and colleagues designed a score based on the Billewicz index to evaluate symptoms and signs in the contemporary thyroid function testing era (Zulewski et al. 1997). Fourteen symptoms and signs of hypothyroidism, similar to the ones described by Billewicz, were evaluated in 332 subjects (50 with overt hypothyroidism, 93 with subclinical hypothyroidism, and 189 euthyroid controls, based on TSH assays). From the original 14 symptoms and signs, two (cold intolerance and reduced pulse rate) were excluded as they had low sensitivity and specificity (Table 2). Using this score, 62% of overtly hypothyroid patients were correctly diagnosed as compared to 42% with the Billewicz index. This score however showed no correlation with TSH in hypothyroid patients though the free TH levels were related in a linear fashion to the score. The authors noted that some patients with severe biochemical hypothyroidism had few symptoms, whereas some with minor biochemical abnormalities had profound manifestations. In addition, the score was directly related to age with older people having a higher score. Thus, an additional point was added for younger (<55 years) patients. Moreover, this score also carries the inconvenience of clinicians asking patients about seven symptoms and also examining them for five signs. The authors therefore concluded that this score is useful to assess the tissue severity of hypothyroidism at peripheral target

Table 2 Accuracy of 12 symptoms and signs in the diagnosis of primary hypothyroidism (Zulewski score)

Symptoms	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Score if present
Hearing impairment	22	98	90	53	1
Diminished sweating	54	86	80	65	1
Constipation	48	85	76	62	1
Paresthesia	52	83	75	63	1
Hoarseness of voice	34	88	73	57	1
Weight increase	54	78	71	63	1
Dry skin	76	64	68	73	1
Physical signs					
Slow movements	36	99	97	61	1
Periorbital puffiness	60	96	94	71	1
Delayed ankle reflex	77	94	92	80	1
Coarse skin	60	81	76	67	1
Cold skin	50	80	71	62	1
Sum of scores if all symptoms and signs are present[a]					12[b]

[a]Add 1 point in women younger than 55 years
[b]Hypothyroid ≥6 points; intermediate = 3–5 points; euthyroid ≤2 points

organ level and that biochemical tests should remain the gold standard tool to diagnose hypothyroidism.

Seshadri and colleagues designed a score using symptoms of hypothyroidism and compared it to biochemical testing for TSH and thyroxine levels. This score had a false-positive result in 45% of euthyroid individuals, and the authors concluded that this score should only be used as a screening tool where resources are limited and biochemical testing difficult (Seshadri et al. 1989).

Biochemical Investigations for Diagnosing Hypothyroidism

TSH, FT3, and FT4

Biochemical testing of thyroid function remains the cornerstone to make a diagnosis of hypothyroidism. These tests include estimating serum TSH and TH (T4 and T3) concentrations. As the functional performance of the TSH assays has improved over the last few decades, this has revolutionized strategies for thyroid testing and firmly established TSH as the first-line thyroid function test to assess TH status in the vast

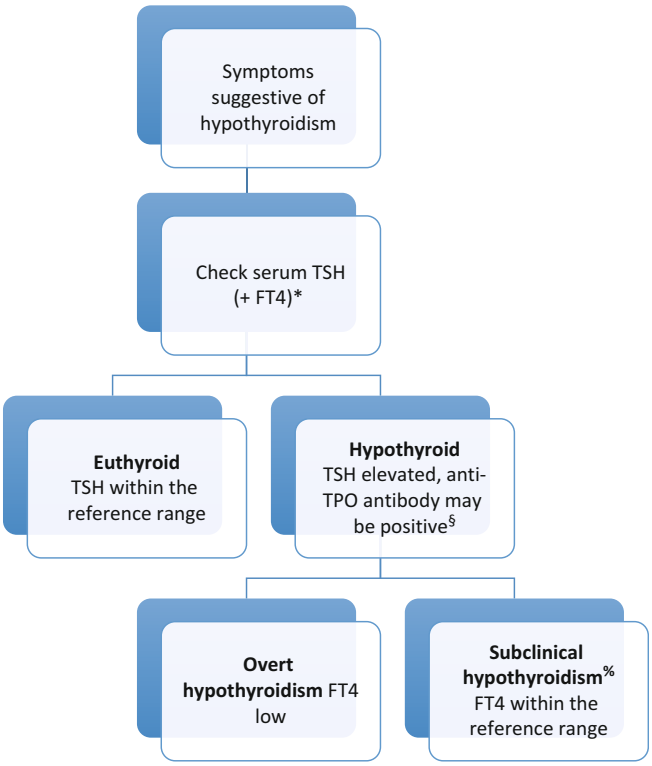

Fig. 3 Algorithm for diagnosing hypothyroidism. *Most laboratories utilize a TSH-first policy in thyroid function testing and will measure FT4 and/or FT3 if TSH is abnormal. This is because it is very rare for thyroid hormones to be outside their reference range when TSH is within its own reference range. §Positive anti-TPO antibody levels indicate underlying autoimmune thyroid disease and a higher risk of progression to overt hypothyroidism (in subclinical hypothyroid patients) but may not influence treatment decision as this is guided primarily by severity of symptoms, age of the patient, and degree of elevation of serum TSH (Pearce et al. 2013). %Older individuals may have a slightly elevated serum TSH that may be a normal response to aging and does not warrant treatment if serum TSH <10 mU/L (Pearce et al. 2013)

majority of patients with suspected thyroid disease (Garber et al. 2012; Okosieme et al. 2016). In fact, measurement of serum TSH has become the single most reliable test for diagnosing abnormalities in thyroid status, provided that patients are not receiving drug therapies that alter TSH secretion or have pituitary disease. Measurement of serum TSH is now considered to be the most important thyroid function test for diagnosing early (also called mild or subclinical) hypo- or hyperthyroidism because of the log/linear relationship between TSH and T4: a twofold change in serum FT4 level leads to a 100-fold alteration in circulating TSH (Andersen et al. 2003a). The reference range for TH is wide for a given population; therefore, in principle, TSH will be the first detected circulating abnormality as the pituitary registers that T4 has changed from its genetically determined set point for that particular individual (Hansen et al. 2004a) (Fig. 3).

Serum TH can be measured either as total (protein-bound) or free (unbound) fractions. TH are bound to three main circulating transport proteins: thyroxine-binding globulin (TBG), transthyretin, and albumin. TBG has the strongest affinity for TH, while albumin is the most abundant protein present in blood (Thienpont et al. 2013). It is argued, however, that the free TH assays better reflect the physiological effects of TH than total hormone concentrations as binding proteins can vary in the population. Although the free hormones are present in small quantity compared to the total T4 and T3, it is believed that the minute free fraction of hormone (0.02% and 0.2% for FT4 and FT3, respectively) is responsible for biological activity at the cellular level and hence reflect the physiological effects of TH better than total hormone concentrations, particularly when binding proteins are abnormal. The impetus to develop free hormone tests has been the high frequency of binding-protein abnormalities encountered in clinical practice, especially the high TBG state of pregnancy. Currently, most clinical laboratories use automated immunoassays to estimate serum FT4 and FT3 concentrations.

TSH Reference Range

Determining the TSH reference range is crucial to diagnose mild as well as overt thyroid dysfunction. Guidelines produced by the American National Academy of Clinical Biochemistry in 2003 state that "TSH reference intervals should be established from the 95% confidence limits of the log-transformed values of at least 120 rigorously screened normal euthyroid volunteers who have: (a) No detectable thyroid autoantibodies, TPOAb or TgAb (measured by sensitive immunoassay); (b) No personal or family history of thyroid dysfunction; (c) No visible or palpable goiter and, (c) Who are taking no medications except oestrogen" (Baloch et al. 2003). In addition, circulating TSH levels have a diurnal variation with a peak late at night/early hours of morning, and, therefore, sample timing and shift work also should be taken into account when defining the TSH reference range (Jensen et al. 2004). Several other factors also influence serum TSH values (discussed in detail in section on Factors impacting TSH).

As the ability of the TSH assays to detect lower levels of the hormone improved with each generation, the lower euthyroid reference limit was set at 0.3–0.4 mU/L. This resulted in overt and subclinical hyperthyroidism being diagnosed with a greater degree of precision without the need for thyrotropin-releasing hormone (TRH) stimulation, irrespective of the population being studied or the method used. There has been a general consensus on the lower (2.5 percentile) TSH reference limit for some time, but, in contrast, the level at which to set the upper (97.5 percentile) reference limit for nonpregnant adults is still a matter of debate (Dickey et al. 2005; Surks et al. 2005). Consequently, it is difficult for manufacturers to cite a TSH reference range appropriate for universal adoption across different populations and various geographic areas.

The most robust data determining the TSH reference range was obtained from the US National Health and Nutritional Examination Survey (NHANES) III study

(Hollowell et al. 2002). This was the largest study (n = 16,088) that analyzed the median and lower and upper reference limits of serum TSH in carefully selected euthyroid individuals using current immunoassays. This study showed that it is not possible to establish an accurate TSH upper limit at an individual level from population data as it is not a sensitive parameter for detecting subtle thyroid dysfunction. This is because TSH has a low index of individuality (the ratio between the within- and between-person variability). Other studies of various markers of TH action on tissues have however suggested that even slightly elevated TSH levels (4–10 mU/L) may increase the risk for atherosclerosis in susceptible individuals (Thvilum et al. 2012a). Similarly, low TSH has also been associated with evidence of end-organ damage: mainly abnormal heart rhythm (atrial fibrillation) and loss of bone structure (osteoporosis) (Biondi and Cooper 2008). The lower and upper limits of the TSH reference range should also take into account the individual variability of the TSH measurement in the same individual. Several studies provide data showing significant variation in repeated TSH measurements over time in the same individuals (Andersen et al. 2003b). Each person appears to have a specific and unique set point for TH concentrations, which is partly genetically determined as shown by twin studies (Hansen et al. 2004b). TSH measurements in any individual vary within 50% of the entire group's TSH distribution, and the variation is large and clinically significant (Andersen et al. 2002).

The distribution of serum TSH is not Gaussian and has a skew to the right. However, more than 95% of healthy euthyroid individuals have serum TSH values between 0.4 and 2.5 mU/L. It is therefore argued that individuals with TSH values higher than 2.5 mU/L have occult autoimmune thyroid disease and contribute to the skewed TSH distribution curve (Wartofsky and Dickey 2005). In support of this contention, individuals with serum TSH > 2.5 mU/L at baseline have a higher risk of progression to subsequent hypothyroidism (Vanderpump et al. 1995; Walsh et al. 2010). The counterargument to keep the upper limit of the TSH reference range around the 4.0–5.0 mU/L mark is that routine screening and treatment for subclinical hypothyroidism is not warranted and therefore, by extension, is not required for values near the upper limit of the reference range. Most patients with thyroid disease with positive anti-TPO antibodies have a TSH value below 2.5 mU/L, and patients with a TSH between 2.5 and 4.5 mU/L probably have minimal thyroid deficiency without any reported adverse health consequences or benefit of treatment with LT4 (Surks et al. 2005). Complicating this issue is the fact that current TSH immunoassays differ in specificity for recognizing circulating TSH isoforms and that this can give rise to a full 1.0 mU/L difference in TSH values reported by different assays – a difference that in some cases is greater than the influence of many of the other variables listed above.

In summary, decreasing the upper limits of normal for serum TSH could have enormous implications for health and health economy as the long-term impact on health is unknown as this has not been tested in prospective trials, and unnecessary treatment prescriptions will lead to a higher economic burden for individuals and/or taxpayers. Furthermore, millions of individuals could be wrongly classed as having thyroid disease based on a biochemical measurement. In addition, a higher serum

TSH level may be normal in older individuals and not associated with adverse outcomes (Waring et al. 2012; Pearce et al. 2016). This has implications not only for diagnosing subclinical hypothyroidism in the elderly but also the level of serum TSH to aim for in treated hypothyroid patients in this age group (Pasqualetti et al. 2014; Razvi et al. 2016). Further research is required before age-specific TSH reference ranges become part of routine clinical practice.

Total or Free T4 and T3 Reference Range

In clinical practice, the reference ranges for T4 and T3 are less important than that for TSH as it is rare to have values that are outside of the reference range without a corresponding abnormality in the TSH level. Such scenarios could occur in central hypothyroidism (e.g., low T4 with an inappropriately normal TSH in hypopituitarism) or when there is interference with either the T4/T3 or TSH immunoassay due to heterophilic antibodies (Burman 2008).

Total T4 reference ranges vary somewhat depending on the methods employed, ranging between 58 and 160 nmol/L (4.5–12.5 μg/dL). For reasons that are unclear, race seems to impact on total T4 levels with Mexican Americans seeming to have higher total T4 than white or black individuals (Hollowell et al. 2002). Serum total T3 values are method dependent and have reference ranges approximating to 1.2–2.7 nmol/L (80–180 ng/dL). The FT4 and FT3 reference range is age dependent, being slightly higher in infants than children or adults.

Factors Impacting TSH

Multiple factors influence the TSH reference limits for a population including time of sampling, age, sex, ethnicity, iodine intake, smoking status, as well as the failure to exclude the presence of subclinical autoimmune thyroid disease using the presence of TPO antibody.

Diurnal Variation

There is evidence of a considerable diurnal variation in serum TSH concentration, with a peak around midnight (Weeke and Gundersen 1978). A decrease of up to 50% occurs till the morning; thereafter the concentration remains relatively constant until evening, with a nadir in the late afternoon. As serum TSH concentrations vary markedly and time of sampling is unknown in most studies, sampling time differences between studies may be one of the main reasons for the discrepancies in published reference intervals (Ehrenkranz et al. 2015). Furthermore, night-shift workers have displaced or reduced diurnal rhythms, a phenomenon that should also be taken into account when establishing reference intervals. Patients with overt hypothyroidism lose the circadian rhythm which is restored with LT4 treatment (Persani et al. 1995).

Age

Thyroid function changes with age due mainly to the upper limit of the TSH reference range increasing (Thvilum et al. 2012a; Aggarwal and The 2013). As serum TSH seems to be influenced by age, it raises the possibility that its reference range may need to be adjusted for the age of the individual. However, prospective studies will be required to show whether treatment of mildly raised TSH in this – is beneficial or not before age-appropriate TSH reference ranges are widely adopted (Wilkes et al. 2013). Several observational studies have shown that older individuals with a slightly raised serum TSH level have no adverse consequences (Waring et al. 2012; Pearce et al. 2016). In fact, one report suggests that a slightly raised TSH may be beneficial for survival in 85-year-old individuals from Leiden (Gussekloo et al. 2004). On the other hand, these mild TSH elevations could be a transient phenomenon and could, in part, be related to polymorphisms of the TSH receptor and therefore cannot be generalized that subclinical hypothyroidism per se is beneficial for elderly population (Amaud-Lopez et al. 2008).

Age also alters the pituitary set point of TSH for reduced FT4 levels so that a lower serum TSH increase is seen in older patients compared to similar falls in FT4 in younger ones (Bremner et al. 2012). An increase in TSH with age could be secondary to an increase in the secretion of biologically inactive TSH isoforms or reduced responsiveness of the thyroid gland to TSH stimulation. It is unclear whether this resetting of the pituitary thyrotroph is an adaptive response to normal aging or maladaptive. The longevity associated with a slightly higher TSH (Gussekloo et al. 2004; Atzmon et al. 2009) suggests that this phenomenon is likely to be adaptive and possibly beneficial. However, an observational longitudinal study of a cohort of older people (70–79 years old) showed a higher risk of heart failure in those with subclinical hypothyroidism – this finding is more in keeping with a maladaptive process (Rodondi et al. 2005). Thus, more evidence in the form of a randomized clinical trial is required before this issue can be settled.

Iodine Intake

Serum TSH levels are poor indicators of iodine status (Zimmermann et al. 2008). In adults, iodine intake may alter serum TSH levels minimally within the reference range. A positive correlation between TSH concentrations and age in iodine sufficient populations but not in iodine-deficient individuals has been noted. The inverse relationship between TSH and age in iodine-deficient areas could represent a failure to exclude individuals with autonomously functioning nodules. In an observational study from two regions in Denmark with mild and moderate iodine deficiency at baseline, salt iodine fortification was associated with a significant increase in serum TSH that was independent of age, but this was only observed in the region with the higher iodine intake (Bjergved et al. 2012).

Gender

The cross-sectional Whickham study noted that serum TSH levels increased markedly in women aged greater than 45 year though they did not vary with age in men. They found no increase in TSH with age in women in the absence of antithyroid antibodies (Tunbridge et al. 1977). A large retrospective database analysis of 465,593 samples also showed that women had a significantly higher serum TSH level albeit by only 0.1 mU/L (Ehrenkranz et al. 2015). However, in the rigorously screened NHANES III population, after those with positive thyroid autoantibodies and those on medications affecting thyroid function were excluded, there was no difference in mean serum TSH levels between men and women (Hollowell et al. 2002). Nevertheless, a slight but significant increase in TSH with age in both men and women was seen in the disease-free population.

Ethnicity

TSH levels were noted to be higher in whites than in blacks in the NHANES III study (Hollowell et al. 2002). This was even in the absence of thyroid antibodies and other risk factors. Despite thyroid antibodies being less frequent in blacks, their association with TSH concentrations was much less in blacks than in whites. However confounding factors like environmental influences may play a role in the observed racial differences. Additionally, ethnic differences in TSH are not observed when populations with the same relative frequency of thyroid antibodies are compared (Spencer et al. 2007).

Anti-TPO Antibodies

There is a correlation between TSH values and anti-TPO antibodies. The presence of anti-TPO antibodies is generally agreed to provide evidence of autoimmune thyroid disease, and it predicts an increased risk for development of subclinical or overt hypothyroidism (Vanderpump et al. 1995). However, there is a cohort of patients who are known to be anti-TPO antibody positive with normal thyroid function but do not progress to any degree of hypothyroidism. In the NHANES III study, anti-TPO antibodies were present in 17.5, 24.9, and 30.0% of patients whose TSH was between 3.0–3.49, 3.5–3.99, and 4.0–4.49 mU/L, respectively (Hollowell et al. 2002). Overall only 22.2% of those subjects with TSH between 3.0 and 4.49 mU/L in the disease-free group had anti-TPO antibodies. However, the prevalence of positive anti-TPO antibody in those with mild subclinical hypothyroidism (serum TSH between 5 and 10 mU/L) was 56.8%.

Smoking

Tobacco smoking has consistently been associated with thyroid function in population-based studies. Current smokers are more likely to have lower TSH levels

and lower risk of hypothyroidism. In a large population-based study of more than 30,000 individuals cigarette smoking was positively associated with hyperthyroidism and negatively with hypothyroidism (Asvold et al. 2007). Furthermore, ex-smokers showed a gradual increase in TSH levels since smoking cessation. There seemed to be an indirect evidence of a dose-response link as moderate smokers had higher TSH levels than those that smoked more. Recent smoking cessation is associated with a higher risk of developing de novo autoimmune thyroid disease (Wiersinga 2013).

It is as yet unclear what the exact mechanism is or what component of tobacco is responsible for the effect on thyroid function. Thiocyanates (a by-product of cyanide that is generated from cigarette smoke that inhibits iodide trapping and is a goitrogen) and/or nicotine (stimulates sympathetic activation) may be the components of tobacco responsible for this effect. Smoking is also noted to exert tissue-specific effects on TH action at both the pretranslational and posttranslational levels.

Body Weight

Overt hypothyroidism is associated with weight gain. In addition, serum TSH, even within the reference range, is positively correlated with body mass index or weight (Knudsen et al. 2005). Change in weight is directly connected to change in TSH although a causal link cannot be determined by these observations. A reduction in serum TSH has been noted in patients undergoing bariatric surgery; however, some others have shown an increase in FT4 but no change in TSH (Dall'Asta et al. 2010; MacCuish et al. 2012; de Moraes et al. 2005).

The mechanism by which body weight influences serum TSH levels is unclear. Several possible theories have been suggested. It may be that the increase in weight is counteracted by an increase in TSH as an adaptive phenomenon, leading to an increased production of bioinactive TSH isomers or resistance to TSH in target organs or maybe related to the effects of changes in adipokines (mainly leptin) (Menendez et al. 2003; F1 et al. 2010).

Genetic Factors

Thyroid function is determined in an individual by both genetic and environmental factors. Twin studies estimate heritability of 49–65% for TSH and 40–90% for FT4 – which suggests that genetic factors play a very strong role (Hansen et al. 2004a). However, genome-wide association studies to identify single nucleotide polymorphisms (SNPs) that are linked to thyroid function have only been able to explain no more than around 5% of total TSH and 2% of FT4 variance (Porcu et al. 2013). The main SNPs for TSH are in the phosphodiesterase type 8B (PDE8B), capping protein muscle Z-line (CAPZB), nuclear receptor subfamily 3, group C, member 2 (NR3C2), and v-maf musculoaponeurotic fibrosarcoma oncogene homolog (MAF/LOC440389) genes. Interestingly, these loci contribute to TSH variation

within and outside the reference range, indicating a genetic role in thyroid dysfunction too. In addition, thyroid autoimmunity also seems to be under significant genetic control in both males and females (Hansen et al. 2006).

Imaging to Diagnose Hypothyroidism

Imaging has very little role to play in diagnosing hypothyroidism in adults but may be useful in elucidating the underlying cause, once the diagnosis is made biochemically. There is a correlation between thyroid hypoechogenicity and higher than average levels of serum TSH, even in subjects without overt thyroid disease. However, a normal sonogram does not preclude hypothyroidism, though there is no clear relationship between the two (Marcocci et al. 1991). Furthermore, there is some evidence that thyroid hypoechogenicity on ultrasound may be a better marker in predicting present and future thyroid function abnormalities than the presence of circulating antithyroid antibodies (Rago et al. 2001).

Treatment of Hypothyroidism

Thyroid Hormone Replacement

Of the available TH replacement preparations, LT4 is presently recommended as the drug of choice in view of its long half-life, ready quantification in the blood, ease of absorption, and the availability of multiple tablet strengths (Garber et al. 2012). It became clear in the early 1970s that biologically active T3 is generated by peripheral conversion of T4 by deiodinasing enzymes (Braverman and Vagenakis 1979). Since then, LT4 monotherapy has become the mainstay of the treatment of hypothyroidism replacing desiccated thyroid extract and combination of T4 and T3 therapies (Table 3).

History of Thyroid Hormone Replacement

There was limited knowledge of thyroid biology until the middle of the nineteenth century. In 1836, Thomas Wilkinson King of Guys Hospital, London, on the basis of observation and experiments he had carried out, wondered at the thyroid's

Table 3 Summary of differences between LT4 and triiodothyronine (LT3)

	LT4	LT3
Absorption after oral ingestion	80%	90%
Peak serum concentration	2–4 h	1–2 h
Half-life	7 days	12–19 h
Affinity for thyroid hormone receptor	Low	High
Percentage bound to plasma proteins	99.98%	99.7%

disproportionately large vascular supply in the absence of any evident mechanical or other local function and described its "peculiar" fluid (King 1836). It was Kocher – who later received the Nobel Prize for his work on the thyroid – who created an understanding of the importance of the thyroid by identifying the late effects of total ablation of goiters (Kocher 1883).

In 1891, George Murray, presented to the local Durham and Northumberland Medical Society his idea of treating myxedema with subcutaneous sheep thyroid extract. He has been credited with the first reported case of replacement of TH after obtaining a fresh sheep's thyroid from a slaughterhouse. George Murray described carefully his method of preparing and subcutaneously injecting the 1.5 ml extract twice weekly to a 46-year-old woman with myxedema (Murray 1891). Following Murray's paper, there were reports from others of success with whole sheep thyroid or thyroid extract taken orally (Slater 2011). Cecil Beadles and Byrom Bramwell are notable in having published reviews of larger numbers of treated patients. Bramwell laid down some basic principles of treatment, most of which we adhere to even today: that treatment should start with a small dose and, if necessary, be gradually and carefully increased, that too large a dose may be dangerous in the elderly and in patients with heart or arterial disease, and that treatment must be lifelong. Desiccated thyroid extract was and still is prepared from animal thyroid glands, usually pig, obtained from slaughterhouses. Replacement with thyroid extract led to a dramatic improvement in symptoms for large number of patients with hypothyroidism.

Thyroxine was isolated in 1915 and its chemical structure determined in 1926 and synthesized in 1927. Thyroxine became commercially available from Glaxo in 1949. However, tablets of desiccated thyroid extract continued to remain the principle source of treatment for many years. In the 1960s, desiccated thyroid extract began to be replaced by LT4, the reasons for which are outlined in the section on *Desiccated Thyroid Extract* below. Triiodothyronine (T3) was later identified, isolated, and synthesized in 1952/1953 but, until relatively recently, used only in the management of myxedema coma. This life-threatening complication of untreated hypothyroidism is now rarely encountered, but T3 has been advocated for use alongside LT4 in the routine management of myxedema.

Levothyroxine (LT4)

LT4 is one of the most widely prescribed medications throughout the world. In England, for instance, prescriptions for LT4 have increased by more than 14 to 29.7 million between 2005 and 2015 – making it the third most prescribed drug (by number of items dispensed). In the same time period, the costs have also increased by £78.7 million although most of this is due to an increase in the cost of the medicine (Prescribing and Primary Care Services, Health and Social Care Information Center). Similarly, in the United States, LT4 is the most prescribed medication (121 million prescriptions in 2015), and its prescriptions have seen a year-on-year increase (Medicines Use and Spending in the U.S 2017).

There have been significant changes to the dosing of TH replacement therapy over the last five decades. Prior to the advent of the sensitive TSH assay, the replacement doses of LT4 were much higher (200–400 mcg/day) as the main aim of treatment was symptom resolution and biomarkers of replacement were inadequate. Later it was successfully argued that optimum dose of TH should be the one that returns the serum TSH level to the reference range. This strategy became widespread, and the average treatment doses of LT4 were more than halved. The rapidity with which normal TH levels should be restored depends on a number of factors, including the age of patient, the duration and severity of the hypothyroidism, and the presence or absence of comorbidities, particularly those of the cardiovascular system. Most patients under the age of 60 years can immediately begin a complete replacement dose of 1.6 to 1.8 μg/kg body weight (Jonklaas et al. 2014) although lean body weight may be a better predictor of dose requirement (Santini et al. 2005). A full starting dose of LT4 (1.6 μg/kg/day) in patients with newly diagnosed hypothyroidism with no cardiac symptoms is safe, more convenient, and cost-effective than a low starting dose regimen.

The bioavailability of LT4 has also been a subject of much study. The standard advice is to take LT4 tablets in the morning half an hour before breakfast (Benvenga et al. 2008). However some studies have shown that LT4 taken at bedtime is associated with higher FT4 and T3 and lower TSH concentrations in serum compared to the same LT4 dose taken in the morning, attributed to better absorption of LT4 during the night (Bolk et al. 2007). The dose of LT4 also depends on the cause of hypothyroidism. Patients who have had a total thyroidectomy or have severe primary hypothyroidism prior to replacement being commenced have slightly higher requirements than patients who become hypothyroid after radioiodine or partial thyroidectomy as they may have some residual thyroid function that is autonomous. A complete replacement dose of LT4 for most women is usually between 75 and 125 μg per day and, for most men, between 100 and 150 μg per day, the difference between the genders probably being related to the variation in lean body mass. Pretreatment serum TSH predicts to a certain extent the daily maintenance dose of LT4 in patients with primary hypothyroidism. The vast majority of patients with hypothyroidism have underlying autoimmune thyroid disease and have a degree of thyroidal hormone secretion. As the autoimmune destruction of the thyroid progresses, LT4 dose requirements increase – the timing for each patient being individualistic and hence different. Once a state of equilibrium is reached, then the LT4 dose is likely to remain stable until the aging process leads to loss of muscle mass and hence a reduction in LT4 requirements (Cunningham and Barzel 1984).

Since age plays an important role in determining the replacement doses, it is advised that in patients over the age of 60 years with history of coronary artery disease, full replacement doses should not be administered initially. In patients who suffer from long-standing severe hypothyroidism, replacement of LT4 should be gradual. In patients with hypothyroidism, replacement of TH improves cardiac function, increases cardiac output, and decreases systemic vascular resistance and end-diastolic volume. Hence patients with coronary artery disease may benefit from reversal of their hypothyroid state. However, administration of TH is also noted to

increase myocardial oxygen consumption, and hence to avoid precipitating acute myocardial ischemia, the dose of LT4 should be titrated cautiously in these patients, aiming for normalization of serum TSH (Cooper and Biondi 2012).

Liothyronine

T3 is the more metabolically active TH than T4. Most of the circulating T3 is produced from peripheral deiodination of T4. Synthetic T3 is also available in the form of a sodium compound and is more readily absorbed than LT4. After oral administration of liothyronine sodium, peak levels of serum T3 are observed within 2 to 4 hours (Table 3). The serum T3 concentration may reach elevated values after a single dose of 50 μg or even 25 μg and are sometimes associated with cardiac symptoms like palpitations. Because the half-life of T3 is approximately 12 hours, the preparations of liothyronine can be useful in the short-term management of patients with thyroid cancer to shorten the period of hypothyroidism required for diagnosis and treatment of remaining tumor tissue with 131I and in myxedema coma when rapid correction of tissue hypothyroidism is required. T3 replacement is currently not recommended for long-term replacement therapy in hypothyroidism (Wiersinga 2004). Given in combination, the pharmacodynamic equivalence of LT4 and liothyronine is achieved at a dose ratio of about 3:1 (Garber et al. 2012).

Pharmacology of Thyroid Hormone Replacement Preparations

LT4

LT4 is prescribed mostly as the sodium salt as its absorption is enhanced. Its absorption occurs along the entire length of the small intestine. Intestinal absorption of oral LT4 is typically 80% and is greater in the fasting state. Serum T4 concentrations peak 2–4 h after an oral dose and remain above normal for approximately 6 h in patients receiving daily replacement therapy. Serum T3 concentrations increase gradually postthyroxine absorption in the hypothyroid patient as the gradual conversion of T4 into T3 in the peripheral tissues increases; however, daily LT4 administration does not significantly change the circulating free T3 levels in patients who have been on LT4 for some time. The long half-life of LT4 – about 7 days – means that once-daily treatment is adequate and omission of an occasional tablet is of little clinical relevance.

LT4 has a narrow therapeutic index. Hence the potential of putting patients who take the tablet at risk for iatrogenic hyperthyroidism or hypothyroidism at doses only 25% less or greater than optimal, based on patient's serum TSH, is high (Hennessey et al. 2010). Up to half of patients on regular LT4 therapy for the treatment of primary hypothyroidism have abnormal TSH levels (Canaris et al. 2000b).

Certain formulations of LT4 could vary in concentrations. Generic and branded LT4 preparations are mostly bioequivalent (Dong et al. 1997). However, altered bioavailability has been reported due to changes in the formulation of preparations.

Hence the different marketed LT4 formulations might not be mutually exchangeable unless their bioequivalence is comparable. Profiles of selected commercial L-thyroxine preparations show considerable reduction in dissolution with increase in pH, with differences in dissolvement between the various preparations (Pabla et al. 2009).

Combinations of T3 and T4

A proportion of patients (10%) with hypothyroidism who are treated with LT4 are dissatisfied and show impaired psychological well-being than age- and gender-matched euthyroid controls – the reasons for which are unclear (Toft 1999; Bunevicius et al. 1999). It has been argued that the normal thyroid gland produces substantially more T4 (90%) than T3 (10%) with the majority of T3 production being extra thyroidal in the periphery due to the action of the deiodinasing enzymes. However, some have reasoned that a combination regimen that replaces both T4 and T3 is likely to be more physiological. Furthermore, experimental data suggests that T4 replacement alone does not return tissue T3 levels to normal and that combination therapy with both T4 and T3 does. In a study of thyroidectomized rats, restoration of the euthyroid state in all tissues was achieved by the combination of T4 and T3, and not by T4 alone (Escobar-Morreale et al. 1996). This finding has aroused new interest in combinations of T4 and T3 in hypothyroid patients on LT4 replacement therapy. However, the clinical significance of low serum T3 in hypothyroid patients is unknown. In support of the animal data, a study demonstrated that combination of T4 and T3 was superior to T4 alone in hypothyroid patients in improving mood and neurocognitive function (Bunevicius et al. 1999). Following on from this, several trials (and meta-analyses obtained thereof) have failed to replicate this finding. These subsequent trials have significant heterogeneity and therefore do not allow a single valid conclusion. However, meta-analysis of these randomized clinical trials found no evidence for superiority of LT4 and liothyronine combination therapy over LT4 monotherapy (Grozinsky-Glasberg et al. 2006). However, some genetic polymorphisms in TH transporters and deiodinases have been postulated as a potential cause for dissatisfaction with LT4 monotherapy. A follow-on subgroup analysis of a trial of LT4 and liothyronine combination therapy, which did not show a benefit of combination therapy over LT4 monotherapy, showed that the combination was effective in participants with a genetic polymorphism in the DIO2 gene (Panicker et al. 2009). This hypothesis needs to be tested in a prospective RCT of combination therapy of primary hypothyroid patients with the specific DIO2 genetic polymorphism in question. Nevertheless, American as well as European guidelines do not recommend combination therapy, and LT4 currently remains the standard treatment modality for hypothyroidism (Jonklaas et al. 2014; Wiersinga et al. 2012). There is consensus that in patients with persistent complaints despite adequate LT4 replacement as evident from normal serum TSH levels, a trial of LT4 + liothyronine could be considered only in an experimental setting. However it should be offered after exclusion of other conditions that might be responsible for the persistent complaints.

Desiccated Thyroid Extract

Desiccated thyroid extract was the mainstay of treatment of hypothyroidism until a few decades ago. However, its use has declined, in favor of synthetic T4 or T3, due to problems with excessive variation in the inter- and intra-batch preparations associated with wide fluctuations in blood T4 and T3 levels (Garber et al. 2012). There was further impetus to increase the use of LT4 when it became apparent that the main hormone of the thyroid gland was T4 and that tissues had the capacity to generate local T3 by a process of deiodination, and this led to improvement of symptoms in the majority of hypothyroid patients (Braverman et al. 1970).

Despite this, some still advocate the use of desiccated thyroid extract as a form of TH replacement for managing hypothyroidism. Proponents of its use argue that the other iodinated molecules (e.g., T2, reverse T3, thyronamines) present in the thyroid, apart from T4 and T3, may have important therapeutic use and that replacement with T4 (or even T3) alone may not be sufficient. In the past, desiccated thyroid was standardized by the organic iodine content. One grain, about 60 mg, of desiccated pig thyroid extract contains approximately 38mcg of T4 and 9mcg of T3, a ratio of around 4 to 1. However, in human thyroid, the normal concentration of these hormones is at a ratio of 14 to 1. In other words, desiccated thyroid extract contains excessive amounts of T3 relative to T4 when used to replace TH in man. Hence patients receiving an amount of this medication adequate to normalize serum TSH generally have serum T4 concentrations in the lower half of the normal range. Because of the short half-life of serum T3, the serum T3 concentrations vary in such patients, depending on the interval between ingestion of the medication and the time of blood sampling. The time course of the absorption of T3 is similar whether it is contained in thyroglobulin or free in the tablet, with peak levels approximately 2 to 4 hours after oral administration. A recent randomized clinical trial compared LT4 replacement with desiccated thyroid extract (Armour Thyroid, of which each grain of 65 mg contained 38 μg T4 and 9 μg T3) reported that the use of desiccated thyroid extract relative to LT4 was associated with modest weight loss and greater patient preference; serum T3 was noted to be higher, and serum FT4 was lower with desiccated thyroid extract (Hoang et al. 2013). Although desiccated thyroid extract may provide satisfactory replacement therapy in some patients with hypothyroidism, it is not recommended in current guidelines for treatment of hypothyroidism due to concerns regarding long-term safety. In summary, evidence supports the traditional view that T3 and T4 are the only biologically important secreted products of the thyroid gland and that none of the other secreted molecules have been definitely shown to have physiologic relevance in humans at endogenous concentrations.

Thyroid Hormone Analogues

TH receptors (TR) are present as two isoforms: TRα and TRβ. The expression of these TR isoforms differs throughout the various tissues such that some have one TR isoform more dominant than the other. For instance, TRα predominates in the brain,

heart, and skeleton, whereas TRβ is the main isoform in the liver and pituitary. TH analogues have varying affinity for TR isoforms, and this property could be potentially useful in targeting tissue selective actions of TH without increased risk of toxicity. For example, eprotirome, sobetirome, and GC-24, among others, have a 10–40-fold higher affinity for TRβ than TRα. As the liver is rich in TRβ, it may be possible to use a TRβ selective analogue to lower cholesterol without inducing toxic effects in cardiac tissue or the skeleton, where TRα predominates. Initial studies have shown some success of TH analogues especially in reducing cholesterol in patients not well controlled on statin therapy (Ladenson et al. 2010a). In this phase II trial, eprotirome treatment was associated with significant reductions in LDL cholesterol when added to statins. However, recent reports of liver and cartilage toxicity have dampened enthusiasm for their use, and no further evaluation of this molecule is currently underway (Sjouke et al. 2014).

Another TH analogue 3,5-diiothyroprionic acid (DITPA) has equal affinity for both TR isoforms but lower than that of T3. Animal studies suggest that DITPA may be more cardioselective by, as yet, an unknown mechanism and, therefore, may lead to less cardiac toxicity than T3. However, further evidence is needed to establish the role of DITPA in the treatment of cardiovascular diseases. DITPA has been utilized in two clinical trials in patients with heart failure. In one study, DITPA did not show any overall benefit (Goldman et al. 2009), whereas the other trial reported reductions in body weight and LDL cholesterol but was associated with adverse skeletal effects and also a high dropout rate (Ladenson et al. 2010b).

Assessing Response to the Treatment

Serum TSH is the best available biomarker of TH adequacy (Jonklaas et al. 2014). Therefore, the dose of LT4 is adjusted according to the serum TSH level. Several factors determine the dose of LT4 that is required to normalize a hypothyroid patient's serum TSH. The patient's weight (particularly lean body mass), pregnancy status, degree of TSH elevation, etiology of hypothyroidism (surgical thyroidectomy or autoimmune), age, and other comorbidities (especially existing untreated coronary artery disease) should be taken into account when deciding LT4 replacement dosing.

An elevated TSH indicates the need for a modest increase in dose. A suppressed TSH indicates a reduction in the LT4 dose is warranted. This is usually done in small (12.5–25 μg) increments, depending on the patient and clinical situation. When a dose change has occurred, TSH and FT4 should be repeated again in 6–8 weeks to assess response and adequacy. After optimum TSH levels have been achieved, the dose of LT4 needs to be continued and monitored on a regular basis. In most patients with severe primary hypothyroidism, few adjustments will be required after the initial titration, although dose may need to be adjusted in certain situations that lead to changes in TH requirements or absorption (loss of muscle with aging or commencing a medication that affects LT4 absorption, for instance). However, patients with Graves' disease who have had radioactive iodine or subtotal thyroidectomy or patients with Hashimoto's thyroiditis may require dosage adjustments up

to as long as 5–10 years after treatment is begun due to the slow deterioration of residual thyroid function. Therapy should be monitored with TSH measurements and estimates of FT4. As the goal of LT4 therapy is to normalize the thyroid status of the patient and as serum TSH provides the most sensitive and readily quantification of thyroid status in the patient with primary hypothyroidism, target TSH values should be within the reference range with improvement in TH concentrations (Jonklaas et al. 2014).

The clinical symptoms and signs could lag behind the biochemical picture. In general, serum T4 normalizes before serum TSH, and both may normalize before the disappearance of all of the symptoms of hypothyroidism. In one study (Winther et al. 2016), many aspects of health-related quality of life improved during the first 6 months of LT4 therapy, but full recovery was not obtained. In the severely hypothyroid patient with long-standing disease, a number of profound alterations may occur as the hypothyroid state is corrected. Symptoms such as moon facies, coarse nasal voice, puffy fingers, deafness, and sleep apnea all improve gradually. Many of the nonspecific symptoms, such as fatigue or cold intolerance, will eventually reverse as well. Hair and skin abnormalities take even longer to improve. Weight loss after commencing LT4 therapy is mainly due to mobilization of interstitial fluid as the glycosaminoglycans are degraded. The modest reduction in weight due to fluid loss, in an obese patient, is not usually more than a 4–5 kg, particularly if serum TSH values are only modestly elevated. Virtually all of the weight loss in hypothyroidism is associated with mobilization of fluid, and significant decreases in body fat rarely occur. While metabolic rate increases, in general appetite increases as well, and a new equilibrium is established.

Satisfaction with treatment and quality of life (QoL) in patients with hypothyroidism can be evaluated using various instruments (Razvi et al. 2005). The most recent patient-reported outcome measure that has been validated for use in patients with thyroid disease is the ThyPRO questionnaire (Watt et al. 2014). ThyPRO assesses quality of life in patients with thyroid disease and consists of 85 items pertaining to physical, mental, and social domains of functioning and well-being. The ThyPRO demonstrated good responsiveness across the whole range of quality-of-life aspects in patients with both hyper- and hypothyroidism and can be utilized as a patient-reported outcome in clinical trials. Another disease-specific quality-of-life questionnaire that has been validated and shown responsiveness to change is the ThyDQoL (McMillan et al. 2004; Razvi et al. 2007). However, in contrast to ThyPRO, the ThyDQoL was only designed for use with hypothyroid patients. A questionnaire to measure treatment satisfaction in hypothyroid patients has also been designed (McMillan et al. 2006).

Treatment Failures

The majority (90%) of hypothyroid patients are satisfied with LT4 replacement therapy (Wiersinga et al. 2012). However there is a small cohort of patients who continue to suffer from symptoms and signs of hypothyroidism despite adherence to

the therapy and normal range of TSH. A small proportion of hypothyroid patients (5–10%) are dissatisfied with LT4 monotherapy, even when their serum TSH is within the reference range (Saravanan et al. 2002). As discussed previously, one of the explanation could be that LT4 replacement therapy fails to mimic precisely the thyroidal secretion rates of T4 and T3 and the serum FT4 and FT3 concentrations of healthy subjects. However, a number of randomized clinical trials comparing T4 monotherapy with T4/T3 combination therapy have failed to show any advantage of combination therapy over standard T4 monotherapy (Grozinsky-Glasberg et al. 2006).

Another issue relates to the target TSH to aim for in managing hypothyroidism. A study in which LT4-replaced patients were asked to continue with their usual T4 dose or take 25 μg less or more resulted in expected changes in serum FT4, TSH, and cholesterol, but no changes were observed in scores of well-being, cognitive function, QoL, and thyroid symptom questionnaires (Walsh et al. 2006). The study concluded that slight over- or undertreatment with LT4 did not provide a reasonable explanation for continuous dissatisfaction with LT4 monotherapy. It appeared more likely that the modality of LT4 replacement itself is involved.

More research is required to definitively confirm if T4 and T3 combination has any role in the management of hypothyroidism. A slow-release formula of T3 might circumvent the marked changes in serum FT3, and proof of principle of such a preparation has been obtained in a study in which the serum FT4-to-FT3 ratio was lower during T4 plus slow-release T3 than during T4 monotherapy but still higher than in controls (Henneman et al. 2004). Furthermore, serum T3 has a circadian rhythm with the acrophase occurring in the early hours of the morning (around 3 AM and about 90 min after the TSH acrophase). In order to replicate the circadian T3 rhythm and to maintain a physiological ratio of serum FT4 to FT3 throughout 24 h in hypothyroid patients, replacement should provide constant FT4 levels accompanied by an early morning rise in serum FT3. This can be reached by the administration of LT4 once daily in combination with a single nighttime dosing of a sustained-release T3 preparation.

Genetic polymorphisms have also been postulated as being useful in deciding the ideal form of treatment. Genetic polymorphisms in deiodinases and TH transporters may not only affect serum TH concentrations but also the biological availability of TH in particular tissues (Dayan and Panicker 2009). SNPs in the gene encoding for deiodinase type 1 influence the serum FT4-to-FT3 ratio but do not have any association with psychological well-being in patients on TH replacement (Saravanan et al. 2006). Another study did not find an association between the Thr92Ala polymorphism in the deiodinase type 2 gene and well-being, neurocognition, or preference for T4/T3 combination therapy, but a study with a much larger sample size observed associations between the CC genotype of the D2 Thr92Ala polymorphism and worse baseline scores for general health and greater improvement on T4/T3 combination therapy (Panicker et al. 2009; Appelhof et al. 2005). The hypothyroid patients dissatisfied with LT4 monotherapy might be frequent carriers of these polymorphisms and might have a better response to T4/T3 combination therapy – however, this concept needs to be proven in large trials.

One of the most common causes of treatment failure is poor compliance with ingestion of thyroxine tablets. In patients whose symptoms do not improve with LT4

therapy, establishing that they are taking and absorbing the medication is important. If nonadherence is suspected, then the patient should be assessed by a thyroxine absorption test. This can be done by supervised administration of LT4 7 days dosage given once weekly for 6 weeks (Grebe et al. 1997).

Adverse Effects of Treatment

LT4 replacement is usually lifelong. Despite being used as primary replacement therapy in hypothyroidism for several decades, the long-term morbidity and mortality of patients on LT4 replacement is unclear (Thvilum et al. 2012b). LT4 treatment in TSH-suppressive doses has been associated with detrimental effects on the heart and the bones. A TSH value of <0.1 mU/l has been identified as a risk factor for the development of atrial fibrillation (Sawin et al. 1994). Furthermore, risk of atrial fibrillation is related to degree of thyroid function as assessed by serum TSH levels, those with lower TSH values being at higher risk than individuals with subclinical hypothyroidism or those with TSH at the upper limit of the reference range (Selmer et al. 2012). Long-term LT4 therapy in TSH-suppressive doses may cause left ventricular hypertrophy and increase the risk of ischemic heart disease in patients under the age of 65 years (Biondi and Cooper 2010). Fracture risk in LT4-treated hypothyroid patients seems to be closely related to the cumulative effects of periods exposed to excessive LT4 therapy and more pronounced in postmenopausal women (Abrahamsen et al. 2015). On the other hand, a database analysis of all patients in Tayside, Scotland, prescribed with LT4 replacement therapy, showed that cardiovascular disease, dysrhythmias, and fractures were increased in patients with a high TSH and in patients with a suppressed TSH when compared to patients with a TSH in the laboratory reference range (Flynn et al. 2010). Patients with only a low but measurable TSH did not have an increased risk of any of these outcomes. Thus, patients on LT4 therapy for hypothyroidism should have their thyroid function evaluated regularly, and both high and low TSH levels should be avoided. However, it is unknown whether the U-shaped curve observed between serum TSH levels and adverse outcomes is a causal relationship or if the TSH is a biomarker of other undefined conditions.

Effects of Thyroid Hormone Replacement Therapy

Effects of Replacement Therapy on Symptoms and Signs

The symptoms and signs of hypothyroidism improve with TH replacement therapy in the vast majority of patients with overt disease. The response to treatment in patients with milder (subclinical) hypothyroidism is less clear (Villar et al. 2007). In one double-blind placebo-controlled study, a higher prevalence of specific symptoms and signs of hypothyroidism in 33 subclinical hypothyroid patients than in euthyroid controls was reported at baseline (Cooper et al. 1984). The study

population was selected from a large cohort of women enrolled in a follow-up study of patients treated for hyperthyroidism. One year after LT4 treatment, the symptom score improved significantly with replacement doses of LT4 (between 50 and 125 mcg daily). Another double-blind placebo-controlled study of 37 patients older than 55 years reported a significant improvement in memory scores but not in overall health related (Jaeschke et al. 1996). Meier and colleagues evaluated 66 women, aged 18–75 years, with subclinical hypothyroidism due to thyroiditis or a history of Graves' disease (Meier et al. 2001). An improvement in two symptom scores (the Billewicz and Zulewski scores) was observed after 48 weeks of replacement therapy vs. baseline values though the comparison of the mean treatment effects between the two treatment groups did not reach the level of significance. However, a subgroup of patients with pretreatment TSH values >12 mU/L showed an improvement in symptom score. The relation between neuropsychological function and subclinical hypothyroidism was investigated in the Tromso study of 89 subjects, a double-blind placebo-controlled study of LT4 therapy given for 1 year. It was reported that there was no significant difference between LT4 therapy and placebo with regard to cognitive function or depression (Jorde et al. 2006). A randomized double-blind placebo crossover study assessed quality of life in 100 patients with subclinical hypothyroidism defined by TSH greater than 4 mU/L (Razvi et al. 2007). In this study, overall disease-specific QoL and tiredness improved. In addition, subscales of the SF36 (apart from the emotional item) tended toward improvement after replacement therapy with LT4, although none reached statistical significance after correction for multiple comparisons. However, in this study, LT4 was administered for a short period (3 months) and at a fixed dosage (100 μg/d), and 10% of LT4 subjects had subnormal serum TSH values at the end of the treatment period.

In summary, symptoms and signs improve with LT4 in most patients with hypothyroidism. However, there are important differences in the study design in terms of duration of therapy, LT4 dosage, and differences in the scores used to assess the symptoms.

Effects of Treatment on Thyroid Volume

In patients with goiter due to Hashimoto's thyroiditis, treatment with LT4 is usually recommended with the aim of decreasing thyroid size. Treatment with LT4 is generally effective in reducing thyroid volume by 30–80% (Hegedus et al. 1991).

Effect on Thyroid Autoantibodies

LT4 replacement can have an effect on the levels of anti-TPO antibodies and other thyroid antibodies (Hegedus et al. 1991). It has been reported that the antibodies may decrease during treatment with LT4 in patients with Hashimoto thyroiditis or idiopathic myxedema. Interestingly, in hypothyroid patients with TSH receptor antibodies, treatment with LT4 can lead to disappearance of these antibodies and achieve euthyroidism (Akamizu et al. 2000).

Effects of Replacement Therapy on Cardiac Function

Cardiac function, especially the diastolic function, improves post-LT4 replacement therapy in patients with hypothyroidism. Isovolumetric relaxation time and late transmitral flow velocity decrease and early-to-late transmitral peak flow velocity ratio (E/A) improves with LT4 replacement therapy. A positive effect on systolic function and endothelial function is also observed after replacement therapy (Cooper and Biondi 2012). However, whether these improvements in cardiac mechanical functions are associated with any benefits in long-term morbidity and mortality is not known.

Effect on Lipid Profile

Hypothyroidism is a recognized risk factor for atherosclerosis and cardiovascular disease. A linear increase in total cholesterol, LDL cholesterol, and triglyceride levels has been observed with increasing TSH. TH induce the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which is the first step in cholesterol biosynthesis. Triiodothyronine (T3) upregulates LDL receptors by controlling the LDL receptor gene activation. This T3-mediated gene activation is done by the direct binding of T3 to specific TH response elements (TREs). It is also noted that T3 controls the sterol regulatory element-binding protein-2, which in turn regulates LDL receptor's gene expression. TH therapy has been consistently shown to reduce total and LDL cholesterol levels (Danese et al. 2000).

Effect of Concomitant Medical Conditions and Drug Interactions in Absorption of LT4

Several medications and medical conditions affect LT4 absorption (Jonklaas et al. 2014) (also see ▶ Chap. 25, "Drugs and other substances interfering with thyroid function"). Patients with impaired gastric acid secretion require a 22–34% higher than usual dose of LT4 to suppress serum TSH, suggesting that normal gastric acid secretion is necessary for effective absorption of LT4. Patients with conditions causing malabsorption such as coeliac disease or those with short bowel syndrome may require an increase in LT4 dosage. As coeliac disease is frequently seen in patients with autoimmune thyroid disease, many authors suggest screening for coeliac disease in patients with hypothyroidism who require higher than expected doses of LT4. Bariatric surgery could result in reduced drug absorption requiring higher doses of LT4. This is seen frequently after jejunoileal bypass and less often after gastric bypass/gastroplasty and rarely after biliopancreatic diversion. In such patients, liquid LT4 preparation may be better absorbed compared to LT4 tablets.

Bile acid sequestering agents bind – at least in vitro – large amounts of LT4 and also interfere with the enterohepatic circulation of TH in which T4 and T3 conjugates are excreted in bile and partially deconjugated in the intestine with the release of small amounts of T4 and T3 for reabsorption. Treatment with colestipol or cholestyramine may cause a slight increase in TSH in LT4-treated patients, but not

in normal subjects. The newer bile acid sequestrant colesevelam reduced absorption of LT4 by 96% in healthy subjects. Hence it is suggested that LT4 and bile acid sequestrants should not be taken at the same time and an interval of at least 4–5 h separating is recommended to attain near-normal absorption of LT4. Sucralfate binds LT4 in vitro, decreases absorption of LT4 in healthy volunteers, and may result in resistant hypothyroidism in some cases. Aluminum may form a complex with LT4, and a dose-related adsorption of LT4 with aluminum hydroxide has been demonstrated in vitro. Ferrous sulfate also increases serum TSH in hypothyroid patients on stable LT4 replacement. Calcium carbonate decreases the bioavailability of LT4 in healthy volunteers. Calcium carbonate therapy in LT4-replaced patients increases serum TSH, sometimes to above the normal range. Phosphate binders may also adsorb LT4, and both sevelamer and lanthanum carbonate reduce bioavailability of LT4 in healthy subjects. The use of sevelamer in hemodialysis patients on LT4 is associated with significant increases of LT4 dose after 6 months of therapy. Raloxifene and orlistat have also been reported to interfere with absorption of LT4.

Estrogens are known to increase the serum concentration of thyroxine-binding globulin (TBG) through increased sialylation of TBG thereby slowing its clearance from the circulation by the liver. The route of administration, the dose, and the chemical structure of the estrogen dictate the effect of estrogens on TBG. Transdermal administration of estradiol causes minimal changes in TBG, in contrast to oral estradiol which raises serum TBG by 50–70%, due to high estrogen levels in the portal vessels and first-pass metabolism in the liver after oral administration. This effect is dose dependent and is usually seen within 6 weeks after initiation of estrogens and reaches its peak at 12 weeks. Selective estrogen receptor modifiers (SERM) may have similar effects: tamoxifen increases serum TBG by 24% and droloxifene by 41%.

Several medications including antiepileptics and drugs used in the treatment of tuberculosis interfere with LT4 bioavailability by inducing hepatic oxygenases responsible for drug metabolism, which accelerates thyroxine clearance via these pathways.

Tyrosine kinase inhibitors can alter TH regulation by mechanisms that are specific to each molecule. Regular assessment of thyroid function is therefore recommended before and during treatment with tyrosine kinase inhibitors.

Pregnancy and Doses of LT4

In pregnant patients with primary hypothyroidism, an increase in thyroxine requirement is noted, probably related to increased lean body mass, increased serum TBG, increased deiodination by the placenta, and increased iodine requirements as well as maternal renal clearance (see also ▶ Chap. 23, “Thyroid Physiology and Thyroid Diseases in Pregnancy”). The reference ranges for thyroid function also change during pregnancy and are trimester specific. Guidelines by both the European Thyroid Association and the American Thyroid Association suggest that local data should be utilized in formulating trimester-specific reference ranges (Stagnaro-Green et al. 2011; Lazarus et al. 2014). In LT4-treated hypothyroid patients, a 25–50% increase in LT4 dosage is required as soon as pregnancy is confirmed. The increase in LT4 dose depends on the etiology of

hypothyroidism, being higher in those with ablative treatments such as radioiodine or near-total thyroidectomy. TSH values should be checked every 4–6 weeks at least during the first trimester and once during the second and third trimesters. Both sets of guidelines recommend that LT4 should be the treatment of maternal hypothyroidism. It is strongly advised not to use other TH preparations such as T_3 or desiccated thyroid extract, which cause lowering of serum T_4 levels. In patients with morning sickness, the administration of LT4 late at night could be tried. The LT4 dose may be reinstituted at its pregestational level immediately after delivery.

The universal screening of asymptomatic pregnant women for hypothyroidism in the first trimester is controversial. Data for universal screening is equivocal, and moreover, results from interventional trials in hypothyroid patients identified by universal screening are negative as assessed by neurocognitive outcomes in the offspring (Lazarus et al. 2012; Casey Brian and Thom 2017). Due to lack of high-quality evidence, and because the criteria for universal screening are not all satisfactory, most professional societies recommend targeted case finding rather than universal screening. The American Thyroid Association recommends measurement of serum TSH in pregnant women who are symptomatic and are from iodine-deficient areas or if they have risk factors including a family or personal history of thyroid disease, type 1 diabetes, history of miscarriage, preterm delivery, history of head and neck radiation, or morbid obesity (BMI >40) (Lazarus et al. 2014). However, this strategy risks missing 30–80% of patients with hypothyroidism (Lazarus et al. 2014).

Interference with Coexisting Conditions

Hypoadrenalism

Autoimmune disorders like autoimmune hypothyroidism, hypoadrenalism, or type 1 diabetes mellitus coexist frequently. Primary hypothyroidism due to chronic autoimmune thyroiditis is present in at least 20% of patients with primary autoimmune adrenocortical insufficiency (Addison's disease) (Betterle et al. 2004). It is important that if the two entities are diagnosed simultaneously, glucocorticoid replacement is commenced first. This is vital for two reasons. First, treatment of hypothyroidism in patients with untreated glucocorticoid deficiency may precipitate an adrenal crisis due to the increased demand for cortisol induced by the rise of the metabolic rate. Second, patients with adrenal insufficiency could have slightly elevated TSH levels without serological evidence of chronic autoimmune thyroiditis and with glucocorticoid therapy the TSH generally normalizes – which shows the small inhibitory effect of cortisol on TSH secretion.

Pernicious Anemia

The LT4 requirement in autoimmune hypothyroidism is about 18% higher in parietal cell antibodies (PCA)-positive patients than in PCA-negative patients, and a

significant positive correlation has been found between LT4 requirement and serum PCA levels (Donaich and Roitt 1964).

Ischemic Heart Disease

Treatment of hypothyroidism with LT4 improves myocardial function and reduces peripheral vascular resistance. Most patients with hypothyroidism and minimal coronary artery disease can be treated with full replacement dose. However, in hypothyroid patients with significant coronary artery disease, LT4 treatment may provoke symptoms of worsening angina due to increased metabolic rate and consequent increase in oxygen demand in the myocardium. In a large series of hypothyroid patients, new-onset angina occurred in 2% upon thyroxine treatment; preexistent angina worsened in 16%, did not change in 46%, and improved in 38% (Keating et al. 1961). Retrospective studies looking at replacement of LT4 in patients with ischemic heart disease suggest that the possibility of myocardial infarction is greater than the possibility of an adverse event during angiography or angioplasty (Hays 1991). In some patients with advanced cardiac disease, particularly if they are older, complete correction of the hypothyroid state might not be possible. In such patients, submaximal amounts of LT4 supplemented by other agents to enhance myocardial function may be safer (Levine 1980).

Growth Hormone Deficiency

With growth hormone (GH) administration, a decrease in serum FT4 and an increase in T3 are seen. The significance of these changes is uncertain, although one study reports a good correlation between changes in serum T3 and resting energy expenditure and cardiac isovolumetric contraction time upon GH treatment (Martins et al. 2007). These changes in thyroid function can be transient and could normalize in a few months. In adults with a new diagnosis of hypopituitarism, GH replacement can unmask central hypothyroidism in 36–47% of apparently euthyroid patients, necessitating thyroxine replacement. At highest risk are patients with organic pituitary disease or multiple pituitary hormone deficiencies. Thyroid function should therefore be monitored in hypopituitary patients who are starting GH therapy.

Summary

The diagnosis and treatment of hypothyroidism have seen great advances in the last century mainly driven by ability to be able to measure hormones biochemically. Yet, many questions remain unanswered. The management of specific subgroups of patients with hypothyroidism, especially in pregnancy, in the older age groups, and in those dissatisfied with current therapy, is still unclear. The reasons behind why some patients on adequate LT4 replacement remain symptomatic are not known

and satisfactory, yet safe, management modality in this group is not currently apparent. The fine-tuning of reference ranges to better reflect what is closer to normal for an individual rather than the population as well as an increase in our understanding of genetics and epigenetics will be the driving forces that will change clinical practice in the next few decades.

References

Abrahamsen B, Jørgensen HL, Laulund AS, Nybo M, Bauer DC, Brix TH, Hegedüs L. The excess risk of major osteoporotic fractures in hypothyroidism is driven by cumulative hyperthyroid as opposed to hypothyroid time: an observational register-based time-resolved cohort analysis. J Bone Miner Res. 2015;30(5):898–905.

Aggarwal N, The RS. thyroid and ageing or the ageing thyroid? An evidence-based analysis of the literature. J Thyroid Res. 2013;2013:481287.

Akamizu T, Amino N, DeGroot LJ. Hashimoto's Thyroiditis. [Updated 2013 Dec 20]. In: De Groot LJ, Chrousos G, Dungan K, et al., editors. Endotext [Internet]. South Dartmouth: MDText.com, Inc.; 2000.

Amaud-Lopez L, Usala G, Ceresini G, Mitchell BD, Pilia MG, Piras MG, Setsu N, Maschio A, Busonero F, Albai G, Dei M, Lai S, Mulas A, Crisponi L, Tanaka T, Bandinelli S, Guralnik JM, Loi A, Balaci L, Sole G, Prinzis A, Mariotti S, Shuldlner AR, Cao A, Schlessinger D, Uda M, Abecasis GR, Nagaraja R, Sanna S, Naitza S. Phosphodiesterase 8B gene variants are associated with serum TSH levels and thyroid function. Am J Hum Genet. 2008;82:1270–80.

Andersen S, Pedersen KM, Bruun NH, Laurberg P. Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. J Clin Endocrinol Metab. 2002;87(3):1068–72.

Andersen S, Bruun NH, Pedersen KM, Laurberg P. Biologic variation is important for interpretation of thyroid function tests. Thyroid. 2003a;13:1069–78.

Andersen S, Bruun NH, Pedersen KM, Laurberg P. Biologic variation is important for interpretation of thyroid function tests. Thyroid. 2003b;13(11):1069–78.

Appelhof BC, Peeters RP, Wiersinga WM, et al. Polymorphisms in type 2 deiodinase are not associated with well-being, neurocognitive functioning, and preference for combined thyroxine/3,5,3′-triiodothyronine therapy. J Clin Endocrinol Metab. 2005;90:6296–9.

Asvold BO, Bjøro T, Nilsen TIL, Vatten LJ. Tobacco smoking and thyroid function: a population-based study. Arch Intern Med. 2007;167(13):1428–32.

Atzmon G, Barzilai N, Hollowell JG, Surks MI, Gabriely I. Extreme Longevity Is Associated with Increased Serum Thyrotropin. J Clin Endocrinol Metab. 2009;94(4):1251–4.

Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, LiVosli VA, Sire PN, John R, Ruf J. Laboratory medicine practice guidelines. Laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid. 2003;13(1):3–126.

Benotti J, Benotti N. Protein-bound iodine, total iodine, and butanol-extractable iodine by partial automation. Clin Chem. 1963;12:408–16.

Benvenga S, Bartolone L, Pappalardo MA, Russo A, Lapa D, Giorgianni G, Saraceno G, Trimarchi F. Altered intestinal absorption of L-thyroxine caused by coffee. Thyroid. 2008;18(3):293–301.

Betterle C, Lazzarotto F, Presotto F. Autoimmune polyglandular syndrome Type 2: the tip of an iceberg? Clin Exp Immunol. 2004;137(2):225–33.

Billewicz WL, Chapman RS, Crooks J. Statistical methods applied to the diagnosis of hypothyroidism. Q J Med. 1969;38:255–66.

Biondi B, Cooper DS. The clinical significance of subclinical thyroid dysfunction. Endocr Rev. 2008;29(1):76–131.

Biondi B, Cooper DS. Benefits of thyrotropin suppression versus the risks of adverse effects in differentiated thyroid cancer. Thyroid. 2010;20(2):135–46.

Bjergved L, Jørgensen T, Perrild H, Carle A, Cerqueira C, Krejbjerg A, Laurberg P, Ovesen L, low Pedersen IB, Rasmussen LB, Knudsen N. Predictors of change in serum TSH after iodine fortification: an 11-year follow-up to the DanThyr Study. J Clin Endocrinol Metab. 2012;97(11):4022–9.

Bolk N, Visser TJ, Kalsbeek A, van Domburg RT, Berghout A. Effects of evening vs morning thyroxine ingestion on serum thyroid hormone profiles in hypothyroid patients. Clin Endocrinol. 2007;66(1):43–8.

Boothby W, Sandiford I. Summary of the basal metabolism data on 8,614 subjects with especial reference to the normal standards for the estimation of the basal metabolic rate. J Biol Chem 1922;54:783–803.

Braverman LE, Vagenakis AG. The thyroid. Clin Endocrinol Metab. 1979;8(3):621–39.

Braverman LE, Ingbar SH, Sterling K. Conversion of thyroxine (T4) to triiodothyronine (T3) in athyreotic human subjects. J Clin Invest. 1970;49(5):855–64.

Bremner AP, Feddema P, Leedman PJ, Brown SJ, Beilby JP, Lim EM, Wilson SG, O'Leary PC, Walsh JP. Age-related changes in thyroid function: a longitudinal study of a community-based cohort. J Clin Endocrinol Metab. 2012 May;97(5):1554–62.

Brent GA. Mechanisms of thyroid hormone action. J Clin Invest. 2012;122(9):3035–43.

Bunevicius R, Kazanavicius G, Zalinkevicius R, Prange Jr AJ. Effects of thyroxine as compared with thyroxine plus triiodothyronine in patients with hypothyroidism. N Engl J Med. 1999;340(6):424–9.

Burman KD. Commentary: discordant measurements of serum triiodothyronine (T4), thyroxine (T3) and thyroid-stimulating hormone (TSH). Clin Chem. 2008;54(7):124.

Canaris GJ, Manowitz NR, Mayor G, Ridgway EC. The Colorado thyroid disease prevalence study. Arch Intern Med. 2000a;160(4):526–34.

Canaris GJ, Manowitz NR, Mayor GM, Ridgway EC. The Colorado thyroid disease prevalence study. Arch Intern Med. 2000b;160:526–34.

Carlé A, Pedersen IB, Knudsen N, Perrild H, Ovesen L, Laurberg P. Hypothyroid symptoms and the likelihood of overt thyroid failure: a population-based case-control study. Eur J Endocrinol. 2014;171(5):593–602.

Casey Brian M, Thom EA. Treatment of subclinical hypothyroidism or hypothyroxinemia in pregnancy. N Engl J Med. 2017;376:815–25.

Cooper DS, Biondi B. Subclinical thyroid disease. Lancet. 2012;379(9821):1142–54.

Cooper DS, Halpern R, Wood LC, Levin AA, Ridgway EC. L-Thyroxine therapy in subclinical hypothyroidism. A double-blind, placebo-controlled trial. Ann Intern Med. 1984;101(1):18–24.

Cunningham JJ, Barzel US. Lean body mass is a predictor of the daily requirement for thyroid hormone in older men and women. J Am Geriatr Soc. 1984;32(3):204–7.

Dall'Asta C, Paganelli M, Morabito A, Vedani P, Barbieri M, Paolisso G, Folli F, Pontiroli AE. Weight loss through gastric banding: effects on TSH and thyroid hormones in obese subjects with normal thyroid function. Obesity. 2010;18(4):854–7.

Danese M, Ladenson PW, Meinert CL, Powe NR. Clinical review 115: effect of thyroxine therapy on serum lipoproteins in patients with mild thyroid failure: a quantitative review of the literature. J Clin Endocrinol Metab. 2000;85:2993–3001.

Dayan CM, Panicker V. Novel insights into thyroid hormones from the study of common genetic variation. Nat Rev Endocrinol. 2009;5:211–8.

de Moraes CMM, Mancini MC, de Melo ME, Figueiredo DA, Villares SMF, Rascovski A, Zilberstein B, Halpern A. Prevalence of subclinical hypothyroidism in a morbidly obese population and improvement after weight loss induced by Roux-en-Y gastric bypass. Obes Surg. 2005;15(9):1287–91.

Dickey RA, Wartofsky L, Feld S. Optimal thyrotropin level: normal ranges and reference intervals are not equivalent. Thyroid. 2005;15(9):1035–9.

Donaich D, Roitt IM. An evaluation of gastric and thyroid auto-immunity in relation to hematologic disorders. Semin Hematol. 1964;1:313.

Dong BJ, Hauck WW, Gambertoglio JG, et al. Bioequivalence of generic and brand-name levothyroxine products in the treatment of hypothyroidism. JAMA. 1997;227:1205–13.

Dufour DR. Laboratory Tests of Thyroid function: Uses and limitations. Endocrinol Metab Clin N Am. 2007;36:579–94.

Dunlap DB. Chapter 142: Thyroid function tests. In: Walker HK, Hall WD, Hurst JW, editors. Clinical methods: the history, physical, and laboratory examinations. 3rd ed. Boston: Butterworths; 1990.

Ehrenkranz J, Bach PR, Snow GL, Schneider A, Lee JL, Ilstrup S, Bennett ST, Benvenga S. Circadian and circannual rhythms in thyroid hormones: determining the TSH and free T4 reference intervals based upon time of day, age, and sex. Thyroid. 2015;25(8):954–61.

Escobar-Morreale HF, Rey F, Obregon MJ, Escobar GM. Only the combined treatment with thyroxine and triiodothyronine ensures euthyroidism in all tissues of the thyroidectomized rat. Endocrinology. 1996;137:2490–502.

Flynn RW, Bonellie SR, Jung RT, MacDonald TM, Morris AD, Leese GP. Serum Thyroid-Stimulating Hormone Concentration and Morbidity from Cardiovascular Disease and Fractures in Patients on Long-Term Thyroxine Therapy. J Clin Endocrinol Metab. 2010;95(1):186–93.

Garber JR, Cobin RH, Gharib H, et al. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical endocrinologists and the American Thyroid association. Thyroid. 2012;22:1200–35.

Goldman S, McCarren M, Morkin E, Ladenson PW, Edson R, Warren S, O'Brien T, Anand I, Warner A, Hattler B, Shih MC, Lavori P. DITPA (3,5-Diiodothyropropionic Acid), a thyroid hormone analog to treat heart failure: phase II trial veterans affairs cooperative study. Circulation. 2009;119(24):3093–100.

Grebe SKG, Cooke RR, Ford HC, et al. Treatment of hypothyroidism with once weekly thyroxine. J Clin Endocrinol Metab. 1997;82:870–5.

Gross J, Pitt-Rivers R. Physiological activity of 3:5:3′-L-triiodothyronine. Lancet. 1952;592.

Grozinsky-Glasberg S, Fraser A, Nahshoni E, Weizman A, Leibovici L. Thyroxine-triiodothyronine combination therapy versus thyroxine monotherapy for clinical hypothyroidism: meta-analysis of randomized controlled trials. J Clin Endocrinol Metab. 2006;91:2592–9.

Gull WW. On a Cretinoid State supervening in Adult Life in Women. Trans Clin Soc Lond. 1874;7:180–5.

Gussekloo J, Exel E, de Craen AJM, Meinders AE, Frölich M, Westendorp RGJ. Thyroid status, disability and cognitive function, and survival in old age. JAMA. 2004;292(21):2591–9.

Hansen PS, Brix TH, Bennedbaek FN, Bonnema SJ, Kyvik KO, Hegedüs L. Genetic and environmental causes of individual differences in thyroid size: a study of healthy Danish twins. J Clin Endocrinol Metab. 2004a;89(5):2071–7.

Hansen PS, Brix TH, Sørensen TIA, Kyvik KO, Hegedüs L. Major genetic influence on the regulation of the pituitary-thyroid axis: A study of healthy Danish twins. J Clin Endocrinol Metab. 2004b;89:1181–7.

Hansen PS, Brix TH, Iachine I, Kyvik KO, Hegedüs L. The relative importance of genetic and environmental effects for the early stages of thyroid autoimmunity: a study of healthy Danish twins. Eur J Endocrinol. 2006;154(1):29–38.

Hays MT. Localization of human thyroxine absorption. Thyroid. 1991;3:241.

Hegedus L, Hansen JM, Feldt-Rasmussen U, Hansen BM, Moier-Madsen M. Influence of thyroxine treatment on thyroid size and anti-thyroid peroxidase antibodies in Hashimoto's thyroiditis. Clin Endocrinol. 1991;35:235–8.

Henneman G, Docter R, Visser TJ, Postema PT, Krenning EP. Thyroxine plus low-dose, slow-release triiodothyronine replacement in hypothyroidism. Thyroid. 2004;14:271–5.

Hennessey JV, Malabanan AO, Haugen BR, Levy EG. Adverse event reporting in patients treated with levothyroxine: results of the pharmacovigilance task force survey of the American Thyroid Association, American Association of Clinical Endocrinologists and The Endocrine Society. Endocr Pract. 2010;11:1–41.

Hoang TD, Olsen CH, Mai VQ, Clyde PW, Shakir MKM. Desiccated thyroid extract compared with levothyroxine in the treatment of hypothyroidism: a randomized, double-blind, crossover study. J Clin Endocrinol Metab. 2013;98:2013.

Hollowell JG, Staehling NW, Hannon WH, Flanders WD, Gunter EW, Spencer CA, Braverman LE. Serum thyrotropin, thyroxine, and thyroid antibodies in the United States population (1988 to 1994): NHANES III. J Clin Endocrinol Metab. 2002;87:489–99.

Jaeschke R, Guyatt G, Gerstein H, Patterson C, Molloy W, Cook D, Harper S, Griffith L, Carbotte R. Does treatment with L-thyroxine influence health status in middle-aged and older adults with subclinical hypothyroidism? J Gen Intern Med. 1996;11(12):744–9.

Jensen E, Petersen PH, Blaabjerg O, et al. Establishment of a serum thyroid stimulating hormone (TSH) reference interval in healthy adults: the importance of environmental factors, including thyroid antibodies. Clin Chem Lab Med. 2004;42:824–32.

Jonklaas J, Bianco AC, Bauer AJ, Burman KD, Cappola AR, Celi FS, Cooper DS, Kim BW, Peeters RP, Rosenthal MS, Sawka AM. Guidelines for the treatment of hypothyroidism: prepared by the american thyroid association task force on thyroid hormone replacement. Thyroid. 2014;24(12):1670–751.

Jorde R, Waterloo K, Storhaug H, Nyrnes A, Jenssen TG. Neuropsychological function and symptoms in subjects with subclinical hypothyroidism and the effect of thyroxine treatment. J Clin Endocrinol Metab. 2006;91(1):145–53.

Keating FR, Parkin TW, Selby JB, Dickinson LS. Treatment of heart disease associated with myxedema. Prog Cardiovasc Dis. 1961;3:364.

King TW. Observations on the thyroid gland. Guy's Hosp Rep. 1836;1:429–46.

Knudsen N, Laurberg P, Rasmussen LB, Bülow I, Perrild H, Ovesen L, Jørgensen T. Small differences in thyroid function may be important for body mass index and the occurrence of obesity in the population. J Clin Endocrinol Metab. 2005;90(7):4019–24.

Kocher T. Ueber kropfextirpation unde ihre folgen. Arc Klin Chir. 1883;29:254–337.

Ladenson PW, Kristensen JD, Ridgway E, Olsson AG, Klein I, Baxter JD, Angelin B. Use of the Thyroid Hormone Analogue Eprotirome in Statin-Treated Dyslipidemia. N Engl J Med. 2010a;362:906–16.

Ladenson PW, McCarren M, Morkin E, Edson RG, Shih MC, Warren SR, Barnhill JG, Churby L, Thai H, O'Brien T, Anand I, Warner A, Hattler B, Dunlap M, Erikson J, Goldman S. Effects of the thyromimetic agent diiodothyropropionic acid on body weight, body mass index, and serum lipoproteins: a pilot prospective, randomized, controlled study. J Clin Endocrinol Metab. 2010b;95(3):1349–54.

Larsen PR, Yamashita K, Dekker A, Field JB. Biochemical observations in functioning human thyroid adenomas. J Clin Endocrinol Metab. 1973;36(5):1009–18.

Lazarus JH, Bestwick JP, Channon S, et al. Antenatal screening for hypothyroidism and childhood cognitive impairment. N Engl J Med. 2012;306(6):10–1056.

Lazarus J, Brown RS, Daumerie C, Hubalewska-Dydejczyk A, Negro R, Vaidya B. European Thyroid Association Guidelines for the management of subclinical hypothyroidism in pregnancy and in children. Eur Thyroid J. 2014 Jun;3(2):76–94.

Levine HD. Compromise therapy in the patient with angina pectoris and hypothyroidism: a clinical assessment. Am J Med. 1980;69:411.

MacCuish A, Razvi S, Syed AA. Effect of weight loss after gastric bypass surgery on thyroid function in euthyroid people with morbid obesity. Clin Obes. 2012 Feb;2(1–2):25–8.

Marcocci C, Vitti P, Cetani F, Catalano F, Concetti R, Pinchera A. Thyroid ultrasonography helps to identify patients with diffuse lymphocytic thyroiditis who are prone to develop hypothyroidism. J Clin Endocrinol Metab. 1991;72(1):209–13.

Martins MR, Doin FC, Komatsu WR, Barros-Neto TL, Moises VA, Abucam J. Growth hormone replacement improves thyroxine biological effects: implications for management of central hypothyroidism. J Clin Endocrinol Metab. 2007;92:4144–53.

McMillan CV, Bradley C, Woodcock A, Razvi S, Weaver JU. Design of new questionnaires to measure quality of life and treatment satisfaction in hypothyroidism. Thyroid. 2004;14 (11):916–25.

McMillan C, Bradley C, Razvi S, Weaver J. Psychometric evaluation of a new questionnaire measuring treatment satisfaction in hypothyroidism: the ThyTSQ. Value Health. 2006;9(2):132–9.

Medicines use and spending in the U.S. – A review of 2015 and Outlook to 2010. http://www.imshealth.com/en/thought-leadership/quintilesims-institute/reports/medicines-use-and-spending-in-the-us-a-review-of-2015-and-outlook-to-2020. Accessed 21 Feb 2017.

Meier C, Staub JJ, Roth CB. TSH-controlled L-thyroxine therapy reduces cholesterol levels and clinical symptoms in subclinical hypothyroidism: a double-blind, placebo-controlled trial (Basel Thyroid Study). J Clin Endocrinol Metab. 2001;86:4860–6.

Menendez C, Baldelli R, Camiña JP, Escudero B, Peino R, Dieguez C, Casanueva FF. TSH stimulates leptin secretion by a direct effect on adipocytes. J Endocrinol. 2003;176(1):7–12.

Moncayo H, Dapunt O, Moncayo R. Diagnostic accuracy of basal TSH determinations based on the intravenous TRH stimulation test: An evaluation of 2570 tests and comparison with the literature. BMC Endocr Disord. 2007;7:5.

Murray GR. Note on the treatment of myxoedema by hypodermic injections of an extract of the thyroid gland of a sheep. BMJ. 1891;2:796–7.

Nicoloff JT, Spencer CA. Clinical review 12: the use and misuse of the sensitive thyrotropin assays. J Clin Endocrinol Metab. 1990;71:553–8.

Nyström E, Caidahl K, Fager G, Wikkelsö C, Lundberg PA, Lindstedt G. A double-blind cross-over 12-month study of L-thyroxine treatment of women with 'subclinical' hypothyroidism. Clin Endocrinol. 1988;29(1):63–75.

Okosieme O, Gilbert JP, Vanderpump M, et al. Management of primary hypothyroidism: statement by the British Thyroid Association Executive Committee. Clin Endocrinol (Oxf). 2016 Jun;84(6):799–808.

Pabla D, Akhlaghi F, Zia H. A comparative pH-dissolution profile study of selected commercial levothyroxine products using inductively coupled mass spectrometry. Eur J Pharm Biopharm. 2009;72:105–10.

Panicker V, Saravanan P, Vaidya B, Evans J, Hattersley AT, Frayling TM, Dayan CM. Common variation in the DIO2 gene predicts baseline psychological well-being and response to combination thyroxine plus triiodothyronine therapy in hypothyroid patients. J Clin Endocrinol Metab. 2009;94(5):1623–9.

Pasqualetti G, Pagano G, Monzani F. Cardiovascular risk and mild thyroid hormone deficiency: are there some differences in the elderly? J Hematol Thrombo Dis. 2014;2:5.

Pearce SH, Brabant G, Duntas LH, Monzani F, Peeters RP, Razvi S, Wemeau JL. 2013 ETA guideline: management of subclinical hypothyroidism. Eur Thyroid J. 2013 Dec;2(4):215–28.

Pearce SH, Razvi S, Yadegarfar ME, Martin-Ruiz C, Kingston A, Collerton J, Visser TJ, Kirkwood TB, Jagger C. Serum thyroid function, mortality and disability in advanced old age: the newcastle 85+ study. J Clin Endocrinol Metab. 2016;101(11):4385–94.

Persani L, Terzolo M, Asteria C, Orlandi F, Angeli A, Beck-Peccoz P. Circadian variations of thyrotropin bioactivity in normal subjects and patients with primary hypothyroidism. J Clin Endocrinol Metab. 1995;80(9):2722–8.

Porcu E, Medici M, Pistis G, Volpato CB, Wilson SG. A meta-analysis of thyroid-related traits reveals novel loci and gender-specific differences in the regulation of thyroid function. PLoS Genet. 2013;9(2):e1003266.

Prescribing and Primary Care Services, Health and Social Care Information Center. Prescriptions dispensed in the community: England 2005–15. http://content.digital.nhs.uk/catalogue/PUB20664/pres-disp-com-eng-2005-15-rep.pdf. Accessed 3 Mar 2017.

Rago L, Chiovato L, Grasso T, Pinchera A, Vitti P. Thyroid ultrasonography as a tool for detecting thyroid autoimmune diseases and predicting thyroid dysfunction in apparently healthy subjects. J Endocrinol Investig. 2001;24(10):763–9.

Razvi S, McMillan CV, Weaver JU. Instruments used in measuring symptoms, health status and quality of life in hypothyroidism: a systematic qualitative review. Clin Endocrinol. 2005;63(6):617–24.

Razvi S, Ingoe L, Keeka G, Oates C, McMillan C, Weaver JU. The beneficial effect of L-thyroxine on cardiovascular risk factors, endothelial function, and quality of life in subclinical hypothyroidism: randomized, crossover trial. J Clin Endocrinol Metab. 2007;92(5):1715–23.

Razvi S, Ingoe L, Ryan V, Pearce SHS, Wilkes S. Study of Optimal Replacement of Thyroxine in the Elderly (SORTED) – results from the feasibility randomised controlled trial. Thyroid Res. 2016;9:5.

Rodondi N, Newman AB, Vittinghoff E, de Rekeneire N, Harris TB, Bauer DC. Subclinical hypothyroidism and the risk of heart failure, other cardiovascular events, and death. Arch Intern Med. 2005;165(21):2460–6.

Santini F, Pinchera A, Marsili A, Ceccarini G, Castagna MG, Valeriano R, Giannetti M, Taddei D, Centoni R, Scartabelli G, Rago T, Mammoli C, Elisei R, Vitti P. Lean body mass is a major determinant of levothyroxine dosage in the treatment of thyroid diseases. J Clin Endocrinol Metab. 2005;90(1):124–7.

Santini F, Galli G, Maffei M, Fierabracci P, Pelosini C, Marsili A, Giannetti M, Castagna MG, Checchi S, Molinaro E, Piaggi P, Pacini F, Elisei R, Vitti P, Pinchera A. Acute exogenous TSH administration stimulates leptin secretion in vivo. Eur J Endocrinol. 2010;163(1):63–7.

Saravanan P, Chau WF, Roberts N, Vedhara K, Greenwood R, Dayan CM. Psychological well-being in patients on 'adequate ' doses of L-thyroxine: results of a large, controlled community-based questionnaire study. Clin Endocrinol. 2002;57:577–85.

Saravanan P, Visser TJ, Dayan CM. Psychological well-being correlates with free thyroxine but not free 3,5,3′-triiodothyronine levels in patients on thyroid hormone replacement. J Clin Endocrinol Metab. 2006;91:3389–93.

Sawin CT, Geller A, Wolf PA, Belanger AJ, Baker E, Bacharach P, Wilson PW, Benjamin EJ, D'Agostino RB. Low serum thyrotropin concentrations as a risk factor for atrial fibrillation in older persons. N Engl J Med. 1994;331(19):1249–52.

Selmer C, Olesen JB, Hansen ML, Lindhardsen J, Olsen AM, Madsen JC, Faber J, Hansen PR, Pedersen OD, Torp-Pedersen C, Gislason GH. The spectrum of thyroid disease and risk of new onset atrial fibrillation: a large population cohort study. BMJ. 2012;345:e7895.

Seshadri MS, Samuel BU, Kanagasabapathy AS, Cherian AM. Clinical scoring system for hypothyroidism: is it useful? J Gen Intern Med. 1989;4(6):490–2.

Sjouke B, Langslet G, Ceska R, Nicholls SJ, Nissen SE, Öhlander M, Ladenson PW, Hovingh G, Kastelein JJP. Eprotirome in patients with familial hypercholesterolaemia (the AKKA trial): a randomised, double-blind, placebo-controlled phase 3 study. Lancet Diabetes Endocrinol. 2014;2(6):455–63.

Slater S. The discovery of thyroid replacement therapy. Part 3: a complete transformation. J R Soc Med. 2011;104(3):100–6.

Spencer CA, Nicoloff JT. Serum TSH measurement: a 1990 status report. Thyroid Today. 1990;13:1.

Spencer CA, Hollowell JG, Kazarosyan M, Braverman LE. National Health and Nutrition Examination Survey III thyroid stimulating hormone (TSH)-thyroperoxidase antibody relationships demonstrate that TSH upper reference limits may be skewed by occult thyroid dysfunction. J Clin Endocrinol Metab. 2007;92(11):4236–40.

Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, Nixon A, Pearce EN, Soldin OP, Sullivan S, Wiersinga W. Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. American Thyroid Association Taskforce on Thyroid Disease during Pregnancy and Postpartum. Thyroid. 2011;21:1081–125.

Surks MI, Schadlow AR, Oppenheimer JH. Citation information. J Clin Invest. 1972;51(12):3104–13.

Surks MI, Goswami G, Daniels GH. The thyrotropin reference range should remain unchanged. J Clin Endocrinol Metab. 2005;90(9):5489–96.

Thienpont LM, Van Uytfanghe K, Beastall G, et al. for the IFCC Working Group on Standardization of Thyroid Function Tests. Clnical Chem. 2010;56:912–20.

Thienpont LM, Van Uytfanghe K, Poppe K, Velkeniers B. Determination of free thyroid hormones. Best Pract Res Clin Endocrinol Metab. 2013;27(5):689–700.

Thvilum M, Brandt F, Brix TH, Hegedüs LA. review of the evidence for and against increased mortality in hypothyroidism. Nat Rev Endocrinol. 2012a Mar 6;8(7):417–24.

Thvilum M, Brandt F, Brix TH, Hegedus L. A review of the evidence for and against increased mortality in hypothyroidism. Nat Rev Endocrinol. 2012b;8:417–24.

Toft AD. Thyroid hormone replacement – one hormone or two? New Engl J Med. 1999;340: 469–70.

Tunbridge WM, Evered DC, Hall R, Appleton D, Brewis M, Clark F, Evans JG, Young E, Bird T, Smith PA. The spectrum of thyroid disease in a community: the Whickham survey. Clin Endocrinol. 1977;7(6):481–93.

Vanderpump MP, Tunbridge WM, French JM, Appleton D, Bates D, Clark F, Evans JG, Hasan DM, Rodgers H, Tunbridge F. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clin Endocrinol. 1995;43(1):55–68.

Villar HC, Saconato H, Valente O, Atallah AN. Thyroid hormone replacement for subclinical hypothyroidism. Cochrane Database Syst Rev. 2007;(3):CD003419.

Walsh JP, Ward LC, Burke V, Bhagat CI, Shiels L, Healey D, Gillett MJ, Gilbert R, Tanner M, Stuckey GA. Small changes in thyroxine dosage do not produce measurable changes in hypothyroid symptoms, well-being, or quality of life: results of a double-blind, randomized clinical trial. J Clin Endocrinol Metab. 2006;91:2624–30.

Walsh JP, Bremner AP, Feddema P, Leedman PJ, Brown SJ, O'Leary P. Thyrotropin and thyroid antibodies as predictors of hypothyroidism: a 13-year, longitudinal study of a community-based cohort using current immunoassay techniques. J Clin Endocrinol Metab. 2010;95(3):1095–104.

Waring AC, Arnold AM, Newman AB, Bùzková P, Hirsch C, Cappola AR. Longitudinal changes in thyroid function in the oldest old and survival: the cardiovascular health study all-stars study. J Clin Endocrinol Metab. 2012;97(11):3944–50.

Wartofsky L, Dickey RA. The evidence for a narrower thyrotropin reference range is compelling. J Clin Endocrinol Metab. 2005;90(9):5483–8.

Watt T, Cramon P, Hegedüs L, Bjorner JB, Bonnema SJ, Rasmussen ÅK, Feldt-Rasmussen U, Groenvold M. The thyroid-related quality of life measure ThyPRO has good responsiveness and ability to detect relevant treatment effects. J Clin Endocrinol Metab. 2014;99(10):3708–17.

Weeke J, Gundersen HJ. Circadian and 30 minutes variations in serum TSH and thyroid hormones in normal subjects. Acta Endocrinol. 1978;89(4):659–72.

Wiersinga M. Chapter 107: Adult hypothyroidism and myxedema coma. In: LJ DG, Jameson JL, editors. Endocrinology. 5th ed. Philadelphia: WB Saunders Company; 2004.

Wiersinga WM. Smoking and thyroid. Clin Endocrinol. 2013;79(2):145–51.

Wiersinga WM, Duntas L, Fadeyev V, Nygaard B, Vanderpump MP. ETA Guidelines: The Use of L-T4 + L-T3 in the Treatment of Hypothyroidism. Eur Thyroid J. 2012 Jul;1(2):55–71.

Wilkes S, Pearce S, Ryan V, Rapley T, Ingoe L, Razvi S. Study of optimal replacement of thyroxine in the Elderly (SORTED): protocol for a mixed methods feasibility study to assess the clinical utility of lower dose thyroxine in elderly hypothyroid patients: study protocol for a randomized controlled trial. Trials. 2013;14(83):22.

Winther KH, et al. PLoS One. 2016;11(6):e0156925.

Yiger RD, Odell WD, Condliffe PG. Immunologic studies of purified human and bovine thyrotropin. Endocrinology. 1963;73:359–65.

Zimmermann MB, Jooste PL, Pandav CS. Iodine-deficiency disorders. Lancet. 2008;372(9645):1251–62.

Zulewski H, Müller B, Exer P. Estimation of tissue hypothyroidism by a new clinical score: evaluation of patients with various grades of hypothyroidism and controls. J Clin Endocrinol Metab. 1997;82:771–6.

Part V

Hyperthyroidism and Thyrotoxicosis

Graves' Disease

14

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Contents

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Abstract

Graves' disease (GD) is a common autoimmune thyroid disorder, affecting 20–30 per 100,000 of the population per year. In keeping with other autoimmune diseases, it exhibits a clear female preponderance (F:M 6–7:1) with approximately 3% of women and 0.5% of men developing GD during their lifetime.

GD is a complex genetic condition, with environmental factors precipitating the disease in genetically predisposed individuals who harbor multiple susceptibility alleles. Thyroid-stimulating hormone receptor (TSHR) antibodies are the immunological hallmark of the disease and the key driver for thyrocyte hyperplasia and the resulting hyperthyroidism. Our understanding of the pathogenesis of the condition has developed significantly in recent years, reflecting advances in human genomics, molecular immunology, and the availability of murine models of disease.

Clinical features in GD are widespread, with a myriad of typical symptoms and physical findings at presentation. Frequently reported symptoms include tremor, palpitations, heat intolerance, weight loss, and anxiety. Physical examination may reveal warm, tremulous extremities, atrial fibrillation, signs of thyroid orbitopathy, and a goiter with a bruit. A series of extrathyroidal manifestations can accompany GD at presentation or appear during the course of the disease; these are associated with elevated titers of circulating autoantibodies. The commonest extrathyroidal manifestation is thyroid orbitopathy, which can be sight-threatening and requires a detailed and careful approach to management. Recent developments in our understanding of the pathogenesis of these conditions may lead to the development of novel therapies in coming years.

Keywords

Autoimmune · Hyperthyroidism

Introduction

Graves' disease (GD) is characterized by circulating thyroid-stimulating autoantibodies which stimulate, rather than destroy, their target tissue. This unique feature makes GD exceptional among autoimmune conditions and has resulted in its role as a paradigm for research into autoimmune endocrinopathies. The distinct pathogenesis of thyroid-associated orbitopathy (TAO), the commonest extra-thyroidal manifestation in GD, has prompted much research into the extended role of circulating stimulating antibodies. This chapter will review the epidemiology of the disease, the contributing genetic and environmental factors, and the immunopathogenic features of the condition.

Recent advances have improved our understanding of the genetic and immune factors which play a role in the development of GD and other autoimmune diseases. A complex interplay of genetic and environmental factors contributes to disease predisposition and overt clinical presentation. A number of environmental triggers have been postulated to play a role in disease, although the significance of each may vary with ethnicity and geographical location.

Epidemiology

Autoimmune thyroid diseases (AITD), namely, Graves' disease (GD) and Hashimoto's thyroiditis (HT), are prevalent conditions. GD, the commonest cause of hyperthyroidism, affects approximately 3% of women and 0.5% of men during their lifetime (Nyström et al. 2013). An incidence of 0.04% per year was found in the 12-year follow-up of the Nurses Health II study in North America (Hollowell et al. 2002). A 20-year follow-up study from the North East of England corroborated this with an incidence of 0.08% per year in women (Vanderpump et al. 1995). Prevalence will vary between populations, reflecting geographical and iodine-dependent factors; these will be discussed later in this chapter. Antibodies to thyroid antigens (thyroid peroxidase, TPO; and thyroglobulin, Tg), inferring risk of overt AITD, are even more common.

GD, in common with other autoimmune diseases, has a clear female preponderance with a female to male ratio of 6–7:1. AITD has a sibling risk ratio (λ_s) of 5.9–10 (lambda sib is an index of the heritability of a disorder, calculated by assessing the risk to a sibling of an affected individual compared to that of the unrelated background population). Twin studies suggest genetic factors account for 80% of the risk of contracting AITD (Brix et al. 2001). Recently, the Thyroid Events Amsterdam Score (THEA) was designed to predict the likelihood of progression to overt hypo- or hyperthyroidism in female relatives of patients with AITD (Strieder et al. 2008). This predictive score weights three risk factors (proportionately according to their relative risks) – TSH level, TPO antibodies, and family history – to estimate the 5-year risk of overt autoimmune thyroid disease in female relatives of affected patients (Strieder et al. 2008), emphasizing the significance of these three factors as the dominant determinants of susceptibility to AITD.

GD can present anytime between childhood and older age, although the majority of patients are diagnosed between the ages of 35–40 years (Holm et al. 2005) reflecting a peak in incidence in the fourth decade. Unsurprisingly, it is quite common to encounter GD in pregnancy. In this setting, it is relatively easy to manage because of the mitigating effect of pregnancy on autoimmune disease activity, although patients will usually relapse following delivery. Its management in this setting does require some additional consideration and is discussed later in the chapter.

Genetics

Graves' disease is a complex genetic condition, with multiple chromosomal loci contributing to disease pathogenesis. These genes encode proteins in biological pathways that regulate immune system activity or thyroid biology (Vaidya et al. 2002; Eschler et al. 2011). The discrete contribution of individual genes is influenced by a wide range of environmental factors and varies between affected individuals. Advances in our understanding of the genetics of AITD have resulted in an appreciation that both immune-modulating and thyroid-specific genes play a role in disease.

Three loci in particular have been identified as having a moderate or large effect in Graves' disease; these are the major histocompatibility complex (MHC), the cytotoxic T lymphocyte antigen-4 (CTLA4) locus, and the protein tyrosine phosphatase-22

(PTPN22) gene. Alleles at these loci also contribute significantly to other complex autoimmune diseases, including type 1 diabetes, rheumatoid arthritis, coeliac disease, and autoimmune Addison's disease. There is a wealth of evidence to demonstrate that these genes determine the "general" predisposition to autoimmunity (and contribute to co-existing autoimmune disease) (Criswell et al. 2005), while more specific "target-organ" genes contribute to the organ-specific presentation of the autoimmune disease.

A previous study of the NHANES database revealed that the prevalence of autoimmune thyroid disease varied depending upon ethnic origin, and other world-wide studies in various populations (Chinese, Caucasian, etc.) have supported the variation in susceptibility genes between populations.

Human Leukocyte Antigen

The HLA complex on chromosome 6 contains sequences which encode highly polymorphic genes responsible for immune regulation. HLA genes are subdivided into three predominant classes (Class I, Class II, and Class III). Class II includes histocompatibility genes which are expressed exclusively on leucocytes and immune competent cells (HLA-DR).

The association of GD with alleles of the MHC on chromosome 6p21 has long been established (Grumet et al. 1974; Farid et al. 1979). The dominant association in white populations of European descent is with HLA-DR3 carrying haplotypes (DRB1*0301-DQB1*0201-DQA1*0501) (Yanagawa et al. 1993; Heward et al. 1998). This haplotype is carried by 25–30% of unaffected individuals, but is consistently over-represented among those with GD, of whom about 50% carry the haplotype. Resequencing of the DRB1 gene in many GD subjects has suggested that a critical amino acid for disease susceptibility is arginine at position 74 (Ban et al. 2004; Simmonds et al. 2005). Even so, the odds ratio for the DR3 haplotype in GD is about 2, and MHC does not have the dominant genetic effect in GD that is found in other autoimmune disorders (for instance type 1 diabetes or rheumatoid arthritis). This is manifest by the lack of strong evidence for genetic linkage to 6p21 in many studies (Simmonds et al. 2005; Vaidya et al. 1999) and suggests that loci other than MHC also have major effects. A total of 50% of GD subjects carry "non-DR3" HLA alleles, which is a likely indication that there is not just one unique immunogenic or pathogenic peptide that is consistently involved in triggering the T-cell response in GD. Other ethnic groups carry different haplotypes, although the available data are somewhat limited (Jacobson et al. 2008; Yanagawa and DeGroot 1996). Although the risk inferred by HLA is well established, it explains a relatively small proportion of overall genetic predisposition to GD.

Cytotoxic T-Lymphocyte Antigen-4

CTLA4 is a T lymphocyte surface protein which has a role in the regulation of the costimulatory ("second") signal in T lymphocyte activation. It is a good candidate

gene for GD and several studies have shown a link between CTLA4 and GD; alleles in the 3′ region of the gene on chromosome 2q33 have been extensively associated with the disease. Initial investigations (Yanagawa et al. 1995) were confirmed with extensive replication studies in differing GD populations (Vaidya and Pearce 2004). The true disease susceptibility allele at CTLA4 remains to be defined but may lie within a 6 kb region including the 3′ untranslated region (UTR) of the gene (Ueda et al. 2003). The susceptibility haplotype at CTLA4 is carried by about 50% of the healthy white population, and its prevalence increases to 60% in subjects with GD, with an odds ratio for the most associated allele of about 1.5 (Ueda et al. 2003). The mechanism by which these noncoding polymorphisms might modulate the immune response is far from clear. CTLA4 polymorphisms may have a role in T-cell differentiation and lineage commitment, with genotypes being correlated to the number of circulating $CD4^+$ and $CD25^+$ T regulatory lymphocytes (Atabani et al. 2005).

What is also apparent is that CTLA4 is an important regulator of T cell function and T cell activation (in combination with the CD28 pathway), although the precise manner in which CTLA4 controls immune responses remains ill-defined. Recent work indicates that quantitative differences in CTLA4 expression may act in competition to the CD28 receptor for CD80/CD86-dependent activation of resting human T cells and that this may be contextual and related to the number of antigen presenting cells (Hou et al., 2015). Unsurprisingly, CTLA4 polymorphisms are also significantly related to disease susceptibility in type 1 diabetes and autoimmune Addison's disease. CTLA4 has also been linked to the production of TPO antibodies and Tg antibodies (Vaidya and Pearce 2004; Ueda et al. 2003; Atabani et al. 2005; Hou et al. 2015) (Fig. 1).

Protein Tyrosine Phosphatase Nonreceptor-22

An additional locus, PTPN22, came to light after the acceptance of the role of HLA in GD and the discovery of CTLA4. PTPN22 encodes the lymphoid tyrosine phosphatase (LYP) molecule, which, like CTLA4, is involved in the regulation of T-cell activation. A coding polymorphism, arginine to tryptophan at codon 620, activates the LYP molecule, paradoxically causing more potent inhibition of the T-cell antigen receptor (CD3)-signaling kinases, following engagement with MHC-antigen (Vang et al. 2005). The tryptophan allele is carried by about 7% of healthy subjects in white populations, but is overrepresented in GD subjects with a prevalence of about 13% (Velaga et al. 2004; Smyth et al. 2004). The odds ratio for the effect of this allele is about 1.8, but because of its comparative rarity (Velaga et al. 2004; Smyth et al. 2004), it contributes slightly less to overall population Graves' disease susceptibility than CTLA4. It remains to be seen how the effect of this variant on T-cell receptor signaling predisposes to autoimmunity. This SNP at codon 620 is also implicated in type 1 diabetes and other autoimmune disorders (Velaga et al. 2004). As with other genetic factors, the association of PTPN22 with autoimmune thyroid disease is influenced by ethnicity (Criswell et al. 2005).

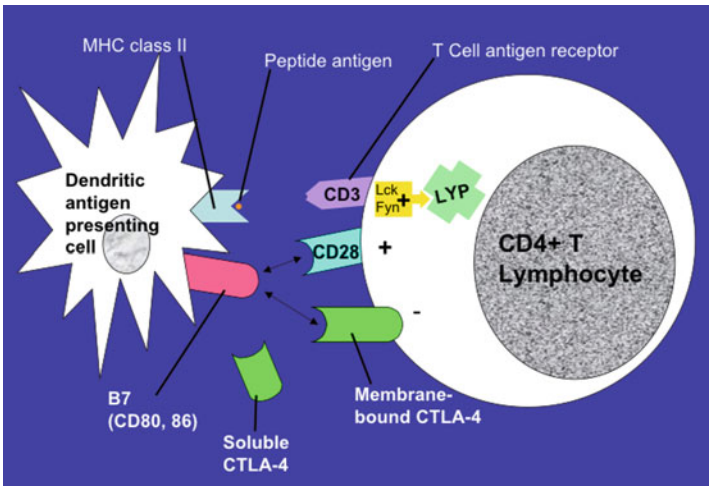

Fig. 1 *An antigen-presenting cell (APC) interacting with a T lymphocyte.* The dendritic APC presents a cleaved peptide antigen bound to the groove of the tetrameric class II MHC molecule. This is recognized by a T lymphocyte with an antigen receptor (CD3 complex) of appropriate affinity for the peptide/MHC combination. For the lymphocyte to become activated, a second signal must be delivered by the interaction of a costimulatory molecule with B7 molecules expressed on the APC. CD28 is a positive costimulator allowing lymphocyte proliferation and activation, but CTLA4 is an inhibitory costimulator, causing the lymphocyte to become quiescent or to apoptose. Soluble CTLA4 (sCTLA4) may have a role as a natural inhibitor of CD28 engagement, by binding to APC B7 molecules with a higher affinity than CD28 and thereby stopping the costimulatory activation of the lymphocyte via CD28-B7 interaction. Subjects with the susceptibility allele for AITD at CTLA4 have relatively less sCTLA4 mRNA (Hou et al. 2015), and if reflected at the protein level, this could allow a greater activation signal to T lymphocytes, by stronger costimulatory activity. Lymphoid tyrosine phosphatase (LYP) is encoded by the PTPN22 gene, and the autoimmunity-associated SNP codes for an arginine (wild-type) to tryptophan change. The LYP molecule inhibits Lck signaling following TCR (CD3) engagement; however, the tryptophan variant of LYP is more highly active than the arginine variant, paradoxically causing more potent downregulation of signaling (Vang et al. 2005)

Several other genes encoding molecules involved in immunoregulation have been demonstrated to have allelic variants associated with GD. These include CD25, CD40, IFIH1, BACH2, SCGB3A2, and FCRL (Brand et al. 2007; Tomer et al. 2002a; Sutherland et al. 2007; Liu et al. 2014; Song et al. 2009; Simmonds et al. 2010), but none confer the same magnitude of susceptibility risk that the HLA, CTLA-4, or PTPN22 variants do. Programmed cell Death-Ligand 1 (PD-L1) is an additional locus encoding a costimulatory molecule, where alleles have been associated with GD in several studies (Hayashi et al. 2008; Mitchell et al. 2009). This is of particular clinical interest as monoclonal antibodies that have anticancer effects through stimulation of the immune system via costimulatory blockade ("Immune checkpoint blockade") are just becoming mainstream therapies for melanoma and lung cancer. These treatments block either the CTLA4 or PD-L1 pathways and have autoimmune thyroid disease as a common side effect from their use (Abdel-Wahab et al. 2016).

In addition to these genetic variants in immunoregulatory pathways, loci specific to GD have also been identified. These thyroid-specific genes include TSHR and TG (but not TPO).

Thyroid-Stimulating Hormone Receptor

After a period of negative investigations into the TSH receptor (TSHR) gene, alleles of SNP markers were subsequently shown to have unequivocal association with GD in two distinct patient cohorts (Hiratani et al. 2005; Dechairo et al. 2005). Single-nucleotide polymorphisms (SNPs) in TSHR have been specifically associated with GD in Caucasian populations (Dechairo et al. 2005). The functional role of these intronic SNPs remains to be elucidated. One theory is that they give rise to RNA splice variants, increasing the level of TSHR-A subunits. Alternatively, they could result in fewer thymic TSHR mRNA transcripts, potentially reducing central tolerance to TSHR (Colobran et al. 2011).

Thyroglobulin

The thyroglobulin (Tg) gene (8q24) encodes the Tg protein, the precursor for the thyroid hormones triiodothyronine (T3) and thyroxine (T4). In 2003, studies began to show weak evidence for association of GD with SNP and microsatellite markers in the thyroglobulin (Tg) gene (Ban et al. 2003; Collins et al. 2003). On aggregate, studies of the Tg gene at this time did not show convincing evidence for association with GD. Tg is a huge, 48 exon gene so further work was carried out to explore and define the enormous diversity of haplotypes. SNPs in the Tg gene have been associated with GD in some Caucasian cohorts, and an epistatic interaction between a SNP in Tg exon 33 and HLA-DR has been postulated. This suggestion has raised the possibility that Tg polymorphisms predispose to GD by modulating Tg peptide presentation by antigen-presenting cells on HLA class II molecules to T-cells.

The mechanism of action of many of these susceptibility loci is still not fully understood. Future examination of the role of gene-gene or gene-environment interactions and epigenetic factors is likely to be important in furthering our understanding of the genetic basis of this disease. Although these studies have led to some insights into pathogenesis, genetic information is currently not robust enough for clinical prediction of GD, nor have novel treatments come from the above pathogenic insights.

Environmental Factors

GD is one of the few autoimmune conditions where environmental factors have been definitively established to play an important role in disease onset.

Iodine

Iodine is one of the most common precipitants of thyroid dysfunction and GD is more prevalent in countries that are iodine replete (Laurberg et al. 1991). Dietary iodine supply is a significant factor in determining the frequency of GD in individuals who are already genetically predisposed to the disorder. A study in Iceland, where iodine intake is high, showed that the incidence of GD was more than double in this environment than in age-matched individuals in East Jutland, Denmark, where iodine intake is low (Laurberg et al. 1991). Salt iodinization is a public health consideration in iodine-deficient countries. Increasing iodine intake in a previously deficient population is associated with a small increase in the prevalence of subclinical hypothyroidism and markers of thyroid autoimmunity, although it is unclear if these increases are transient (Zimmermann and Boelaert 2015).

Smoking

Cigarette smoking is a well-defined risk factor for GD and has a clear association with TAO (Wiersinga 2013). It has long been established that smokers are overrepresented among patients with GD (odds ratio, 1.9; 95% confidence intervals 1.1–3.2) and that smoking will greatly increase the risk of TAO (odds ratio, 7.7; 95% confidence intervals 3–13.7) (Prummel and Wiersinga 1993). TAO will be more severe in smokers (Prummel and Wiersinga 1993; Bartalena et al. 1989) and smokers will have a poorer outcome from immunosuppressive treatment (Bartalena et al. 1998; Eckstein et al. 2003). Smoking cessation can improve outcomes in TAO (Pfeilschifter and Ziegler 1996), and therefore, patients should be encouraged to stop, particularly in the presence of eye disease.

Stress

The role of stress in GD pathogenesis is not clearly defined, although it has been implicated in the disease for many decades. Previous studies during spells of national conflict have shown increased rates of the condition, although other, major changes that occur simultaneously could be confounding factors, for example dietary changes during wartime (Mizokami et al. 2004).

In many different populations, negative life events (such as the death of a close family member in the year before the onset of the disease) have been linked to the onset of GD (Winsa et al. 1991; Yoshiuchi et al. 1998). The exact relationship between stress and GD is unclear, but there are many potential mechanisms (Mizokami et al. 2004) whereby stress could influence immune function in a predisposed individual. No correlation has been demonstrated between stressful life events and TPO Ab status (Strieder et al. 2005).

Hormones

Pregnancy is a well-recognized risk factor for GD. The mechanism for this presumably relates to a relative suppression of immune function during pregnancy and a rebound of immune activity in the postpartum period. This may be similar to the mechanisms of GD in immune reconstitution, which is discussed below. In contrast, estrogen use (e.g., while on the combined contraceptive pill) is protective against GD (Strieder et al. 2003a). This protective effect remains even when corrected for the lack of pregnancy in those on estrogen.

Immune Reconstitution

The relationship between immune reconstitution and an increased incidence of GD has been strengthening for some time. An increased use of monoclonal antibodies and newer therapies in a wide range of diseases has coincided with relatively high numbers of patients presenting with GD following treatment for the original condition. Presumably, the immunosuppressive and relatively long-term effect on immune function of these agents precedes a "re-exposure" to thyroid antigens, which result in GD. This was noted in patients with HIV who have received effective Highly Active AntiRetroviral Therapy and in those with multiple sclerosis or rheumatological disease who have received lymphocyte-depleting alemtuzumab (Campath-H1) treatment (Coles et al. 1999; Chen et al. 2005). Increasing experience with and more widespread usage of this drug is revealing more and more patients developing GD in the months and years following therapy. Experience managing these patients to date suggests they should be managed with conventional therapy (Weetman 2014), although emerging evidence suggests they are probably at least as likely to relapse as those with spontaneous GD.

Allergic Illness/Infective Trigger

A minority of GD cases appear to have a clear link to an allergic illness (typically allergic rhinitis), with high serum IgE levels preceding the onset of, or relapse, symptoms (Hidaka et al. 1993; Sato et al. 1999). It has been speculated that the Th2 (humoral) arm of the immune system is active in these circumstances and provides a permissive environment for thyroid antibody generation. There are many reports citing varying infectious agents (Strieder et al. 2003b; Munakata et al. 2005; Tomer and Davies 1993), although many of these studies are small and their findings have not been convincingly reproduced (Arscott et al. 1992). Importantly, GD patients represent a population with a distinct immunogenetic profile (50% of Caucasians with GD are HLA-DR3 positive), and studies examining antibody responses to infectious triggers need to be designed with an HLA-matched healthy control group or using large twin cohorts (Brix et al. 2008) before reports can be considered credible.

The influence of seasonality on disease incidence has been explored and conflicting data have been published so far (Facciani and Kazim 2000; Krassas et al. 2007; Hamilton et al. 2014). The bearing of month of birth on disease incidence later in life may be related to the increased exposure to viral or bacterial illnesses after birth in those born during the autumn/winter period. Increased exposure to viral or bacterial infection in this period could predispose to immune dysregulation.

Vitamin D/Selenium

Vitamin D deficiency has been reported in patients with GD and linked to relapse following a course of treatment with ATDs (Yasuda et al. 2012; Yasuda et al. 2013). One study has demonstrated that selenium supplementation was beneficial in patients with mild TAO (Marcocci et al. 2011), likely due to its antioxidant action, and selenium deficiency has also been reported in GD patients (Bülow Pedersen et al. 2013). Further studies are awaited in this field.

Immunopathogenesis

Graves' disease (GD) is characterized by TSH receptor antibodies (TSHR Abs), the principal driver for thyrocyte hyperplasia and hyperthyroidism (Adams et al. 1974). These stimulating antibodies (TSAbs) are detected by sensitive immunoassays in >95% patients with GD (Smith et al. 2004).

TSAbs are predominantly of the IgG1 subclass and target a conformational and discontinuous epitope in the amino-terminal region of the leucine-rich repeat motif in the extracellular domain of the TSHR (Costagliola et al. 2004) (Fig. 2). The TSHR has the unique feature for a glycoprotein hormone receptor of being cleaved and re-formed from two disulfide-linked subunits (termed A and B) during its processing for cell surface expression. Curiously, it appears that TSAbs have a higher affinity for the cleaved-off, "shed" extracellular A domain of the receptor than for the intact "holoreceptor", and indeed immunization with the A domain also generates higher TSHR stimulating activity in murine models than with intact receptor (Chazenbalk et al. 2002; Chen et al. 2003). Binding of these TSAbs activates the intracellular G proteins coupled to the TSHR, and this induces transcription of the genes encoding Tg, the thyroid hormone-generating enzymes (including TPO), and the sodium iodide transporter via the cyclic-AMP and phospholipase-C pathways. Thyroid hormone production, secretion, and thyrocyte growth results.

As well as TSAbs, subjects with Graves' disease and other autoimmune thyroid diseases may have circulating antibodies that block the effects of TSH (or of TSHR-stimulating antibodies) on TSHR activation. The presence of these blocking antibodies, co-existing with circulating TSAbs, is partly responsible for the fluctuating nature of the hyperthyroidism that is sometimes seen in Graves' disease. In addition, high titer blocking antibodies are responsible for the occasional individual with hyperthyroid Graves' disease who presents after a prolonged period of hypothyroidism.

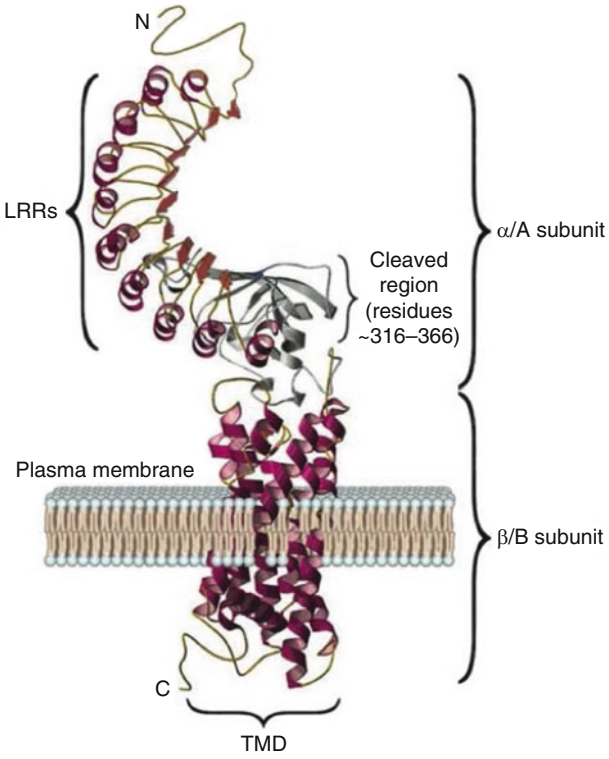

Fig. 2 *Ribbon diagram of the putative structure of the TSH receptor.* The large extra-cellular "A" domain contains nine leucine-rich repeat (LRR) regions, which is attached to the transmembrane and intracellular tail of the receptor ("B" domain) by disulfide bonds. The sites of TSHR-stimulating antibody binding have been mapped to discontinuous and conformational epitopes involving the amino-terminal portion of the LRR domain

Whether the immune response directed against the TSHR is the initiating immunological event for Graves' disease may never be fully clarified; however, there are circulating antibodies to several other thyroid antigens in the majority of subjects with Graves' disease. These include antibodies directed against TPO, thyroglobulin, the sodium iodide symporter (NIS), the apical iodide channel (known as pendrin), and the insulin-like growth factor receptor-1 (IGF1R) (Brix et al. 2014; Minich et al. 2013). Anti-TPO (formerly known as microsomal) antibodies are the most frequently found of these antibodies (found in up to 90% of Graves' subjects using sensitive assays) (Beever et al. 1989) and are pathogenically important. These anti-TPO antibodies may be of various subclasses (IgG1, −2 or −4) and uniquely among thyroid autoantibodies have the capacity to fix complement and to mediate thyrocyte damage through antibody dependent cell-mediated cytotoxicity (Weetman and Cohen 1986; Khoury et al. 1981). In contrast to the low-concentration THSR-stimulating antibodies, which circulate in nanogram per ml quantities in Graves'

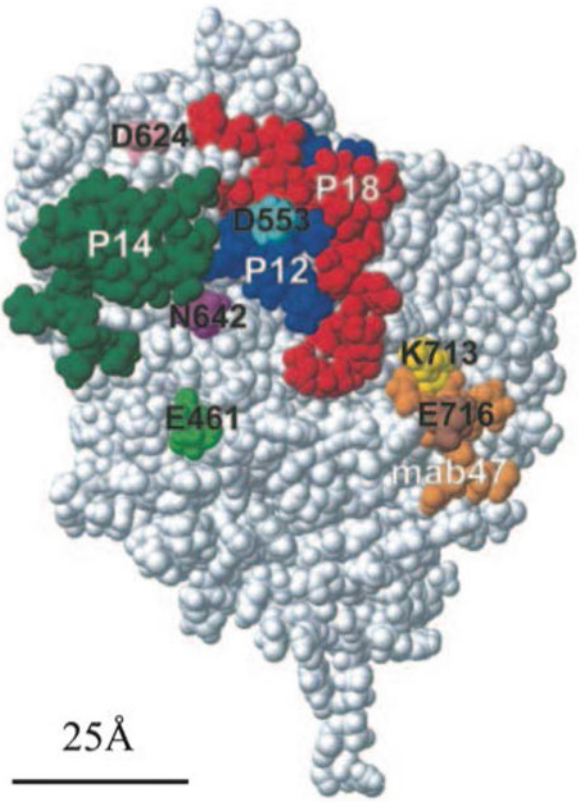

Fig. 3 *Space-filling diagram of the putative structure of the myeloperoxidase-like domain of thyroid peroxidase*. Thyroid peroxidase (TPO) acts as a cell surface homodimer and has a large myeloperoxidase (MPO)-like domain, with 2 smaller, complement control protein (CCP)-like and EGF-like domains (not shown). There are specific TPO epitopes that are predominantly recognized by autoantibodies in human autoimmune thyroid diseases (Chazenbalk et al. 1993). The TPO peptides that block autoantibody binding to these immunodominant regions are shown mapped out on the surface of the MPO-like domain, but in reality these epitopes involve additional residues on the CCP-like domain in a 3-dimensional conformation (Gora et al. 2004)

disease, TPO antibodies are commonly found at 1000-fold or higher concentrations. Naturally occurring TPO antibodies tend to be directed against certain specific and highly conformational "immunodominant" epitopes of TPO (Chazenbalk et al. 1993; Arscott et al. 1996) (Fig. 3). Interestingly, these patterns of TPO antibody response (epitopic fingerprints) are stable, persisting for many years; they are also inherited in a dominant fashion (Jaume et al. 1999). In contrast to anti-TSHR antibodies, anti-TPO antibodies do not seem to affect the enzymatic function of TPO in vivo.

Thyroglobulin is a major autoantigen and could be implicated in the pathogenesis of GD and TAO (Marinò et al. 2004). Genome-wide association studies (GWAS) have shown a link between a locus on chromosome 8q24, where the Tg gene is located, and autoimmune thyroid diseases (Tomer et al. 2002b). More recently, a genetic/epigenetic mechanism by which a SNP variant of the Tg promoter predisposes to AITD (by an altered interaction with interferon regulatory factor-1) has been proposed (Stefan et al. 2011). In contrast, circulating antibodies to NIS, pendrin, and IGF1R are less frequent in GD and currently of uncertain pathogenic significance (Brix et al. 2014; Minich et al. 2013).

In the untreated state, the thyroid gland in Graves' disease, as well as being enlarged by thyrocyte hyperplasia, also contains an extensive B- and T-cell

lymphocytic infiltrate. The thyroid autoantibodies are produced predominantly from these intrathyroidal B cells/plasma cells (Weetman et al. 1982), which cluster into lymphoid follicles. Antibodies are also produced to a lesser degree from activated B cells in adjacent lymph nodes, spleen, or thymus. These B cells, in turn, are regulated by activated intrathyroidal T-cells, which are of restricted T-cell antigen receptor diversity (Nakashima et al. 1996), suggesting derivation from a limited number of precursor T-cells. Once established, it is likely that the immune response in the thyroid is maintained by a number of factors, including the ability of thyrocytes themselves, as well as the intrathyroidal B cells, to present antigen to T-cells and the overexpression of TPO and Tg that is driven by TSHR stimulation. It can certainly be envisaged that a circle of perpetuation might occur, whereby antibody-mediated TSHR stimulation triggers increased TPO and Tg expression, leading to formation of anti-TPO and Tg antibodies and progressive thyrocyte antigen release, which re-primes the immune response.

Clinical Presentation

Symptoms and Signs

The symptoms of thyrotoxicosis are often nonspecific, so patients with Graves' disease may present in numerous ways (Burch and Cooper 2015; Vaidya and Pearce 2014; Smith and Hegedüs 2016). The onset of symptoms is gradual and often poorly defined with most subjects having felt unwell for 3 to 6 months before seeking medical attention. If the onset of thyrotoxic symptoms is rapid or can be pinned down to a single day or few days, then the diagnosis is most frequently that of a destructive thyroiditis rather than Graves' disease. Weight loss despite an increase in appetite is found in 80% of subjects, although in a minority the increase in appetite, coupled with the free availability of calorie-dense food, leads to weight gain. Pervasive exhaustion may alternate with periods of restlessness and hyperactivity. Heat intolerance is also common, with the need to wear fewer clothes and sweating at night being characteristic. Palpitations at rest or on minimal exertion or shortness of breath during light exercise are common at all ages. Tremor of the hands may be noted, along with inappropriate feelings of anxiety, apprehension, or jumpiness. Poor sleep with mental overactivity and physical hyperkinesis at night may be a problem. Intestinal transit-time is shortened, leading to more frequent defecation. Menstrual bleeding may be light, decreased in frequency, or absent.

Thyroid tenderness or pain is not a feature. Less commonly reported symptoms are thirst, nausea, generalized itch, and scalp hair loss which can be diffuse. In 5–10% of people, the first symptoms are due to Graves' orbitopathy with itchy, gritty, or watering eyes, or an abnormal appearance (Mitchell et al. 2015). Individuals with asymmetrical orbitopathy tend to present earlier with change in appearance. In the elderly, there may be little to suggest thyrotoxicosis. Nonspecific symptoms or feelings of lethargy and a reduced appetite may lead to a diagnosis of depression. Alternatively, the onset of atrial fibrillation may precipitate a cardiac

presentation with dyspnea and/or congestion. In childhood, hyperactivity, short attention span, behavioral problems, and increased linear growth are found.

The subject may have difficulty sitting still, with constant fidgeting of the hands. The face, neck, and upper chest wall are often flushed. The palms may be warm and sweaty, with a symmetrical fine tremor when hands are outstretched. A diffuse goiter can be visible or palpable, with a systolic phase bruit found over it. There is often tachycardia unless beta-blockers are being taken, and rapid atrial fibrillation may be present, particularly in the elderly. Systolic blood pressure may be elevated. There may be hepatomegaly or splenomegaly. The ankles may be swollen with pitting edema or rarely because of infiltrative thyroid dermopathy (pretibial myxedema). However, the latter is more commonly manifest as discrete violaceous plaques on the shin or dorsum of the foot. Hyperreflexia is common and proximal musculature can be weak. Frank spasticity and pseudobulbar paresis are late features. Rapid onset of severe and generalized muscle weakness suggests hypokalemic periodic paralysis, a syndrome most common in men of Asian descent that is precipitated by thyrotoxicosis. Signs of Graves' orbitopathy, including lid retraction, lid or conjunctival redness and edema, proptosis, and restricted ocular motility may be present. Rare signs of Graves' disease include chorea, onycholysis, or acropachy of the nails.

Investigation and Diagnosis

Elevation of one or both serum-free thyroid hormones together with an undetectable TSH (on a third generation assay) confirms the diagnosis of thyrotoxicosis (Fig. 4). About 5% of subjects, most commonly elderly, present with elevation of free T3 alone, with normal free T4 and undetectable TSH. This "T3 thyrotoxicosis" is often a manifestation of relatively mild hyperthyroidism that may respond well to medical treatment. Elevation of free T4 alone, with normal free T3 and undetectable TSH, may be found in someone with co-existing major illness (a combination of thyrotoxicosis and sick-euthyroid syndrome), but is also typical of iodine-induced thyrotoxicosis or exogenous levothyroxine use. If there is doubt about the chronicity or severity of symptoms, then it is good practice to repeat the abnormal thyroid function tests after a short period, as a rapid fluctuation may be the clue to the diagnosis of destructive (silent) thyroiditis. If the TSH is low but detectable, the diagnosis is almost certainly not Graves' disease and further investigations are needed (Mitchell and Pearce 2010). Individuals with a persistently undetectable TSH but normal free thyroid hormones (in the absence of pituitary disease and drug effects) are said to have subclinical hyperthyroidism (SH) and need further investigation (i.e., serum thyroid antibodies, Holter monitor, DEXA bone scan) (Mitchell and Pearce 2010). There is little clear evidence to guide treatment in this situation, although recent guidelines have been produced (Mitchell and Pearce 2010). Intervention depends on the degree of SH and the sequelae (Mitchell and Pearce 2010; Biondi et al. 2015); but in the presence of atrial fibrillation or established osteoporosis, patients may warrant treatment.

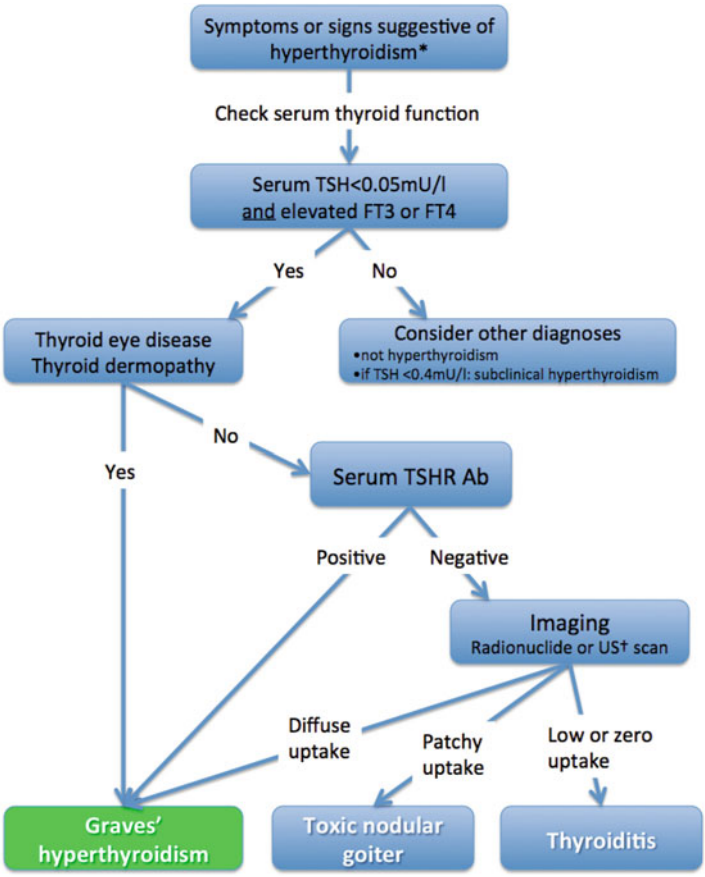

Fig. 4 Algorithm for confirmation of the diagnosis of Graves' hyperthyroidism

In the presence of clear extrathyroidal signs of Graves' disease (e.g., proptosis, dermopathy), no further testing beyond free thyroid hormone(s) and TSH is necessary. In the absence of these features, an attempt to secure an etiological diagnosis should be made. The gold-standard test is a highly sensitive TSHR-stimulating antibody assay (Burch and Cooper 2015; Smith and Hegedüs 2016) (Fig. 4). Other serum antibody tests, including indirect assay of TSH-stimulating antibodies by TSH-binding inhibitory immunoglobulin (TBI or TBII) or TPO antibody assay, are commonly employed and have >90% sensitivity for Graves' disease. In the absence of a positive antibody test, or in the presence of a nodular thyroid on palpation, the thyroid gland should be imaged. Although an ultrasound examination may give a diagnosis of multinodular goiter, a radionuclide image with either $^{99-}$Tc or $^{123-}$I gives functional data as to the presence and distribution of functioning thyroid tissue. However, both Graves' disease and multinodular goiter may coexist on occasions causing confusion if only imaging is used to make an etiological

diagnosis. Other investigations may be worthwhile depending upon the clinical situation and likely treatment plan. If there is significant tachycardia, it is good practice to document the rhythm by ECG and subjects with atrial fibrillation should proceed to a more detailed cardiac evaluation. If antithyroid drug treatment is planned, then a blood count and white cell differential at baseline may be helpful for the future. A negative pregnancy test is mandatory if radioiodine treatment is to be undertaken. Microcytosis, elevation of serum alkaline phosphatase, and mildly deranged liver enzymes are often found; mild hypercalcemia can also be present.

Treatment

The treatment options for hyperthyroid Graves' disease include thionamide antithyroid drug treatment, radioiodine therapy, or thyroid surgery. All thyrotoxic patients may gain symptomatic benefit from beta blockade, but this is contraindicated in those with asthma. Currently, there is no perfect treatment for Graves' hyperthyroidism. Each modality has its own pros and cons, and patient preference is frequently a deciding factor. These issues are elucidated in detail ► Chap. 16, "Treatment of Graves' Disease".

Future Developments

Current exome or whole genome sequencing strategies have yet to be applied wholeheartedly to complex genetic traits such as Graves' disease, as the analysis of such large datasets where multiple missense or noncoding variants are likely to have biological significance is problematic. Nevertheless, the identification of multiple but individually rare variants at a single genetic locus could lead to a leap in understanding of disease pathogenesis. Furthermore, large-scale expression analysis using RNA sequencing "RNA-seq" approaches in various relevant tissues including thyroid is expected to yield much more robust quantitative information, as well as hitherto unachievable details about expressed isoforms (RNA splice variants) that are starting to be explored (Qin et al. 2015). Development of an anti-TSHR antibody or a small molecule antagonist that could block binding of TSHR Abs (and therefore prevent TSHR over-stimulation) would ameliorate hyperthyroidism and could provide an effective future therapy for GD (Neumann et al. 2015).

References

Abdel-Wahab N, Shah M, Suarez-Almazor ME. Adverse events associated with immune checkpoint blockade in patients with cancer: a systematic review of case reports. PLoS One. 2016; 11(7):e0160221.

Adams DD, Fastier FN, Howie JB, Kennedy TH, Kilpatrick JA, Stewart RD. Stimulation of the human thyroid by infusions of plasma containing LATS protector. J Clin Endocrinol Metab. 1974;39(5):826–32.

Arscott P, Rosen ED, Koenig RJ, Kaplan MM, Ellis T, Thompson N, et al. Immunoreactivity to Yersinia enterocolitica antigens in patients with autoimmune thyroid disease. J Clin Endocrinol Metab. 1992;75(1):295–300.

Arscott PL, Koenig RJ, Kaplan MM, Glick GD, Baker Jr JR. Unique autoantibody epitopes in an immunodominant region of thyroid peroxidase. J Biol Chem. 1996;271(9):4966–73.

Atabani SF, Thio CL, Divanovic S, Trompette A, Belkaid Y, Thomas DL, et al. Association of CTLA4 polymorphism with regulatory T cell frequency. Eur J Immunol. 2005;35(7):2157–62.

Ban Y, Greenberg DA, Concepcion E, Skrabanek L, Villanueva R, Tomer Y. Amino acid substitutions in the thyroglobulin gene are associated with susceptibility to human and murine autoimmune thyroid disease. Proc Natl Acad Sci USA. 2003;100(25):15119–24.

Ban Y, Davies TF, Greenberg DA, Concepcion ES, Osman R, Oashi T, et al. Arginine at position 74 of the HLA-DR β1 chain is associated with Graves' disease. Genes Immun. 2004;5(3):203–8.

Bartalena L, Martino E, Marcocci C, Bogazzi F, Panicucci M, Velluzzi F, et al. More on smoking habits and Graves' ophthalmopathy. J Endocrinol Investig. 1989;12(10):733–7.

Bartalena L, Marcocci C, Tanda ML, Manetti L, Dell'Unto E, Bartolomei MP, et al. Cigarette smoking and treatment outcomes in Graves ophthalmopathy. Ann Intern Med. 1998;129(8):632–5.

Beever K, Bradbury J, Phillips D, McLachlan SM, Pegg C, Goral A, et al. Highly sensitive assays of autoantibodies to thyroglobulin and to thyroid peroxidase. Clin Chem. 1989;35(9):1949–54.

Biondi BBL, Cooper DS, Hegedus L, Laurberg P, Kahaly GJ. The 2015 European Thyroid Association guidelines on diagnosis and treatment of endogenous subclinical hyperthyroidism. Eur Thyr J. 2015;4(3):149–63.

Brand OJ, Lowe CE, Heward JM, Franklyn JA, Cooper JD, Todd JA, et al. Association of the interleukin-2 receptor alpha (IL-2Rα)/CD25 gene region with Graves' disease using a multilocus test and tag SNPs. Clin Endocrinol. 2007;66(4):508–12.

Brix TH, Kyvik KO, Christensen K, Hegedüs L. Evidence for a major role of heredity in Graves' disease: a population-based study of two Danish twin cohorts. J Clin Endocrinol Metab. 2001;86(2):930–4.

Brix TH, Hansen PS, Hegedüs L, Wenzel BE. Too early to dismiss Yersinia enterocolitica infection in the aetiology of Graves' disease: evidence from a twin case-control study. ClinEndocrinol. 2008;69:491–6.

Brix TH, Hegedüs L, Weetman AP, Kemp HE. Pendrin and NIS antibodies are absent in healthy individuals and are rare in autoimmune thyroid disease: evidence from a Danish twin study. Clin Endocrinol. 2014;81(3):440–4.

Bülow Pedersen I, Knudsen N, Carlé A, Schomburg L, Köhrle J, Jørgensen T, et al. Serum selenium is low in newly diagnosed Graves' disease: a population-based study. Clin Endocrinol. 2013;79(4):584–90.

Burch HB, Cooper DS. Management of graves disease a review. JAMA. 2015;314(23):2544–54.

Chazenbalk GD, Portolano S, Russo D, Hutchison JS, Rapoport B, McLachlan S. Human organ-specific autoimmune disease Molecular cloning and expression of an autoantibody gene repertoire for a major autoantigen reveals an antigenic immunodominant region and restricted immunoglobulin gene usage in the target organ. J Clin Investig. 1993;92(1):62–74.

Chazenbalk GD, Pichurin P, Chen CR, Latrofa F, Johnstone AP, McLachlan SM, et al. Thyroid-stimulating autoantibodies in Graves disease preferentially recognize the free A subunit, not the thyrotropin holoreceptor. J Clin Investig. 2002;110(2):209–17.

Chen CR, Pichurin P, Nagayama Y, Latrofa F, Rapoport B, McLachlan SM. The thyrotropin receptor autoantigen in Graves disease is the culprit as well as the victim. J Clin Investig. 2003;111(12):1897–904.

Chen F, Day SL, Metcalfe RA, Sethi G, Kapembwa MS, Brook MG, et al. Characteristics of autoimmune thyroid disease occurring as a late complication of immune reconstitution in patients with advanced human immunodeficiency virus (HIV) disease. Medicine. 2005;84(2):98–106.

Coles AJ, Wing M, Smith S, Coraddu F, Greer S, Taylor C, et al. Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. Lancet. 1999;354(9191):1691–5.

Collins JE, Heward JM, Carr-Smith J, Daykin J, Franklyn JA, Gough SCL. Association of a rare thyroglobulin gene microsatellite variant with autoimmune thyroid disease. J Clin Endocrinol Metab. 2003;88(10):5039–42.

Colobran R, MdP A, Faner R, Gärtner M, Tykocinski L, Lucas A, et al. Association of an SNP with intrathymic transcription of TSHR and Graves' disease: a role for defective thymic tolerance. Hum Mol Genet. 2011;20(17):3415–23.

Costagliola S, Bonomi M, Morgenthaler NG, Van Durme J, Panneels V, Refetoff S, et al. Delineation of the discontinuous-conformational epitope of a monoclonal antibody displaying full in vitro and in vivo thyrotropin activity. Mol Endocrinol. 2004;18(12):3020–34.

Criswell LA, Pfeiffer KA, Lum RF, Gonzales B, Novitzke J, Kern M, et al. Analysis of families in the multiple autoimmune disease genetics consortium (MADGC) collection: the PTPN22 620 W allele associates with multiple autoimmune phenotypes. Am J Hum Genet. 2005;76(4):561–71.

Dechairo BM, Zabaneh D, Collins J, Brand O, Dawson GJ, Green AP, et al. Association of the TSHR gene with Graves' disease: the first disease specific locus. Eur J Hum Genet. 2005; 13(11):1223–30.

Eckstein A, Quadbeck B, Mueller G, Rettenmeier AW, Hoermann R, Mann K, et al. Impact of smoking on the response to treatment of thyroid associated ophthalmopathy. Br J Ophthalmol. 2003;87(6):773–6.

Eschler DC, Hasham A, Tomer Y. Cutting edge: the etiology of autoimmune thyroid diseases. Clin Rev Allergy Immunol. 2011;41(2):190–7.

Facciani JM, Kazim M. Absence of seasonal variation in Graves disease. Ophthal Plast Reconstr Surg. 2000;16(1):67–71.

Farid NR, Sampson L, Noel EP, Barnard JM, Mandeville R, Larsen B, et al. A study of human leukocyte D locus related antigens in Graves' disease. J Clin Investig. 1979;63(1):108–13.

Gora M, Gardas A, Wiktorowicz W, Hobby P, Watson PF, Weetman AP, et al. Evaluation of conformational epitopes on thyroid peroxidase by antipeptide antibody binding and mutagenesis. Clin Exp Immunol. 2004;136(1):137–44.

Grumet FC, Payne RO, Kinishi J, Kriss JP. HL A antigens as markers for disease susceptibility and autoimmunity in Grave's disease. J Clin Endocrinol Metab. 1974;39(6):1115–9.

Hamilton A, Newby PR, Carr-Smith JD, Disanto G, Allahabadia A, Armitage M, et al. Impact of month of birth on the development of autoimmune thyroid disease in the United Kingdom and Europe. J Clin Endocrinol Metab. 2014;99(8):E1459–E65.

Hayashi M, Kouki T, Takasu N, Sunagawa S, Komiya I. Association of an A/C single nucleotide polymorphism in programmed cell death-ligand 1 gene with Graves' disease in Japanese patients. Eur J Endocrinol. 2008;158(6):817–22.

Heward JM, Allahabadia A, Daykin J, Carr-Smith J, Daly A, Armitage M, et al. Linkage disequilibrium between the human leukocyte antigen class II region of the major histocompatibility complex and Graves' disease: replication using a population case control and family-based study. J Clin Endocrinol Metab. 1998;83(10):3394–7.

Hidaka Y, Amino N, Iwatani Y, Itoh E, Matsunaga M, Tamaki H. Recurrence of thyrotoxicosis after attack of allergic rhinitis in patients with Graves' disease. J Clin Endocrinol Metab. 1993; 77(6):1667–70.

Hiratani H, Bowden DW, Ikegami S, Shirasawa S, Shimizu A, Iwatani Y, et al. Multiple SNPs in intron 7 of thyrotropin receptor are associated with Graves' disease. J Clin Endocrinol Metab. 2005;90(5):2898–903.

Hollowell JG, Staehling NW, Dana Flanders W, Harry Hannon W, Gunter EW, Spencer CA, et al. Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab. 2002;87 (2):489–99.

Holm IA, Manson JE, Michels KB, Alexander EK, Willett WC, Utiger RD. Smoking and other lifestyle factors and the risk of graves' hyperthyroidism. Arch Intern Med. 2005;165(14):1606–11.

Hou TZ, Qureshi OS, Wang CJ, Baker J, Young SP, Walker LSK, et al. A transendocytosis model of CTLA-4 function predicts its suppressive behavior on regulatory T cells. J Immunol. 2015;194 (5):2148–59.

Jacobson EM, Huber A, Tomer Y. The HLA gene complex in thyroid autoimmunity: from epidemiology to etiology. J Autoimmun. 2008;30(1–2):58–62.
Jaume JC, Guo J, Pauls DL, Zakarija M, McKenzie JM, Egeland JA, et al. Evidence for genetic transmission of thyroid peroxidase autoantibody epitopic 'fingerprints'. J Clin Endocrinol Metab. 1999;84(4):1424–31.
Khoury EL, Hammond L, Bottazzo GF, Doniach D. Presence of the organ-specific 'microsomal' autoantigen on the surface of human thyroid cells in culture: its involvement in complement-mediated cytotoxicity. Clin Exp Immunol. 1981;45(2):316–28.
Krassas GE, Tziomalos K, Pontikides N, Lewy H, Laron Z. Seasonality of month of birth of patients with Graves' and Hashimoto's diseases differ from that in the general population. Eur J Endocrinol. 2007;156(6):631–6.
Laurberg P, Pedersen KM, Vestergaard H, Sigurdsson G. High incidence of multinodular toxic goitre in the elderly population in a low iodine intake area vs. high incidence of Graves' disease in the young in a high iodine intake area: comparative surveys of thyrotoxicosis epidemiology in East-Jutland Denmark and Iceland. J Intern Med. 1991;229(5):415–20.
Liu W, Wang HN, Gu ZH, Yang SY, Ye XP, Pan CM, et al. Identification of BACH2 as a susceptibility gene for Graves' disease in the Chinese Han population based on a three-stage genome-wide association study. Hum Genet. 2014;133(5):661–71.
Marcocci C, Kahaly GJ, Krassas GE, Bartalena L, Prummel M, Stahl M, et al. Selenium and the course of mild Graves' orbitopathy. N Engl J Med. 2011;364(20):1920–31.
Marinò M, Chiovato L, Lisi S, Altea MA, Marcocci C, Pinchera A. Role of thyroglobulin in the pathogenesis of Graves' ophthalmopathy: the hypothesis of Kriss revisited. J Endocrinol Investig. 2004;27(3):230–6.
Minich WB, Dehina N, Welsink T, Schwiebert C, Morgenthaler NG, Köhrle J, Eckstein A, Schomburg L. Autoantibodies to the IGF1 receptor in Graves' orbitopathy. J Clin Endocrinol Metab. 2013;98(2):752–60.
Mitchell AL, Pearce SH. How should we treat patients with low serum thyrotropin concentrations? Clin Endocrinol. 2010;72(3):292–6.
Mitchell AL, Cordell HJ, Soemedi R, Owen K, Skinningsrud B, Wolff AB, et al. Programmed death ligand 1 (PD-L1) gene variants contribute to autoimmune Addison's disease and Graves' disease susceptibility. J Clin Endocrinol Metab. 2009;94(12):5139–45.
Mitchell AL, Goss L, Mathiopoulou L, Morris M, Vaidya B, Dickinson AJ, Quinn A, Dayan C, McLaren J, Hickey JL, Lazarus JH, Rose GE, Foley P, MacEwen CJ, Perros P. Diagnosis of Graves' orbitopathy (DiaGO): results of a pilot study to assess the utility of an office tool for practicing endocrinologists. J Clin Endocrinol Metab. 2015;100(3):E458–62.
Mizokami T, Li AW, El-Kaissi S, Wall JR. Stress and thyroid autoimmunity. Thyroid. 2004;14(12): 1047–55.
Munakata Y, Kodera T, Saito T, Sasaki T. Rheumatoid arthritis, type 1 diabetes, and Graves' disease after acute parvovirus B19 infection. Lancet. 2005;366(9487):780.
Nakashima M, Martin A, Davies TF. Intrathyroidal T cell accumulation in Graves' disease: delineation of mechanisms based on in situ T cell receptor analysis. J Clin Endocrinol Metab. 1996;81(9):3346–51.
Neumann S, Place RF, Krieger CC, Gershengorn MC. Future prospects for the treatment of Graves' hyperthyroidism and eye disease. Horm Metab Res. 2015;47(10):789–96.
Nyström HF, Jansson S, Berg G. Incidence rate and clinical features of hyperthyroidism in a long-term iodine sufficient area of Sweden (Gothenburg) 2003–2005. Clin Endocrinol. 2013;78(5):768–76.
Pfeilschifter J, Ziegler R. Smoking and endocrine ophthalmopathy: impact of smoking severity and current vs lifetime cigarette consumption. Clin Endocrinol. 1996;45(4):477–81.
Prummel MF, Wiersinga WM. Smoking and risk of Graves' disease. JAMA. 1993;269(4):479–82.
Qin Q, Wang X, Yan N, Song RH, Cai TT, Zhang W, Guan LJ, Muhali FS, Zhang JA. Aberrant expression of miRNA and mRNAs in lesioned tissues of Graves' disease. Cell Physiol Biochem. 2015;35(5):1934–42.
Sato A, Takemura Y, Yamada T, Ohtsuka H, Sakai H, Miyahara Y, et al. A possible role of immunoglobulin E in patients with hyperthyroid Graves' disease. J Clin Endocrinol Metab. 1999;84(10):3602–5.

Simmonds MJ, Howson JMM, Heward JM, Cordell HJ, Foxall H, Carr-Smith J, et al. Regression mapping of association between the human leukocyte antigen region and Graves disease. Am J Hum Genet. 2005;76(1):157–63.

Simmonds MJ, Brand OJ, Barrett JC, Newby PR, Franklyn JA, Gough SCL. Association of Fc receptor-like 5 (FCRL5) with Graves' disease is secondary to the effect of FCRL3. Clin Endocrinol. 2010;73(5):654–60.

Smith TJ, Hegedüs L. Graves' Disease. N Engl J Med. 2016;375(16):1552–65.

Smith BR, Bolton J, Young S, Collyer A, Weeden A, Bradbury J, et al. A new assay for thyrotropin receptor autoantibodies. Thyroid. 2004;14(10):830–5.

Smyth D, Cooper JD, Collins JE, Heward JM, Franklyn JA, Howson JMM, et al. Replication of an association between the lymphoid tyrosine phosphatase locus (LYP/PTPN22) with type 1 diabetes, and evidence for its role as a general autoimmunity locus. Diabetes. 2004;53(11):3020–3.

Song HD, Liang J, Shi JY, Zhao SX, Liu Z, Zhao JJ, et al. Functional SNPs in the SCGB3A2 promoter are associated with susceptibility to Graves' disease. Hum Mol Genet. 2009;18(6):1156–70.

Stefan M, Jacobson EM, Huber AK, Greenberg DA, Li CW, Skrabanek L, et al. Novel variant of thyroglobulin promoter triggers thyroid autoimmunity through an epigenetic interferon α-modulated mechanism. J Biol Chem. 2011;286(36):31168–79.

Strieder TGA, Prummel MF, Tijssen JGP, Endert E, Wiersinga WM. Risk factors for and prevalence of thyroid disorders in a cross-sectional study among healthy female relatives of patients with autoimmune thyroid disease. Clin Endocrinol. 2003a;59(3):396–401.

Strieder TGA, Wenzel BE, Prummel MF, Tijssen JGP, Wiersinga WM. Increased prevalence of antibodies to enteropathogenic Yersinia enterocolitica virulence proteins in relatives of patients with autoimmune thyroid disease. Clin Exp Immunol. 2003b;132(2):278–82.

Strieder TGA, Prummel MF, Tijssen JGP, Brosschot JF, Wiersinga WM. Stress is not associated with thyroid peroxidase autoantibodies in euthyroid women. Brain Behav Immun. 2005;19(3):203–6.

Strieder TGA, Tijssen JGP, Wenzel BE, Endert E, Wiersinga WM. Prediction of progression to overt hypothyroidism or hyperthyroidism in female relatives of patients with autoimmune thyroid disease using the thyroid events Amsterdam (THEA) score. Arch Intern Med. 2008;168(15):1657–63.

Sutherland A, Davies J, Owen CJ, Vaikkakara S, Walker C, Cheetham TD, et al. Brief report: genomic polymorphism at the interferon-induced helicase (IFIH1) locus contributes to Graves' disease susceptibility. J Clin Endocrinol Metab. 2007;92(8):3338–41.

Tomer Y, Davies TF. Infection, thyroid disease, and autoimmunity. Endocr Rev. 1993;14(1):107–20.

Tomer Y, Concepcion E, Greenberg DA. A C/T single-nucleotide polymorphism in the region of the CD40 gene is associated with Graves' disease. Thyroid. 2002a;12(12):1129–35.

Tomer Y, Greenberg DA, Concepcion E, Ban Y, Davies TF. Thyroglobulin is a thyroid specific gene for the familial autoimmune thyroid diseases. J Clin Endocrinol Metab. 2002b;87(1):404–7.

Ueda H, Howson JMM, Esposito L, Heward J, Snook H, Chamberlain G, et al. Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. Nature. 2003;423(6939):506–11.

Vaidya B, Pearce S. The emerging role of the CTLA-4 gene in autoimmune endocrinopathies. Eur J Endocrinol. 2004;150(5):619–26.

Vaidya B, Pearce SH. Diagnosis and management of thyrotoxicosis. BMJ. 2014;349:g5128.

Vaidya B, Imrie H, Perros P, Young ET, Kelly WF, Carr D, et al. The cytotoxic T lymphocyte antigen-4 is a major Graves' disease locus. Hum Mol Genet. 1999;8(7):1195–9.

Vaidya B, Kendall-Taylor P, Pearce SHS. Genetics of endocrine disease: the genetics of autoimmune thyroid disease. J Clin Endocrinol Metab. 2002;87(12):5385–97.

Vanderpump MPJ, Tunbridge WMG, French JM, Appleton D, Bates D, Clark F, et al. The incidence of thyroid disorders in the community: a twenty-year follow-up of the Whickham Survey. Clin Endocrinol. 1995;43(1):55–68.

Vang T, Congia M, Macis MD, Musumeci L, Orrú V, Zavattari P, et al. Autoimmune-associated lymphoid tyrosine phosphatase is a gain-of-function variant. Nat Genet. 2005;37(12):1317–9.
Velaga MR, Wilson V, Jennings CE, Owen CJ, Herington S, Donaldson PT, et al. The codon 620 tryptophan allele of the Lymphoid Tyrosine Phosphatase (LYP) gene is a major determinant of Graves' disease. J Clin Endocrinol Metab. 2004;89(11):5862–5.
Weetman AP. Graves' disease following immune reconstitution or immunomodulatory treatment: should we manage it any differently? Clin Endocrinol. 2014;80(5):629–32.
Weetman AP, Cohen S. The IgG subclass distribution of thyroid autoantibodies. Immunol Lett. 1986;13(6):335–41.
Weetman AP, McGregor AM, Lazarus JH, Hall R. Thyroid antibodies are produced by thyroid derived lymphocytes. Clin Exp Immunol. 1982;48(1):196–200.
Wiersinga WM. Smoking and thyroid. Clin Endocrinol. 2013;79(2):145–51.
Winsa B, Karlsson A, Bergstrom R, Adami HO, Gamstedt A, Jansson R, et al. Stressful life events and Graves' disease. Lancet. 1991;338(8781):1475–9.
Yanagawa T, DeGroot LJ. HLA class II associations in African-American female patients with Graves' disease. Thyroid. 1996;6(1):37–9.
Yanagawa T, Mangklabruks A, Chang YB, Okamoto Y, Fisfalen ME, Curran PG, et al. Human histocompatibility leukocyte antigen-DQA1*0501 allele associated with genetic susceptibility to graves' disease in a caucasian population. J Clin Endocrinol Metab. 1993;76(6):1569–74.
Yanagawa T, Hidaka Y, Guimaraes V, Soliman M, DeGroot LJ. CTLA-4 gene polymorphism associated with Graves' disease in a Caucasian population. J Clin Endocrinol Metab. 1995;80 (1):41–5.
Yasuda T, Okamoto Y, Hamada N, Miyashita K, Takahara M, Sakamoto F, et al. Serum vitamin D levels are decreased and associated with thyroid volume in female patients with newly onset Graves' disease. Endocrine. 2012;42(3):739–41.
Yasuda T, Okamoto Y, Hamada N, Miyashita K, Takahara M, Sakamoto F, et al. Serum vitamin D levels are decreased in patients without remission of Graves' disease. Endocrine. 2013; 43(1):230–2.
Yoshiuchi K, Kumano H, Nomura S, Yoshimura H, Ito K, Kanaji Y, et al. Stressful life events and smoking were associated with Graves' disease in women, but not in men. Psychosom Med. 1998;60(2):182–5.
Zimmermann MB, Boelaert K. Iodine deficiency and thyroid disorders. Lancet Diabet Endocrinol. 2015;3(4):286–95.

Graves' Ophthalmopathy

15

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Abstract

Thyroid-associated ophthalmopathy aka Graves' orbitopathy (GO), represents the periocular component of Graves' disease (GD), for which safe and effective therapies remain elusive. The central pathogenic event in GD is loss of immune tolerance to the thyrotropin receptor (TSHR) and generation of activating anti-TSHR antibodies. Recent evidence suggests that TSHR might collaborate with other proteins, such as the insulin-like growth factor-I receptor in the pathogenesis of GO. In this chapter we have attempted to review the most most recent insights into disease mechanisms underlying GO, strategies for evaluating patients, and currently available therapeutic strategies and those remedies that are in development.

Keywords

Ophthalmopathy · Autoimmune · Inflammation

Abbreviations

AKT	Protein kinase B
Arg	Arginine
CAS	Clinical activity score
CD	Cluster of differentiation
CTLA-4	Cytotoxic T-lymphocyte-associated protein **4**, also known as CD152
DON	Dysthyroid optic neuropathy
FRAP	FK506-binding protein 12-rapamycin-associated protein
GD	Graves' disease
GO	Graves' orbitopathy, also known as thyroid-associated ophthalmopathy
HA	Hyaluronan
HLA-DRβ	Human leukocyte antigen DRβ
IGF-IR	Insulin-like growth factor-I receptor
IL	Interleukin
mTOR	Mechanistic target of rapamycin
OR	Orbital radiotherapy
PGE2	Prostaglandin E2
PGHS-2	Prostaglandin endoperoxide H synthase-2
PTPN22	Protein tyrosine phosphatase, non-receptor type 22
QoL	Quality of life
RANTES	Regulated on activation, normal T cell expressed and secreted
TGF-beta	Transforming growth factor beta
THYPRO	Thyroid patient reported outcome
TNF	Tumor necrosis factor
TSHR Ab	Thyrotropin receptor antibodies
TSHR	Thyrotropin receptor
TSI	Stimulating TSHR Ab
UDP	Uridine diphosphate

Introduction

Thyroid-associated ophthalmopathy aka Graves' orbitopathy (GO), a disfiguring and potentially sight-threatening condition, is the periocular component of Graves' disease (GD) (Smith and Hegedüs 2016; Wang and Smith 2014). It remains a vexing condition for which limited therapies proven to be effective and safe currently exist. It represents the most common and serious extra-thyroidal manifestation of the autoimmunity connected to GD. GO was first described in the late nineteenth century and is recognized as most likely resulting from the systemic autoimmune process also underlying the thyroid involvement in GD. It affects not only the orbital contents but also the upper face. Tying together these anatomically dispersed manifestations of GD has proven to be challenging. Only in the past few years have coherent theoretical models of the disease been proposed. At the heart of GD is the loss of immune tolerance to the thyrotropin receptor (TSHR) against which activating antibodies (TSHR Ab) are directed (Rapoport and McLachlan 2016). It is precisely the activities of these stimulating TSHR Ab (TSI) that account for the thyroid growth and hyperthyroidism that frequently lead to the initial diagnosis of GD. The molecular events surrounding TSHR expression, mutations, processing, activation, and downstream signaling in thyroid epithelial cells have been well described (Kleinau et al. 2016; Vassart and Kleinau 2014). But a relatively recent awareness that TSHR expression is not limited to the thyroid and is expressed widely in many tissues, has led to identification of an increasingly diverse role for the receptor in health and disease. Extra-thyroidal levels of TSHR are considerably lower than those in thyroid epithelium, but the protein appears to be functional (Tsui et al. 2008). Its activation initiates signaling through both canonical and untraditional pathways. It stands to reason that a shared antigen such as TSHR might explain, at least in part, the manifestation of GD in thyroid and orbit, two tissues with embryological dissimilarities and anatomical separation. But TSIs cannot be detected in a small proportion of individuals developing GD and manifesting GO. While the issue of assay detection limits might account for these cases, it remains possible that other antigens could also play roles in GO. Absence of animal and *in vitro* experimental models with high-degree fidelity to the human disease has posed significant barriers to solving GD. Thus, significant gaps in our understanding of GO remain. This in turn has resulted in inadequate specific and safe therapeutic options. In this chapter, we have attempted to provide an overview of the currently held concepts of GO pathogenesis, describe the pathological and clinical features of the disease and strategies for its diagnosis. We also, summarize medical and surgical therapies currently available. Finally we examine the prospects for additional treatments for GO that should improve the lives of patients with this disease.

Pathogenesis

Details concerning the mechanisms underlying GO are now becoming clearer. Investigative strategies developed while studying closely related diseases, including those with autoimmune bases and involving connective tissue remodeling, have been applied to the orbit in GD.

Factors Conveying Disease Risk

The factors initiating GD and GO remain uncertain. The orbital disease appears to be autoimmune in nature rather than the consequence of abnormalities of thyroid function *per* se. GO is presumed to occur in genetically susceptible individuals who are exposed to an as yet unidentified environmental factor and then manifest the clinical disease. It is unclear whether individuals with GD who do not manifest GO are genetically or immunologically distinct from those with ocular involvement. In fact, to our knowledge, no compelling evidence has thus far been advanced that the genetic or molecular underpinnings of GD vary in those without and with clinically apparent GO. Many workers in the field contend that virtually all individuals with *bona fide* GD manifest at least minimal alterations in the orbit and upper face. These might be limited to dry eye symptoms or minimal eyelid retraction. Changes in several genes conveying susceptibility to GD have been identified, including polymorphisms of the TSH receptor, thyroglobulin, CTLA-4, CD25, HLA-DRβ-Arg74, PTPN22, and CD40 (Tomer 2014). Limited evidence also supports a role for epigenetic factors in disease initiation and promotion. Several genes are hyper-methylated, notably TSHR and those encoding key signaling proteins in T cells (Limbach et al. 2016). Triggering of GO, like that of GD, in many cases can be linked to distinct antecedent episodes of emotional (examples include death of loved ones, incarceration, loss of a job) and physical stress (myocardial infarction, major surgery, severe infections, motor automobile accidents). Despite the common threads connecting the onset of hyperthyroidism and ocular processes, the initial manifestations of the two can be simultaneous or separated by many years. Dietary iodine intake and exposure to tobacco smoke are thought to represent important determinants of risk. Of particular note is the remarkable incidence of GD among individuals undergoing treatment with the CD52-targeting drug, alemtuzumab (aka Campath), in the context of multiple sclerosis or chronic lymphocytic leukemia (Coles et al. 1999). Clearly a vast array of intersecting factors emerge as important candidates for provoking the development of GO (Effraimidis and Wiersinga 2014). This panoply has made the task of piecing together the initiating factors in this disease extremely challenging.

Role of Orbital Fibroblasts in GO

A key cellular participant in the pathogenesis of GO is the orbital fibroblast (Fig. 1) (Smith et al. 1995). It is widely but not universally accepted that these cells, rather than extraocular muscle cells, represent the dominant target for the autoimmunity occurring within the orbit in GO. These fibroblasts exhibit unique characteristics that set them apart from similar cells in other anatomic regions. Further, fibroblasts derived from connective tissue in GO diverge from those inhabiting the healthy, unaffected orbit (Young et al. 1998; Smith et al. 1994). Unlike certain fibroblast types which serve primarily structural roles, subsets of orbital fibroblasts exhibit a complex set of phenotypic attributes that suggest important roles in host defense and immune surveillance. Among the distinguishing characteristics of these cells are

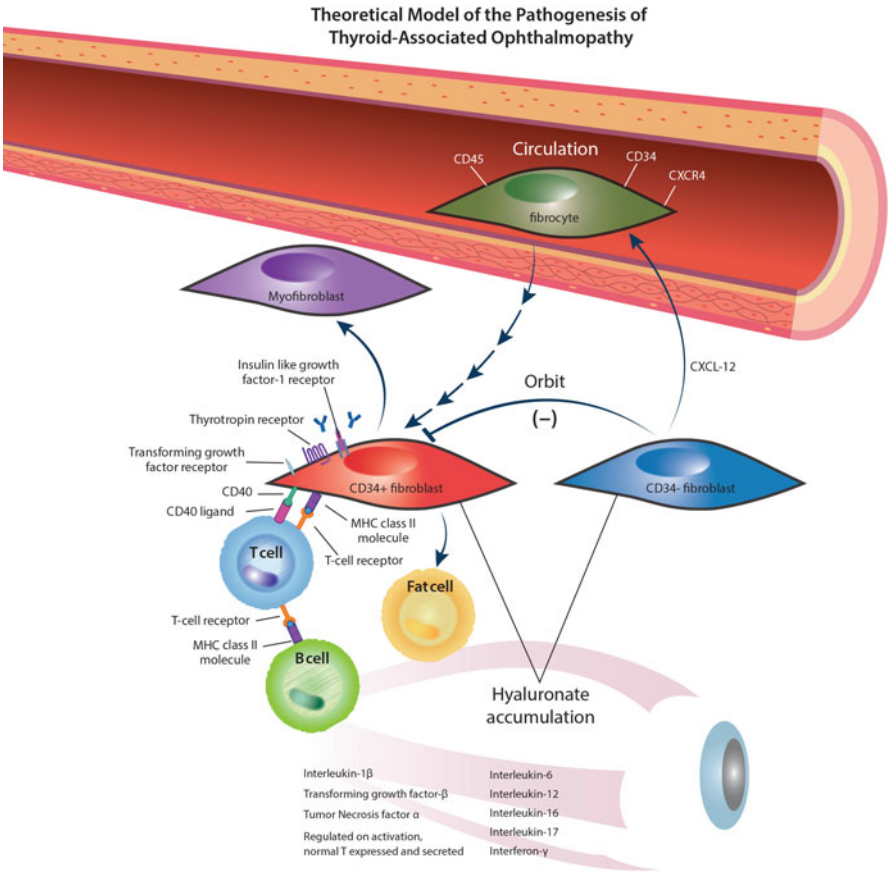

Fig. 1 Theoretical model of the pathogenesis of GO. The orbit becomes infiltrated by B and T cells and $CD34^+$ fibrocytes. The bone marrow-derived fibrocytes differentiate into $CD34^+$ fibroblasts which in turn can further differentiate into myofibroblasts or adipocytes. $CD34^+$ fibroblasts cohabit the orbit with residential $CD34^-$ fibroblasts. These cells produce several different cytokines. Their expression repertoire depends on the environment that the fibroblasts are subjected to, including the array of lymphocytes, mast cells, and macrophages that are recruited to the orbit. Interleukins 1β, 6, 8, and 16, tumor necrosis factor-α, regulated on activation, normal T expressed and secreted (RANTES), and CD40 ligand (CD154) appear to play integral roles in promoting inflammation and tissue remodeling. $CD34^+$ fibroblasts, putatively derived from circulating fibrocytes, express thyroglobulin, thyroperoxidase, sodium iodide symporter, and TSHR. These are expressed because $CD34^+$ orbital fibroblasts and fibrocytes express autoimmune regulator protein. Immunoglobulins activate the thyrotropin/insulin-like growth factor receptor-1 complex and in so doing induce expression of several inflammatory molecules. They also express the molecular machinery that produces hyaluronan and other abundant glycosaminoglycans. These are extremely hydrophilic and voluminous and tend to expand the tissues in which they accumulate. They are thought to promote expansion of orbital tissue volume in GO, causing anterior displacement of the globe and compression of the optic nerve. Orbital fat expansion also results from *de novo* adipogenesis

their remarkably robust responses to inflammatory cytokines such as IL-1β, CD40 ligand (CD154), leukoregulin, insulin-like growth factor-I (IGF-I), and platelet-derived growth factor (Cao et al. 1998; van Steensel et al. 2012; Han et al. 2002). Cognate receptors for most of these factors have been detected on the fibroblast surface, and their functions have been at least partially characterized. Further, these cells utilize a diverse set of signaling pathways downstream from cell-surface-displayed cytokine and growth factor receptors. A number of target genes and their products are dramatically upregulated in orbital fibroblasts by cytokines and growth factors and thus account in part for the pathological changes occurring in the disease. Among these target genes, the inflammatory cyclooxygenase, prostaglandin endoperoxide H synthase-2 (PGHS-2) and the terminal enzyme, microsomal prostaglandin E_2 synthase, are especially responsive to many of these stimuli, accounting for an exaggerated generation of PGE_2 (Young et al. 1998; Smith et al. 1994; Cao et al. 1998; van Steensel et al. 2012; Han et al. 2002). Orbital fibroblasts themselves are important sources of several key cytokines involved in the genesis of GO, including IL-1α, IL-1β, IL-6, RANTES, IL-8, and IL-16 (Pritchard et al. 2003; Sciaky et al. 2000; Li and Smith 2013). Other factors generated by orbital fibroblasts, such as IL-1 receptor antagonist and IL-10, serve as "brakes" that modulate the inflammatory processes in which these cells participate (Li and Smith 2013). The expression of genes encoding these and other inflammatory mediators are regulated at both the transcriptional and posttranscriptional levels. Fundamental differences in patterns of transcription factor expression and mRNA stability appear to play important roles in shaping the unique attributes associated with orbital fibroblast phenotypes. Several of the particularly robust inductions demonstrated in cultured orbital fibroblasts coincide with genes that have been found to be upregulated *in situ* in orbital tissues retrieved from individuals with GO, including PGHS-2 and IL-1β.

Orbital Fibroblast Heterogeneity and Recognition of Fibrocyte Involvement

GO orbital fibroblasts comprise a heterogeneous population of cells. They can be parsed into discrete subsets based on their expression and display of surface markers such as Thy-1 (Smith et al. 2002) and CD34 (Douglas et al. 2010). These markers are of substantial importance because they provide clues as to the embryonic and anatomic origins of these cells and because they also inform about the potential of fibroblasts for terminal differentiation. For instance, Thy-1-displaying cells are particularly responsive to TGF-β and can differentiate under its influence into myofibroblasts playing important roles in fibrosis and wound repair (Smith et al. 2002). Thy-1-negative fibroblasts, on the other hand, differentiate into lipid-accumulating adipocytes when exposed to peroxisome proliferator-activated receptor γ (PPARγ) PPARγ-activating factors. It is currently believed that the adipogenic potential of orbital fibroblast subsets may play a major role in the tissue expansion occurring in GO. Fibroblasts expressing CD34, which can be uniquely identified in the GO orbit, are currently believed to derive from circulating $CD34^+$ fibrocytes (Douglas

et al. 2010). These cells are derived from bone marrow and are thought to be of monocyte lineage. They have established roles in the fibrotic changes occurring following tissue injury and are trafficked to sites of wound repair in experimental models of lung fibrosis (Mehred et al. 2007). Circulating fibrocytes are relatively rare in peripheral blood mononuclear cells of healthy individuals. In contrast, they become substantially more abundant in many individuals with GD and especially those with active GO (Douglas et al. 2010). Their definitive identification rests on display of an array of specific cell markers, including collagen I, the chemokine receptor, CXCR4, CD34, and CD45. $CD34^+$ orbital fibroblasts appear to be peculiar to the GO orbit (Douglas et al. 2010). They express relatively high levels of functional TSHR as well as other thyroid-specific antigens including thyroglobulin, sodium iodide symporter, and thyroperoxidase (Fernando et al. 2012, 2014). The expression of these proteins appears to be dependent on the autoimmune regulator protein (AIRE), a nontraditional transcription factor with an established role in thymic education and the depletion of autoreactive T cells. $CD34^+$ orbital fibroblasts coexist in the GO orbit with $CD34^-$ fibroblasts which appear to be identical to the fibroblasts uniformly found in the healthy orbit. Recent reports from a single laboratory group suggest that these fibroblasts exert a set of influences on $CD34^+$ orbital fibroblasts that tend to down-regulate the distinctive features of their reactive phenotype (Fernando et al. 2012). Thus it has been postulated that an imbalance between $CD34^+$ and $CD34^-$ orbital fibroblasts in favor of the former may lead to particularly aggressive GO.

An important attribute of orbital fibroblasts is their capacity to synthesize large amounts of hyaluronan. They express a characteristic profile of hyaluronan synthase isoenzymes and the UDP glucose dehydrogenase gene (Kaback and Smith 1999). These are highly inducible in GO orbital fibroblasts by IL-1β, TSH, IGF-I, and CD40 ligand (Cao et al. 1998; Kaback and Smith 1999; Spicer et al. 1998). In addition, other abundant glycosaminoglycans such as chondroitin sulfate and dermatan sulfate may also accumulate in this disease. It is the extreme hydrophilic nature of these molecules that contributes to the volume expansion associated with GO.

Cytokines and GO

A wide range of soluble inflammatory mediators have been implicated in the pathogenesis of GO. These include eicosanoids (prostaglandins, thromboxanes, leukotrienes, lipoxins), mitogenic growth factors (IGF-I, platelet-derived growth factor), and cytokines. None of these factors has been the focus of study in the context of GO more than the medley of cytokines thus far detected in affected orbital fat. The specific cytokines found in a particular case may suggest skew toward a helper T cell paradigm, such as Th1, Th2, or Th17. It would appear that the stage of the orbital disease may represent an important determinant of which T cell subset might play a primary role in the tissue remodeling that occurs during that period. Among those cytokines that have been detected are IL-1α, IL-1β, IL-6, interferon γ, TGF-β, and TNFα (Heufelder and Bahn 1993). Rather than a particular cytokine likely exhibiting primacy in the disease, it seems more likely that the medley of many of these factors,

changing in relative abundance with disease progression, serves to guide the inflammatory response followed by the tissue repair processes. Although not focused on in most studies, molecules with regulatory roles that modulate rather than promoting inflammation and tissue reactivity are likely to play important roles in terminating tissue remodeling in the orbit. These include activating ligands of PPAR-γ and members of the cytokine family including IL-1 receptor antagonists and IL-10.

Lymphocytes and the Development of GO

Immune reactivity occurring in the orbit is thought to be similar or identical to that within the thyroid gland in GD. The adaptive immune system has been convincingly implicated in the development of GD and GO. Both T and B cells are required and have been identified in the diseased orbit and have been partially characterized although antigen-specific cells have yet to be identified in the GO orbit (Grubeck-Loebenstein et al. 1994; de Carli et al. 1993; Kahaly et al. 2011; Jaume et al. 1994). Included in these cells are those exhibiting effector phenotypes as well as others with regulatory characteristics. It is the balance between these populations of effector and regulatory T and B cells that determines whether immune reactivity will culminate in disease or be suppressed. T cells are required to endorse critical B cell functions such as immunoglobulin class switching. The infiltrating lymphocytes as well as mast cells and monocyte subsets determine in large part the cytokine milieu in the GO orbit and therefore the pattern of tissue reactivity and remodeling that occurs. Lymphocytes are capable of cross talk with orbital fibroblasts through molecular portals such as the CD40/CD40 ligand pathway and a variety of cytokines and their cognate receptors. Thus these pathways represent potentially important therapeutic targets. In fact, these have already been exploited in allied autoimmune disease such as rheumatoid arthritis and are being repurposed for the treatment of GO.

Expression of TSHR in the Orbit

Shortly after its molecular cloning by Parmentier et al. (1989), transcripts encoding TSHR were detected by PCR, albeit at extremely low levels, in orbital fat (Feliciello et al. 1993). This finding and those that followed demonstrated TSHR mRNA expression in fat depots in an anatomically dispersed pattern (Agretti et al. 2002) and suggested the possibility that the receptor might play a role in both normal physiological regulation and in the pathogenesis of GO. Subsequent studies revealed low-level expression by cultured orbital fibroblasts that were fractionally higher in cells derived from patients with the disease than those from healthy donors (Valyasevi et al. 1999). Later studies have revealed that subjecting fibroblasts to culture conditions favoring their differentiation into adipocytes increases the expression of TSHR. TSHR expressed by orbital fibroblasts has been shown to be functional since both TSH and TSIs can induce the expression of several gene products in these cells,

including pro-inflammatory cytokines such as IL-1 and IL-6 [Li and Smith 2013; Raychaudhuri et al. 2013). Thus, the leading candidate for an antigen shared by the thyroid and orbit appears to be TSHR. This strong possibility is not a certainty since TSHR has been detected in many if not all fatty tissue deposits thus far examined in human beings and in rodents (Agretti et al. 2002; Shimura et al. 1998). Some of these fatty tissues inhabit anatomic regions not obviously manifesting GD. From the evidence thus far generated, it would appear that infiltrating fibrocytes may account for most if not the entire presence of TSHR in the GO orbit.

Implicating Other Antigens as Potentially Participating in GO

The complexities surrounding the pathogenesis of GO have provoked inquiry into potential roles for antigens other than TSHR in the disease process. Among the initial studies attempting to identify the pathogenic autoantigen(s) in GO were those conducted by Kriss and his associates more than 40 years ago (Kriss 1970). His group reported the detection of thyroglobulin in affected orbital tissues. He postulated that the protein traveled to the orbit from the thyroid by retrograde lymphatic flow. More recent findings by Lisi and colleagues (2002) have disclosed that thyroglobulin can bind hyaluronan (HA) produced by orbital fibroblasts in a non-specific manner.

Much more recently, IGF-IR has emerged as a potentially attractive antigenic candidate. Its insinuation into the disease dates back to the observations of Weightman et al. (1993) who demonstrated that IgGs from patients with GO could displace radiolabeled IGF-I from surface binding on orbital fibroblasts. Although the studies fell short of identifying IGF-IR as containing the binding site to which the IgGs and IGF-I were competing, they for the first time implicated the IGF-1 pathway in the disease. Subsequently, Pritchard et al. (2003) reported that IGF-1R was overexpressed by orbital fibroblasts from patients with the disease. They also found that IgGs purified from these patients could induce chemokines such as IL-16 and RANTES. These responses were absent in fibroblasts from healthy individuals and were mediated through the FRAP/mTOR/AKT/$p70^{s6kinase}$ pathway and were thus inhibited by rapamycin (Pritchard et al. 2002). Transfecting fibroblasts with a dominant negative IGF-1R could block the induction as could monoclonal blocking antibodies directed at IGF-IR (Pritchard et al. 2003).

Pathological Features of GO

General, Anatomical, and Functional Aspects of Orbital Pathology

The human orbit represents a boney cone-shaped space where multiple structures and soft tissues must function in close proximity. Thus any enlargement of its occupants can easily distort the normal physical relationships existing within the orbit. The orbit is filled with a fatty connective tissue depot which surrounds the

vascular investments, nerves, extraocular muscles, and globe. Initial presentation of GO is typically dominated by signs of inflammation and local congestion. These are frequently coupled to the symptoms of ocular pain, irritation, and dry eyes. This constellation of signs and symptoms heralds the onset of characteristic tissue remodeling, including the disordered accumulation of the glycosaminoglycan, HA, and the expansion of orbital fat (Smith et al. 1989). HA stands out among similar complex sugars as the most abundant, lacking a core protein, and being non-sulfated. Unlike most related glycosaminoglycan molecules, HA is synthesized at the cell membrane. Its rheological properties, including an enormous Stokes radius and extreme hydrophilic nature, result in a frequently dramatic expansion of the orbital contents as the molecule accumulates. Besides the volume expansion directly resulting from HA accumulation, evidence suggests that accelerated *de novo* adipogenesis may also occur in GO (Kumar et al. 2004). While the majority of individuals with GO exhibit both expansion of fatty connective tissue and increased muscle volume, the disease can be dominated in certain individuals with either. Whether primarily involving the fat, muscles, or both, swelling of the orbital contents in GO can cause the anterior propulsion of the globe in the process known as proptosis. Further, venous drainage from the orbit can be impaired. The optic nerve can be compromised by crowding of the adjacent tissues and can in extreme cases result in dysthyroid optic neuropathy (DON).

Microscopic examination of the orbital tissues reveals accumulation of extracellular metachromatic material and prominent cells accumulating triglyceride. Typically the native architecture of the extraocular muscles is spared until very late in the disease process when fibrosis diminishes ocular motility and distorts muscle morphology (Hufnagel et al. 1984). This stage of the disease is currently considered irreversible. The enlargement of the muscles is a consequence of intercalating fibroblasts expanding the muscle volume and generating HA and thus increasing the space between intact muscle fibers as a consequence of water accumulation. The changes observed in the muscles can impose restrictions on eye movement, regardless of whether diplopia is present or not. Further, the increased muscle volume can contribute to crowding of other orbital structures, critically including the optic nerve. Besides the changes observed in the orbital contents, many individuals with GO manifest inflammation of the upper face. These changes can be subtle and limited to mild hyperemia or dramatic with substantial deposition of subcutaneous HA and appreciable swelling. The eyelids can be affected and appear edematous, sometimes disrupting their motility. Abnormalities in eyelid function can result in their dysfunction and poor coverage of the globe, leaving the anterior eye surface exposed.

Ocular Surface and Tear Film Abnormalities in GO

The anterior surface of the eye frequently becomes victimized by the architectural and biochemical abnormalities occurring in GO. These changes can be among the earliest signs of GO and can present as dry eye (Gupta et al. 2009). The anterior surface comprises the cornea, conjunctiva, and margins of the lids. Anterior

propulsion of the eye, resulting in proptosis of variable extent, can result in significant lagophthalmos from inadequate eyelid coverage. These surfaces are covered by the protective fluid coating known as tear film. Further, tear composition and quantity in GO change, resulting in inadequate protection of surface epithelium (Boehm et al. 2013). Alterations in tear production may result from the direct actions of TSIs on the acinar cells of the lacrimal gland mediated through the TSHR (Eckstein et al. 2004). Levels of factors involved in surface protection and modulation of inflammation, including proline-rich proteins, annexin A1, cavin, and cystatins, are altered in GO (Matheis et al. 2015). On the other hand, inflammatory mediators are more abundant in active GO. In addition, tears evaporate more rapidly in the disease, resulting in increased osmolarity. These factors result in anterior eye surface inflammation which presents clinically as dry eye symptoms, including gritty sensations of foreign bodies, irritation, and eye redness. These symptoms can be among the very earliest found in GO and can dramatically reduce the quality of life in affected patients. Thus, therapy improves but does not fully restore quality of life in GD and GO patients, as measured by a disease-specific quality of life instrument (the ThyPRO) (Cramon et al. 2016). If the condition remains inadequately treated, the integrity of the epithelium is disturbed and can result in infection and irreversible corneal changes affecting visual quality.

Epidemiology

Clinical manifestations of GO are evident in about 40–50% of patients with GD, even though, using orbital imaging, subclinical evidence of eye involvement can be detected in the large majority (Smith and Hegedüs 2016; Wang and Smith 2014). Moderate-to-severe and very severe GO are observed in about 5% of cases of GD. GO usually occurs simultaneously with the onset of hyperthyroidism but these usual appears within 18 months of each other. In a very few cases, GO can precede or follow hyperthyroidism by many years (Smith and Hegedüs 2016; Menconi et al. 2014a). Rarely GO may occur in patients with autoimmune thyroiditis without or with hypothyroidism (euthyroid/hypothyroid GO).

The incidence of GO in a population-based study in Olmsted County, MN, United States, was 16.0/100,000 persons/year for women and 2.9/100,000 person/year for men (Bartley et al. 1995), The majority of patients manifested mild GO. More recent studies in Europe show lower incidence rates. In Sweden the incidence of GO was 4.2/100,000 person/year, and about one fifth of the patients exhibited infiltrative signs (Abraham-Nordling et al. 2011). In Denmark, the incidence of moderate-to-severe GO was 1.6/100,000 person/year, with a rate of 2.7/100,000 person/year in women and 0.5/100,000 person/year in men (Laurberg et al. 2012). That study revealed a peak incidence between 40 and 60 years of age. The incidence of GO is relatively lower in patients younger than 40 years. Most studies show a greater prevalence in females than in males, with a relative predominance of more severe cases in males.

Radioiodine therapy and, particularly, cigarette smoking are well-recognized risk factors for *de novo* development or deterioration of GO (Bartalena 2012), particularly in patients with hyperthyroidism of recent onset (Traisk et al. 2009). The risk associated with radioiodine administration is further increased in smokers and in patients with severe hyperthyroidism. The harmful effect of radioiodine is substantially mitigated by glucocorticoid prophylaxis (Bartalena et al. 2015).

Several lines of evidence support the association between tobacco smoking and GO. Among these is an increased prevalence of smokers among patients with GD and GO when compared with those without GO. Further, more severe eye disease is found in smokers than nonsmokers (Bartalena 2012). In a retrospective study, patients with GO who stopped smoking had a better outcome of their eye disease (Pfeilschifter and Ziegler 1996). Accordingly, all patients with GD should be advised to stop smoking, irrespective of the presence or absence of GO.

Clinical Presentation

GO is the most frequent extra-thyroidal manifestation of GD. It is a disfiguring, potentially sight-threatening, and emotionally invalidating disease that impairs, even in its milder forms, the quality of life (QoL) of affected patients (Wiersinga 2010b). GO is typically bilateral, but may present as asymmetrical manifestations, and is rarely unilateral (Fig. 2).

Symptoms

The initial symptoms, even in the milder forms, are due to alteration in the tear film that coats the anterior surface of the cornea. They are characterized by gritty or burning sensations in the eye without or with retro-ocular pressure due to expansion of retrobulbar structures, excess tearing, photophobia, and visual blurring (Smith and Hegedüs 2016; Wang and Smith 2014; Dickinson and Perros 2001). Patients with GO experience ocular pain, without or with eye movements, and eventually may develop diplopia in the primary and/or the lateral gazes. Ocular motility abnormalities are caused by the failure of antagonist muscles to relax. Thus diplopia in upgaze is typically due to involvement of the inferior rectus. Patients with DON can complain of blurred vision, reduced color perception, and sight loss with alteration in visual acuity in one or more visual field quadrants. Older diabetic men are at increased risk for DON. Marked proptosis with prolonged exposure of cornea and sclera to air dust can lead to keratitis and corneal ulcers.

Signs

The most common clinical features of GO include eyelid retraction (90%) with bright-eyed stare which can be asymmetrical and edema without or with erythema of

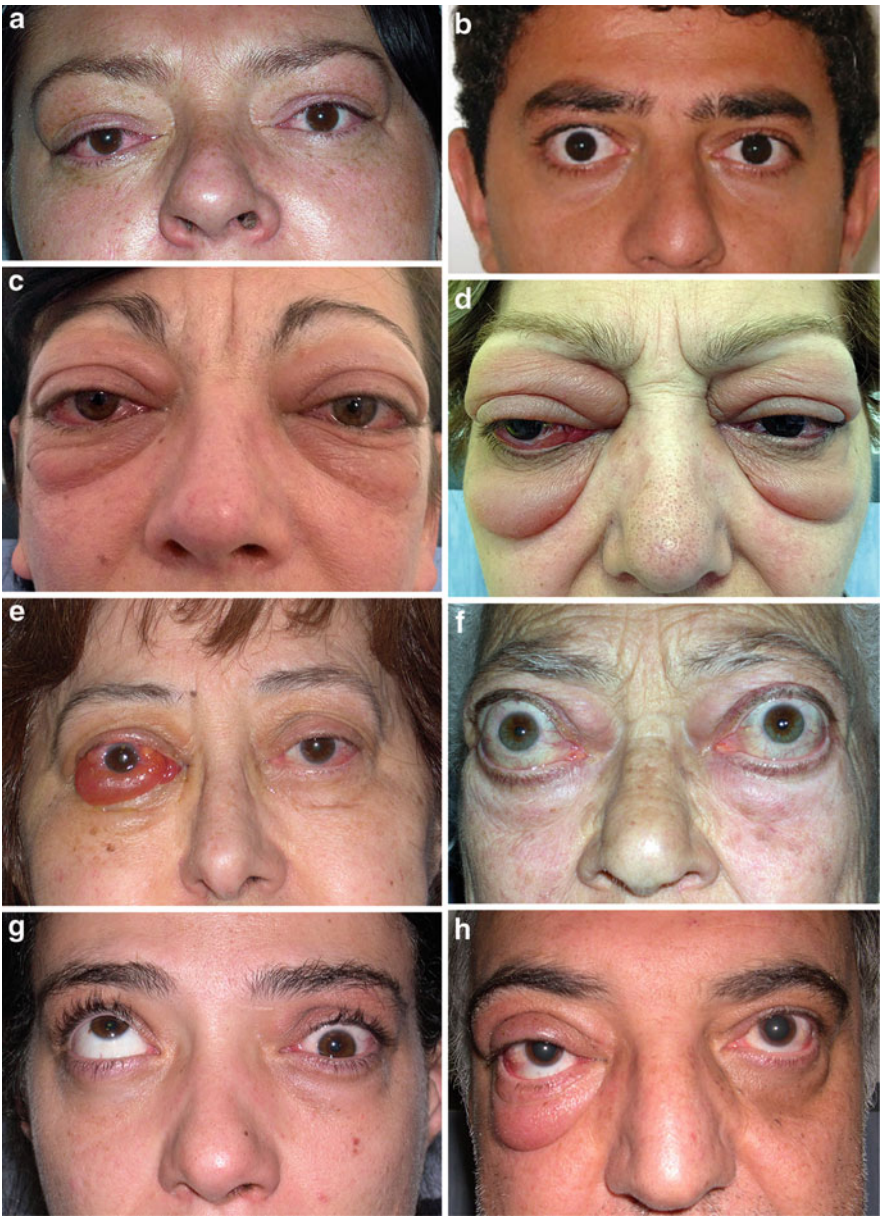

Fig. 2 Clinical manifestations of GO. (**a**) Mild and active GO: mild periorbital edema and conjunctival redness are present; (**b**) mild inactive GO; (**c, d**) moderately severe and active GO; (**e**) unilateral severe chemosis; (**f**) inactive GO with residual proptosis and lid retraction; (**g**) inactive GO with residual eye muscle restriction; (**h**) unilateral GO

periorbital tissue, conjunctival erythema, chemosis, and proptosis (Fig. 2). Increased orbital content volume and the related venous engorgement account for most of these manifestations. Orbital and periocular soft tissues are primarily affected, with secondary effects on the eye. Upper eyelid retraction can be explained by exophthalmos itself and by increased β-adrenergic stimulation of the levator Muller's muscle resulting from thyrotoxicosis. Patients with significant dysmotility may exhibit a compensatory head tilt. Lagophthalmos may be present in patients with severe proptosis

Patient Evaluation

The aim of patient evaluation is to verify the diagnosis, assess severity and activity of the disease, and determine the management strategy.

Differential Diagnosis

Making the diagnosis is quite simple in patients with classical signs and symptoms of hyperthyroidism, elevated TSHR Ab, and bilateral eye involvement. Most features of GO, however, are nonspecific, being shared with other conditions also characterized by increased orbital content and venous stasis. Therefore in selected cases such as those with unilateral or asymmetrical involvement, no current or past history of autoimmune thyroid disease, or diplopia as the only manifestation, other causes of orbitopathy should be considered. These include primary or metastatic orbital tumors, vascular abnormalities, myasthenia gravis, and inflammatory conditions (Table 1). The latter group includes the recently described IgG4-related disease, a fibro-inflammatory condition characterized by the presence of multi-organ tumefactive lesions, lymphoplasmacytic infiltration (due to IgG4-positive plasma cells), and storiform fibrosis, frequently associated

Table 1 Most frequent causes of proptosis and/or extraocular muscle enlargements other than Graves' orbitopathy

Pseudotumor
Osteoma
Mucocele
Metastases (melanoma, breast cancer, lung cancer, prostate)
Meningioma
Melanoma
Lymphoma and leukemia
Lachrymal gland tumors
IgG4-related ophthalmic disease
Hemangioma
Fibrous dysplasia
Cysts
Cushing's syndrome
Carotid-cavernous fistula
Acute myositis

with elevated serum IgG4 levels (Bartalena and Chiovato 2014; Stone et al. 2012). When the diagnosis of GO is uncertain, imaging studies including computerized tomography (CT) or magnetic nuclear imaging (MRI) should be performed. Typical features of GO include enlarged extraocular muscles and/or increased orbital fibroadipose tissue.

Eye Evaluation

The eye evaluation should focus on objective assessment, but there is no consensus on how it should be scored and defined. Table 2 lists the various parameters that should be evaluated to assess GO severity. A complete examination protocol and photographic color atlas for grading soft tissue changes can be found at the website of the European Group on GO (EUGOGO) www.eugogo.eu.

Eyelid and Periorbital Changes

Retraction of upper and/or lower lids causes widening of the palpebral aperture (Fig. 2b). Proptosis is usually responsible for both upper and lower lid retraction. The former may also be due to contraction of elevator and Muller's muscles and rigidity of inferior rectus muscle. Palpebral aperture at the level of the mid-pupil

Table 2 Ophthalmologic evaluation[a]

Lid aperture
Distance between the lid margins in mm with the patient looking in the primary position, sitting relaxed and with distant fixation
Swelling of the eyelids (absent, mild, moderate, severe)
Redness of the eyelids (absent, present)
Redness of the conjunctivae (absent, mild, moderate, severe)
Conjunctival edema (absent, present)
Inflammation of the caruncle or plica (absent, present)
Exophthalmos
Measured in mm using the same Hertel exophthalmometer and same intercanthal distance for an individual patient
Subjective diplopia (Gorman's Score)[b]
Eye muscle involvement (ductions in degrees)
Corneal involvement (absent/punctate keratopathy/ulcer)
Optic nerve involvement
Best corrected visual acuity
Color vision
Visual fields (to be included only if optic nerve compression is suspected)
Optic disc
Relative afferent pupillary defect (absent/present)

[a]To standardize the assessments of the subjective components of GO (soft tissue and inflammatory features), EUGOGO recommends the use of an examination protocol with a comparative photographic Color Atlas (Ref. Dickinson and Perros 2001; www.eugogo.eu), which provides precise definitions for clinical signs and examples for grading their severity

[b]See Table 3

should be measured under standardized conditions (head position, distance fixation in a relaxed state, and closure of the contralateral eye in cases of strabismus). Inability to completely close the eyelid is referred to as lagophthalmos. It is usually associated with marked lid retraction and proptosis.

Eyelid swelling may be due to orbital fat prolapse or lacrimal gland enlargement; the finding of eyelid erythema with subcutaneous fluid accumulation and skin thickening is indicative of active GO.

Conjunctival erythema, particularly the presence of dilated superficial vessels at the insertion of the lateral rectus and conjunctival edema (chemosis), denotes acute orbital congestion. Superior limbic keratoconjunctivitis (inflammation of the upper bulbar and eyelid conjunctiva) is usually associated with upper lid retraction. Inflammation of the caruncle (the small, reddish body at the medial canthus of the eye) or plica (a small fold of bulbar conjunctiva on the medial canthus immediately lateral to the caruncle) is present in patients with active GO (Fig. 2c and 2d).

Proptosis

Proptosis, namely, the protrusion of eye, is usually called exophthalmos when due to GD. It can be measured with an exophthalmometer and represents the distance between the lateral angle of the bony orbit and the cornea. Normal values are race, age, and gender dependent and may vary when measured with different instruments. Thus, measurement by the same observer using the same instrument may improve accuracy. More accurate measures may be obtained using CT or MRI.

Eye Motility

Extraocular muscle involvement is characterized by myositis in the active phase, followed by fibrosis, which renders the muscle stiff and inextensible, thus limiting rotation of the eye in the direction opposite from the muscle involved (restrictive strabismus). Eye dysmotility can be evaluated in several ways. Subjective diplopia can be graded using the Gorman's score (Table 3). An objective assessment of eye motility can be made by evaluating the uniocular field of fixation (which independently defines the involvement of each eye) or by the prism cover test or the field of binocular single vision (the area in which the patient has no double vision), which reflect the limitation in both eyes.

A simple, but inaccurate, evaluation of eye motility can involve the Hirschberg test (Eskridge et al. 1988), which can be performed by the endocrinologist. Initially, the patient is asked to fix on a penlight in the straight gaze. The light reflex should lie slightly nasal from the center of the cornea. The patient is then asked to follow the penlight, which is moved in the four cardinal positions of gaze. If both eyes move

Table 3 Subjective diplopia evaluation (Gorman's Score)

Absent	No diplopia
Intermittent	Diplopia in primary position of gaze, when tired or when first awakening
Inconstant	Diplopia at extremes of gaze
Constant	Continuous diplopia in primary or reading position

normally and symmetrically, the light reflex should remain in the pupil. If the light locates to the cornea, an abnormality of eye movement is present. Abnormalities include exotropia (abnormal eye is turned out), esotropia (abnormal eye is turned in), hypertropia (abnormal eye is higher than the normal fellow eye), or hypotropia (abnormal eye is lower than the normal fellow eye).

Cornea

Mild degrees of punctate keratopathy are rather common in patients with GO and can be highlighted by fluorescein staining. Lagophthalmos increases the risk of corneal lesions, particularly if Bell's phenomenon (upward rotation of the eye upon eyelid closure) is poor.

Optic Nerve

DON is usually bilateral (up to 70% of cases) and can be present in any patients with active GO, particularly in those with severe eye muscle dysfunction or severe proptosis. DON is most frequently due to compression of the optic nerve at the orbital apex by enlarged extraocular muscles, especially the inferior and medial rectus (apical crowding, Fig. 3) and also can be present in the absence of proptosis. Alternatively, it can be due to stretching of the optic nerve by severe proptosis.

Corrected visual acuity, color discrimination, pupillary responses, and optic disc appearance should be evaluated in suspected DON. DON can be present in the absence of visual loss; indeed most patients (up to 70%) have visual acuity of 20/40 or better. Color discrimination defects can be detected in most cases. A relative afferent pupillary defect (RAPD) can be detected and indicates an asymmetrical optic nerve involvement; however its absence does not exclude DON. Optic disc swelling, in the absence of other causes, may strongly suggest DON, but the optic

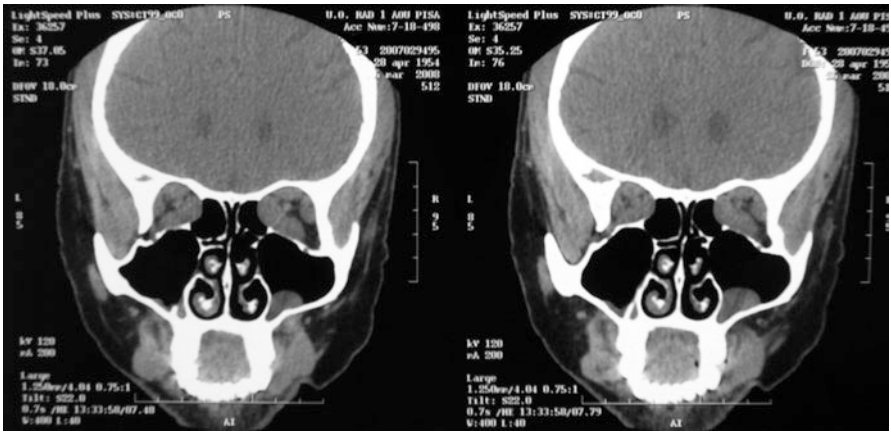

Fig. 3 CT scan of the orbit in a case of dysthyroid optic neuropathy due to optic nerve involvement. Coronal CT scan showing a crowded orbital apex with an almost complete absence in the left orbit of perineural orbital fat

disc may be normal in up to half of patients with unequivocal DON. Visual field evaluation and visual evoked potentials may support the diagnosis of DON. Importantly, no single test can establish or exclude the diagnosis of DON. The presence of confounding pathologies, particularly in older patients (cataract, maculopathy, and glaucoma), should be taken into account. A EUGOGO study has shown that impaired color discrimination and optic disc swelling have the greatest specificity for the diagnosis of DON (McKeag et al. 2007).

Intraocular Pressure

Intraocular pressure can be measured by tonometry using a variety of instruments. Orbital venous congestion and globe compression by a firm, inelastic inferior rectus may cause high intraocular pressure, which may be further increased in upward gaze (up to 15 mm). The inability of the inferior rectus to relax *vis a vis* the globe when the eyes rotate upward causes compression, thus increasing orbital pressure. The elevated intraocular pressure rarely progresses to glaucoma. As a matter of fact, a retrospective study has shown no difference in the prevalence of primary open angle glaucoma between patients with GO and the general population (Kalmann and Mourits 1998).

Disease Severity

The magnitude of functional and cosmetic abnormalities defines disease severity (Fig. 2). Grading the severity is arbitrary and fraught with difficulties, but the presence of sight-threatening manifestation, such as DON, especially if associated with visual loss, severe proptosis with corneal ulceration, or subluxation of the globe, is sufficient to score GO as severe.

In 1977, Werner introduced a numerical score (named ophthalmopathy index (OI)) based on the NOSPECS classification of GO (N, no signs or symptoms; O, only signs, no symptoms; S, soft tissue involvement; P, proptosis; E, extraocular muscle involvement; C, corneal involvement; S, sight loss due to optic nerve involvement) (Werner 1977). Each class of eye change was scored from 0 to 3 according to the degree of involvement. For many years, OI represented the best tool for assessing severity and the effects of treatment. OI was criticized for being dependent on examiner subjectivity and because it gave the same relevance to eye manifestations of different severity. Despite these limitations, the NOSPECS represents a useful mnemonic aid for bedside evaluation of the patient. More recently, authorities from the American Thyroid Association, European Thyroid Association, Asia-Oceania Thyroid Association, and Latin-American Thyroid Association reached consensus on evaluating characteristics of GO (Pinchera et al. 1992).

Recently, a more detailed protocol for evaluating the severity of GO has gained consensus within EUGOGO, but worldwide agreement has yet to be reached. Patients are classified into three groups: mild, moderate-to-severe, and very severe (sight-threatening) GO (Table 4). This classification is mostly based primarily on the presence of DON and the impact of the eye disease on QoL.

Table 4 GO severity assessment according to EUGOGO

Mild GO
Patients whose features of GO have only a minor impact on daily life insufficient to justify immunosuppressive or surgical treatment. They usually have one or more of the following: minor lid retraction (<2 mm), mild soft tissue involvement, exophthalmos <3 mm above normal for race and gender, no or intermittent diplopia, and corneal exposure responsive to lubricants
Moderate-to-severe GO
Patients without sight-threatening GO whose eye disease has sufficient impact on daily life to justify the risks of immunosuppression (if active) or surgical intervention (if inactive). They usually have two or more of the following: lid retraction ≥2 mm, moderate or severe soft tissue involvement, or exophthalmos ≥3 mm above normal for race and gender, inconstant or constant diplopia
Sight-threatening GO (very severe GO)
Patients with DON and/or corneal breakdown

Modified from Bartalena et al. (2016)

Table 5 Clinical activity score[a]

Ocular pain at rest
Ocular pain on attempted up, side, or down gaze
Redness of the eyelids
Swelling of the eyelids
Redness of the conjunctiva
Chemosis of the conjunctiva
Chemosis of the caruncle
Increase in proptosis by ≥2 mm
Decrease in eye movements in any directions by ≥5°
Decrease in pinhole visual acuity by ≥ one line on the Snellen chart

[a]The first seven items were scored at the first visit and the last three evaluated on subsequent assessment. One point is given to each manifestation, if present. GO is considered active if the CAS is ≥4 or ≥3, according to whether all the 10 or the first 7 items are scored

Disease Activity

The activity of GO is an estimate of inflammation intensity underlying signs and symptoms characterizing the initial phase of GO (Fig. 2).

The clinical activity score (CAS), introduced by Mourits et al. in 1989 (Mourits et al. 1989), is a convenient tool for evaluating disease activity (Table 5). In its original formulation, it included 10 items, giving one point to each manifestation. The first seven items were scored at the first visit and the last three evaluated on subsequent assessment. GO is considered active if the CAS is ≥4 or ≥3, according to whether all the 10 (Mourits et al. 1997) or the first seven (Bartalena et al. 2008) items are scored. A CAS ≥4/10 was shown to predict a favorable response to either radiotherapy or oral glucocorticoids (Mourits et al. 1997). Other markers of disease activity have been proposed, including eye muscle reflectivity on A-mode ultrasonography, serum or urine concentration of glycosaminoglycan, T2 relaxation time at MRI, and octreoscan (Bartalena et al. 2000).

VISA Classification

The VISA classification, developed by Dolman and Rootman, uses a clinical recording form that scores severity and activity on the basis of both subjective and objective features (Dolman and Rootman 2006). Four items are scored: vision (V), inflammation/congestion (I), strabismus/motility (S), and appearance/exposure (A). The VISA classification has been adopted particularly in North America. Its advantages include a single-page layout and its simple design.

Orbital Imaging

Orbital imaging should not be performed routinely in patients with typical subjective and objective clinical features of GO. It usually fails to provide additional diagnostic clues and is frequently of little help for therapeutic decisions. It is however essential in patients with asymmetric eye manifestations to rule out other inflammatory orbital disorders or orbital tumors. Both CT and MRI can be used. CT is more widely available and quickly performed but carries the burden of high radiation exposure and therefore should not be used in children or for follow-up studies. CT allows visualization of both soft tissue and bony structures, being the preferred technique of surgeons performing orbital decompression surgery. MRI allows a better evaluation of soft tissues and may offer information concerning disease activity. Both CT and MRI allow measuring orbital tissue volume and defining eye muscle involvement. GO is characterized, in most but not all patients, by absence of muscle tendon involvement. In fact, a recent study has reported tendon thickening in 6.4% of GO patients (Ben Simon et al. 2004).

Quality of Life

GO, particularly in its severe form, may impair the quality of life, mostly as a consequence of altered facial appearance and visual dysfunction such as diplopia. Two disease-specific quality of life questionnaires have been developed for GO (GO-QoL (Terwee et al. 1998) and ThyPRO (Watt et al. 2010)). The former includes 16 questions, eight that evaluate the consequences of the eye disease (decreased visual acuity and diplopia) on visual functioning and eight that assess psychological consequences of altered facial appearance. GO-QoL is available in several languages and can be downloaded at www.eugogo.eu.

Assessment by the Primary Care Physicians, General Internists and General Endocrinologists

Clinical features of GO are present in about 40–50% of patients with GD, but not all patients need a full ophthalmologic evaluation or referral to specialized eye centers. Selection of patients may be challenging for primary care physicians, general internists, and general endocrinologists. A survey performed in Europe in 2006 has shown that some patients with GO are never seen at specialized centers or are referred late in the disease course, resulting in suboptimal care (Perros et al. 1995). However, a more recent study indicates that the time from the diagnosis of GO to referral to specialized centers was halved (Perros et al. 2015). EUGOGO

Table 6 Features requiring urgent referral of patients with GO to specialize centers

Symptoms
Unexplained deterioration in vision
Awareness of change in intensity or quality of color vision in one or both eyes
History of eye(s) suddenly "popping out" (globe subluxation)
Signs
Obvious corneal opacity
Papilledema

recommends that, with the exception of the mildest cases, all patients with GO, particularly those with unusual presentation (unilateral or euthyroid/hypothyroid GO), be referred to specialized tertiary centers for further evaluation and management (European Group on Graves' Orbitopathy (EUGOGO) et al. 2006). Table 6 indicates clinical features to identify patients who need urgent referral.

Natural History

The severity of GO follows a multi-phasic course, with an initial phase lasting a few months and characterized by progressive worsening of eye manifestations, up to a peak of severity. This is followed by a phase of spontaneous improvement lasting up to 1 year or longer and a period of quiescence during which the ocular manifestations stabilize but very rarely resolves completely. The changes in the severity of GO over the course of disease were first described many years ago and is referred to as Rundle's curve (Rundle and Wilson 1945) (Fig. 4). Inflammatory signs and symptoms, which characterize the progressive (active) phase of GO, diminish or disappear in the quiescent (stable) phase. Thus, according to this model, the changes in inflammation would parallel those of severity, but the peak of disease activity would precede that of severity (Smith and Hegedüs 2016; Wang and Smith 2014). Very rarely GO reactivates after reaching quiescence.

Since Rundle's publication, a few studies have investigated the course of GO in untreated patients. In 1977 Teng and Yeo described 56 euthyroid patients with mild to moderately severe GO at baseline evaluation (Teng and Yeo 1977). Eye manifestations spontaneously improved in 37 (66.1%), remained unchanged in 12 (21.4%), and worsened and required treatment with steroids or orbital irradiation in 7 (12.5%). Interestingly, seven patients became hypothyroid during follow-up. GO worsened in one patient during transition from euthyroidism to hypothyroidism and improved following thyroid hormone replacement therapy in four of the six remaining patients. Subsequently, Perros et al. reported 59 patients with GO who were followed without any treatment of their eye disease for a median of 12 months (Perros et al. 1995). GO spontaneously improved in 38 (64.4%), remained stable 13 in (22.0%), and progressively deteriorated and required immunosuppressive therapy in 8 (13.6%). In 2001 Noth et al. followed 53 patients for a median of about 3 years (Noth et al. 2001). GO improved substantially in 25 (47.2%), did not change in 26 (49.0%), and progressively

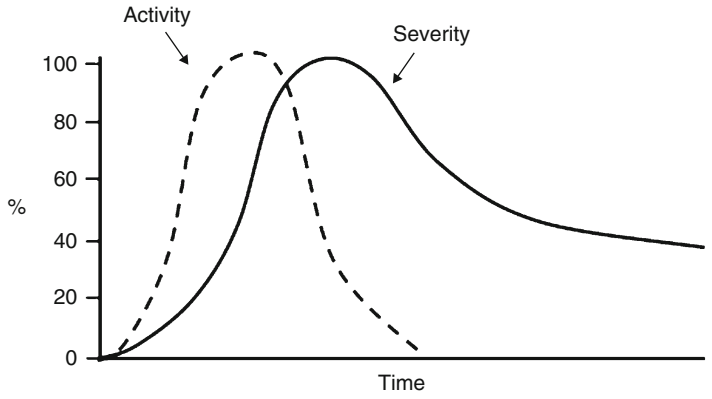

Fig. 4 Rundle's curve. Relationship between activity and severity of GO

deteriorated in 2 (3.8%). Additional insight may be drawn from recent studies. In a EUGOGO study examining the effect of selenium on the course of mild GO, the disease improved in 19 (38%) patients treated with placebo, remained unchanged in 18 (36%), and worsened in 13 (26%) over a follow-up of 12 months (Marcocci et al. 2001). Tanda et al. followed 43 patients with mild GO over an 18-month course of methimazole therapy (Tanda et al. 2013). At the final evaluation, GO was no longer present in 25 (58.1%), unchanged in 17 (39.5%), and had deteriorated in 1 (2.3%). Interestingly, patients in whom GO improved were significantly younger and had lower TSHR Ab as compared with those not improving. The low rate of deterioration of GO in this cohort could be partially explained by relatively low (30%) prevalence of smokers. Finally, Menconi et al. (2014b) reported 65 patients with recently onset, mild and minimally active GO followed without treatment for GO for a median of 40 months. GO improved in 33 (50.8%), remained stable in 22 (33.8%), and deteriorated in 10 (15.4%). The course of GO was not significantly affected by age, gender, smoking habits, duration, and thyroid treatment. In summary, GO either improves or does not change in the majority of patients whose GO does not require specific treatment.

The natural course of GO may be affected by the treatment of Graves' hyperthyroidism. No effect has been reported with treatment of either antithyroid drugs or surgical thyroidectomy, although restoration of euthyroidism may be associated with an improvement of ocular manifestations (Marcocci et al. 1999). In contrast, treatment with radioactive iodine is associated with deterioration or *de novo* development of GO in 15–35% of patients, particularly smokers (Bartalena 2011; Hegedus et al. 2012).

Treatment

The treatment of GO is can be difficult and requires a multidisciplinary approach, with collaboration between endocrinologists, ophthalmologists, radiologists, radiotherapists, and orbital surgeons (Bartalena and Tanda 2009).

The treatment plan for thyroid dysfunction and eye disease in patients with GD should be individually tailored. This can be accomplished most successfully in a combined thyroid-eye clinic, where the endocrinologist and the ophthalmologist evaluate patients at the same visit, preferably in the same room (Wiersinga 2010a). Patients should be counseled regarding the thyroid and eye treatment plans, anticipated outcomes, timing, and treatment-related risks.

The following variables should be considered when planning therapy: choice of thyroid treatment, severity and activity of GO, age, risk factors, and associated conditions.

Choice of Thyroid Treatment

Euthyroidism should be promptly restored and maintained in all patients with GO, with close monitoring that avoids periods of hyper- or hypothyroidism that may worsen eye disease (Marcocci and Pinchera 2010).

Administration of antithyroid drugs to hyperthyroid patients is frequently followed by improvement of GO, likely due to normalization of thyroid function.

Whether the presence of GO should influence the subsequent treatment of hyperthyroidism is still a matter of controversy, and two approaches have been suggested. The first advocates a definitive therapy with radioiodine or surgical thyroidectomy, followed shortly by treatment of GO as appropriate (Marcocci et al. 1998). The second suggests initiating treatment of GO if needed and continuing antithyroid drug until the eye disease becomes inactive. Definitive treatment of hyperthyroidism would be delayed until the treatment of GO is completed (Wiersinga 1998).

Several studies have investigated whether treatment of hyperthyroidism may influence the course of GO. The available data, although somewhat conflicting, suggest that antithyroid drug and thyroidectomy are not disease-modifying treatments. In contrast, radioiodine is associated with a small but definite risk of *de novo* appearance or worsening of GO. This is particularly true in smokers and in patients with hyperthyroidism of recent onset and high levels of free thyroid hormones or TSHR Ab (Bartalena et al. 2015; Marcocci and Pinchera 2010). Prophylaxis with oral glucocorticoids started shortly after radioiodine administration eliminates almost completely this risk (Bartalena 2012).

Treatment Options for GO

The management of patients with GO depends on disease severity and activity as well as age, risk factors, and associated conditions (Marcocci and Marinò 2012). EUGOGO has recently reviewed the guidelines for management of GO (Bartalena et al. 2016) (Fig. 5). Extensive counseling is necessary to inform the patient that (i) the primary goal of therapy is to inactivate the disease, (ii) the therapeutic response may be quite variable independent of treatment used, and (iii) depending on the therapeutic response, one or more rehabilitative surgical procedures might be considered to further improve appearance and function. Thus, patients should be informed that several months may be required to achieve the optimal outcome.

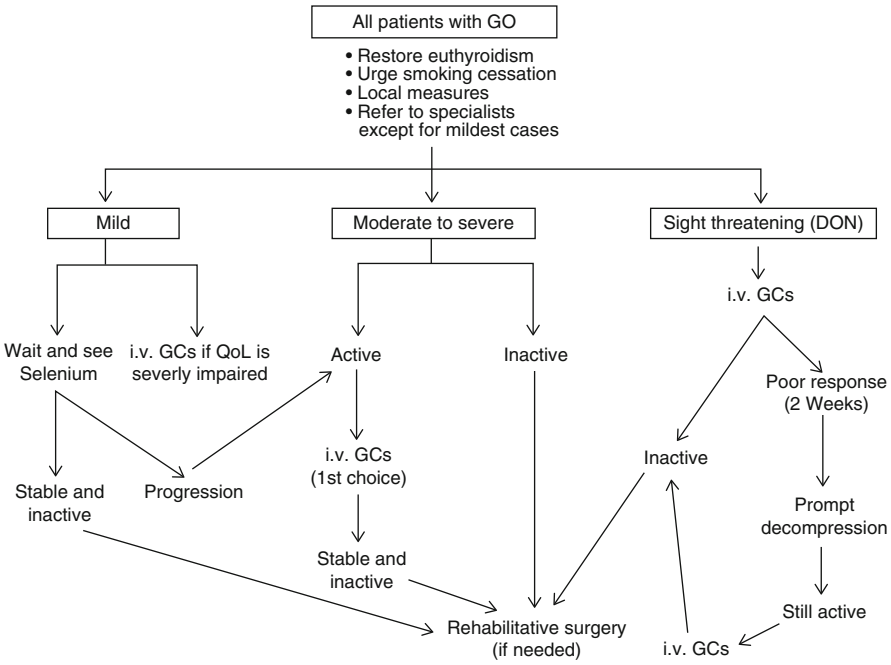

Fig. 5 Management of patients with GO according to 2016 EUGOGO Guidelines. For definition of activity and severity, see text and Tables 4 and 5; for local measures, see text (From Bartalena et al. (2016))

Mild GO

Most patients with GD have mild GO, which often improves spontaneously. Thus, a watchful waiting strategy may be sufficient. Simple supportive measures are usually adequate to achieve symptomatic relief (Smith and Hegedüs 2016; Bartalena et al. 2016). Photophobia can be reduced by wearing sunglasses; symptoms of corneal exposure and defective tear film may be controlled by artificial tears during daytime and ointments at night. The desiccating effects of wind should be avoided by using appropriate eye wear. In patients with lagophthalmos, taping the eyelids closed during the night is useful for preventing nocturnal corneal drying. Prisms may be beneficial for correction of symptomatic diplopia, although the patient may not tolerate them. Local botulinum toxin injection may lessen upper lid retraction. Elimination of risk factors, such as smoking, may decrease the risk of disease progression (Pfeilschifter and Ziegler 1996). Low-dose glucocorticoid prophylaxis should be considered in patients with active GO undergoing radioiodine therapy, particularly if smoking or other risk factors are present (Bartalena 2012; Bonnema and Hegedus 2012).

Immunosuppressive therapy is usually not recommended in patients with mild active GO. However, its use or rehabilitative surgery (inactive disease) may be considered in patients who experience diminished quality of life (Bartalena et al. 2016).

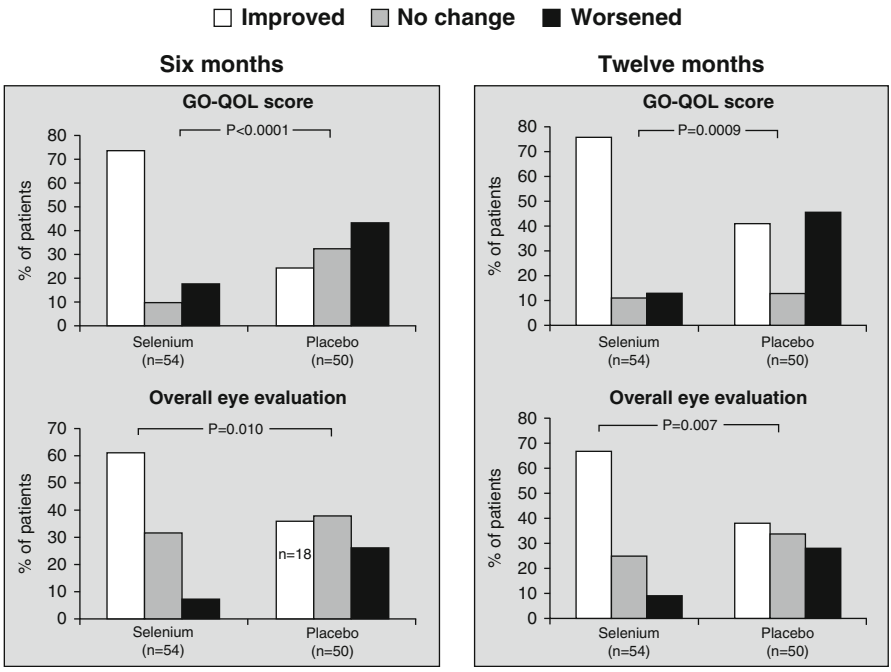

Fig. 6 Effect of a 6-month course of selenium vs placebo in patients with mild GO. Changes in GO-specific quality of life (GO-QOL) questionnaire and overall eye evaluation at 6 months (end of treatment; *left* panel) and 12 months (6 month after treatment withdrawal; *right* panel) months (Modified from Marcocci et al. (2001))

A multicenter, randomized, double-blind, placebo controlled trial performed in Europe by EUGOGO has shown that a 6-month course of sodium selenite (100 μg twice daily, corresponding to 93 μg elemental selenium daily) is associated (compared to placebo) with a greater rate of improvement of QoL and overall eye involvement and a lower rate of deterioration (Fig. 6) (Marcocci et al. 2001). The benefit of selenium was maintained 6 months after treatment withdrawal. No patient treated with selenium reported side effects. It is worth noting that patients included in the study were living in areas with marginal selenium deficiency. Thus the question of whether selenium may benefit patients living in selenium-sufficient areas remains to be established. Importantly, many physicians, as recently reported from Italy (Negro et al. 2016), have already adopted this strategy. However, confirmation is awaited, and concern in relation to indiscriminate use has been raised (Hegedüs et al. 2016).

Moderate-to-Severe GO

The therapeutic approach in patients with moderate-to-severe GO depends on whether it is active (CAS $\geq$3/7 or $\geq$4/10 items) or inactive. In the former, medical treatment (immunosuppressive/anti-inflammatory) should be considered, whereas

rehabilitative surgery can be offered to the latter. Presence of clinical activity may predict a favorable response to medical therapy (Terwee et al. 2005).

Active Eye Disease

Glucocorticoids

Glucocorticoids (GC) represent the most widely used treatment for GO and can be administered locally (subconjunctival or periocular) or systemically (oral or intravenous). The systemic route is currently regarded as first-line treatment, intravenous route being preferred since it is more effective and better tolerated than orally administered drug (Marcocci and Marinò 2012; Bartalena et al. 2016). Intravenous GC administration should be performed in centers with experience in this approach so as to properly select patients and manage adverse events (Fig. 7).

Patients selected for intravenous GC therapy should be carefully evaluated. Recent viral hepatitis, severe liver dysfunction, and substantial cardiovascular disease or psychiatric disorders represent contraindications to their use. Hypertension and diabetes mellitus should be controlled before commencing therapy. A wide range of schedules (daily, alternate days, or weekly) and dosages has been used, and a favorable response rate has been reported in 70–80% of cases (Kahaly 2010). A large, multicenter EUGOGO study, comparing three cumulative doses of GC (about 2.5, 5.0, and 7.5 g) delivered by weekly infusions over a 12-week period, has shown a comparable significant decrease of the CAS but a better overall ophthalmic response in patients

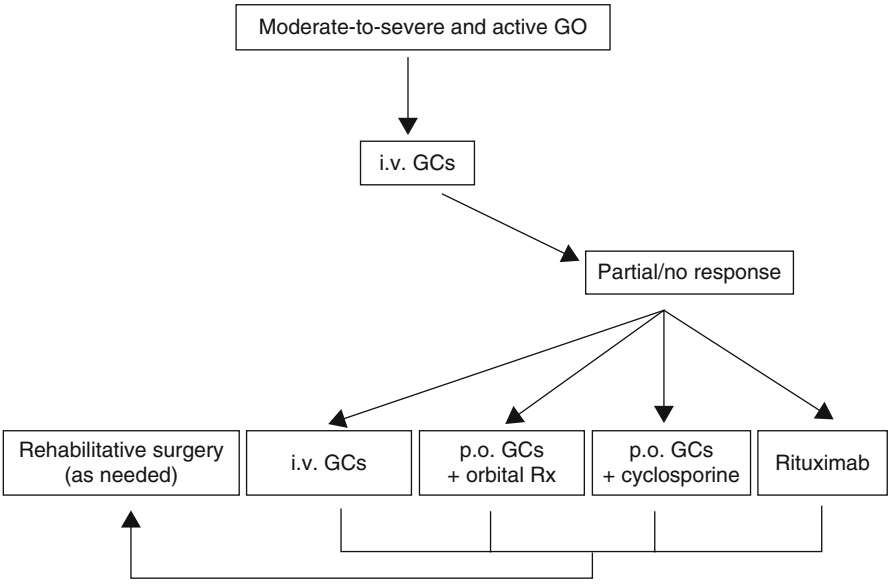

Fig. 7 Second-line treatment OF GO according to 2016 EUGOGO Guidelines. Several options are available for patients with GO and partial or no response to first-line therapy (i.v. GCs) or with recurrence (Modified from Bartalena et al. (2016))

receiving the highest dose (Bartalena et al. 2012). However the latter schedule was most often associated with adverse events. On the basis of these findings, the use of the intermediate 4.5 g cumulative dose schedule (0.5 g once weekly for 6 weeks, followed by 0.25 g once weekly for 6 weeks) in most patients with moderate-to-severe and active GO is advocated. The use of a high-dose regimen (7.5 g cumulative dose, with a starting dosage of 0.830 g weekly for 6 weeks, followed by 0.415 g weekly for 6 weeks) should be reserved for the most severe cases. A second course of intravenous GCs could be considered in patients with insufficient response and residual active GO, provided patient tolerance. In any case the cumulative dose should not exceed 8 g in a single course, and doses greater than 0.75 g should not be administered on consecutive days (Zang et al. 2011). A recent survey performed among members of the European Thyroid Association has reported seven fatal events (four due to acute liver failure and three to cerebral or cardiovascular events) likely related to intravenous GC therapy in patients with GO who were given high daily or alternate-day infusions of large dosages (1 g methylprednisolone). With one exception, all patients had received a cumulative dose of 8 g or more (Marcocci et al. 2012). Liver enzymes, glucose, and blood pressure should be monitored every 2–4 weeks. Proton pump inhibitor and bone protective therapy should be used as appropriate. Some authors recommend a 4–6 week course of alternate-day oral GC therapy at the end of the intravenous course to avoid rebound of autoimmunity (Sisti et al. 2015). In summary, intravenous GC therapy can be considered a relatively safe medical approach for the management of patients with moderate-to-severe and active GO, provided that patients are appropriately selected, that preventive measures are taken, and that treatment is performed in experienced centers.

Oral GCs are commonly started with relatively high doages (60–100 mg daily or 1 mg/kg/day prednisone or equivalent dosages of other GC) (Marcocci and Marinò 2012; Kahaly 2010). The starting dosage is maintained for 1–2 weeks and then tapered by 5–10 mg/weekly according to the clinical response. The total course usually lasts 4–5 months. Several open or randomized trials have shown favorable responses in up to 50–60% of patients. Side effects, particularly cushingoid features and metabolic derangement, are rather frequent. Bone protective therapy should be administered because of the relatively high GC dosage used and the prolonged duration of treatment.

Local administration of GC has also been used with favorable results. Two randomized trials comparing peribulbar injections of triamcinolone acetate (20 mg in each orbit weekly for four consecutive weeks) vs control (Alkawas et al. 2010) or oral GC (Ebner et al. 2004) have shown benefit on eye muscle involvement, with no related adverse events. In another randomized study, one to three injections of 20 mg triamcinolone into the subconjunctival eyelid was effective compared to control in improving recently onset eyelid swelling and mild lid retraction (Lee et al. 2013).

Orbital Radiotherapy

The rationale for orbital radiotherapy (OR) in patients with GO derives from its suppressive effect on lymphocytes and fibroblasts infiltrating the orbital tissues, with decreased production of cytokines and glycosaminoglycans (Kahaly et al.

1999). The standard cumulative dose (20 Gy) is delivered in 10 daily doses over a 2-week period, using lateral radiation field with asymmetric beam to avoid irradiating the contralateral lens. Different protocols, namely, lower doses (10 Gy) or 1 Gy dose given weekly for 20 weeks, have also been proposed (Kahaly 2010). OR has proved to be more effective than sham irradiation, particularly on eye muscle dysfunction and diplopia. Moreover, randomized clinical studies have shown that the efficacy of OR was comparable to that of oral GC and that it potentiates the beneficial effects of oral GC. A good response to OR has been observed in about 60% of treated patients (Bartalena et al. 2000). OR is usually well tolerated and safe (Marcocci et al. 2003). Cataract is a possible, but rare, complication, and radiation retinopathy is extremely rare. The risk of retinopathy is increased in patients with diabetes mellitus, particularly if associated with severe hypertension (Wakelkamp et al. 2004). This modality should be avoided in patients previously treated with cancer chemotherapy. Although long-term data on the risk of secondary tumors are reassuring, it seems prudent to restrict the use of OR to patients older than 35 years (Kahaly 2010).

Cyclosporine

Cyclosporine inhibits cytotoxic T cell activation and antigen presentation by monocytes and macrophages, activates T suppressor cells, and inhibits cytokine production.

Two randomized clinical trials have shown that cyclosporine combined with oral GC was more effective than either treatment alone (Kahaly et al. 1986; Prummel et al. 1989). One study directly compared oral GC with cyclosporine in combination with GC and showed a better outcome of GO and a lower recurrence rate in the latter group (Kahaly et al. 1986). The other study showed that about 60% of nonresponders to either prednisone or cyclosporine used alone had a positive response when given a second course of combined therapy (Prummel et al. 1989). Gingival hyperplasia and liver and renal toxicity are the most common adverse events.

Rituximab

Rituximab (RTX), a chimeric mouse-human anti-CD20 monoclonal antibody, inhibits the activation and differentiation of B cells and B cell antigen presentation (Boross and Leusen 2012; Nielsen et al. 2007).

In open-label studies, RTX has been associated with favorable outcomes in patients with moderately severe and active GO (Hegedüs et al. 2011). Recently, two single-center, small randomized clinical trials in patients with active and moderate-to-severe GO have been completed (Salvi et al. 2015; Stan et al. 2015). One study, which included 15 patients treated with RTX and 16 with intravenous GC, showed that RTX was more effective in decreasing the CAS, preventing disease reactivation, and improving ocular motility and quality of life (Salvi et al. 2015). Conversely, the other study, which included 13 patients treated with RTX and 12 with placebo, failed to show any significant benefit of RTX (Stan et al. 2015). Short-term side effects of RTX included infusion-related reactions, which may occur in 10–30% of patients, and transient exacerbation of orbital edema. Notably, two

patients treated with RTX developed optic neuropathy (Stan et al. 2015). The reasons for the contradictory results remain unclear. Some features, namely, shorter disease duration, younger age, female predominance and lower levels of TSHR Ab in the study cohort of Salvi et al., could account for the better therapeutic response. Because of these conflicting results, larger multicenter studies would be required to unambiguously determine whether a therapeutic role for RTX exists in GO. In the meantime, based on its side effects (Salvi and Campi 2015; El Fassi et al. 2011), use of RTX in GO should be restricted to patients with recent disease onset by experienced physicians. Moreover, patients in whom the drug is used should be carefully monitored for development of DON.

Other Treatments

Other drugs, including azathioprine, ciamexone, somatostatin analogues, etanercept (anti-TNFα analog), and tocilizumab (interleukin IL-6 receptor antagonist), have been used with minimal or unconfirmed beneficial effects on GO (Salvi and Campi 2015). Very recently, a randomized clinical trial has compared the efficacy of mycophenolate mofetil, an immunosuppressive drug largely used in patients undergoing organ transplantation (up to 500 mg twice daily for 24 weeks), vs GC (500 mg intravenously daily for three consecutive days per week for 2 weeks followed by oral prednisone, 60 mg daily for 8 weeks followed by gradual reduction, and withdrawal after 14 weeks) (Ye et al. 2016). Patients treated with mycophenolate mofetil showed a greater overall ophthalmologic response rate at 24 weeks (91.3% vs. 67.9%) and a lower recurrence rate after treatment withdrawal, with a good safety profile.

Inactive Eye Disease

Patients with moderate-to-severe and inactive GO require rehabilitative surgery, which should be performed when the disease has been inactive for at least 6 months. The choice and extent of the surgical procedures depend upon the type and severity of residual eye manifestation that persist in the postinflammatory inactive phase. Orbital decompression, strabismus surgery, and eyelid surgery may be considered, and, when multiple procedures are required, they should be performed in that staged order (Baldeschi 2010).

Very Severe (Sight-Threatening) GO

Sight-threatening GO requires immediate treatment. Corneal breakdown should be initially treated with frequent topical lubricants, moisture chambers, and eventually with tarsorrhaphy until the corneal lesions are healed. Antibiotic treatment should be initiated in case of infection. Orbital decompression is indicated if the above measures are ineffective.

Most cases of DON are due to apical crowding and compression of the optic nerve by enlarged extraocular muscles. High-dose intravenous GC (500–1000 mg methylprednisolone on three consecutive days or on alternate days) should be used initially (Wakelkamp et al. 2005; Curro et al. 2014). A small, randomized prospective study has shown that orbital decompression as initial treatment is not advantageous when compared with high-dose intravenous GC (Wakelkamp et al. 2005).

If an initial positive response is achieved, this course can be repeated after 1 week, eventually followed by lower doses of methylprednisolone weekly, up to a total dose of 8 g. This approach is effective in about 40% of cases with complete or almost complete recovery of vision. If the response is inadequate within 2 weeks, urgent orbital decompression should be carried out. On the other hand, orbital decompression remains the treatment of choice when DON is due to optic nerve stretching (where the nerve dysfunction is due to very severe proptosis rather than from compression by extraocular muscle), subluxation of the globe, or recently developed choroidal folds.

EUGOGO Guidelines

The EUGOGO has recently revised the guidelines for the management of GO (Bartalena et al. 2016) (Fig. 5). All patients with GO should stop smoking, and, with the exception of the mildest cases, all patients should be referred to specialized centers.

Patients with mild disease should be managed with local treatment and measures to control risk factors. A 6-month course of selenium may be offered to GO patients living in selenium-deficient areas, since selenium supplementation may improve the eye manifestations and QoL and decrease the likelihood of progression of GO (Marcocci et al. 2001). General recommendations must await results of ongoing, large-scale studies investigating the consequences of selenium as an add-on therapy in GD, with or without GO (Watt et al. 2013). Conversely, if GO, even if mild, causes a major impact on QoL, major treatments could be justified (immunosuppression if GO is active or rehabilitative surgery if GO is inactive).

High-dose intravenous GC should be the first-line treatment in patients with moderate-to-severe GO. This treatment should be administered in experienced centers and in appropriately selected patients because of the risk of severe adverse events. A cumulative dose of 4.5 g methylprednisolone, given in 12 weekly infusions, should be used in most patients, higher doses being reserved for the most severe cases. The cumulative dose of intravenous GO in a single course should not exceed 8 g. Second-line treatments may include a second course of intravenous GCs, OR alone, or combined with systemic GCs, cyclosporin in combination with oral GCs, and RTX (Fig. 6). A shared decision-making approach is recommended, where benefits and potential risks of each therapeutic option, as well as comorbidities and impact of GO on QoL, are carefully balanced and evaluated in concert with the individual patient.

High-dose intravenous GC (500–1000 mg methylprednisolone on three consecutive days or on alternate days) should be the initial treatment of very severe, sight-threatening GO, but urgent orbital decompression should be performed in cases of inadequate response within 2 weeks.

The Therapeutic Pipeline

As collective wisdom continues to accumulate regarding the underlying causes and disease mechanisms involved in GO, potential treatments based on physiological reality continue to be developed. These are largely agents that are repurposed from

other autoimmune diseases. Prominent among these are molecules that interrupt the production or action of inflammatory cytokines. A good example is the IL-6 pathway which is currently being investigated as a target. An IL-6 receptor antagonist, tocilizumab, was developed for the treatment of rheumatoid arthritis and has recently undergone investigation in a pilot clinical trial for GO (Pérez-Moreiras et al. 2014). Antitumor necrosis factor-α drugs, also widely used in rheumatologic diseases, have undergone limited study (Shin et al. 2009). A recently concluded placebo controlled trial (http://clinicaltrials.gov/show/NCT01868997) examining the safety and efficacy of teprotumumab, an IGF-IR antagonist monoclonal antibody, focused on active, moderate-to-severe GO. The outcome of that study is forthcoming. The rationale for its use rests on the observations made *in vitro* where IGF-IR has been implicated in the disease process (Tsui et al. 2008; Pritchard et al. 2003; Chen et al. 2014). It is anticipated that several additional biological agents will be subjected to clinical trials in the coming years. Among these may be small-molecule TSHR inhibitors (Neumann et al. 2008) and monoclonal blocking Abs (Furmaniak et al. 2012). The ultimate goal of therapy for GD and GO is the restoration of immune tolerance to the relevant autoantigens in this disease.

Surgical Approaches to Therapy

Several surgical strategies are now available to remediate GO. Each must be considered in the specific context of personalized therapeutic, functional, and cosmetic goals. In many oculoplastic practices, the approach to surgical rehabilitation of the eye in GO is mapped out as a staged, sequential process. The process typically begins with decompression followed by strabismus surgery (if necessary) and finally eyelid repair.

Orbital Decompression

While orbital decompression surgery dates back to the nineteenth century, many date the modern era for these procedures to the 1957 paper of Walsh and Ogura (1957) who first described the transantral approach. Several different approaches to surgical decompression have been described since then, and these have been refined with time and experience. Shorr et al. (2000) promoted the transcaruncular approach to the medial orbital wall, while they described the lid crease approach to the lateral orbit wall in the 1990s. Recently, removal of orbital fat while leaving the boney orbit intact has found favor. The typical timing of decompression surgeries occurs once the disease has entered the stable phase and when inflammation and congestion have waned. The most common goal of this surgery is to reduce the intraorbital volume that has resulted in proptosis. The particular approach depends on the goals of the surgeon and his/her patient. For those without extreme proptosis, fat decompression may be sufficient. Its advantages include less invasiveness, potential for diminishing congestion, reduced recovery time, and considerably reduced incidence of postoperative diplopia. Medial wall decompression can reduce optic nerve compression and reduce proptosis and congestion. It carries a high risk of diplopia, sinus bleeding,

and cerebral spinal fluid leak. Lateral wall decompression is also associated with substantial reduction in proptosis without the high risk of diplopia, but recovery from this procedure can be relatively lengthy. Further, it can result in facial anesthesia, and requires skin incision. In a small proportion of patients with clinically significant GO, compromise of the optic nerve can occur during the active phase of the disease. Should this develop, it must be corrected with urgency. Should medical intervention (high-dose corticosteroids, B cell depletion) fail to offer rapid improvement, emergent surgical decompression is indicated.

Strabismus Surgery

GO can result in the misalignment of the extraocular muscles resulting in diplopia. Further, surgical orbital decompression can result in iatrogenic double vision. This result can be enormously debilitating and frequently requires surgical correction. Recessions of the extraocular muscles can be successfully performed to correct even the most severe cases of diplopia.

Surgical Repair of the Eyelids

The final stage of the typical surgical algorithm in the rehabilitation involves restoring eyelid function and appearance. In moderate-to-severe GO, the normal drape provided by the eyelids is disturbed. This is the frequent consequence of eyelid retraction. In fact, upper eyelid retraction is the most common sign of GO. Further, the surgical procedures performed to correct proptosis can result in abnormal relationships between the lids and the globe. Several different procedures have thus far been developed to correct the malpositioned lid. Unfortunately most of these techniques are associated with unpredictable outcomes with respect to lid height and contour. The full-thickness blepharotomy described initially by Elner et al. (2004) can correct even severe retraction. Basic tarsorrhaphy procedures have been advocated for the correction of mildly increased scleral show. With regard to aesthetic outcome, it would appear that the tarsal platform show, eyelid margin position, and marginal reflex distance are all of importance. Besides surgical approaches, those avoiding operations have been proposed. These include injections of botulinum toxin for correction of lid retraction and HA gel fillers to treat upper lid asymmetry.

Unmet Needs of the Patient with GO

Substantial uncertainty remains concerning details of pathogenesis in GO. This has resulted from an absence of comprehensive animal models of the disease and relative inaccessibility of patient-derived tissues during the active phase. It in turn has led to imprecision in diagnosing and disease staging. Further, no laboratory-based biomarkers for disease severity and activity have been identified to date. Predictors of disease progression and outcome are greatly needed. The absence of validated therapeutic end points has hampered the design and implementation of robust clinical trials that could yield unambiguous results. These deficiencies have resulted

in an absence of disease-modifying therapies with proven safety and efficacy. Besides traditional therapies, greater emphasis must be placed on emotional support of individuals with severe GO and their families. Patient advocacy groups have been increasingly active in addressing these inadequately addressed needs.

References

Abraham-Nordling M, Byström K, Törring O, Lantz M, et al. Incidence of hyperthyroidism in Sweden. Eur J Endocrinol. 2011;165:899–905.

Agretti P, Chiovato L, De Marco G, et al. Real-time PCR provides evidence for thyrotropin receptor mRNA expression in orbital as well as in extraorbital tissues. Eur J Endocrinol. 2002;147:733–9.

Alkawas AA, Hussein AM, Shahien EA. Orbital steroid injection versus oral steroid therapy in management of thyroid-related ophthalmopathy. Clin Exp Ophthalmol. 2010;38:692–7.

Baldeschi L. Rehabilitative surgery. In: Wiersinga WM, Kahaly GJ, editors. Graves' orbitopathy: a multidisciplinary approach – questions and answers. Basel: Karger; 2010. p. 167–70.

Bartalena L. The dilemma of how to manage Graves' hyperthyroidism in patients with associated orbitopathy. J Clin Endocrinol Metab. 2011;96:592–9.

Bartalena L. Prevention of Graves' ophthalmopathy. Best Pract Res Clin Endocrinol Metab. 2012;26:371–9.

Bartalena L, Chiovato L. Graves'-like orbitopathy: do not forget IgG4-related disease. J Endocrinol Invest. 2014;37:1233–5.

Bartalena L, Tanda ML. Clinical practice. Graves' ophthalmopathy. N Engl J Med. 2009;360:994–1001.

Bartalena L, Pinchera A, Marcocci C. Management of Graves' ophthalmopathy: reality and perspectives. Endocr Rev. 2000;21:168–99.

Bartalena L, Baldeschi L, Dickinson A, et al. Consensus statement of the European Group on Graves' Orbitopathy (EUGOGO) on management of GO. Eur J Endocrinol. 2008;158: 273–85.

Bartalena L, Krassas GE, Wiersinga W, et al. Efficacy and safety of three different cumulative doses of intravenous methylprednisolone for moderate to severe and active Graves' orbitopathy. J Clin Endocrinol Metab. 2012;97:4454–63.

Bartalena L, Macchia PE, Marcocci C, et al. Effects of treatment modalities for Graves' hyperthyroidism on Graves' orbitopathy: a 2015 Italian Society of Endocrinology Consensus Statement. J Endocrinol Invest. 2015;38:481–7.

Bartalena L, Baldeschi L, Boboridis K, et al. The 2016 European Thyroid Association/European Group on Graves' Orbitopathy guidelines for the management of Graves' orbitopathy. Eur Thyroid J. 2016;5:9–26.

Bartley GB, Fatourechi V, Kadrmas EF, et al. The incidence of Graves' ophthalmopathy in Olmsted County, Minnesota. Am J Ophthalmol. 1995;120:511–7. doi:10.1016/S0002-9394(14)72666-2.

Ben Simon GJ, Syed HM, Douglas R, et al. Extraocular muscle enlargement with tendon involvement in thyroid-associated orbitopathy. Am J Ophthalmol. 2004;137:1145–7.

Boehm N, Funke S, Wiegand M, et al. Alterations in the tear proteome of dry eye patients – a matter of the clinical phenotype. Invest Ophthalmol Vis Sci. 2013;54:2385–92.

Bonnema SJ, Hegedus L. Radioiodine therapy in benign thyroid diseases: effects, side effects, and factors affecting therapeutic outcome. Endocr Rev. 2012;33:920–80.

Boross P, Leusen JH. Mechanisms of action of CD20 antibodies. Am J Cancer Res. 2012;2:676–90.

Cao HJ, Wang HS, Zhang Y, et al. Activation of human orbital fibroblasts through CD40 engagement results in a dramatic induction of hyaluronan synthesis and prostaglandin endoperoxide H synthase-2 expression. Insights into potential pathogenic mechanisms of thyroid-associated ophthalmopathy. J Biol Chem. 1998;273:29615–25.

Chen H, Mester T, Raychaudhuri N, et al. Teprotumumab, an IGF-1R blocking monoclonal antibody inhibits TSH and IGF-1 action in fibrocytes. J Clin Endocrinol Metab. 2014;99:E1635–40.

Coles AJ, Wing M, Smith S, et al. Pulsed monoclonal antibody treatment and autoimmune thyroid disease in multiple sclerosis. Lancet. 1999;354:1691–5.

Cramon P, Winther KH, Watt T, et al. Nodular goiter: a prospective cohort study. Thyroid. 2016;26:1010–8.

Curro N, Covelli D, Vannucchi G, et al. Therapeutic outcomes of high-dose intravenous steroids in the treatment of dysthyroid optic neuropathy. Thyroid. 2014;24:897–905.

de Carli M, D'Elios MM, Mariotti S, et al. Cytolytic T cells with Th1-like cytokine profile predominate in retroorbital lymphocytic infiltrates of Graves' ophthalmopathy. J Clin Endocrinol Metab. 1993;77:1120–4.

Dickinson AJ, Perros P. Controversies in the clinical evaluation of active thyroid-associated orbitopathy: use of a detailed protocol with comparative photographs for objective assessment. Clin Endocrinol. 2001;55:283–303.

Dolman PJ, Rootman J. VISA Classification for Graves orbitopathy. Ophthal Plast Reconstr Surg. 2006;22:319–24.

Douglas RS, Afifiyan NF, Hwang CJ, et al. Increased generation of fibrocytes in thyroid-associated ophthalmopathy. J Clin Endocrinol Metab. 2010;95:430–8.

Ebner R, Devoto MH, Weil D, et al. Treatment of thyroid associated ophthalmopathy with periocular injections of triamcinolone. Br J Ophthalmol. 2004;88:1380–6.

Eckstein AK, Finenrath A, Heiligenhaus A, et al. Dry eye syndrome in thyroid-associated ophthalmopathy: lacrimal expression of TSH receptor suggests involvement of TSHR-specific autoantibodies. Acta Ophthalmol Scand. 2004;82:291–7.

Effraimidis G, Wiersinga WM. Mechanisms in endocrinology: autoimmune thyroid disease: old and new players. Eur J Endocrinol. 2014;170:R241–52.

El Fassi D, Nielsen CH, Junker P, et al. Systemic adverse events following rituximab therapy in patients with Graves' disease. J Endocrinol Invest. 2011;34:e163. doi:10.3275/7411.

Elner VM, Hassan AS, Frueh BR. Graded full-thickness anterior blepharotomy for upper eyelid retraction. Arch Ophthalmol. 2004;122:55–60.

Eskridge JB, Wick B, Perrigin D. The Hirschberg test: a double-masked clinical evaluation. Am J Optom Physiol Opt. 1988;65:745–50.

European Group on Graves' Orbitopathy (EUGOGO), Wiersinga WM, Perros P, et al. Clinical assessment of patients with Graves' orbitopathy: the European Group on Graves' Orbitopathy recommendations to generalists, specialists and clinical researchers. Eur J Endocrinol. 2006;155:387–9.

Feliciello A, Porcellini A, Ciullo I, et al. Expression of thyrotropin-receptor mRNA in healthy and Graves' disease retro-orbital tissue. Lancet. 1993;342:337–8.

Fernando R, Atkins S, Raychaudhuri N, et al. Human fibrocytes coexpress thyroglobulin and thyrotropin receptor. Proc Natl Acad Sci U S A. 2012;109:7427–32.

Fernando R, Lu Y, Atkins SJ, et al. Expression of thyrotropin receptor, thyroglobulin, sodium-iodide symporter, and thyroperoxidase by fibrocytes depends on AIRE. J Clin Endocrinol Metab. 2014;99:E1236–44.

Furmaniak J, Sanders J, Rees Smith B. Blocking type TSH receptor antibodies. Autoimmun Highlights. 2012;4:11–26.

Grubeck-Loebenstein B, Tribe K, Sztankay A, et al. Retrobulbar T cells from patients with Graves' ophthalmopathy are $CD8^+$ and specifically recognize autologous fibroblasts. J Clin Invest. 1994;96:2783–43.

Gupta A, Sadeghi PB, Akpek EK. Occult thyroid eye disease in patients presenting with dry eye symptoms. Am J Ophthalmol. 2009;147:919–23.

Han R, Tsui S, Smith TJ. Up-regulation of prostaglandin E2 synthesis by interleukin-1beta in human orbital fibroblasts involves coordinate induction of prostaglandin-endoperoxide H synthase-2 and glutathione-dependent prostaglandin E2 synthase expression. J Biol Chem. 2002;277:16355–64.

Hegedüs L, Smith TJ, Douglas RS, et al. Targeted biological therapies for Graves' disease and thyroid-associated ophthalmopathy. Focus on B-cell depletion with Rituximab. Clin Endocrinol. 2011;74:1–8.

Hegedus L, Bonnema SJ, Smith TJ, et al. Treating the thyroid in the presence of Graves' Ophthalmopathy. Best Pract Res Clin Endocrinol Metab. 2012;26:313–24. https://doi.org/10.1016/j.beem.2011.09.005.

Hegedüs L, Bonnema SJ, Winther KH. Selenium in the treatment of thyroid diseases: an element in search of the relevant indications. Eur Thyroid J. 2016;5:149–51.

Heufelder AE, Bahn RS. Detection and localization of cytokine immunoreactivity in retro-ocular connective tissue in Graves' ophthalmopathy. Eur J Clin Invest. 1993;23:10–7.

Hufnagel TJ, Hickey WF, Cobbs WH, et al. Immunohistochemical and ultrastructural studies on the exenterated orbital tissues of a patient with Graves' disease. Ophthalmology. 1984;91:1411–9.

Jaume JC, Portolano S, Prummel FF, et al. Molecular cloning and characterization of genes for antibodies generated by orbital tissue-infiltrating B-cells in Graves' ophthalmopathy. J Clin Endocrinol Metab. 1994;78:348–52.

Kaback LA, Smith TJ. Expression of hyaluronan synthase messenger ribonucleic acids and their induction by interleukin-1beta in human orbital fibroblasts: potential insight into the molecular pathogenesis of thyroid-associated ophthalmopathy. J Clin Endocrinol Metab. 1999;84:4079–84.

Kahaly G, Schrezenmeir J, Krause U, et al. Ciclosporin and prednisone v. prednisone in treatment of Graves' ophthalmopathy: a controlled, randomized and prospective study. Eur J Clin Invest. 1986;16:415–22.

Kahaly GJ, Roesler HP, Kutzner J, et al. Radiotherapy for thyroid-associated orbitopathy. Exp Clin Endocrinol Diabetes. 1999;107(Suppl 5):S201–7.

Kahaly GJ. Management of moderately severe Graves' orbitopathy. In: Wiersinga WM, Kahaly GJ, editors. Graves' orbitopathy: a multidisciplinary approach – questions and answers. 2nd ed. Basel: Karger; 2010. p. 120–58.

Kahaly GJ, Shimony O, Gellman YN, et al. Regulatory T-cells in Graves' orbitopathy: baseline findings and immunomodulation by anti-T lymphocyte globulin. J Clin Endocrinol Metab. 2011;96:422–9.

Kalmann R, Mourits MP. Prevalence and management of elevated intraocular pressure in patients with Graves' orbitopathy. Br J Ophthalmol. 1998;82:754–7.

Kleinau G, Kalveram L, Köhrle J, et al. Minireview: insights into the structural and molecular consequences of the TSH-β mutation C105Vfs114X. Mol Endocrinol. 2016;30:954–64.

Kriss JP. Radioisotopic thyroidolymphography in patients with Graves' disease. J Clin Endocrinol Metab. 1970;31:315–23.

Kumar S, Coenen MJ, Scherer PE, et al. Evidence for enhanced adipogenesis in the orbits of patients with Graves' ophthalmopathy. J Clin Endocrinol Metab. 2004;89:930–5.

Laurberg P, Berman DC, Bülow Pedersen I, et al. Incidence and clinical presentation of moderate to severe graves' orbitopathy in a Danish population before and after iodine fortification of salt. J Clin Endocrinol Metab. 2012;97:2325–32.

Lee SJ, Rim TH, Sy J, et al. Treatment of upper eyelid retraction related to thyroid-associated ophthalmopathy using subconjunctival triamcinolone injections. Graefes Arch Clin Exp Ophthalmol. 2013;251:261–70. doi:10.1007/s00417-012-2153-y.

Li B, Smith TJ. Divergent expression of IL-1 receptor antagonists in $CD34^{+}$ fibrocytes and orbital fibroblasts in thyroid-associated ophthalmopathy: contribution of fibrocytes to orbital inflammation. J Clin Endocrinol Metab. 2013;98:2783–90.

Limbach M, Saare M, Tserel L, et al. Epigenetic profiling in CD4+ and CD8+ T cells from Graves' disease patients reveals changes in genes associated with T cell receptor signaling. J Autoimmun. 2016;67:46–56.

Lisi S, Marinò M, Pinchera A, et al. Thyroglobulin in orbital tissues from patients with thyroid-associated ophthalmopathy: predominant localization in fibroadipose tissue. Thyroid. 2002;12:351–60.

Marcocci C, Pinchera A. Thyroid treatment. In: Wiersinga WM, Kahaly GJ, editors. Graves' orbitopathy: a multidisciplinary approach – questions and answers. 2nd ed. Basel: Karger; 2010. p. 100–10.

Marcocci C, Marinò M. Treatment of mild, moderate-to-severe and very severe Graves' orbitopathy. Best Pract Res Clin Endocrinol Metab. 2012;26:13.
Marcocci C, Bartalena L, Pinchera A. Ablative or non-ablative therapy for Graves' hyperthyroidism in patients with ophthalmopathy? J Endocrinol Invest. 1998;21:468–71.
Marcocci C, Bruno-Bossio G, Manetti L, et al. The course of Graves' ophthalmopathy is not influenced by near total thyroidectomy: a case-control study. Clin Endocrinol. 1999;51: 503–8.
Marcocci C, Kahaly GJ, Krassas GE, et al. Selenium and the course of mild Graves' orbitopathy. N Engl J Med. 2001;364:1920–31.
Marcocci C, Bartalena L, Rocchi R, et al. Long-term safety of orbital radiotherapy for Graves' ophthalmopathy. J Clin Endocrinol Metab. 2003;88:3561–6.
Marcocci C, Watt T, Altea MA, et al. Fatal and non-fatal adverse events of glucocorticoid therapy for Graves' orbitopathy: a questionnaire survey among members of the European Thyroid Association. Eur J Endocrinol. 2012;166:247–53.
Matheis N, Lantz M, Grus FH, et al. Proteomics of orbital tissue in thyroid-associated orbitopathy. J Clin Endocrinol Metab. 2015;100:E1523–30.
McKeag D, Lane C, Lazarus JH, et al. Clinical features of dysthyroid optic neuropathy: a European Group on Graves' Orbitopathy (EUGOGO) survey. Br J Ophthalmol. 2007;91:455–8.
Mehred B, Burdick MD, Zisman DA, et al. Circulating peripheral blood fibrocytes in human fibrotic interstitial lung disease. Biochem Biophys Res Commun. 2007;353:104–8.
Menconi F, Marcocci C, Marinò F. Diagnosis and classification of Graves' disease. Autoimmun Rev. 2014a;13:398–402.
Menconi F, Profilo MA, Leo M, et al. Spontaneous improvement of untreated mild Graves' ophthalmopathy: Rundle's curve revisited. Thyroid. 2014b;24:60–6.
Mourits MP, Koornneef L, Wiersinga WM, et al. Clinical criteria for the assessment of disease activity in Graves' ophthalmopathy: a novel approach. Br J Ophthalmol. 1989;73:639–44.
Mourits MP, Prummel MF, Wiersinga WM, et al. Clinical activity score as a guide in the management of patients with Graves' ophthalmopathy. Clin Endocrinol. 1997;47:9–14.
Negro R, Attanasio R, Grimaldi F, on behalf of AME (Associazione Medici Endocrinologi) and AACE (American Association of Clinical Endocrinologists) Italian Chapter, et al. A 2016 Italian survey about the clinical use of selenium in thyroid disease. Eur Thyroid J. 2016;5:164–70.
Neumann S, Neumann S, Kleinau G, et al. A low-molecular-weight antagonist for the human thyrotropin receptor with therapeutic potential for hyperthyroidism. Endocrinology. 2008;149:5945–50.
Nielsen CH, El Fassi D, Hasselbalch HC, et al. B-cell depletion with rituximab in the treatment of autoimmune diseases. Graves' ophthalmopathy the latest addition to an expanding family. Expert Opin Biol Ther. 2007;7:1061–78.
Noth D, Gebauer M, Müller B, et al. Graves' ophthalmopathy: natural history and treatment outcomes. Swiss Med Wkly. 2001;131:603–9.
Parmentier M, Libert F, Maenhaut C, et al. Molecular cloning of the thyrotropin receptor. Science. 1989;246:1620–2.
Pérez-Moreiras JV, Alvarez-López A, Gómez EC. Treatment of active corticosteroid-resistant graves' orbitopathy. Ophthal Plast Reconstr Surg. 2014;30:162–7.
Perros P, Crombie AL, Kendall-Taylor P. Natural history of thyroid associated ophthalmopathy. Clin Endocrinol. 1995;42:45–50.
Perros P, Žarković M, Azzolini C, et al. PREGO (presentation of Graves' orbitopathy) study: changes in referral patterns to European Group On Graves' Orbitopathy (EUGOGO) centres over the period from 2000 to 2012. Br J Ophthalmol. 2015;99:1531–5.
Pfeilschifter J, Ziegler R. Smoking and endocrine ophthalmopathy: impact of smoking severity and current vs lifetime cigarette consumption. Clin Endocrinol. 1996;45:477–81.
Pinchera A, Viersinga W, Glinoer D, et al. Classification of eye changes of Graves' disease. Thyroid. 1992;2:235–6.

Pritchard J, Horst N, Cruikshank W, et al. Igs from patients with Graves' disease induce the expression of T cell chemoattractants in their fibroblasts. J Immunol. 2002;168:942–50.
Pritchard J, Han R, Horst N, et al. Immunoglobulin activation of T cell chemoattractant expression in fibroblasts from patients with Graves' disease is mediated though the insulin-like growth factor I receptor pathway. J Immunol. 2003;170:6348–54.
Prummel MF, Mourits MP, Berghout A, et al. Prednisone and cyclosporine in the treatment of severe Graves' ophthalmopathy. N Engl J Med. 1989;321:1353–9.
Rapoport B, McLachlan SM. TSH receptor cleavage into subunits and shedding of the a-subunit; a molecular and clinical perspective. Endocr Rev. 2016;37:114–34.
Raychaudhuri N, Fernando R, Smith TJ. Thyroptropin regulates IL-6 expression in CD34+ fibrocytes: clear delineation of its cAMP-independent actions. PLoS One. 2013;8:e75100.
Rundle FF, Wilson CW. Development and course of exophthalmos and ophthalmoplegia in Graves' disease with special reference to the effect of thyroidectomy. Clin Sci. 1945;5:177–94.
Salvi M, Campi I. Medical treatment of Graves' orbitopathy. Horm Metab Res. 2015;47:779–88.
Salvi M, Vannucchi G, Currò N, et al. Efficacy of B-cell targeted therapy with rituximab in patients with active moderate to severe Graves' orbitopathy: a randomized controlled study. J Clin Endocrinol Metab. 2015;100:422–31.
Sciaky D, Brazer W, Center DM, et al. Cultured human fibroblasts express constitutive IL-16 mRNA: cytokine induction of active IL-16 protein synthesis through a caspase-3 dependent mechanism. J Immunol. 2000;164:3806–14.
Shimura H, Miyazaki A, Haraguchi K, et al. Analysis of differentiation-induced expression mechanisms of thyrotropin receptor gene in adipocytes. Mol Endocrinol. 1998;12:1473–86.
Shin JI, Kim MJ, Lee JS. Graves' disease, rheumatoid arthritis, and anti-tumor necrosis factor-alpha therapy. J Rheumatol. 2009;36:449–50.
Shorr N, Baylis HI, Goldberg RA, et al. Transcaruncular approach to the medial orbit and orbital apex. Ophthalmology. 2000;107:1459–63.
Sisti E, Coco B, Menconi F, et al. Intravenous glucocorticoid therapy for Graves' ophthalmopathy and acute liver damage: an epidemiological study. Eur J Endocrinol. 2015;172:269–76.
Smith TJ, Hegedüs L. Graves' disease. N Engl J Med. 2016;375:1552–65.
Smith TJ, Bahn RS, Gorman CA. Connective tissue, glycosaminoglycans, and diseases of the thyroid. Endocr Rev. 1989;10:366–91.
Smith TJ, Wang HS, Hogg MG, et al. Prostaglandin E2 elicits a morphological change in cultured orbital fibroblasts from patients with Graves ophthalmopathy. Proc Natl Acad Sci U S A. 1994;91:5094–8.
Smith TJ, Sempowski GD, Wang HS, et al. Evidence for cellular heterogeneity in primary cultures of human orbital fibroblasts. J Clin Endocrinol Metab. 1995;80:2620–5.
Smith TJ, Koumas L, Gagnon A, et al. Orbital fibroblast heterogeneity may determine the clinical presentation of thyroid-associated ophthalmopathy. J Clin Endocrinol Metab. 2002;87:385–92.
Spicer AP, Kaback LA, Smith TJ, et al. Molecular cloning and characterization of the human and mouse UDP-glucose dehydrogenase genes. J Biol Chem. 1998;273:25117–24.
Stan MN, Garrity JA, Carranza Leon BG, et al. Randomized controlled trial of rituximab in patients with Graves' orbitopathy. J Clin Endocrinol Metab. 2015;100:432–41.
Stone JH, Zen Y, Deshpande V. IgG4-related disease. N Engl J Med. 2012;366:539–51.
Tanda ML, Piantanida E, Liparulo L, et al. Prevalence and natural history of Graves' orbitopathy in a large series of patients with newly diagnosed graves' hyperthyroidism seen at a single center. J Clin Endocrinol Metab. 2013;98:1443–9.
Teng CS, Yeo PP. Ophthalmic Graves's disease: natural history and detailed thyroid function studies. Br Med J. 1977;1:273–5.
Terwee CB, Gerding MN, Dekker FW, et al. Development of a disease specific quality of life questionnaire for patients with Graves' ophthalmopathy: the GO-QOL. Br J Ophthalmol. 1998;82:773–9.
Terwee CB, Prummel MF, Gerding MN, et al. Measuring disease activity to predict therapeutic outcome in Graves' ophthalmopathy. Clin Endocrinol. 2005;62:145–55.

Tomer Y. Mechanisms of autoimmune thyroid disease: from genetics to epigenetics. Annu Rev Pathol Mech Dis. 2014;9:147–56.
Traisk F, Tallstedt L, Abraham-Nordling M, et al. Thyroid-associated ophthalmopathy after treatment for Graves' hyperthyroidism with antithyroid drugs or iodine-131. J Clin Endocrinol Metab. 2009;94:3700–7.
Tsui S, Naik V, Hoa N, et al. Evidence for an association between thyroid-stimulating hormone and insulin-like grown factor 1 receptors: a tale of two antigens implicated in Graves' disease. J Immunol. 2008;181:4397–405.
Valyasevi RW, Erickson DZ, Harteneck DA, et al. Differentiation of human orbital preadipocyte fibroblasts induces expression of functional thyrotropin receptor. J Clin Endocrinol Metab. 1999;84:2557–62.
van Steensel L, Hooijkaas H, Paridaens D, et al. PDGF enhances orbital fibroblast responses in TSHR stimulating autoantibodies in Graves' ophthalmopathy patients. J Clin Endocrinol Metab. 2012;97:E944–53.
Vassart G, Kleinau G. TSH receptor mutations and disease. In: De Groot LJ, Chrousos G, Dungan K, et al., editors. Endotext [Internet]. South Dartmouth: MDText.com; 2014. https://www.ncbi.nlm.nih.gov/books/NBK279140/
Wakelkamp IM, Tan H, Saeed P, et al. Orbital irradiation for Graves' ophthalmopathy: is it safe? A long-term follow-up study. Ophthalmology. 2004;111:1557–62.
Wakelkamp IM, Baldeschi L, Saeed P, et al. Surgical or medical decompression as a first-line treatment of optic neuropathy in Graves' ophthalmopathy? A randomized controlled trial. Clin Endocrinol. 2005;63:323–8.
Walsh TE, Ogura JH. Transantral orbital decompression for malignant exophthalmos. Laryngoscope. 1957;67:544–68.
Wang Y, Smith TJ. Current concepts in the molecular pathogenesis of thyroid-associated ophthalmopathy. Invest Ophthalmol Vis Sci. 2014;55:1735–48.
Watt T, Hegedüs L, Groenvold M, et al. Validity and reliability of the novel thyroid-specific quality of life questionnaire, ThyPRO. Eur J Endocrinol. 2010;162:161–7.
Watt T, Cramon P, Bjorner JB, et al. Selenium supplementation for patients with Graves' hyperthyroidism (the GRASS trial): study protocol for a randomized controlled trial. Trials. 2013;14:119.
Weightman DR, Perros P, Sherif IH, et al. Autoantibodies to IGF-1 binding sites in thyroid associated ophthalmopathy. Autoimmunity. 1993;16:251–7.
Werner SC. Modification of the classification of the eye changes of Graves' disease: recommendations of the Ad Hoc Committee of the American Thyroid Association. J Clin Endocrinol Metab. 1977;44:203–4.
Wiersinga WM. Preventing Graves' ophthalmopathy. N Engl J Med. 1998;338:121–2.
Wiersinga WM. Combined thyroid-eye clinic. In: Wiersinga WM, Kahaly GJ, editors. Graves' orbitopathy: a multidisciplinary approach – questions and answers. Basel: Karger; 2010a. p. 96–9.
Wiersinga WM. Quality of life. In: Wiersinga WM, Kahaly GJ, editors. Graves' orbitopathy: a multidisciplinary approach – questions and answers. 2nd ed. Basel: Karger; 2010b. p. 211–20.
Ye X, Bo X, Hu X, et al. Efficacy and safety of mycophenolate mofetil in patients with active moderate to severe Graves' orbitopathy. Clin Endocrinol. 2017;86:247–55.
Young DA, Evans CH, Smith TJ. Leukoregulin induction of protein expression in human orbital fibroblasts: evidence for anatomical site-restricted cytokine-target cell interactions. Proc Natl Acad Sci U S A. 1998;95:8904–9.
Zang S, Ponto KA, Kahaly GJ. Clinical review: intravenous glucocorticoids for Graves' orbitopathy: efficacy and morbidity. J Clin Endocrinol Metab. 2011;96:320–32.

Treatment of Graves' Disease

16

Luigi Bartalena

Contents

Abstract

Treatment of hyperthyroidism due to Graves' disease relies on the use of antithyroid drugs, radioiodine treatment, or thyroidectomy. None of these treatments are perfect, because they are not therapies targeting pathogenic mechanisms of the disease. Selection of either treatment is based on several criteria, but the choice should be shared with the informed patient. The major extrathyroidal manifestation of Graves' disease, i.e., Graves' orbitopathy, when moderate and active should be treated with high doses of intravenous glucocorticoids. This chapter provides an overview of treatment options for both hyperthyroidism and extra-

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thyroidal manifestations, underscoring advantages and disadvantages of therapies, as well as preferred strategies in case of under particular circumstances, such as pregnancy and childhood.

Keywords
Graves' hyperthyroidism · Antithyroid drugs · Thionamides · Methimazole · Propylthiouracil · Radioiodine · Thyroidectomy · Pregnancy · Childhood · Subclinical hyperthyroidism · Graves' orbitopathy · Thyroid dermopathy

Introduction

Graves' disease is an autoimmune disorder and the most frequent cause of hyperthyroidism in iodine-sufficient areas (Bartalena 2013). Although recent years have witnessed relevant improvement in understanding the pathogenic mechanisms underpinning thyroid and extrathyroidal manifestations of this disease (see ▶ Chap. 14, "Graves' Disease" and ▶ 15, "Graves' Ophthalmopathy"), progress in the management has not been equally impressive. As a matter of fact, management of Graves' hyperthyroidism is still based on treatments used for the last 60–70 years (Bartalena 2013). All of these treatments are imperfect, because they do not target pathogenic mechanisms of disease, and either are associated with a high rate of recurrent hyperthyroidism (antithyroid drugs (ATDs)) or cause lifelong hypothyroidism (radioactive iodine (RAI) or thyroidectomy) (Fig. 1). Recently published guidelines (Ross et al. 2016) or reviews (Burch and Cooper 2015; Bartalena et al. 2016c; De Leo and Braverman 2016; Smith and Hegedus 2016) are a helpful guidance for the reader, but reflect the limited evidence currently available. The same considerations can be

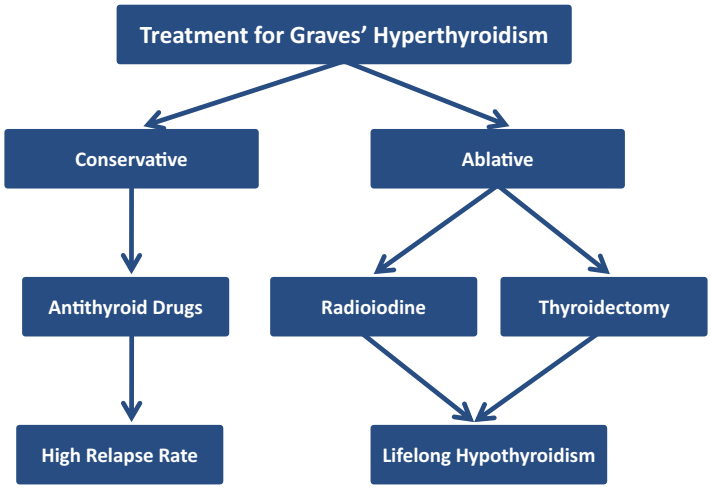

Fig. 1 Current treatments for hyperthyroidism due to Graves' disease and their limitations

applied to the major extrathyroidal expression of Graves' disease, Graves' orbitopathy (GO), although guidelines for its management have recently been published (Bartalena et al. 2016a), the very rare cutaneous manifestation, thyroid dermopathy (or pretibial myxedema), and the exceptional thyroid acropachy.

Treatment of Hyperthyroidism

None of the available treatments for Graves' hyperthyroidism fulfill all of the criteria for an ideal treatment for hyperthyroidism, which should eliminate the pathogenic factors, restore euthyroidism, avoid permanent hypothyroidism, and prevent development or progression of GO. Ideally, it should also be devoid of side effects, while having no negative impact on quality of life. Therefore, the choice of ATDs, RAI, or thyroidectomy as first-line treatment is often conditioned by physician's preference and experience, availability of facilities for RAI treatment, availability of a skilled surgeon, and costs (Bartalena 2013). Of particular importance is a shared decision-making process involving the informed patient and taking into account not only advantages and disadvantages, safety, and side effects of different treatments but also patient expectations and values (Brito et al. 2015). There are geographical differences in selecting treatments. While ATDs are the first choice in Europe, Asia, Oceania, and Latin America, RAI is preferred in North America (Burch et al. 2012; Bartalena et al. 2016b). However, recent reports suggest that ATDs are becoming increasingly popular as first-line treatment also in the USA (Emiliano et al. 2010; Brito et al. 2016). Worldwide, thyroidectomy is rarely used as first-line treatment (Bartalena 2013).

Antithyroid Drugs

Drugs and Regimens

Three thionamide-derived ATDs are currently used: *carbimazole* (CBZ), rapidly converted to its active (and more widely used) metabolite, *methimazole* (thiamazole, MMI), and *propylthiouracil* (PTU). These drugs mainly exert their effect by reducing thyroid hormone synthesis through inhibition of the enzyme thyroperoxidase (Cooper 2005). In addition, they may have some immunosuppressive actions, either direct or indirect, i.e., mediated by restoration of euthyroidism (Cooper 2005). PTU exerts also a peripheral effect consisting in the inhibition of dediodinase-mediated thyroxine (T4) to triiodothyronine (T3) conversion. In the initial phases of treatment, β-blockers (propranolol, atenolol, metoprolol) are useful to control heart rate prior to restoration of euthyroidism by ATDs, but should not be used in patients suffering from asthma (Cooper 2005). It is widely accepted that MMI should be preferred to PTU, except for particular situations, such as the first trimester of pregnancy (see section "Pregnancy"), thyroid storm (see section "Thyroid Storm"), or in patients experiencing minor side effects during MMI treatment and unwilling to undergo definitive treatment by RAI or surgery (Ross et al. 2016). MMI can be taken once daily, while PTU requires fractionated doses because of its shorter half-life.

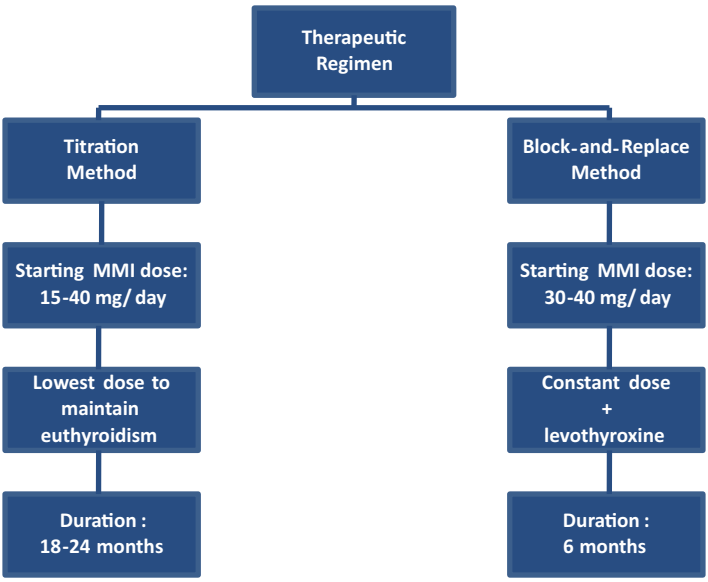

Fig. 2 Regimens of antithyroid drug treatment. *MMI* methimazole

There are two main regimens of ATD treatment: the "titration method" and the "block-and-replace method" (Fig. 2**)**. In both cases MMI is started with an initial daily dose of 10–40 mg (100–400 mg of PTU), depending on the severity of hyperthyroidism (Ross et al. 2016). After restoration of euthyroidism (usually in 4–6 weeks), in the titration method, the dose of MMI is gradually reduced to the lowest dose maintaining euthyroidism (usually 2.5–10 mg/day), and the treatment is continued for 12–18 months, because shorter courses have been reported to increase the likelihood of relapsing hyperthyroidism (Abraham et al. 2005). Longer periods of treatment may be considered in patients in whom serum TSH-receptor antibodies (TRAb), the ultimate cause of Graves' hyperthyroidism, are still detectable in serum, or in particular situations, such as in the elderly with important comorbidities. In the block-and-replace regimen, after restoration of euthyroidism, MMI is continued at the initial high doses, but levothyroxine is added to prevent hypothyroidism. This treatment is usually not continued for more than 6 months, because longer courses do not seem to increase the probability of achieving a permanent remission of hyperthyroidism (Abraham et al. 2005). The two regimens do not differ substantially in terms of risk of recurrences after drug withdrawal; the block-and-replace seems to bear a slightly higher risk of side effects (Abraham et al. 2005). In countries where high-dose (20–30 mg) tablets of MMI are unavailable, the high number of tablets per day in the block-and-replace regimen may jeopardize patient adherence to therapy. The block-and-replace regimen should not be used in pregnancy. In a recent European questionnaire-based survey, the titration method was preferred by 36% of respondents and the block-and-replace by 26%, while the remaining 38% would use the block-and-replace method *only* in selected cases (Bartalena et al. 2016b). The recent American guidelines underscore that the block-and-replace regimen is generally not recommended (Ross et al. 2016).

Table 1 Predictive factors of recurrence of hyperthyroidism after antithyroid drug treatment

Factor	Impact on the risk of recurrence
Age	Uncertain, probably higher risk of recurrence in the young
Gender	Uncertain
Thyroid size	Higher risk when goiter is large
Severity of hyperthyroidism	Uncertain
TSH-receptor antibody (TRAb)	High risk if TRAb is still positive at the end of treatment
Graves' orbitopathy (GO)	Uncertain, but high risk of relapse if GO is severe
Long-term treatment	Possible higher remission rate after long-term treatment
Smoking	Higher risk of relapse in smokers
Postpartum period	High risk of relapse in the postpartum period, also in women in remission

Outcome of Treatment

One of the major limitations of ATD treatment is the high rate of recurrences after treatment withdrawal (Piantanida et al. 2015). Relapses occur in 30–70% of ATD-treated patients (Bartalena 2013). Predictive factors of relapsing hyperthyroidism include large thyroid volume, young age, smoking, the postpartum period (Piantanida et al. 2015) (Table 1**)**. Probably TRAb is the most important predictor of ATD treatment outcome. High TRAb levels at diagnosis have been associated with an 84% risk of relapse over a 4-year period of follow-up (Tun et al. 2016). Even more important is the autoantibody status at the end of treatment, particularly using highly sensitive immunoassays. The large majority of TRAb-positive patients relapse (Tun et al. 2016), indicating that these patients should either continue ATD treatment longer or be switched to a definitive treatment with RAI or surgery (Piantanida et al. 2015). However, it should be underscored that also patients who are TRAb negative at the end of treatment relapse in about 30% of cases (Törring et al. 1996; Barbesino and Tomer 2013). Most relapses occur within 6–12 months after ATD discontinuation (Vitti et al. 1997), but regular monitoring of thyroid function is advised also after that period.

Side Effects

ATDs are generally well tolerated, but they may be associated with side effects (Table 2). These are usually mild and, at least in the case of MMI, most commonly observed during the initial phase of treatment, when relatively higher doses of the drug are given (Cooper 2005). Frequent side effects, such a skin reaction or itching, do not require treatment withdrawal, are usually transient, and can be controlled with antihistamines (Bartalena et al. 2016c). The most serious adverse events are hepatotoxicity, agranulocytosis, and vasculitis. *Hepatotoxicity* is rare using methimazole (0.03%), usually, although not always, with cholestatic characteristics (Wang et al. 2014), while it is more frequent, usually with features of hepatocellular necrosis, in PTU-treated patients (Wang et al. 2014; Yang et al. 2015). Hepatocellular necrosis may occur abruptly and be rapidly progressive and potentially lethal, requiring liver transplantation (Ruiz et al. 2003; Wang et al. 2014; Yang et al. 2015; Bartalena et al. 2016c; Ross et al. 2016). PTU hepatotoxicity is more common in children, and therefore, some authors discourage its

Table 2 Side effects of antithyroid drugs

	Side effect	Frequency
Blood	Mild leukopenia	Relatively frequent
	Agranulocytosis	Rare (0.2–1.2%)
	Aplastic anemia	Very rare
	Thrombocytopenia	Very rare
Skin	Skin rash	Relatively frequent (>5%)[a]
	Urticaria	Relatively frequent (>5%)[a]
	Itching	Relatively frequent (>5%)[a]
	Aplasia cutis	Very rare (MMI)
Liver	Hepatocellular necrosis	Very rare (PTU) (0.07%)
	Cholestasis	Very rare (MMI/CBZ) (0.03%)
Collagen	Polyarthritis	Uncommon
	SLE-like syndrome	Very rare (PTU>MMI)
	Vasculitis	Very rare (PTU>MMI) (<1%)
Miscellaneous	Loss of taste	Rare (MMI)
	Hypothrombinemia	Rare (PTU)
	Hypoglycemia	Very rare (MMI)

MMI methimazole, *CBZ* carbimazole, *PTU* propylthiouracil
[a]These side effects are usually transient, do not require antithyroid drug withdrawal, and can be controlled with anti-histamines

use, particularly in the pediatric population (Rivkees and Szarfman 2010). It should be mentioned that hyperthyroidism per se may account for an increase in serum transaminase or other liver enzyme levels that usually normalize upon restoration of euthyroidism. If, however, a sudden increase of more than threefold in serum transaminase concentrations is found during PTU (or, more rarely, MMI) treatment, ATDs should be immediately withdrawn. The usefulness of routine monitoring of liver function tests, in the absence of suspicious symptoms or signs (such as anorexia, nausea, vomiting, fatigue, jaundice, abdominal pain, dark urine, acholic feces), has not been demonstrated (Kim et al. 2001; Ross et al. 2016). *Agranulocytosis*, i.e., a granulocyte count $<0.5 \times 10^9$/l, is an infrequent (0.2–1.2%) (Watanabe et al. 2012; Yang et al. 2016) but potentially lethal adverse event. Its onset is usually abrupt, making the value of routine white blood cell (WBC) count monitoring questionable (Bartalena et al. 2016c). The patient should be informed that an urgent WBC count should be obtained in the presence of high fever, sore throat, or other signs/symptoms of infection (Cooper 2005). If agranulocytosis develops, ATDs should be immediately discontinued. It is uncertain whether therapy with granulocyte colony-stimulating factor may shorten the time of recovery from agranulocytosis (Fukata et al. 1999; Yang et al. 2016). *Vasculitis* associated with positive antineutrophil cytoplasmic antibody (ANCA) tests is observed in <1% of ATD-treated patients, more frequently with PTU than with MMI (Bartalena et al. 2016c). It is heralded by fever, polyarthralgias, and renal and lung involvement and is more frequently observed during long-term therapy (Bartalena et al. 2016c).

MMI should be preferred to PTU for its more favorable safety profile. If minor side effects using either MMI or PTU occur, the other thionamide can be used, but

Table 3 Advantages and disadvantages of available treatments for Graves' hyperthyroidism

Treatment	Advantages	Disadvantages
Antithyroid drugs	Conservative treatment	High relapse rate
	No hospitalization required	Frequent visits and testing
	Low risk of hypothyroidism	Lack of compliance
	No radiation	Adverse events (rarely major)
	No negative effect on GO	
	Use during pregnancy and breastfeeding	
Radioiodine	Definitive treatment	Lifelong hypothyroidism
	Relatively low cost	Radiation risk
	No hospitalization required	Slow control of hyperthyroidism
	No anesthetic/surgical risk	Possible progression or de novo occurrence of GO
Thyroidectomy	Definitive treatment	Lifelong hypothyroidism
	No radiation risk	Anesthetic/surgical risk
	Prompt control of hyperthyroidism	Hospitalization
	No negative effect on GO	Cost Permanent scar

cross-reactivity between the two drugs is common. Therefore, in case of serious side effects, exposure to the alternative thionamide is contraindicated. For ATD treatment in pregnancy, see section Pregnancy.

Advantages and Disadvantages

As illustrated in Table 3, ATD treatments have advantages and disadvantages. The major advantage is that it is a conservative treatment, thus avoiding destruction (by RAI) or removal (by thyroidectomy) of the thyroid (Bartalena et al. 2016c). Therefore, there is no risk of permanent hypothyroidism, unless the latter reflects the natural evolution of the underlying autoimmune thyroid disorder. Overall, thionamides have an acceptable safety profile, particularly if low doses of the drug are sufficient, as in the majority of cases, to maintain stable euthyroidism. As pointed out above, careful surveillance is, however, required for potential treatment-related adverse events. No hospitalization is required, neither radiation risks nor detrimental effects on GO are associated with ATDs. These drugs can be used, with some precautions, during pregnancy and breastfeeding (Ross et al. 2016) (see section Pregnancy). The major drawback of ATD treatment is the high rate of recurrences that requires either a second course of pharmacological treatment or an ablative treatment, thereby simply postponing the final treatment of hyperthyroidism (Bartalena et al. 2016c). The lack of compliance with treatment is not infrequent, but this is a general problem that can occur also with levothyroxine treatment for hypothyroidism following RAI treatment of thyroidectomy. Frequent visit and testing is required during the treatment course.

Radioiodine Treatment

Modalities of Radioiodine Administration

RAI therapy is performed using ^{131}I. This radioisotope (β and γ radiation emitter) is rapidly concentrated into the thyroid, where it causes progressive thyrocyte necrosis, fibrosis, and glandular atrophy (Ross 2011; Bonnema and Hegedus 2012). RAI treatment is still the preferred treatment in North America (Burch et al. 2012). ^{131}I can be administered in fixed amounts or as calculated doses based on thyroidal RAI uptake and half-life determination and thyroid size (Bonnema and Hegedus 2012). However, evidence of the superiority of such calculations and consensus on the use of either approach are missing. In a survey among UK endocrinologists, it appeared that fixed doses are employed by 70% of respondents (Vaidya et al. 2008). Whether RAI should be administered after restoration of euthyroidism with ATDs is also a matter of argument. The recent American guidelines state that pretreatment with MMI might be considered only in patients who are at risk of complications due to exacerbation of hyperthyroidism after ^{131}I treatment (e.g., patients with relevant cardiovascular comorbidities, or in the elderly) (Ross et al. 2016). In European countries, RAI treatment is carried out after ATD pretreatment in the large majority of cases (Bartalena et al. 2016b). MMI, in any case, is preferred to PTU, because the latter might have radioprotective effects and reduce the efficacy of RAI treatment (Bartalena 2013). ATDs are usually withdrawn 5–7 days before RAI administration; they are resumed 7–10 days after treatment only in patients whose hyperthyroidism, because of associated comorbidities, must be promptly controlled, before RAI stably controls thyroid hyperfunction (Bartalena 2013). Lithium carbonate, administered concomitantly with RAI and continued for a few weeks, may facilitate prompt control of hyperthyroidism in these at-risk patients, although it does not increase the long-term cure rate achieved with RAI therapy (Bogazzi et al. 2010).

Outcome of Treatment

RAI treatment is given with the deliberate purpose of inducing hypothyroidism, which, indeed, represents the final outcome in the large majority of patients (Vaidya et al. 2008; Bonnema and Hegedus 2012). Doses of RAI should be generous enough to achieve this goal. The use of low activities, aiming at restoring euthyroidism without inducing hypothyroidism, is associated with an increased risk of retreatment (Bonnema and Hegedus 2012). As a matter of fact, a small proportion (5–10%) of patients require a second dose of RAI, which should not be given earlier than 6 months after the first treatment (Burch and Cooper 2015).

Contraindications and Safety Measures

RAI is absolutely contraindicated during pregnancy and breastfeeding. A pregnancy test should be obtained 2 days before treatment in women with childbearing potential (Ross et al. 2016). Pregnancy should be postponed for 4–6 months in women, also allowing for stable restoration of euthyroidism, and for 3–4 months in men to permit sperm production turnover (Ross et al. 2016). Practice guidelines from the American Thyroid Association recommend avoiding RAI treatment if the woman has

Table 4 Safety of radioiodine

Side effect	Action
Exacerbation of hyperthyroidism	Debated. Pretreat with antithyroid drugs
Actinic thyroiditis	Rare and usually transient. Treat with steroids
Sialadenitis	Lemon juice
Teratogenicity	Not relevant. Pregnancy postponed for 6 months after RAI treatment
Cardiovascular and cerebrovascular risk	Recent data are reassuring. Related to hyperthyroidism per se rather than to RAI treatment
Cancer	Slight increase in the risk of thyroid and renal cancer. Likely role of hyperthyroidism per se
Graves' orbitopathy	Possible de novo occurrence or progression in at-risk patients (mainly smokers). Preventable by low-dose oral prednisone given concomitantly with RAI

interrupted breastfeeding since less than 6 weeks, to reduce radiation exposure of the breast tissue (Sisson et al. 2011). RAI is also contraindicated if thyroidal iodine uptake is low, e.g., because of iodine contamination, or there are nodules suspicious for being malignant. Very large goiters may require more than one treatment and should probably be preferably treated surgically.

Radiation safety measures, such as sleeping alone for 3–6 days or keeping a distance of 1 m from adults and 2 m from pregnant women and children, have been recommended (Sisson et al. 2011), but vary among countries and are dependent on the administered activities (Bonnema and Hegedus 2012).

Side Effects

In the early period after RAI therapy, transient *exacerbation of hyperthyroidism* may occur because of the cytolytic effect of RAI (Table 4). Whether ATD pretreatment, which reduces intrathyroidal thyroid hormone stores, may avoid this phenomenon is debated (Bonnema et al. 2003; Bonnema and Hegedus 2012). In a recent European survey, 61% of expert thyroidologists always treat patients with ATDs prior to RAI treatment, and only 4% never do so, while the remaining 35% pretreat with ATDs in the presence of relevant comorbidities (Bartalena et al. 2016b). Sometimes RAI therapy may be followed by *actinic thyroiditis*, heralded by thyroid pain and swelling. This is usually mild and transient, not requiring treatment (Bonnema and Hegedus 2012). *Sialadenitis* may also occur, involving submandibular glands and parotids, but these phenomena are permanent in a minority of patients (Bonnema and Hegedus 2012). Lemon juice may help reduce this side effect. *Teratogenicity* does not seem to be an issue in thyroid cancer patients treated with RAI doses higher than those used for hyperthyroidism (Sawka et al. 2008). Doses of RAI used for hyperthyroidism are not not associated with a decreased male gonadal function (Ceccarelli et al. 2006). Graves' hyperthyroidism per se is associated with increased morbidity and mortality (Brandt et al. 2011, 2013a, b). While some studies reported an increased risk of *cardiovascular* (Franklyn et al. 1998; Metso et al. 2007) and *cerebrovascular* events, as well as occurrence of *cancer* (La Cour et al. 2015) following

RAI treatment, a recent meta-analysis failed to reveal any overall increased risk of cancer, except for a slight increase in the risk of thyroid and renal cancer (Hieu et al. 2012). A recent Finnish study observed, however, an increased risk of gastric and respiratory tract cancer due to hyperthyroidism per se, with no difference between patients treated with RAI or thyroidectomy (Ryodi et al. 2015). In addition, a recent study from the UK showed that all-cause mortality (including cardiovascular mortality) was increased during ATD treatment when control of hyperthyroidism was poor or when RAI treatment did not cause hypothyroidism, but not in RAI-treated patients developing hypothyroidism (Boelaert et al. 2013).

RAI treatment can cause **progression or de novo development of** GO, particularly in smokers (Träisk et al. 2009) in patients with preexisting GO (Bartalena et al. 1998), or with high-serum TRAb levels (Eckstein et al. 2006). This occurs in approximately 15% of cases, is often transient, and can be prevented by a concomitant short-term course of low-dose oral prednisone (steroid prophylaxis) (Shiber et al. 2014). Steroid prophylaxis is recommended in at-risk patients, but can be avoided in patients with long-term inactive GO, or in those without risk of progression (Bartalena et al. 2016a). Post-RAI hypothyroidism should be avoided or promptly controlled by levothyroxine replacement, because it is an important risk factor for RAI-associated progression of GO (Tallstedt et al. 1994; Perros et al. 2005).

Advantages and Disadvantages

RAI has advantages and disadvantages (Table 3). The former include the relatively low cost, no need for hospitalization, and the lack of anesthetic and surgical risk. It is, however, an ablative treatment, and the patient is bound to lifelong thyroid hormone replacement therapy to correct hypothyroidism. Control of hyperthyroidism is not immediate, there is a small radiation risk, and GO may progress or de novo occur, particularly in smokers (Bonnema and Hegedus 2012).

Thyroidectomy

Thyroidectomy is the least commonly used among the three available therapies for newly diagnosed Graves' hyperthyroidism, since it is selected in no more than 1–2% of cases (Burch et al. 2012; Bartalena et al. 2016b). It is, however, indicated in the presence of suspicious nodules, when hyperthyroidism relapses after ATD treatment and goiter is large or the patient refuses RAI treatment, or if facilities for RAI treatment are not available (Bartalena 2013). A recent meta-analysis showed greater effectiveness of surgical treatment compared to RAI treatment in terms of risk of relapsing hyperthyroidism (Genovese et al. 2013). This is, however, controversial, because another systematic review failed to show any significant difference between the two definitive treatments (Sundaresh et al. 2013).

Extent of Thyroidectomy

If thyroidectomy is the selected treatment, near-total or total thyroidectomy should be the procedure of choice, because subtotal thyroidectomy is associated with a

higher chance of relapse of hyperthyroidism, while the rate of complications is not significantly different (Genovese et al. 2013; Guo et al. 2013).

Preparation to Surgery

Patients should be rendered euthyroid by ATDs prior to surgery, to avoid the risk of exacerbation of hyperthyroidism caused by anesthetic and surgical stress, as well as by thyroid manipulation (Ross et al. 2016). Although recent guidelines recommend that iodine drops (KI, saturated solution of KI, Lugol's solution) be given preoperatively for 10 days (Ross et al. 2016), in clinical practice, at least in Europe, they are used by no more than one third of respondents to a recent survey (Bartalena et al. 2016b). Indeed, they are useful to reduce thyroid vascularity and intraoperative blood loss (Erbil et al. 2008). In the event that thyroidectomy is an emergency procedure, and to achieve a rapid control of hyperthyroidism, in addition to ATDs and iodinated drops, glucocorticoids, β-blockers, and, possibly, cholestyramine can be used (Ross et al. 2016).

Complications

Thyroid surgery may be complicated by hypoparathyroidism (transient or permanent) and cause hypocalcemia and recurrent laryngeal nerve palsy (transient or permanent), causing hoarseness, bleeding, or wound infection (Bartalena et al. 2016c) (Table 5). Calcium and vitamin D status should be assessed preoperatively and repleted, if required, or even given prophylactically (Ross et al. 2016). If the patient is inadequately prepared by ATDs, thyroid surgery may be followed by a marked worsening of thyrotoxicosis. The rate of complications following thyroidectomy is inversely correlated with the surgeon's experience (Sosa et al. 2008). Accordingly, to minimize the risks of thyroid surgery, it is fundamental to select a skilled surgeon with a high annual volume of thyroidectomies (Bartalena et al. 2016c).

Advantages and Disadvantages

Advantages of thyroidectomy include the absence of radiation risk, the prompt control of hyperthyroidism, the lack of detrimental effects on GO, the absence of radiation risk (Table 3). On the other hand, it implies an anesthetic and surgical risk and requires hospitalization, a permanent scar will be left, and costs are higher than

Table 5 Complications of thyroidectomy

Complication	Action
Exacerbation of hyperthyroidism	Render the patient euthyroid with antithyroid drugs prior to surgery
Hypoparathyroidism	May be transient or permanent. Give vitamin D and calcium preoperatively in at-risk patients
Laryngeal nerve palsy	May be transient or permanent. Intraoperative neuromonitoring of laryngeal nerve helps to detect early damage
Blood loss and hemorrhage	Preoperative treatment with saturated solution of KI or Lugol's solution reduces thyroid vascularity
Wound infection	Accurate care of the wound, drainage, antibiotics

using the other modalities of treatment (Table 3) (Bartalena 2016c). However, in one US study, surgery was more cost-effective than lifelong ATDs or RAI treatment (In et al. 2009). As for RAI treatment, total thyroidectomy is inevitably bound to permanent hypothyroidism and lifelong levothyroxine replacement therapy.

Role of Patients in Selecting Therapy

Because all of the available therapeutic options for Graves' hyperthyroidism have limitations, patient choice may eventually constitute the reason for the choice of treatment. In this regard, shared decision-making is an essential process, because it puts the patient at the center of healthcare taking into account his/her wishes, values, expectations, impact on quality of life, and comorbidities (Ting et al. 2014). The level of involvement of the informed patient can be increased by encounter tools for shared decision-making, such as that recently developed by the Mayo Clinic Specialists (Brito et al. 2015).

Special Situations

Pregnancy (see also ▶ Chap. 23, "Thyroid Physiology and Thyroid Diseases in Pregnancy")

Because RAI treatment is contraindicated, and thyroidectomy should be performed (during the second trimester) only in exceptional cases, such as intolerance to or major adverse events due to ATDs, thionamides represent the treatment of choice for Graves' hyperthyroidism in pregnant women (DeGroot et al. 2012; Stagnaro-Green et al. 2011). According to recent guidelines, PTU should be used during the first trimester and replaced by MMI during the second and third trimester (Ross et al. 2016). This approach is motivated by the observation that exposure to MMI in early pregnancy is associated with an increased risk of fetal malformations (CBZ/MMI embryopathy) (Bowman et al. 2012), but, on the other hand, PTU can cause severe hepatotoxicity in the mother (Cooper and Rivkees 2009) (Table 6). Recent studies from Denmark reported that both MMI and PTU can indeed cause fetal malformations, although those caused by PTU are probably milder (Linding Andersen et al. 2013, 2014). A recent retrospective Italian study found that the rate of malformations in the offspring of women exposed to MMI was not higher than in the general population (Gianetti et al. 2015). A large American insurance database study reported a 13% increase in fetal malformations in hyperthyroid women, but no association with ATD treatment (Korelitz et al. 2013). In addition, a study from Japan did not find any increase in hyperthyroidism-associated malformations (Yoshihara et al. 2012). Despite these controversial results, it seems advisable to follow current guidelines, using PTU in the first trimester and switching to MMI in the second and third trimesters (Bartalena et al. 2016c). Furthermore, the lowest dose of ATDs should be employed during pregnancy, keeping serum FT4 in the upper third of normal range (Bartalena et al. 2016c).

Table 6 Birth defects that have been associated with exposure to carbimazole/methimazole and propylthiouracil in early pregnancy

Carbimazole/methimazole	Propylthiouracil
Choanal atresia	Face and neck (preauricular sinus/cyst)
Omphalocele	Fistula of branchial cleft
Esophageal atresia	Congenital hydronephrosis
Omphalomesenteric duct anomalies	Single cyst of the kidney
Aplasia cutis	Posterior urethral valve
Malformations of the nipples	Megaureter
Anomalies of the eyes	
Malformations of the circulatory system (heart septal defect, ventricular septal defect, pulmonary valve stenosis, pulmonary artery stenosis)	
Malformations of the urinary system	

Derived from Linding Andersen et al. (2013, 2014)

Childhood

Graves' disease is the most frequent etiology of hyperthyroidism in childhood (Rivkees 2016). ATDs represent the first-line therapy in children, and MMI is the preferred thionamide, based on a better safety profile, just as in adults (Rivkees and Szarfman 2010; Rivkees 2016). Unfortunately, the rate of relapses after ATD withdrawal is higher than in adults (Havgaard Kjaer et al. 2015). Although it is unsettled whether long-term ATD treatment for many years may increase the chance of a permanent remission, this approach is reasonable for children who are too young for RAI treatment or surgery, but in the end most of the children will need an ablative treatment (Rivkees 2016). Although data are reassuring on long-term safety of RAI, according to recent American guidelines, RAI treatment should be avoided if children are <5 years of age (Ross et al. 2016). Thyroidectomy is a valid option in children, although it seems to be associated with a higher risk of complications (Rivkees 2016). Therefore, thyroidectomy should be opted for in children who are too young to be treated with RAI, and it should be performed by a skilled surgeon, as in adults (Ross et al. 2016).

Presence of Graves' Orbitopathy (see also ▶ Chap. 15, "Graves' Ophthalmopathy")

Management of hyperthyroidism in patients with associated GO is challenging (Table 7). Patients with mild GO can be treated with either of therapeutic options (ATDs, RAI treatment, or thyroidectomy). If RAI is selected and the patient has mild signs or symptoms of activity, steroid prophylaxis is recommended in most of the patients (see Radioiodine Treatment) (Bartalena et al. 2015). When GO is very severe and sight-threatening, priority should be given to the cure of GO (medically or surgically), and hyperthyroidism should be controlled with ATDs (Bartalena et al. 2015). If GO is moderate-to-severe, but stably inactive, hyperthyroidism can be treated by any of the available treatments without any particular precaution

Table 7 Treatment of Graves' hyperthyroidism in the presence of Graves' orbitopathy

Severity of GO	Activity of GO	Treatment for hyperthyroidism
Mild	Active	Any treatment for hyperthyroidism, depending on standard criteria and patient choice. If RAI is selected, steroid prophylaxis (see text) is advised
Mild	Inactive	Any treatment for hyperthyroidism, as above. Steroid prophylaxis after RAI is not necessary in the absence of risk factors for RAI-associated progression of GO (smoking, high TRAb levels)
Moderate to severe	Active	Priority should be given to prompt management of GO with intravenous glucocorticoids. It is unsettled whether hyperthyroidism should be in the meanwhile treated conservatively or whether the thyroid should be concomitantly ablated
Moderate to severe	Inactive	Any treatment for hyperthyroidism, as above. No steroid prophylaxis is required
Sight threatening (dysthyroid optic neuropathy and/or corneal breakdown)	Active	Emergent treatment for GO (high doses of intravenous glucocorticoids and/or orbital decompression). Keep the patient euthyroid with antithyroid drugs, and postpone definitive treatment until GO is cured

concerning GO. If moderate-to-severe GO is in its active phase, it should be treated promptly, because efficacy of treatment is inversely related to duration of GO (Bartalena et al. 2016c). Under these circumstances, it is still unsettled whether ATDs, given for prolonged periods of time, are preferable to thyroid ablation performed concomitantly with the management of GO (Bartalena et al. 2015).

Subclinical Hyperthyroidism (see also ▶ Chap. 14, "Graves' Disease")

Although hyperthyroidism due to Graves' disease is usually overt, subclinical hyperthyroidism is not infrequent at diagnosis, involving up to 20–30% of early diagnosed patients (Bartalena et al. 2016d; Zhyzhneuskaya et al. 2016). These patients may either remain subclinical hyperthyroid, revert to euthyroidism, or progress to overt hyperthyroidism (rule of thirds) (Zhyzhneuskaya et al. 2016). The choice between active treatment of subclinical hyperthyroid patients versus strict surveillance depends on the degree of TSH suppression, the age of the patient, and the presence of comorbidities that make this condition a risky situation (Biondi et al. 2015).

Management of Graves' Hyperthyroidism During Immune Reconstitution

Graves' disease my occur during immune reconstitution from a lymphopenic disorder, such as highly active antiretroviral therapy (HAART) for HIV infection, alemtuzumab for multiple sclerosis, and bone marrow or stem cell transplantation

(Weetman 2014). Evidence is limited on the management of Graves' hyperthyroidism in these conditions, but ATDs are indicated as first-line treatment, also because they are probably associated with a higher rate of remission of hyperthyroidism compared to the general Graves' patients (Weetman 2014).

Thyroid Storm

Thyroid storm is an endocrine emergency, characterized by an exacerbation of thyrotoxic symptoms with possible systemic decompensation (heart failure, liver failure, psychosis, coma), usually triggered by precipitant events (e.g., infections or other acute illnesses, thyroid or nonthyroidal surgery in undiagnosed or inadequately treated hyperthyroid patient), and with a substantial mortality rate (Angell et al. 2015). Management of this life-threatening condition should be aggressive and include high doses of ATDs (PTU rather than MMI in view of its peripheral effect on T4 to T3 conversion), β- blockers, inorganic iodine, glucocorticoids, cooling with acetaminophen and cooling blankets, blood volume respiratory and nutritional support, and treatment of the underlying disorder precipitating thyroid storm (Ross et al. 2016).

Treatment of Graves' Orbitopathy

Severe and sight-threatening forms of GO are rare nowadays, and even mild GO is found in only 25% of newly diagnosed Graves' patients, is often remitting upon restoration of euthyroidism (Tanda et al. 2013), and rarely progresses to more severe forms (Piantanida et al. 2013). Moderate-to-severe forms account for approximately 5% of cases (Bartalena and Fatourechi 2014).

Mild GO

In most cases of mild GO, a watchful strategy is sufficient, supported by local measures (artificial tears, ointments) and removal of risk factors (particularly smoking). In a randomized placebo-controlled clinical trial, selenium supplementation improved mild GO and prevented its progression to moderate-to-severe forms (Marcocci et al. 2011). In rare patients, whose quality of life is deeply impaired despite GO being objectively mild, intravenous glucocorticoid treatment, as for moderate-to-severe GO, may be considered, although, under these circumstances, risks likely outweigh benefits (Bartalena et al. 2016a).

Moderate-To-Severe GO

Management of these forms depend on the degree of activity of the disease. In active GO, treatment is medical, whereas in inactive forms treatment is surgical.

Active

First-Line Treatment

High-dose intravenous glucocorticoids are the first-line treatment for moderate-to-severe and active GO (Zang et al. 2011) (Fig. 3), because they are more effective and better tolerated than oral glucocorticoids (Marcocci et al. 2001; Kahaly et al. 2005). Intravenous glucocorticoids are usually given in 12 slow (2–3 h), weekly infusions. Modalities of pulse administration and cumulative doses of the drug are extremely variable throughout Europe (Lazarus et al. 2010), but the most common regimen consists in the administration of a total of 4.5 g of methylprednisolone (6 infusions of 500 mg, followed by 6 infusions of 250 mg) (Kahaly et al. 2005). In a large randomized clinical trial by EUGOGO, three different cumulative doses of methylprednisolone (2.25 g, 4.98 g, 7.47 g) were assessed (Bartalena et al. 2012): although the highest dose was slightly more effective, it was also associated with more frequent side effects (Bartalena et al. 2012). In view of the potential serious adverse events of this treatment (Marcocci et al. 2012), EUGOGO recommended a medium dose (4.5 g) for most cases, reserving the higher dose (7.5 g) to most severe forms within the spectrum of moderate-to-severe GO (Bartalena et al. 2016a). The cumulative dose of glucocorticoids should not be higher than 8 g to reduce the risk of hepatotoxicity (Le Moli et al. 2007; Sisti et al. 2015a), and the single dose should not exceed 0.75 g (Riedl et al. 2015). Contraindications to high-dose intravenous glucocorticoids are represented by severe cardiovascular problems, psychiatric disorders, uncontrolled hypertension or diabetes, liver dysfunction, and recent viral hepatitis (Zang et al. 2011). Soft tissue changes and extraocular muscle dysfunction usually respond very well to treatment, while exophthalmos is less responsive (Bartalena and Fatourechi 2014). To improve final outcome, treatment should be started within 1 year from the onset of GO (Bartalena et al. 2016a). Unfortunately GO flares up in about one third of patients after glucocorticoid withdrawal (Bartalena et al. 2012). In these cases, patients should be either submitted to a second course of intravenous glucocorticoids or to a second-line treatment (Bartalena 2011) (Fig. 3).

Second-Line Treatments

Orbital radiotherapy is particularly effective on disturbances of ocular motility (Tanda and Bartalena 2012). It is commonly given in 10 daily fractions over a 2-week period, using a cumulative dose of 20 Gy per eye (Tanda and Bartalena 2012), but different protocols with lower doses or more prolonged regimens have been proposed (Kahaly et al. 2000). The association of orbital radiotherapy and *oral* glucocorticoids is more effective than either treatment alone (Tanda and Bartalena 2012). Randomized clinical trials showing that the combination of orbital radiotherapy and *intravenous* glucocorticoids is more effective than either treatment alone are missing, but a recent retrospective study showed the effectiveness of combination therapy (Sisti et al. 2015b) Orbital radiotherapy is safe, but should not be used in patients with diabetic or hypertensive retinopathy (Bartalena et al. 2016a).

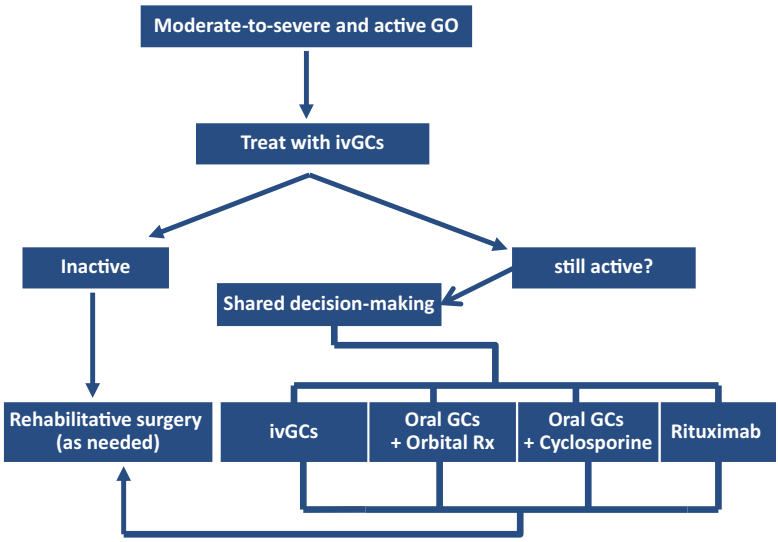

Fig. 3 Treatment of moderate-to-severe and active Graves' orbitopathy (*GO*). *ivGCs* intravenous glucocorticoids, *Rx* radiotherapy, *Rehabilitative surgery* orbital decompression, squint surgery, eyelid surgery (Derived from Bartalena et al. 2016a)

The combination of **cyclosporine** and oral glucocorticoids can be considered a valid alternative. This is based on the results of two randomized clinical trials (Kahaly et al. 1986; Prummel et al. 1989) reporting that the combined treatment was more effective than either drug alone. The starting dose of cyclosporine was 5 mg/kg body weight in one study (Kahaly et al. 1986) and 7.5 mg/kg body weight in the other one (Prummel et al. 1989). Cyclosporine treatment may cause side effects, including dose-dependent liver and renal toxicity, infections, and gingival hyperplasia (Bartalena et al. 2016a).

The use of **rituximab**, CD20+ B cell-depleting monoclonal antibody has been reported, after a few uncontrolled studies (Hegedus et al. 2011; Salvi et al. 2013), in two small randomized clinical trials, comparing rituximab with placebo (Stan et al. 2015) or with intravenous glucocorticoids (Salvi et al. 2015). Results were conflicting, because in the first study, the effects of rituximab did not differ from those of placebo (Stan et al. 2015), whereas in the other one rituximab inactivated GO as well as intravenous glucocorticoids, without any relapse after treatment discontinuation, at variance with glucocorticoids (Salvi et al. 2015). The reasons for this discrepancy between the two studies remain elusive, but in the first study, the duration of disease was longer and patients were older, possibly making these patients less responsive to treatment (Stan and Salvi 2016). In the absence of larger, multicenter randomized clinical trials, for the time being rituximab cannot be recommended as first-line treatment for GO. Rituximab treatment is not devoid of side effects. In particular, in the above studies, progression of dysthyroid optic neuropathy (DON) cumulatively occurred in 4 of 25 patients (16%) during rituximab

treatment (Wiersinga 2016). Accordingly, rituximab should not be administered to patients with impending or overt DON (Bartalena et al. 2016a).

Because all of the available treatments for moderate-to-severe and active GO are often unsatisfactory, pros and cons of each treatment should be discussed with the patient in a shared decision-making dialogue (Stiggelbout et al. 2012).

Inactive

Medical treatment is ineffective in patients whose GO is inactive. In such patients, residual manifestations (exophthalmos, strabismus, eyelid malposition) can be corrected surgically by orbital decompression, squint surgery, and eyelid surgery, respectively. GO should be inactive for at least 6 months prior to rehabilitative surgery. Should all of the three procedures be needed, they should be performed in the above order.

Very Severe (Sight-Threatening) Graves' Orbitopathy

Dysthyroid optic neuropathy (DON) and corneal breakdown are sight-threatening and constitute an emergency situation. Very high doses of intravenous methylprednisolone (500–1000 mg for 3 consecutive days or on alternate days during the first week, to be repeated during the second week) are the first-line treatment, but, if the response is inadequate, patients should urgently be submitted to orbital decompression (Bartalena et al. 2016a).

Treatment of Thyroid Dermopathy and Acropachy

Thyroid dermopathy (also known as pretibial myxedema) is a very rare manifestation, affecting approximately 4% of Graves' patients with GO (Bartalena and Fatourechi 2014). Thyroid acropachy is even rarer, occurring in 0.3% of Graves' patients (Bartalena and Fatourechi 2014). In most cases, thyroid dermopathy is mild and may remit spontaneously or following treatment with local glucocorticoids (fluocinolone acetonide, clobetasol propionate, or triamcinolone cream base 0.05–0.1%) covered by Saran plastic wrap occlusive dressing (12 h/day for 4–6 weeks) (Bartalena and Fatourechi 2014). Compression stockings (20–40 mmHg pressure), complete decompressive physiotherapy, and manual lymphatic drainage or massage may help also in severe (elephantiasic) forms. Surgery is contraindicated, because it may worsen skin lesions (Bartalena and Fatourechi 2014). The benefit of intralesional injections of a solution of lidocaine, dexamethasone, and saline needs to be confirmed in larger studies (Bartalena and Fatourechi 2014). As to the effectiveness of systemic immunosuppressive treatments, as well as of plasmapheresis or intravenous immunoglobulins, evidence of benefit is anecdotal (Bartalena and Fatourechi 2014).

For acropachy there is no treatment, except for pain-relieving drugs in the case of periostitis (Bartalena and Fatourechi 2014).

References

Abraham P, Avenell A, Park CM, Watson WA, Bevan JS. A systematic review of drug therapy for Graves' hyperthyroidism. Eur J Endocrinol. 2005;153:489–98.

Angell TE, Lechner MG, Nguyen CT, Salvato VL, Nicoloff JT, LoPresti JS. Clinical features and hospital outcome in thyroid storm: a retrospective cohort study. J Clin Endocrinol Metab. 2015;100:451–9.

Barbesino G, Tomer Y. Clinical utility of TSH receptor antibodies. J Clin Endocrinol Metab. 2013;98:2247–55.

Bartalena L. The dilemma of how to manage Graves' hyperthyroidism in patients with associated orbitopathy. J Clin Endocrinol Metab. 2011;96:592–9.

Bartalena L. Diagnosis and management of Graves disease: a global overview. Nat Rev Endocrinol. 2013;9:724–34.

Bartalena L, Fatourechi V. Extrathyroidal manifestations of Graves' disease: a 2014 update. J Endocrinol Invest. 2014;38:691–700.

Bartalena L, Marcocci C, Bogazzi F, Manetti L, Tanda ML, Dell'Unto E, et al. Relation between therapy for hyperthyroidism and the course of Graves' ophthalmopathy. N Engl J Med. 1998;338:73–8.

Bartalena L, Krassas GE, Wiersinga WM, Marcocci C, Salvi M, Daumerie C, et al. Efficacy and safety of three different cumulative doses of intravenous methylprednisolone for moderate to severe and active Graves' orbitopathy. J Clin Endocrinol Metab. 2012;97:4454–63.

Bartalena L, Macchia PE, Marcocci C, Salvi M, Vermiglio F. Effects of treatment modalities for Graves' hyperthyroidism on Graves' orbitopathy: a 2015 Italian Society of Endocrinology consensus statement. J Endocrinol Invest. 2015;38:481–7.

Bartalena L, Baldeschi L, Boboridis K, Eckstein A, Kahaly GJ, Marcocci C, et al. The 2016 European Thyroid Association/European Group on Graves' Orbitopathy guidelines for the management of Graves' orbitopathy. Eur Thyroid J. 2016a;5:9–26.

Bartalena L, Burch HB, Burman HD, Kahaly GJ. A 2013 European survey of clinical practice patterns in the management of Graves' disease. Clin Endocrinol (Oxf). 2016b;84:115–20.

Bartalena L, Chiovato L, Vitti P. Management of hyperthyroidism due to Graves' disease: frequently asked questions and answers (if any). J Endocrinol Invest. 2016c;39:1105–14.

Bartalena L, Masiello E, Magri F, Veronesi G, Bianconi E, Zerbini F, et al. The phenotype of newly diagnosed Graves' disease in Italy in recent years is milder than in the past: results of a large observational longitudinal study. J Endocrinol Invest. 2016d;39:1445–1451.

Biondi B, Bartalena L, Coopper DS, Hegedus L, Laurberg P, Kahaly GJ. The 2015 European Thyroid Association guidelines on diagnosis and treatment of endogenous subclinical hyperthyroidism. Eur Thyroid J. 2015;4:149–63.

Boelaert K, Maisonneuve P, Torlinska B, Franklyn JA. Comparison of mortality in hyperthyroidism during periods of treatment with thionamides and after radioiodine. J Clin Endocrinol Metab. 2013;98:1869–82.

Bogazzi F, Giovannetti C, Fessehatsion R, Tanda ML, Campomori A, Compri E, et al. Impact of lithium on efficacy of radioactive iodine therapy for Graves' disease: a cohort study on cure rate, time to cure, and frequency of increased serum thyroxine after antithyroid drug withdrawal. J Clin Endocrinol Metab. 2010;95:201–8.

Bonnema SJ, Hegedus L. Radioiodine therapy in benign thyroid diseases: effects, side effects, and factors affecting therapeutic outcome. Endocr Rev. 2012;33:920–80.

Bonnema SJ, Bennedbaek FN, Gram J, Veje A, Marving J, Hegedus L. Resumption of methimazole after 131I therapy of hyperthyroid diseases: effect on thyroid function and volume evaluated by a randomized clinical trial. Eur J Endocrinol. 2003;149:485–92.

Bowman P, Osborne NJ, Sturley R, Vaidya B. Carbimazole embriopathy: implications for the choice of antithyroid drugs in pregnancy. Q J Med. 2012;105:189–93.

Brandt F, Green A, Hegedus L, Brix TH. A critical review and meta-analysis of the association between overt hyperthyroidism and mortality. Eur J Endocrinol. 2011;165:491–7.

Brandt F, Thvilum M, Almind D, et al. Graves' disease and toxic nodular goiter are both associated with increased mortality but differ with respect to the cause of death: a Danish population-based register study. Thyroid. 2013a;23:408–13.

Brandt F, Thvilum M, Almind D, et al. Morbidity before and after the diagnosis of hyperthyroidsm: a nationwide register-based study. PLoS One. 2013b;8:e66711.

Brito JP, Castaneda-Guarderas A, Gionfriddo NR, Singh Ospina N, Maraka S, Dean DS, et al. Development and pilot testing of an encounter tool for shared decision making about the treatment of Graves' disease. Thyroid. 2015;25:1191–8.

Brito JP, Stephanie S, Singh Ospina N, Rodriguez-Gutierrez R, Maraka S, Sangaralingham R, et al. Antithyroid drugs – the most common treatment for Graves' disease in the United states: a nationwide population-based study. Thyroid. 2016;26:1144–5.

Burch HB, Cooper DS. Management of Graves disease. A review. JAMA. 2015;314:2544–54.

Burch HB, Burman KD, Cooper DS. A 2011 survey of clinical practice patterns in the management of Graves' disease. J Clin Endocrinol Metab. 2012;97:4549–58.

Ceccarelli C, Canale D, Battisti P, Cagliaresi C, Moscatelli A, Gavioli S, et al. Testicular function after ^{131}I therapy for hyperthyroidism. Clin Endocrinol (Oxf). 2006;65:446–52.

Cooper DS. Antithyroid drugs. N Engl J Med. 2005;352:905–17.

Cooper DS, Rivkees SA. Putting propylthiouracil in perspective. J Clin Endocrinol Metab. 2009;94:1881–2.

De Leo S, Braverman LE. Hyperthyroidism. Lancet. 2016;338:906–18.

DeGroot L, Abalovich M, Alexander EK, Amino N, Barbour L, Cobin RH, et al. Management of thyroid dysfunction during pregnancy and postpartum: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2012;97:2543–65.

Eckstein AK, Plicht M, Lax H, Neuhäuser M, Mann K, Lederbogen S, Heckmann C, Esser J, Morgenthaler NG. Thyrotropin receptor autoantibodies are independent risk factors for Graves' ophthalmopathy and help to predict severity and outcome of the disease. J Clin Endocrinol Metab. 2006;91:3464–70.

Emiliano AB, Governale L, Parks M, Cooper DS. Shifts in propylthiouracil and methimazole prescribing practices: antithyroid drug use in the United States from 1991 to 2008. J Clin Endocrinol Metab. 2010;95:2227–33.

Erbil Y, Giriş M, Salmaslioglu A, Ozluk Y, Barbaros U, Yanik BT, Kapran Y, Abbasoglu SD, Ozarmagan S. The effect of anti-thyroid drug treatment duration on thyroid gland microvessel density and intraoperative blood loss in patients with Graves' disease. Surgery. 2008;143:216–25.

Franklyn JA, Maisonneuve P, Sheppard MC, Betteridge J, Boyle P. Mortality after the treatment of hyperthyroidism with radioactive iodine. N Engl J Med. 1998;338:712–8.

Fukata S, Kuma K, Sugawara M. Granulocyte colony-stimulating factor (G-CSF) does not improve recovery from antithyroid drug-induced agranulocytosis: a prospective study. Thyroid. 1999;9:29–31.

Genovese BM, Noureldine SI, Gleeson EM, Tufano RP, Kandil E. What is the best definitive treatment for Graves' disease? A systematic review of the existing literature. Am Surg Oncol. 2013;20:660–7.

Gianetti E, Russo L, Orlandi F, Chiovato L, Giusti M, Benvenga S, et al. Pregnancy outcome in women treated with methimazole or propylthiouracil during pregnancy. J Endocrinol Invest. 2015;38:977–85.

Guo Z, Yu P, Liu Z, Si Y, Jin M. Total thyroidectomy vs bilateral subtotal thyroidectomy in patients with Graves' disease: a meta-analysis of randomized clinical trials. Clin Endocrinol (Oxf). 2013;79:739–46.

Havgaard Kjaer R, Smedegard Hansen M, Hansen D. Increasing incidence of juvenile thyrotoxicosis in Denmark: a nationwide study, 1998–2012. Horm Res Paediatr. 2015;84:102–7.

Hegedus L, Smith TJ, Douglas RS, Nielsen CH. Targeted biological therapies for Graves' disease and thyroid-associated ophthalmopathy. Focus on B-cell depletion with Rituximab. Clin Endocrinol (Oxf); 2011;74:1–8.

Hieu TT, Russell AW, Cuneo R, Clark J, Kron T, Hall P, et al. Cancer risk after medical exposure to radioactive iodine in benign thyroid disease: a meta-analysis. Endocr Relat Cancer. 2012;19:645–55.

In H, Pearce EN, Wong AK, Burgess JF, McAneny DB, Rosen JE. Treatment options for Graves' disease: a cost-effectiveness analysis. J Am Coll Surg. 2009;209:170–9.

Kahaly G, Schrezenmeir J, Krause U, Schweikert B, Meuer S, Muller W, et al. Ciclosporin and prednisone vs. prednisone in treatment of Graves' ophthalmopathy: a controlled, randomized and prospective study. Eur J Clin Invest. 1986;16:415–22.

Kahaly GJ, Rosler HP, Pitz S, Hommel G. Low- versus high-dose orbital radiotherapy for Graves' ophthalmopathy: a randomized, single blind trial. J Clin Endocrinol Metab. 2000;85:102–8.

Kahaly GJ, Pitz S, Hommel G, Dittmar M. Randomized, single blind trial of intravenous versus oral steroid monotherapy in Graves' orbitopathy. J Clin Endocrinol Metab. 2005;90:5234–40.

Kim HJ, Kim BH, Yan JS, et al. The incidence and clinical characteristics of symptomatic propylthiouracil-induced hepatic injury in patients with hyperthyroidism: a single-center retrospective study. Am J Gastroenterol. 2001;96:165–9.

Korelitz JJ, McNally DL, Masters MN, Li SX, Xu Y, Rivkees SA. Prevalence of thyrotoxicosis, antithyroid drug use and neonatal outcomes with an integrated healthcare delivery system. Thyroid. 2013;23:758–65.

La Cour JL, Jensen LT, Vej-Hansen A, Nygaard B. Radioiodine therapy increases the risk of cerebrovascular events in hyperthyroid and euthyroid patients. Eur J Endocrinol. 2015;172:771–8.

Lazarus JH, Bartalena L, Marcocci C, Kahaly GJ, Krassas G, Wiersinga WM. Glucocorticoid administration for Graves' hyperthyroidism treated by radioiodine. A questionnaire survey among members of the European Thyroid Association. J Endocrinol Invest. 2010;33:409–13.

Le Moli R, Baldeschi L, Saeed P, Regensburg N, Mourits MP, Wiersinga WM. Determinants of liver damage associated with intravenous methylprednisolone pulse therapy in Graves' ophthalmopathy. Thyroid. 2007;17:357–62.

Linding Andersen S, Olsen J, Wu CS, Laurberg P. Birth defects after early pregnancy use of antithyroid drugs: a Danish nationwide study. J Clin Endocrinol Metab. 2013;98:4373–81.

Linding Andersen S, Olsen J, Wu CS, Laurberg P. Severity of birth defects after propylthiouracil exposure in early pregnancy. Thyroid. 2014;10:1533–40.

Marcocci C, Bartalena L, Tanda ML, Manetti L, Dell'Unto E, Rocchi R, et al. Comparison of the effectiveness and tolerability of intravenous or oral glucocorticoids associated with orbital radiotherapy in the management of severe Graves' ophthalmopathy: results of a prospective. single-blind, randomized study. J Clin Endocrinol Metab. 2001;86:3562–7.

Marcocci C, Kahaly GJ, Krassas GE, Bartalena L, Prummel M, Stahl M, et al. Selenium and the course of mild Graves' orbitopathy. N Engl J Med. 2011;364:1920–32.

Marcocci C, Watt T, Altea MA, Rasmussen AK, Feldt-Rasmussen, Orgiazzi J, et al. Fatal and non-fatal adverse events of glucocorticoid therapy for Graves' orbitopathy: a questionnaire survey among members of the European Thyroid Association. Eur J Endocrinol. 2012;166:247–53.

Metso S, Jaatinen P, Huhtala H, Auvinen A, Oksala H, Salmi J. Increased cardiovascular and cancer mortality after radioiodine treatment for hyperthyroidism. J Clin Endocrinol Metab. 2007;92:2190–6.

Perros P, Kendall-Taylor P, Neoh C, Frewin S, Dickinson J. A prospective study of the effects of radioiodine therapy for hyperthyroidism in patients with minimally active Graves' ophthalmopathy. J Clin Endocrinol Metab. 2005;90:5321–3.

Piantanida E, Tanda ML, Lai A, Sassi L, Bartalena L. Prevalence and natural history of Graves' orbitopathy in the XXI century. J Endocrinol Invest. 2013;36:444–9.

Piantanida E, Lai A, Sassi L, Gallo D, Spreafico M, Tanda ML, Bartalena L. Outcome prediction of treatment of Graves' hyperthyroidism with antithyroid drugs. Horm Metab Res. 2015;47:767–72.

Prummel MF, Mourits MP, Berghout A, Krenning EP, van der Gaag R, Koornneef L, et al. Prednisone and cyclosporine in the treatment of severe Graves' ophthalmopathy. N Engl J Med. 1989;321:1353–9.

Riedl M, Kolbe E, Kampmann E, Kramer I, Kahaly GJ. Prospectively recorded and MedDRA-coded safety data of intravenous methylprednisolone therapy in Graves' orbitopathy. J Endocrinol Invest. 2015;38:177–82.

Rivkees SA. Controversies in the management of Graves' disease in children. J Endocrinol Invest. 2016;39:1247–57.

Rivkees SA, Szarfman A. Dissimilar hepatoxicity profiles of propylthiouracil and methimazole in children. J Clin Endocrinol Metab. 2010;95:3260–7.

Ross DS. Radioiodine therapy for hyperthyroidism. N Engl J Med. 2011;364:542–50.

Ross DS, Burch HB, Copper DS, Greenlee MC, Laurberg P, Maia AL, et al. American Thyroid Association guidelines for diagnosis and management of hyperthyroidism and other causes of thyrotoxicisos. Thyroid. 2016;26:1343–421.

Ruiz JK, Rossi GV, Vallejos HA, Brener RW, Lopez IB, Escribano AA. Fulminant hepatic failure associated with propylthiouracil. Ann Pharmacother. 2003;37:224–8.

Ryodi E, Metso S, Jaatinen P, Huhtala H, Saaristo R, Valimaki M, et al. Cancer incidence and mortality in patients treated either with RAI or thyroidectomy for hyperthyroidism. J Clin Endocrinol Metab. 2015;100:3710–7.

Salvi M, Vannucchi G, Beck-Peccoz P. Potential utility of rituximab for Graves' orbitopathy. J Clin Endocrinol Metab. 2013;98:4291–9.

Salvi M, Vannucchi G, Currò N, Campi I, Covelli D, Dazzi D, et al. Efficacy of B-cell targeted therapy with rituximab in patients with active moderate to severe Graves' orbitopathy: a randomized controlled study. J Clin Endocrinol Metab. 2015;100:422–31.

Sawka AM, Lakra DC, Lea J, Alshehri B, Tsang RW, Brierley JD, et al. A systematic review examining the effects of therapeutic radioactive iodine on ovarian function and future pregnancy in female thyroid cancer survivors. Clin Endocrinol (Oxf). 2008;69:479–90.

Shiber S, Stiebel-Kalish H, Shimon I, Grossman A, Robenshtok E. Glucocorticoid regimens for prevention of Graves' ophthalmopathy progression following radioiodine treatment: systematic review and meta-analysis. Thyroid. 2014;24:1515–23.

Sisson JC, Freitas J, McDougall IR, Dauer LT, Hurley JR, Brierley JD, et al. Radiation safety in the treatment of patients with thyroid diseases by radioiodine 131I: practice recommendations of the American Thyroid Association. Thyroid. 2011;21:335–46.

Sisti E, Coco B, Menconi F, Leo M, Rocchi R, Latrofa F, et al. Intravenous glucocorticoid therapy for Graves' ophthalmopathy and acute liver damage: an epidemiological study. Eur J Endocrinol. 2015a;172:269–76.

Sisti E, Menconi F, Leo M, Profilo MA, Mautone T, Mazzi B, et al. Long-term outcome of Graves' orbitopathy following high-dose intravenous glucocorticoids and orbital radiotherapy. J Endocrinol Invest. 2015b;38:661–8.

Smith TJ, Hegedus L. Graves' disease. N Engl J Med. 2016;375:1552–65.

Sosa IA, Tuggle CT, Wabg TS, Thomas DC, Boudourakis L, Rivkees S, et al. Clinical and economic outcomes of thyroid and parathyroid surgery in children. J Clin Endocrinol Metab. 2008;93:3058–65.

Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, et al. Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. Thyroid. 2011;21:1081–125.

Stan M, Salvi M. Rituximab therapy for Graves' orbitopathy. Lessons from randomized clinical trials. Eur J Endocrinol. 2017;176:R101–9.

Stan MN, Garrity JA, Carranza Leon BG, Prabin T, Bradley EA, Bahn RS. Randomized controlled trial of rituximab in patients with Graves' orbitopathy. J Clin Endocrinol Metab. 2015;100:432–41.

Stiggelbout AM, Van der Weijden T, De Wit MPT, Frosch D, Légare F, Montori V, et al. Shared decision making: really putting patients at the centre of healthcare. Br Med J. 2012;344:e256.

Sundaresh V, Brito JP, Wang Z, Prokop LJ, Stan MN, Murad MH, et al. Comparative effectiveness of therapies for Graves' hyperthyroidism: a systematic review and network meta-analysis. J Clin Endocrinol Metab. 2013;98:3671–7.

Tallstedt L, Lundell G, Blomgren H, Bring J. Does early administration of thyroxine reduce the development of Graves' ophthalmopathy after radioiodine treatment? Eur J Endocrinol. 1994;130:494–7.

Tanda ML, Bartalena L. Efficacy and safety of orbital radiotherapy for Graves' orbitopathy. J Clin Endocrinol Metab. 2012;97:3857–65.

Tanda ML, Piantanida E, Liparulo L, Veronesi G, Lai A, Sassi L, et al. Prevalence and natural history of Graves' orbitopathy in a large series of patients with newly diagnosed Graves' hyperthyroidism seen at a single center. J Clin Endocrinol Metab. 2013;98:1443–9.

Ting HH, Brito JP, Montori VM. Shared decision making. Science and action. Circ Cardiovasc Qual Outcomes. 2014;7:323–7.

Törring O, Tallstedt L, Wallin G, Lundell G, Ljunggren JG, Taube A, et al. Graves' hyperthyroidism: treatment with antithyroid drugs, surgery, or radioiodine – a prospective, randomized study. J Clin Endocrinol Metab. 1996;81:2986–93.

Träisk F, Tallstedt L, Abraham-Nordling M, Andersson T, Berg G, Calissendorff J, et al. Thyroid associated ophthalmopathy after treatment for Graves' hyperthyroidism with antithyroid drugs or iodine-131. J Clin Endocrinol Metab. 2009;94:3700–7.

Tun NN, Beckett G, Zammitt NN, Strachan MW, Seckl JR, Gibb FW. Thyrotropin receptor antibody levels at diagnosis and after thionamide course predict Graves' disease relapse. Thyroid. 2016;26:1004–9.

Vaidya B, Williams GR, Abraham P, Pearce SH. Radioiodine treatment for benign thyroid disorders: results of a nationwide survey of UK endocrinologists. Clin Endocrinol (Oxf). 2008;68:814–20.

Vitti P, Rago T, Chiovato L, Pallini S, Santini F, Fiore E, et al. Clinical features of patients with Graves' disease undergoing remission after antithyroid drug treatment. Thyroid. 1997;7:369–75.

Wang MT, Lee WJ, Huang TY, Chu CL, Hsieh CH. Antithyroid drug-related hepatotoxicity in hyperthyroidism patients: a population-based cohort study. Br J Clin Pharmacol. 2014;78:619–29.

Watanabe N, Narimatsu H, Yoshimura Noh J, Yamaguchi T, Kobayashi K, Kami M, et al. Antithyroid drug-induced hematopoietic damage: a retrospective cohort study of agranulocytosis and pancytopenia involving 50, 385 patients with Graves' disease. J Clin Endocrinol Metab. 2012;97:E49–53.

Weetman AP. Graves' disease following immune reconstitution of immunomodulatory treatment: should we manage it any differently? Clin Endocrinol (Oxf). 2014;80:629–32.

Wiersinga W. Advances in treatment of active, moderate-to-severe Graves' ophthalmopathy. Lancet Diabetes Endocrinol. 2016; doi:10.1016/S2213-8587(16)30046-8 (e-pub ahead of print).

Yang J, Li LF, Xu Q, Zhang J, Weng W-W, Zhu YI, et al. Analysis of 90 cases of antithyroid drug-induced severe hepatotoxicity over 13 years in China. Thyroid. 2015;25:278–83.

Yang J, Zhu Y-J, Zhong JJ, Zhang J, Weng W-W, Z-f L, et al. Characteristics of drug-induced agranulocytosis in patients with hyperthyroidism: a retrospective analysis of 114 cases in a single institution in China involving 9690 patients referred for radioiodine treatment over 15 years. Thyroid. 2016;26:627–33.

Yoshihara A, Noh J, Yamaguchi T, Ohye H, Sato S, Seiya K, et al. Treatment of Graves' disease with antithyroid drugs in the first trimester of pregnancy and the prevalence of congenital malformation. J Clin Endocrinol Metab. 2012;97:2396–403.

Zang S, Ponto KA, Kahaly GJ. Clinical Review: intravenous glucocorticoids for Graves' orbitopathy: efficacy and morbidity. J Clin Endocrinol Metab. 2011;96:320–32.

Zhyzhneuskaya S, Addison C, Tsatlidis V, Weaver JU, Razvi S. The natural history of subclinical hyperthyroidism in Graves' disease: the rule of thirds. Thyroid. 2016;26:765–9.

Toxic Adenoma and Multinodular Toxic Goiter

17

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Abstract

Toxic thyroid adenoma (TA) is a well-encapsulated homogeneous neoplasia secreting thyroid hormones in the absence of a TSH stimulus, in an otherwise normal gland. The diagnosis involves the ability to take up iodide autonomously and the decreased or suppressed uptake in the rest ofthe thyroid, as demonstrated by scintigraphy. Toxic multinodular goiter (TMNG) encompasses a spectrum of pathologies ranging from a single hyperfunctioning nodule within an enlarged thyroid gland, which has additional normal or nonfunctioning nodules, to multiple hyperfunctiong nodules.Toxic adenoma is more common in women, and can occur at any age, being more frequent between the ages of 30 and 60 years, while TMNG tends to occur at older age. The prevalence of TA and TMNG as a cause of thyrotoxicosis varies throughout the world, and higher prevalences are observed in areas with mild to moderate iodine deficiency. Both TA and TMNG are characterized by the presence of autonomous tissues. TA are monoclonal benign encapsulated tumors that grow, metabolize iodide and secrete thyroid hormones independently of TSH control. The metabolism in autonomous adenomas is characterized mainly by a greatly increased iodine accumulation and consequently by a high iodination rate. Activating TSHR mutations and with lower frequencies Gs-alpha mutations are the main causes of TA or in hyperfunctioning nodules within TMNGs. Furthermore, a recent study has identified a second hit mutation in enhancer of zeste homolog 1 (EZH1) in TA. A significant proportion of patients with TA or TMNG develops thyrotoxicosis, and this is directly related to the duration the goiter has been present. Typically, the thyrotoxicosis comes about insidiously, hence the patient is often unaware of the symptoms. This is particularly seen in the elderly. The symptoms of thyrotoxicosis are those observed with other causes of thyroid hormone excess. The diagnosis of TA and TMNG is based on clinical examination, thyroid function tests, thyroid ultrasound and scintiscanning. Due to the underlying molecular defect, there is no spontaneous resolution of TA and TMNG. Hence ablative treatment is generally indicated, once thyroid autonomy is diagnosed with subclinical or overt hyperthyroidism. The two most widely used ablation modalities are thyroid surgery and radioiodine. Both options, their advantages and potential risks should be openly discussed with the patient and the final decision will be based on patient characteristics (age, the severity of hyperthyroidism, goitre size and extent of nodular thyroid disease, concomitant non-thyroid illness), patient's preferences, possibly costs and and also logistics.

Keywords

Toxic adenoma · Toxic multinodular goiter · Goiter · TSH receptor mutations · Cell proliferation · Hyperthyroidism · 131-I therapy · Surgical treatment · Antithyroid drugs

Introduction

Toxic thyroid adenoma (TA) is a well-encapsulated homogeneous neoplasia secreting thyroid hormones in the absence of a TSH stimulus, in an otherwise normal gland. The diagnosis involves the ability to take up iodide autonomously and the decreased or suppressed uptake in the rest of the thyroid, as demonstrated by scintigraphy. Toxic multinodular goiter (TMNG) encompasses a spectrum of pathologies ranging from a single hyperfunctioning nodule within an enlarged thyroid gland, which has additional normal or nonfunctioning nodules, to multiple hyperfunctiong nodules.

TA and TMNG are mainly present in iodine-deficient areas and TMNG may represent the natural evolution of diffuse or nodular goiters (Holzapfel et al. 1997; Tonacchera et al. 1998a; Krohn et al. 2005). Nodular goiters (NGs) are clinically recognizable enlargements of the thyroid gland. In the absence of thyroid dysfunction, autoimmune thyroid disease and thyroid malignancy, they constitute an entity described as nontoxic NG (Hegedus et al. 2003; Krohn et al. 2005; Studer et al. 1989). NG occurs both endemically, mainly related to iodine deficiency, and sporadically. In the early phase of goitrogenesis goiters are diffuse and, with time, tend not only to grow but also to become nodular. In general, NG can be divided into solitary nodular and multinodular thyroid disease. Concomitantly with growth, thyroid function often becomes autonomous. That is, thyroid hormone secretion becomes independent of thyrotropin secretion (thyroid autonomy) and therefore the patients gradually develop subclinical and eventually overt hyperthyroidism. Thus, the natural history is characterized clinically by thyroid growth, nodule formation, and the development of functional autonomy (Elte et al. 1990). The clinical forms include both TA and TMNG and are responsible for the development of non-autoimmune hyperthyroidism (Krohn et al. 2005).

Clinical Aspects and Epidemiology

In the clinical setting, TA often presents as a palpable thyroid nodule, able to concentrate radioiodine avidly with functional inhibition of the extranodular thyroid tissue at thyroid scintiscan. Rarely two or more toxic adenomas coexist in an otherwise normal thyroid gland (multiple adenomatosis, multifocal autonomy (Fig. 1)) (Krohn et al. 2005). TA is more common in women, with a female:male ratio ranging from 6:1 to 15:1, and can occur at any age, being most frequent between the ages of 30 and 60, while TMNG tends to occur at an older age (Reinwein et al. 1988; Krohn et al. 2005). TMNG has also been termed “Plummer’s disease.” The prevalence of TA, as a cause of thyrotoxicosis, varies throughout the world and the determination of the true prevalence may be hampered by the difficulty in distinguishing between true TA and hyperfunctioning nodules within a goiter (see below) (Krohn et al. 2005). In the United States, an iodine-sufficient

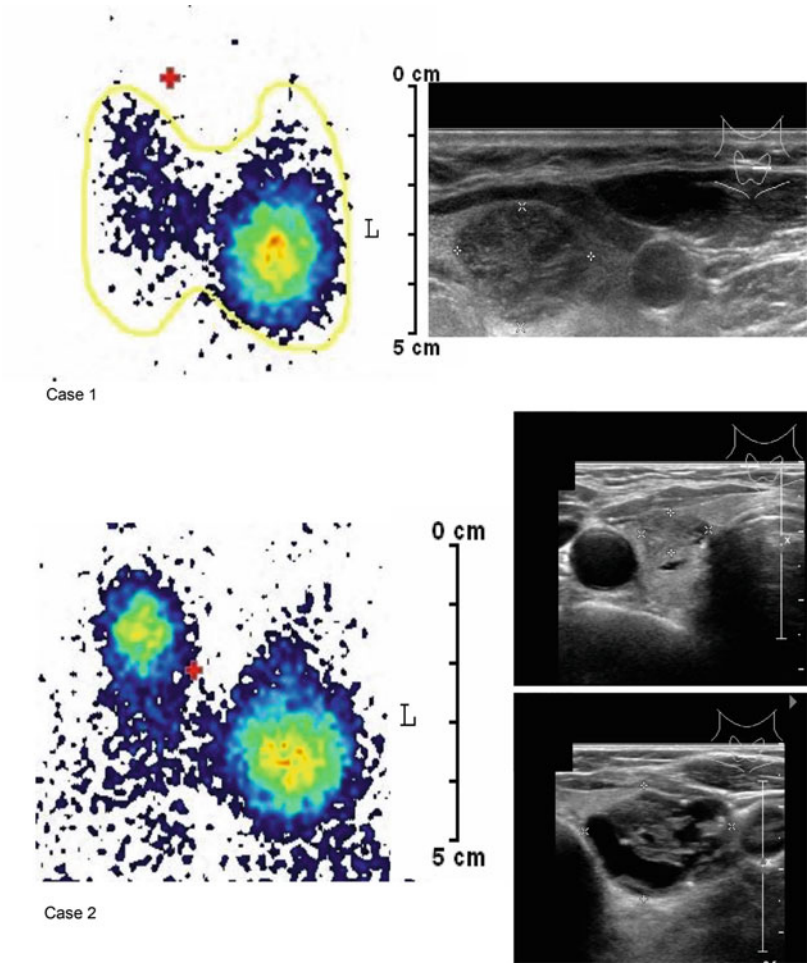

Fig. 1 **^{99m}technecium scintiscan of a TA and a TMNG.** Increased focal uptake in the thyroid lobe with suppression of the surrounding thyroid tissue and contralateral lobe (TA) and increased multifocal radionuclide uptake in both the right and the left thyroid lobe, with suppression of surrounding tissue, is seen. Both patients presented with overt hyperthyroidism and a left-sided nodule (case 1) and multinodular goiter (case 2) with a partially cystic nodule in the left and a small nodule in the right upper lobe on ultrasound

country, TAs are reported to account for only 2% of all cases of hyperthyroidism (Hamburger 1987), whereas higher prevalences are observed in Europe (areas with mild to moderate iodine deficiency) (Reinwein et al. 1988; Lauberg et al. 1991). Before iodine prophylaxis, in some areas of Switzerland and Germany, TAs accounted for more than 30% of cases of thyrotoxicosis (Baltisberger et al. 1995).

In a prospective multicenter study performed in six European countries, 9% of thyrotoxic patients had a TA, with a prevalence more than three times higher in areas with a relatively low iodine intake compared with areas in which iodine intake was

higher (Reinwein et al. 1988). In particular, the prevalence of TA in the United Kingdom was 10% in iodine-deficient areas and 3% in iodine-sufficient areas. Similarly, in a retrospective study in Sicily, TA was twice as common in an iodine-deficient compared to an iodine-sufficient area (Belfiore et al. 1985). Denmark was an area of iodine deficiency, when mandatory iodine fortification of table salt and salt in bread was initiated in 2000 and a program for monitoring iodine intake and thyroid diseases developed (Dan Thyr program) (Laurberg et al. 2006). In years 1997–2000 the overall standardized incidence rate per 100,000 person-years for hyperthyroidism was 81.6 higher in Aalborg (moderate iodine-deficient area) compared to Copenhagen (mild iodine-deficient area). TA represented 5.7% of all cases of hyperthyroidism (Carle et al. 2011) and the incidence rate of TA rose with age and peaked in the sixth decade (Carle et al. 2011).

TA and TMNG have a multifaced clinical presentation. It spans from a single hyperfunctioning nodule, where several normal or nonfunctioning nodules also coexist, to a nodular goiter where multiple hyperfunctioning areas, not confined to distinct nodules, are barely distinguishable from other nodules (Krohn et al. 2005; Tonacchera et al. 2010). TMNG is the most common cause of hyperthyroidism in iodine-deficient areas. In a landmark study the incidence of different types of hyperthyroidism was compared between East Jutland, an area of low iodine intake, and Iceland, a country with adequate iodine intake (Lauberg et al. 1998). In the area of low iodine intake, toxic multinodular goiter was the most common cause of hyperthyroidism, while it was infrequent in Iceland, where Graves' disease was the dominant cause of hyperthyroidism. The age distribution of new cases of hyperthyroidism also demonstrated large differences between the two regions. In Iceland, the majority of cases of hyperthyroidism were observed in people aged 20–60 years. The typical hyperthyroid patient was a young to middle-aged woman with Graves' disease. In Jutland, there was a peak of hyperthyroidism in the elderly, the typical patient being an elderly woman with toxic multinodular goiter. Furthermore, the incidence of hyperthyroidism was significantly higher in Jutland (38.7/100000/year) than in Iceland (23.6/100000/year).

In the Dan Thyr program, TMNG constituted 44% of all cases of hyperthyroidism (Graves' diseases accounted for 37% of cases), with a higher incidence in Aalborg (moderate iodine deficient) than in Copenhagen (mild iodine deficient). With advancing age above 45 years, TMNG increasingly outnumbered Graves' disease (Carle et al. 2011).

The critical role of iodine deficiency in the development of thyroid autonomy is also demonstrated by the sustained decreased incidence of this disease that occurred in several countries including Switzerland (Baltisberger et al. 1995), Denmark (Carle et al. 2011), and many others (Hegedus et al. 2003; Krohn et al. 2005) after institution and implementation of iodine prophylaxis.

In a cross-sectional study on the spectrum of thyroid disorders occurring in a community with mild-to-moderate iodine deficiency in the South of Italy (Aghini-Lombardi et al. 1999), it was shown that the prevalence of goiter, thyroid nodularity, and functional autonomy increased with age. Thus, the prevalence of goiter rose from 16% in children to 60% in adults. Presence of nodular goiter was negligible in the 15–25 year age group, but increased up to 29% in the 56–65 year

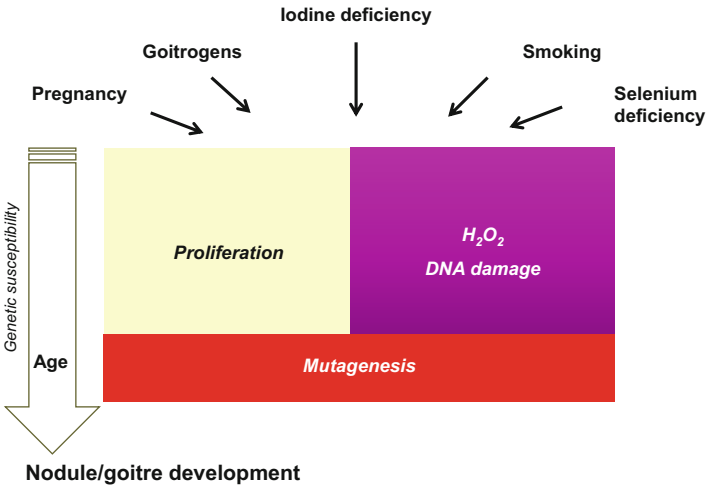

Fig. 2 Exogenous and endogeneous factors contributing to nodule and goiter development. Both are characterized by increased thyrocyte proliferation, which together with increased oxidative stress augments the likelihood of increased mutagenesis

age group. Thyroid autonomy was rare in children and progressively increased with age to reach 15% in the older age group. Hyperthyroidism was mainly due to toxic nodular goiter and was more frequent in aged people. These data indicate that the natural history of functional autonomy and nodular goiter is that they evolve together (Fig. 2). The few longitudinal studies in patients with a multinodular goiter confirm these cross-sectional data. In one study, 11% of 90 patients with nontoxic multinodular goiter developed hyperthyroidism within 1–7 years (Als et al. 1995).

Pathology of TA and TMNG

TAs are well-defined encapsulated lesions (adenomas) that are characteristically surrounded by a complete fibrous capsule and have a homogeneous pattern of growth. Variations in architecture mostly depend on the degree of cellularity and presence and size of follicles. Pseudo-papillary formations may also be present. Adenomas are classified as microfollicular, normofollicular, or macrofollicular. Cells lining the follicle tend to be tall cuboidal rather than flattened, and the nuclear/cytoplasmatic ratio is likely to be decreased due to cytoplasmic prominence (Viacava et al. 2007).

The basic process in the pathogenesis of TMNG is the proliferation of follicular epithelial cells resulting in the formation of new follicles (Studer et al. 1989; Krohn et al. 2005, 2007; Roger et al. 2010). During goitrogenesis new daughter follicles are growing out from single cells or small families of cells of mother follicles. If the progenitor cells are different (with respect to their iodination capacity and their growth rate) clusters of follicles of different size and different function must necessarily arise with time. Autonomously hyperfunctioning follicles may occur in clusters adjacent to each other or they

may be intermingled with normally functioning follicles giving the typical patchy scintigraphic patterns seen at thyroid scintiscan (Fig. 1). The nodular growth pattern is the ultimate outcome and the nodular areas may contain hyper- and hypofunctional follicles. More frequently nodules are constituted by multiple microfollicular and macrofollicular aggregates not confined by a capsule (hyperplastic nodules) (Studer et al. 1992; Viacava et al. 2007; Krohn et al. 2005). Nodules are separated by irregular strands of apparently normal micro-macrofollicular parenchyma. Secondary changes that can be seen at the gross level include hemorrhage, fibrosis, calcification, ossification, and cystic degeneration, all of which contribute to heterogeneous appearance of growing goiters.

Biochemical and Growth Properties of Autonomous Tissue

Both TA and TMNG are characterized by the presence of autonomous tissues (Corvilain et al. 2000; Dremier et al. 1996; Deleu et al. 2000; Krohn et al. 2000). TAs are monoclonal benign encapsulated tumors that grow, metabolize iodide, and secrete thyroid hormones independently of TSH control. The metabolism in autonomous adenomas is characterized mainly by a greatly increased iodine accumulation and consequently by a high iodination rate. TAs are characterized by an increased iodide transport, increased expression of thyroperoxidase (TPO), and sodium iodine symporter (NIS) mRNAs while thyroglobulin (TG) and TSH receptor (TSHR) gene expression are unchanged (Corvilain et al. 2000; Deleu et al. 2000). The normal expression of E-cadherin gene fits well with the fact that TAs are not invasive and only exceptionally cancerous. As tumors, autonomous adenomas seem to grow rather slowly and adenomatous cells seem to have a longer lifespan than normal cells. TAs have a Ki67 labeling index, a marker of cells in the active phases of the cell cycle, that is higher with respect to both the quiescent tissue surrounding the tumor and the normal thyroid tissue, but definitely lower than that observed in other neoplastic lesions (Corvilain et al. 2000; Krohn et al. 2005).

While in a normal thyroid gland, follicles are functioning in a similar way, respond to TSH in a highly predictable manner, and contain identically iodinated thyroglobulin, as shown by 125-I autoradiographs after equilibrium labeling, follicles in TMNG have a heterogeneous morphology and a heterogenous pattern of iodine uptake. On the one hand, a follicle may no longer participate in iodine turnover (cold follicle), while on the other hand a follicle may display normal or even high iodine turnover (hot follicle), which may be independent of TSH regulation (autonomy, Fig. 3) (Krohn et al. 2000; Corvilain et al. 2000).

Constitutive cAMP Activation as a/the Molecular Hallmark of Autonomous Tissue

The group of Dumont and Vassart were among the first to suggest that any molecular lesion leading to constitutive activity of the cAMP cascade (TSH receptor, G protein, cyclase, protein kinase) could be responsible for the growth and functional

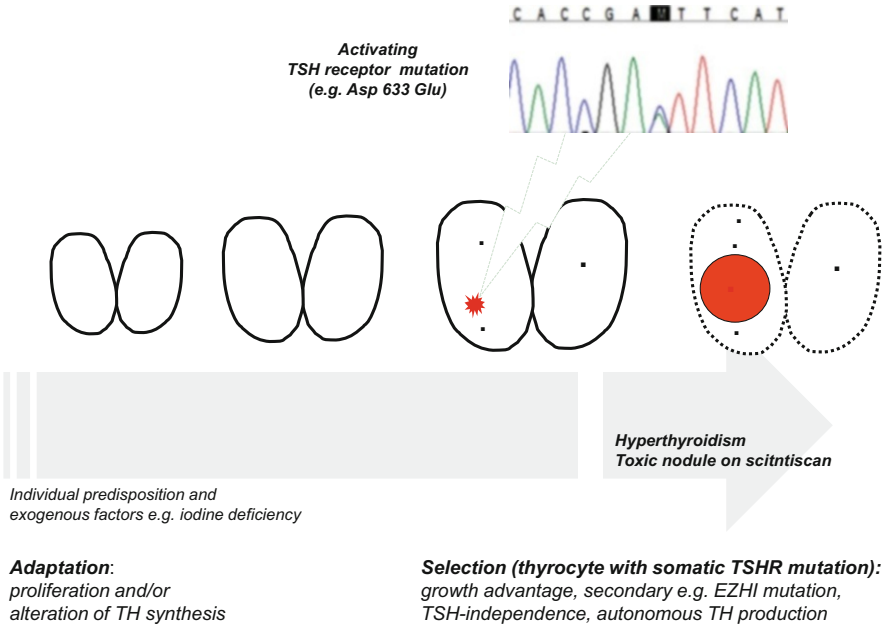

Fig. 3 Development of thyroid autonomy. Mutagenesis, which results in a somatic gain-of-function TSH receptor mutation in a thyrocyte, confers growth and functional advantage over other thyrocytes that harbor the wild-type TSHR. Over time an autonomous nodule develops and increasingly contributes to thyroid hormone production, independent of TSH, the latter in turn starts to decrease. When the nodule reaches a critical mass, hyperthyroidism becomes clinically manifest. TSH levels are low and the surrounding tissue is suppressed on scintiscan with the toxic adenoma appearing as a "hot" nodule" (see also Fig. 1)

properties of toxic nodules (Dumont et al. 1992). In support of this, it was shown that transgenic mice expressing adenosine A2 receptor in the thyroid mimic the phenotype of thyroid autonomy in humans (Ledent et al. 1992). After somatic mutations impairing GTPase activity of Gs alpha had been found in some TA, it was logical to investigate the TSHR gene. In the first study from the group of Dumont and Vassart (Parma et al. 1993), 9 out of 11 TAs studied were shown to harbor an activating TSHR mutation. Subsequent studies have confirmed this observation, describing mutations in other residues (Parma et al. 1997; Porcellini et al. 1994; Paschke et al. 1994; Russo et al. 1996; Tassi et al. 1999; Tonacchera et al. 1996, 1999; Fuhrer et al. 1997, 1998) and with other amino acid exchanges. All the mutations found are heterozygous, as expected from gain-of-function mutations with a dominant effect, and confined to the adenomatous tissue (somatic mutations).

In vitro studies confirmed that these mutants result in constitutive stimulation of adenylylcylase and intracellular cAMP accumulation. These experiments also showed that the wild-type TSH receptor displays a certain/considerable degree of basal constitutive activity (Parma et al. 1993; Van Sande et al. 1995; Paschke et al. 1994; Tonacchera et al. 1996). Furthermore, some mutants were also shown

to activate the phospholipase C-dependent cascade (Parma et al. 1995; Van Sande et al. 1995; Fuhrer et al. 1997).

Interestingly, different mutant receptors varied in their level of constitutive activity, and while showing decreased level of expression when transfected in COS-7 cells, bound radiolabeled bovine TSH with higher affinity than the wild-type TSHR (Parma et al. 1995; Tonacchera et al. 1996). Most mutants respond to stimulation by TSH by further increasing both cAMP and inositolphosphate accumulation, but the magnitude is highly variable, with some mutations behaving as if they were fully activated (e.g., mutant 486F for cAMP) (Parma et al. 1995) or displaying very little stimulation properties (e.g., mutation 672 for inositol phosphates) (Van Sande et al. 1995). Other mutants (N670S for example) clearly show a dissociation in the ability of the receptor to respond to bovine TSH for the Gs alpha and Gq alpha–dependent regulatory cascades, favoring the idea of the existence of multiple active conformations of the TSH receptor, with differential capabilities to couple to Gs alpha and Gq alpha (Tonacchera et al. 1996).

Distinct biological properties of various TSHR mutations have also been demonstrated in rat thyroid follicular cells and human thyrocytes with characteristics not identical to analysis of these mutations in nonthyroid COS-7 cells. This demonstrates the importance of the cell context for biological assessment (Fuhrer et al. 2003). In addition, proteome analyses have demonstrated that the signaling properties of the mTSHR are similar, but not identical, and include involvement of other non-PKA pathways, at least in the rat FRTL-5 cells (Krause et al. 2012). Furthermore, using mTSHR stably expressing FRTL-5 cells as in vitro model of thyroid autonomy, it was shown that exposure to iodine in thyroid autonomy may down-regulate transcription of genes involved in cell proliferation (Muller et al. 2011). This is in line with epidemiological observations from the Pescopagano study (Aghini-Lombardi et al. 1999; Aghini-Lombardi et al. 2013), suggesting that improved iodine supply in early stage of thyroid autonomy may actually decrease the likelihood of progression of clinically relevant thyroid autonomy.

Subsequent studies confirmed that activating TSHR mutations are the main cause of TA, with prevalence of somatic TSHR mutations in up to 82% of TA (Krohn et al. 1998; Nogueira et al. 1999; Tonacchera et al. 1998a, b; 2000; Georgopoulos et al. 2003; Gozu et al. 2006; Palos-Paz et al. 2008). Interestingly, a much lower frequency of TSHR mutations was found in other studies, in particular from Japan and the USA (Takeshita et al. 1995; Gabriel et al. 1999). This discrepancy might be due to differences in the population studied, to different criteria used to define toxic thyroid adenoma, or most likely due to differences in the methodological approaches applied for TSHR mutation detection. Furthermore, Gs-alpha mutations (gsp oncogene) were identified in up to 30% of toxic thyroid adenomas showing that alterations of other proteins may also contribute to activation of the cAMP pathway (Krohn et al. 2005).

Similarly to TAs, activating TSHR mutations have also been demonstrated in hyperfunctioning nodules (either adenomas or hyperplastic nodules) within TMNGs (Duprez et al. 1997; Holzapfel et al. 1997; Tonacchera et al. 1998b, 2000). Moreover, at autoradiography microscopic foci of increased uptake of 125-I have been

demonstrated in euthyroid goiters in iodine-deficient areas. Using archival tissues of euthyroid goiters, which had originally been prepared for autoradiography, somatic TSHR mutations were identified in areas with high 125-I labeling (Krohn et al. 2000). These data indicate that TSHR mutations are implicated in the evolution of the majority of hyperfunctioning areas of toxic multinodular goiter (Fig. 3).

The molecular mechanisms, whereby cAMP stimulation results in sustained proliferation and differentiation in human thyrocytes, are still not fully understood (Dremier et al. 1996). Cyclic AMP is known to stimulate the cAMP-dependent protein kinase A (PKA), which in turn phosphorylates cytoplasmic and nuclear target proteins (Dumont et al. 1992; Roger et al. 2010). One of the best characterized PKA substrates is the nuclear transcription factor cAMP response element binding protein (CREB) which stimulates the transcription of cAMP-responsive genes after its phosphorylation by PKA. In a series of TAs studied by Brunetti et al. (Brunetti et al. 2000), Western blot analysis of the phosphorylated form of CREB (P-CREB) revealed that P-CREB content was significantly lower in tumor thyroid tissue compared with that of the surrounding normal tissue. These data suggest that CREB phosphorylation was specifically reduced in TAs. It has also been shown that the reduced phosphorylation of CREB in toxic adenomas neither reflects a reduced level of adenylate cyclase nor PKA activities, but suggests that CREB phosphorylation can be modulated by other mechanisms independent of cAMP/PKA pathways. Moreover, microinjection of an activated TSH receptor mutation into dog thyreocytes was clearly sufficient to stimulate the proliferation of cells and to enhance the expression of thyroglobulin and thyroperoxidase genes (Dremier et al. 1997). In contrast, microinjection of the catalytic subunit of PKA in dog thyrocytes triggered TPO overexpression, but neither enhanced TG gene expression nor induced thyroid proliferation. These data suggest that, in addition to PKA activation, other cAMP-dependent mechanism(s) or cAMP-regulated binding proteins could be involved in the TSHR-dependent stimulation of mitogenesis and gene expression in thyroid cells, and this is also supported by data from proteomic studies (Krause et al. 2012). In the same context, it has been shown that cAMP directly activates a new guanine nucleotide exchange factor named EPAC, acting as a stimulus of the small G protein Rap1, which in turn would stimulate proliferation of thyrocytes (Dremier et al. 1997). Furthermore, a recent study applying whole-exome sequencing has identified a second hit mutation in enhancer of zeste homolog 1 (EZH1) in TAs (Calebiro et al. 2016). This mutation occurs recurrently in one codon (571 Gln–Arg) of EZH1, a catalytic subunit of the polycomb repressive complex 2, which is involved in embryonic stem cell pluripotency and plasticity and has been linked to cancer aggressiveness. Functional characterization of this EZH1 mutation in rat thyroid cells showed that it confers increased histone H3 trimethylation and promotes thyroid proliferation. Interestingly, by screening a large cohort of 123 TAs, EZH1 mutations were found in 27% of TAs, and only in tumors, which also harbored a somatic TSHR mutation. This novel finding adds a first piece to the puzzle and ongoing conceptual discussion that additional alterations, besides constitutive TSHR or Gs alpha mutations, may be required for development of a clinically relevant TA. However, not all TAs displayed an EZH1 mutation, and from

screening of other benign and malignant thyroid tumors it appears that EZH1 mutations at least in codon 571 are not principally involved in regulation of proliferation in these tumors (Calebiro et al. 2016).

TA and TMNG in Children and Adolescents

Juvenile thyrotoxicosis is a rare disorder with an incidence of person-years (ages 0–15 years) in Caucasians. In a recent Danish nationwide study the overall incidence was 1.58/100,000 person-years (Havgaard Kjaer et al. 2015). Graves' disease accounts for more than 96% of cases, while hyperfunctional thyroid nodules and TA account for less than 3% (Lavard et al. 1994; Havgaard Kjaer et al. 2015). Constitutive activation of the TSHR resulting from germline mutations is responsible for most familial forms of non-autoimmune hyperthyroidism and more than 35 families harboring different activating TSHR germline mutations have been identified to date (Fuhrer et al. 2000; Gozu et al. 2010). In addition occurrence of a TSHR germline mutation can cause sporadic congenital hyperthyroidism (Kopp et al. 1995; Gozu et al. 2010). Only few cases of toxic thyroid adenomas in childhood have been reported and the incidence of this pathology is unknown. Somatic TSHR mutations have been identified in these TAs with the first case of congenital hyperthyroidism caused by a large solitary adenoma harboring a somatic TSHR mutation described in 1997 (Kopp et al. 1997; Kraemer et al. 2009). More recent case studies have also reported somatic TSHR mutations in a girl with three TAs and in a boy with two TAs (Ly et al. 2016). In contrast, analysis of TSHR and Gs alpha genes in eight Italian children subjected to surgery for TAs showed a somatic TSHR mutation only in one patient (Agretti et al. 2013). Thus, the prevalence of TSHR mutations in toxic adenomas from children could differ from that in adulthood, but similar to studies in the adult cohort, methodological differences could also influence this and hence the issue remains unresolved.

Clinical Aspects and Treatment of TA and TMNG

Signs and Symptoms

A significant proportion of patients with TA or TMNG develops hyperthyroidism, and this is directly related to goiter duration. Typically, hyperthyroidism develops insidiously, hence the patient is often unaware of the symptoms. This is particularly seen in the elderly (Krohn et al. 2005). The symptoms of hyperthyroidism are those observed with other causes of thyroid hormone excess, and are also discussed in ► Chap. 14, "Graves' Disease". In general, symptoms are more frequent in young patients and typically include tremor, sweating, hyperkinesis, nervousness, increased metabolic rate, tachycardia, cardiac arrhythmias (extrasystoly, atrial fibrillation), and increased motility of the intestines. In contrast to Graves' disease, inflammatory ophthalmopathy is absent (Smith and Hegedus 2016). Occasionally, muscle

Table 1 Signs and symtoms of TA and TMNG

Symptoms of thyrotoxicosis
Nervousness
Fatigue
Sweating
Heat intolerance
Difficulty concentrating
Tremor
Palpitation
Weight loss
Loose stools
Menstrual irregularities
Signs of thyrotoxicosis
Hyperactivity
Irritability
Tachycardia/arrhythmia
Systolic hypertension
Warm, moist skin
Tremor
Hyperreflexia
Muscle weakness
Oedema
Shortness of breath
Compression symptoms in case of large TA or TMNG
Increase in collar size
Neck swelling
Difficulty swallowing
Shortness of breath
Pemberton's sign

weakness is so severe that the patient is unable to climb stairs, or even to walk, when few other symptoms or signs of the disease have become manifest. Emotional lability is often prominent. Irritability, emotional fatigue, depression, and crying episodes may lead to the suspicion of an agitated depression. Frequently the symptoms are confusing because they often coincide with those of the menopause in women. Hyperthyroidism is often oligosymptomatic in the elderly and in this population atrial fibrillation, congestive heart failure, and anorexia may prevail (Table 1).

Subclinical hyperthyroidism, defined by low or suppressed serum TSH with normal serum free T4 (FT4) and free T3 (FT3) concentrations, is also more commonly observed in older patients and it confers increased risk of atrial fibrillation (Biondi and Cooper 2008). Recurrent or permanent atrial fibrillation or recurrent episodes of atrial tachycardia may dominate the picture. In fact, thyrotoxicosis should be carefully excluded in any adult with goiter and congestive heart failure or tachyarrhythmia. In as many as 46%, hyperthyroidism may only involve elevation of FT3 but not FT4 (T3 hyperthyroidism). Subclinical or overt hyperthyroidism may be associated with negative impact on quality of life. It is preceded and followed by an increased somatic as well as psychiatric morbidity (Brandt et al. 2013a, b). Furthermore, a critical review and meta-analysis has demonstrated an association between overt hyperthyroidism and mortality in six of the eight eligible studies, and

a trend toward higher mortality in patients with hyperthyroidism compared with controls was also found in the remaining two studies (Brandt et al. 2011).Whether this is influenced by the clinical phenotype, i.e., Graves' disease or TMNG, and/or environmental and genetic confounders is not fully clarified. A Danish study of twin pairs discordant for hyperthyroidism suggested that genetic confounding influences the association between hyperthyroidism and mortality (Brandt et al. 2012). Furthermore, unfortunately without being able to differentiate between the etiology of thyrotoxicosis, it was recently shown that therapy and normalization of serum TSH significantly reduced this excess mortality (Lillevang-Johansen et al. 2017).

Subclinical and overt hyperthyroidism contributes to reduced bone density (Biondi and Cooper 2008). The association between thyroid status and major osteoporotic fractures was also demonstrated in a Danish population-based observational register cohort. Here, a single low TSH at baseline was associated with an increased risk of hip fractures in females over a median follow-up of 7.5 years. Also a significant association between duration of thyrotoxicosis and risk of major osteoporotic fractures was found. The increased risk in men was of the same magnitude as in females, but not statistically significant (Abrahamsen et al. 2014).

Some patients with TA or TMNG complain of cosmetic disfigurement of the anterior neck and local discomfort is common. Obstructive symptoms, in particular in TMNG patients, range from very slight to severe caused by compression of the upper airways, the esophagus and the veins in the thoracic inlet. There may be dysphagia and cough and swelling of the neck. Paralysis of the recurrent laryngeal nerve is most unusual and if unilateral vocal cord paralysis is demonstrated, cancer should be suspected and ruled out.

Hyperthyroidism in multinodular goiter can occur for other reasons than nodular autonomy. First, any patient with long-standing Graves' disease may develop nodules in an initially diffuse thyroid gland. Additionally, Graves' disease may develop in a multinodular goiter. Using a sensitive method to measure TSH receptor antibodies, a high prevalence of activating TSH receptor antibodies were found in 17% of patients previously classified as TMNG (Pedersen et al. 2001). If circulating TSH-receptor antibodies, pathognomonic for Graves' disease, are present, this indicates autoimmune thyroid disease. If Graves' disease develops in a multinodular goiter, this may be difficult to separate from the classical type. This is especially a problem in areas with a low iodine intake because of the high prevalence of nontoxic multinodular goiter. Several clinical observations support a fundamental distinction between Graves' disease and Plummer's disease. Frequently the hyperactive tissue in the latter is confined to one or a few nodules, as demonstrated by scintiscan. Ophthalmopathy is not present in TA/TMNG, unless there is concomitant Graves' disease. Usually, TMNG patients are older, and thyrotoxicosis is milder and often exists for a long time without overt symptoms, as compared to Graves' disease (Smith and Hegedus 2016).

A characteristic and clinical risk in TMNG patients is the sudden induction of hyperthyroidism following an iodine overload (iodine-induced hyperthyroidism, IIH) (Bourdoux et al. 1996; Reinwein et al. 1988). In a series of euthyroid Belgian patients with autonomous thyroid nodules, a daily dose of 0.5 mg iodide caused hyperthyroidism within a few weeks (Ermans and Camus 1972). Similar data have been reported

in Germany, where autonomous tissue was found to be rather common in nontoxic goiter (Bähre et al. 1988) and responsible for subclinical or overt hyperthyroidism after incidental exposure to excessive iodine, e.g., iodine-containing drugs, such as amiodarone or contrast media, or iodine-rich food (algae). The sequence of events, leading to IIH, involves the presence of autonomously functioning thyroid nodules, which, upon iodine overload may increase iodine incorporation and thyroid hormone synthesis. IIH occurs most often, but not exclusively, in populations exposed to long-standing iodine deficiency and in subjects of older age who harbor autonomously functioning thyroid nodules (Bourdaux et al. 1996). Thus, the cardinal prerequisite for the development of IIH is thyroid autonomy. Importantly, iodine overload first results in blockade of thyroid hormone (TH) synthesis and thyroid vascularization ("Wolff-Chaikoff effect"). Later, escape from the "Wolff-Chaikoff effect" occurs. IIH typically starts after 3–6 weeks of iodine overload (Stanbury et al. 1998).

Diagnosis

The diagnosis of TA and TMNG is based on clinical examination, thyroid function tests, thyroid ultrasound, and scintiscan. The evaluation of a patient suspected of having TA and TMNG comprises a careful history, including information on possible iodine overload and physical examination focusing on inspection of the neck and upper thorax, palpation of the goiter to determine its size and nodularity, and assessment of regional lymph nodes and signs of thyrotoxicosis.

Standard thyroid function tests (TSH, FT4 and FT3) will confirm overt or subclinical hyperthyroidism, but depending on the autonomous cell mass, euthyroidism may still prevail. In a retrospective study, files of nontoxic goiter patients were reviewed (Fast et al. 2008). In the group of patients with uninodular goiter 46% of patients and in the group of multinodular goiter patients 68% had a serum TSH below 1 mIU/L, suggesting presence of partial autonomy. In any patient with goiter, serum TSH is by far the most frequently used test in the initial evaluation of thyroid function (Tonacchera et al. 2010). If TSH concentration is low, measurement of FT4 is warranted to determine whether the patient has subclinical or overt hyperthyroidism. In patients with low serum TSH and normal FT4 concentrations, serum FT3 should be measured (Fig. 4).

Measurement of thyroid antibodies is not routinely indicated in thyroid autonomy. However, in iodine-deficient areas distinction between Graves' disease and TMNG can be difficult if extrathyroidal manifestations of autoimmune thyroid disease are absent and ultrasound shows the presence of thyroid nodules (Pedersen et al. 2001). In this case, the determination of TSH receptor antibodies is helpful to establish the correct diagnosis. Anti-thyroperoxidase antibodies have been reported to predict the development of hypothyroidism after 131-I therapy of hyperthyroid diseases. In fact presence of anti-TPO antibodies is associated with a higher risk of developing permanent hypothyroidism, thereby reflecting the coexistence of autoimmune thyroiditis (Hegedus et al. 2003; Bonnema and Hegedus 2012). Urinary iodine excretion can be measured in cases of suspected iodine contamination.

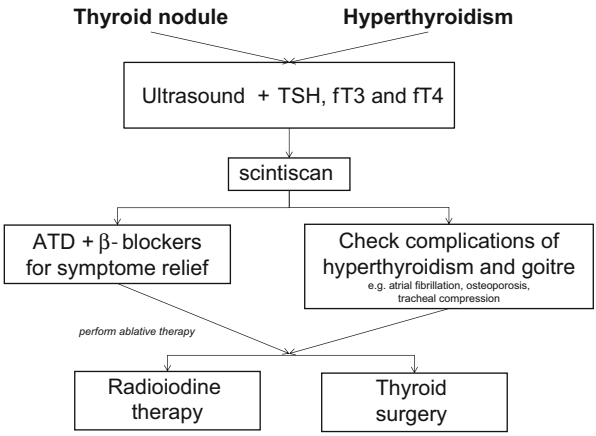

Fig. 4 Management algorithm in TA and TMNG

Localization, size, and number of thyroid nodules as well as goiter volume can be determined by ultrasound scanning. Radionuclide scintigraphy with 123-I or 99mTc is very helpful in determining the nodules' functionality. Nodules with a high uptake on scintigraphy almost never harbor clinically significant malignancy, although exceptions have been reported. Using 99mTc as a tracer may result in false positive uptake in 3–8% of thyroid nodules ("the trapping only nodules") (Reschini et al. 2006), but 99mTc is more widely available and less costly than 123-I. The a priori risk of malignancy among nonfunctioning nodules may be higher than in normal functioning tissue. Hence, fine needle aspiration in TMNG, where functioning, normal, and nonfunctioning thyroid nodules often coexist, should be considered in hypofunctioning goiter areas, based on the ultrasound appearance of the nodules (Tonacchera et al. 1999). If thyroid autonomy is suspected in a euthyroid patient, a "suppression" scan can be performed after administration of thyroid hormone to induce exogenous hyperthyroidism. Thereby, nonautonomous tissue will be suppressed and thyroid autonomy unmasked (Bähre et al. 1988; Krohn et al. 2005).

Other Imaging Techniques

It is often necessary to establish whether thyroid enlargement, particularly if nodular, causes any significant compression and/or displacement of neighboring structures. Lung function testing may aid in revealing evidence of tracheal narrowing. In addition, CT scanning (without contrast media) or MRI will provide imaging of the mediastinal involvement and the relationship between the goiter and the surrounding structures. Laryngoscopy can be performed to assess vocal cord mobility, but this is very rarely impaired in a patient with TA or TMNG. A systematic review (Sorensen et al. 2014) has shown that the trachea may be significantly affected in individuals with goiter. Clinically this can lead to upper airway obstruction, which primarily diminishes the inspiratory capacity, ultimately with stridor as a clinical characteristic. Tracheal compression is less common than tracheal deviation. Data

Table 2 Pros and cons of radioiodine therapy versus surgical treatment of TA and TMNG

Radioiodine	Surgery
PRO	
Non-invasive, easy applicability	Rapid resolution of thyrotoxicosis
No risk of hypoparathyroidism	Rapid and effective volume reduction with large goitres
No risk of laryngeal nerve damage	Histological diagnosis
No risk of anesthesia	removal of non-functioning nodular tissue
Out-patient treatment	100% cure rate
CON	
In some countries confinement to "isolation ward"	In-patient treatment
Intermittent ATD may be required until euthyroid	Risk of acute surgical complications (bleeding, infection) and anaesthesia
Slow and variable volume reduction	Transient or persistent hypoparathyroidism or laryngeal nerve damage
Requires compliant patient	Outcome depends on experience of surgeon
Not effective in iodine induced hyperthyroidism	
Long-term risk of hypothyroidism	

from the swallowing questionarre studies reveal that esophageal dysfunction is also common among people with goiter (Sorensen et al. 2014).

Treatment of TA and TMNG

Due to the underlying molecular defect, there is no spontaneous resolution of TA and TMNG. Hence ablative treatment is generally indicated, once thyroid autonomy is diagnosed with subclinical or overt hyperthyroidism (Fig. 4). The most widely used modalities are ablation via thyroid surgery (Cirocchi et al. 2015; Porterfield 2008) or radioiodine (Bonnema and Hegedus 2012), alternatively long-term maintenance of euthyroidism with methimazole. The options, as well as their advantages and potential risks, should be openly discussed with the patient allowing the final decision to be based on patient characteristics (age, the severity of hyperthyroidism, goiter size and extent of nodular thyroid disease, concomitant nonthyroid illness), patient preference, cost, and availability (Table 2).

Antithyroid Drugs

Often first-line treatment in patients with overt hyperthyroidism is antithyroid drug therapy (ATD). For this, the thioamides methimazole or its prodrug carbimazole, which is converted into methimazole in the body, and less frequently propylthiouracil (PTU) are used. These drugs inhibit thyroid hormone synthesis by blocking thyroid peroxidase mediated iodination of TG-bound tyrosine in the thyroid follicles. PTU also inhibits

peripheral conversion of T4 to T3 by blocking the type 1 deiodinase. In their recently updated guidelines (Ross et al. 2016), the ATA suggests initial dosing of methimazole according to FT4 serum concentrations, i.e., 5–10 mg/d if FT4 is 1–1.5 times the upper limit of normal (ULN), 10–20 mg if FT4 elevation is 1.5–2 times the ULN, and 30–40 mg if FT4 elevation is >2–3 times the ULN. Methimazole and carbimazole are administered once daily. Higher ATD doses will not speed up restoration of euthyroidism but will result in more frequent adverse effects (Reinwein et al. 1993). Due to its shorter half-life, PTU has to be given two to three times per day. The usual starting dose is 3 × 50–100 mg/ PTU daily. At present PTU is only recommended for treatment of overt hyperthyroidism during the first trimester of pregnancy (mostly in a woman with Graves' disease) and should side effects to thiamazole occur (Ross et al. 2016).

Importantly, ATDs do not block secretion of already presynthesized T4 and T3. The mode of action of these drugs, in addition to the long half-life of T4 (7 days), explain why restoration of euthyroidism is slow. For these reasons combination of ATDs with β-blockers, either nonselective or selective β-1 blockers, is recommended in symptomatic patients, unless the patient suffers from asthma. In a randomized trial, addition of β-blockers ameliorated symptoms and function in daily life, compared to monotherapy with ATD (Tagami et al. 2012).

Depending on the clinical severity of disease, a first assessment of ATD treatment is recommended after 2–3 weeks and should include measurement of serum FT4 and FT3 concentrations as well as a clinical assessment. Usually ATD dose can be tapered and normalization of FT4 and FT3 concentrations should be accomplished within 6–8 weeks. β-blockers can gradually be tapered at this time. Importantly, TSH often remains suppressed for weeks or even months and therefore cannot stand alone as a marker of thyroid function.

ATDs are associated with several minor side effects. For methimazole these are dose-dependent and include rashes and urticaria, gastrointestinal discomfort, arthralgias and elevated liver function tests (the latter, however, may also be due to thyrotoxicosis). These ATD side-effects occur in 5–13% of patients. Most data are derived from Graves' disease patients and may thus not reflect the situation in TAs and TMNGs. For PTU, the dose-relation is not as obvious and besides the same minor side effects as observed with methimazole, liver failure – from fulminant hepatic necrosis resulting in need for transplantation – and risk of death has led to an FDA warning in 2010 and advocation for restricting the use of this drug worldwide.

Agranulocytosis remains a feared complication of all ATDs. A recent survey employing a Danish national database (Andersen et al. 2016) reported agranulocytosis in 0.11% of methimazole and 0.27% of PTU-treated patients. No consistent risk factors for development of agranulocytosis or PTU-induced hepatotoxicity could be identified. Most cases occur within the first 3 months of treatment. All patients need to be informed, if possible also in writing, about ATD side effects. Full blood count and/or liver function tests are obtained prior to ATD treatment by many, but no study convincingly demonstrates that patients benefit from routine full blood count or liver function testing during ATD treatment.

In conditions of IIH, which in previous studies has been reported to account for up to 50% of all cases of new onset overt hyperthyroidism, release of free iodide may

continue even days to weeks after the use of organified iodine contrast media (Stanbury et al. 1998). In this situation, efficacy of ATDs is hampered and higher starting doses of methimazole (40–60 mg/d) or even iv methimazole administration are required. Patients with IIH have an increased likelihood of severe thyrotoxicosis and early surgery is an option, if response to ATD is insufficient (Scholz et al. 2003). Potassium perchlorate (3–4 × 200–400 mg/d) can be given as an additional treatment, since it effectively inhibits iodine uptake via NIS into the thyroid gland. Potassium perchlorate is also used prophylactically, in countries with high prevalence of thyroid autonomy in patients with low TSH, prior to medically indicated use of iodine-containing contrast media. For example, in Germany and Italy, a common practice is to administer potassium perchlorate 2–4 h prior to giving iodine-containing contrast media to patients suspected of having TA or TMNG, and to continue the treatment for 2–3 weeks with follow-up of thyroid function at 3, 6, and 12 weeks (Scholz et al. 2003; Krohn et al. 2005).

In TAs and TMNGs, it is apparent that the role of ATDs can only be to achieve euthyroidism prior to surgery or radioiodine therapy (Table 2). Antithyroid treatment, on this indication, is also recommended in patients >65 years who have subclinical hyperthyroidism, because of the known adverse effects of subclinical hyperthyroidism on the cardiovascular system and bone. Very old patients and patients with limited life expectancy may be an exception to this rule and low-dose ATD (5–10 mg methimazole/day) may be justified as the only treatment in these patients. Importantly, ATDs are not contraindicated as life-long maintenance therapy in patients who for any reason decline ablative therapy.

Surgery

The purpose of thyroid surgery is to cure hyperthyroidism by removing all autonomously functioning thyroid tissue and other clinically relevant nodular thyroid tissue. Therefore, the extent of surgery depends on the preoperative ultrasound combined with the findings during surgery.

For TAs, hemithyroidectomy or even isthmectomy may be adequate, while in TMNG a subtotal, near-total, or total thyroidectomy is performed. If possible, surgery should be carried out in a euthyroid patient to minimize the surgical risk. In the era of ATDs, “plummering” by administration of high dose potassium iodide, e.g., 5–7 drops Lugol’s solution (8 mg iodide/drop) three times daily for 10 days, is only rarely employed, and it is not recommended as preparation before surgery in the present ATA guidelines (Ross et al. 2016). On the other hand, if the patient has IIH it is highly efficient in blocking thyroid hormone synthesis and release, resulting in a rapid drop in FT4 and FT3 concentrations. In selected inpatients, with severe thyrotoxicosis not responsive to ATD, it may be considered if thyroid surgery is carried out within the following 10 days. Usually at that time escape from the Wolff-Chaikoff effect starts to occur (Stanbury et al. 1998).

The advantages of surgery are rapid restoration of euthyroidism and immediate and sustained resolution of compression symptoms in patients with a large TMNG. Potential

risks, besides immediate surgical complications such as bleeding, include postoperative hypoparathyroidism and/or vocal cord paralysis. The risk for either is declines with increasing surgeon expertise, and increases with the extent of surgery and if the patient has had previous neck surgery. It is therefore paramount that all patients be operated by an experienced thyroid surgeon. In this setting, complications are to be expected in <2% for permanent hypoparathyroidism and <1–2% for laryngeal nerve palsy, while transient impairments may be much more prevalent. TH replacement is required after near-total or total thyroidectomy, while following hemithyroidectomy, it will depend on functionality of the remaining thyroid tissue. Often TH replacement is required if the remaining thyroid tissue volume is less than 10 ml. TH replacement is commenced immediately after surgery, unless the patient was hyperthyroid at time of operation, and is dosed according to the patient's body weight (in case of total thyroidectomy: 1.6–1.8 μg LT4/kg body weight/day). Serum calcium concentrations (preferably ionized calcium) should be obtained prior to surgery as well as postoperatively. Should postoperative hypoparathyroidism occur, patients need to be treated for according to international recommendations (Brandi et al. 2016).

Surgery is usually preferred in large symptomatic TMNG (e.g., >80 ml) with retrosternal extension and is recommended in case of suspicion of concomitant thyroid cancer. It is also advocated in patients with overt hyperthyroidism, those with side effects of ATD, in case of IIH not responding to ATD, and as an early emergency procedure in patients with thyroid storm (Scholz et al. 2003; Stanbury et al. 1998; Ross et al. 2016).

Radioiodine Therapy

Radioiodine is highly effective in eradicating hyperthyroidism and reducing autonomous tissue volume in TAs and TMNGs (Bonnema et al. 1999; Bonnema and Hegedus 2012). A cure rate for hyperthyroidism can be expected in up to 94% of TAs and 81% of TMNGs (Nygaard et al. 1999a, b; Ceccarelli et al. 2005). Reduction in thyroid volume is observed already after 3 months and continues over 2 years, reaching 30–50% even in large TMNGs (Nygaard et al. 1999a; b). 131-I doses for TAs and TMNGs are usually calculated on the basis of the amount of autonomous tissue (TA) or goiter size (TMNG) and corrected for the 24 h 131-I uptake, even sometimes for effective half-life. However, some centers prefer to apply fixed activities, e.g., 370–740 MBq 131-I in TAs and TMNG. Different algorithms exist for dose calculation, and low- and high-dose 131-I protocols have been suggested to reduce the risk of post-ablative hypothyroidism or to reach an optimal ablation rate of hyperthyroidism, respectively, but the data are inconsistent. If hyperthyroidism persists over 6 months, a second course of 131-I may be administered. In selected cases, some offer this already after 3 months (Bonnema and Hegedus 2012; Ross et al. 2016). As an alternative surgery may be considered.

Pretreatment with methimazole may not be necessary and certainly is not mandatory in younger patients, but generally these patients benefit from β-blockers to antagonize thyroid hormone excess. In the elderly and those with severe

hyperthyroidism, pretreatment with ATD and β-blockers is recommended. In that case ATDs need to be stopped again 3–7 days before 131-I administration to ensure sufficient TSH-suppression and hence selective targeting of autonomous tissue (Walter et al. 2007). Advantages of radioiodine therapy are low cost, ease of application, and the fact that it is an outpatient procedure in most countries. The major disadvantage, compared to surgery, is the insidious onset of autonomous tissue destruction and hence resolution of hyperthyroidism (in TMNG 55% at 3 months and 80% at 6 months; in TAs 75% at 3 months). For this reason, β-blockers should be continued and ATD treatment resumed, e.g., at 3–7 days after 131-I therapy in patients with severe hyperthyroidism, age older than 60 years or with cardiovascular disease (Bonnema and Hegedus 2012).

Post 131-I administration, thyroid function has to be monitored regularly. Measurement of FT4, FT3, and TSH is recommended at 4–6 week intervals for 6–12 months, until hypothyroidism occurs or stable euthyroidism is demonstrated. Hereafter annual follow-up should suffice.

Radioiodine treatment is contraindicated in pregnancy and during lactation. Furthermore a pregnancy test must be carried out with a negative result prior to 131-I administration. In addition, contraception is advocated for 4 months after radioiodine administration, primarily because of changes in thyroid function after 131-I therapy, which may negatively impact pregnancy, but also side effects on the gonads, in particular spermatogenesis.

Side effects of radioiodine treatment may include transient local pain and tenderness while exacerbation of thyrotoxicosis due to destructive thyroiditis is very rare. Development of thyroid stimulating antibodies and Graves' disease following 131-I therapy has been reported in up to 4% in some series (Meller et al. 2006; Nygaard et al. 1997). Another "side-effect" is post-radioiodine hypothyroidism, which develops insidiously. In TAs, subclinical or overt hypothyroidism was found in 8% and 60% of patients at 1 and 20 years follow-up, respectively (Ceccarelli et al. 2005), in TMNG it was reported in 3% at 1 year and 64% at 24-year follow-up, respectively (Holm et al. 1982). However, many have found much lower prevalences (Bonnema and Hegedus 2012). Development of post 131-I hypothyroidism is linked to the degree of TSH suppression at treatment, increases with older age and coexistence or evolution of autoimmune thyroid disease (Hegedus et al. 2003; Bonnema and Hegedus 2012). In any given individual, the risk and occurrence of hypothyroidism are unpredictable and therefore warrants life-long follow-up after radioiodine therapy.

Alternative Techniques for Ablation of Thyroid Autonomy

Nonsurgical ultrasound-guided ablation modalities have been explored for treating TAs and TMNGs. These include percutaneous ethanol injection therapy (PEIT), radiofrequency ablation (RFA), and laser therapy. The methods have been studied in a limited number of expert centers, mainly in Europe and South Korea (Monzani et al. 1997; Tarantino et al. 2008; Gharib et al. 2013; Ha et al. 2015; Zingrillo et al. 2000).

In experienced hands, they have been shown to eliminate autonomy in a tissue and thyroid function-sparing manner and to decrease nodule size with very few complications, except pain and local discomfort during the procedure. However, since these techniques are not widely available they may, at present, best be reserved for further exploration in trials and patients for whom no other treatment option is feasible or for those who decline the traditional options (Gharib et al. 2013).

Management of Familial Nonautoimmune Hyperthyroidism

In principle, treatment follows the same recommendations as for TAs and MTGs. However, since the affected individuals harbor a germline activating TSHR mutation, complete ablation of thyroid tissue should be the prime target to efficiently prevent recurrence of hyperthyroidism (Kopp et al. 1997; Fuhrer et al. 2000). In the published case series, usually thyroid surgery was performed for ablation but in some cases this was followed by radioiodine ablation (Gozu et al. 2010). Whether this sequence should be recommended for all patients, or 131-I treatment postponed until disease recurs, is unknown and may be individually discussed with the patient. Since familial nonautoimmune hyperthyroidism is inherited, all first-degree relatives should undergo molecular diagnostics.

Follow-Up

The long-term management of TA and TMNG patients must pay attention to thyroid function, in particular development of hypothyroidism after 131-I, and securing the correct thyroid hormone substitution to obtain and maintain euthyroidism and reduce excess morbidity and mortality due to thyroid dysfunction (Brandt et al. 2011; Thvilum et al. 2013). In addition, patients should be monitored for recurrence of symptomatic nodular thyroid disease. After surgery, detecting and adequately managing hypoparathyroidism is important for quality of life and to prevent long-term morbidity (Brandi et al. 2016). Combination therapy of thyroxine and iodine is often administered after thyroid surgery with the concept that recurrence of goiter/nodules may be efficiently prevented in regions with iodine deficiency, but this has never been proven and is certainly questioned by some (Hegedus et al. 1999; Krohn et al. 2005). Satisfactory follow-up arrangements must be made, e.g., on an annual basis for long-term follow-up, depending on patient characteristics and local infrastructure.

Summary and Conclusions

According to our current understanding, thyroid autonomy results from increased thyroid cell proliferation and oxidative stress, both of which contribute to increased mutagenesis and likelihood of somatic TSHR mutations, found in microscopic

areas of autonomous tissue in nontoxic goiters (Krohn et al. 2007). These follicles, with a mutant TSHR, display high rates of iodine turnover and autonomous growth and function and appear to gradually evolve towards functionally detectable (by scintiscan) or ultimately clinically recognizable thyroid autonomy (hyperthyroidism). The risk of thyroid autonomy is significantly increased in populations exposed to iodine deficiency, which may contribute to increase thyroid proliferation and oxidative burden in the thyroid.

The observation that single hyperfunctioning thyroid nodules within TMNGs, similar to that seen in TAs, harbor a TSHR gene mutation suggests that the basic mechanism leading to the formation of TAs and hyperfunctioning nodules in TMNGs is the same. However, how autonomy evolves from its molecular initiation is still unclarified and a research topic. The recent identification of additional mutational events provides exciting novel insights and adds to the debate on whether TSHR-driven cAMP stimulation is sufficient for sustaining thyroid proliferation towards a nodule. It also remains open whether TAs in children and TAs in iodine-sufficient areas follow the same principles of constitutive TSHR and less frequently Gs alpha protein activation.

In TAs and TMNGs, it is apparent that the role of ATDs can only be for maintaining euthyroidism as long-term therapy, or more often to prepare the patient for ablative surgery or radioiodine therapy. The purpose of thyroid surgery is to cure hyperthyroidism by removing all autonomously functioning thyroid tissue and other clinically relevant nodular thyroid tissue. Thus, the extent of surgery will vary depending on a combination of findings at preoperative ultrasound and morphology revealed at intraoperative inspection. For TAs, hemithyroidectomy or even isthmectomy may be adequate, while in TMNG a subtotal, near-total, or total thyroidectomy is performed.

Radioiodine is highly effective in eradicating hyperthyroidism and reducing autonomous tissue volume in TAs and TMNGs. Other nonsurgical, ultrasound-guided, ablation modalities have been explored for treating TAs and TMNGs. As the most prominent these include percutaneous ethanol injection therapy, radiofrequency ablation, and laser therapy. However, since these techniques are not widely available, and still under development, they may best be reserved for further exploration in trials and in patients for whom no other treatment option seems feasible or who decline traditional therapy.

References

Abrahamsen B, Jorgensen H, Laulund AS, et al. Low serum thyrotropinlevel and duration of suppression as a predictor of major osteporotic fractures – the OPENTHYRO register cohort. J Bone Miner Res. 2014;29:2040–50.

Aghini-Lombardi F, Antonangeli L, Martino E, et al. The spectrum of thyroid disorders in an iodine-deficient community: the Pescopagano survey. J Clin Endocrinol Metab. 1999;84:561–8.

Aghini-Lombardi F, Fiore E, Tonacchera M, et al. The effect of voluntary iodine prophylaxis in a small rural community: the Pescopagano survey 15 years later. J Clin Endocrinol Metab. 2013;98(3):1031–9.

Agretti P, Segni M, De Marco G, et al. Prevalence of activating thyrotropin receptor and Gsα gene mutations in paediatric thyroid toxic adenomas: a multicentric Italian study. Clin Endocrinol. 2013;79(5):747–9.

Als C, Listewnik M, Roesler H, et al. Immunogenic and non-immunogenic hyperthyroidism. Recent trends in prealpine Switzerland and in coastal Poland. Nuklearmedizin. 1995;34:92–7.

Andersen SL, Olsen J, Laurberg P. Antithyroid drug side effects in the population and in pregnancy. J Clin Endocrinol Metab. 2016;101(4):1606–14.

Bähre M, Hilgers R, Lindemann C, et al. Thyroid autonomy: sensitive detection in vivo and estimation of its functional relevance using quantified high-resolution scintigraphy. Acta Endocrinol. 1988;117(2):145–53.

Baltisberger BL, Minder CE, Burgi H. Decrease of incidence of toxic nodular goiter in a region of Switzerland after full correction of mild iodine deficiency. Eur J Endocrinol. 1995;132:546–51.

Belfiore A, Sava L, Runello F, et al. Solitary autonomously functioning thyroid nodules and iodine deficiency. J Clin Endocrinol Metab. 1985;56:283–7.

Biondi B, Cooper DS. The clinical significance of subclinical thyroid dysfunction. Endocr Rev. 2008;29(1):76–131.

Bonnema SJ, Hegedus L. Radioiodine therapy in benign thyroid diseases: effects, side effects, and factors affecting therapeutic outcome. Endocr Rev. 2012;33:1–61.

Bonnema SJ, Bertelsen H, Mortensen J, et al. The feasibility of high dose iodine 131-I treatment as an alternative to surgery in patients with a very large goiter: effect on thyroid function and size and pulmonary function. J Clin Endocrinol Metab. 1999;84:3636–41.

Bourdoux P, Ermans AM, Mukalay WA, et al. Iodine induced thyrotoxicosis in Kivu Zaire. Lancet. 1996;347:552–3.

Brandi ML, Bilezikian JP, Shoback D, et al. Management of hypoparathyroidism: summary statement and guidelines. J Clin Endocrinol Metab. 2016;101(6):2273–83.

Brandt F, Green A, Hegedus L, et al. A critical review and meta-analysis of the association between overt hyperthyroidism and mortality. Eur J Endocrinol. 2011;165:491–7.

Brandt F, Almind D, Christensen K, et al. Excess mortality in hyperthyroidism: the influence of preexisting comorbidity and genetic confounding: a Danish nationwide register-based cohort study of twins and singletons. J Clin Endocrinol Metab. 2012;97:4123–9.

Brandt F, Thvilum M, Almind D. Morbidity before and after the diagnosis of hyperthyroidism: a nation-wide register-based study. PLoS One. 2013a;8:e66711.

Brandt F, Thvilum M, Almind D. Hyperthyroidism and psychiatric morbidity: evidence from a Danish nation-wide register study. Eur J Endocrinol. 2013b;170:341–8.

Brunetti A, Chiefari E, Filetti S, et al. The 3′-5′ cyclic adenosine monophosphate response element binding protein (CREB) is functionally reduced in human toxic thyroid adenomas. Endocrinology. 2000;141:722–30.

Calebiro D, Grassi ES, Eszlinger M, et al. Recurrent EZH1 mutations are a second hit in autonomous thyroid adenomas. J Clin Invest. 2016;126(9):3383–8.

Carle A, Pedersen IB, Knudsen N, et al. Epidemiology of subtypes of hyperthyroidism in Denmark: a population-based study. Eur J Endocrinol. 2011;164:801–9.

Ceccarelli C, Bencivelli W, Vitti P, et al. Outcome of radioiodine-131 therapy in hyperfunctioning thyroid nodules: a 20 years' retrospective study. Clin Endocrinol. 2005;62(3):331–5.

Cirocchi R, Trastulli S, Randolph J, et al. Total versus subtotal thyroidectomy for multinodular non toxic goitre in adults. Cochrane Database Syst Rev. 2015;8:CD013070.

Corvilain B, Dumont JE, Vassart G Toxic adenoma and toxic multinodular goiter. In: the Thyroid eds Braverman LE, Utiger RD Werner and Ingbar's. Philadelphia: Lippincott-Ravn, 2000; 564–572.

Deleu S, Allory Y, Radulescu A, et al. Characterization of autonomous thyroid adenoma: metabolism, gene expression, and pathology. Thyroid. 2000;10:131–40.

Dremier S, Coppee F, Delange F, et al. Thyroid autonomy; mechanism and clinical effects. J Clin Endocrinol Metab. 1996;81:4187–93.
Dremier S, Pohl V, Poteet-Smith C, et al. Activation of cyclic AMP-dependent kinase is required but may not be sufficient to mimic cyclic AMP-dependent DNA synthesis and thyroglobulin expression in dog thyroid cells. Mol Cell Biol. 1997;17:6717–26.
Dumont JE, Lamy F, Roger P, et al. Physiological and pathological regulation of thyroid cell proliferation and differentiation by thyrotropin and other factors. Physiol Rev. 1992;72:667–97.
Duprez L, Hermans J, Van Sande J, et al. Two autonomous nodules of a patient with multinodular goiter harbor different activating mutations of the thyrotropin receptor gene. J Clin Endocrinol Metab. 1997;82:306–8.
Elte JW, Bussemaker JK, Haak A. The natural history of euthyroid multinodular goiter. Postgrad Med J. 1990;66:186–90.
Ermans AM, Camus M. Modifications of thyroid function induced by chronic administration of iodide in the presence of "autonomous" thyroid tissue. Acta Endocrinol. 1972;70(3):463–75.
Fast S, Bonnema SJ, Hegedus L. The majority of Danish non-toxic goitre patients are ineligible for levothyroixine suppressive therapy. Clin Endocrinol. 2008;69:653–8.
Fuhrer D, Holzapfel HP, Wonerow P, et al. Somatic mutations in the thyrotropin receptor gene and not in the Gs alpha protein gene in 31 toxic thyroid nodules. J Clin Endocrinol Metab. 1997;82:3885–91.
Fuhrer D, Kubisch C, Scheibler U, et al. The extracellular thyrotropin receptor domain is not a major candidate für mutations in toxic thyroid nodules. Thyroid. 1998;8:997–1001.
Fuhrer D, Warner J, Sequeira M, et al. Novel TSHR germline mutation (Met463val) masquerading as Graves' disease in a large Welsh kindred with hyperthyroidism. Thyroid. 2000;10:1035–41.
Fuhrer D, Lewis MD, Alkhafaji F, et al. Biological activity of activating thyroid-stimulating hormone receptor mutants depends on the cellular context. Endocrinology. 2003;144(9):4018–30.
Gabriel EM, Bergert ER, Grant CS, Van Heerden JA, Thompson GB, Morris JC. Germline polymorphism of codon 727 of human thyroid-stimulating hormone receptor is associated with toxic multinodulkar goiter. J Clin Endocrinol Metab. 1999;84:3328–3335.
Georgopoulos NA, Sykiotis GP, Sgourou A, et al. Autonomously functioning thyroid nodules in a former iodine deficient area commonly harbor gain-of-function mutations in the thyreotropin signaling pathway. Eur J Endocrinol. 2003;149:287–92.
Gharib H, Hegedus L, Pacella CM, et al. Clinical review: nonsurgical, image-guided, minimally invasive therapy for thyroid nodules. J Clin Endocrinol Metab. 2013;98:3949–7.
Gozu HI, Bircan R, Krohn K, et al. Similar prevalence of somatic TSH receptor and Gs alpha mutations in toxic thyroid nodules in geographical regions with different iodine supply in Turkey. Eur J Endocrinol. 2006;155:535–45.
Gozu HI, Lublinghoff J, Bircan R, et al. Genetics and phenomics of inherited and sporadic non-autoimmune hyperthyroidism. Mol Cell Endocrinol. 2010;322(1–2):125–34.
Hamburger JI. The autonomously functioning thyropid nodule: Goetsch's disease. Endocr Rev. 1987;8:439–450.
Ha EJ, Baek JH, Kim KW, et al. Comparative efficacy of radiofrequency and laser ablation for the treatment of benign thyroid nodules: systematic review including traditional pooling and bayesian network meta-analysis. J Clin Endocrinol Metab. 2015;100:1903–11.
Havgaard Kjaer R, Andersen MS, Hansen D. Increasing incidence of juvenile thyrotoxicosis in Denmark: a nationwide study, 1998–2012. Horm Res Paediatr. 2015;84:102–7.
Hegedus K, Nygaard B, Hansen JMI. Routine thyroxine treatment to hinder postoperative recurence of nontoxic goiter justified. J Clin Endocrinol Metab. 1999;84:756–60.
Hegedus L, Bonnema SJ, Bennedbaek FN. Management of simple nodular goiter: current status and future perspectives. Endocr Rev. 2003;24:102–32.
Holm LE, Lundell G, Israelsson A, et al. Incidence of hypothyroidism occurring long after iodine-131-I therapy for hyperthyroidism. J Nucl Med. 1982;23(2):103–7.

Holzapfel HP, Fuhrer D, Wonerow P, et al. Identification of constitutively activating somatic thyrotropin receptor mutations in a subset of toxic multinodular goiter. J Clin Endocrinol Metab. 1997;82:4229–33.

Kopp P, van Sande J, Parma J, Duprez L, Gerber H, Joss E, Jameson JL, Dumont JE, Vassart G. Brief report: congenital hyperthyroidism caused by a mutation in the thyrotropin-receptor gene. N Engl J Med. 1995;332(3):150–4.

Kopp P, Muirhead S, Jourdain N, et al. Congenital hyperthyroidism caused by a solitary toxic adenoma harboring a novel somatic mutation (serine281→isoleucine) in the extracellular domain of the thyrotropin receptor. J Clin Invest. 1997;100(6):1634–9.

Kraemer S, Rothe K, Pfaeffle R, et al. Activating TSH-receptor mutation (Met453Thr) as a cause of adenomatous non-autoimmune hyperthyroidism in a 3-year-old boy. J Pediatr Endocrinol Metab. 2009;22(3):269–74.

Krause K, Boisnard A, Ihling C, et al. Comparative proteomic analysis to dissect differences in signal transduction in activating TSH receptor mutations in the thyroid. Int J Biochem Cell Biol. 2012;44(2):290–301.

Krohn K, Führer D, Holzapfel HP, et al. Clonal origin of toxic thyroid nodules with constitutively activating thyrotropin receptor mutations. J Clin Endocrinol Metab. 1998;83(1):130–4.

Krohn K, Wohlgemuth S, Gerber H, et al. Hot microscopic areas of iodine deficient euthyroid goiters contain constitutively activating TSH receptor mutations. J Pathol. 2000;192:37–42.

Krohn K, Führer D, Bayer Y, et al. Molecular pathogenesis of euthyroid and toxic multinodular goiter. Endocr Rev. 2005;26(4):504–24.

Krohn K, Maier J, Paschke R. Mechansims of disease: hydrogene peroxide, DNA damage and mutagenesis in the development of thyroid tumours. Nat Clin Pract Endocrinol Metab. 2007;3:713–20.

Lauberg P, Pedersen KM, Vestergard H, et al. High incidence of multinodular toxic goiter in the elderly population in a low iodine intake area vs. high incidence of Graves' disease in the young in a high iodine intake area: comparative surveys of thyrotoxicosis epidemiology in East-Jutland Denmark and Iceland. J Intern Med. 1991;229:415–20.

Lauberg P, Pedersen KM, Hreidarsson A, et al. Iodine intake and the pattern of thyroid disorders: a comparative epidemiological study of thyroid abnormalities in the elderly in Iceland and in Jutland, Denmark. J Clin Endocrinol Metab. 1998;83:765–9.

Laurberg P, Jorgensen T, Perrild H, et al. The Danish investigation on iodine intake and thyroid disease, DanThyr: status and Perspectives. Eur J Endocrinol. 2006;155:219–28.

Lavard L, Ranlov I, Perrild H, et al. Incidence of juvenile thyrotoxicosis in Denmark, 1982–1988. A nation-wide study. Eur J Endocrinol. 1994;130:563–8.

Ledent C, Dumont JE, Vassart G, et al. Thyroid expression of an A2 adenosine receptor transgene induces thyroid hyperplasia and hyperthyroidism. EMBO J. 1992;11:537–42.

Lillevang-Johansen M, Abrahamsen B, Jørgensen HL, Brix TH, Hegedüs L. Excess mortality in treated and untreated hyperthyroidism is related to cumulative periods of low serum TSH. J Clin Endocrinol Metab. 2017;102:2301. (epub).

Ly S, Frates MC, Benson CB, et al. Features and outcome of autonomous thyroid nodules in children: 31 consecutive patients seen at a single center. J Clin Endocrinol Metab. 2016; 101(10):3856–62.

Meller J, Siefker U, Hamann A, et al. Incidence of radioiodine induced Graves' disease in patients with multinodular toxic goiter. Exp Clin Endocrinol Diabetes. 2006;114(5):235–9.

Monzani F, Caraccio N, Goletti O, et al. Five-year follow-up of percutaneous ethanol injection for the treatment of hyperfunctioning thyroid nodules: a study of 117 patients. Clin Endocrinol. 1997;46:9–15.

Müller K, Krohn K, Eszlinger M, et al. Effect of iodine on early stage thyroid autonomy. Genomics. 2011;97(2):94–100.

Nogueira CR, Kopp P, Arseven OK, et al. Thyreotropin receptor mutations in hyperfunctioning thyroid adenomas from Brazil. Thyroid. 1999;11:1063–8.

Nygaard B, Knudsen J, Hegedus L, et al. Thyrotropin receptor antibodies and Graves' disease, a side effect of 131-I treatment in patients with non-toxic goiter. J Clin Endocrinol Metab. 1997;82:2926–30.

Nygaard B, Hegedüs L, Ulriksen P, et al. Radioiodine therapy for multinodular toxic goiter. Arch Intern Med. 1999a;28(12):1364–8.

Nygaard B, Hegedus L, Nielsen KG, et al. Long-term effect of radioactive iodine on thyroid function and size in patients with solitary autonomously functioning toxic thyroid nodules. Clin Endocrinol. 1999b;50:197–202.

Palos-Paz F, Perez-Guerra O, Cameselle Teijero J, et al. Prevalence of mutations in TSHR, GNAS, PRKAR1A, and RAS genes in a large series of toxic thyroid adenomas from Galicia, an iodine deficient area in NW Spain. Eur J Endocrinol. 2008;159:623–31.

Parma J, Duprez L, Van Sande J, et al. Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. Nature. 1993;365:649–51.

Parma J, Van Sande J, Swillens S, et al. Somatic mutations causing constitutive activity of the thyrotropin receptor are the major cause of hyperfunctioning thyroid adenomas: identification of additional mutations activating both the cyclic adenosine 3′,5′-monophosphate and inositolphosphate-Ca^{++} cascades. Mol Endocrinol. 1995;9:725–33.

Parma J, Duprez L, Van Sande J, et al. Diversity and prevalence of somatic mutations in the thyrotropin receptor and Gs alpha genes as a cause of toxic thyroid adenoma. J Clin Endocrinol Metab. 1997;82:2695–701.

Paschke R, Tonacchera M, Van Sande J, et al. Identification and functional characterization of two new somatic mutations causing constitutive activation of the TSH receptor in hyperfunctioning autonomous adenomas of the thyroid. J Clin Endocrinol Metab. 1994;79:1785–9.

Pedersen IB, Knudsen N, Perrild H, et al. TSH receptor antibody measurement for differentiation of hyperthyroidism into Graves' disease and multinodular toxic goiter: a comparison of two competitive binding assays. Clin Endocrinol. 2001;55:381–90.

Porcellini A, Ciullo I, Laviola L, et al. Novel mutations of thyrotropin receptor gene in thyroid hyperfunctioning adenomas. J Clin Endocrinol Metab. 1994;79:657–61.

Porterfield JR Jr, Thompson GB, Farley DR, et al. Evidence-based management of toxic multinodular goiter (Plummer's disease). World J Surg. 2008; 32:1278–84.

Reinwein D, Benker G, Konig MP, et al. The different types of hyperthyroidism in Europe. Results of a prospective survay of 924 patients. J Endocrinol Investig. 1988;11:193–200.

Reinwein D, Benker G, Lazarus JH, et al. A prospective randomized trial of antithyroid drug dose in Graves' disease therapy. European Multicenter Study Group on antithyroid drug treatment. J Clin Endocrinol Metab. 1993;76(6):1516–21.

Reschini E, Ferrari C, Castellani M, et al. The trapping-only nodules of the thyroid gland: prevalence study. Thyroid. 2006;16(8):757–62.

Roger PP, van Staveren WC, Coulonval K, et al. Signal transduction in the human thyrocyte and its perversion in thyroid tumors. Mol Cell Endocrinol. 2010;321(1):3–19.

Ross DS, Burch HB, Cooper DS, et al. American Thyroid Association guidelines for diagnosis and management of hyperthyroidism and other causes of thyrotoxicosis. Thyroid. 2016;26(10):1343–421.

Russo D, Arturi F, Suarez HG, et al. Thyrotropin receptor gene alterations in thyroid hyperfunctioning adenomas. J Clin Endocrinol Metab. 1996;81:1548–51.

Scholz GH, Hagemann E, Arkenau C, et al. Is there a place for thyroidectomy in older patients with thyrotoxic storm and cardiorespiratory failure? Thyroid. 2003;13:933–40.

Smith TJ, Hegedus L. Graves' disease. N Engl J Med. 2016;375:1552–65.

Sorensen JR, Hegedus L, Kruse-Andersen S, et al. The impact of goitre and its treatment on the trachea, airflow, oesophagus and swallowing function. A systematic review. Best Pract Res Clin Endocrinol Metab. 2014;28:1–14.

Stanbury JB, Ermans AS, Bourdoux P, et al. Iodine-induced hyperthyroidism: occurrence and epidemiology. Thyroid. 1998;8:83–100.

Studer H, Peter HJ, Gerber H. Natural heterogeneity of thyroid cells: the basis for understanding thyroid function and nodular goiter growth. Endocr Rev. 1989;10:125–35.

Studer H, Gerber H, Zbaeren J, et al. Histomorphological and immunohistochemical evidence that human nodular goiters grow by episodic replication of multiple clusters of thyroid follicular cells. J Clin Endocrinol Metab. 1992;875:1151–8.

Tagami T, Yambe Y, Tanaka T, et al. Short-term effects of beta-adrenergic antagonists and methimazole in new-onset thyrotoxicosis caused by Graves' disease. Intern Med. 2012;51:2285–90.

Takeshita A, Nagayama Y, Yokoyama N, et al. Rarity of oncogenic mutations in the thyrotropin receptor of autonomously functioning thyroid nodules in Japan. J Clin Endocrinol Metab. 1995;80:2607–10.

Tarantino L, Francica G, Sordelli I, et al. Percutaneous ethanol injection of hyperfunctioning thyroid nodules: long-term follow-up in 125 patients. AJR Am J Roentgenol. 2008;190(3):800–8.

Tassi V, Di Cerbo A, Porcellini A, et al. Screening of thyrotropin receptor mutations by fine-needle aspiration biopsy in autonomous functioning thyroid nodules in multinodular goiters. Thyroid. 1999;4:353–7.

Thvilum M, Brandt F, Almind D, et al. Type and extent of somatic morbidity before and after the diagnosis of hypothyroidism. A nationwide register study. PLoS One. 2013;8:e75789.

Tonacchera M, Van Sande J, Parma J, et al. TSH receptor and disease. Clin Endocrinol. 1996;44:621–33.

Tonacchera M, Chiovato L, Pinchera A, et al. Hyperfunctioning thyroid nodules in toxic multinodular goiter share activating somatic thyrotropin receptor mutations with solitary toxic adenoma. J Clin Endocrinol Metab. 1998a;83:492–8.

Tonacchera M, Vitti P, Agretti P, et al. Activating thyrotropin receptor mutations in histologically heterogeneous hyperfunctioning nodules of multinodular goiter. Thyroid. 1998b;7:559–64.

Tonacchera M, Vitti P, Agretti P, et al. Functioning and nonfunctioning thyroid adenomas involve different molecular pathogenetic mechanisms. J Clin Endocrinol Metab. 1999;84:4155–8.

Tonacchera M, Agretti P, Chiovato L, et al. Activating Thyrotropin receptor mutations are present in nonadenomatous hyperfunctioning nodules of toxic or autonomous multinodular goiter. J Clin Endocrinol Metab. 2000;85:2270–4.

Tonacchera M, Pinchera A, Vitti P. Assessment of nodular goiter. Best Pract Res Clin Endocrinol Metab. 2010;24(1):51–61.

Van Sande J, Parma J, Tonacchera M, et al. Somatic and germline mutations of the TSH receptor gene in thyroid diseases. J Clin Endocrinol Metab. 1995;80:2577–85.

Viacava P, Bocci G, Tonacchera M, et al. Markers of cell proliferation, apoptosis, and angiogenesis in thyroid adenomas: a comparative immunohistochemical and genetic investigation of functioning and non-functioning nodules. Thyroid. 2007;17:191–7.

Walter MA, Briel M, Crain MC, et al. Effects of antithyroid drugs on radioiodine treatment: systematic review and meta-analysis of randomized controlled trials. Br Med J. 2007;334:514.

Zingrillo M, Torlontano M, Ghiggi MR, et al. Radioiodine and percutaneous ethanol injection in the treatment of large toxic thyroid nodule: a long-term study. Thyroid. 2000;10:985–9.

Part VI

Thyroid Carcinoma

Pathogenesis of Thyroid Carcinoma

18

Massimo Santoro and Francesca Carlomagno

Contents

Abstract

This chapter summarizes our current knowledge about molecular lesions driving the most common subtypes of thyroid carcinoma. Genetic lesions in the RET receptor tyrosine kinase and in RAS family GTPases are present in a large proportion of sporadic medullary thyroid carcinomas (MTC). RAS mutations are also common in well-differentiated thyroid carcinomas of the papillary (PTC) and follicular (FTC) type. Genetic lesions, most commonly the V600E point mutation, in the BRAF serine/threonine kinase are common in PTC; the PAX8-PPARG gene fusion is present in FTC. Finally, aggressive thyroid cancer types are enriched in several additional mutations including those targeting the TP53 tumor suppressor and the TERT (telomerase-reverse transcriptase) gene

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promoter. This knowledge is being translated into novel diagnostic and prognostic markers as well as molecular targets for novel therapeutic options.

Keywords
Kinase · Neoplastic progression · Differentiation · MAPK · TP53 · TERT

Abbreviations

4E-BP1	4E-binding protein 1
AKT	AKR mice tyhymoma oncogene
ALK	Anaplastic lymphoma kinase
APC	Adenomatous polyposis coli
ARID	AT-rich interaction domain
ART	Artemin
ATC	Anaplastic thyroid carcinoma
ATRX	ATP-dependent helicase X
BRAF	B-type rapidly accelerated fibrosarcoma oncogene
CCDC6	Coiled coil domain containing protein 6
CEA	Carcinoembryonic antigen
CLA	Cutaneous lichen amyloidosis
CLD	Cadherin like domain
CRD	Cysteine rich domain
CREBBP	Cyclic AMP responsive element binding binding protein
CTNNB1	Catenin beta 1
CV-PTC	Classical variant-papillary thyroid carcinoma
DDR	DNA damage response
DICER1	Dicer 1 ribonuclease III
DTC	Differentiated thyroid carcinoma
EIF1AX	Eukaryotic translation initiation factor 1A, X-linked
ERK	Extracellular regulated kinase
ETS	E-twenty six
FAP	Familial adenomatous polyposis of colon
FNMTC	Familial non medullary thyroid carcinoma
FOXE1	Forkhead box E1
FTA	Follicular thyroid adenoma
FTC	Follicular thyroid carcinoma
FTEN	Familial thyroid epithelial neoplasia
FV-PTC	Follicular variant-papillary thyroid carcinoma
GABP	GA binding protein
GAP	GTPase activating protein
GDNF	Glial cell-derived neurotrophic factor
GEF	Guanine nucleotide exchange factor
GTP	Guanosine triphosphate
HCC	Hürthle cell carcinoma
HD	Hirschsprung's disease
Indels	Insertion/deletions

KD	Kinase domain
KMT2A/C/D	Lysine methyltransferase 2 A/C/D
MAPK	Mitogen-activated protein kinase
MEN	Multiple endocrine neoplasia syndrome
MLH1	Mut L homolog 1
MLH3	Mut L homolog 3
MMR	Mismatch repair
MNG1	Multinodular goiter 1
MSH2	Mut S homolog 2
MSH6	Mut S homolog 6
MTC	Medullary thyroid carcinoma
mTOR	mammalian target of rapamycin
mTORC	mammalian target of rapamycin complex
NCOA4	Nuclear coactivator 4
NF1	Neurofibromatosis 1
NF2	Neurofibromatosis 2
NIFTP	Non invasive follicular thyroid neoplasm with papillary like nuclear features
NKX2-1	NK2 homeobox 1
NMTC	Non-medullary thyroid carcinoma
NMTC1	Non-medullary thyroid carcinoma 1
NRT	Neurturin
NTRK	Neurotrophic tyrosine kinase
PAX8	Paired box gene 8
PBMR1	Polybromo-1, BRG1-associated factor
PDK1	Phosphoinositide-dependent kinase 1
PDTC	Poorly differentiated thyroid carcinoma
PI3K	Phosphatidylinositol 3 kinase
PI3KCA	Phosphatidylinositol 3 kinase catalytic subunit
PIP3	Phosphatidylinositol (3,4,5)-trisphosphate
PKB	Protein kinase B
PPARG	Peroxisome proliferator-activated receptor gamma
PPFP	PAX8-PPARG fusion protein
PSP	Persephin
PTC	Papillary thyroid carcinoma
PTEN	Phosphatase and tensin homolog
RAS	Rat sarcoma oncogene
RBD	Ras binding domain
RET	Rearranged during transfection
RSK	Ribosomal protein S6 kinase
RTK	Receptor tyrosine kinase
SBS	Single base substitution
SETD2	SET domain containing 2 protein
SMARCB1	SWI/SNF related matrix associated actin dependent regulator of chromatin B1

SOS	Son of sevenless
SWI/SNF	SwItch/sucrose non-fermentable
TC	Thyroid carcinoma
TCGA	The cancer genome atlas
TCO	Thyroid tumors with cell oxyphilia
TCV-PTC	Tall cell variant-papillary thyroid carcinoma
TERT	Telomerase reverse transcriptase
THADA	Thyroid adenoma associated protein
TP53	Tumor protein p53
TSC2	Tuberous sclerosis 2
TSH	Thyroid stimulating hormone
WNT	Wingless-related integration site
YAP	Yes-associated protein

Epidemiology of Thyroid Carcinoma

In 2015, the estimated incidence of thyroid carcinoma (TC) is 64,300 new cases in the United States, rendering TC the fifth most common cancer type in women if non-melanoma skin cancers are excluded (Cabanillas et al. 2016; Siegel et al. 2016). TC incidence is highest in countries with a high human development index (HDI), and it has been stably rising over the last 10 years. This increase is, at least in part, related to increased surveillance and use of high-resolution diagnostic imaging methods (Cabanillas et al. 2016; Siegel et al. 2016; Dralle et al. 2015; Fagin and Wells 2016).

Classification of Thyroid Carcinoma

TC is classified based on the cell type of origin (C cells or follicular cells) and the expression of differentiated features (Giordano 2016; Nikiforov and Nikiforova 2011). TC arising from neuroendocrine calcitonin-producing C cells is called medullary thyroid carcinoma (MTC), while TC arising from endoderm-derived follicular cells is called non-medullary thyroid carcinoma (NMTC). MTC accounts for approximately 3–5% of all TC cases (Fagin and Wells 2016; Howlader et al. 2016; Wells et al. 2013, 2015). NMTC is further subdivided in four major types (PTC, FTC, PDTC, ATC), whose relative prevalence depends on geographic factors, including iodine intake in the region of interest (Cabanillas et al. 2016; Fagin and Wells 2016). Papillary (PTC) and follicular (FTC) TC, together with other rare types, such as Hürthle cell (oncocytic) carcinomas (HCC), belong to the differentiated thyroid carcinoma (DTC) category. PTC is the most common TC subtype overall (Cabanillas et al. 2016; Fagin and Wells 2016). Poorly differentiated (PDTC) and anaplastic (ATC) carcinomas are rare TC subtypes characterized by a partial or total loss of differentiation, respectively. TC histological classification is strongly related to disease outcome. DTC is generally associated with a favorable outcome and with a long-term survival rate >90%, though up to 30% of patients may relapse after

initial treatment (Mazzaferri 1999; Liebner and Shah 2011; Xing et al. 2013). ATC has a rapid onset and fulminant disease course with a mean survival of only 6 months (Fagin and Wells 2016; Smallridge et al. 2009; Smallridge and Copland 2010). PDTC has a behavior that is intermediate between DTC and ATC, with a mean survival of approximately 3 years (Fagin and Wells 2016; Xing et al. 2013).

Etiology of Thyroid Carcinoma

Both environmental and genetic factors are relevant for the etiology of TC.

The incidence of FTC, in particular, and possibly ATC, is highest in regions with iodine deficiency (Zimmermann and Galetti 2015). Mechanistically, iodine deficiency can lead to cancer promotion secondary to chronic TSH stimulation due to impaired thyroid hormones biosynthesis (Dumont et al. 2015). High iodine content has also been related to increased TC incidence (Dralle et al. 2015). Exposure to ionizing radiation, particularly in childhood, has been recognized as a risk factor for PTC. Persons who have received external radiotherapy for the treatment of various head and neck diseases, or have been exposed to radiation after the atomic bomb explosions in Hiroshima and Nagasaki, or to radioisotopes after the Chernobyl accident have shown increased PTC incidence (Williams 2008, 2015). Mechanistically, ionizing radiation can lead to oncogenic rearrangements, secondary to double-strand DNA breaks, and illegitimate recombination of genes that map in spatial proximity in the interphase chromatin of thyrocytes (Gandhi et al. 2010). Chromosome fragility may further contribute to these recombination events (Dillon et al. 2013). Exposure to environmental pollutants may also play a role in TC. Increased TC incidence has been reported in volcanic areas, such as Hawaii, Iceland, and Sicily, possibly due to inorganic elements present in pollution (Duntas and Doumas 2009; Malandrino et al. 2016). Accordingly, in the area of Mount Etna in Sicily, a twofold increase in incidence of TC has been associated with increased levels of several trace elements in the water and in the residents' urine samples (Malandrino et al. 2016).

About one quarter of MTC cases have a monogenic pattern of inheritance (see below). Familial inheritance has also been described in up to 9% of the NMTC cases (Fagin and Wells 2016; Mazeh and Sippel 2013; Navas-Carrillo et al. 2014). Accordingly, first-degree relatives of TC patients carry a risk that is four to tenfold higher than the general population (Hsiao and Nikiforov 2014). Familial NMTC can either occur as one of the components of familial multicancer syndromes (familial adenomatous polyposis of colon (FAP), Cowden's disease, Carney complex, Werner's syndrome, McCune-Albright syndrome) or as a predominant feature (isolated FNMTC) (Navas-Carrillo et al. 2014; Bonora et al. 2010). Most FNMTC are PTC; some cases show specific histological features, such as trabecular struma with oxyphilia or cribriform pattern in FAP patients (Mazeh and Sippel 2013; Navas-Carrillo et al. 2014). Increased multifocality, invasion, lymph-node metastases, and recurrence as well as early age at onset have been described in some FNMTC cases with respect to sporadic counterparts. An autosomal dominant pattern of inheritance with incomplete penetrance and variable expressivity has been documented in most

isolated FNMTC. However, FNMTC is a genetically heterogeneous condition, and different candidate susceptibility loci have been involved in different families. These include MNG1 (chr. 14q31), TCO (chr. 19p13.2), fPTC/PRN (chr. 1q21), NMTC1 (chr. 2q21), FTEN (chr. 8p23.1), NKX2–1 (chr. 14q13.3), and FOXE1 (chr. 9q22.33) (Mazeh and Sippel 2013; Navas-Carrillo et al. 2014; Gudmundsson et al. 2009). In one pedigree with multiple TC cases, a highly penetrant germline mutation was found to target the DICER1 gene (chr. 14q31) whose protein product is involved in microRNA processing (Rio Frio et al. 2011).

Molecular Pathogenesis of Thyroid Carcinoma

Currently, more than 90% of TC cases have at least one recognized pathogenetic genetic lesion (Hsiao and Nikiforov 2014). Simplistically, most of the targeted genes code for proteins that function along two particular signaling pathways: the ERK (extracellular regulated kinase) and the PI3K (phosphatidylinositol 3-kinase) signaling cascades (Mendoza et al. 2011). However, as discussed below, other molecular circuits are involved in TC, as well.

The ERK pathway is one of several MAPK (mitogen-activated protein kinases) signaling modules in eukaryotes (Dhillon et al. 2007). Its activation is typically initiated by the binding of a growth factor to its cognate membrane receptor endowed with tyrosine kinase catalytic activity (RTK) (Fig. 1). This is followed by the activation of the kinase activity of the RTK and the recruitment of the guanine nucleotide exchange factor (GEF) SOS, which mediates GTP nucleotide loading on RAS small GTPases. Secondary to recruitment to GTP-bound RAS, serine/threonine RAF kinases (ARAF, BRAF, CRAF) are activated through dimerization and phosphorylation (on serine 338 and other sites) and phosphorylate (on serine 217/serine 221) MEK proteins. MEKs, in turn, are dual (tyrosine and serine/threonine) activity kinases which phosphorylate (on threonine 202/tyrosine 204) the serine/threonine kinases p44 and p42 ERKs. Finally, activated ERKs, directly or via other downstream kinases such as p90RSK, potently affect cellular transcriptional output by targeting a large number of transcription factors (Roberts and Der 2007; Schubbert et al. 2007). Different components of the ERK system are targeted by genetic lesions in TC (Fig. 1). As discussed in the next paragraphs, these lesions include: (i) single base substitutions (SBS), small insertion/deletions (indels) or rearrangements of RET or other RTKs (NTRK, ALK, and others); (ii) SBS, indels, and, more rarely, rearrangements of BRAF; and (iii) SBS of RAS.

The PI3K pathway is another signaling circuit that is commonly upregulated in TC, particularly in the most aggressive forms (Fig. 1) (Fagin and Wells 2016; Xing et al. 2013). Similarly to the MAPK system, also PI3K activation is initiated by a RTK or directly by RAS (Mendoza et al. 2011). PI3K produces the second intracellular messenger phosphatidylinositol (3,4,5)-trisphosphate (PIP3). The kinase PDK1 is activated by PIP3 binding and mediates activation of the AKT/PKB (hereafter referred to as AKT) serine/threonine kinase via phosphorylation of its threonine 308. The PTEN (phosphatase and tensin homolog) phosphatase functions as a gatekeeper of this circuit by hydrolyzing PIP3. Active AKT leads to the inhibition of tuberous

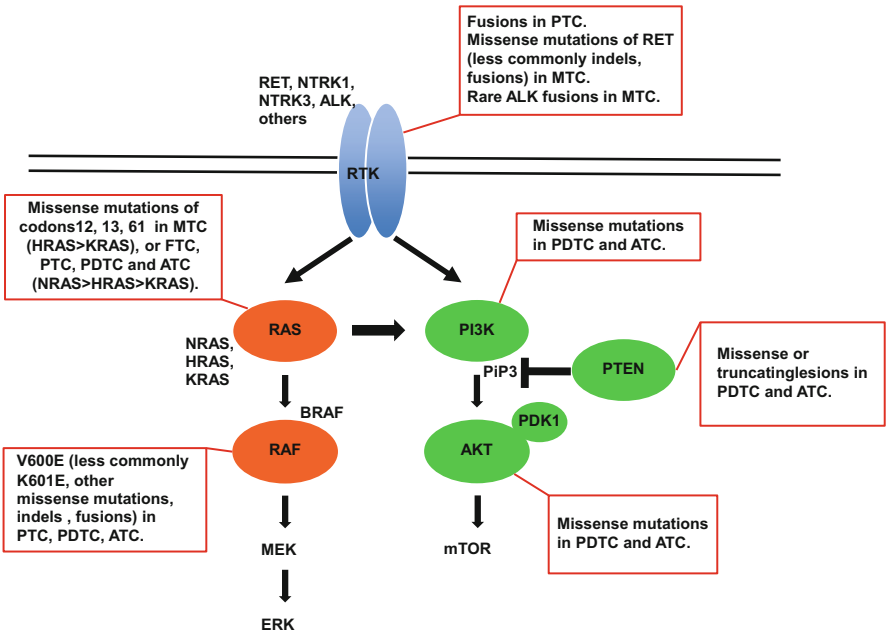

Fig. 1 Schematic representation of the ERK and PI3K signaling pathways. Next the two pathways, individual proteins most commonly involved in thyroid cancer are indicated. Type(s) of genetic lesions associated to the different types of thyroid cancer (MTC, PTC, FTC, PDTC, ATC) are indicated in the *red boxes*

sclerosis complex 2 (TSC2), thereby activating Rheb (Ras homolog enriched in brain) small GTPase, which, finally, activates the serine/threonine kinase mTOR (mammalian target of rapamycin), in the context of the mTORC1 complex. mTOR triggers protein synthesis, cell growth, and proliferation by phosphorylating p70-S6 kinase (p70S6K or S6K1) that, in turn, phosphorylates the ribosomal protein S6 and the eukaryotic translation initiation factor 4E–binding protein 1 (4E–BP1) (Hay and Sonenberg 2004; Moritz et al. 2010). In a positive feedback loop, AKT is also activated by serine 473 phosphorylation mediated by mTOR in the context of the mTORC2 complex. Different components of the PI3K signaling system are targeted by genetic lesions in TC (Fig. 1). As discussed in the next paragraphs, besides those targeting RTKs and RAS, these include single base substitutions (SBS) of the catalytic subunit of PI3K (PI3KCA) or of AKT and inactivating mutations of PTEN.

As described hereafter, genetic lesions targeting pathways other than the ERK and PI3K ones are also found in specific subtypes of TC. These include PAX8-PPARG fusion (mainly in FTC), THADA gene fusions (Drieschner et al. 2007), and mutations in TP53, EIF1AX, TERT promoter, or in components of mismatch repair, histone methyltransferase, and SWI/SNF chromatin remodeling systems (mainly in PDTC and ATC) (Fagin and Wells 2016).

Molecular Lesions in Papillary Thyroid Carcinoma (PTC)

Papillary thyroid carcinoma (PTC) is the most common TC subtype and the increased overall incidence of TC recorded in the past few years is mainly due to increased detection of PTC (Cabanillas et al. 2016; Fagin and Wells 2016). Several clinicopathological variants of PTC are identified. Small volume PTC (<1 cm) are called microcarcinomas. Approximately 30% of the PTC cases belong to classical (CV-PTC) variant, characterized by papillary architecture and specific nuclear features (grooves, pseudoinclusions, and optical clearing) (Giordano 2016). Follicular (FV-PTC) variant accounts for another 30% of the PTC cases and is characterized by the presence of nuclear features in the context of a follicular growth pattern (Giordano 2016; Asa et al. 2015). Rare and aggressive PTC variants include tall cell (TCV-PTC), columnar cell, hobnail cell, and diffuse sclerosing variants (Papp and Asa 2015).

As shown in Fig. 2, molecular pathogenesis of PTC is dominated by genetic lesions of the ERK pathway; these lesions are alternative PTC driver events as they

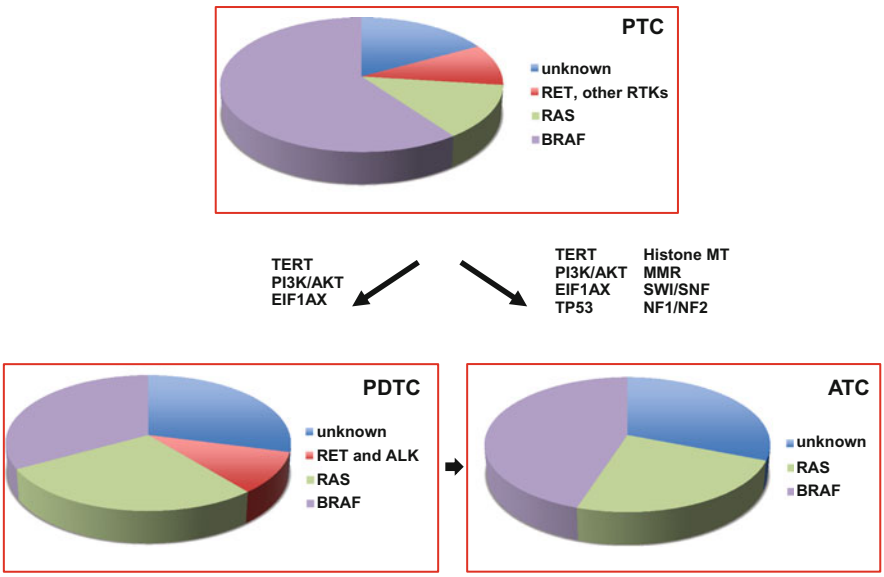

Fig. 2 Prevalence of the major genetic driver lesions in thyroid cancer. The prevalence of driver mutations (BRAF, RAS, and RET or other RTKs) in papillary, poorly differentiated, and anaplastic thyroid carcinomas is shown in the pie charts (Fagin and Wells 2016; Cancer Genome Atlas Research Network 2014). In the case of PTC, lesions in RET and other RTKs (NTRK1, NTRK3, ALK, MET, LTK, and FGFR2) are pooled together. Shown BRAF lesions include valine-to-glutamate mutation at residue 600 (V600E) only. RAS lesions are single amino acid substitutions of glycine 12, glycine 13, and glutamine 61 of HRAS, NRAS, and more rarely KRAS. Unknown areas include samples without any identified driver lesion. Lesions that co-occur with the above mentioned driver lesions in the progression to aggressive cancer subtypes (PDTC and ATC) are indicated. TERT (telomerase-reverse transcriptase) lesions include promoter mutations; *MMR* mismatch repair genes, *histone MT* histone methyltransferases. See text for additional details

generally do not overlap in individual patients (Fagin and Wells 2016). Accordingly, the analysis of several hundred PTC samples by the Cancer Genome Atlas (TCGA) has demonstrated that the most common PTC lesions are those targeting BRAF (about 60% of the cases), mainly the V600E point mutation, and RAS (about 15% of the cases), mainly point mutations in NRAS and HRAS (Fig. 3) (Cancer Genome Atlas Research Network 2014). Most BRAF- or RAS-negative cases bear chromosomal rearrangements affecting RTKs (about 10% of the cases) like RET (the receptor for glial-derived neurotrophic factor family of neurotrophic growth factors: GDNF, NRT, ART, PSP) or, less frequently, ALK, NTRK1, NTRK3, or others (Fagin and Wells 2016; Cancer Genome Atlas Research Network 2014). These rearrangements typically cause the fusion of the 5′-terminal portion, containing an active transcriptional promoter and a protein dimerization encoding motif, of one of several different genes, to the 3′-portion of a RTK gene encoding the catalytic domain. In the case of RET, these rearrangements have been

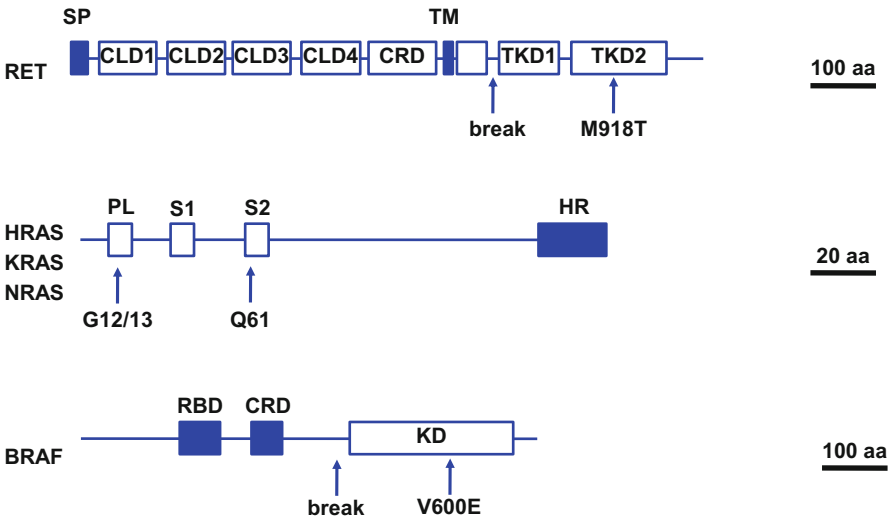

Fig. 3 Schematic representation of RET, RAS, and BRAF proteins and their most common genetic lesions in thyroid cancer. RET: SP (signal peptide), CLD1 to 4 (cadherin-like domains 1 to 4), CRD (cysteine-rich domain), TM (transmembrane domain), TKD1 and 2 (tyrosine kinase domain, subdomain 1 and 2). The position of the breakpoint (break) of RET in RET/PTC fusions and the most common RET point mutation in medullary thyroid carcinoma (methionine-to-threonine mutation at residue 918) are indicated. RAS: PL (P loop, involved in binding the γ phosphate of GTP), S1/2 (switch regions 1 and 2, which regulate binding to RAS regulators and effectors), HR (hypervariable region, which specifies membrane localization and post-translational modifications). The position of the RAS residues (glycine 12, glycine 13, and glutamine 61) most commonly targeted by point mutations in thyroid cancer is indicated. BRAF: RBD (RAS-binding domain), CRD (cysteine-rich domain), KD (kinase domain). The position of the breakpoint (break) of BRAF in fusions identified in papillary thyroid cancer and the most common BRAF point mutation in thyroid carcinoma (valine-to-glutamate mutation at residue 600) are indicated. Scale bars for each protein are reported on the right end side

termed "RET/PTCs," the most common variants being represented by RET/PTC1 (the CCDC6-RET fusion) and RET/PTC3 (the NCOA4-RET fusion) (Fig. 3) (Santoro and Carlomagno 2013).

Ionizing radiation-associated childhood PTC cases, in particular, those occurred early after the Chernobyl disaster, have a very high prevalence (up to 80%) of fusion oncogenes involving RET or other (other RTKs, BRAF or PPARG) genes (Ricarte-Filho et al. 2013). Accordingly, exposure to X-rays induces RET/PTC fusions in cultured thyrocytes (Ito et al. 1993).

The TCGA study has subdivided PTC in two major classes named BRAFV600E-like (BVL) and RAS-like (RL) PTC. BVL-PTC include samples with BRAFV600E mutation (or more rarely BRAF fusions), low differentiation, with high levels of MAPK signaling, and enriched for classical (CV-PTC) and tall cell (TCV-PTC) subtypes (Fagin and Wells 2016; Cancer Genome Atlas Research Network 2014; Ciampi et al. 2005). RL-PTC include samples with RAS (NRAS>HRAS>KRAS) mutations, well differentiated, and belonging to the follicular variant (FV-PTC) (Fagin and Wells 2016; Cancer Genome Atlas Research Network 2014; Zhu et al. 2003). When positive for BRAF lesions, FV-PTC commonly harbor the K601E mutation or mutations other than the V600E one (Afkhami et al. 2016). Mechanistically, the intensity of MAPK signaling (higher in the case of BRAF V600E and BRAF fusions and lower in the case of other BRAF lesions or RAS mutations) correlates with the different tumor phenotypes (Cancer Genome Atlas Research Network 2014). Similar to FTC, FV-PTC may also harbor the PAX8-PPARG fusion (1–5% of the cases) (Armstrong et al. 2014).

Thus, the morphological (follicular architecture) and genetic similarities render FV-PTC closely related to the FTC category (Giordano 2016; Asa et al. 2015). FV-PTC can be infiltrative or encapsulated (Hodak et al. 2016). Encapsulated FV-PTC have an indolent disease course, and it has been recently proposed that these tumors should be renamed NIFTP (noninvasive follicular thyroid neoplasms with papillary-like nuclear features) and handled as neoplasms with very low malignant potential (Hodak et al. 2016; Nikiforov et al. 2016).

Molecular Lesions in Follicular Thyroid Carcinoma (FTC)

Tumors that recapitulate the follicular architecture of the normal gland, and that lack the nuclear features of PTC, are divided into follicular thyroid adenomas (FTA), which are noninvasive, and follicular thyroid carcinomas (FTC), that demonstrate capsular and/or vascular invasion (Giordano 2016). Similar to FV-PTC, FTC are characterized by mutually exclusive SBS in RAS (approximately 50% of the cases), the PAX8-PPARG (peroxisome-proliferator activated receptor gamma), or other rare PPARG variant fusions (approximately 35% of the cases) (Fagin and Wells 2016; Nikiforova et al. 2003). The PAX8-PPARG fusion (also referred to as PPFP) arises as the consequence of a reciprocal translocation between chromosome regions 2q13 and 3p25. This juxtaposes the PAX8 transcriptional promoter (that is highly active in thyrocytes) and the PAX8 coding sequence (just missing the C-ter activating

domain) to the entire PPARG coding sequence (Eberhardt et al. 2010; Raman and Koenig 2014). PAX8 is a transcription factor of the paired box family that is necessary for thyroid development (Pasca di Magliano et al. 2000). PPARG is a member of the nuclear receptor family of transcription factors and it is involved in regulation of adipogenesis and insulin sensitivity (Krishnan et al. 2007). The PPFP chimeric protein exerts oncogenic activity, probably as the consequence of its dominant negative inhibitor activity on endogenous PPARG and perturbed regulation of PAX8- and PPARG-regulated genes (Raman and Koenig 2014). FTC positive for PAX8-PPARG fusion present at a younger age are smaller and almost always overtly invasive with respect to RAS mutant ones (Nikiforova et al. 2003).

It is still unknown whether FTA has any potential of evolving into malignant lesions (Raman and Koenig 2014). Approximately half of them have RAS mutations; less than 5% have the PAX8-PPARG fusion (Nikiforova et al. 2003). Among the three RAS oncogenes (KRAS, HRAS, NRAS), the KRAS codon 12/codon13 mutation was found associated with a significantly lower carcinoma risk than HRAS codon 61 and NRAS codon 61 mutations (Radkay et al. 2014).

Hürthle cell (oncocytic) carcinomas (HCC) are considered FTC variants; however, only infrequently they feature FTC-related genetic lesions, e.g., RAS mutations or PAX8-PPARG fusion (Nikiforova et al. 2003). Mutations of GRIM-19 gene, a component of complex I mitochondrial chain, as well as of other proteins of this complex encoded by the mitochondrial DNA, have been described in HCC (Nikiforov and Nikiforova 2011; Máximo et al. 2005; Gasparre et al. 2007). A particular type of genomic instability has been found in HCC, with copy number gains of large regions of chromosomes 5, 7, 12, and 17 that can represent the genetic driver lesion for this type of tumor (Ganly et al. 2013). Moreover, gene expression profiling has highlighted a potential role of the PI3K pathway in HCC (Ganly et al. 2013).

Molecular Lesions in Anaplastic (ATC) and Poorly Differentiated (PDTC) Thyroid Carcinoma

Though rare, poorly differentiated, and anaplastic thyroid carcinomas represent an important clinical issue, being intrinsically refractory to radiometabolic treatment (Smallridge et al. 2009; Smallridge and Copland 2010). Criteria for PDTC diagnosis listed in the Turin proposal include architectural and high-grade (mitosis and necrosis) features, solid/nested/insular pattern of growth, absence of nuclear features of PTC, and presence either of convoluted nuclei, or mitotic activity $\geq 3 \times 10$ high power fields, or tumor necrosis (Volante et al. 2007). PDTC and ATC frequently arise from pre-existing DTC, as indicated by the patients' history, presence of areas of differentiation within the cancer bulk, and the overlapping of some molecular lesions with those present in DTC. Accordingly, BRAFV600E has been found in 33% and 45% of PDTC and ATC, respectively, while RAS gene mutations have been found in approximately 25% of both PDTC and ATC (Landa et al. 2016; Kunstman et al. 2015) (Fig. 2). RAS mutations were overrepresented in PDTC fulfilling the Turin criteria, while BRAFV600E was

overrepresented in PDTC only featuring high mitotic rate and necrosis (irrespective of the growth pattern) (Landa et al. 2016).

As shown in Fig. 2, progression from DTC is believed to depend on the acquisition of additional mutations. Accordingly, mutations exclusively associated with PDTC and ATC, and thus virtually absent in DTC, are known. These include TP53 mutations in up to 70% ATC (Landa et al. 2016; Fagin et al. 1993), mutations in PI3K pathway components (Ricarte-Filho et al. 2009), mutations in the WNT signaling pathway (CTNNB1/βCatenin, AXIN1 and APC) (Garcia-Rostan et al. 1999), and mutations in the TERT transcriptional promoter (Landa et al. 2016; Landa et al. 2013).

TERT (telomerase-reverse transcriptase) represents the catalytic subunit of the telomerase complex whose activity is necessary to overcome telomeric DNA shortening and replicative senescence (Kumar et al. 2016). Mutations in TERT transcriptional promoter have been found in melanoma, urothelial bladder and liver cancer, and glioblastoma (Kumar et al. 2016; Vinagre et al. 2013). Most common mutations are the cytidine-to-thymidine transitions named C228T and C250T. These mutations have a gain-of-function effect releasing TERT from the epigenetic silencing that normally occurs in adult cells by promoting recruitment to the TERT promoter of the GA-binding protein (GABP) transcription factor; GABP, in turn, belongs to the ETS (E-twenty six) family transcription factors that are prototypic MAPK-responsive factors (Akıncılar et al. 2016; Liu et al. 2016a). Importantly, TERT mutations co-occurred with BRAF and RAS mutations in PDTC and ATC (Landa et al. 2016; Melo et al. 2014). Importantly, co-occurrence of BRAF and TERT mutations correlated with increased mortality in PTC patients (Liu et al. 2016b).

Recent genome-wide next-generation sequencing approaches have contributed to the identification of further genetic lesions and novel pathways involved in PDTC and ATC (Landa et al. 2016; Kunstman et al. 2015) (Fig. 2). Alterations of additional players in the ERK pathway, such as mutations of NF1 and deletions of NF2, have been identified (Landa et al. 2016). NF1 (Neurofibromatosis 1) codes for a tumor suppressor called Neurofibromin, a GTPase-activating protein (GAP) that functions as a gatekeeper of RAS activity (Schubbert et al. 2007). NF2 (Neurofibromatosis 2) codes for a tumor suppressor called Merlin that negatively regulates the Hippo-YAP signal transduction pathway. Thus, Merlin loss in TC unleashes the ability of the YAP transcription factor to stimulate RAS genes expression (Garcia-Rendueles et al. 2015). The EIF1AX gene codes for a eukaryotic translation initiation factor that is required for the transfer of methionyl-tRNA molecules to ribosomes in order to initiate protein translation. Mutations in EIF1AX have reported in about 1% of PTC in a mutually exclusive manner with respect to BRAF and RAS mutations (Cancer Genome Atlas Research Network 2014). EIF1AX mutations are more common in PDTC and ATC (approximately 10% of the cases) and are strongly associated with RAS mutations (Landa et al. 2016; Kunstman et al. 2015).

Besides ERK and PI3K signaling networks, novel oncogenic pathways have been identified in PDTC and ATC (Fig. 2). Besides TP53 mutations, alterations of several other genes encoding proteins involved in DDR (DNA damage response) are present in advanced TC, probably concurring to its genomic instability. They include players

of the mismatch repair (MMR) pathway, such as MSH2, MSH6, MLH1, and MLH3, and, more rarely, the checkpoint kinase ATM, involved in DNA double-strand break repair. In addition, genes encoding proteins involved in the epistatic control of gene expression and chromatin remodeling are frequently mutated in ATC. As an example, components of the SWI/SNF complex (ARID1A, ARID2, ARID5B, SMARCB1, PBMR1, and ATRX) are mutated in 36% of ATC and 6% of PDTC, and diverse histone methyltransferases (KMT2A, KMT2C, KMT2D, and SETD2) are mutated in 24% of ATC and 7% of PDTC. The acetyltransferase CREBBP has been frequently found altered in advanced thyroid cancer (Landa et al. 2016; Kunstman et al. 2015). Finally, somatic gene copy number alterations are much more represented in advanced thyroid cancer, particularly ATC (mainly 8p and 17p losses and 20q gains) than in DTC (Kunstman et al. 2015).

As previously discussed, gene rearrangements are found in DTC, including RET and, less frequently, other RTK gene fusions in PTC and PAX8-PPARG fusion in FTC. These rearrangements are scarcely represented in ATC indicating that, for as yet unknown reasons, they are associated to cancers that have a reduced propensity to progression (Kunstman et al. 2015).

Molecular Lesions in Medullary Thyroid Carcinoma (MTC)

Medullary thyroid carcinoma (MTC) is a rare cancer that originates from thyroid parafollicular C cells that are responsible for the synthesis of the calcitonin (Wells et al. 2013; Tuttle et al. 2014). Recent studies have attributed to anterior endoderm rather than neuroectoderm the origin of C cells (Johansson et al. 2015). MTC can be either sporadic (75% of cases) or familial (25% of cases). Familial cases occur as part of the autosomal dominant inherited cancer syndrome identified as multiple endocrine neoplasia type 2 (MEN2) (Wells et al. 2013; Tuttle et al. 2014). In MEN2, MTC can be isolated or associated to other phenotypes that delineate two different MEN2 subtypes: MEN2A (the most common one) and MEN2B (Table 1). Besides MTC, MEN2A patients can also display pheochromocytoma and parathyroid hyperplasia; more rarely, they develop cutaneous lichen amyloidosis (CLA),

Table 1 Phenotype of MEN2 syndromes

Syndrome	Neoplasms			Other manifestations
MEN2A	Medullary thyroid carcinoma	Pheochromocytoma	Parathyroid hyperplasia	Hirschsprung's disease
				Cutaneous lichen amyloidosis
MEN2B	Medullary thyroid carcinoma	Pheochromocytoma	Ganglioneuromatosis	Marfanoid habitus
				Ocular abnormalities

Hirschsprung's disease (HD), or prominent corneal nerves (Wells et al. 2013). Besides MTC, MEN2B patients develop pheochromocytoma, generalized ganglioneuromatosis, marfanoid habitus, and ocular abnormalities (Wells et al. 2013). FMTC (familial MTC) is characterized by the presence of only MTC; recently, FMTC has been proposed to be a variant of MEN2A (Wells et al. 2015). In MEN2 patients, MTC is preceded by a preneoplastic C cell hyperplasia which is generally present in the first decade in MEN2A patients or at birth in MEN2B ones and can be revealed by increased levels of pentagastrin-stimulated serum calcitonin (Machens et al. 2003). Serum calcitonin levels are also used to monitor persistent or recurrent disease and response to therapy, as they are directly proportional to tumor bulk. CEA (carcinoembryonic antigen) is another tumor marker for MTC (Wells et al. 2013).

The gene responsible for MEN2 maps on chromosome 10 (10q11.2) and encodes the receptor tyrosine kinase RET (Santoro and Carlomagno 2013). More than 100 different germline point mutations of RET gene have been found in MEN2 patients, accounting for over 95% of carriers. In most MEN2A cases, specific extracellular cysteines in the cysteine-rich domain of RET (C609, 611, 618, 620 in exon 10 and C634 in exon 11) (Fig. 3) are mutated to different amino acids, with C634 mutations mainly associated to the MEN2A complete phenotype (MTC, pheochromocytoma, and parathyroid hyperplasia). Mutations of other residues, mainly in exons 13, 14, and 15 of RET, can also be associated to MEN2A and FMTC (Fagin and Wells 2016; Wells et al. 2013). MEN2B is caused by mutations in RET exon 16 (M918 T) (Fig. 3), less often exon 15 (A883F), or, rarely, double RET mutations (e.g., V804M plus Y806C or, alternatively, E805K, S904C, Q781R) (Fagin and Wells 2016; Wells et al. 2013; Frank-Raue et al. 2011). Genetic testing in MEN2 family members is mandatory to identify carriers that can be treated with preventive thyroidectomy (Wells et al. 2013). Such screening is important also in sporadic cases because some of them are, in fact, familial (Elisei et al. 2007). RET MEN2 mutations convert RET in a dominant oncogene by activating RET autophosphorylation and downstream signaling in a ligand-independent manner and generating, indeed, a compelling therapeutic target for MEN2-associated tumors (Santoro and Carlomagno 2013; Mulligan 2014).

Approximately half of sporadic MTC also carry RET mutations, prevalently M918T, and more rarely other RET SBSs (like V804M/L) or Indels or finally, as recently described, RET fusion to the MYH13 gene (Fagin and Wells 2016; Wells et al. 2013; Grubbs et al. 2015). RET-negative sporadic MTC bear commonly activating mutations in RAS (principally HRAS and KRAS) genes (Moura et al. 2011; Boichard et al. 2012; Ciampi et al. 2013; Agrawal et al. 2013; Ji et al. 2015). Additional rare driver events, still targeting the ERK cascade, have been identified in RET- and RAS-negative MTC cases, such as fusions of GFPT1 and EML4 genes to the ALK RTK (Ji et al. 2015) and fusion of PARP12 to the BRAF gene (Kasaian et al. 2016). Besides those described above, other recurrent driver mutations have not yet been described in MTC (Agrawal et al. 2013; Ji et al. 2015).

Conclusions

Recent studies, mostly based on next-generation sequencing approaches, have contributed to depict the genetic landscape of the major thyroid cancer histotypes such as PTC, FTC, PDTC, ATC, and MTC (Fagin and Wells 2016). Besides the ERK and PI3K pathways, novel genetic players have emerged in thyroid tumorigenesis which includes proteins involved in translation, mismatch repair, and in chromatin remodeling (Cancer Genome Atlas Research Network 2014; Landa et al. 2016; Kunstman et al. 2015).

Translation of this knowledge to clinical practice is being very rapid. Probably, the most striking example of this concept is the diagnosis of MEN2 carriers based on the identification of germline RET mutations (Wells et al. 2015). In addition, a negative prognostic role of the BRAF V600E mutation which is associated to increased risk of nodal recurrences for PTC has been proposed by some authors (Xing et al. 2015). Novel molecular tests either based on a gene expression classifier or next-generation sequencing of a panel of TC driver genes have been proposed to be able to complement pathology diagnosis of TC (Hsiao and Nikiforov 2014).

Finally, molecular characterization of TC has encouraged the initiation of several targeted therapy clinical trials which have led to the approval of novel drugs for radioiodide refractory TC (lenvatinib and sorafenib) or for locally advanced or metastatic MTC (vandetanib and cabozantinib) (Fagin and Wells 2016; Haraldsdottir and Shah 2014; Dunn and Fagin 2015). It is feasible that these progresses may continue to be translated into better diagnosis and treatment of TC in the next future.

Summary

Thyroid cancer is classified in different subtypes based on the type of cell of origin (C cell or follicular cell) and on the expression of differentiation markers. Several genetic lesions have been associated to the different types of thyroid cancer, with the RAS/MAPK pathway almost invariably activated in all types. Advanced thyroid cancers display additional mutations in pathways controlling genome stability, survival and senescence. Such distinctive features might help designing new therapies and diagnostic strategies.

Cross-References

▶ Anaplastic and Other Forms of Thyroid Carcinoma
▶ Differentiated Thyroid Carcinoma of Follicular Origin
▶ Medullary Carcinoma
▶ New (Medical) Treatment for Thyroid Carcinoma
▶ Thyroid Nodule

References

Afkhami M, Karunamurthy A, Chiosea S, Nikiforova MN, Seethala R, Nikiforov YE, et al. Histopathologic and clinical characterization of thyroid tumors carrying the BRAF(K601E) mutation. Thyroid. 2016;26(2):242–7.

Agrawal N, Jiao Y, Sausen M, Leary R, Bettegowda C, Roberts NJ, et al. Exomic sequencing of medullary thyroid cancer reveals dominant and mutually exclusive oncogenic mutations in RET and RAS. J Clin Endocrinol Metab. 2013;98(2):E364–9.

Akıncılar S, Khattar E, Boon PL, Unal B, Fullwood MJ, Tergaonkar V. Long-range chromatin interactions drive mutant tert promoter activation. Cancer Discov. 2016;6(11):1276–91.

Armstrong MJ, Yang H, Yip L, Ohori NP, McCoy KL, Stang MT, et al. PAX8/PPARγ rearrangement in thyroid nodules predicts follicular-pattern carcinomas, in particular the encapsulated follicular variant of papillary carcinoma. Thyroid. 2014;24(9):1369–74.

Asa SL, Giordano TJ, LiVolsi VA. Implications of the TCGA genomic characterization of papillary thyroid carcinoma for thyroid pathology: does follicular variant papillary thyroid carcinoma exist? Thyroid. 2015;25(1):1–2.

Boichard A, Croux L, Al Ghuzlan A, Broutin S, Dupuy C, Leboulleux S, et al. Somatic RAS mutations occur in a large proportion of sporadic RET-negative medullary thyroid carcinomas and extend to a previously unidentified exon. J Clin Endocrinol Metab. 2012;97(10):E2031–5.

Bonora E, Tallini G, Romeo G. Genetic predisposition to familial nonmedullary thyroid cancer: an update of molecular findings and state-of-the-art studies. J Oncol. 2010;2010:385206.

Cabanillas ME, McFadden DG, Durante C. Thyroid cancer. Lancet. 2016 Dec 3;388(10061): 2783–2795.

Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. Cell. 2014;159(3):676–90.

Ciampi R, Knauf JA, Kerler R, Gandhi M, Zhu Z, Nikiforova MN, et al. Oncogenic AKAP9-BRAF fusion is a novel mechanism of MAPK pathway activation in thyroid cancer. J Clin Invest. 2005;115(1):94–101.

Ciampi R, Mian C, Fugazzola L, Cosci B, Romei C, Barollo S, et al. Evidence of a low prevalence of RAS mutations in a large medullary thyroid cancer series. Thyroid. 2013;23(1):50–7.

Dhillon AS, Hagan S, Rath O, Kolch W. MAP kinase signalling pathways in cancer. Oncogene. 2007;26(22):3279–90.

Dillon LW, Pierce LC, Lehman CE, Nikiforov YE, Wang YH. DNA topoisomerases participate in fragility of the oncogene RET. PLoS One. 2013;8(9):e75741.

Dralle H, Machens A, Basa J, Fatourechi V, Franceschi S, Hay ID, et al. Follicular cell-derived thyroid cancer. Nat Rev Dis Primers. 2015;1:15077.

Drieschner N, Kerschling S, Soller JT, Rippe V, Belge G, Bullerdiek J, Nimzyk R. A domain of the thyroid adenoma associated gene (THADA) conserved in vertebrates becomes destroyed by chromosomal rearrangements observed in thyroid adenomas. Gene. 2007;403(1–2):110–7.

Dunn L, Fagin JA. Therapy: lenvatinib and radioiodine-refractory thyroid cancers. Nat Rev Endocrinol. 2015;11(6):325–7.

Duntas LH, Doumas C. The 'rings of fire' and thyroid cancer. Hormones (Athens). 2009;8(4): 249–53.

Eberhardt NL, Grebe SK, McIver B, Reddi HV. The role of the PAX8/PPARgamma fusion oncogene in the pathogenesis of follicular thyroid cancer. Mol Cell Endocrinol. 2010;321 (1):50–6.

Elisei R, Romei C, Cosci B, Agate L, Bottici V, Molinaro E, et al. RET genetic screening in patients with medullary thyroid cancer and their relatives: experience with 807 individuals at one center. J Clin Endocrinol Metab. 2007;92(12):4725–9.

Fagin JA, Matsuo K, Karmakar A, Chen DL, Tang SH, Koeffler HP. High prevalence of mutations of the p53 gene in poorly differentiated human thyroid carcinomas. J Clin Invest. 1993;91(1): 179–84.

Fagin JA, Wells Jr SA. Biologic and clinical perspectives on thyroid cancer. N Engl J Med. 2016;375(11):1054–67.
Frank-Raue K, Rybicki LA, Erlic Z, Schweizer H, Winter A, Milos I, et al. Risk profiles and penetrance estimations in multiple endocrine neoplasia type 2A caused by germline RET mutations located in exon 10. Hum Mutat. 2011;32(1):51–8.
Gandhi M, Evdokimova V, Nikiforov YE. Mechanisms of chromosomal rearrangements in solid tumors: the model of papillary thyroid carcinoma. Mol Cell Endocrinol. 2010;321(1):36–43.
Ganly I, Ricarte Filho J, Eng S, Ghossein R, Morris LG, Liang Y, et al. Genomic dissection of Hurthle cell carcinoma reveals a unique class of thyroid malignancy. J Clin Endocrinol Metab. 2013;98(5):E962–72.
Garcia-Rendueles ME, Ricarte-Filho JC, Untch BR, Landa I, Knauf JA, Voza F, et al. NF2 loss promotes oncogenic RAS-induced thyroid cancers via YAP-dependent transactivation of RAS proteins and sensitizes them to MEK inhibition. Cancer Discov. 2015;5(11):1178–93.
Garcia-Rostan G, Tallini G, Herrero A, D'Aquila TG, Carcangiu ML, Rimm DL. Frequent mutation and nuclear localization of beta-catenin in anaplastic thyroid carcinoma. Cancer Res. 1999; 59(8):1811–5.
Gasparre G, Porcelli AM, Bonora E, Pennisi LF, Toller M, Iommarini L, et al. Disruptive mitochondrial DNA mutations in complex I subunits are markers of oncocytic phenotype in thyroid tumors. Proc Natl Acad Sci U S A. 2007;104(21):9001–6.
Giordano TJ. Follicular cell thyroid neoplasia: insights from genomics and the cancer genome atlas research network. Curr Opin Oncol. 2016;28(1):1–4.
Grubbs EG, Ng PK, Bui J, Busaidy NL, Chen K, Lee JE, et al. RET fusion as a novel driver of medullary thyroid carcinoma. J Clin Endocrinol Metab. 2015;100(3):788–93.
Gudmundsson J, Sulem P, Gudbjartsson DF, Jonasson JG, Sigurdsson A, Bergthorsson JT, et al. Common variants on 9q22.33 and 14q13.3 predispose to thyroid cancer in European populations. Nat Genet. 2009;41(4):460–4.
Haraldsdottir S, Shah MH. New era for treatment in differentiated thyroid cancer. Lancet. 2014;384 (9940):286–8.
Hay N, Sonenberg N. Upstream and downstream of mTOR. Genes Dev. 2004;18(16):1926–45.
Hodak S, Tuttle RM, Maytal G, Nikiforov YE, Randolph G. Changing the cancer diagnosis: the case of follicular variant of papillary thyroid cancer-primum non nocere and NIFTP. Thyroid. 2016;26(7):869–71.
Howlader N, Noone AM, Krapcho M, Miller D, Bishop K, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA, editors. SEER cancer statistics review, 1975–2013. Bethesda: National Cancer Institute. http://seer.cancer.gov/csr/1975_2013/, based on November 2015 SEER data submission, posted to the SEER web site. 2016.
Hsiao SJ, Nikiforov YE. Molecular approaches to thyroid cancer diagnosis. Endocr Relat Cancer. 2014;21(5):T301–13.
Ito T, Seyama T, Iwamoto KS, Hayashi T, Mizuno T, Tsuyama N, et al. In vitro irradiation is able to cause RET oncogene rearrangement. Cancer Res. 1993;53(13):2940–3.
Ji JH, Oh YL, Hong M, Yun JW, Lee HW, Kim D, et al. Identification of driving ALK fusion genes and genomic landscape of medullary thyroid cancer. PLoS Genet. 2015;11(8):e1005467.
Johansson E, Andersson L, Örnros J, Carlsson T, Ingeson-Carlsson C, Liang S, et al. Revising the embryonic origin of thyroid C cells in mice and humans. Development. 2015;142(20):3519–28.
Kasaian K, Wiseman SM, Walker BA, Schein JE, Hirst M, Moore RA, et al. Putative BRAF activating fusion in a medullary thyroid cancer. Cold Spring Harb Mol Case Stud. 2016;2(2): a000729.
Krishnan A, Nair SA, Pillai MR. Biology of PPAR gamma in cancer: a critical review on existing lacunae. Curr Mol Med. 2007;7(6):532–40.
Kumar M, Lechel A, Güneş Ç. Telomerase: the devil inside. Genes (Basel). 2016;7(8).
Kunstman JW, Juhlin CC, Goh G, Brown TC, Stenman A, Healy JM, et al. Characterization of the mutational landscape of anaplastic thyroid cancer via whole-exome sequencing. Hum Mol Genet. 2015;24(8):2318–29.

Landa I, Ganly I, Chan TA, Mitsutake N, Matsuse M, Ibrahimpasic T, et al. Frequent somatic TERT promoter mutations in thyroid cancer: higher prevalence in advanced forms of the disease. J Clin Endocrinol Metab. 2013;98(9):E1562–6.

Landa I, Ibrahimpasic T, Boucai L, Sinha R, Knauf JA, Shah RH, et al. Genomic and transcriptomic hallmarks of poorly differentiated and anaplastic thyroid cancers. J Clin Invest. 2016;126(3): 1052–66.

Liebner DA, Shah MH. Thyroid cancer: pathogenesis and targeted therapy. Ther Adv Endocrinol Metab. 2011;2(5):173–95.

Liu T, Yuan X, Xu D. Cancer-Specific Telomerase Reverse Transcriptase (TERT) Promoter Mutations: Biological and Clinical Implications. Genes (Basel). 2016a Jul 18;7(7). pii: E38.

Liu T, Yuan X, Xu D. Cancer-specific telomerase reverse transcriptase (TERT) promoter mutations: biological and clinical implications. Genes (Basel). 2016b;7(7):38.

Machens A, Niccoli-Sire P, Hoegel J, Frank-Raue K, van Vroonhoven TJ, Roeher HD, et al. Early malignant progression of hereditary medullary thyroid cancer. N Engl J Med. 2003;349(16): 1517–25.

Maenhaut C, Christophe D, Vassart G, Dumont J, Roger PP, Opitz R. Ontogeny, Anatomy, Metabolism and Physiology of the Thyroid. [Updated 2015 Jul 15]. In: De Groot LJ, Chrousos G, Dungan K, et al., Editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000. https://www.ncbi.nlm.nih.gov/books/NBK285554/

Malandrino P, Russo M, Ronchi A, Minoia C, Cataldo D, Regalbuto C, et al. Increased thyroid cancer incidence in a basaltic volcanic area is associated with non-anthropogenic pollution and biocontamination. Endocrine. 2016;53(2):471–9.

Máximo V, Botelho T, Capela J, Soares P, Lima J, Taveira A, et al. Somatic and germline mutation in GRIM-19, a dual function gene involved in mitochondrial metabolism and cell death, is linked to mitochondrion-rich (Hurthle cell) tumours of the thyroid. Br J Cancer. 2005;92(10): 1892–8.

Mazeh H, Sippel RS. Familial nonmedullary thyroid carcinoma. Thyroid. 2013;23(9):1049–56.

Mazzaferri EL. An overview of the management of papillary and follicular thyroid carcinoma. Thyroid. 1999;9(5):421–7.

Melo M, da Rocha AG, Vinagre J, Batista R, Peixoto J, Tavares C, et al. TERT promoter mutations are a major indicator of poor outcome in differentiated thyroid carcinomas. J Clin Endocrinol Metab. 2014;99(5):E754–65.

Mendoza MC, Er EE, Blenis J. The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. Trends Biochem Sci. 2011;36(6):320–8.

Moritz A, Li Y, Guo A, Villén J, Wang Y, MacNeill J, et al. Akt-RSK-S6 kinase signaling networks activated by oncogenic receptor tyrosine kinases. Sci Signal. 2010;3(136):ra64.

Moura MM, Cavaco BM, Pinto AE, Leite V. High prevalence of RAS mutations in RET-negative sporadic medullary thyroid carcinomas. J Clin Endocrinol Metab. 2011;96(5):E863–8.

Mulligan LM. RET revisited: expanding the oncogenic portfolio. Nat Rev Cancer. 2014;14(3): 173–86.

Navas-Carrillo D, Ríos A, Rodríguez JM, Parrilla P, Orenes-Piñero E. Familial nonmedullary thyroid cancer: screening, clinical, molecular and genetic findings. Biochim Biophys Acta. 2014;1846(2):468–76.

Nikiforov YE, Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. Nat Rev Endocrinol. 2011;7(10):569–80.

Nikiforov YE, Seethala RR, Tallini G, Baloch ZW, Basolo F, Thompson LD, et al. Nomenclature revision for encapsulated follicular variant of papillary thyroid carcinoma: a paradigm shift to reduce overtreatment of indolent tumors. JAMA Oncol. 2016;2(8):1023–9.

Nikiforova MN, Lynch RA, Biddinger PW, Alexander EK, Dorn 2nd GW, Tallini G, et al. RAS point mutations and PAX8-PPAR gamma rearrangement in thyroid tumors: evidence for distinct molecular pathways in thyroid follicular carcinoma. J Clin Endocrinol Metab. 2003;88(5): 2318–26.

Papp S, Asa SL. When thyroid carcinoma goes bad: a morphological and molecular analysis. Head Neck Pathol. 2015;9(1):16–23.

Pasca di Magliano M, Di Lauro R, Zannini M. Pax8 has a key role in thyroid cell differentiation. Proc Natl Acad Sci U S A. 2000;97(24):13144–9.
Radkay LA, Chiosea SI, Seethala RR, Hodak SP, LeBeau SO, Yip L, et al. Thyroid nodules with KRAS mutations are different from nodules with NRAS and HRAS mutations with regard to cytopathologic and histopathologic outcome characteristics. Cancer Cytopathol. 2014;122(12): 873–82.
Raman P, Koenig RJ. Pax-8-PPAR-γ fusion protein in thyroid carcinoma. Nat Rev Endocrinol. 2014;10(10):616–23.
Ricarte-Filho JC, Ryder M, Chitale DA, Rivera M, Heguy A, Ladanyi M, et al. Mutational profile of advanced primary and metastatic radioactive iodine-refractory thyroid cancers reveals distinct pathogenetic roles for BRAF, PIK3CA, and AKT1. Cancer Res. 2009;69(11):4885–93.
Ricarte-Filho JC, Li S, Garcia-Rendueles ME, Montero-Conde C, Voza F, Knauf JA, et al. Identification of kinase fusion oncogenes in post-Chernobyl radiation-induced thyroid cancers. J Clin Invest. 2013;123(11):4935–44.
Rio Frio T, Bahubeshi A, Kanellopoulou C, Hamel N, Niedziela M, Sabbaghian N, et al. DICER1 mutations in familial multinodular goiter with and without ovarian Sertoli-Leydig cell tumors. JAMA. 2011;305(1):68–77.
Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene. 2007;26(22):3291–310.
Santoro M, Carlomagno F. Targeting the Raf-MEK-ERK mit. Cold Spring Harb Perspect Biol. 2013;5(12):a009233.
Schubbert S, Shannon K, Bollag G. Hyperactive Ras in developmental disorders and cancer. Nat Rev Cancer. 2007;7(4):295–308.
Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin. 2016;66(1):7–30.
Smallridge RC, Copland JA. Anaplastic thyroid carcinoma: pathogenesis and emerging therapies. Clin Oncol (R Coll Radiol). 2010;22(6):486–97. doi:10.1016/j.clon.2010.03.013.
Smallridge RC, Marlow LA, Copland JA. Anaplastic thyroid cancer: molecular pathogenesis and emerging therapies. Endocr Relat Cancer. 2009;16(1):17–44.
Tuttle RM, Haddad RI, Ball DW, Byrd D, Dickson P, Duh QY, et al. Thyroid carcinoma, version 2.2014. J Natl Compr Canc Netw. 2014;12(12):1671–80.
Vinagre J, Almeida A, Pópulo H, Batista R, Lyra J, Pinto V, et al. Frequency of TERT promoter mutations in human cancers. Nat Commun. 2013;4:2185.
Volante M, Collini P, Nikiforov YE, Sakamoto A, Kakudo K, Katoh R, et al. Poorly differentiated thyroid carcinoma: the Turin proposal for the use of uniform diagnostic criteria and an algorithmic diagnostic approach. Am J Surg Pathol. 2007;31(8):1256–64.
Wells Jr SA, Pacini F, Robinson BG, Santoro M. Multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma: an update. J Clin Endocrinol Metab. 2013;98(8):3149–64.
Wells Jr SA, Asa SL, Dralle H, Elisei R, Evans DB, Gagel RF, et al. American thyroid association guidelines task force on medullary thyroid carcinoma. revised American thyroid association guidelines for the management of medullary thyroid carcinoma. Thyroid. 2015;25(6):567–610.
Williams D. Radiation carcinogenesis: lessons from Chernobyl. Oncogene. 2008;27(Suppl 2): S9–18.
Williams D. Thyroid growth and cancer. Eur Thyroid J. 2015;4(3):164–73.
Xing M, Haugen BR, Schlumberger M. Progress in molecular-based management of differentiated thyroid cancer. Lancet. 2013;381(9871):1058–69.
Xing M, Alzahrani AS, Carson KA, Shong YK, Kim TY, Viola D, et al. Association between BRAF V600E mutation and recurrence of papillary thyroid cancer. J Clin Oncol. 2015;33(1):42–50.
Zhu Z, Gandhi M, Nikiforova MN, Fischer AH, Nikiforov YE. Molecular profile and clinical-pathologic features of the follicular variant of papillary thyroid carcinoma. An unusually high prevalence of ras mutations. Am J Clin Pathol. 2003;120(1):71–7.
Zimmermann MB, Galetti V. Iodine intake as a risk factor for thyroid cancer: a comprehensive review of animal and human studies. Thyroid Res. 2015;8:8.

Differentiated Thyroid Carcinoma of Follicular Origin

19

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Abstract

Differentiated thyroid carcinoma (DTC) includes the papillary and follicular histotypes and their variants accounting for more than 90% of all thyroid cancers. The incidence of thyroid cancer has been increasing in many countries over the last 30 years, and it is the human solid cancer at the highest increase worldwide.

Both papillary and follicular differentiated thyroid cancer have usually a good prognosis with an overall mortality of less than 10%. This excellent prognosis results from the combination of the biological properties of most thyroid carcinomas and effective primary therapy. When compared with other malignancies, thyroid cancer is probably the most curable cancer, with very high long-term survival rates, at least in the well-differentiated histotypes. However, some patients are at high risk for recurrent disease or even death. Most of these patients can be identified at the time of diagnosis with the use of well-defined prognostic indicators.

Keywords

Radioiodine · Thyroid ablation · Thyroid cancer · Thyroglobulin · rhTSH

Epidemiology of Differentiated Thyroid Carcinoma

Thyroid cancer accounts for only 5–10% of all thyroid nodules and represents about 1–2% of all solid human malignancies (Agate et al. 2012). Differentiated thyroid carcinoma (DTC) includes the papillary and follicular histotypes and their variants accounting for more than 90% of all thyroid cancers. The incidence of thyroid cancer has been increasing in many countries over the last 30 years, and it is the human solid cancer at the highest increase worldwide. In the USA, the incidence of thyroid carcinoma in 2012 was nearly 15 cases per 100,000 persons, and this rate may rise worldwide in the next years (Davies et al. 2015; Vaccarella et al. 2016). The incidence of papillary thyroid cancer (PTC) in the USA is 13 per 100,000 person-years. The incidence of follicular thyroid cancer (FTC) in the USA is 1.15 per 100,000 person-years (Davies et al. 2015; Vaccarella et al. 2016). Thyroid carcinoma is two to four times more frequent in females than in males (Vaccarella et al. 2016). The same trend is confirmed also in adolescents where girls have a higher incidence of PTC than boys (Holmes et al. 2012). Despite the increasing incidence, the mortality from thyroid cancer tended to decline over the last three decades (Davies et al. 2015). The prevalence of thyroid cancer in autopsy series is around 11% and is stable since

the 1970s. The increasing incidence is attributable to better detection of small PTC as a result of improved diagnostic accuracy [neck ultrasound (US) and fine needle aspiration cytology) (FNAC)]. It is common experience in thyroid cancer referral centers that nearly 60–80% of thyroid carcinomas detected nowadays are micro-PTC (less than 1 cm in size) carrying an excellent long-term prognosis. However, an increased incidence for all size of thyroid tumor has been reported in the USA and may also be the result of more extended screening (Bosetti et al. 2008). Although in 1997–2005 the annual percentage change (APC) for primary tumor <1.0 cm has been 9.9 in man and 8.6 in women, a significant increase was also observed for tumor >4 cm among men (APC 3.7) and women (APC 5.7) (Bosetti et al. 2008).

When compared with other malignancies, thyroid cancer is probably the most curable cancer, with very high long-term survival rates, at least in the well-differentiated histotypes. However, some patients are at high risk for recurrent disease or even death. Most of these patients can be identified at the time of diagnosis with the use of well-defined prognostic indicators.

Pathology

Histologic diagnosis of malignancy is usually very simple, but in some tumors it is challenging.

Papillary and follicular carcinomas are the two most common entities, usually referred to as differentiated thyroid cancer.

Papillary Thyroid Carcinoma: Classic Type

The diagnosis of PTC is based on the presence of typical features (Hedinger et al. 1988). Microscopically, papillary carcinomas contain papillary areas with a focal distribution or with a diffuse pattern. The papillae consist of a stromal vascular axis lined by characteristic cells. Other aspects may be associated with the papillae: follicles filled with colloid or a trabecular or lobular aspect, squamous metaplasia, and psammoma bodies are other distinguishing features present in 40–50% of tumors. Nuclei are characteristic. They are larger than those found in normal follicular cells and overlap, contain hypodense chromatin, irregular in shape, an may be “fissured” like “coffee grains” and well-delimited intranuclear inclusions, an expression of cytoplasmic invagination, are present (Hedinger et al. 1988). In the absence of other features of the tumor, the diagnosis of PTC is based on typical features of the nuclei (Hedinger et al. 1988).

PTC is often multicentric in one lobe and bilateral, with a frequency varying between 20% and 80% in different series (Hedinger et al. 1988). Commonly and early in the course of the disease, PTC invades lymphatic vessels. Invasion progresses from the peri-thyroid chains to more distant chains. Vascular invasion and distant metastases (most often to the lung and bone) are rare and account for 2–5% of cases (Hedinger et al. 1988).

Variants of Papillary Thyroid Carcinoma

The most frequent variant of PTC is the *follicular variant of papillary thyroid carcinoma* (FVPTC). The FVPTC was broadly recognized in the mid-1970s as a tumor composed only of neoplastic follicles rather than papillae, but with follicular cells showing nuclear features characteristic of PTC. Lymph node and distant metastases are rare (Hedinger et al. 1988) and respond well to conventional treatment. The prognosis is similar to that of the classic variant.

Two main subtypes are known to occur: infiltrative (or nonencapsulated) and encapsulated follicular variant of papillary thyroid carcinoma (EFVPTC). Studies over the past decade have demonstrated that EFVPTC has an indolent behavior and is genetically distinct from infiltrative tumors. Recently, an international, multidisciplinary, retrospective study of patients with thyroid nodules diagnosed as EFVPTC, including 109 patients with noninvasive EFVPTC and 101 patients with invasive EFVPTC, was performed (Baloch et al. 2016). Based on the better outcome of noninvasive EFVPTC, the name "noninvasive follicular thyroid neoplasm with papillary-like nuclear features" (NIFTP) was adopted. The authors concluded that thyroid tumors currently diagnosed as noninvasive EFVPTC have a very low (or no) risk of adverse outcome and should be termed NIFTP. This reclassification (that needs the complete histological examination of all the tumor capsule to exclude any capsular invasion) will affect a large population of patients worldwide and result in a significant reduction in psychological and clinical consequences associated with the diagnosis of cancer (Baloch et al. 2016).

The rare *diffuse sclerosing variant* is found most often in children and young adults (Hedinger et al. 1988). At microscopy, this form is often multicentric. Tumor papillae are associated with squamous metaplasia without keratinization and abundant psammoma bodies. Extensive lymphocytic infiltration of the gland is often found, and lymph node metastases are present in 100% of cases. Also, lung metastases are common. The prognosis is less favorable than for classic PTC, although the response to treatment may be excellent.

In the *tall cell variant* (Hedinger et al. 1988), the tumor is usually large and extends outside the thyroid gland. These tumors have a papillary pattern, and more than 30% of cells are tall and have a granular, eosinophilic cytoplasm. Vascular invasion is commonly seen, and the tumors are typical of older patients. A poor prognosis has been reported with this variant as well as for the rare *columnar cell variant* (Hedinger et al. 1988).

Follicular Thyroid Carcinoma

The diagnosis of FTC is based on the presence of follicular differentiation without the typical nuclear features of papillary cancer (Hedinger et al. 1988). At variance with the papillary histotype, FTC usually is seen as a solitary, more or less encapsulated nodule in the thyroid gland. Depending on the degree of invasiveness, the tumor is classified as minimally invasive (encapsulated) or widely invasive

(Hedinger et al. 1988). The distinction has great prognostic impact because the prognosis is more severe when more extensive vascular invasion is present (Hedinger et al. 1988).

Minimally invasive carcinomas represent more than 50% of cases. The diagnosis of malignancy is based totally on the demonstration of unequivocal vascular invasion and/or invasion of the full thickness of the capsule. In case of capsular invasion only, the prognosis is excellent; in case of vascular invasion, the risk of metastases and of death increases with the number of foci of vascular invasion.

Widely invasive tumors present few diagnostic problems. They show widespread infiltration of blood vessels and surrounding thyroid tissue. The capsule, when present, is infiltrated in several areas and is grossly disrupted. FTC invades blood vessels but rarely invades lymphatics. Metastases are spread hematogenously to the lungs, bones, and, less commonly, the brain and liver (Hedinger et al. 1988). Metastases are common with the widely invasive variant, less common with the minimally invasive variant.

Variants of Follicular Carcinoma

Clear cell tumor is a rare variant, with architectural and clinical features similar to those of the usual follicular carcinomas. The cells are clear because of the formation of intracytoplasmic vesicles, glycogen, or fat accumulation. These tumors must be distinguished from clear cell adenoma, from parathyroid adenoma or carcinoma, and particularly from metastatic clear cell renal carcinoma (Hedinger et al. 1988).

Insular carcinoma is also a rare variant (Hedinger et al. 1988). It is a poorly differentiated, invasive follicular cancer with a solid aspect and follicular differentiation represented by small vesicles with very little colloid. The cells are very homogeneous in shape and smaller and more dense than in typical follicular cancer. The general picture may resemble that of carcinoid tumors. Metastases, very common, are found in lymph nodes and in distant organs (Hedinger et al. 1988). The prognosis is poor. Poorly differentiated carcinomas may also have a trabecular or a solid feature, and the presence of necrosis or a high mitotic rate may also indicate tumor aggressiveness.

Hürthle Cell Carcinoma

The Hürthle cell type (or oxyphilic cell type) is composed of cells derived from the follicular epithelium and characterized by large size with abundant granular, eosinophilic cytoplasm, large nuclei, and prominent nucleoli. The granular appearance of the cytoplasm is conferred by the large number of mitochondria inside the cell.

Because Hürthle cells can be found in papillary carcinomas and in a number of benign conditions (e.g., nodular goiter, hyperthyroidism, Hashimoto's thyroiditis, benign nodules), the same criteria for malignancy mentioned for follicular tumors (i.e., invasion) apply to oxyphilic cell tumors. As with follicular carcinoma,

macroscopically, the oxyphilic variant is seen as a solitary thyroid nodule with complete or partial encapsulation. In several series, the prognosis for this variant has been reported as less favorable than for the follicular cell type.

Pathogenesis of Differentiated Thyroid Carcinoma

Genetic Alterations

Recent advances in molecular biology have resulted in significant improvement in our understanding of the pathogenesis of DTC. Several proto-oncogenes have been identified. They are transformed in active oncogene by rearrangements or point mutations able to confer a growth advantage to a cell, eventually leading to malignant transformation. The three most frequently found driver mutations are RET/PTC rearrangements and RAS and BRAF point mutations. They are mutually exclusive. The other possible event is the inactivation of a tumor suppressor gene.

The most frequent mutational event in DTC is represented by point mutations of the *BRAF* gene. This mutation is associated specifically with *PTC* with a frequency of nearly around 40% (Nikiforov 2011a). BRAF point mutation is found in only 10% of FTC, while it can be seen also in anaplastic and poorly differentiated thyroid neoplasms (Nikiforov 2011a). The presence of this mutation is related with more unfavorable tumor behavior. In many studies, BRAF correlates with aggressive tumor characteristics like tall cell variant, extrathyroidal extension, advanced tumor stage at presentation and the evidence of lymph node or distant metastases (Li et al. 2012), and tumor dedifferentiation, including the loss of iodine uptake.

Rearrangements involving the *RET* and *TRK* proto-oncogenes have been demonstrated as causative events specific for a subset of PTC. Oncogenic activation of these genes is accomplished by fusion of their tyrosine kinase domain with the N-terminal promoter sequences of other genes in the same or other chromosomes.

In the case of *RET* rearrangements, the resulting chimeric oncogenes have been called *PTC*, an acronym for papillary thyroid carcinoma (Nikiforov 2002). Several chimeric forms have been identified, the most common being *RET/PTC* 1, 2, and 3. Although strictly associated with PTC, *RET/PTC* is found in only 20–30% of cases of PTC. In PTC occurring after irradiation, the frequency of *RET/PTC* activation is between 60% and 70%, either in Belarus children heavily exposed to radiation after the Chernobyl nuclear disaster (Nikiforov 2002) or in patients who received external radiation treatment during childhood (Agate et al. 2012). Worthy of note, these radiation-induced tumors are often of the solid variant of papillary cancer, and the oncogene involved is mainly *RET/PTC* 3, particularly in the youngest subjects. In spontaneous tumors or in classic papillary variants of radiation-induced cancers, *RET/PTC* 1 is the predominant rearrangement (Nikiforov 2002). Based on this finding, one can speculate that *RET/PTC* 3 is linked specifically to radiation and to solid papillary tumors arising in young patients (most Belarus cancers were diagnosed in children) with or without the cooperation of radiation. This second hypothesis is supported by data showing a significant correlation

between high rates of *RET/PTC* activation and lower age at diagnosis in Italian patients not exposed to radiation (Bongarzone et al. 1996).

Mutated forms of the *H-ras*, *K-ras*, and *N-ras* proto-oncogenes are found in differentiated FTC and in the follicular variant of PTC; however, these mutations are not specifically restricted to malignant lesions, because they have been found in about 30% of follicular thyroid adenomas (Nikiforov 2011a). Ras-associated cancers are less aggressive and less dedifferentiated than BRAF-associated cancers.

As far as follicular neoplasms are concerned, a specific oncogene originating from a rearrangement with a gene with tumor suppressor function, *PAX8/PPARγ*, has been associated with the malignant phenotype with high frequency (Nikiforov 2011a) but is also present in some benign follicular tumors.

Telomerase reverse transcriptase (TERT) mutations have a prominent role in the tumorigenesis and progression of thyroid cancer. TERT mutations have been reported on overage of 11.3 % in PTC, 17.1% in FTC, 43.2% in poorly differentiated thyroid cancer, and 40.1% in anaplastic thyroid cancer. TERT promoter mutations are associated with aggressive thyroid tumor characteristics, tumor recurrence, and patient mortality as well as BRAF V600E mutation (Liu and Xing 2016).

Inactivating mutations of the *p53* tumor suppressor gene are rare in patients with differentiated thyroid carcinoma but common in those with undifferentiated thyroid carcinoma (Nikiforov 2011a).

Ionizing Radiation

External irradiation of the neck during childhood increases the risk for PTC (Ron et al. 2012). The latency period between exposure and diagnosis is usually 5 years, is maximal at about 20 years, remains high for about 20 years, and then decreases gradually. A linear dose-response relationship is found between external irradiation and thyroid cancer, starting with radiation doses as low as 10 cGy and up to 1,500 cGy. Beyond this point, the risk for thyroid cancer decreases, probably because of thyroid cell killing. A major risk factor is young age at the time of irradiation; after the age of 15 or 20 years, the risk is much reduced. In children exposed to a dose of 1 Gy (100 rad) to the thyroid, excess risk for thyroid cancer is 7.7-fold (Ron et al. 1995). Diagnostic or therapeutic administration of ^{131}I to adults does not seem to be associated with an increased risk for thyroid cancer (Angusti et al. 2000). However, the increased incidence of PTC in children in the Marshall Islands after atomic bomb testing and, more recently, in Belarus and Ukraine after the Chernobyl nuclear reactor accident (Baverstok et al. 1992; Pacini et al. 1997) indicates a direct carcinogenic effect of radioactive isotopes, both ^{131}I and/or short-lived isotopes, on the thyroid gland. The post-Chernobyl cancers diagnosed in Belarus and Ukrainian children and young adults developed after a very short mean latency period (6.5 years on average) from exposure to diagnosis (Pacini et al. 1997). Whether these discrepancies are caused by different radiation doses to the thyroid, by the very young age of the patients, when the growing thyroid is particularly sensitive to radiation, or by a combination of these and other environmental factors (iodine deficiency) is still a matter of discussion.

Genetic Factors

Thyroid carcinomas are present in several familial syndromes, including Cowden's disease (hamartomas, multinodular goiters, and thyroid, breast, colon, and lung cancer) (Lloyd and Dennis 1963), familial adenomatous polyposis (where incidence is estimated to be increased 100-fold above baseline) (de Mestier 1990), Gardner's syndrome (Camiel et al. 1968), and familial chemodectomas (Albores-Saavedra and Duran 1968). However, these syndromes are very rare. The large majority of familial cancers (almost always papillary) occur as isolated, non-syndromic, PTC, in which no candidate predisposing oncogene has so far been detected. This form of familial cancer has been reported in 3–10% of patients in different series and should be differentiated from familial aggregation related to screening (Capezzone et al. 2008a). Epidemiologic studies have shown that these pedigrees exhibit the phenomenon of "genetic anticipation," consisting of the appearance of thyroid carcinoma at an earlier age with increased aggressiveness in the second and subsequent generations (Capezzone et al. 2008a). A germline alteration, consisting of short telomeres and increased telomerase activity, has been detected in familial cases, leading to genomic instability and possibly predisposing to the risk for thyroid carcinoma (Capezzone et al. 2008b).

Diagnosis

The most frequent presentation of DTC is the discovery of a thyroid nodule at neck US performed for non-thyroidal diseases or for benign thyroid disorders. The malignant nature is demonstrated by a positive cytology of the nodule (Bongiovanni et al. 2012). Sometimes, particularly in children, one or more metastatic neck lymph nodes may be the first sign of the disease. More rarely, distant metastases in the lung or the bone from FTC may be the initial symptom. Hoarseness, dysphagia, and dyspnea are seldom hallmarks of the tumor; these findings are suggestive of advanced stages of the disease. At physical examination, the nodule, usually single, is firm, movable during swelling, and often is not distinguishable from a benign lesion. Carcinoma should be suspected when the nodule is single in an otherwise normal thyroid, when it is found in children or adolescents, in males, or in association with ipsilateral enlarged lymph nodes, and, particularly, when a history of previous exposure to external radiation during childhood is present. Whatever the manifestation, the final diagnosis of malignancy must rely on the results of FNAC. Thyroid ultrasonography, although not able to differentiate benign and malignant lesions, is useful to stratify the risk of malignancy in thyroid nodules and aid decision-making about whether FNAC is indicated (Horvath et al. 2009). At ultrasonography thyroid nodules are classified as follows:

1. High suspicious (estimated risk of malignancy >70–90%) and FNAC is recommended for all nodules >1.0 cm.
2. Intermediate suspicion (estimated risk of malignancy 10–20%) and FNAC is recommended in all nodules >1.0 cm.

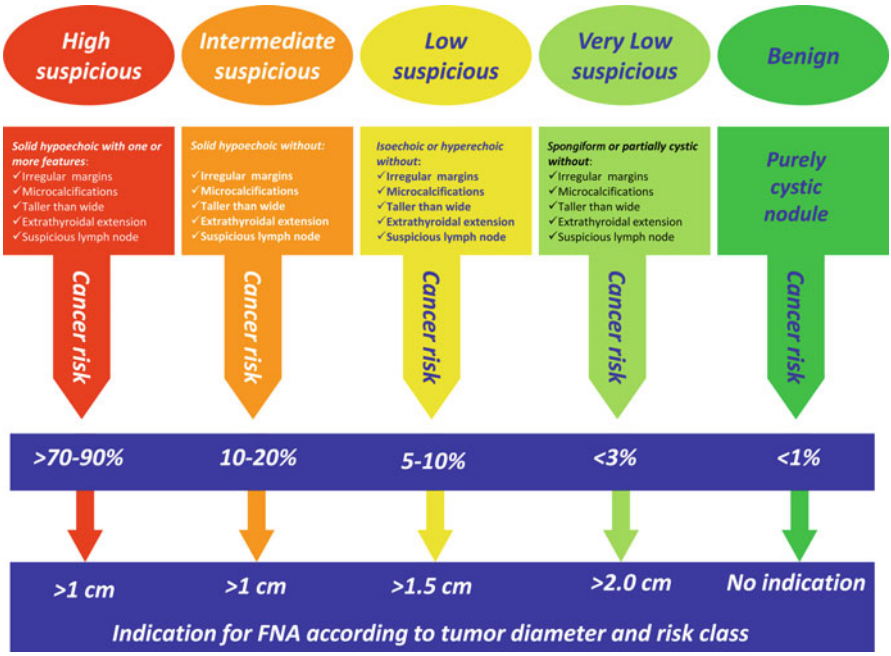

Fig. 1 Risk of malignancy according to US features and indication for FNA

3. Low suspicion (estimated risk of malignancy 5–10%) and FNAC is recommended for all nodules >1.5 cm.
4. Very low suspicion (estimated risk of malignancy <3 %) and FNAC have to be considered in nodules >2.0 cm.
5. Benign (estimated risk of malignancy <1 %) and FNAC is not indicated (Haugen et al. 2016) (Fig. 1).

Using diagnostic groups outlined in the Bethesda System for Reporting Thyroid Cytopathology (Baloch et al. 2008), six cytologic results are possible

1. Nondiagnostic or unsatisfactory
2. Benign
3. Atypia of undetermined significance or follicular lesion of undetermined significance
4. Follicular neoplasm or suspicious for a follicular neoplasm
5. Suspicious for malignancy
6. Malignant

False-negative and false-positive results are rare and more frequent in the group of atypia of undetermined significance or follicular lesion of undetermined significance (estimated risk of malignancy 5–15%) and follicular neoplasm or suspicious

for a follicular neoplasm (estimated risk of malignancy 15–30%). To reduce that, a new approach is used based on the search of genetic profiles associated to thyroid carcinoma (Nikiforov et al. 2011b; Alexander et al. 2012).

Thyroid sonography with survey of the cervical lymph nodes should be performed in all patients with known or suspected thyroid nodules in order to evaluate the presence or absence of any suspicious cervical lymph nodes in the central or lateral compartments. Although US is currently the most useful method to detect cervical lymphadenopathy, none of the suspicious US findings (round shape, cystic changes, hyperechoic foci or microcalcifications, irregular chaotic vascularization, and loss of the hilum) is highly specific (Kim et al. 2013). FNAC is usually required to confirm or exclude metastasis because reactive lymph node enlargement is common in the neck region. The Tg measurements in the needle washout (FNA-Tg) have been reported to increase the sensitivity of FNAC in identifying lymph node metastases from DTC, particularly in the case of very small cervical lymph nodes (Haugen et al. 2016).

Clinical Course

PTC occur at any age. They are rare in children and increase in frequency in the fourth and fifth decades (Agate et al. 2012). Lymph node metastases are found in 12–64% of patients, about 10% have extrathyroidal invasion, and 3–6% have distant metastases (Elisei et al. 2010). These tumors may exist for decades without producing serious symptoms or causing death. They tend to metastasize to the cervical lymph nodes and, ultimately, to the lungs. It is an especially benign process in young adults and rarely causes death in persons younger than 40 years. In older patients, the disease is more invasive and behaves in some instances like undifferentiated carcinoma (Elisei et al. 2010). Positive cervical nodes do not seem to carry an adverse risk of death in young individuals, but they do imply a worse prognosis in patients older than 40 years. Pulmonary metastasis may be manifested as large "snowballs" or may give a diffuse mottling appearance on chest radiography. Almost all PTC metastases in young patients have some ability to take up radioiodine (^{131}I) when first diagnosed. The mortality from PTC is 8–20%, mainly among older patients who have fixed or invasive cervical lesions or distant metastases at the time of diagnosis (Bosetti et al. 2008; Elisei et al. 2010). About half of patients who die of this disease succumb because of local invasion.

FTC occur in an older age group, with peak incidence in the fifth decade of life. In the widely invasive variant, the tumor tends to metastasize to the lungs and bones. Prognosis of minimally invasive FTC is favorable, especially when there is only capsular invasion; in case of vascular invasion the risk of distant metastases increases with the number of vascular foci of invasion. Commonly, lesions retain the ability to accumulate radioactive iodide and thus are theoretically susceptible to ^{131}I treatment. FTC are slightly more lethal than PTC, and mortality over the 10–15 years following diagnosis is 10–20%, again primarily in patients with widely invasive disease, with vascular invasion or distant metastases at the time of initial diagnosis (Elisei et al. 2010).

Prognostic Factors

Most patients, particularly those with the most differentiated histotypes, have high cure rates after initial treatment, but some are at risk for recurrence or death. Univariate analysis of the risk for recurrence or death has considered several potential prognostic factors that are based on epidemiologic, biological, clinical, pathologic, and, more recently, molecular features of the tumor. Factors more commonly associated with an adverse prognosis are reported in Table 1.

Prognostic Scoring Systems

Both papillary and follicular differentiated thyroid cancer have usually a good prognosis with an overall mortality of less than 10% (Elisei et al. 2010; Agate et al. 2012). This excellent prognosis results from the combination of the biological properties of most thyroid carcinomas and effective primary therapy. In the last several years, an increased emphasis has been posed on using individual estimates of risk to guide treatment and follow-up in DTC patients. Prognostic scoring systems based on multiple regression analysis of prognostic factors are intended to distinguish between low-risk patients to be treated with less aggressive protocols and high-risk patients to be treated with the most aggressive therapy. Several different risk stratification systems have been published in the past years, and the most popular is the TNM system (by the International Union Against Cancer) that is based on the extent of the primary tumor (T), lymph node status (N), the presence of distant metastases (M), and age at diagnosis (younger or older than 45 years). All of them have been developed to predict the risk of death but not of recurrence and, being based on clinical pathologic factors available soon after diagnosis and initial surgical therapy, do not change over time (AJCC 7th ed. 2009).

To overcome this limitation, the American Thyroid Association (ATA) in recently published guidelines (Haugen et al. 2016) graduated the risk of recurrence in three

Table 1 Factors associated with adverse prognosis

Older age
Distant metastases
Less well-differentiated histologic variant
Follicular widely invasive or with vascular invasion, tall cells, columnar cells, Hürthle cells, insular features, necrosis, and high mitotic rate
Large tumor size
Extrathyroidal invasion
Multicentricity
Lymph node metastases, with increasing risk with larger number, larger size, and extra nodal extension
Male sex
BRAF(*V600E*) and TERT mutation

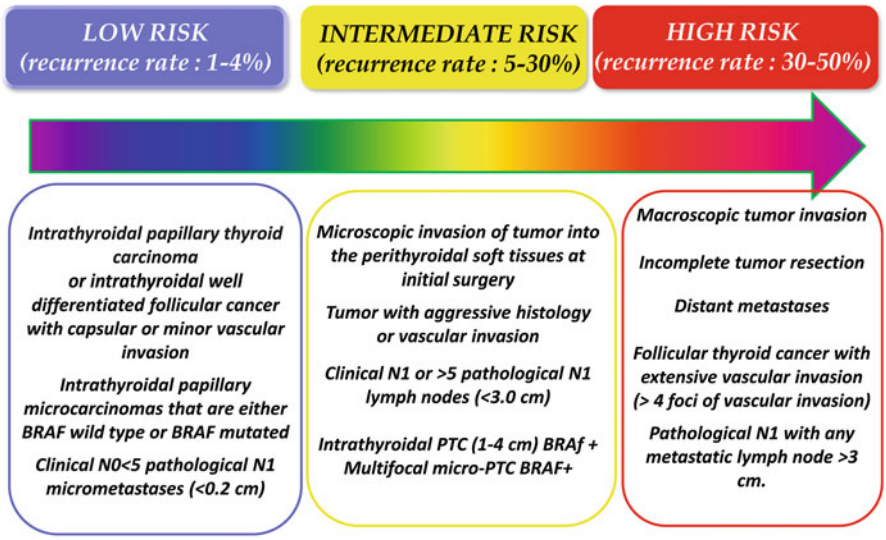

Fig. 2 American Thyroid Association (ATA) risk classification in differentiated thyroid carcinoma (Haugen et al. 2016)

categories (low, intermediate, high risk) based on tumor-related parameters (pTNM and histological variant) integrated with other clinical features, including the result of the first post-therapy radioiodine whole-body scan (WBS) and serum thyroglobulin (Tg) measurement (Fig. 2). Age is not taken into account, and the significance of lymph node metastases increases with their number, size, location, and the presence of extracapsular extension.

Recent reports have developed a new concept of "ongoing risk stratification" or "delayed risk stratification (DRS)" which better define the patient risk based on the results of the initial treatment (Tuttle et al. 2010; Castagna et al. 2011). This concept is based on the integration of the initial risk stratification (at the time of diagnosis) with the clinical, radiologic, and laboratory data becoming available during follow-up. As a matter of fact, although the ATA risk stratifications is a good starting point for initial decision-making, they have a very low positive predictive value due to the fact that a large number of patients (about 60%) classified as intermediate/high-risk are in complete remission at the end of follow-up (Castagna et al. 2011). This drawback is probably due to the lack of consideration of the effects of the initial therapy. When patients are re-stratified according to the results of the 8–12 months control after initial treatment (and then at each subsequent control), a significant number of patients who were initially considered as high risk were reclassified as low risk, and almost all of these patients continued to be in apparent remission up to the end of follow-up. This DRS allows better modulation of the subsequent follow-up excluding a significant number of intermediate/high-risk patients from unnecessary intensive work-up.

Therapy of Differentiated Thyroid Carcinoma

Surgery

Surgery is based on total or near-total thyroidectomy when the diagnosis was made before surgery for patients with thyroid cancer >4 cm, or with gross extrathyroidal extension, clinically lymph node metastases or distant metastases at diagnosis. Less extensive surgical procedures (lobectomy) may be accepted in the case of patients with thyroid cancer >1 cm and <4 cm without extrathyroidal extension, and without clinical evidence of lymph node metastases (Haugen et al. 2016). In case of unifocal DTC diagnosed at final histology after a partial surgical procedure performed for benign thyroid disorders, provided that the tumor is small, intrathyroidal, and of a favorable histotype (classical papillary or follicular variant of papillary or minimally invasive follicular), less extensive surgical procedure may be accepted. In the case of widely invasive follicular cancer at final histology, completion thyroidectomy is indicated (Haugen et al. 2016) (Table 2).

The major problem concerns the treatment of papillary thyroid microcarcinoma (PTMC), because, up to now, there is no universal agreement about the natural course, thus creating controversy concerning its diagnosis and treatment. Following thyroid surgery for PTMC, defined as a tumor 1 cm or less in size, disease-specific mortality rates have been reported to be <1%, locoregional recurrence rates are 2–6%, and distant recurrence rates are 1–2% (Mazzaferri 2007). It is quite likely that these excellent outcomes are more related to the indolent nature of the disease rather than to the effectiveness of treatment. Accordingly, thyroid lobectomy alone is suggested as a sufficient treatment for small, unifocal, intrathyroidal carcinomas in the absence of prior head and neck irradiation, familial thyroid carcinoma, or detectable cervical nodal metastases (Haugen et al. 2016) (Table 2).

More recently, prospective studies of active surveillance from Japan reported that most patients showed stable tumor size on average follow-up of 5 years (Ito et al. 2010, 2014). At 10 year follow-up, only 8% showed tumor enlargement (>3 mm) by US, and lymph node metastases were observed in 3.8% of patients. Interestingly, the older patients (age >60 years) had the lowest rate of clinical progression (1.6%),

Table 2 Surgical treatment of differentiated thyroid cancer

Total thyroidectomy	Whenever the diagnosis is presurgical for patients with thyroid cancer >4 cm, or with gross extrathyroidal extension, clinical lymph node metastases or distant metastases at diagnosis
Lobectomy	May be allowed in case of:
	1. Incidental finding at histology for surgery performed for other thyroid disease
	2. Patients with thyroid cancer >1 cm and <4 cm without extrathyroidal extension, and without clinical evidence of any lymph node metastases
	3. For small, unifocal, intrathyroidal microcarcinomas in the absence of prior head and neck irradiation, familial thyroid carcinoma, or detectable cervical nodal metastases

whereas the highest rate of clinical progression was observed in the youngest patients (<40 years old; 8.9% rate of progression) (Ito et al. 2014). These results suggest that cautious observation is a safe and effective alternative to immediate surgical resection at least in patients older than 40 years.

Before surgery, a careful exploration of the neck by US to assess the status of lymph node chains is mandatory because cervical lymph node metastases are frequent and occur early in PTC. They are much less frequent in FTC patients. Clinically apparent locoregional metastases are present in up to 35% of patients with PTC at presentation, with higher rates in younger and older patients (Randolph et al. 2012). Therapeutic central and lateral compartment neck dissection for patients with clinically involved central nodes should accompany total thyroidectomy in DTC patients.

Extensive neck dissection coupled with meticulous pathologic examination reveals locoregional lymph node metastases in 12–81% of patients with PTCs (Randolph et al. 2012). Although regional lymph node metastases are frequent at the time of diagnosis in PTC patients, prophylactic central node dissection, without clinical and US evidence of nodal disease, is controversial. As a matter of fact, even though small volume microscopic lymph node metastases appear to be frequently present in patients with PTC, locoregional recurrence rates range from 2% to 6% regardless of the extent of lymph nodes dissection and whether or not radioactive iodine was given as adjuvant therapy after surgical resection (Randolph et al. 2012). For these reasons, thyroidectomy without prophylactic central neck dissection may be appropriate for small (T1 or T2), noninvasive, clinically node-negative PTC (cN0) and for most FTC (Haugen et al. 2016). Prophylactic central compartment neck dissection should be considered in patients with PTC with clinically uninvolved central neck lymph nodes (cN0) who have advanced primary tumors (T3 or T4) or clinically involved lateral neck nodes (cN1b) (Haugen et al. 2016).

Radioiodine Ablative Therapy

Surgery may be followed by the administration of ^{131}I activities aimed to ablate any remnant thyroid tissue and potential microscopic residual tumor.

Postsurgical ablation of thyroid remnant with radioactive iodine (RAI) is aimed to facilitate the early detection of recurrence based on serum thyroglobulin measurement and to obtain a post-therapy whole-body scan (WBS), whose results may change the initial staging by identifying previously undiagnosed disease. In addition, RAI ablation may represent an *adjuvant therapy* by cleaning persistent microscopic foci of cancer, which can be present in the thyroid remnant especially in PTC, which is frequently multifocal, and by destroying small volume microscopic lymph node metastases (present in up to 80% of PTC) (Castagna et al. 2016; Haugen et al. 2016).

In past years, RAI ablation was indicated in almost every patient with a diagnosis of DTC. Nowadays, careful revision of patients' outcome has introduced the concept of risk-based selection of patients, candidates to RAI ablation (Castagna et al. 2016; Haugen et al. 2016). The individual risk depends on initial prognostic indicators

Table 3 Postoperative administration of radioactive iodine (RAI): indication and procedures

Risk class	Indication for remnant ablation	Activity of ^{131}I when indicated	Preparation
Low	Not routinely recommended	30 mCi	Recombinant human TSH (rhTSH)
Intermediate	May be considered	30 mCi (if low volume central neck nodal metastases with no other known residual disease are present)	Recombinant human TSH (rhTSH)
		30–150 mCi (if extensive lymph node disease, multiple clinically involved lymph nodes or suspected or documented microscopic residual disease are present)	Thyroid hormone withdrawal or recombinant human TSH (rhTSH)
High	Is routinely recommended	100–150 mCi	Thyroid hormone withdrawal or rhTSH

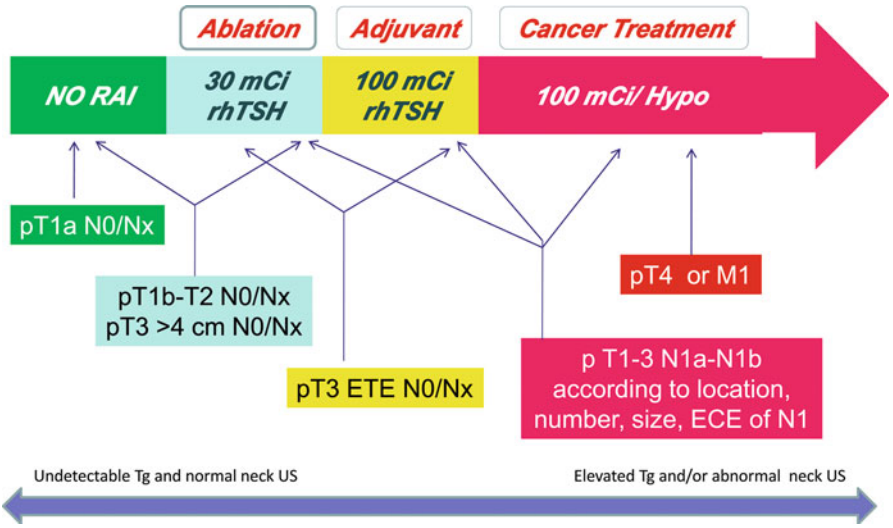

Fig. 3 Indication for postsurgical RAI administration

obtained at surgery and on results of serum Tg measurements and neck ultrasonography obtained after surgery (Table 3 and Fig. 3). RAI ablation is indicated in high-risk patients. In low-risk patients, it is unclear whether RAI administration has any benefit after complete resection; thus, it is not recommended in these patients (Haugen et al. 2016) but may be considered when serum Tg values are >5–10 ng/ml or when the likelihood of finding foci of radioiodine uptake outside the thyroid bed is significant (Webb et al. 2012; Haugen et al. 2016).

In intermediate-risk patients, RAI remnant ablation may be indicated, but the decision must be individualized (Castagna et al. 2016; Haugen et al. 2016). The

greatest potential benefit may be observed with adverse thyroid cancer histologies, increasing volume of nodal disease, lymph node metastases outside the central neck, and advanced patient age (Castagna et al. 2016; Haugen et al. 2016). In the other conditions (i.e., minimal extrathyroidal invasion, microscopic or few lymph node metastases, and intrathyroidal PTC with BRAFV600E mutation), postoperative Tg together with neck US can be used to select intermediate patients for RAI ablation (Castagna et al. 2016; Haugen et al. 2016).

Effective thyroid ablation requires adequate stimulation by TSH. This may be achieved by thyroid hormone withdrawal (THW) or after recombinant human TSH (rhTSH) administration. The last procedure is considered the method of choice based on several reports demonstrating equal efficacy compared to THW but better acceptance from the patients (Schlumberger et al. 2012; Mallick et al. 2012). In light of these data, the use of rhTSH for post-thyroidectomy ^{131}I ablation represents a safe and effective option for the postoperative management of patients with thyroid cancer (Pacini et al. 2016; Castagna et al. 2016; Haugen et al. 2016).

There is no consensus regarding the optimal activity of post-thyroidectomy RAI. Postoperative activity may vary from low "ablation" activities (1.1 GBq or 30 mCi) to high "treatment" (5.5 GBq or 150 mCi) activities (Pacini et al. 2016; Haugen et al. 2016). However, the recent publication of two large randomized controlled trials of low-risk patients, in which the rate of successful remnant ablation was the same with either 1.1 GBq or 3.7 GBq activities of ^{131}I and with either thyroid hormone withdrawal or using recombinant human thyroid-stimulating hormone, suggests that low activities after preparation with rhTSH should be treatment of choice in low-risk patients (Schlumberger et al. 2012; Mallick et al. 2012), also in view of the reduction of treatment-related toxicities associated with low activities.

Follow-Up of Differentiated Thyroid Carcinoma

Levothyroxine Therapy (LT4) After Initial Therapy

Immediately after surgery thyroid hormone therapy is initiated with two aims: to replace thyroid hormone and to suppress the potential growth stimulus of TSH on thyroid tumor cells (TSH-suppressive therapy). Thyroid hormone suppression therapy is an important part of the treatment of thyroid cancer. The drug of choice is LT4, and the suppressive dose varies according to age and body mass index (Santini et al. 2005). TSH-suppressive treatment with LT4 is of benefit in high-risk thyroid cancer patients in whom it may decrease progression of metastatic disease thus reducing cancer-related mortality (Diessl et al. 2012) while no significant benefits are demonstrated in low-risk patients (Jonklaas et al. 2006; Hovens et al. 2007). For high-risk and intermediate-risk thyroid cancer patients, initial TSH suppression to below 0.1 mU/L and to 0.1–0.5 mU/L, respectively, is recommended. For low-risk patients (regardless they have or not remnant ablation) with undetectable serum Tg levels, TSH may be maintained at the lower end of the reference range (0.5–2 mU/L), while

continuing surveillance for recurrence (Haugen et al. 2016). On the contrary, if low-risk patients have low-level serum Tg levels, TSH may be maintained at or slightly below the lower limit of normal (0.1–0.5 mU/L) while continuing surveillance for recurrence. During follow-up in patients free of disease, regardless of their initial risk class, LT4 therapy may be shifted from suppressive to replacement (Haugen et al. 2016).

The Response to Therapy Assessment and Subsequent Follow-Up in DTC Patients Treated with Total Thyroidectomy and Radioiodine Ablation

The aim of follow-up is to discover and to treat persistent or recurrent locoregional or distant disease. The large majority of recurrences are detected in the first 5 years after diagnosis. However, in a minority of cases, local or distant recurrence may develop in late follow-up, even 20 years after the initial treatment.

About 2 months after initial treatment, thyroid hormone therapy should be evaluated to check the adequacy of LT4 daily dose (Haugen et al. 2016). At 6–12 months, physical examination, neck US, and basal (using an ultrasensitive serum Tg assay) and/or rhTSH-stimulated serum Tg measurement are the most useful tools to define the clinical status of DTC patients (Table 4). At this time, patients with no clinical, biochemical, or structural evidence of disease are classified has having an "excellent response" to initial therapy (Haugen et al. 2016). 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, and it is associated with a very low risk of recurrence in the long-term follow-up (ranging from 1% to 4%) (Tuttle et al. 2010; Castagna et al. 2011; Momesso and Tuttle 2014). On these bases, ATA low- and intermediate-risk patients with "excellent response" can be reclassified as having a very low risk of recurrent disease and an early decrease in the intensity and frequency of follow-up, and the degree of TSH suppression can be considered (Table 4). In initial high-risk patients who achieve an "excellent response," mild TSH suppression could be appropriate for at least 3–5 years after they achieve excellent response (Haugen et al. 2016).

Patients who have persistently abnormal suppressed and/or stimulated Tg values or rising anti-Tg antibodies without structural evidence of disease are classified has having a "biochemical incomplete response" (Haugen et al. 2016) (Table 4). A "biochemical incomplete response" is seen in 11–19% of ATA low-risk patients, 21–22% of ATA intermediate-risk patients, and 16–18% of ATA high-risk patients (Tuttle et al. 2010; Momesso and Tuttle 2014). Despite the persistent evidence of biochemical disease, the clinical outcome is very good, and 56–68% of these patients do not show evidence of disease during follow-up; in these patients the serum Tg level will decrease with time to undetectable values in the absence of any subsequent treatment (Momesso and Tuttle 2014). Patients with "biochemical incomplete response" should be followed with 6–12 months follow-up visit maintaining a

Table 4 Management of DTC patients according to the response to initial therapy (total thyroidectomy and radioiodine ablation)

Response	Definition	Outcomes	Management of DTC patients
Excellent	Basal Tg <0.2 ng/ml	Very low recurrence rate (<2%)	Follow-up visit: 12–18 months
	Stim Tg <1 ng/ml		
	TgAb negative		TSH levels: 0.5–2.0 mU/L
	Imaging negative		
Biochemical incomplete response	Basal Tg >1 ng/ml	NED: 56–68%	Follow-up visit: 6–12 months
	Stim Tg >10 ng/ml	Biochemical disease: 10–27%	TSH levels: 0.1–0.4 mU/L
	TgAb increasing		Cross-sectional imaging based on Tg doubling time
	Imaging negative	Structural disease: 8–17%	
		No death	
Structural incomplete response	Persistent or newly identified locoregional or distant metastases	NED: 4%	TSH levels: <0.1 mU/L
		Persistent disease: 45%	Additional therapy
		Deaths: 12%	
Indeterminate response	Basal Tg 0.2–1.0 ng/ml	NED: 80%	Follow-up visit: 12 months
	Stim Tg 1–10 ng/ml	Biochemical disease: 20%	TSH levels: 0.5–1.0 mU/L
	TgAb stable or declining		Cross-sectional imaging:
	Imaging negative	No structural disease	1–2 year interval
		No death	

NED: no evidence of disease

mild TSH suppression (0.1–0.4 mU/l) with appropriate cross-sectional imaging based on the serum Tg levels over time (Haugen et al. 2016) (Table 4).

Persistent or newly identified locoregional or distant metastases is defined as "structural incomplete response" (Table 4). A structural incomplete response to initial therapy is seen in 2–6% of ATA low-risk patients, 19–28% of ATA intermediate-risk patients, and 67–75% of ATA high-risk patients (Tuttle et al. 2010; Momesso and Tuttle 2014). The management of these patients must be individualized based on their specific features and TSH suppression is indicated (<0.1 mU/l). Despite additional treatments, the majority of patients classified as having a structural incomplete response will have persistent structural and/or biochemical evidence of persistent disease at final follow-up (Tuttle et al. 2010; Momesso and Tuttle 2014) (Table 4).

Patients with an "indeterminate response" have biochemical, structural, or functional findings that cannot be confidently classified as either excellent response or persistent disease (Table 4). An indeterminate response to initial therapy is seen in 12–29% of ATA low-risk patients, 8–23% of ATA intermediate-risk patients, and 0–4% of ATA high-risk patients (Tuttle et al. 2010; Momesso and Tuttle 2014). Patients with indeterminate response should be followed with yearly follow-up visit with serum Tg and TgAb measurement and neck US, maintaining TSH levels in the range of 0.5–1.0 mIU/l (Haugen et al. 2016) (Table 4). During follow-up, only 13–20% of patients with an *indeterminate response* to therapy are reclassified as persistent/recurrent disease, whereas, in the remaining 80–90% of patients, the non-specific findings either remain stable or resolve with observation alone (Tuttle et al. 2010; Momesso and Tuttle 2014) (Table 4).

The Response to Therapy Assessment and Subsequent Follow-Up in DTC Patients Treated with Surgery Alone

While the response to therapy assessment is validated for DTC patients treated with total thyroidectomy and RAI (Tuttle et al. 2010; Castagna et al. 2011), it has less been studied in patients treated with lobectomy or total thyroidectomy without RAI. Recently, the response to therapy in DTC patients treated without RAI was proposed and validated (Momesso and Tuttle 2016). In this multicenter analysis with 507 DTC patients initially treated with lobectomy or total thyroidectomy without RAI, response to therapy assessment was able to effectively identify patients with an increased risk of recurrent/persistent disease during follow-up (Momesso et al. 2016).

Metastatic Disease

Recurrent disease occurs in 10–15%, mostly in patients with extensive disease (large thyroid tumor, extension beyond the thyroid capsule, and lymph node metastases) and in those with an aggressive histologic type (Elisei et al. 2010; Agate et al. 2012). They carry an unfavorable prognosis, and most patients dying of differentiated thyroid cancer are included in this group. In three-fourths of cases, recurrent disease is located in the neck only, mostly in lymph nodes or in the thyroid bed.

Distant metastases are observed in about 10% of patients with clinical DTC (and are extremely rare in those with micro-PTC), half of them detected at presentation. They are usually located in the lungs (50%), bones (25%), lungs and bones (20%), or at other sites (5%). Treatment of distant metastases includes LT4 treatment at doses that suppress TSH secretion, local treatment modalities (such as surgery, radiation therapy, and thermal (radiofrequency or cryoablation)), and radioiodine in the two-thirds of patients who demonstrate significant radioiodine uptake in their metastases. These methods provide a complete remission in only one-third of patients with distant metastases (Durante et al. 2006).

Treatment of Metastatic Disease

Surgery

After primary surgery, recurrences in the neck may develop in the thyroid bed and in surrounding soft tissues or in the regional lymph nodes. The prognosis is better when recurrent cancer is diagnosed by ^{131}I scintigraphy rather than clinically and when the tumor is able to concentrate iodine (Pacini et al. 1994). Any clinically detectable local recurrence and/or larger than 8 mm in the central neck or 10 mm in the lateral neck should be treated by surgery if possible, although radical reoperation involving central dissection is difficult and risks complications to the parathyroid glands and recurrent laryngeal nerve.

Recurrent disease in the lateral cervical nodes is easier to treat surgically because the operative field has not been dissected previously. The preferred surgical procedure is a modified radical neck dissection.

When lymph nodes concentrate iodine, treatment with ^{131}I is a partially effective adjunct to reoperation. Two or three therapeutic courses of ^{131}I are effective in treating more than 60% of patients, and mainly those with small tumor foci (Pacini et al. 1994). Local recurrences that cannot be excised completely and that do not take up ^{131}I can benefit from external radiotherapy (Tubiana et al. 1985).

The decision to treat distant metastases by surgery depends on their location, spread, ability to concentrate radioiodine, and radiologic pattern. Lung metastases are typically treated by radioiodine therapy, with the choice of surgical therapy left to a minority of selected cases. Patients eligible for surgery are those with a single macronodular lesion or more than one in the same lobe, but lung metastases are often multiple and bilateral, with or without mediastinal lymph node involvement, particularly when they are devoid of radioiodine uptake.

The intent of bone surgery may be palliative or curative. Palliation is required for pathologic fractures or to ameliorate neurologic symptoms resulting from spinal cord compression by vertebral metastases. Curative surgery is possible in single, localized metastases. For large metastases not radically resectable, surgery may be of help in reducing tumor mass to allow more effective action of radioiodine therapy.

Thermal ablation with radiofrequency or cryotherapy is an alternative treatment for distant metastases that is as effective but less aggressive than surgery. It may be completed by cement injection in case of bone metastases. Similarly, stereotactic external radiation therapy may be used in patients with brain, lung, or bone metastases.

In patients with bone metastases, treatment with bisphosphonates or denosumab may be beneficial in reducing the risk of subsequent skeletal events.

Radioactive Iodine Therapy

^{131}I therapy is indicated in patients with gross residual disease that is inoperable and in patients with pulmonary metastases. Indeed, it is the only approach that has been demonstrated to significantly improve disease-free survival (Samaan et al. 1992)

and, most important, *overall* survival (Durante et al. 2006), which represents the main outcome in clinical oncology.

Micronodular diffuse lung metastases (not visible at X-rays) and, to a lesser extent, small metastatic bone foci revealed by WBS in the absence of radiographic changes have the greatest chance of cure (Durante et al. 2006). This observation is particularly true in children, who often have a diffuse pattern of metastatic pulmonary spread and do exceptionally well with radioiodine therapy. In adult patients, the treatment activity is usually 100–200 mCi, repeated every 6–8 months. Lower activities (about 1 mCi/kg body weight) are used in children with lung metastases, particularly of the diffuse type, to avoid the risk for radiation-induced pulmonary fibrosis (Rall et al. 1957; Ceccarelli et al. 1988).

Lung macronodules may benefit from radioiodine therapy, but the definitive cure rate is low (Durante et al. 2006). Bone metastases with radiologic abnormalities usually do not respond to radioiodine therapy alone, and the addition of focal treatment modality may improve the control rate. The prognosis of these patients is linked to the dimension of the lesions and the presence of tumor cells that do not concentrate ^{131}I (Marcocci et al. 1989).

Brain metastases are relatively rare and usually carry a poor prognosis. Surgical resection and stereotactic external beam radiotherapy represent the best therapeutic options.

External Beam Radiotherapy

Radiotherapy is appropriate if complete surgical excision is not possible as it has been suggested by various retrospective studies (Haugen et al. 2016).

It is indicated also if there is no significant radioiodine uptake in the tumor, and for painful osseous metastases. Brain metastases and predominant lung metastases may benefit from stereotactic external radiation therapy (Haugen et al. 2016).

Chemotherapy

Various traditional chemotherapeutic approaches have been attempted with minimal success. Doxorubicin (Adriamycin) has been reported to provide a higher percentage of remission (20–33%) (Shimaoka 1980). However, responses were partial and of short duration, with limitation imposed by toxicity of the medication. Chemotherapeutic agents given in combination appear to be slightly more effective than doxorubicin alone (Haugen et al. 2016). An Italian study reported an improvement in the rate of success when an administered chemotherapy scheme was based on the use of epirubicin and cis-platinum, administered while the patient was under endogenous or exogenous TSH stimulation (Santini et al. 2002). The rationale for this protocol is based on the assumption that tumor cells may be more prone to be killed if they are in a state of active replication, as can be obtained by stimulating them with TSH, rather than in a quiescent state, as may be observed during suppression of circulating TSH.

Treatment of Patients Refractory to Conventional Therapy

Treatment of distant metastases with radioiodine provides a complete remission in only one-third of metastatic patients. The other patients have radioiodine refractory disease defined as no RAI avid lesions or RAI avid lesion that do not benefit from repeated treatment courses of radioiodine. These patients are candidate to systemic therapies especially in case of progressive disease.

The recent advances in the molecular biology have identified several genetic events related to the genesis of the DTC (Nikiforov 2016; Giordano 2016).

In more than 80% of PTC, activating mutations of MAPK pathway have been found and are believed to be the initiating events. This includes RET/PTC rearrangement and point mutations of RAS and BRAF, with no overlap between these mutations in primary tumors. Acquisition of additional mutations and gene amplifications that activate the PI3K pathway may be a common event in poorly DTCs (Nikiforov 2016; Giordano 2016).

Angiogenesis represents another set of potential molecular targets for therapy. Various vascular endothelial growth factors (VEGF) and VEGF receptors (VEGFR-1 (FLT1) and VEGFR-2 (KDR)) as well as receptors for the fibroblast growth factor (FGF) and for the platelet-derived growth factor (PDGF) are often overexpressed in the vascular endothelium of thyroid cancer tissues where they also trigger the MAP kinase signaling pathway (Bunone et al. 1999; Klein et al. 2001).

Recently, molecules that block kinase activity at distal steps in the MAP kinase pathway have been identified as logical candidate drugs for refractory thyroid cancer. These drugs, tyrosine kinase inhibitors (TKI), are multi-kinase inhibitors that are mostly anti-angiogenic and share the ability of inhibiting Ret and VEGFR.

TKIs being tested against differentiated thyroid cancer in clinical trials include motesanib, axitinib, sorafenib, sunitinib, pazopanib, lenvatinib, cabozantinib, and vandetanib (Cohen et al. 2008; Gupta-Abramson et al. 2008; Sherman et al. 2008; Hoftijzer et al. 2009; Kloos et al. 2009; Bible et al. 2010; Carr et al. 2010; Ahmed et al. 2011; Leboulleux et al. 2012; Brose et al. 2014; Cabanillas et al. 2014, 2015; Schlumberger et al. 2015; Bikas et al. 2016) (Table 5).

Partial response rate ranges from 14% to 65% and stable disease from 34% to 68% (Brilli and Pacini 2011). Two large phase III trials versus placebo have been completed in patients with RAI refractory advanced disease, and documented progression demonstrated a significant improvement in the median progression-free survival, of 5 months with sorafenib and of 15 months with lenvatinib. This led to their labelization by the FDA and EMA for these patients.

TKIs may also block the mutated driver oncogene and may use one of two aims: either as short-term treatment with a MEK or BRAF inhibitor (4–6 weeks) for redifferentiation and then treatment with radioiodine (Ho et al. 2013) or as long-term treatment to inhibit tumor growth with a BRAF inhibitor (Brose et al. 2016).

TKIs are generally quite well tolerated; the most common adverse events are fatigue, weight loss, diarrhea and nausea, hypertension, mucositis, and hand-foot skin reaction. Another common side effect is the increase of serum TSH, due to interference in thyroid hormone metabolism or malabsorption of LT4 that often requires an adjustment of LT4 therapy (Daimon et al. 2012).

Table 5 Kinase inhibitors used in patients with radioiodine refractory thyroid cancer

Drug	Target	Reference	Patients	PR (%)	PFS (months)
Sorafenib	VEGFR, PDGFR, RET, RET/PTC, BRAF	Gupta-Abramson et al. 2008	30	23	20
		Kloos et al. 2009	41	15	15
		Hoftijzer et al. 2009	32	25	13.3
		Ahmed et al. 2011	19	18	>19
		Brose et al. 2014	417	12	11
Vandetanib	VEGFR, EGFR, RET, RET/PTC	Leboulleux et al. 2012	145	8.3	11.1
Motesanib	VEGFR, PDGFR, RET, cKIT	Sherman et al. 2008	93	14	9
Axitinib	VEGFR	Cohen et al. 2008	45	31	18.1
Pazopanib	VEGFR, PDGFR, cKIT	Bible et al. 2010	37	49	11.7
Sunitinib	VEGFR, PDGFR, RET, RET/PTC, cKIT	Carr et al. 2010	28	29	12.8
		Bikas et al. 2016	23	6	8
Lenvatinib	VEGFR, PDGFR, FGFR	Cabanillas et al. 2015	58	50	12.6
		Schlumberger et al. 2015	261	63	18.3
Cabozantinib	VEGFR, RET, cKIT, cMET	Cabanillas et al. 2014	15	53	NE

PR partial response, *PFS* progression-free survival, *NE* not estimated

References

Agate L, Lorusso L, Elisei R. J Endocrinol Invest. 2012;35:3–9.

Ahmed M, Barbachano Y, Riddell A, Hickey J, Newbold KL, Viros A, Harrington KJ, Marais R, Nutting CM. Eur J Endocrinol. 2011;165(2):315–22.

Albores-Saavedra J, Duran ME. Am J Surg. 1968;116:887–90.

Alexander EK, Kennedy GC, Baloch ZW, Cibas ES, Chudova D, Diggans J, Friedman L, Kloos RT, LiVolsi VA, Mandel SJ, Raab SS, Rosai J, Steward DL, Walsh PS, Wilde JI, Zeiger MA, Lanman RB, Haugen BR. N Engl J Med. 2012;367:705–15.

American Joint Committee on Cancer (AJCC). In: Edge SB, Byrd DR, Carducci MA, et al., editors. *AJCC Cancer Staging Manual*. 7th ed. New York: Springer; 2009. p. 117–26.

Angusti T, Codegone A, Pellerito R, Favero A. J Nucl Med. 2000;41:1006–9.

Baloch ZW, LiVolsi VA, Asa SL, Rosai J, Merino MJ, Randolph G, Vielh P, DeMay RM, Sidawy MK, Frable WJ. Diagn Cytopathol. 2008;36:425–37.

Baloch ZW, Seethala RR, Faquin WC, Papotti MG, Basolo F, Fadda G, Randolph GW, Hodak SP, Nikiforov YE, Mandel SJ. Cancer Cytopathol. 2016;124(9):616–20.

Baverstock K, Egloff B, Pinchera A, Ruchti C, Williams D. Nature. 1992;359:21–2.

Bible KC, Suman VJ, Molina JR, Smallridge RC, Maples WJ, Menefee ME, Rubin J, Sideras K, Morris 3rd JC, McIver B, Burton JK, Webster KP, Bieber C, Traynor AM, Flynn PJ, Goh BC, Tang H, Ivy SP, Erlichman C. Lancet Oncol. 2010;11:962–72.

Bikas A, Kundra P, Desale S, Mete M, O'Keefe K, Clark BG, Wray L, Gandhi R, Barett C, Jelinek JS, Wexler JA, Wartofsky L, Burman KD. Eur J Endocrinol. 2016;174(3):373–80.
Bongarzone I, Fugazzola L, Vigneri P, Mariani L, Mondellini P, Pacini F, Basolo F, Pinchera A, Pilotti S, Pierotti MA. J Clin Endocrinol Metab. 1996;81:2006–9.
Bongiovanni M, Spitale A, Faquin WC, Mazzucchelli L, Baloch ZW. Acta Cytol. 2012;56:333–9.
Bosetti C, Bertuccio P, Levi F, Lucchini F, Negri E, La Vecchia C. Ann Oncol. 2008;19:631–40.
Brilli L, Pacini F. Future Oncol. 2011;7:657–68.
Brose MS, Nutting CM, Jarzab B, Elisei R, Siena S, Bastholt L, de la Fouchardiere C, Pacini F, Paschke R, Shong YK, Sherman SI, Smit JW, Chung J, Kappeler C, Peña C, Molnár I, Schlumberger MJ. Lancet. 2014;384(9940):319–28.
Brose MS, Cabanillas ME, Cohen EE, Wirth LJ, Riehl T, Yue H, Sherman SI, Sherman EJ. Lancet Oncol. 2016;17(9):1272–82.
Bunone G, Vigneri P, Mariani L, Butó S, Collini P, Pilotti S, Pierotti MA, Bongarzone I. Am J Pathol. 1999;155:1967–76.
Cabanillas ME, Brose MS, Holland J, Ferguson KC, Sherman SI. Thyroid. 2014;24(10):1508–14.
Cabanillas ME, Schlumberger M, Jarzab B, Martins RG, Pacini F, Robinson B, McCaffrey JC, Shah MH, Bodenner DL, Topliss D, Andresen C, O'Brien JP, Ren M, Funahashi Y, Allison R, Elisei R, Newbold K, Licitra LF, Sherman SI, Ball DW. Cancer. 2015;121(16):2749–56.
Camiel MR, Mule JE, Alexander LL, Benninghoff DL. N Engl J Med. 1968;278:1056–9.
Capezzone M, Marchisotta S, Cantara S, Busonero G, Brilli L, Pazaitou-Panayiotou K, Carli AF, Caruso G, Toti P, Capitani S, Pammolli A, Pacini F. Endocr Relat Cancer. 2008a;15:1075–81.
Capezzone M, Cantara S, Marchisotta S, Filetti S, De Santi MM, Rossi B, Ronga G, Durante C, Pacini F. J Clin Endocrinol Metab. 2008b;93:3950–7.
Carr LL, Mankoff DA, Goulart BH, Eaton KD, Capell PT, Kell EM, Bauman JE, Martins RG. Clin Cancer Res. 2010;16:5260–8.
Castagna MG, Maino F, Cipri C, Belardini V, Theodoropoulou A, Cevenini G, Pacini F. Eur J Endocrinol. 2011;165:441–6.
Castagna MG, Cantara S, Pacini F. J Endocrinol Investig. 2016;39(10):1087–94.
Ceccarelli C, Pacini F, Lippi F, Elisei R, Arganini M, Miccoli P, Pinchera A. Surgery. 1988;104:1143–8.
Cohen EE, Rosen LS, Vokes EE, Kies MS, Forastiere AA, Worden FP, Kane MA, Sherman E, Kim S, Bycott P, Tortorici M, Shalinsky DR, Liau KF, Cohen RB. J Clin Oncol. 2008;26:4708–13.
Daimon M, Kato T, Kaino W, Takase K, Karasawa S, Wada K, Kameda W, Susa S, Oizumi T, Tomita Y, Kato T. Jpn J Clin Oncol. 2012;42:756–7.
Davies L, Morris LG, Haymart M, Chen AY, Goldenberg D, Morris J, Ogilvie JB, Terris DJ, Netterville J, Wong RJ, Randolph G, AACE Endocrine Surgery Scientific Committee. P Endocr Pract. 2015;21(6):686–96.
de Mestier P. Chirurgie. 1990;116:514–6.
Diessl S, Holzberger B, Mäder U, Grelle I, Smit JW, Buck AK, Reiners C, Verburg FA. Clin Endocrinol. 2012;76(4):586–92.
Durante C, Haddy N, Baudin E, Leboulleux S, Hartl D, Travagli JP, Caillou B, Ricard M, Lumbroso JD, de Vathaire F, Schlumberger M. J Clin Endocrinol Metab. 2006;91:2892–9.
Elisei R, Molinaro E, Agate L, Bottici V, Masserini L, Ceccarelli C, Lippi F, Grasso L, Basolo F, Bevilacqua G, Miccoli P, Di Coscio G, Vitti P, Pacini F, Pinchera A. J Clin Endocrinol Metab. 2010;95:1516–27.
Giordano TJ. Curr Opin Oncol. 2016;28(1):*1*–4.
Gupta-Abramson V, Troxel AB, Nellore A, et al. J Clin Oncol. 2008;26:4714–9.
Haugen BR, Alexander EK, Bible KC, et al. Thyroid. 2016;26:1–133.
Hedinger C, Williams ED, Sobin LH. Histological typing of thyroid tumours. In: International Histological Classification of Tumors. 2nd ed. 11 Berlin: Springer-Verlag; 1988.
Ho AL, Grewal RK, Leboeuf R, Sherman EJ, Pfister DG, Deandreis D, Pentlow KS, Zanzonico PB, Haque S, Gavane S, Ghossein RA, Ricarte-Filho JC, Domínguez JM, Shen R, Tuttle RM, Larson SM, Fagin JA. N Engl J Med. 2013;368(7):623–32.

Hoftijzer H, Heemstra KA, Morreau H, et al. Eur J Endocrinol. 2009;161:923–31.
Holmes L, Hossain J, Opara F. ISRN Oncol. 2012;2012:439070.
Horvath E, Majlis S, Rossi R, Franco C, Niedmann JP, Castro A, Dominguez M. J Clin Endocrinol Metab. 2009;94:1748–51.
Hovens GC, Stokkel MP, Kievit J, et al. J Clin Endocrinol Metab. 2007;92:2610–5.
II Lloyd KM, Dennis M. Ann Intern Med. 1963;58:136–42.
Ito Y, Miyauchi A, Inoue H, Fukushima M, Kihara M, Higashiyama T, Tomoda C, Takamura Y, Kobayashi K, Miya A. World J Surg. 2010;34:28–35.
Ito Y, Miyauchi A, Kihara M, Higashiyama T, Kobayashi K, Miya A. Thyroid. 2014;24:27–34.
Jonklaas J, Sarlis NJ, Litofsky D, Ain KB, Bigos ST, Brierley JD, Cooper DS, Haugen BR, Ladenson PW, Magner J, Robbins J, Ross DS, Skarulis M, Maxon HR, Sherman SI. Thyroid. 2006;16:1229–42.
Kim DW, Choo HJ, Lee YJ, Jung SJ, Eom JW, Ha TK. J Ultrasound Med. 2013;32(7):1173–80.
Klein M, Vignaud JM, Hennequin V, Toussaint B, Bresler L, Plénat F, Leclère J, Duprez A, Weryha G. J Clin Endocrinol Metab. 2001;86:656–8.
Kloos RT, Ringel MD, Knopp MV, Hall NC, King M, Stevens R, Liang J, Wakely Jr PE, Vasko VV, Saji M, Rittenberry J, Wei L, Arbogast D, Collamore M, Wright JJ, Grever M, Shah MH. J Clin Oncol. 2009;27:1675–84.
Leboulleux S, Bastholt L, Krause T, de la Fouchardiere C, Tennvall J, Awada A, Gómez JM, Bonichon F, Leenhardt L, Soufflet C, Licour M, Schlumberger MJ. Lancet Oncol. 2012;13:897–905.
Li C, Lee KC, Schneider EB, Zeiger MA. J Clin Endocrinol Metab. 2012;97:4559–70.
Liu R, Xing M. Endocr Relat Cancer. 2016;23(3):R143–55.
Mallick U, Harmer C, Yap B, Wadsley J, Clarke S, Moss L, Nicol A, Clark PM, Farnell K, McCready R, Smellie J, Franklyn JA, John R, Nutting CM, Newbold K, Lemon C, Gerrard G, Abdel-Hamid A, Hardman J, Macias E, Roques T, Whitaker S, Vijayan R, Alvarez P, Beare S, Forsyth S, Kadalayil L, Hackshawk A. N Engl J Med. 2012;366:1674–85.
Marcocci C, Pacini F, Elisei R, Schipani E, Ceccarelli C, Miccoli P, Organini M, Pinchera A. Surgery. 1989;106:960–6.
Mazzaferri EL. Endocr Pract. 2007;13:498–512.
Momesso DP, Tuttle RM. Endocrinol Metab Clin N Am. 2014;43(2):401–21.
Momesso DP, Vaisman F, Yang SP, Bulzico DA, Corbo R, Vaisman M, Tuttle RM. J Clin Endocrinol Metab. 2016;101(7):2692–700.
Nikiforov YE. Endocr Pathol. 2002;13:3–16.
Nikiforov YE. Mod Pathol. 2011;24:S34–43.
Nikiforov YE. Nat Rev Endocrinol. 2016;12(2):64.
Nikiforov YE, Ohori NP, Hodak SP, Carty SE, LeBeau SO, Ferris RL, Yip L, Seethala RR, Tublin ME, Stang MT, Coyne C, Johnson JT, Stewart AF, Nikiforova MN. J Clin Endocrinol Metab. 2011;96:3390–7.
Pacini F, Cetani F, Miccoli P, Mancusi F, Ceccarelli C, Lippi F, Martino E, Pinchera A. World J Surg. 1994;18:600–4.
Pacini F, Vorontsova T, Demidchik EP, Molinaro E, Agate L, Romei C, Shavrova E, Cherstvoy ED, Ivashkevitch Y, Kuchinskaya E, Schlumberger M, Ronga G, Filesi M, Pinchera A. J Clin Endocrinol Metab. 1997;82:3563–9.
Pacini F, Brianzoni E, Durante C, Elisei R, Ferdeghini M, Fugazzola L, Mariotti S, Pellegrini G. J Endocrinol Investig. 2016;39:487–8.
Rall JE, Alpers JB, Lewallen CG, Sonenberg M, Berman M, Rawson RW. J Clin Endocrinol Metab. 1957;17:1263–76.
Randolph GW, Duh QY, Heller KS, et al. Thyroid. 2012;22:1144–52.
Ron E, Lubin JH, Shore RE, Mabuchi K, Modan B, Pottern LM, Schneider AB, Tucker MA, Boice Jr JD. Radiat Res. 1995;141:259–77.
Ron E, Lubin JH, Shore RE, Mabuchi K, Modan B, Pottern LM, Schneider AB, Tucker MA, Boice Jr JD. Radiat Res. 2012;178(2):AV43–60.
Samaan NA, Schultz PN, Hickey RC, Goepfert H, Haynie TP, Johnston DA, Ordonez NG. J Clin Endocrinol Metab. 1992;75:714–20.

Santini F, Bottici V, Elisei R, Montanelli L, Mazzeo S, Basolo F, Pinchera A, Pacini F. J Clin Endocrinol Metab. 2002;87:4160–5.
Santini F, Pinchera A, Marsili A, Ceccarini G, Castagna MG, Valeriano R, Giannetti M, Taddei D, Centoni R, Scartabelli G, Rago T, Mammoli C, Elisei R, Vitti P. J Clin Endocrinol Metab. 2005;90:124–7.
Schlumberger M, Catargi B, Borget I, Deandreis D, Zerdoud S, Bridji B, Bardet S, Leenhardt L, Bastie D, Schvartz C, Vera P, Morel O, Benisvy D, Bournaud C, Bonichon F, Dejax C, Toubert ME, Leboulleux S, Ricard M, Benhamou E. N Engl J Med. 2012;366:1663–73.
Schlumberger M, Tahara M, Wirth LJ, Robinson B, Brose MS, Elisei R, Habra MA, Newbold K, Shah MH, Hoff AO, Gianoukakis AG, Kiyota N, Taylor MH, Kim SB, Krzyzanowska MK, Dutcus CE, delasHeras B, Zhu J, Sherman SI, N Engl J Med. 2015;372:621–30.
Sherman SI, Wirth LJ, Droz JP, Hofmann M, Bastholt L, Martins RG, Licitra L, Eschenberg MJ, Sun YN, Juan T, Stepan DE, Schlumberger MJ. N Engl J Med. 2008;359:31–42.
Shimaoka K. J Surg Oncol. 1980;15:283–6.
Tubiana M, Haddad E, Schlumberger M, Hill C, Rougier P, Sarrazin D. Cancer. 1985;55:2062–71.
Tuttle RM, Tala H, Shah J, Leboeuf R, Ghossein R, Gonen M, Brokhin M, Omry G, Fagin JA, Shaha A. Thyroid. 2010;20:1341–9.
Vaccarella S, Franceschi S, Bray F, Wild CP, Plummer M, Dal ML. N Engl J Med. 2016;375:614–7.
Webb RC, Howard RS, Stojadinovic A, Gaitonde DY, Wallace MK, Ahmed J, Burch HB. J Clin Endocrinol Metab. 2012;97:2754–63.

Medullary Carcinoma

20

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Abstract

Medullary thyroid cancer (MTC) is a rare neuroendocrine tumor that can be either sporadic or familial. In both cases, the pathogenesis is due to constitutively activating mutations, somatic or germline, of *RET* oncogene. The familial form of MTC can be associated with other endocrine neoplasias such as pheochromocytoma (PHEO) and/or multiple adenomatosis of parathyroids (PTHAd). According to the phenotype, three different syndromes are distinguished: the multiple endocrine neoplasia (MEN) type 2A, characterized by the association of MTC, PHEO, and PTHAd; the MEN 2B in which MTC and PHEO are associated with other nonendocrine diseases such as multiple mucosal neuromas, marfanoid habitus, and megacolon; and the familial form of MTC (FMTC) with hereditary MTC not associated with other neoplasias. The familial form, but not the sporadic, can affect children, and the *RET* genetic screening is the only diagnostic tool able to identify gene carriers when the tumor is not yet developed. As all thyroid tumors, MTC clinical manifestation is represented by a thyroid nodule, either isolated or in the context of a multinodular goiter. The cytological diagnosis is not always straightforward and can be facilitated by the measurement of serum calcitonin (Ct) that when >100 pg/ml is the most specific and sensitive serum marker of MTC. An early diagnosis of MTC, when the tumor is still intrathyroid, is needed to definitively cure the patient with the first surgical treatment. The presence of distant metastases at diagnosis is, together with the presence of a somatic *RET* mutation in the tumor tissue, the most important prognostic factor for a poor outcome. If the first surgery will not be curative, other local or systemic therapies are currently available, and their use can have a positive impact on the progression-free survival of MTC patients. Since MTC is a rare tumor, with several peculiarities such as the possibility to be hereditary, the management of MTC patients should be performed in referral centers and by a multidisciplinary team.

Keywords

Medullary thyroid cancer · Calcitonin · *RET* · Multiple endocrine neoplasia type 2 · Vandetanib · Cabozantinib

Introduction

Medullary thyroid carcinoma (MTC) is a very rare thyroid tumor which derives from parafollicular or calcitonin-producing C cells (Schmid 2015), which are located in the thyroid gland but are different from follicular cells. At variance with follicular cells, C cells are of neuroendocrine origin; they do not respond to thyrotropin-stimulating hormone (TSH), do not produce thyroglobulin (Tg), and are not able

to take up iodine. However, recently a new theory about an endodermal origin of mammalian C cell progenitors has been put forward (Johansson et al. 2015; Nilsson and Williams 2016), and the expression of E-cadherin, which is consistent with an origin different from the neural crest-derived mesenchyme, seems to support this hypothesis (Kameda et al. 2007).

The overall frequency of MTC is not established, but it has recently been demonstrated that it increased from 0.14 to 0.21 per 100,000 population between 1983 and 2012 in the USA, regardless of the stage at diagnosis (Randle et al. 2017). The prevalence is 5–10% of all thyroid malignancies, 0.4–1.4% of all thyroid nodules, and about 0.14% in the thyroids of subjects submitted to autopsy (Valle and Kloos 2011). Contrary to papillary thyroid carcinoma (PTC) and follicular thyroid carcinomas (FTC), no difference in gender distribution is observed. The clinical appearance is mainly in the fourth and fifth decades with a small, but statistically significant, increase in the mean age at diagnosis from 50 to 54 years during the last 30 years (Randle et al. 2017). Children are rarely affected, and usually when this occurs, the probability of facing a familial/hereditary form is very high (Pelizzo et al. 2007).

Ethnic or environmental risk factors for MTC development are unknown. At variance, the pathogenic mechanism responsible for MTC development has been recognized in the genetic alteration of the *RET* proto-oncogene, mainly activating point mutations (Romei et al. 2016b). *RET* mutations can be somatic or germline according to the sporadic or familial nature of the MTC, respectively. The sporadic form is the most prevalent (75%), while the hereditary or familial form accounts for the remaining 25%. The hereditary form is an autosomal dominant inherited syndrome with a variable degree of expressivity and an age-related penetrance. Three different hereditary syndromes are classified according to the involved organs (Table 1): (A) multiple endocrine neoplasia type 2A (MEN 2A), characterized by the presence of MTC associated with pheochromocytoma (PHEO) (50% of cases) and/or multiple adenomatosis or hyperplasia of parathyroids (PTHAd) (30% of cases) (Keiser et al. 1973) and in about 10% of cases also with an interscapular itching cutaneous lichen amyloidosis that, when present, is diagnostic of MEN 2A; (B) multiple endocrine neoplasia type 2B (MEN 2B), characterized by MTC, PHEO (50% of cases), mucosal neuromas particularly of the conjunctiva and/or tongue (98–100% of cases), ganglioneuromatosis (98% of cases), and an almost invariable typical marfanoid habitus, with long arms and legs in comparison to the trunk of the body (Cunliffe et al. 1970); (C) familial medullary thyroid carcinoma (FMTC),

Table 1 Prevalence of the different components of the multiple endocrine neoplasia type 2 (MEN 2) syndromes

	MTC + CCH	PHEO	PTHAd	CLA	Marfanoid habitus	Mucosal neuromas	Scheletric alterations	Megacolon
MEN 2A	100%	45%	30%	30%	0	0	0	0
MEN 2B	100%	50%	0	0	100%	98–100%	40%	50%
FMTC	100%	0	0	0	0	0	0	0

MTC medullary thyroid cancer, *PHEO* pheochromocytoma, *PTHAd* parathyroid adenomas, *MEN* multiple endocrine neoplasia, *FMTC* familial medullary thyroid cancer

which is characterized by the presence of an inheritable MTC with no association with other endocrine neoplasias (Farndon et al. 1986).

The biological behavior of MTC is more aggressive when compared with that of the other well-differentiated thyroid carcinomas (i.e., PTC and FTC) even though it is not as aggressive as that of anaplastic carcinomas (ATC). A 10-year survival of about 50% in MTC patients is reported in several series. Both the cure and survival of these patients are positively affected by an early diagnosis (Pelizzo et al. 2007). A recent study showed that the 5-year disease-specific survival improved from 86% to 89%, and this improvement was particularly true for MTC patients with regional (from 82% to 91%) and distant (from 40% to 51%) metastases. The most favored hypothesis for this improvement is the increase in the percentage of cases treated with a more appropriate and extensive primary surgery, including thyroidectomy and lymphadenectomy (Randle et al. 2017).

Pathogenesis

While risk factors for the development of MTC are unknown, the molecular pathogenesis is almost completely clarified. In the hereditary form, *RET* oncogene is the major player. So far, 98% of kindred with this disease are characterized by the presence of a germline *RET* mutation, and only 2% are still orphan of genetic alterations. *RET* is also involved, at the somatic level, in sporadic MTC cases. Somatic *RAS* mutations represent the second most important genetic alteration in sporadic MTC (Ciampi et al. 2013). Currently, only few other private mutations have been reported (Heilmann et al. 2016), and 30% of sporadic cases are still orphans for genetic alterations.

RET Oncogene

The *RET* proto-oncogene is a 21-exon gene located on chromosome 10q11-2 that encodes for a tyrosine kinase (TK) transmembrane receptor, the activation of which induces the activation of downstream signaling pathways (Romei et al. 2016b). *RET* is expressed in a variety of neuronal cell lineages, including thyroid C cells and the adrenal medulla. Activating mutations can determine a ligand-independent dimerization of *ret* protein that induces the autophosphorylation of the TK domain with a subsequent stimulation of proliferation and tumoral transformation of *ret* protein-expressing cells (Romei et al. 2016b) (Fig. 1).

In 1993, two independent groups reported that activating germline point mutations of the *RET* proto-oncogene are causative events in MEN 2A and in FMTC (Donis-Keller et al. 1993; Mulligan et al. 1993). One year later, also MEN 2B was found associated with germline *RET* proto-oncogene mutations (Eng et al. 1994). Over the years, many *RET* mutations have been found to be associated with MEN 2, and currently the genotype-phenotype correlation is almost completely clarified (Table 2).

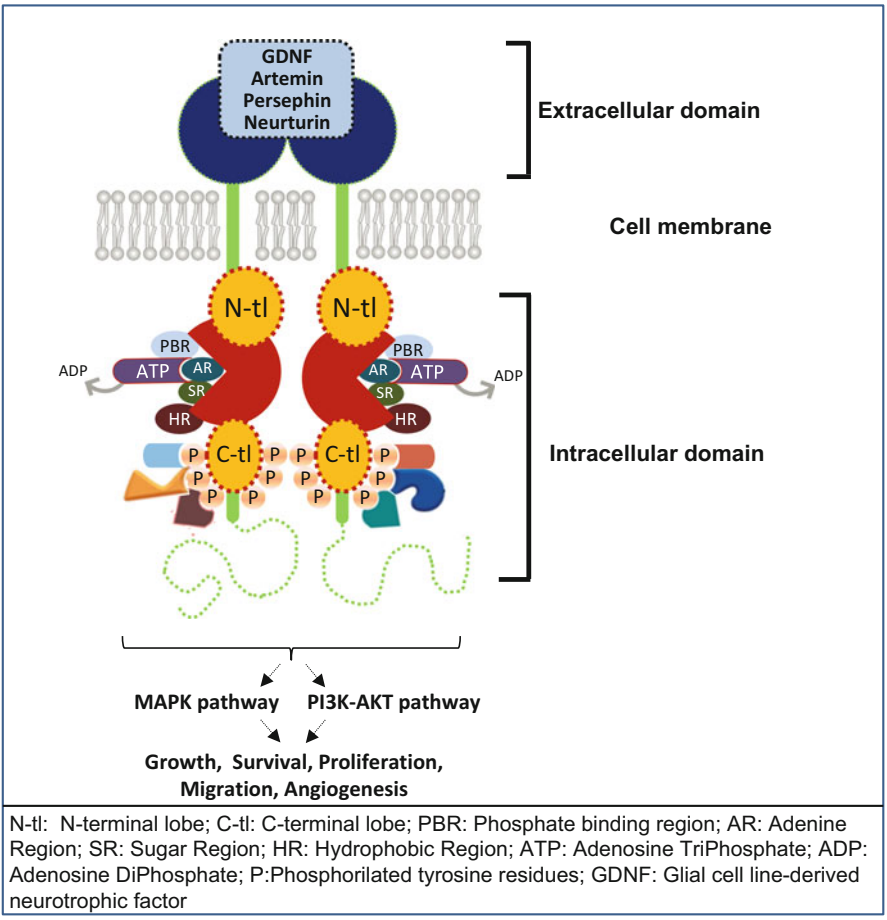

Fig. 1 Schematic representation of ret receptor encoded by *RET* oncogene. The receptor is a tyrosine kinase transmembrane receptor. Its physiological activation, due to the binding with several different ligands (i.e., GDNF, neurturin, artemin, and perseptin), stimulates cell growth, angiogenesis, and proliferation and promotes cell migration and survival through the activation of intracellular pathways. The constitutive activation of ret receptor, due to activating mutations, is responsible of an uncontrolled cell stimulation leading to tumor transformation

About 98% of MEN 2A are associated with *RET* mutations in the cysteine-rich extracellular domain and in particular in codons 609, 611, 618, 620, and 634 of exons 10 and 11. Germline mutations at codon 634 of exon 11 account for 85% of MEN 2A cases. Interestingly, mutation of cysteine 634 significantly correlates with the presence of PHEO, PTHAd, and cutaneous lichen amyloidosis (CLA) (Eng et al. 1996; Raue and Frank-Raue 2009) (Fig. 2).

A specific mutation in exon 16, M918T, is almost invariably associated with MEN 2B. The M918T mutation is associated with a very aggressive biological behavior of the tumor that commonly develops a few years after birth. Other mutations rarely associated

Table 2 Classification of *RET* mutations according to their degree of aggressiveness and penetrance of the different MEN 2 components

RET mutation	Exon	MTC risk level	PHEO	PTHAd	CLA
M918T	16	HST	+++	–	N
A883F	15	H	+++	–	N
C634F/G/R/S/W/Y	11	H	+++	++	Y
C609F/G/R/S/Y	10	MOD	+/++	+	N
C611F/G/S/Y/W	10	MOD	+/++	+	N
C618F/R/S	10	MOD	+/++	+	N
C620F/R/S	10	MOD	+/++	+	N
C630R/Y	10	MOD	+/++	+	N
D631Y	11	MOD	+++	–	N
K666E	11	MOD	+	–	N
E768D	13	MOD	–	–	N
L790F	13	MOD	+	–	N
V804L	14	MOD	+	+	Y
V804M	14	MOD	+	+	N
S891A	15	MOD	+	+	N
G533C	8	MOD	+	–	N
R912P	16	MOD	–	–	N

HST highest risk, *H* high risk, *MOD* moderate risk (according to ATA 2015 guidelines) to develop MTC, + = 10%, ++ = 20–30%, +++ = 50% probability to develop PHEO and/or PTHAd, *MTC* medullary thyroid cancer, *PHEO* pheochromocytoma, *PTHAd* parathyroid adenomas, *CLA* cutaneous lichen amyloidosis, *N* negative occurrence, *Y* positive occurrence

with MEN 2B have been reported at codon 883 of exon 15, but MTC of A883F carriers seems to have a more indolent course compared with that of M918T carriers (Mathiesen et al. 2017). A double *RET* mutation at codons 804 and 904 has also been described (Menko et al. 2002) in MEN 2B.

In FMTC, the mutations are widely distributed among the five cysteine codons 609, 611, 618, 620, and 634 but also in other non-cysteine codons, such as codon 804 in exon 14, 891 in exon 15, and others. A different biological behavior, characterized by a lower aggressiveness and an older mean age at diagnosis, has been described for FMTC associated with mutations in non-cysteine codons with respect to both MEN 2A and FMTC with mutations in cysteine codons (Raue and Frank-Raue 2009).

In about 4–10% of MEN 2A or FMTC patients, and in about 95% of those with MEN 2B, the germline *RET* mutation is a "de novo" mutation as demonstrated by the negative finding of the *RET* genetic analysis in the patients' parents. In such cases, the mutation is usually located in the allele inherited from the patients' father (Schuffenecker et al. 1997).

A subgroup of *RET* variants of unknown significance (VUS) have been sporadically reported in a few families. Their transforming ability is questionable and in many cases not demonstrated in in vitro experiments. The pathogenic role of VUS has not been demonstrated, and subjects carrying these alterations must be carefully monitored but not necessarily treated if there is no evidence of disease (Cosci et al. 2011; Lebeault et al. 2017).

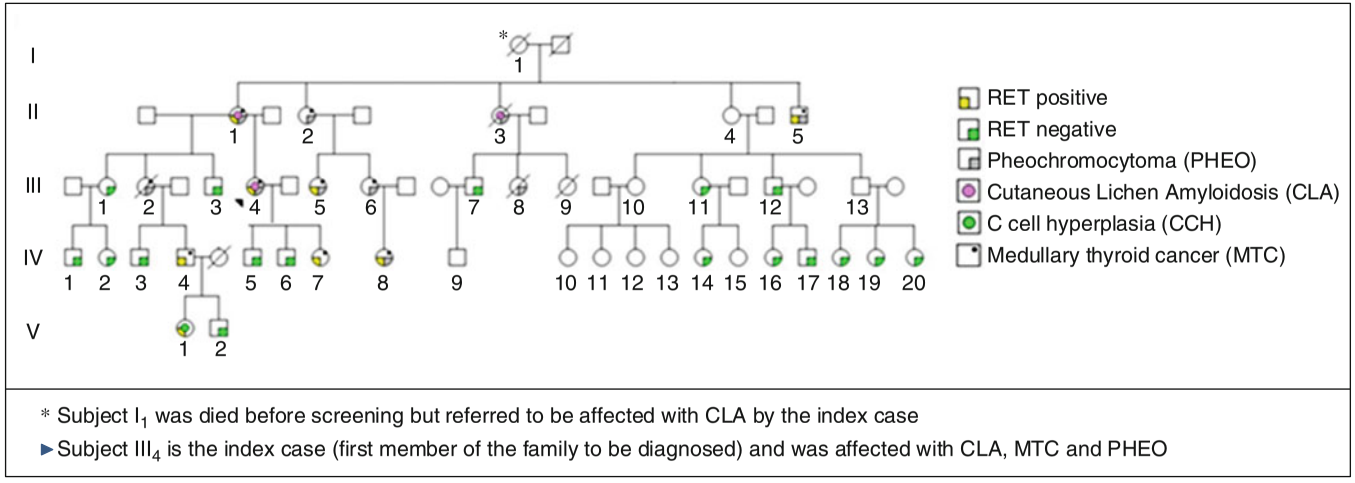

Fig. 2 Genealogical tree of a big family with a MEN 2A syndrome due to a C634Y germline *RET* mutation. The Mendelian autosomal transmission is clearly demonstrated by the evidence that all five generations are interested by the disease and that males and females are equally affected. The tree clearly shows the different penetrances of the components of the syndrome in *RET*-positive cases with a 100% penetrance of MTC, 50% penetrance of PHEO, and 30% penetrance of CLA and, in this family, no cases with parathyroid adenomas. A late onset of these pathologies cannot be excluded

Somatic *RET* mutations are found in about 40% of sporadic cases of MTC mainly consisting of a M918T mutation in exon 16, which is the same mutation seen in MEN 2B (Elisei et al. 2007). Other somatic *RET* mutations, and some small deletions and/or insertions, have been reported in other codons, especially in advanced MTC that are somatically *RET* mutated in >80% of cases (Heilmann et al. 2016; Romei et al. 2016a). Several studies indicate that MTC patients with somatic *RET* mutations have an advanced disease at diagnosis and a poorer prognosis than those with no evidence of *RET* mutations (Elisei et al. 2008; Moura et al. 2009). A positive correlation has also been demonstrated between the presence of a somatic *RET* mutation and a higher Ki 67 proliferation index (Mian et al. 2011).

Several *RET* gene polymorphisms have been found, both in MTC-affected patients and in normal subjects. It is still controversial whether some of these polymorphisms have a higher prevalence in MTC compared with normal individuals or if they play any role in the development of MTC (Elisei et al. 2004b; Colombo et al. 2015; Lebeault et al. 2017).

RAS Oncogenes

About 15% of *RET*-negative MTC carry a somatic *RAS* mutation, mainly H- and K-*RAS* activating point mutations. With very few exceptions, *RET* and *RAS* mutations are mutually exclusive. MTC with *RAS* mutations appear to have a less aggressive biological behavior (Ciampi et al. 2013; Simbolo et al. 2014).

Although several studies with advanced and very sensitive techniques have already been performed, no other oncogenes have been found to be mutated. So far, only few alterations, mainly copy number variations, have been reported in some genes, the pathogenic role of which is still to be defined (Heilmann et al. 2016).

Clinical Presentation

As previously noted, MTC can be either sporadic or familial. The familial medical history is of great help in identifying hereditary forms, as is the coexistence – in the same patient or in other members of the family – of other endocrine neoplasias, such as PHEO and/or PTHAd, which can be associated with MTC in the hereditary forms (Wells et al. 2013). Presently, *RET* screening allows making a definitive diagnosis based on the presence of a germline *RET* mutation and even discovers the hereditary nature of about 10% of cases presenting as apparently sporadic (Romei et al. 2011).

Sporadic Form

The most common clinical presentation of sporadic MTC is a thyroid nodule, either single or belonging to a multinodular goiter. No specific clinical manifestations or symptoms are present in patients affected with MTC, but in rare cases, diarrhea

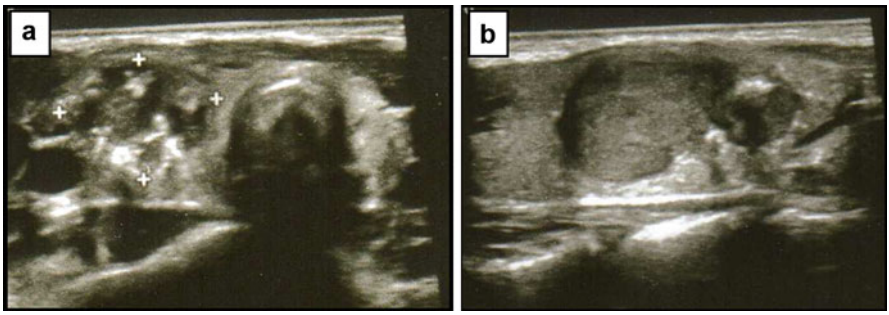

Fig. 3 Neck ultrasound imaging of a medullary thyroid cancer (MTC): cross (panel **a**) and longitudinal (panel **b**) sections of an intrathyroidal MTC. The ultrasound characteristics are clearly and highly suspicious for malignancy, but no specific features for MTC do exist to induce a specific presurgical suspicion

and/or flushing syndrome can be present in very advanced cases with elevated serum Ct levels (Alam 1994; Hannah-Shmouni et al. 2016). Thyroid function is usually normal, and suspicion of malignancy, but not specifically of MTC, is due to the presence of a suspicious nodule at neck ultrasound (US). Importantly, the US features of MTC nodules are not specific for this tumor but very similar to those of other thyroid cancer histotypes (Lee et al. 2010) (Fig. 3).

Familial Form

The clinical appearance of MTC in the familial/hereditary form is that of nodular thyroid disease, similar to that of the sporadic form with the exception that it is usually bilateral, multicentric, and almost invariably associated with C cell hyperplasia (Schmid 2015).

Very rarely MEN 2A and MEN 2B syndromes are diagnosed because the PHEO or PTHAd are discovered first, since the latency period of development is much longer for these two diseases than for MTC. About 30% of patients with MEN 2A also develop hyperparathyroidism due to the multiple PTHAd (Alevizaki and Saltiki 2015). The mean age at diagnosis is the third to fourth decades of life. The clinical findings are completely similar to those of the sporadic form of hyperparathyroidism, and very often there are no specific symptoms. At variance with the sporadic form, multiple hyperplasia or adenomatosis is most commonly found. Hyperparathyroidism has only occasionally been reported in patients with MEN 2B (Cunliffe et al. 1970).

About 50% of MEN 2A and 40–45% of MEN 2B patients develop PHEO, which shares the same characteristics in both syndromes (Mucha et al. 2017). At variance with the sporadic form, the adrenal tumors of MEN 2 syndromes are usually bilateral and multicentric but not necessarily synchronous, and a mean period of 10 years usually exists between the developments of the tumor in the two adrenal glands.

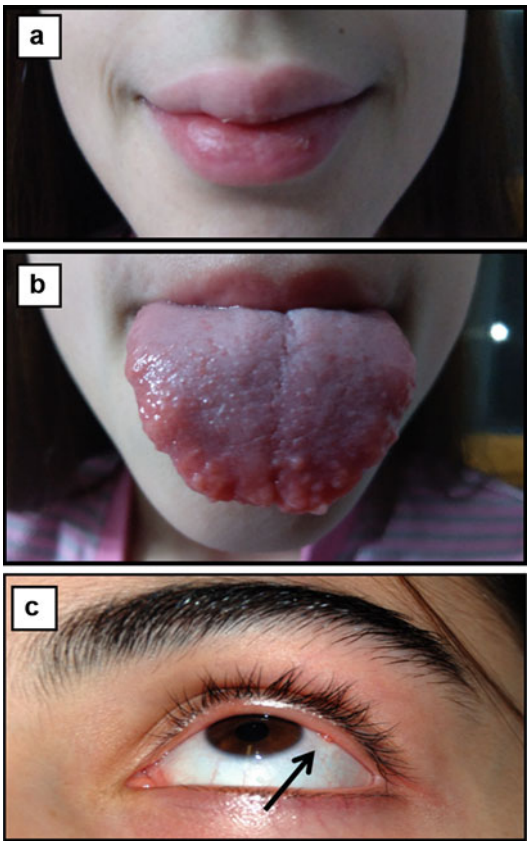

Fig. 4 Typical and pathognomonic face features of a multiple endocrine neoplasia type 2B patient. (Panel **a**) thick lips; (panel **b**) macroglossia and multiple mucosal neuromas of the tongue; (panel **c**) conjuntival neuroma (indicated by the arrow)

MEN 2B patients may be easily recognized at physical examination by the typical marfanoid habitus characterized by thin and inappropriately long extremities and pectus excavatum. In a minority of cases, severe skeletal alterations, such as shorter legs and/or scoliosis of the vertebral spine and/or poli- or oligodactylies, may be present (Brauckhoff et al. 2004). Thick lips are frequently observed in the presence of mucosal and/or conjunctival neuromas and are usually clearly visible when the eyes and mouth are explored (Fig. 4). The disease is very aggressive, and frequently children less than 5 years have already an advanced disease at the time of diagnosis (Fig. 5). Gastrointestinal disorders including obstructive symptoms, cramping, and diarrhea are frequently observed in early childhood. These symptoms are mainly related to the presence of megacolon, owing to the intestinal neuromas throughout the intestinal tract (Erdogan et al. 2006) (Fig. 5, panel a).

An association with CLA, a characteristically pigmented and itchy skin lesion specifically localized in the interscapular region of the back, has been reported in less than 10% of MEN 2A families (Ceccherini et al. 1994). The development of CLA may precede the development of MTC, and when present, it is almost invariably diagnostic of MEN 2A.

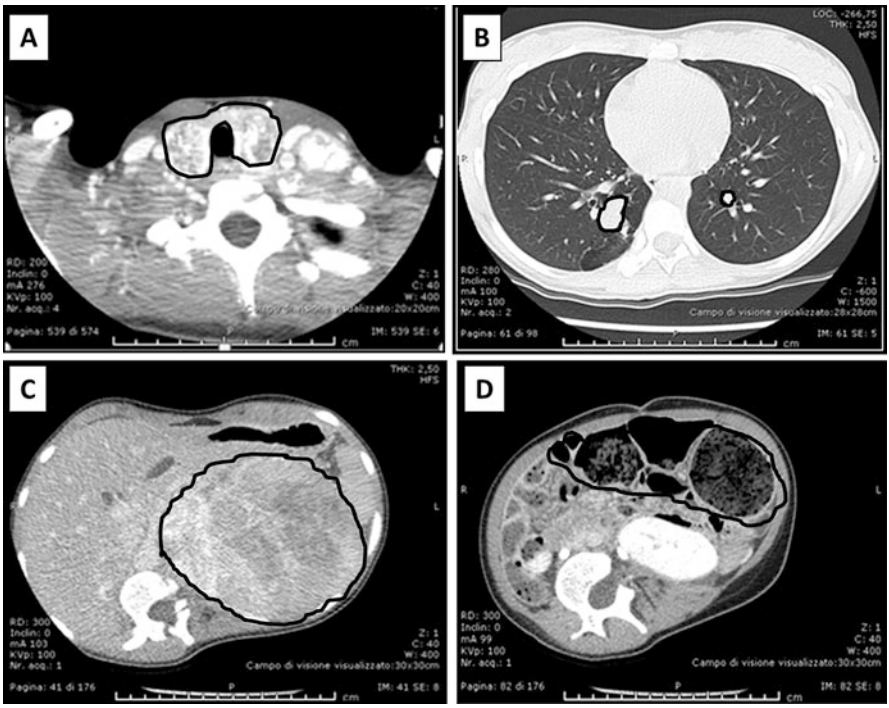

Fig. 5 CT scans with contrast medium of a multiple endocrine neoplasia 2B patient before surgery: (panel **a**) neck scan showing the bilateral involvement of the thyroid gland; (panel **b**) lung scan showing multiple and bilateral pulmonary metastases; (panel **c**) huge pheochromocytoma of the left adrenal gland; (panel **d**) severe and abnormal dilatation of the colon (i.e., megacolon) frequently present in MEN 2B patients

Diagnosis

An early diagnosis, likely when the tumor is still intrathyroidal, is highly desirable since this represents the only possibility to cure MTC patients. In the majority of cases, especially when sporadic, the clinical presentation of MTC is a thyroid nodule, which may be solitary or appear in the context of a multinodular goiter. Thus, the diagnosis is performed through the typical diagnostic work-up of thyroid nodules (Hegedus 2004).

Neck Exploration

Physical examination of the neck rarely offers any specific diagnostic advantage especially today when the majority of nodules are impalpable and often incidentally discovered by neck US performed on other indications (Acar et al. 2014).

Thyroid US usually shows a hypoechoic nodule, sometimes with microcalcifications and other suspicious features for malignancy (Fig. 3). However, the echographic pattern is similar to those of other thyroid malignancies (Lee et al. 2010). As for all thyroid nodular diseases (Gharib et al. 2016), thyroid scintiscan is not indicated unless TSH is low or low normal and a hyperfunctioning thyroid nodule or multinodular goiter is suspected. This way superfluous FNAB can be avoided, and the extension of a large goiter and its eligibility for radioiodine therapy can be evaluated (Hegedus et al. 2003). In any case, MTC nodule will appear as a "cold" nodule with no difference from any other thyroid malignancies.

Fine Needle Aspiration Cytology

US-guided fine needle aspiration cytology (FNAC) is considered the gold standard for the presurgical diagnosis of thyroid nodules. However, a recent multicentric international study, involving 12 different referral centers located in 7 different countries, demonstrated that FNAC was able to make a correct presurgical diagnosis of MTC in $<50\%$ of 313 analyzed cases. This limit of FNAC had a negative impact when planning the extension of the surgical treatment that was incorrect or inadequate in $>60\%$ of cases (Essig et al. 2013). Over the years, several series showing a high percentage of FNAC failure in making a presurgical diagnosis of MTC have been reported (Pacini et al. 1994; Rieu et al. 1995; Niccoli et al. 1997; Forrest et al. 1998). The most plausible reasons for this inadequacy are related to the not invariably well-defined cytological aspects of MTC cells which in certain cases can be misinterpreted. The final cytology may even indicate a benign, an indeterminate, or even a PTC or FTC lesion (Essig et al. 2013).

The results of FNAC could be significantly improved by performing immunocytochemistry for Ct (Fig. 6). This is, however, not a standard procedure and only done in selected cases, mainly in those with known elevated serum Ct levels. Sometimes cytologically negative results might be due to the fact that MTC could be present in one nodule, in the context of a multinodular goiter, not submitted to FNAC. In this condition, serum Ct measurement is more reliable, since it is elevated even in the presence of microfoci of MTC (Pacini et al. 1994; Vierhapper et al. 1997).

Serum Calcitonin and Other Peptides

Calcitonin (Ct) is the most specific and sensitive MTC marker, both before and after thyroidectomy (Melvin and Tashjian 1968). It is a small polypeptide hormone of 32 amino acids normally produced almost exclusively by C cells. The release and secretion of Ct are mainly regulated by extracellular calcium concentration. Other substances, such as pentagastrin, B-adrenergic agonists, growth hormone-releasing hormone, and other gastrointestinal peptides (Emmertsen et al. 1980), can stimulate Ct release from C cells.

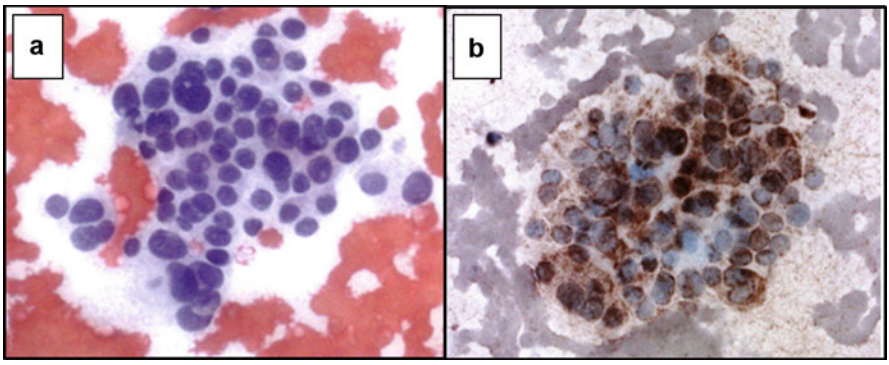

Fig 6 Cytological smears of a medullary thyroid cancer: (panel **a**) standard papanicolau staining of C cells showing a typical "salt-and-pepper" chromatin and plasmacytoid cytoplasm (40×); (panel **b**) immunocytochemistry for calcitonin showing a typical cytoplasmic granular positivity (40×)

Table 3 Other causes of hypercalcitoninemia: pathologies and interferences

Nonthyroidal diseases	Thyroidal diseases	Interferences/technical problems
Small cell lung carcinoma[a]	Lymphocytic thyroiditis[b]	Heterophilic antibodies
Breast cancer[a]	Micropapillary thyroid cancer[b]	Too high sensitivity of the assay
Other neuroendocrine tumors		Macrocalcitonin
Chronic renal failure		Chronic therapies with omeprazole
Pernicious anemia		
Zollinger's syndrome		
Pancreatitis		

[a]Usually when the tumoral disease is very advanced
[b]In these pathologies, the hypercalcitoninemia is due to the presence of an accompanying C cell hyperplasia

In 1968, 10 years after the recognition of MTC as a distinct histological type of thyroid carcinoma, high levels of serum Ct were demonstrated both in the tumor and the serum of patients with MTC (Melvin and Tashjian 1968). Elevated basal levels of serum Ct (>100 pg/ml) are diagnostic of MTC (Costante et al. 2007). However, there are other conditions, both physiological and pathological, in which basal levels of serum Ct may be found to be elevated and a differential diagnosis needs to be considered (Elisei 2008) (Table 3). Since the release of Ct in these diseases does not appear to be regulated by the same factors that stimulate Ct release in the C cells, differential diagnosis can be done by performing a stimulation test with a calcium infusion (25 mg/Kg of calcium gluconate or 2.5 m/Kg of calcium element, diluted up to 50 ml with saline solution ev in 5 min) that will provide different levels of stimulations depending on the disease (Fugazzola 2013).

Routine measurement of serum Ct in nodular thyroid diseases allows the preoperative diagnosis of unsuspected sporadic MTC (Pacini et al. 1994; Rieu et al. 1995;

Niccoli et al. 1997; Vierhapper et al. 1997; Kaserer et al. 1998; Ozgen et al. 1999; Hahm et al. 2001). Calcitonin screening determines the early diagnosis of MTC, usually when the tumor is still at stage I, thus favoring successful surgical treatment. A comparison of the outcome of two groups of patients, one diagnosed by serum Ct screening and the other by cytology or histology, has demonstrated a significantly better prognosis of the first group (Elisei et al. 2004a). However, despite this evidence, there is still a lot of resilience in performing routine serum Ct measurement in nodular thyroid disease, and when comparing current guidelines, it is clear that there is no consensus (Wells et al. 2015). There have been major concerns in applying this screening. As for cost-benefit, this has recently been demonstrated to be acceptable (Cheung et al. 2008), and the risk of false positives, especially at medium-low levels (<100 pg/ml), can be overcome with the calcium stimulation test. Moreover, if serum Ct is elevated, but <100 pg/ml, it should be interpreted as a potential MTC, and further diagnostic procedures, such as immunocytochemistry for Ct on cytological smears (Fig. 6), and/or the Ct measurement in the washout of the needle used for the puncture of a suspected thyroid nodule (Boi et al. 2007), should be carried out. The latter approach is of particular diagnostic utility to ascertain the nature of neck lymph nodes, especially before thyroidectomy, to plan the most appropriate therapeutic strategies.

Other Secretory Products

Although Ct is the most reliable tumor marker due to its high sensitivity and specificity, there are some other proteins that are released by the malignant C cell. Serum carcinoembryonic antigen (CEA) is usually elevated when the disease is disseminated and distant metastases are present (Rougier et al. 1983). Cases with advanced local disease, demonstrated at neck US and associated with elevated serum CEA levels, should be studied by computerized tomography (CT) to better evaluate the relationship of the disease with the gross veins, trachea, and esophagus and plan the most appropriate surgical treatment (Kodama et al. 1980; Jackson et al. 1987). However, CEA is most useful in monitoring the progression of the disease since its level increases when the burden of the disease is rapidly increasing.

Serum chromogranin-A may also be elevated in patients with MTC but it is highly unspecific (Baudin et al. 2001). Recently, a comparable diagnostic accuracy, as with serum Ct, has been demonstrated for serum procalcitonin (Machens et al. 2014), but further studies are needed before including this measurement in clinical practice.

As in many other neuroendocrine tumors, somatostatin (SMS), calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP), neuron-specific enolase (NSE), and other neuroendocrine substances may be produced abnormally, but none of these peptides are useful for diagnosis of MTC (Pacini et al. 1991; Barakat et al. 2004). At variance, some of them, such as CGRP, VIP, serotonin, and prostaglandins, together with serum Ct may contribute to flushing and diarrhea syndrome (Jaffe 1979; Wyon et al. 1998).

RET Genetic Analysis

Since at least 5–7% of apparently sporadic MTC are found to be hereditary, not only the family history should be carefully considered, with particular regard to the occurrence of PHEO and PTHAd in other family members, but genetic screening for germline *RET* mutations is always indicated (Elisei et al. 2013a). This finding is of great relevance for the early discovery of the other gene carriers who are unaware of their condition. At present, *RET* screening is mandatory in documented hereditary cases to allow for screening of all first-degree relatives.

Screening for *RET* Gene Mutations in Apparently Sporadic Cases

One single blood sample collected in EDTA, or even salivary smears, is sufficient for DNA extraction and genetic analysis. If a *RET* mutation is identified, the case can be reclassified as hereditary despite the absence of a family history. All the first-degree relatives (i.e., parents, brothers and sisters, sons and daughters) should be invited for a screening test (Elisei et al. 2013a).

Although not yet a standard of care procedure, *RET* gene analysis should be performed also in the tumor tissue, both for its prognostic value and for a more accurate tissue characterization that could turn out to be very useful if a drug, specifically aimed at inhibiting the altered *RET* gene, should become necessary.

Screening for *RET* Gene Mutations in MEN 2 Family Members

The pathogenic role of *RET* mutations in MEN 2 provides the rationale for screening family members of any affected proband carrying a germline mutation. From a practical point of view, once the germline *RET* mutation of the index case has been recognized, blood is taken from all first-degree family members. Informed consent and an adequate genetic counseling are requested. This allows the identification of "gene carriers" at a time when they are still clinically unaffected or at an early stage of the disease. It also has the advantage of excluding "nongene carriers" from further testing for life. Although the presence of a germline *RET* mutation is diagnostic of the MEN 2 syndrome, gene carriers must be submitted to further clinical and biochemical evaluations to ascertain the actual development of the MTC and its extension, if already present. The involvement of other endocrine organs must also be assessed (Elisei et al. 2013a).

Histology

Under macroscopic examination, MTC shows a hard and firm consistency and is either chalky white or red in color on cross section (Schmid 2015). Histologically, MTC is pleomorphic with spindle-shaped or rounded cells characteristically organized in a nested pattern. Mitoses are not very frequent, nuclei are usually uniform, and the eosinophilic cytoplasm is characterized by the presence of secretory granules. Deposits of amyloid substance are frequently (60–80%) observed between tumor cells (Sletten et al. 1976).

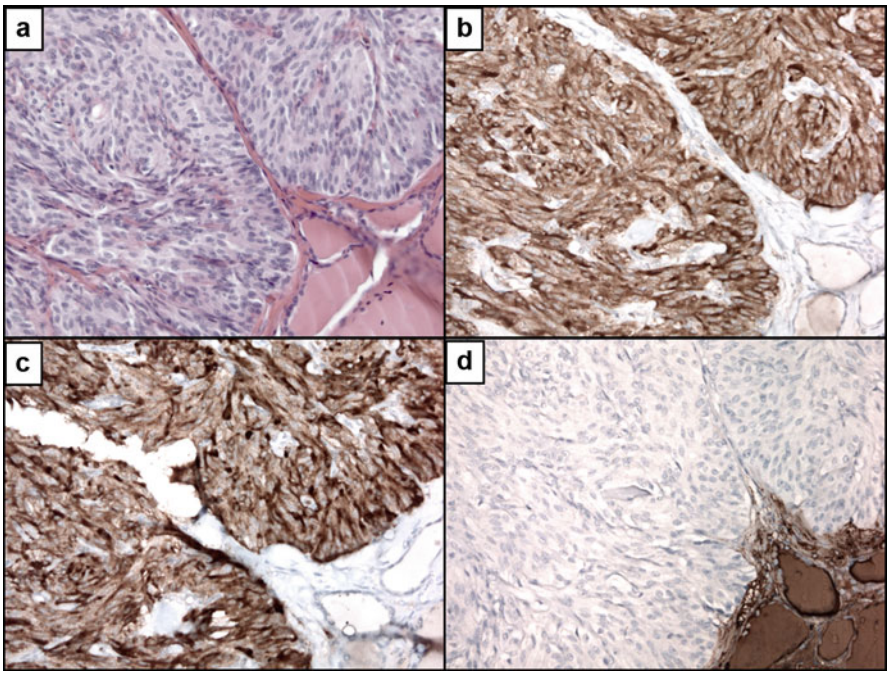

Fig. 7 Histology of a medullary thyroid cancer: (panel **a**) standard hematoxylin/eosin staining (20×); (panel **b**) positive immunohistochemistry for calcitonin (20×); (panel **c**) positive immunohistochemistry for chromogranin (20×); (panel **d**) negative immunohistochemistry for thyroglobulin (20×)

Sometimes it may be difficult to distinguish MTC from ATC, Hürthle cell carcinomas, or insular carcinomas, especially if pseudopapillary elements or giant cells are present. Positive immunohistochemistry for Ct is diagnostic for MTC (Fig. 7). Immunohistochemistry for chromogranin-A and CEA may also be useful, especially in those few cases with low or no production of Ct (Giovanella et al. 2008; Trimboli and Giovanella 2015).

Histopathological description of MTC should include the number and the distribution of tumor foci, as well as the simultaneous presence of C cell hyperplasia. This information is of practical usefulness because bilaterality, multicentricity, and C cell hyperplasia are considered the histological hallmarks of the hereditary MTC forms (Schmid 2015). Regarding C cell hyperplasia diagnosis, the most widely accepted definition is when >50 C cells per single low-power field are found, even though this criterion may not be respected in the presence of cytologically evident atypias (LiVolsi 1997). Diffuse, focal, or nodular C cell hyperplasia can be distinguished on the basis of the number and distribution of C cells. It is likely that they represent progressive stages through which the normal C cell is transformed into a malignant cell. While there is general agreement in considering C cell hyperplasia the preneoplastic lesion of the hereditary form of MTC, little is known about the

relationship between C cell hyperplasia and the sporadic form. Nevertheless, about 30% of sporadic MTC are associated with C cell hyperplasia (Nikiforov et al. 2012).

A mixed form of MTC and PTC is also described (Matias-Guiu 1999). It is characterized by the simultaneous presence of parafollicular and follicular cell features, with positive immunohistochemistry for both Ct and Tg. In this respect, it is worth noting that the occurrence of MTC and PTC in the same thyroid gland seems to be quite frequent (Biscolla et al. 2004; Wong et al. 2012). It is still questioned whether the mixed MTC/PTC is a genuine separate histological entity, originating from an ancestral stem cell able to differentiate into both follicular and parafollicular cells, or the consequence of the collision of two distinct tumors, MTC and PTC, originating in the same thyroid gland. The latter of the two hypotheses seems to be supported by evidence of the two entities not sharing common classical point mutations (Ciampi et al. 2017).

Follow-Up

After the initial therapy (see the following paragraph), serum basal and stimulated Ct should be measured to verify the completeness of the treatment. Neck US and other imaging techniques may be useful to map the metastatic lesions, but they are almost invariably negative when serum Ct is <150 pg/ml. Most important in the follow-up of MTC patients is the doubling time of both serum Ct and CEA (Gawlik et al. 2010) for predicting the outcome of the disease.

Biochemical Monitoring

Initial postsurgical control of serum Ct should be done 3 months after surgery and include physical examination, neck US, and measurement of serum FT3, FT4, TSH, and CEA. Due to the prolonged half-life, if performed too early, measurement of serum Ct may be misleading, especially if a high serum concentration was present preoperatively (Fugazzola et al. 1994). If basal Ct is undetectable, patients have a high probability of being cured, with an estimated 10% risk of recurrence in the long term (Pellegriti et al. 2003). This probability is reduced to 3.3% in patients with a negative postoperative stimulation test (Franc et al. 2001). The follow-up of these "negative" patients should include Ct measurement on a 12–18-month basis, together with a neck US. In patients with undetectable levels of serum Ct, measurement of CEA is not necessary, unless undifferentiated MTC is suspected (Trimboli and Giovanella 2015).

About 50% of patients not cured at surgery have no evidence of metastatic disease when studied with the traditional imaging techniques. In this condition of "biochemical disease," the most widely accepted strategy is to "wait and see." A detectable serum Ct level is in fact compatible with long-term survival, during which serum Ct may remain stable or slowly increase over time. Such patients are monitored at intervals of 6–12 months. A rapid increase of serum Ct and/or CEA indicates a poor

prognosis, both for recurrence and for death, especially if the doubling time is <0.5–1 year (Meijer et al. 2010). Facing this scenario, imaging control must be intensified to verify the progression of the disease and to decide if it is time to initiate active therapy (Schlumberger et al. 2012a).

Imaging Techniques

Because serum Ct is a very sensitive and specific MTC marker, if detectable after surgery, it is highly suggestive of persistent disease. In this case, serum CEA should be monitored because both high and increasing levels are strongly suggestive of progressive disease (Busnardo et al. 1984). In the majority of cases, the challenge is to find the source of production of Ct and CEA, and there is evidence that if serum Ct is <150 pg/ml, it is very unlikely to find this source with the current imaging techniques. No single sensitive diagnostic imaging method can reveal all MTC recurrences or metastases. A neck US is the first localization technique to be performed due to the high likelihood of local recurrence and cervical node metastases. Depending on the region to be explored, CT scan, MRI, US, or scintigraphy may be the most appropriate to use (Giraudet et al. 2007). Other imaging techniques such as octreoscan, 123-meta-iodobenzylguanidine (MIBG), and 18-fluorodesossiglucose (FDG)-positron emission tomography (PET) may be useful although at present they do not appear to be particularly sensitive, especially in the presence of micrometastases and low levels of serum Ct (Baudin et al. 1996; Giraudet and Taieb 2017). Selective catheterization for venous sampling is an invasive and relatively insensitive technique and therefore no more applied in clinical practice. New tracers for PET/CT scan are emerging since the sensitivity of PET, using (18)F-fluorodihydroxyphenylalanine (18F-DOPA) (Fig. 8) or 68Ga-labeled somatostatin analogues (68Ga-DOTATATE or DOTATOC), is greater than that of older radiotracers (Skoura 2013).

Therapy

Medullary thyroid cancer, both sporadic and familial, is a challenging tumor at high risk not to be successfully cured and to be lethal within 5–10 years in 50% of cases with distant metastases at the time of diagnosis. However, the prognosis in the most aggressive forms, whether sporadic or hereditary, has been improved over the last 20 years (Randle et al. 2017; Raue et al. 2018). Several reasons can explain this, including earlier diagnosis and better therapeutic strategies. In the following, a detailed description of the therapeutic strategy for primary as well as metastatic tumors is given.

Initial Surgical Treatment

Early diagnosis and a complete surgical treatment are the bases for a definitive cure of patients affected by MTC. Conventionally, the minimal standard surgical

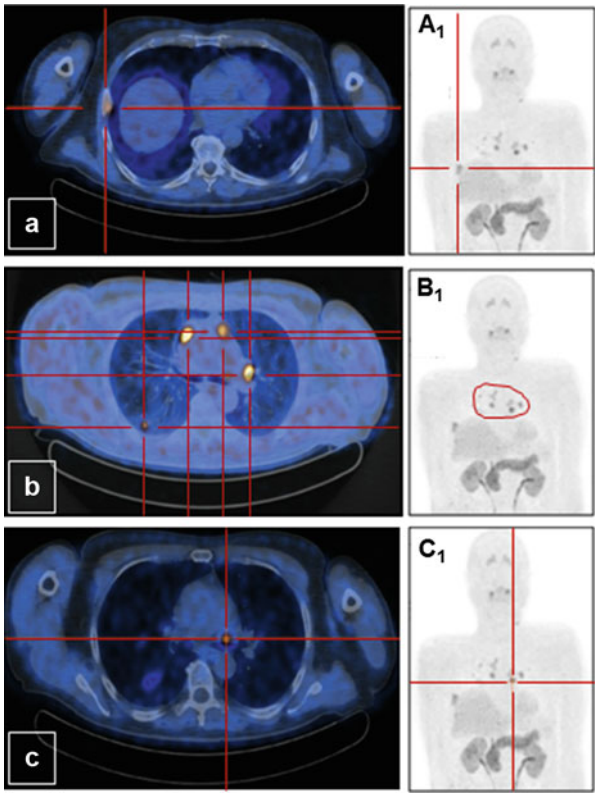

Fig. 8 18Fluoro-di-idrossiphenyl-alanina positron emission tomography (18F-DOPA-PET) integrated with computerized tomography (CT) scan (panels **A**–**C**) in a patient affected with metastatic medullary thyroid cancer: 18F-DOPA-PET is at present the most sensitive imaging technique, and the integration with the CT scan allows to better localize the lesions that are difficult to be distinguished in the planar scans (panels $\mathbf{A_1}$-$\mathbf{B_1}$-$\mathbf{C_1}$); (panel **A**) metastasis in the fifth right rib; (panel **B**) several metastatic lesions in the mediastinum – the integration with CT scan clearly shows that one lesion is in the lung while the others are lymph nodes, one of which is localized in the lung left hilum (panel **C**)

procedure is total thyroidectomy with central neck lymph node dissection, both in sporadic and familial forms. The need for total thyroidectomy is supported by the multicentricity and bilaterality of the MTC, occurring in about 100% of the hereditary and 20–30% of the sporadic form (Essig et al. 2016). Furthermore, C cell hyperplasia, which is considered a preneoplastic lesion, is almost invariably associated with the hereditary form of MTC and, to a lesser extent, with the sporadic form (Schmid 2015). An additional reason favoring total thyroidectomy is that 5–7% of apparently sporadic cases are in fact hereditary forms, which almost invariably have a bilateral disease (Romei et al. 2011) (Fig. 5, panel a).

Central node neck dissection is part of the initial surgical treatment, independent of the size of the primary tumor and the presurgical evidence of lymph node involvement. This node compartment represents in fact the primary lymphatic station of the thyroid, and 50–60% of MTC show micro and/or macro node metastases in this area at initial surgery (Moley and DeBenedetti 1999; Ukkat et al. 2004). A correlation between the presurgical values of serum Ct and the presence of neck node metastases has been demonstrated with a probability of less than 10% or zero of finding central neck node metastases when serum Ct is lower than 50 pg/ml or 20 pg/ml, respectively (Machens and Dralle 2010). For many years, this surgical approach has

also been suggested in *RET* gene carriers without clinical evidence of the disease, since they can be completely cured by surgery. However, according to the most recent American Thyroid Association guidelines (Wells et al. 2015), *RET* gene carriers with serum Ct < 40 pg/ml can be treated with total thyroidectomy alone because of the evidence that neck node metastases in the central compartment are rare or absent in this condition (Rohmer et al. 2011; Elisei et al. 2012). Whether, as a principle, a modified radical neck dissection with the removal of nodes in the ipsilateral or bilateral compartments should be performed is still debated. Several authors strongly suggest an "en bloc" dissection of both central and bilateral neck compartments together with the thyroid gland (Scollo et al. 2003). The rationale for this kind of strategy is that uni- or bilateral cervical nodal metastases occur in up to 90% of patients with MTC, especially when the primary tumor is >2 cm and presurgical serum Ct is >200 pg/ml (Machens and Dralle 2010). This is of great clinical significance because the adequacy of the initial surgical treatment is a prerequisite for the effective cure of the MTC. Thus, choice of the most appropriate initial procedure is fundamental. However, it is worth noting that radical neck dissection may result in significant morbidity and not clearly been shown to improve the prognosis, which is also dependent on factors such as the local extension of the disease at the time of diagnosis, the presence or absence of other endocrine neoplasias, and the cervical lymph node metastases. In particular, it has been demonstrated that despite the radicality of the surgical treatment, if the MTC is extrathyroidal at the initial surgery, it is almost impossible to obtain biochemical cure (i.e., undetectable levels of postoperative serum Ct) of the disease (Gimm et al. 1998; Franc et al. 2001; Weber et al. 2001). For this reason, when presurgical serum Ct is <200 pg/ml, the lateral lymph node compartments should be removed only if neck US has clearly shown presence of metastatic lymph nodes.

Gene Carrier Initial Treatment

Once a gene carrier has been diagnosed by genetic analysis, the therapeutic strategy should be defined according to the guidelines for the management of multiple endocrine neoplasia (Elisei et al. 2013a; Wells et al. 2015). These guidelines take into account the varying biological behaviors of the MTC in the three forms of MEN syndromes and according to the type of *RET* mutation and level of risk (Table 2). In MEN 2B, total thyroidectomy should be performed as soon as possible, even within the first months of life. In MEN 2A, total thyroidectomy should be performed at 5 years of age or earlier if the stimulation test for Ct is positive. There is still dispute on the management of gene carriers in families with FMTC. Since, in the majority of cases and especially in those with non-cysteine *RET* gene mutations, the risk is quite low, we and others (Rohmer et al. 2011; Elisei et al. 2012) suggest to perform a Ct stimulation test immediately after the discovery of the positive genetic screening and, if negative, an annual follow-up with repetition of the stimulation test aiming at thyroid surgery immediately after the first positive test.

Surgery should be total thyroidectomy, with or without central neck dissection according to the level of serum Ct (Wells et al. 2015). Much evidence suggests that when serum Ct is <30–40 pg/ml, the probability of lymph node involvement is almost null (Machens and Dralle 2010; Rohmer et al. 2011; Elisei et al. 2012) allowing avoidance of central neck dissection and thereby reducing surgical complication rate (Viola et al. 2015).

Parathyroid and adrenal gland morphology and function must be assessed and an adequate treatment offered if needed. Importantly, if no abnormalities of these glands are found at the time of diagnosis, their morphology and function should be monitored annually because both PTHAd and PHEO may occur later in life.

Further Local Treatments in Patients Not Cured by Surgery

Second Surgery

In the first years following surgical treatment, the regional lymph nodes of the neck and mediastinum are the most frequent sites of recurrences, especially in patients with postoperative biochemical persistence of the disease (i.e., elevated values of Ct without evidence of structural disease). In such cases, a second surgical treatment with a curative intent is recommended, and to this purpose, an extensive modified neck dissection involving microdissection of all node-bearing compartments is recommended. Unfortunately, less than 40% of patients affected by MTC with extrathyroidal invasion can be cured by a second surgical treatment (Tisell et al. 1986; Moley et al. 1998). Capsular invasion and more than ten lymph node metastases (Scollo et al. 2003; Miccoli et al. 2007) in the primary surgical specimens are significant predictors of poor response to operation. In the clinical management of patients with MTC, the identification of those who might benefit from this treatment is of great practical importance to avoid inappropriate expectations (Moley et al. 1998). Moreover, according to the latest ATA guidelines, when the first surgical treatment is performed in a referral center, fewer cancer reoperations for MTC are required (Verbeek et al. 2015).

A second surgery, with palliative rather than curative intent, may also be strongly indicated in patients with compressive symptoms who can benefit from a surgical debulking (Chen et al. 1998). Even if definitive cure is not foreseen, a second surgical treatment should be performed for symptomatic lesions or when their growth may cause significant morbidity as may happen for lymph nodes of the mediastinum adjacent to the great vessels, tracheoesophageal groove, carotid sheath, and brachial plexus. Patients with widely metastatic MTC often live for several years with acceptable quality of life (QoL), and palliative surgical resection of symptomatic lesions can offer significant long-term relief from such symptoms.

External Radiotherapy

In patients with local aggressive disease not completely removed by the primary resection, surgical treatment should be followed by external beam radiotherapy (ERT) as adjuvant treatment. Although MTC has very low sensitivity to ERT,

there is evidence of potential benefit from radiotherapy in terms of a lower risk (two- to fourfold) of local recurrence in patients with residual disease (Schwartz et al. 2008). Radiation therapy after thyroidectomy and node dissection is not generally recommended on a prophylactic basis, and the procedure should be reserved to patients who, although having undergone extensive surgery, still have local disease. In patients who have had less aggressive primary resection, the ERT should be postponed until after a second surgical treatment.

Radiofrequency Thermoablation (RFA)

This procedure is based on the use of electromagnetic waves that produce heat. A needlelike RFA probe is introduced in the tumor mass, and the high temperature results in the destruction of the tissue. This treatment is applicable to local disease but also to bone, liver, and lung lesions if technically accessible (Lencioni et al. 2008; Eisele 2016; Ringe et al. 2016). This treatment is of particular benefit when the lesion to be treated is the only site of disease or the only one, among several others, that is growing. It is also indicated if there are contraindications to surgery or when repeat surgery due to MTC recurrence is unfeasible or afflicted with a high surgical risk.

Treatment of Distant Metastases

Local Treatments

Local treatment for distant metastases is indicated for single lesions and should be taken into consideration if the metastatic lesion is unique or, if multiple, only one of them represents a clinical problem for any of the following reasons: pain, local compression of other organs, risk of fracture, or blood vessel invasion. The possibility of performing a local treatment should always be considered before starting systemic therapy, which should be reserved for cases with multiple metastatic lesions, involving multiple organs and simultaneously growth (Schlumberger et al. 2012b). As shown in Table 4, local treatments can vary according to the site and the number of the metastatic lesions. Therapy decisions require involvement of a multidisciplinary team.

Brain Metastases

Brain metastases are relatively rare, occur late in MTC, and are usually a hallmark of a poor prognosis. They can be treated with external radiotherapy (ERT), either whole-brain radiotherapy or stereotaxic radiosurgery or both, with a rapid and reliable response (Simoes-Pereira et al. 2016). If solitary and localized in an approachable site, they may be treated neurosurgically. Corticosteroid therapy is usually employed to reduce the edema that can be present. Antiepileptic drugs may be warranted.

Lung Metastases

Surgery is indicated only if the lesion is solitary and located in an approachable site or if the lesion is at risk of infiltrating a bronchus or a blood vessel. External radiotherapy

Table 4 Local treatments for MTC metastases to be considered before starting systemic therapy

Surgery (especially for isolated lesions or when the disease is confined to the neck)
External radiotherapy (palliative on the neck and/or mediastinum, pain control for bone lesions)
Whole-brain irradiation (for stabilization of multiple brain metastases)
Radiosurgery (for single small brain metastases)
Intra-arterial chemo (TACE)- or radioembolization (TARE) (especially for liver metastases)
Radiofrequency ablation (lung, bone, liver, local disease if solitary and accessible)
Laser or cryoablation (as for radiofrequency)
Endotracheal/bronchial laser ablation (to maintain vital functions)

of lung metastases should be avoided since it carries a risk of radiation fibrosis and may lead to respiratory insufficiency. The possibility of performing thermoablation (RFA) should be considered if the lesions are few and no bigger than 3 cm. No data have been reported so far on RFA in MTC lung metastases. However, this procedure, as well as laser-induced interstitial thermotherapy and microwave ablation, has been used with success in several other histotypes of lung metastases (Nour-Eldin et al. 2017).

Mediastinal Lymph Nodes

Metastatic spread commonly occurs to cervical and mediastinal lymph nodes. The latter are frequently involved and very often represent the major bulk of the disease, and being close to major blood vessels, they represent a major risk of complications. Thoracic surgery is the most effective option for curative therapy, reduction in tumor burden, and/or effective palliation but should only be offered if the thoracic surgeon feels that the surgery can be complete or almost complete. A "berry-picking" approach is strongly discouraged (Machens and Dralle 2015). In some cases, ERT alone or following surgery can be considered for these metastases.

Liver Metastases

Surgical resection may be indicated for liver metastases, although at present there are other local highly effective treatments such as transarterial chemoembolization (TACE). This procedure has been found of particular benefit and should absolutely be taken into consideration. Recent data from a specialized center demonstrated a 100% response rate with a median time to tumor progression of 38 months, even in big lesions (median size 4 cm), and with considerable involvement of the hepatic tissue (up to 50%) (Grozinsky-Glasberg et al. 2017). Thermoablation (RFA) can also be considered for liver metastases from thyroid cancer and successfully applied (Wertenbroek et al. 2008). Radioembolization (TARE) with selective internal radiation microspheres is used for neuroendocrine liver metastases (King et al. 2008) and can also be used in MTC liver metastases, especially if small, disseminated, and well vascularized. A hepatic artery angiography, for diagnostic evaluation of liver metastases, and to exclude a pulmonary shunt, which represents a contraindication to TARE, should always be performed before TARE (Fig. 9).

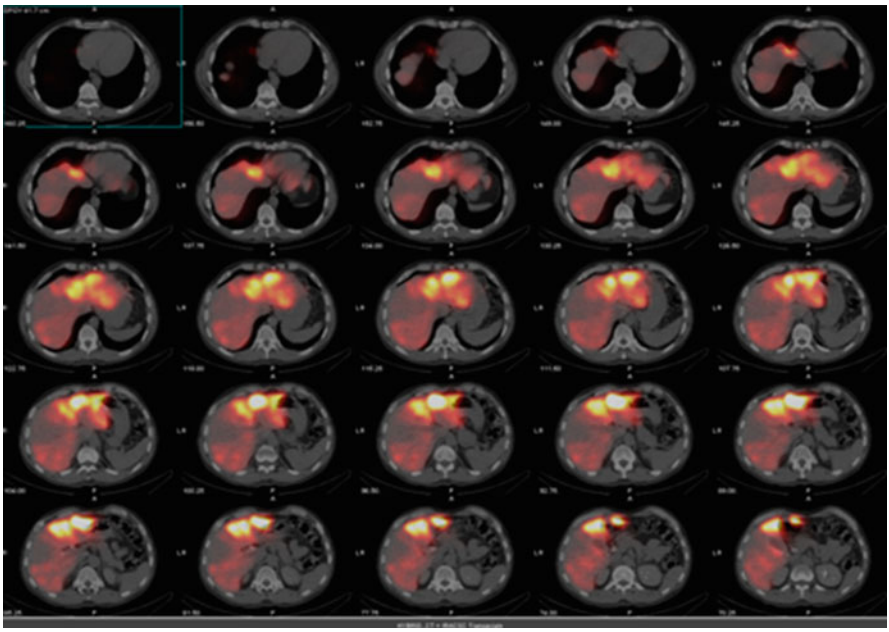

Fig. 9 Consecutive acquisitions during a hepatic artery angiography for diagnostic evaluation of liver metastases in a patient affected with metastatic medullary thyroid cancer. The procedure is commonly performed to evaluate the feasibility of transarterial radioembolization (TARE) with 90Y-microspheres. In this case, the procedure was considered feasible since the metastases were well vascularized and there was no evidence of pulmonary shunt that represents a contraindication to the procedure

Bone Metastases

About 20% of MTC patients develop bone metastases with potentially devastating skeletal-related events. In about 90% of cases, they are associated with other distant metastases (Xu et al. 2016). Bone lesions can be successfully treated by surgery dependent on their localization. In particular surgery can be effective if metastatic lesions involve long bones (i.e., femur and humerus) or the pelvic bones. Surgical debulking of vertebral metastases that might impair spinal cord function is an example of a non-curative but appropriate surgical procedure. Sometimes, spinal cord stabilization can be indicated if a vertebral collapse is anticipated. If surgical treatment is performed for a bone lesion, a subsequent ERT is indicated as adjuvant therapy. Moreover, ERT is indicated for bone metastases both to prevent pathological fractures and as palliation of symptoms. The combination of ERT with hyperthermia appears to significantly increase the pain control rate and extends response duration compared with ERT alone for painful bony metastases (Chi et al. 2018).

Cutaneous Lesions

The appearance of cutaneous metastases is a poor prognostic factor, since all the cases reported so far have died within 1 year of the diagnosis of metastases.

Although a few cases of MTC discovered with cutaneous metastases as first clinical manifestation have been reported (Santarpia et al. 2008), usually cutaneous metastases develop in the presence of known distant metastases, indicating systemic spread of MTC. Fortunately they are rare (Nashed et al. 2010). No specific therapy is available, and surgery is not recommended unless the lesion represents a clinical problem for the patient being painful. The detection of cutaneous metastases is a strong indication to start systemic therapy.

Systemic Therapy

Conventional Chemotherapy

Chemotherapy for advanced, metastatic MTC has shown limited response rates in several small-scale trials (Orlandi et al. 2001). Chemotherapy should not generally be used anymore but be reserved for patients with disseminated and well-documented progressive disease who, for any reason, cannot be treated with tyrosine kinase inhibitors (TKI) that represent the first-choice systemic therapy. A high dose of doxorubicin has been demonstrated to be the most effective chemotherapeutic agent with a response rate of 15–20% in terms of stabilization of the disease either when used alone or in combination with other drugs such as 5-fluorouracil, dacarbazine, streptozocin, cyclophosphamide, and vincristine (Nocera et al. 2000). However, since major toxic effects are frequently observed, and the response is only partial and short lived, chemotherapy should be used only as a "last option" therapy.

Other Systemic Therapies

Medullary thyroid carcinoma is a neuroendocrine tumor, and 30–50% of cases express somatostatin (SMS) receptors as documented by octreoscan (Baudin et al. 1996). Over the years, different types of octreotide, from the native to the long-acting analogues, have been explored as potential therapeutic agents. In the majority of cases, a significant reduction in serum Ct has been demonstrated (Lupoli et al. 2003). Unfortunately, no evidence of a parallel reduction of the structural disease has been shown. Inconsistent and transient effects in reducing symptoms, such as flushing and diarrhea, are not sufficient to recommend the administration of SMS analogues in metastatic MTC patients. In cases with severe diarrhea, uncontrolled with any other conventional drugs, SMS long-acting analogues may be tried. Specific SMS receptors have been identified both in cell lines deriving from human MTC and in surgical tissue specimens of MTC (Papotti et al. 2001; Zatelli et al. 2002). The possibility of using analogues that specifically recognize these receptors is under evaluation. No improvement in the therapeutic effect of SMS analogues has been observed when combined with gamma interferon (Lupoli et al. 2003).

Although there are no specific studies in MTC patients, treatment with either bisphosphonates or the receptor activator of nuclear factor kappa-B ligand (RANKL) inhibitor, denosumab, has been recognized as valid in the treatment of bone metastases (Wells et al. 2015). This therapeutic procedure has been demonstrated to be effective in controlling the bone pain and delaying the occurrence of skeletal-related events in patients with bone metastases due to differentiated

thyroid cancer (Vitale et al. 2001; Orita et al. 2015). Both pamidronate and zoledronate are administered monthly i.v., while denosumab is subcutaneously administered every 6 months. The side effects of these potent antiresorptive agents, although rare, include osteonecrosis of the jaw (Khosla et al. 2007), atypical subtrochanteric fractures (Abrahamsen et al. 2009), and hypocalcemia and must be carefully taken into consideration before starting therapy and during long-term treatment.

Radionuclide Therapy

Treatment with several radioactive elements has been widely explored, including that of radioiodine (131-I). Although some anecdotic reports indicate a beneficial effect of 131-I treatment of the postsurgical remnant, presumably due to death of C cells adjacent to follicular cells as a consequence of a bystander effect (Nusynowitz et al. 1982; Nieuwenhuijzen Kruseman et al. 1984), C cells are unable to actively concentrate iodine. As a consequence, 131-I is neither indicated for thyroid remnant ablation nor for metastatic lesions in MTC.

A more promising use of 131-I has been hoped when radioiodine was linked to meta-iodobenzylguanidine (MIBG) (Maiza et al. 2012). However, only a small proportion of patients (30%) are positive, and the treatment has been shown to be virtually ineffective. Thus, 131-I MIBG therapy represents an alternative in metastatic MTC patients only when there is significant uptake on MIBG scintigraphy and if TKI are ineffective or contraindicated.

Other radioimmunotherapeutic agents have been explored. In particular, the bi-specific antibodies directed against CEA, which are expressed on the surface of the majority of metastatic MTC cells. Controversial data have been reported in different studies, and while in some studies no significant benefits were found (Kraeber-Bodere et al. 2003), a phase II study showed good disease control in 76.2% of treated cases in another (Salaun et al. 2012). However, the same phase II clinical trial showed a high grade of hematologic toxicity affecting about 55% of patients. As for other systemic therapeutic approaches, also the anti-CEA pretargeted radioimmunotherapy may be taken into consideration in advanced and progressive cases that cannot be treated with other strategies.

Data have reported on the use of SMS analogues labeled with yttrium 90 or lutetium 177, in patients with metastatic MTC showing octreotide uptake at octreoscan (Budiawan et al. 2013). A few studies have offered promising but not enthusiastic results (Bodei et al. 2004; Kaltsas et al. 2004). However, the authors hypothesized that a possible reason for the modest results could be too advanced disease at the time of treatment and thus need of performing studies in less advanced disease.

Tyrosine Kinase Inhibitors

The first-choice systemic therapy for advanced and progressive MTC is currently represented by two oral drugs, vandetanib and cabozantinib, which belong to the family of TKI. They have been approved by both the Food and Drug Administration (FDA) and European Medicines Agency (EMA) after promising results, in terms of a

significant prolongation of the progression-free survival time, obtained in the phase III clinical ZETA and EXAM studies (Wells et al. 2012; Elisei et al. 2013b).

Both vandetanib and cabozantinib are small molecules able to block, with different activities and different patterns, multiple tyrosine kinases (Matrone et al. 2017). Nevertheless, both of them, among other properties, are able to block *RET* which is the major pathogenic event in MTC. The drugs should be started at the time of evidence of disease progression, as assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) or according to clinical judgment in very advanced cases. Vandetanib can be used also in symptomatic patients and in those with ectopic Cushing's syndrome (Nella et al. 2014; Pitoia et al. 2015; Paepegaey et al. 2017). The choice of one or the other drug mainly depends on local availability, since not all countries have both drugs approved and reimbursed. However, if both vandetanib and cabozantinib can be prescribed, the choice is dictated by the patient's clinical features, location of metastatic lesions, and drug characteristics (Table 5). According to the results of the mentioned phase III studies (Wells et al. 2012; Elisei et al. 2013b), by comparing the effects of the two drugs, it appears that cabozantinib action is more rapid but with a series of adverse events (AE) more severe than with vandetanib. Taking into account this observation, cabozantinib should be preferred when a rapid shrinkage of the tumor mass is required, although running the risk of AE. In the phase III study, patients who had previously been treated with other TKI could be enrolled and treated with cabozantinib, and the results showed that it works in terms of prolongation of the progression-free survival. Taking into account this finding, vandetanib should be used as first choice to reserve cabozantinib as second choice when, for any reason, vandetanib needs to be stopped. Vandetanib, but not cabozantinib, has been successfully tested also in children affected by advanced and metastatic MTC in MEN 2, mainly MEN 2B (Fox et al. 2013). The outcome in these children demonstrated that the treatment with vandetanib is safe and results in sustained responses (Kraft et al. 2018). Moreover, there are several reports showing that the ectopic ACTH secretion and the paraneoplastic Cushing's syndrome, which is frequently present when the disease is multimetastatic and advanced, are completely reverted and cured by vandetanib (Nella et al. 2014; Pitoia et al. 2015; Paepegaey et al. 2017). A limitation to the use of vandetanib, but not of cabozantinib, is the presence of a prolonged QTc (>450 ms in men and >470 ms in females). Therefore, in these patients, cabozantinib is the first-choice drug. Side effects of both drugs are very similar but the prevalence varies, and this, as well as other morbidities, must be taken into consideration when deciding which drug to use first. Although both drugs show a significant increase in the progression-free survival time, the overall survival (OS) is as yet not increased (Table 5). However, exploratory analyses suggest that patients with *RET* M918T-positive tumors may benefit more from treatment with cabozantinib than do those with M918T-negative tumors, especially in terms of OS (Schlumberger et al. 2017).

As all TKI, both vandetanib and cabozantinib are cytostatic but not cytotoxic. This means that they can block the cell proliferation and growth but cannot kill the tumor cells and therefore must be continued until evidence of clinical benefit. However, from the results of two studies, as well as from real-life experience, it is

Table 5 Comparison between the most significant data regarding vandetanib and cabozantinib phase III clinical trials

Trial name	Drug	Phase	Study design	Enrolled patients (N)	Inclusion	Median PFS (months)	ORR	MDR (months)	OS
Exam	Cabozantinib	III	Drug vs placebo NO crossover at progression	330	Disease progression	11.2 vs 4.0 ($p < 0.0001$)	$p < 0.0001$	14.7	No difference
ZETA	Vandetanib	III	Drug vs placebo Crossover at progression	331	Disease progression or symptoms	30.5 vs 19.3 ($p < 0.001$)	$p < 0.001$	22	No difference

PFS progression-free survival, *ORR* objective response rate, *MDR* median duration response, *OS* overall survival

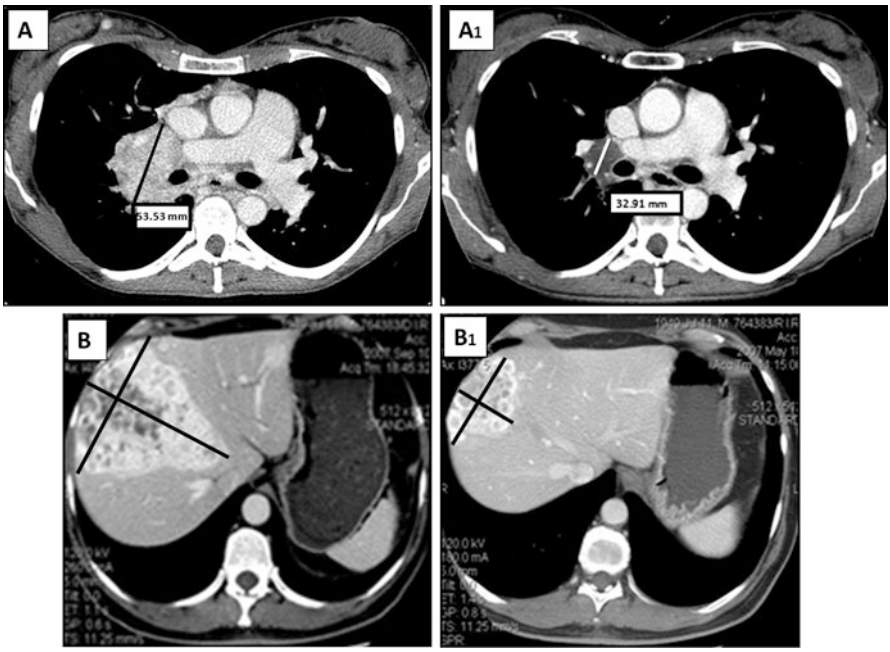

Fig. 10 Two cases of MTC metastatic lesions treated with tyrosine kinase inhibitors: (panel **A**) big lymph node metastasis of the lung hilum before (panel **A**) and after 3 months of vandetanib therapy (panel $\mathbf{A_1}$). A significant reduction of the size associated with a change of the tumor density, likely due to devascularization, is evident when comparing the two scans. (Panel **B**) big liver metastasis before (panel **B**) and after 3 months of therapy with cabozantinib (panel $\mathbf{B_1}$). An impressive reduction of the size of the lesion is evident when comparing the two scans: a significant reduction of symptoms related to this metastasis (i.e., pain and abdominal compression) was also referred by the patient

evident that the lesions can be significantly reduced in size although no complete response has ever been observed (Wells et al. 2012; Elisei et al. 2013b) (Fig. 10). The development of drug resistance is a major problem. If/when this occurs, clinicians must decide whether to continue or stop the drug. At present, only "off-label" drugs demonstrated to be useful in the treatment of MTC in phase II clinical trials (Schlumberger et al. 2009, 2016; Lam et al. 2010; Bible et al. 2014; Locati et al. 2014; Ravaud et al. 2017) may be used after cabozantinib and vandetanib (Table 6). Further studies to analyze the possibility of using the two drugs in an alternating way or in combination between them or with other drugs, either targeting the same mechanisms or by modulating the immunosystem, will be one of the many challenges of the immediate future.

Novel *RET*-specific inhibitors with an improved anti-*RET* activity and, at the same time, a reduced toxicity are currently under investigation at the clinical and preclinical level. A phase I/Ib study of RXDX-105, a *RET* and BRAF inhibitor that relatively spares VEGFR2 and VEGFR1, with a planned expansion at the recommended phase II dose, is ongoing (NCT0187781). Other *RET*-specific

Table 6 Tyrosine kinase inhibitors already tested in phase II clinical trials in medullary thyroid cancer patients and their activities, expressed as IC_{50}, against different tyrosine kinase receptors

TKI	VEGFR1	VEGFR2	VEGFR3	RET	MET	KIT	BRAF	Others
Imatinib	19.500[a]	10.700	5.700	–	>100.000	410	–	ABL (38[a])
Axitinib	1.2	0.25	0.29	–	–	–	–	–
Vandetanib	1.600	40	108	130	–	–	–	EGFR (500)
Motesanib	2	3	6	59	–	8	–	PDGFR (84)
Sunitinib	15	38	30	224	–	1–10	–	FLT3 (21)
Gefitinib	–	–	–	–	3.200	–	–	EGFR (14)
Sorafenib	–	90	20	5.9	–	68	22	CRAF (6)
Lenvatinib	22	4	–	35	–	–	–	FGFR1 (25)
Cabozantinib	–	0.035	–	4.5	1.8	–	–	–

[a]All numbers express the half maximal inhibitory concentration (IC50) that is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. IC50 indicates how much of the drug is needed to inhibit a given biological process

inhibitors under investigation in phase I studies are LOXO-292 (NCT03157128) and BLU-667 (NCT03037385). They are both potent KDR/VEGF2-sparing *RET* inhibitors with preclinical specificity for *RET* and demonstrated to be active also against *RET*-resistant mutants. The results of these studies are very much awaited since a better and definitive cure of advanced MTC is still an unmet need.

Treatment of Iatrogenic Hypothyroidism

Hormone replacement therapy with L-thyroxine (LT4) should be started immediately after thyroidectomy. At variance with PTC and FTC, MTC is not dependent on TSH, and there is no need to treat patients with LT4 suppressive therapy: the daily dose should be tailored by measuring serum FT3, FT4, and TSH aiming to keep their values within the normal range.

Treatment of Symptoms in Advanced MTC

Diarrhea and Flushing

Diarrhea is the most frequent symptom in patients affected by advanced MTC and frequently associated with flushing. It is likely due to the peptides, some or all, produced by the tumor cells, and the higher the serum Ct, the higher the probability of having such symptoms. Sometimes the QoL of patients is severely affected because of the high number of bowel frequencies, with up to 15–20 discharges per day. Loperamide hydrochloride is the first-choice drug. In severely affected patients, it should be taken daily. As an alternative to loperamide, the diphenoxylate-atropine can be used. Diosmectite can be added to the previous drugs if they are unable to control the diarrhea. Long acting SMS can be tried in very resistant cases. Hydration

by drinking at least 2 l of water should be always suggested. Incurable diarrhea can motivate the initiation of a systemic therapy with TKI, especially with vandetanib (Table 5), even if there is no evidence of progression according to RECIST.

Flushing syndrome is rarer than diarrhea and less devastating. When present, histamine receptor inhibitors may be employed for symptom relief.

Hypercortisolism Due to the Ectopic ACTH Syndrome

As for all types of ectopic ACTH-induced hypercortisolism, the treatment options consist of tumor management, SMS analogues, adrenocortical steroidogenesis inhibitors (e.g., ketoconazole), and bilateral adrenalectomy (Deldycke et al. 2017). However, vandetanib has been demonstrated to be very effective in the clinical and biochemical control of the ectopic Cushing's syndrome related to MTC. Today this secondary hypercortisolism represents an indication for starting vandetanib therapy (Nella et al. 2014; Pitoia et al. 2015; Paepegaey et al. 2017).

Treatment of the Other Endocrine Neoplasias in MEN 2 Syndromes

MEN 2 syndromes, both 2A and 2B, are characterized by the association of MTC with PHEO and/or iperPTH due to either multiple PTHAd or hyperplasia (Table 1). Both of them require specific treatments independent of the MTC treatment.

Pheochromocytoma

Uni- or bilateral adrenalectomy must be performed before total thyroidectomy, when a PHEO is documented simultaneously with the MTC. In fact, the risk of a life-threatening hypertensive crisis during the induction of anesthesia for the neck surgical treatment is very high, and the PHEO must be removed first. For the same reason, a preoperative screening for the presence of a PHEO should be carried out in all patients with a diagnosis of MTC, since the patient may be an index case of a familial form, presented as apparently sporadic (Romei et al. 2011). PHEO is usually bilateral but very often metachronous. A 10-year interval is the mean period between appearance of the first and the contralateral adrenal mass. Different approaches to the management of adrenal gland disease have been suggested when only one gland is involved at the time of the diagnosis. In principle, bilateral adrenalectomy eliminates the need for a second intervention later in life but implies a risk associated with the corticosteroid deficiency that usually does not occur when only one gland is removed. After introduction of the laparoscopic surgical approach, the preferred strategy is to remove only the affected adrenal gland and monitor the other adrenal gland morphology and function periodically. Whatever the final decision, all patients submitted to adrenalectomy should be treated preoperatively with pharmacologic A- and B-adrenergic antagonists (van der Zee and de Boer 2014).

Multiple Adenomatosis or Hyperplasia of the Parathyroids

In patients with hereditary forms of MTC, and documented clinical primary hyperparathyroidism, grossly enlarged parathyroid glands should be resected during the

first operation. As recommended by the American Association of Endocrine Surgeons, intraoperative serum PTH measurement should be performed to ensure the precise and total removal of the affected gland(s) (Wilhelm et al. 2016). This procedure is of practical importance, especially when the macroscopic appearance of the removed parathyroid is not indicative of adenoma, suggesting the presence of multiple adenomatosis or diffuse hyperplasia (Libansky et al. 2017). In some centers, normal or hyperplastic parathyroid glands of patients with hereditary forms are always removed, even in the presence of normal serum PTH levels. They are appropriately marked, for making their localization easier whenever it might be necessary, and totally or partially implanted in a muscle (Niederle et al. 1982). It is worth noting that an aggressive management of normal parathyroid glands is associated with a higher incidence of hypoparathyroidism. In this context, a greater concern is represented by young *RET* gene carriers who if rendered hypoparathyroid would be exposed to the need of calcium and vitamin D supplementation for the rest of their life. The genotype-phenotype correlation among the numerous *RET* mutations and the probability of developing iperPTH is rather well known (Frank-Raue and Raue 2015) (Table 2). While patients with *RET* mutations at codon 634 have a high probability (up to 30%) to develop parathyroid disease, patients with other *RET* mutations that have never been described to be associated with iperPTH will probably never develop such disease. Surgeons must be aware of this correlation when they are planning the surgical treatment.

Conclusions

Medullary thyroid cancer is a very rare cancer with a relatively poor prognosis, especially if it is diagnosed too late and the disease is already extrathyroidal. In 25% of cases, it is inherited as an autosomal dominant trait disease, and children can be affected. Genetic screening is recommended in this latter form. The initial therapy is complete removal of the thyroid gland accompanied by at least central neck lymph node dissection, except in cases of prophylactic thyroidectomy. Patients must be followed up over the years due to the possibility of recurrence, especially when serum Ct is still detectable after surgery. Several local and systemic therapy modalities are available for metastatic lesions. The management of MTC patients should be performed in referral centers and by a multidisciplinary team who needs to include an expert endocrinologist, especially when the disease involves other endocrine glands as seen in the familial forms.

References

Abrahamsen B, Eiken P, et al. Subtrochanteric and diaphyseal femur fractures in patients treated with alendronate: a register-based national cohort study. J Bone Miner Res. 2009;24(6): 1095–102.

Acar T, Ozbek SS, et al. Incidentally discovered thyroid nodules: frequency in an adult population during Doppler ultrasonographic evaluation of cervical vessels. Endocrine. 2014;45(1):73–8.
Alam MJ. Chronic refractory diarrhoea: a manifestation of endocrine disorders. Dig Dis. 1994; 12(1):46–61.
Alevizaki M, Saltiki K. Primary hyperparathyroidism in MEN2 syndromes. Recent Results Cancer Res. 2015;204:179–86.
Barakat MT, Meeran K, et al. Neuroendocrine tumours. Endocr Relat Cancer. 2004;11(1):1–18.
Baudin E, Lumbroso J, et al. Comparison of octreotide scintigraphy and conventional imaging in medullary thyroid carcinoma. J Nucl Med. 1996;37(6):912–6.
Baudin E, Bidart JM, et al. Impact of chromogranin A measurement in the work-up of neuroendocrine tumors. Ann Oncol. 2001;12(Suppl 2):S79–82.
Bible KC, Suman VJ, et al. A multicenter phase 2 trial of pazopanib in metastatic and progressive medullary thyroid carcinoma: MC057H. J Clin Endocrinol Metab. 2014;99(5):1687–93.
Biscolla RP, Ugolini C, et al. Medullary and papillary tumors are frequently associated in the same thyroid gland without evidence of reciprocal influence in their biologic behavior. Thyroid. 2004;14(11):946–52.
Bodei L, Handkiewicz-Junak D, et al. Receptor radionuclide therapy with 90Y-DOTATOC in patients with medullary thyroid carcinomas. Cancer Biother Radiopharm. 2004;19(1):65–71.
Boi F, Maurelli I, et al. Calcitonin measurement in wash-out fluid from fine needle aspiration of neck masses in patients with primary and metastatic medullary thyroid carcinoma. J Clin Endocrinol Metab. 2007;92(6):2115–8.
Brauckhoff M, Gimm O, et al. Multiple endocrine neoplasia 2B syndrome due to codon 918 mutation: clinical manifestation and course in early and late onset disease. World J Surg. 2004; 28(12):1305–11.
Budiawan H, Salavati A, et al. Peptide receptor radionuclide therapy of treatment-refractory metastatic thyroid cancer using (90)Yttrium and (177)Lutetium labeled somatostatin analogs: toxicity, response and survival analysis. Am J Nucl Med Mol Imaging. 2013;4(1):39–52.
Busnardo B, Girelli ME, et al. Nonparallel patterns of calcitonin and carcinoembryonic antigen levels in the follow-up of medullary thyroid carcinoma. Cancer. 1984;53(2):278–85.
Ceccherini I, Romei C, et al. Identification of the Cys634→Tyr mutation of the RET proto-oncogene in a pedigree with multiple endocrine neoplasia type 2A and localized cutaneous lichen amyloidosis. J Endocrinol Investig. 1994;17(3):201–4.
Chen H, Roberts JR, et al. Effective long-term palliation of symptomatic, incurable metastatic medullary thyroid cancer by operative resection. Ann Surg. 1998;227(6):887–95.
Cheung K, Roman SA, et al. Calcitonin measurement in the evaluation of thyroid nodules in the United States: a cost-effectiveness and decision analysis. J Clin Endocrinol Metab. 2008;93:2173–80.
Chi MS, Yang KL, et al. Comparing the effectiveness of combined external beam radiation and hyperthermia versus external beam radiation alone in treating patients with painful bony metastases: a phase 3 prospective, randomized, controlled trial. Int J Radiat Oncol Biol Phys. 2018;100(1):78–87.
Ciampi R, Mian C, et al. Evidence of a low prevalence of RAS mutations in a large medullary thyroid cancer series. Thyroid. 2013;23(1):50–7.
Ciampi R, Romei C, et al. Classical point mutations of RET, BRAF and RAS oncogenes are not shared in papillary and medullary thyroid cancer occurring simultaneously in the same gland. J Endocrinol Investig. 2017;40(1):55–62.
Colombo C, Minna E, et al. The modifier role of RET-G691S polymorphism in hereditary medullary thyroid carcinoma: functional characterization and expression/penetrance studies. Orphanet J Rare Dis. 2015;10:25.
Cosci B, Vivaldi A, et al. In silico and in vitro analysis of rare germline allelic variants of RET oncogene associated with medullary thyroid cancer. Endocr Relat Cancer. 2011;18(5):603–12.
Costante G, Meringolo D, et al. Predictive value of serum calcitonin levels for preoperative diagnosis of medullary thyroid carcinoma in a cohort of 5817 consecutive patients with thyroid nodules. J Clin Endocrinol Metab. 2007;92(2):450–5.

Cunliffe WJ, Hudgson P, et al. A calcitonin-secreting medullary thyroid carcinoma associated with mucosal neuromas, marfanoid features, myopathy and pigmentation. Am J Med. 1970;48(1): 120–6.

Deldycke A, Haenebalcke C, et al. Paraneoplastic Cushing syndrome, case-series and review of the literature. Acta Clin Belg. 2017; 1–7. http://www.tandfonline.com/loi/yacb20.

Donis-Keller H, Dou S, et al. Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. Hum Mol Genet. 1993;2(7):851–6.

Eisele RM. Advances in local ablation of malignant liver lesions. World J Gastroenterol. 2016; 22(15):3885–91.

Elisei R. Routine serum calcitonin measurement in the evaluation of thyroid nodules. Best Pract Res Clin Endocrinol Metab. 2008;22(6):941–53.

Elisei R, Bottici V, et al. Impact of routine measurement of serum calcitonin on the diagnosis and outcome of medullary thyroid cancer: experience in 10,864 patients with nodular thyroid disorders. J Clin Endocrinol Metab. 2004a;89(1):163–8.

Elisei R, Cosci B, et al. RET exon 11 (G691S) polymorphism is significantly more frequent in sporadic medullary thyroid carcinoma than in the general population. J Clin Endocrinol Metab. 2004b;89(7):3579–84.

Elisei R, Romei C, et al. RET genetic screening in patients with medullary thyroid cancer and their relatives: experience with 807 individuals at one center. J Clin Endocrinol Metab. 2007;92(12): 4725–9.

Elisei R, Cosci B, et al. Prognostic significance of somatic RET oncogene mutations in sporadic medullary thyroid cancer: a 10-year follow-up study. J Clin Endocrinol Metab. 2008;93(3): 682–7.

Elisei R, Romei C, et al. The timing of total thyroidectomy in RET gene mutation carriers could be personalized and safely planned on the basis of serum calcitonin: 18 years experience at one single center. J Clin Endocrinol Metab. 2012;97(2):426–35.

Elisei R, Alevizaki M, et al. 2012 European thyroid association guidelines for genetic testing and its clinical consequences in medullary thyroid cancer. Eur Thyroid J. 2013a;1(4):216–31.

Elisei R, Schlumberger MJ, et al. Cabozantinib in progressive medullary thyroid cancer. J Clin Oncol. 2013b;31(29):3639–46.

Emmertsen KK, Nielsen HE, et al. Pentagastrin, calcium and whisky stimulated serum calcitonin in medullary carcinoma of the thyroid. Acta Radiol Oncol. 1980;19(2):85–9.

Eng C, Smith DP, et al. Point mutation within the tyrosine kinase domain of the RET proto-oncogene in multiple endocrine neoplasia type 2B and related sporadic tumours. Hum Mol Genet. 1994;3(2):237–41.

Eng C, Clayton D, et al. The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. JAMA. 1996;276(19):1575–9.

Erdogan MF, Gulec B, et al. Multiple endocrine neoplasia 2B presenting with Pseudo-Hirschsprung's disease. J Natl Med Assoc. 2006;98(5):783–6.

Essig Jr GF, Porter K, et al. Fine needle aspiration and medullary thyroid carcinoma: the risk of inadequate preoperative evaluation and initial surgery when relying upon FNAB cytology alone. Endocr Pract. 2013;19(6):920–7.

Essig Jr GF, Porter K, et al. Multifocality in sporadic medullary thyroid carcinoma: an international multicenter study. Thyroid. 2016;26(11):1563–72.

Farndon JR, Leight GS, et al. Familial medullary thyroid carcinoma without associated endocrinopathies: a distinct clinical entity. Br J Surg. 1986;73(4):278–81.

Forrest CH, Frost FA, et al. Medullary carcinoma of the thyroid: accuracy of diagnosis of fine-needle aspiration cytology. Cancer. 1998;84(5):295–302.

Fox E, Widemann BC, et al. Vandetanib in children and adolescents with multiple endocrine neoplasia type 2B associated medullary thyroid carcinoma. Clin Cancer Res. 2013;19(15): 4239–48.

Franc S, Niccoli-Sire P, et al. Complete surgical lymph node resection does not prevent authentic recurrences of medullary thyroid carcinoma. Clin Endocrinol. 2001;55(3):403–9.

Frank-Raue K, Raue F. Hereditary medullary thyroid cancer genotype-phenotype correlation. Recent Results Cancer Res. 2015;204:139–56.
Fugazzola L, Pinchera A, et al. Disappearance rate of serum calcitonin after total thyroidectomy for medullary thyroid carcinoma. Int J Biol Markers. 1994;9(1):21–4.
Fugazzola L. Stimulated calcitonin cut-offs by different tests. Eur Thyroid J. 2013;2(1):49–56.
Gawlik T, d'Amico A, et al. The prognostic value of tumor markers doubling times in medullary thyroid carcinoma – preliminary report. Thyroid Res. 2010;3(1):10.
Gharib H, Papini E, et al. American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi medical guidelines for clinical practice for the diagnosis and management of thyroid nodules – 2016 update. Endocr Pract. 2016;22(5):622–39.
Gimm O, Ukkat J, et al. Determinative factors of biochemical cure after primary and reoperative surgery for sporadic medullary thyroid carcinoma. World J Surg. 1998;22(6):562–7. discussion 567–568.
Giovanella L, Crippa S, et al. Serum calcitonin-negative medullary thyroid carcinoma: role of CgA and CEA as complementary markers. Int J Biol Markers. 2008;23(2):129–31.
Giraudet AL, Taieb D. PET imaging for thyroid cancers: current status and future directions. Ann Endocrinol (Paris). 2017;78(1):38–42.
Giraudet AL, Vanel D, et al. Imaging medullary thyroid carcinoma with persistent elevated calcitonin levels. J Clin Endocrinol Metab. 2007;92(11):4185–90.
Grozinsky-Glasberg S, Bloom AI, et al. The role of hepatic trans-arterial chemoembolization in metastatic medullary thyroid carcinoma: a specialist center experience and review of the literature. Eur J Endocrinol. 2017;176(4):461–8.
Hahm JR, Lee MS, et al. Routine measurement of serum calcitonin is useful for early detection of medullary thyroid carcinoma in patients with nodular thyroid diseases. Thyroid. 2001;11(1):73–80.
Hannah-Shmouni F, Stratakis CA, et al. Flushing in (neuro)endocrinology. Rev Endocr Metab Disord. 2016;17(3):373–80.
Hegedus L, Bonnema SJ, et al. Management of simple nodular goiter: current status and future perspectives. Endocr Rev. 2003;24(1):102–32.
Hegedus L. Clinical practice. The thyroid nodule. N Engl J Med. 2004;351(17):1764–71.
Heilmann AM, Subbiah V, et al. Comprehensive genomic profiling of clinically advanced medullary thyroid carcinoma. Oncology. 2016;90(6):339–46.
Jackson CE, Norum RA, et al. Clinical value of calcitonin and carcinoembryonic antigen doubling times in medullary thyroid carcinoma. Henry Ford Hosp Med J. 1987;35(2–3):120–1.
Jaffe BM. Prostaglandins and serotonin: nonpeptide diarrheogenic hormones. World J Surg. 1979;3(5):565–78.
Johansson E, Andersson L, et al. Revising the embryonic origin of thyroid C cells in mice and humans. Development. 2015;142(20):3519–28.
Kaltsas G, Rockall A, et al. Recent advances in radiological and radionuclide imaging and therapy of neuroendocrine tumours. Eur J Endocrinol. 2004;151(1):15–27.
Kameda Y, Nishimaki T, et al. Expression of the epithelial marker E-cadherin by thyroid C cells and their precursors during murine development. J Histochem Cytochem. 2007;55(10):1075–88.
Kaserer K, Scheuba C, et al. C-cell hyperplasia and medullary thyroid carcinoma in patients routinely screened for serum calcitonin. Am J Surg Pathol. 1998;22(6):722–8.
Keiser HR, Beaven MA, et al. Sipple's syndrome: medullary thyroid carcinoma, pheochromocytoma, and parathyroid disease. Studies in a large family. NIH conference. Ann Intern Med. 1973;78(4):561–79.
Khosla S, Burr D, et al. Bisphosphonate-associated osteonecrosis of the jaw: report of a task force of the American Society for Bone and Mineral Research. J Bone Miner Res. 2007;22(10):1479–91.
King J, Quinn R, et al. Radioembolization with selective internal radiation microspheres for neuroendocrine liver metastases. Cancer. 2008;113(5):921–9.
Kodama T, Fujino M, et al. Identification of carcinoembryonic antigen in the C-cell of the normal thyroid. Cancer. 1980;45(1):98–101.

Kraeber-Bodere F, Faivre-Chauvet A, et al. Pharmacokinetics and dosimetry studies for optimization of anti-carcinoembryonic antigen x anti-hapten bispecific antibody-mediated pretargeting of Iodine-131-labeled hapten in a phase I radioimmunotherapy trial. Clin Cancer Res. 2003; 9(10 Pt 2):3973S–81S.

Kraft IL, Akshintala S, et al. Outcomes of children and adolescents with advanced hereditary medullary thyroid carcinoma treated with vandetanib. Clin Cancer Res. 2018;24:753–765.

Lam ET, Ringel MD, et al. Phase II clinical trial of sorafenib in metastatic medullary thyroid cancer. J Clin Oncol. 2010;28(14):2323–30.

Lebeault M, Pinson S, et al. Nationwide French study of RET variants detected from 2003 to 2013 suggests a possible influence of polymorphisms as modifiers. Thyroid. 2017;27(12):1511–22.

Lee S, Shin JH, et al. Medullary thyroid carcinoma: comparison with papillary thyroid carcinoma and application of current sonographic criteria. AJR Am J Roentgenol. 2010;194(4):1090–4.

Lencioni R, Crocetti L, et al. Response to radiofrequency ablation of pulmonary tumours: a prospective, intention-to-treat, multicentre clinical trial (the RAPTURE study). Lancet Oncol. 2008;9(7):621–8.

Libansky P, Adamek S, et al. Measurement of intact parathormone during operation for primary hyperparathyroidism. Bratisl Lek Listy. 2017;118(5):255–7.

LiVolsi VA. C cell hyperplasia/neoplasia. J Clin Endocrinol Metab. 1997;82(1):39–41.

Locati LD, Licitra L, et al. Treatment of advanced thyroid cancer with axitinib: phase 2 study with pharmacokinetic/pharmacodynamic and quality-of-life assessments. Cancer. 2014;120(17): 2694–703.

Lupoli GA, Fonderico F, et al. The role of somatostatin analogs in the management of medullary thyroid carcinoma. J Endocrinol Investig. 2003;26(8 Suppl):72–4.

Machens A, Dralle H. Biomarker-based risk stratification for previously untreated medullary thyroid cancer. J Clin Endocrinol Metab. 2010;95(6):2655–63.

Machens A, Lorenz K, et al. Utility of serum procalcitonin for screening and risk stratification of medullary thyroid cancer. J Clin Endocrinol Metab. 2014;99(8):2986–94.

Machens A, Dralle H. Surgical treatment of medullary thyroid cancer. Recent Results Cancer Res. 2015;204:187–205.

Maiza JC, Grunenwald S, et al. Use of 131 I-MIBG therapy in MIBG-positive metastatic medullary thyroid carcinoma. Thyroid. 2012;22(6):654–5.

Mathiesen JS, Habra MA, et al. Risk profile of the RET A883F germline mutation: an international collaborative study. J Clin Endocrinol Metab. 2017;102(6):2069–74.

Matias-Guiu X. Mixed medullary and follicular carcinoma of the thyroid. On the search for its histogenesis. Am J Pathol. 1999;155(5):1413–8.

Matrone A, Valerio L, et al. Protein kinase inhibitors for the treatment of advanced and progressive radiorefractory thyroid tumors: from the clinical trials to the real life. Best Pract Res Clin Endocrinol Metab. 2017;31(3):319–34.

Meijer JA, le Cessie S, et al. Calcitonin and carcinoembryonic antigen doubling times as prognostic factors in medullary thyroid carcinoma: a structured meta-analysis. Clin Endocrinol. 2010;72(4): 534–42.

Melvin KE, Tashjian Jr AH. The syndrome of excessive thyrocalcitonin produced by medullary carcinoma of the thyroid. Proc Natl Acad Sci U S A. 1968;59(4):1216–22.

Menko FH, van der Luijt RB, et al. Atypical MEN type 2B associated with two germline RET mutations on the same allele not involving codon 918. J Clin Endocrinol Metab. 2002;87(1): 393–7.

Mian C, Pennelli G, et al. Combined RET and Ki-67 assessment in sporadic medullary thyroid carcinoma: a useful tool for patient risk stratification. Eur J Endocrinol. 2011;164(6):971–6.

Miccoli P, Minuto MN, et al. Clinically unpredictable prognostic factors in the outcome of medullary thyroid cancer. Endocr Relat Cancer. 2007;14(4):1099–105.

Moley JF, DeBenedetti MK. Patterns of nodal metastases in palpable medullary thyroid carcinoma: recommendations for extent of node dissection. Ann Surg. 1999;229(6):880–7. discussion 887–888.

Moley JF, Debenedetti MK, et al. Surgical management of patients with persistent or recurrent medullary thyroid cancer. J Intern Med. 1998;243(6):521–6.
Moura MM, Cavaco BM, et al. Correlation of RET somatic mutations with clinicopathological features in sporadic medullary thyroid carcinomas. Br J Cancer. 2009;100(11):1777–83.
Mucha L, Leidig-Bruckner G, et al. Phaeochromocytoma in multiple endocrine neoplasia type 2: RET codon-specific penetrance and changes in management during the last four decades. Clin Endocrinol. 2017;87(4):320–6.
Mulligan LM, Kwok JB, et al. Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature. 1993;363(6428):458–60.
Nashed C, Sakpal SV, et al. Medullary thyroid carcinoma metastatic to skin. J Cutan Pathol. 2010;37(12):1237–40.
Nella AA, Lodish MB, et al. Vandetanib successfully controls medullary thyroid cancer-related Cushing syndrome in an adolescent patient. J Clin Endocrinol Metab. 2014;99(9):3055–9.
Niccoli P, Wion-Barbot N, et al. Interest of routine measurement of serum calcitonin: study in a large series of thyroidectomized patients. The French Medullary Study Group. J Clin Endocrinol Metab. 1997;82(2):338–41.
Niederle B, Roka R, et al. The transplantation of parathyroid tissue in man: development, indications, technique, and results. Endocr Rev. 1982;3(3):245–79.
Nieuwenhuijzen Kruseman AC, Bussemaker JK, et al. Radioiodine in the treatment of hereditary medullary carcinoma of the thyroid. J Clin Endocrinol Metab. 1984;59(3):491–4.
Nikiforov Y, Biddinger P, Thompson LDR, editors. Diagnostic pathology and molecular genetics of the thyroid: a comprehensive guide for practicing thyroid pathology. In: Wolters Kluwer/ Lippincott Williams & Wilkins (eds.), 2nd ed. 2012. Philadelphia PA 19103.
Nilsson M, Williams D. On the origin of cells and derivation of thyroid cancer: C cell story revisited. Eur Thyroid J. 2016;5(2):79–93.
Nocera M, Baudin E, et al. Treatment of advanced medullary thyroid cancer with an alternating combination of doxorubicin-streptozocin and 5 FU-dacarbazine. Groupe d'Etude des Tumeurs a Calcitonine (GETC). Br J Cancer. 2000;83(6):715–8.
Nour-Eldin NA, Exner S, et al. Ablation therapy of non-colorectal cancer lung metastases: retrospective analysis of tumour response post-laser-induced interstitial thermotherapy (LITT), radiofrequency ablation (RFA) and microwave ablation (MWA). Int J Hyperth. 2017;33(7):820–9.
Nusynowitz ML, Pollard E, et al. Treatment of medullary carcinoma of the thyroid with I-131. J Nucl Med. 1982;23(2):143–6.
Orita Y, Sugitani I, et al. Prospective evaluation of zoledronic acid in the treatment of bone metastases from differentiated thyroid carcinoma. Ann Surg Oncol. 2015;22(12):4008–13.
Orlandi F, Caraci P, et al. Treatment of medullary thyroid carcinoma: an update. Endocr Relat Cancer. 2001;8(2):135–47.
Ozgen AG, Hamulu F, et al. Evaluation of routine basal serum calcitonin measurement for early diagnosis of medullary thyroid carcinoma in seven hundred seventy-three patients with nodular goiter. Thyroid. 1999;9(6):579–82.
Pacini F, Basolo F, et al. Medullary thyroid cancer. An immunohistochemical and humoral study using six separate antigens. Am J Clin Pathol. 1991;95(3):300–8.
Pacini F, Fontanelli M, et al. Routine measurement of serum calcitonin in nodular thyroid diseases allows the preoperative diagnosis of unsuspected sporadic medullary thyroid carcinoma. J Clin Endocrinol Metab. 1994;78(4):826–9.
Paepegaey AC, Cochand-Priollet B, et al. Long-term control of hypercortisolism by vandetanib in a case of medullary thyroid carcinoma with a somatic RET mutation. Thyroid. 2017;27(4): 587–90.
Papotti M, Kumar U, et al. Immunohistochemical detection of somatostatin receptor types 1–5 in medullary carcinoma of the thyroid. Clin Endocrinol. 2001;54(5):641–9.
Pelizzo MR, Boschin IM, et al. Natural history, diagnosis, treatment and outcome of medullary thyroid cancer: 37 years experience on 157 patients. Eur J Surg Oncol. 2007;33(4):493–7.

Pellegriti G, Leboulleux S, et al. Long-term outcome of medullary thyroid carcinoma in patients with normal postoperative medical imaging. Br J Cancer. 2003;88(10):1537–42.
Pitoia F, Bueno F, et al. Rapid response of hypercortisolism to vandetanib treatment in a patient with advanced medullary thyroid cancer and ectopic Cushing syndrome. Arch Endocrinol Metab. 2015;59(4):343–6.
Randle RW, Balentine CJ, et al. Trends in the presentation, treatment, and survival of patients with medullary thyroid cancer over the past 30 years. Surgery. 2017;161(1):137–46.
Raue F, Frank-Raue K. Genotype-phenotype relationship in multiple endocrine neoplasia type 2. Implications for clinical management. Hormones (Athens). 2009;8(1):23–8.
Raue F, Dralle H, et al. Long-term survivorship in multiple endocrine neoplasia type 2B diagnosed before and in the new millennium. J Clin Endocrinol Metab. 2018;103(1):235–43.
Ravaud A, de la Fouchardiere C, et al. A multicenter phase II study of sunitinib in patients with locally advanced or metastatic differentiated, anaplastic or medullary thyroid carcinomas: mature data from the THYSU study. Eur J Cancer. 2017;76:110–7.
Rieu M, Lame MC, et al. Prevalence of sporadic medullary thyroid carcinoma: the importance of routine measurement of serum calcitonin in the diagnostic evaluation of thyroid nodules. Clin Endocrinol. 1995;42(5):453–60.
Ringe KI, Panzica M, et al. Thermoablation of bone tumors. RoFo. 2016;188(6):539–50.
Rohmer V, Vidal-Trecan G, et al. Prognostic factors of disease-free survival after thyroidectomy in 170 young patients with a RET germline mutation: a multicenter study of the Groupe Francais d'Etude des Tumeurs Endocrines. J Clin Endocrinol Metab. 2011;96(3):E509–18.
Romei C, Cosci B, et al. RET genetic screening of sporadic medullary thyroid cancer (MTC) allows the preclinical diagnosis of unsuspected gene carriers and the identification of a relevant percentage of hidden familial MTC (FMTC). Clin Endocrinol. 2011;74(2):241–7.
Romei C, Casella F, et al. New insights in the molecular signature of advanced medullary thyroid cancer: evidence of a bad outcome of cases with double RET mutations. J Med Genet 2016a;53:729–734.
Romei C, Ciampi R, et al. A comprehensive overview of the role of the RET proto-oncogene in thyroid carcinoma. Nat Rev Endocrinol. 2016b;12(4):192–202.
Rougier P, Calmettes C, et al. The values of calcitonin and carcinoembryonic antigen in the treatment and management of nonfamilial medullary thyroid carcinoma. Cancer. 1983;51(5):855–62.
Salaun PY, Campion L, et al. Phase II trial of anticarcinoembryonic antigen pretargeted radio-immunotherapy in progressive metastatic medullary thyroid carcinoma: biomarker response and survival improvement. J Nucl Med. 2012;53(8):1185–92.
Santarpia L, El-Naggar AK, et al. Four patients with cutaneous metastases from medullary thyroid cancer. Thyroid. 2008;18(8):901–5.
Schlumberger MJ, Elisei R, et al. Phase II study of safety and efficacy of motesanib in patients with progressive or symptomatic, advanced or metastatic medullary thyroid cancer. J Clin Oncol. 2009;27(23):3794–801.
Schlumberger M, Bastholt L, et al. 2012 European thyroid association guidelines for metastatic medullary thyroid cancer. Eur Thyroid J. 2012a;1(1):5–14.
Schlumberger M, Bastholt L, Dralle H, Jarzab B, Pacini F, Smit JWA, the European Task Force. 2012 European Thyroid Association Guidelines for metastatic medullary thyroid cancer. Eur Thyroid J. 2012b;1:5–14.
Schlumberger M, Jarzab B, et al. A phase II trial of the multitargeted tyrosine kinase inhibitor lenvatinib (E7080) in advanced medullary thyroid cancer. Clin Cancer Res. 2016;22(1):44–53.
Schlumberger M, Elisei R, et al. Overall survival analysis of EXAM, a phase III trial of cabozantinib in patients with radiographically progressive medullary thyroid carcinoma. Ann Oncol. 2017;28(11):2813–9.
Schmid KW. Histopathology of C cells and medullary thyroid carcinoma. Recent Results Cancer Res. 2015;204:41–60.
Schuffenecker I, Ginet N, et al. Prevalence and parental origin of de novo RET mutations in multiple endocrine neoplasia type 2A and familial medullary thyroid carcinoma. Le Groupe d'Etude des Tumeurs a Calcitonine. Am J Hum Genet. 1997;60(1):233–7.

Schwartz DL, Rana V, et al. Postoperative radiotherapy for advanced medullary thyroid cancer – local disease control in the modern era. Head Neck. 2008;30(7):883–8.
Scollo C, Baudin E, et al. Rationale for central and bilateral lymph node dissection in sporadic and hereditary medullary thyroid cancer. J Clin Endocrinol Metab. 2003;88(5):2070–5.
Simbolo M, Mian C, et al. High-throughput mutation profiling improves diagnostic stratification of sporadic medullary thyroid carcinomas. Virchows Arch. 2014;465(1):73–8.
Simoes-Pereira J, Macedo D, et al. Clinical outcomes of a cohort of patients with central nervous system metastases from thyroid cancer. Endocr Connect. 2016;5(6):82–8.
Skoura E. Depicting medullary thyroid cancer recurrence: the past and the future of nuclear medicine imaging. Int J Endocrinol Metab. 2013;11(4):e8156.
Sletten K, Westermark P, et al. Characterization of amyloid fibril proteins from medullary carcinoma of the thyroid. J Exp Med. 1976;143(4):993–8.
Tisell LE, Hansson G, et al. Reoperation in the treatment of asymptomatic metastasizing medullary thyroid carcinoma. Surgery. 1986;99(1):60–6.
Trimboli P, Giovanella L. Serum calcitonin negative medullary thyroid carcinoma: a systematic review of the literature. Clin Chem Lab Med. 2015;53(10):1507–14.
Ukkat J, Gimm O, et al. Single center experience in primary surgery for medullary thyroid carcinoma. World J Surg. 2004;28(12):1271–4.
van der Zee PA, de Boer A. Pheochromocytoma: a review on preoperative treatment with phenoxybenzamine or doxazosin. Neth J Med. 2014;72(4):190–201.
Valle LA, Kloos RT. The prevalence of occult medullary thyroid carcinoma at autopsy. J Clin Endocrinol Metab. 2011;96(1):E109–13.
Verbeek HH, Meijer JA, et al. Fewer cancer reoperations for medullary thyroid cancer after initial surgery according to ATA guidelines. Ann Surg Oncol. 2015;22(4):1207–13.
Vierhapper H, Raber W, et al. Routine measurement of plasma calcitonin in nodular thyroid diseases. J Clin Endocrinol Metab. 1997;82(5):1589–93.
Viola D, Materazzi G, et al. Prophylactic central compartment lymph node dissection in papillary thyroid carcinoma: clinical implications derived from the first prospective randomized controlled single institution study. J Clin Endocrinol Metab. 2015;100(4):1316–24.
Vitale G, Fonderico F, et al. Pamidronate improves the quality of life and induces clinical remission of bone metastases in patients with thyroid cancer. Br J Cancer. 2001;84(12):1586–90.
Weber T, Schilling T, et al. Impact of modified radical neck dissection on biochemical cure in medullary thyroid carcinomas. Surgery. 2001;130(6):1044–9.
Wells Jr SA, Robinson BG, et al. Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. J Clin Oncol. 2012;30(2):134–41.
Wells Jr SA, Pacini F, et al. Multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma: an update. J Clin Endocrinol Metab. 2013;98(8):3149–64.
Wells Jr SA, Asa SL, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. Thyroid. 2015;25(6):567–610.
Wertenbroek MW, Links TP, et al. Radiofrequency ablation of hepatic metastases from thyroid carcinoma. Thyroid. 2008;18(10):1105–10.
Wilhelm SM, Wang TS, et al. The American Association of Endocrine Surgeons guidelines for definitive management of primary hyperparathyroidism. JAMA Surg. 2016;151(10):959–68.
Wong RL, Kazaure HS, et al. Simultaneous medullary and differentiated thyroid cancer: a population-level analysis of an increasingly common entity. Ann Surg Oncol. 2012;19(8):2635–42.
Wyon Y, Frisk J, et al. Postmenopausal women with vasomotor symptoms have increased urinary excretion of calcitonin gene-related peptide. Maturitas. 1998;30(3):289–94.
Xu JY, Murphy Jr WA, et al. Bone metastases and skeletal-related events in medullary thyroid carcinoma. J Clin Endocrinol Metab. 2016;101(12):4871–7.
Zatelli MC, Tagliati F, et al. Somatostatin receptor subtype 1-selective activation reduces cell growth and calcitonin secretion in a human medullary thyroid carcinoma cell line. Biochem Biophys Res Commun. 2002;297(4):828–34.

Anaplastic and Other Forms of Thyroid Carcinoma

21

Leonard Wartofsky

Contents

Abstract

This chapter describes the more relatively rare thyroid malignancies other than the well-differentiated thyroid cancers and medullary thyroid carcinoma that are discussed above in their specific chapters. Included in this section are the separate entities of anaplastic thyroid carcinoma, primary thyroid lymphoma, teratoma of the thyroid, squamous cell carcinoma, mucoepidermoid carcinoma, sarcoma, and the SETTLE (spindle epithelial tumor with thymus-like elements) tumor. Aspects of the epidemiology, clinical presentation, pathology, and treatment including surgery, chemotherapy, and external radiation are discussed. Most of these tumors are quite poorly differentiated, and unless diagnosed and treated aggressively early, the prognosis is bleak, because the therapeutic armamentarium is relatively limited.

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Anaplastic thyroid carcinoma · Primary thyroid lymphoma · Teratoma of the thyroid · Squamous cell carcinoma · Mucoepidermoid carcinoma · Sarcoma · SETTLE tumor · CASTLE tumor · Cytology · Chemotherapy · External radiation

Anaplastic Thyroid Carcinoma

Anaplastic carcinoma of the thyroid is a highly undifferentiated and highly aggressive tumor that constitutes less than 2% of all thyroid cancers and is almost invariably fatal within 1–2 years of diagnosis (Akaishi et al. 2011; Besic and Gazic 2013; Ito et al. 2012; Segerhammar et al. 2012; Swaak-Kragten et al. 2009; Yau et al. 2008). Based on the American Joint Committee on Cancer (AJCC) TNM cancer classification, these tumors are always considered Stage IV and can be subclassified as Stage IV-A when caught early and are still intrathyroidal, Stage IV-B when there is involvement of regional lymph nodes due to extrathyroidal extension, and Stage IV-C when distant metastases are present. An age of onset of <50 is quite unusual, and many patients have an antecedent history of multinodular goiter or even of a well-differentiated thyroid carcinoma. Microscopic pathology will demonstrate residual elements of a more well-differentiated thyroid tumor, as well as giant cells, spindle cells, and intranuclear cytoplasmic inclusions. Like most thyroid tumors, it is more common in women than in men (1.9:1) and typically presents as a rapidly enlarging mass in the neck in an older individual. There may be symptoms of hoarseness, dysphagia, dysphonia, dyspnea, and signs of neck tenderness and superior vena cava syndrome. Hoarseness and dysphonia indicate invasion of the recurrent laryngeal nerve, while dysphagia indicates esophageal involvement which is present in about a third of patients at presentation. Survival statistics are best with Stage IV-A and clearly worst in Stage IV-C patients. Decisions regarding approaches to initial management such as extent of surgery and external radiation therapy demand the use of cross-sectional imaging (CT, MRI) to define the extent of disease, and FDG-PET (fluorodeoxyglucose positron emission tomography) may be useful to detect distant metastases. As these tumor cells lack the sodium iodide symporter (NIS), treatment with radioactive iodine is not feasible.

The most success with treatment has occurred in those patients caught early whose disease was amenable to aggressive surgical management, but unfortunately this is the case in very few patients. The American Thyroid Association (ATA) has recently published guidelines for the management of anaplastic thyroid carcinoma that tend to recommend aggressive surgical management when it may be possible to resect the bulk of the disease in Stage IV-A and IV-B patients (Smallridge et al. 2012). The recommendation is based on experience indicating that survival is best with a greater extent of surgery, but this conclusion is biased by the fact that it is the lower-stage patients who are candidates for surgery (Smallridge and Copland 2010). In one review of 100 cases of anaplastic carcinoma, survival at 1 year was 73% for Stage IV-A, 23% for Stage IV-B, and 7% for Stage IV-C with only 9/100 patients

surviving more than 1 year (Akaishi et al. 2011). Survival was best in those early-stage patients treated with aggressive surgery and both external radiation and chemotherapy. Even more aggressive or so-called super-radical surgery including laryngectomy, pharyngectomy, tracheal-esophageal resection, and mediastinal dissection was shown to potentially improve survival in highly selected Stage IV-B cases (Sugitani et al. 2014). The ATA Guidelines recommend external radiation and chemotherapy for Stage IV-C disease rather than an initial aggressive surgical approach (Smallridge et al. 2012), but some centers have shown slightly better survival with more extensive debulking of tumor (Brignardello et al. 2014).

External beam radiation therapy combined with chemotherapy has been reported with variable but far from encouraging results. In one series of 91 patients, there was a complete response to radiation therapy with local control achieved in 40% of those treated. However, the poor overall survival of 11% at 3 years indicates long-term control is not achieved and patients die of their distant metastases (Junor et al. 1992). Failure of conventional radiotherapy has led to innovations employing novel hyperfractionated and accelerated radiation regimens either alone or in combination with chemotherapy. In a series of 55 patients so treated in whom surgery was possible in 40, there was local control free of recurrence in 60% of the patients, but median survival was still only 3.5 months with 2-year survival of 9% (Tennvall et al. 2002). In a Mayo Clinic series of 134 patients, multimodality therapy was not seen to improve survival, but patients who received radiation had a small but nonsignificant improvement in median survival. The likelihood that hyperfractionated radiation and chemotherapy may produce better outcomes in those patients with small tumors amenable to surgical excision is suggested by the small cohort of the total group like this whose 1-year survival was 23% compared to the 9% overall survival (McIver et al. 2001).

The most common sites for distant metastasis are the lung, mediastinum, liver, and bone. The tumor cells do not trap iodine and hence radioactive iodine scanning or treatment is of no use in these patients. External beam radiation may be palliative for peripheral bone metastases; brain metastases may also be temporarily controlled although elderly patients are known to not tolerate whole-brain radiation well. Given the bleak prognosis with extensive disease, management often turns from attempts at aggressive therapy with external radiation and chemotherapy to that of palliative care. Death is secondary to either extension of locoregional disease to vital structures or to the distant metastases.

The results of an aggressive combined modality therapeutic approach were reported for 26 patients undergoing surgery, adjuvant radiotherapy, and chemotherapy with doxorubicin or paclitaxel for local disease or higher-dose (70 Gy) radiotherapy when surgery was not feasible and either low- or higher-dose (30 vs 50 Gy) palliative radiotherapy for metastatic disease depending on the clinical state of the patients (Nachalon et al. 2015). They observed some survival benefit for both chemotherapy and higher-dose radiotherapy although perhaps statistically significant, the clinical benefit was marginal. Given the lack of success with conventional therapy for anaplastic carcinoma (including surgery, radioiodine, thyroxine-suppressive therapy, conventional chemotherapy, and external radiation), efforts

have turned to identify specific molecular targets for personalized therapy (Smith and Nucera 2015). The molecular mutation pathways that are typically altered in this tumor include $BRAF^{V600E}$, P13K-AKT, and ERK 1/2-MEK 1/2, and they have been the targets in several recent clinical trials, albeit with very limited numbers of patients. One such drug, sorafenib, a multi-tyrosine kinase inhibitor (TKI), has had some success with metastatic differentiated thyroid carcinoma but not with anaplastic cancer (Thomas et al. 2014; Savvides et al. 2013).

In conclusion, outcomes remain quite bleak for this highly lethal tumor, and despite the failure of multimodality therapy reported from the Mayo Clinic (McIver et al. 2001), other centers have observed moderate success with a combined surgical and external radiation approach (Conzo et al. 2014; Lowe et al. 2014; Dumke et al. 2014). Nonetheless, hope for these patients in the future rests upon our greater understanding of the underlying genetic mutations present in the tumor that will allow the development of effective personalized targeted therapies (Smith and Nucera 2015; Hsu et al. 2014).

Squamous Cell Carcinoma of the Thyroid

Squamous cell carcinoma of the thyroid is an extremely rare tumor of squamous epithelium, accounting for less than 0.5% of thyroid malignancies. These primary tumors are distinguished from the more common secondary type of squamous cell cancer of the thyroid that may be metastatic to the gland from a regional extension of a cancer from the head and neck. It is believed that the origin of these tumors may be from squamous cell metaplasia, e.g., from cells in a thyroglossal duct cyst (Syed et al. 2011), and squamous cell carcinoma accounts for about 1% of thyroglossal duct tumors. The cells are characterized by forming intercellular bridges and keratin. The tumor occurs in the later decades of life with a female/male ratio of 1.7/1 (Burman et al. 2016), may be associated with thyroiditis, and is typically as aggressive as anaplastic carcinoma. Pathologically, these tumors are poorly differentiated and should be distinguished from anaplastic carcinoma with squamoid elements.

Surgical resection may be quite difficult and incomplete due to its invasiveness, and outcome is poor with a median survival of only 8.6 months even with surgery and combined chemoradiotherapy (Booya et al. 2006). Clinically, patients present with a rapidly enlarging neck mass, and more advanced tumors will demonstrate regional lymphadenopathy and local invasion into both strap muscles and soft tissues with pain, compressive symptoms of stridor, dysphagia, hoarseness, and dyspnea. Care should be taken to distinguish the tumor from a local head and neck or lung carcinoma metastatic to the thyroid gland. Patients may present with systemic symptoms such as hypercalcemia, weight loss, fever, night sweats, and leukocytosis, possibly mediated by secretion of interleukin-1 and colony-stimulating factor. The utility of FNA cytology is limited, and thyroglobulin immunostaining will be negative except in the surrounding normal thyroid tissue, with positive staining more likely for cytokeratin 19 and negative for cytokeratins 1, 4, 10, 13,

and 20 (Lam et al. 2001a, b). Ultrasonography will usually define the extent of tumor (Chen et al. 2010; Hwang et al. 2009), and soft tissue calcifications may be noted. Cross-sectional imaging (CT and MRI) are more definitive (Kinshuck et al. 2012; Weber et al. 2000), and imaging with FDG-PET tends to be less sensitive (Hwang et al. 2009). Gallium^{67} may concentrate in these tumors and has been employed to monitor recurrence of disease.

In regard to treatment, radioiodine has no role as the tumors do not take up iodine. The mainstay of therapy is largely supportive after thyroidectomy to resect tumor burden and relieve obstruction, along with tracheostomy and gastrostomy followed by external beam radiation. In spite of such aggressive management, most patients are dead within 5–9 months (Booya et al. 2006; Sarda et al. 1988). Nor have chemotherapy results been encouraging (Syed et al. 2011) with attempts at treatment with bleomycin, cisplatin, and doxorubicin. Death is due to local complications of invasive disease or from distant metastases. Longer-term survivors were likely fortunate to have been detected early in their course and to have had surgically resectable disease.

Teratoma

Malignant thyroid teratoma is a rare and aggressive disease. The typical clinical presentation of this disease is a rapidly enlarging neck mass, sometimes associated with dyspnea, stridor, or both (Thompson et al. 2000; Tsang et al. 2003). When teratomas arise in the neck, they may have been derived from pluripotent germ layer from cells of the thyroglossal duct tract and the ultimobranchial body (Chen et al. 1998). A teratoma in the neck is considered to be of thyroid origin if any of the tumor is intrathyroidal or if the thyroid gland is continuous with the tumor (Silbermann and Mendelson 1960).

Definitive diagnosis usually depends upon surgical pathology after resection (Djalilian et al. 2000), as FNA cytology is often indeterminate. Aggressiveness of the tumor relates to the underlying histology with Grade 1 teratomas having only one immature element per low-power field, Grade 2 with $>1-<4$ elements per low-power field, and malignancy (Grade 3) defined as having >4 immature elements with mitoses and atypia (Thompson et al. 2000), often with a predominant component of primitive neuroepithelium (Djalilian et al. 2000; Thompson et al. 2000). The tumors can consist of a multiloculated cystic structure and contain pilosebaceous adnexal structures, cartilage or bone, neural tissue such as neuroblastomal elements, and choroid plexus.

Thyroid teratoma is most frequently seen in women of childbearing age, and the age at presentation correlates with tumor histology, with adults more likely to present with malignant disease (Thompson et al. 2000). A reduced survival time is associated with larger tumor size.

If the 25 cases of teratoma reported in the literature to date are summarized, it appears that the average age was 32.7 years (range 15–68), 20% were male, and 80% (20/25) were female. All patients underwent thyroidectomy with 6 (24%) having

subtotal thyroidectomy and 19 (76%) having total thyroidectomies. Twenty patients (80%) received adjuvant chemotherapy. Six patients (24%) received chemotherapy alone after surgery, while 14 patients (56%) underwent chemotherapy plus radiation. Treatment of malignant thyroid teratoma typically consists of complete surgical excision, followed by adjuvant therapy with chemotherapy, radiation, or both. Various combinations of chemotherapy have been tried, including vincristine, methotrexate, bleomycin, and cisplatin; cyclophosphamide, adriamycin, and cisplatin; actinomycin D, cyclophosphamide, and etoposide; bleomycin, etoposide, and cisplatin; and vincristine, doxorubicin, dactinomycin, and cyclophosphamide. Although some patients appear to be disease-free for as long as 10 years after aggressive chemotherapy (Tsang et al. 2003), other patients die of their disease within months (Buckley et al. 1986).

External radiation therapy does not appear to have an impact on patient survival and is largely palliative (Kim et al. 2007). A total of 15 patients received postoperative radiation therapy, and only 6/15 (40%) were noted to be alive at the last follow-up (range 9–120 months), with the remaining nine patients having an average survival of 8 months (range 2–14 months). Patients receiving maximal therapy including thyroidectomy, chemotherapy, radiation, and neck dissection ($n = 5$) were all alive at the last follow-up (mean 36 months, range 16–120 months). It may be that multiple modality therapy with thyroid surgery and adjuvant chemoradiotherapy has the greatest potential for long-term survival.

Lymphoma

Primary thyroid lymphoma is rare, comprising clearly <5% of thyroid malignancies (Sangalli et al. 2001). Women are more commonly affected than men (2–8:1) (Thieblemont et al. 2002), with the typical patient being a woman aged 60–80 with underlying Hashimoto's thyroiditis. It should be suspected when a patient with a history of Hashimoto's thyroiditis presents with an enlarging neck mass. The WHO categorizes lymphomas by their dominant cell type such as B-cell lymphomas, T-cell lymphomas, natural killer cell lymphomas, and Hodgkin's lymphomas (Jaffe 2009), and most of the tumors are of the non-Hodgkin's type of B-cell origin with the most common subtype (>50%) being diffuse large B-cell lymphoma (DLBCL) and about 10–23% being mucosa-associated lymphoid tissue (MALT) lymphoma (Alzouebi et al. 2012; Graff-Baker et al. 2009; Onal et al. 2011; Thieblemont et al. 2002). Pure MALT lymphomas are more indolent but can transform into DLBCL, and therefore both tumors can co-occur in the same gland in which case the clinical behavior tends to be that of DLBCL (Derringer et al. 2000). Rarer subtypes of thyroid lymphoma include follicular (10%), small lymphocytic (3%), and Hodgkin's lymphoma (2%), along with Burkitt's, T-cell, mantle cell, and lymphoblastic lymphomas each of which accounting for <1% of cases (Graff-Baker et al. 2009).

FNA cytology in DLBCL demonstrates a uniform population of large, abnormal lymphoid cells with the presence of lymphoepithelial lesions and decreased/absent

colloid (Sangalli et al. 2001) with nuclear abnormalities such as segmentation or micronucleoli. FNA cytology of MALT lymphoma is highly cellular but has a more heterogeneous appearance with a prominent population of intermediate-sized lymphoid cells, lymphoepithelial lesions, reactive lymphoid follicles, and large plasma cells (Sangalli et al. 2001). About a third of patients will have a mixed cytology of DLBCL and MALT lymphoma (Derringer et al. 2000). The majority of DLBCLs are B-cell lymphoma (Bcl)-6 positive and approximately half are Bcl-2 positive (Niitsu et al. 2007). Monotypic surface immunoglobulin is often detected by flow cytometry. Seen with MALT lymphomas are the presence of immunoglobulin light chains, Bcl-2, and immunoglobulin-M heavy chain staining in the plasma cell component (Rawal et al. 2007).

Clinically, DLBCL behaves more aggressively than MALT and follicular lymphomas. Patients present with a rapidly growing neck mass in >70% of cases, most commonly in those with DLBCL (Derringer et al. 2000; Nam et al. 2012; Onal et al. 2011; Thieblemont et al. 2002). A third of patients experience compressive symptoms such as dyspnea, dysphagia, stridor, and hoarseness (Alzouebi et al. 2012; Derringer et al. 2000; Onal et al. 2011; Watanabe et al. 2011) with a duration prior to diagnosis of as little as a few days up to 36 months with the shorter duration seen in those with DLBCL (Derringer et al. 2000).

On physical examination, the tumor can be unilateral or bilateral, averages 7 cm (0.5–19.5 cm) (Derringer et al. 2000), and is hard with a smooth surface. Thyroid ultrasound will disclose potentially three types of findings based on internal echoes, borders, and posterior echoes as either nodular, diffuse, or mixed (Ota et al. 2006). Enhanced posterior echoes are present in all three types and help distinguish lymphoma from other types of thyroid lesions. In the nodular type, the goiter is typically unilateral with internal echoes that are hypoechoic, homogeneous, and pseudocystic. Well-defined borders separate lymphomatous from non-lymphomatous tissues. In the diffuse type, the goiter is bilateral, hypoechoic, with indistinct borders between the lymphomatous and non-lymphomatous tissues. The positive predictive value of ultrasound was higher for the nodular type at 64.9% (37/57) and the mixed type at 63.2% (12/19) than for the diffuse type at 33.7% (30/89) (Ota et al. 2006). In a retrospective review of ultrasounds from 13 patients with PTL, 2/13 (15.4%) showed a nodular pattern, 10/13 (76.9%) diffuse, and 1/13 (7.7%) mixed (Nam et al. 2012). FNA may have an accuracy rate of 80–100% that in many cases may be improved if combined with immunophenotyping (Sangalli et al. 2001). Although core needle or surgical biopsies are less often needed today for the diagnosis of PTL, they may have a role in distinguishing thyroiditis from low-grade MALT lymphoma and ensuring that aggressive histologies such as those tumors representing a mixed MALT lymphoma and DLBCL are not missed.

Staging of thyroid lymphoma is based on the Ann Arbor system (Carbone et al. 1971). In Stage I-E, the lymphoma is limited to the thyroid, while Stage II-E indicates lymphoma has extended to regional lymph nodes. Stage III-E disease applies when there are lymph nodes on both sides of the diaphragm, and Stage IV-E is characterized by systemic dissemination. Most patients present with either Stage I-E (30–66%) or II-E (25–66%) disease (Derringer et al. 2000; Graff-Baker

et al. 2009; Onal et al. 2011; Watanabe et al. 2011). Stage III-E and IV-E disease is seen about 2–7% of the time.

Imaging to determine the stage of disease at presentation is important to establish prognosis and potential response to treatment. Computerized tomographic (CT) scans are superior to ultrasound in defining extent of disease, while FDG-PET scanning may be useful to monitor response to therapy (Arabi et al. 2011).

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. 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, largely for alleviation of compressive symptoms and tracheostomy for relief of airway compromise (Klyachkin et al. 1998). In addition to radiation, the mainstay of therapy for disseminated disease or tumors with aggressive histology is chemotherapy, usually of the CHOP regimen (cyclophosphamide, doxorubicin, vincristine, and prednisone). Surgery and RT generally suffice for localized, indolent disease with no improvement in outcome between those patients with disease confined to the neck who received RT both to the neck and mediastinum and those receiving combined modality treatment (CMT) with chemotherapy and RT (Doria et al. 1994).

Patients with low-grade localized MALT lymphomas appear to do very well with RT alone. 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 (Laing et al. 1994). Thyroidectomy plus RT does not appear associated with better outcomes than RT alone, and thyroidectomy may not benefit patients with aggressive histologic subtypes such as DLBCL. Instead, patients with DLBCL as well as those with non-localized indolent subtypes should be treated with both chemotherapy and RT (Miller et al. 1998). Although there are no randomized controlled trials, efficacy of CHOP plus RT has been reported, with somewhat improved results with the addition of rituximab to CHOP compared to CHOP alone (Jonak et al. 2010; Onal et al. 2011).

In a population-based study from the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute database of 1408 patients with thyroid lymphoma over 32 years of follow-up, 5-year disease-specific survival was 86%, 81%, and 64% for Stage I, II, and III/VI disease, respectively (Graff-Baker et al. 2009). 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 non-Hodgkin's lymphomas (NHL). 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 (Derringer et al. 2000; Graff-Baker et al. 2009; Onal et al. 2011).

Miscellaneous Rare Tumors

Mucoepidermoid Carcinoma

Mucoepidermoid carcinoma is a rare thyroid malignancy of uncertain cellular lineage that demonstrates mucinous and squamous differentiation and typically occurs in the salivary glands. It is more common in women than in men (2.9:1) and thought to arise either from heterologous transformation and dedifferentiation of papillary thyroid carcinoma (PTC) or from metaplasia of thyroid follicular epithelium (Prichard et al. 2012; Wenig et al. 1995). Several cases of combined mucoepidermoid carcinoma with a classic PTC have been reported (Monroe et al. 2009; Nath et al. 2014). Although many of the cases appear to have indolent behavior and be of low malignant potential, still about 25% of patients die of the disease, and it has been known to further transform into anaplastic carcinoma with its usual dim prognosis. Advanced age is a poor prognostic sign, as is locoregional metastatic disease. There are less than 50 cases reported in the literature, many of whom have underlying Hashimoto's thyroiditis. There may be a female predominance, and a few cases have had an earlier history of childhood irradiation. Because these tumors may arise from a prior well-differentiated thyroid carcinoma, the presence of background thyroiditis would not be unusual, especially in the sclerosing variant of this tumor (Lopez et al. 2008). FNA cytology is not often diagnostic, and on gross pathology, the tumors are usually solid but sometimes cystic, and mucus may be seen on the cut surface. Histochemically, a sclerosing variant with eosinophilia may be noted (Quiroga-Garza et al. 2015; Baloch et al. 2000), and both types of tumor usually stain negative for TTF-1, calcitonin, and thyroglobulin and will stain positively for cytokeratin (Baloch et al. 2000).

Half of the patients reported already had disease outside of the thyroid in the neck at the time of diagnosis. FDG-PET scanning may be useful to detect metastatic disease (Wissmeyer et al. 2007). No role for adjuvant chemotherapy has been shown, and the tumor does not take up iodine and so radioiodine scanning and/or treatment are not of use. Successful outcome depends upon early diagnosis and appropriate surgery including thyroidectomy and regional dissection of all involved nodes followed by external radiation therapy. Distant metastases may occur but are relatively less common, and remission rates can be relatively high.

Sarcoma

Primary sarcoma of the thyroid is quite rare, with some authorities suggesting that these tumors are variants of anaplastic thyroid carcinoma (Lindahl 1976). However, there are convincing immunohistochemical data and ultrastructural findings that support the specific diagnosis of a variety of subtypes of sarcoma, including Ewing sarcoma (Chan et al. 2013), angiosarcoma or hemangiosarcoma (Del Rio et al. 2007), osteosarcoma (Tong et al. 2008), chondrosarcoma and synovial sarcoma (Jang et al. 2007), Kaposi's sarcoma (Poniecka et al. 2007), histiocytic sarcoma (Hsu

et al. 2008; Munoz et al. 2012; Yu and Yang 2010), and leiomyosarcoma of the thyroid gland (Wang et al. 2008). Nevertheless, secondary metastasis from a sarcoma elsewhere in the patient should be ruled out. Immunohistochemistry may be positive for vimentin and CD34 and CD31 (factor VII-related antigens) and negative for cytokeratin, TTF-1, thyroglobulin, calcitonin, synaptophysin, chromogranin, and S-100 protein (Tong et al. 2008). Histiocytic sarcoma may stain positively for S-100 protein and for macrophage-associated protein, CD45, CD68, and CD163 (Yu and Yang 2010). Patients typically have had a long-standing history of goiter, live in an area of iodine insufficiency, or have a history of prior childhood irradiation. There is at least one case of coincident Hashimoto's thyroiditis (Yu and Yang 2010). Biopsy will reveal spindle cells in most variants and osteoid in the case of osteosarcoma. Most of the cases reported had large tumors and already had locoregional metastases at the time of presentation and went on to die of their disease. Cross-sectional imaging may help identify metastases with the tumors usually hypoechoic on ultrasound and hypodense on CT scanning and with MRI scanning demonstrating heterogeneous signal on T2-weighted images and decreased signal on T1-weighted images. ^{18}F-FDG PET/CT may be useful to localize metastases (Treglia et al. 2015). The tumors do not take up iodine, and so radioiodine scanning and/or treatment is to no avail, and no palliative or effective chemotherapy has been identified. Rather, similar to other poorly differentiated thyroid tumors, the best outcomes occur with early diagnosis, meticulous thyroidectomy, and lymph node resection, followed by external radiation therapy.

SETTLE Tumor

This is an extremely rare tumor primarily occurring in children and adolescents that has been named the SETTLE tumor (Grushka et al. 2009) and is a subtype of the CASTLE (carcinoma showing thymus-like differentiation) tumors (Huang et al. 2013). It is thought to arise from ectopic thymus tissue within the thyroid gland or from remnants of the branchial pouch. Cytologically, there are scant mitotic figures, and thyroid epithelial cells will be mixed with spindle cells having bland nuclear chromatin (Misra et al. 2013). Histologically, the tumors need to be distinguished from spindle cell anaplastic carcinoma, spindle cell medullary thyroid carcinoma, teratoma, or sarcoma. Immunohistochemistry will reveal positive staining for cytokeratin, smooth muscle actin, muscle-specific actin, and vimentin, but the tumors are negative for TTF-1, thyroglobulin, calcitonin, S-100 protein, desmin, chromogranin, synaptophysin, or carcinoembryonic antigen (Misra et al. 2013). The number of cases in the literature is so few, probably less than 40, that it is hard to summarize or generalize the typical presentation, findings, and course of the disease. It has been said to be more common in men with a male/female ratio of 1.8:1.0 (Misra et al. 2013), although this was not the case in the series reported by Folpe (Folpe et al. 2009). After initial subtotal or total thyroidectomy, adjuvant chemotherapy may be attempted but has met with mixed responses (Grushka et al. 2009). While a few cases have been relatively indolent in their behavior, others have been

quite aggressive, presenting with both local spread and distant metastases. There can be late metastasis to lung and lymph nodes (Folpe et al. 2009), and two-thirds of patients will have distant metastases at 5 years of follow-up (Cheuk et al. 2000). Although there is one reported case in which technetium^{99m} was accumulated by the tumor, without a marker for residual or recurrent tumor (like thyroglobulin for differentiated thyroid cancer), patients need to be periodically and rigorously screened for metastases by CT, MRI, or FDG-PET imaging.

Summary

The various malignancies described in this chapter are relatively speaking rare, and can generally be easily distinguished from the more common well-differentiated thyroid carcinoma or medullary thyroid carcinoma by either cytologic or histologic examination, with or without immunohistochemistry. These tumors do not make thyroglobulin, and consequently this serum tumor marker is not available for detection of residual or recurrent disease. Moreover, this family of tumors does not demonstrate NIS, the sodium iodine symporter, and hence radioactive iodine has no role for either scanning or treatment. Some are quite invasive and aggressive, such as anaplastic cancer or squamous cell carcinoma, and success with either external radiation, chemotherapy, or combined therapy is quite limited and mortality rates are high. Even thyroid lymphoma which can be responsive to both radiation and chemotherapy still has a high recurrence rate and poor survival. For virtually all of these tumors, the best successful management has rested upon very early diagnosis and appropriate surgery.

Cross-References

► New (Medical) Treatment for Thyroid Carcinoma

References

Akaishi J, Sugino K, Kitagawa W, et al. Prognostic factors and treatment outcomes of 100 cases of anaplastic thyroid carcinoma. Thyroid. 2011;21:1183–9.

Alzouebi M, Goepel JR, Horsman JM, Hancock BW. Primary thyroid lymphoma: the 40 year experience of a UK lymphoma treatment centre. Int J Oncol. 2012;40(6):2075–80.

Arabi M, Dvorak R, Smith LB, Kujawski L, Gross MD. Fluorodeoxyglucose positron emission tomography in primary thyroid lymphoma with coexisting lymphocytic thyroiditis. Thyroid. 2011;21(10):1153–6.

Baloch ZW, Solomon AC, LiVolsi VA. Primary mucoepidermoid carcinoma and sclerosing mucoepidermoid carcinoma with eosinophilia of the thyroid gland: a report of nine cases. Mod Pathol. 2000;13:802–7.

Besic N, Gazic B. Sites of metastases of anaplastic thyroid carcinoma: autopsy findings in 45 cases from a single institution. Thyroid. 2013;23:709–13.

Booya F, Sebo TJ, Kasperbauer JL, Fatourechi V. Primary squamous cell carcinoma of the thyroid: report of ten cases. Thyroid. 2006;16:89–93.
Brignardello E, Palestini N, Felicetti F, Castiglione A, Piovesan A, Gallo M, et al. Early surgery and survival of patients with anaplastic thyroid carcinoma: analysis of a case series referred to a single institution between 1999 and 2012. Thyroid. 2014;24:1600–6.
Buckley NJ, Burch WM, Leight GS. Malignant thyroid teratoma in the thyroid of an adult. A case report and a review of the literature. Surgery. 1986;100:932–6.
Burman KD, Ringel MD, Shmookler BM. Miscellaneous and unusual tumors of the thyroid gland. In: Wartofsky L, Van Nostrand D, editors. Thyroid cancer: a comprehensive guide to clinical management. 3rd ed. New York: Springer; 2016 (in press).
Carbone PP, Kaplan HS, Musshoff K, Smithers DW, Tubiana M. Report of the committee on Hodgkin's disease staging classification. Cancer Res. 1971;31(11):1860–1.
Chan JM, Bilodeau E, Celin S, Nikiforov Y, Johnson JT. Ewing sarcoma of the thyroid: report of 2 cases and review of the literature. Head Neck. 2013;35:E346–50.
Chen JS, Lai G, Hsueh S. Malignant thyroid teratoma of an adult: a long-term survival after chemotherapy. Am J Clin Oncol. 1998;21:212–4.
Chen CY, Tseng HS, Lee CH, Chan WP. Primary squamous cell carcinoma of the thyroid gland with eggshell calcification: sonographic and computed tomographic findings. J Ultrasound Med. 2010;29:1667–70.
Cheuk W, Jacobson AA, Chan JK. Spindle epithelial tumor with thymus-like differentiation (SETTLE): a distinctive malignant thyroid neoplasm with significant metastatic potential. Mod Pathol. 2000;13:1150–5.
Conzo G, Polisten A, Calo PG, Bononi P, Gambardella C, Mauriello C, Tartaglia E, et al. Efficacy of combined treatment for anaplastic thyroid carcinoma: results of a multiinstitutional retrospective analysis. Int J Surg. 2014;12 Suppl 1:S178–82.
Del Rio P, Cataldo S, Sommaruga L, Corcione L, Guazzi A, Sianesi M. A rare case of thyroid haemangiosarcoma. Chir Ital. 2007;59:747–9.
Derringer GA, Thompson LD, Frommelt RA, Bijwaard KE, Heffess CS, Abbondanzo SL. Malignant lymphoma of the thyroid gland: a clinicopathologic study of 108 cases. Am J Surg Pathol. 2000;24(5):623–39.
Djalilian HR, Bradley L, Maisel RH. Malignant teratoma of the thyroid: review of literature and report of a case. Am J Otolaryngol. 2000;21:112–5.
Doria R, Jekel JF, Cooper DL. Thyroid lymphoma: the case for combined modality therapy. Cancer. 1994;73(1):200–6.
Dumke AK, Pelz T, Vordermark D. Long-term results of radiotherapy in anaplastic thyroid cancer. Radiat Oncol. 2014;9:90.
Folpe AL, Lloyd RV, Bacchi CE, Rosai J. Spindle epithelial tumor with thymus-like differentiation: a morphologic, immunohistochemical, and molecular genetic study of 11 cases. Am J Surg Path. 2009;33:1179–88.
Graff-Baker A, Roman SA, Thomas DC, Udelsman R, Sosa JA. Prognosis of primary thyroid lymphoma: demographic, clinical, and pathologic predictors of survival in 1,408 cases. Surgery. 2009;146(6):1105–15.
Grushka JR, Ryckman J, Mueller C, Roessingh AB, Walton JM, Vil DS, Laberge J-M, Bernard C, Nguyer V-H, Puligandla PS. Spindle epithelial tumor with thymus-like elements of the thyroid: a multi-institutional case series and review of the literature. J Pediatr Surg. 2009;44:944–8.
Hsu KF, Lin YS, Hsieh CB, Yu JC, Duh QY, Sheu LF, Jen YM, Shih ML. Primary malignant fibrous histiocytoma of the thyroid: review of the literature with two new cases. Thyroid. 2008;18:51–5.
Hsu K-T, Yu X-M, Audhya AW, Jaume JC, Lloyd RV, Miyamoto S, Prolla TA, Chen H. Novel approaches in anaplastic thyroid cancer therapy. Oncologist. 2014;19:1148–55.
Huang C, Wang L, Wang Y, Yang X, Li Q. Carcinoma showing thymus-like differentiation of the thyroid (Castle). Pathol Res Pract. 2013;209:662–5.
Hwang HS, Perez DA, Orloff LA. Comparison of positron emission tomography/computed tomography imaging and ultrasound in staging and surveillance of head and neck and thyroid cancer. Laryngoscope. 2009;119:1958–65.

Ito KI, Hanmura T, Murayama K, et al. Multimodality therapeutic outcomes in anaplastic thyroid carcinoma: improved survival in subgroups of patients with localized primary tumors. Head Neck. 2012;34:230237.

Jaffe ES. The 2008 WHO classification of lymphomas: implications for clinical practice and translational research. Hematology Am Soc Hematol Educ Program. 2009;2009:523–31.

Jang KS, Min KW, Jang SH, Plk SS, Tae K, Jang SJ, Park MH. Primary synovial sarcoma of the thyroid gland. J Korean Med Sci. 2007;22(Suppl):S154–8.

Jonak C, Troch M, Mullauer L, Niederle B, Hoffmann M, Raderer M. Rituximab plus dose-reduced cyclophosphamide, mitoxantrone, vincristine, and prednisolone are effective in elderly patients with diffuse large B-cell lymphoma of the thyroid. Thyroid. 2010;20:425–7.

Junor E, Paul J, Reed N. Anaplastic thyroid carcinoma: 91 patients treated by surgery and radiotherapy. Eur J Surg. 1992;18:83–8.

Kim E, Bae TS, Kwon Y, Kim TH, Chung KW, Kim SW, Ro J, Lee ES. Primary malignant teratoma with a primitive neuroectodermal tumor component in thyroid gland: a case report. J Korean Med Sci. 2007;22:568–71.

Kinshuck AJ, Goodyear PW, Lancaster J, Roland NJ, Jackson S, Hanlon R, et al. Accuracy of magnetic resonance imaging in diagnosing thyroid cartilage and thyroid gland invasion by squamous cell carcinoma in laryngectomy patients. J Laryngol Otol. 2012;126:302–6.

Klyachkin ML, Schwartz RW, Cibull M, Munn RK, Regine WF, Kenady DE, et al. Thyroid lymphoma: is there a role for surgery? Am Surg. 1998;64(3):234–8.

Laing RW, Hoskin P, Hudson BV, Hudson GV, Harmer C, Bennett MH, et al. The significance of MALT histology in thyroid lymphoma: a review of patients from the BNLI and royal marsden hospital. Clin Oncol (R Coll Radiol). 1994;6(5):300–4.

Lam KY, Cy L, Liu MC. Primary squamous cell carcinoma of the thyroid gland: an entity with aggressive clinical behaviour and distinctive cytokeratin expression profiles. Histopathology. 2001a;39:279–86.

Lam KY, Lui MC, Lo CY. Cytokeratin expression profiles in thyroid carcinomas. Eur J Surg Oncol. 2001b;27:631–5.

Lindahl F. Sarcoma of the thyroid gland: twenty-two cases in Denmark 1943–1968. Dan Med Bull. 1976;23:103–7.

Lopez JI, Gaafar A, Garmendia M, Velasco V, Ortega FJ, del Cura JL. Sclerosing mucoepidermoid carcinoma of the thyroid gland: cytohistological findings of a case. Hematol Oncol Stem Cell Ther. 2008;1:62–5.

Lowe NM, Loughran S, Slevin NJ, Yap BK. Anaplastic thyroid cancer: the addition of systemic chemotherapy to radiotherapy led to an observed improvement in survival – a single centre experience and review of the literature. Sci World J. 2014;67:674583.

McIver B, Hay ID, Giuffrida DF, et al. Anaplastic thyroid carcinoma: a 50 year experience at a single institution. Surgery. 2001;130:1028–34.

Miller TP, Dahlberg S, Cassady JR, Adelstein DJ, Spier CM, Grogan TM, et al. Chemotherapy alone compared with chemotherapy plus radiotherapy for localized intermediate- and high-grade non-Hodgkin's lymphoma. N Engl J Med. 1998;339(1):21–6.

Misra RK, Mitra S, Yadav R, Bundela A. Spindle epithelial tumor with thymus-like differentiation: a case report and review of literature. Acta Cytol. 2013;57:303–8.

Monroe MM, Sauer DA, Samuels MH, Gross ND. Coexistent conventional mucoepidermoid carcinoma of the thyroid (MECT) and papillary thyroid carcinoma. Arch Otolaryngol Head Neck Surg. 2009;135:720–2.

Munoz J, Sanchez BE, Wang D. Histiocytic sarcoma of the thyroid. Am J Hematol. 2012;87:531.

Nachalon Y, Stem-Shavit S, Bachar G, Shvero J, Limon D, Popovtzer A. Aggressive palliation and survival in anaplastic thyroid carcinoma. JAMA Otolaryngol Head Neck Surg. 2015;141:1128–32.

Nam M, Shin JH, Han BK, Ko EY, Ko ES, Hahn SY, et al. Thyroid lymphoma: correlation of radiologic and pathologic features. J Ultrasound Med. 2012;31(4):589–94.

Nath V, Parks GE, Baliga M, Hartle EO, Geisinger KR, Shenoy V. Mucoepidermoid carcinoma of the thyroid with concomitant papillary carcinoma: comparison of findings on fine-needle aspiration biopsy and histology. Endocr Pathol. 2014;25:427–32.

Niitsu N, Okamoto M, Nakamura N, Nakamine H, Bessho M, Hirano M. Clinicopathologic correlations of stage IE/IIE primary thyroid diffuse large B-cell lymphoma. Ann Oncol. 2007;18(7):1203–8.
Onal C, Li YX, Miller RC, Poortmans P, Constantinou N, Weber DC, et al. Treatment results and prognostic factors in primary thyroid lymphoma patients: a rare cancer network study. Ann Oncol. 2011;22(1):156–64.
Ota H, Ito Y, Matsuzuka F, Kuma S, Fukata S, Morita S, et al. Usefulness of ultrasonography for diagnosis of malignant lymphoma of the thyroid. Thyroid. 2006;16(10):983–7.
Poniecka A, Ghorab Z, Arnold D, Khaled A, Ganjel-Azar P. Kaposi's sarcoma of the thyroid gland in an HIV-negative woman: a case report. Acta Cytol. 2007;51:421–3.
Prichard RS, Lee JC, Gill AJ, Sywak MS, Fingleton L, Robinson BG, Sidhu SB, Delbridge LW. Mucoepidermoid carcinoma of the thyroid: a report of three cases and postulated histogenesis. Thyroid. 2012;22:205–9.
Quiroga-Garza G, Lee JH, El-Naggar A, Black JO, Amrikachi M, Zhai QJ, Ayala AG, Ro JY. Sclerosing mucoepidermoid carcinoma with eosinophilia of the thyroid: more aggressive than previously reported. Human Pathol. 2015;46:725–31.
Rawal A, Finn WG, Schnitzer B, Valdez R. Site-specific morphologic differences in extranodal marginal zone B-cell lymphomas. Arch Pathol Lab Med. 2007;131(11):1673–8.
Sangalli G, Serio G, Zampatti C, Lomuscio G, Colombo L. Fine needle aspiration cytology of primary lymphoma of the thyroid: a report of 17 cases. Cytopathology. 2001;12(4):257–63.
Sarda AK, Bal S, Arunabh, Singh MK, Kapur MM. Squamous cell carcinoma of the thyroid. J Surg Oncol. 1988;39:175–8.
Savvides P, Nagaish G, Lavertu P, et al. Phase II trial of sorafenib in patients with advance anaplastic carcinoma of the thyroid. Thyroid. 2013;23:600–4.
Segerhammar I, Larsson C, Nilsson IL, et al. Anaplastic carcinoma of the thyroid gland: treatment and outcome over 13 years at one institution. J Surg Oncol. 2012;106:981–6.
Silbermann R, Mendelson IR. Teratoma of the neck: report of two cases and review of the literature. Arch Dis Child. 1960;35:159–70.
Smallridge RC, Copland JA. Anaplastic thyroid carcinoma: pathogenesis and emerging therapies. Clin Oncol. 2010;22:486–97.
Smallridge RC, Ain KB, Asa SL, Bible KC, Brierley JD, Burman KD, et al. American Thyroid Association guidelines for management of anaplastic thyroid cancer. Thyroid. 2012;22:1104–39.
Smith N, Nucera C. Personalized therapy in patients with anaplastic thyroid cancer: targeting genetic and epigenetic alterations. J Clin Endocrinol Metab. 2015;100:35–42.
Sugitani I, Hasegawa Y, Sugasawa M, Tori M, Higashiyama T, Miyazaki M, et al. Super-radical surgery for anaplastic thyroid carcinoma: a large cohort study using the anaplastic thyroid carcinoma research consortium of Japan database. Head Neck. 2014;36:328–33.
Swaak-Kragten AT, de Wilt JH, Schmitz PI, et al. Multimodality treatment for anaplastic thyroid carcinoma- treatment outcome in 75 patients. Radiother Oncol. 2009;92:100–4.
Syed MI, Stewart M, Syed S, Dahill S, Adams C, McLellan DR, Clark LJ. Squamous cell carcinoma of the thyroid gland: primary or secondary disease? J Laryngol Otol. 2011;125:3–9.
Tennvall J, Lundell G, Wahlberg P, et al. Anaplastic thyroid carcinoma: three protocols combining doxorubicin, hyperfractionated radiotherapy and surgery. Br J Cancer. 2002;86:1848–53.
Thieblemont C, Mayer A, Dumontet C, Barbier Y, Callet-Bauchu E, Felman P, et al. Primary thyroid lymphoma is a heterogeneous disease. J Clin Endocrinol Metab. 2002;87(1):105–11.
Thomas L, Lai SY, Dong W, Geng L, Dadu R, Regone RM, Cabanillas ME. Sorafenib in metastatic thyroid cancer: a systematic review. Oncologist. 2014;19:251–8.
Thompson DR, Rosai J, Heffess CS. Primary thyroid teratomas. A clinicopathologic study of 30 cases. Cancer. 2000;88:1149–58.
Tong GX, Hamele-Bena D, Liu JC, Horst B, Ramotti F. Fine-needle aspiration biopsy of primary osteosarcoma of the thyroid: report of a case and review of the literature. Diagn Cytopathol. 2008;36:589–94.

Treglia G, Bongiovanni M, Paone G, Ceriani L, Giovanella L. Metastatic undifferentiated spindle cell sarcoma of the thyroid gland evaluated by ^{18}F-FDG PET/CT. Clin Nucl Med. 2015;40: e208–10.

Tsang RW, Brierly JD, Asa SL, Sturgeon JF. Malignant teratoma of the thyroid: aggressive chemoradiation therapy is required after surgery. Thyroid. 2003;13:401–4.

Wang TS, Ocal IT, Oxley K, Sosa JA. Primary leiomyosarcoma of the thyroid gland. Thyroid. 2008;18:425–8.

Watanabe N, Noh JY, Narimatsu H, Takeuchi K, Yamaguchi T, Kameyama K, et al. Clinicopathological features of 171 cases of primary thyroid lymphoma: a long-term study involving 24553 patients with Hashimoto's disease. Br J Haematol. 2011;153(2):236–43.

Weber AL, Randolph G, Aksoy FG. The thyroid and parathyroid glands. CT and MR imaging and correlation with pathology and clinical findings. Radiol Clin N Am. 2000;38:1105–29.

Wenig BM, Adair CF, Heffess CS. Primary mucoepidermoid carcinoma of the thyroid gland: a report of six cases and a review of the literature of follicular epithelial derived tumor. Hum Pathol. 1995;26:1099–108.

Wissmeyer M, Weidner S, Muggli B, Weimann R, Junengling FD, Krause T. FDG uptake in metastatic spreading mucoepidermoid carcinoma of the thyroid. Clin Nucl Med. 2007;32:383–7.

Yau T, Lo CY, Epstein RJ, et al. Treatment outcomes in anaplastic thyroid carcinoma: survival improvement in young patient s with localized disease treated by combination of surgery and radiotherapy. Ann Surg Oncol. 2008;15:2500–5.

Yu L, Yang SJ. A case of primary histiocytic sarcoma arising from the thyroid gland. Pathol Oncol Res. 2010;16:127–32.

New (Medical) Treatment for Thyroid Carcinoma

22

Sebastiano Filetti and Steven I. Sherman

Contents

Abstract

Treatment of locally advanced/metastatic thyroid cancer patients poses several challenges. The disease course can be rapidly progressive or can spontaneously

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remain stable over time. Periodic (3–12 months) imaging assessments are indicated. The tumor burden, location, and the pace of volume growth should be taken into account for treatment decision (Fig. 1). When small disease burden and no symptomatic/threatening lesion are present, the patient should be actively surveilled. In case of single/few threatening lesions, local treatment should be preferred. Large tumor burden and/or rapidly (<12 months) progressing disease are indicators for systemic treatments (Haugen et al. 2016). Approved (Tables 1 and 2) and investigational drugs (Table 3) for the treatment of thyroid cancer are presented in this chapter. Figure 2 shows all the cited kinase inhibitors and their targets in thyroid cancer cells and in endothelial cells.

Keywords
Advanced Thyroid Cancer · Tyrosine Kinase Inhibitors · Antiangiogenic Drugs · MAPK Pathway · Phosphatidylinositol 3-Kinase/AKT Pathway · Immunotherapy

Treatment of locally advanced/metastatic thyroid cancer patients poses several challenges. The disease course can be rapidly progressive or can spontaneously remain stable over time. Periodic (3–12 months) imaging assessments are indicated. The tumor burden, location, and the pace of volume growth should be taken into account for treatment decision (Fig. 1). When small disease burden and no symptomatic/threatening lesion are present, the patient should be actively surveilled. In case of single/few threatening lesions, local treatment should be preferred. Large tumor burden and/or rapidly (<12 months) progressing disease are indicators for systemic treatments (Haugen et al. 2016). Approved (Tables 1 and 2) and investigational drugs (Table 3) for the treatment of thyroid cancer are presented in this chapter. Figure 2 shows all the cited kinase inhibitors and their targets in thyroid cancer cells and in endothelial cells.

Antiangiogenic Therapies

Rationale for the Use of Antiangiogenic Drug Therapy in Thyroid Cancer

Thyroid carcinomas, regardless of whether they originate from follicular or parafollicular cells, are richly vascularized tumors, with microvessel densities exceeding those in the surrounding normal thyroid tissue (Akslen and Livolsi 2000; Durante et al. 2011; Jebreel et al. 2007; Verrienti et al. 2016). Moreover, increased angiogenesis is clearly correlated with more aggressive tumor behavior and metastasis (Dhar et al. 1998; Ishiwata et al. 1998). These findings suggest that, to grow and metastasize,

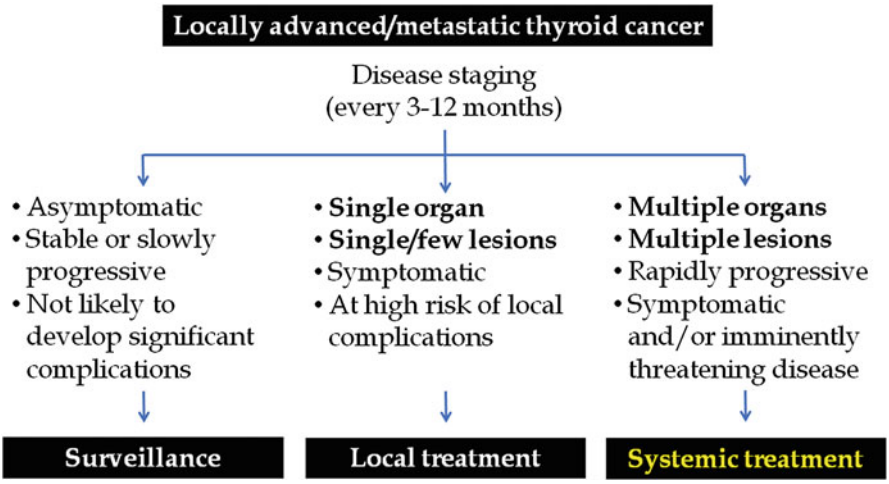

Fig. 1 Management of patients with locally advanced/metastatic thyroid cancer

Table 1 Inhibitory concentrations (IC_{50} [nM]) for the relevant targets of the four drugs approved for advanced thyroid cancer

	DTC		MTC	
Drug targets	Sorafenib[a, b]	Lenvatinib[c, d]	Vandetanib[e, f]	Cabozantinib[g]
VEGFR-1	–	22 nM	–	–
VEGFR-2	15 nM	4 nM	40 nM	0.035 nM
VEGFR-3	20 nM	5.2 nM	110 nM	–
PDGFRalfa	–	51 nM	–	–
PDGFRbeta	57 nM	39 nM	1100 nM	–
BRAF	22 nM	–	–	–
RET	5.9 nM	35 nM	100 nM	5.2 nM
Others (IC_{50})	Raf-1 (6 nM)	FGFR1 (46 nM), KIT (100 nM)	EGFR (500 nM)	MET (1.3 nM), KIT (4.6 nM)

Drug Target References:
[a]Wilhelm et al. (2004)
[b]Carlomagno et al. (2006)
[c]Matsui et al. (2008)
[d]Okamoto et al. (2013)
[e]Wedge et al. (2002)
[f]Carlomagno et al. (2002)
[g]Yakes et al. (2011)

thyroid cancers require an "angiogenic switch" that tips the balance between angiogenic stimulators and inhibitors toward the former (Fallahi et al. 2015).

Neovascularization is controlled by complex molecular mechanisms. The main stimulating pathways are those activated by the vascular endothelial growth factors (VEGFs) and platelet-derived growth factors (PDGFs) expressed on endothelial

Table 2 Summary of efficacy results and treatment-related adverse events occulting in the DECISION, SELECT, ZETA, and EXAM trials

Indication in thyroid cancer	Locally advanced or metastatic RAI-R DTC		Locally advanced or metastatic MTC	
Trial[a]	DECISION	SELECT	ZETA	EXAM
Treatment arm	Sorafenib vs placebo	Lenvatinib[c] vs placebo	Vandetanib[c] vs placebo	Cabozantinib vs placebo
N	207 vs 210	261 vs 131	231 vs 100	219 vs 111
PFS, months	10.8 vs 5.8	18.3 vs 3.6	30.5 vs 19.3	11.2 vs 4.0
(HR, 95% CI; p value)	(0.59, 0.45–076; $p < 0.0001$)	(0.21, 0.14–0.31; $p < 0.001$)	(0.46, 0.31–0.69; $p < 0.001$)	(0.28, 0.19–0.40; $p < 0.001$)
Response rate (%)				
Complete response	0 vs 0	1.5 vs 0	0 vs 0	0 vs 0
Partial response	12.2 vs 0.5	63.2 vs 1.5	45 vs 13	28 vs 0
Stable disease	41.8 vs 33.2	23.0 vs 54.2	NA	48.1 vs 50
Grade 3–4 AEs (%)[b]				
Hypertension	9.7 vs 2.4	41.8 vs 2.3	9 vs 0	8.4 vs 0.9
ECG QT prolonged	0 vs 0	0 vs 0	8 vs 1	0 vs 0
Fatigue or asthenia	5.3 vs 1.4	9.2 vs 2.3	6 vs 1	9.3 vs 2.8
Decreased appetite	2.4 vs 0	5.4 vs 0	4 vs 0	4.7 vs 0.9
Dysgeusia	0 vs 0	0 vs 0	0 vs 0	0.5 vs 0
Nausea	0 vs 0	2.3 vs 0.8	0 vs 0	1.4 vs 9
Diarrhea	5.3 vs 1	8 vs 0	11 vs 2	15.9 vs 1.8
Decreased weight	5.8 vs 1	9.6 vs 0	0 vs 0	4.7 vs 0
Hand-foot skin reaction	20.3 vs 0	3.4 vs 0	0 vs 0	12.6 vs 0
Rash	4.8 vs 0	0.4 vs 0	4 vs 1	0.9 vs 0
Death due to adverse event (%)	5.7 vs 2.8	7.7 vs 4.6	2.1 vs 2	7.9 vs 7.3
Dose interruption due to AEs	18.8 vs 3.8	14.2 vs 2.3	12 vs 3	16 vs 8

RAI-R radioactive iodine refractory, *DTC* differentiated thyroid cancer *MTC* medullary thyroid cancer, *AEs* adverse events *HR* hazard ratio *CI* confidence interval

[a]Crossover to the study drug upon progression was allowed in all trials except EXAM

[b]Adverse events are reported according to National Cancer Institute–Common Terminology Criteria for Adverse Events. The grade 3–4 adverse events reported in the ZETA trials only included those that occurred with an incidence ≥2% on either arm

[c]The efficacy and safety of vandetanib are also being assessed in patients with differentiated thyroid cancer. The phase III, randomized, placebo-controlled, "registration" trial initiated for this purposed (VERIFY, NCT01876784) is expected to be completed in May 2017 (ClinicalTrials.gov, https://clinicaltrials.gov)

Table 3 Investigational drugs currently not approved for thyroid cancer

Drug	Targeted pathways or selective gene	Main targets	Phase of clinical studies	Ongoing trials[a]
Vemurafenib	MAPK	RAF	II	NCT01286753 (completed)
Dabrafenib		RAF	I	NCT01947023
				NCT02465060
Selumetinib		MEK	II	NCT00970359
				NCT00559949
				NCT02393690
Trametinib		MEK	I/II	NCT03085056
				NCT02152995
Everolimus	PI3K/AKT	mTOR	II	NCT02143726
				NCT03139747
Sirolimus temsirolimus		mTOR	II	NCT03099356
RXDX-105	RET	RET	I	–
LOXO-292		RET	I	NCT03157128
BLU-667		RET	I	NCT03037385
LOXO-101 (larotrectinib)	TRK	TRK	II	NCT02576431

[a]https://clinicaltrials.gov/

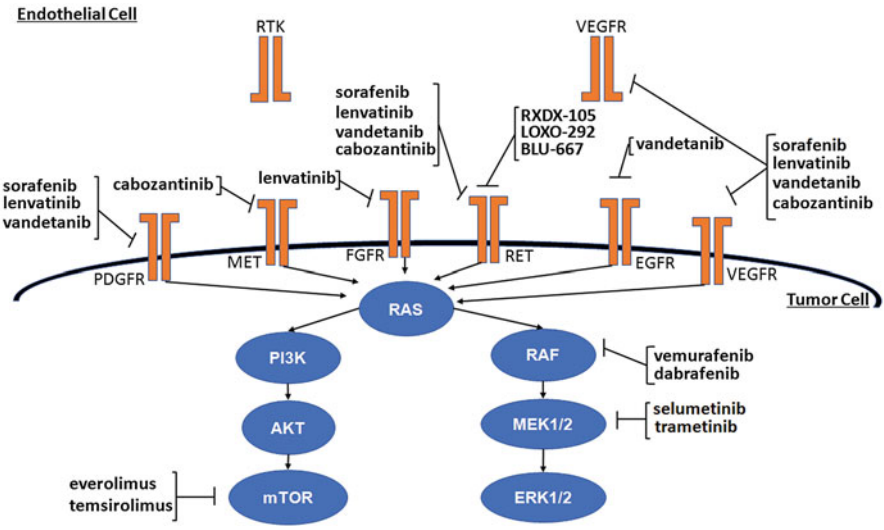

Fig. 2 Kinase inhibitors and their targets in thyroid cancers. Key molecular signaling pathways involved in thyroid cancers are shown, along with clinically relevant kinase inhibitors. *RTK* receptor tyrosine kinase

cells. After binding to its receptors (VEGFR-1, VEGFR-2, and VEGFR-3), VEGF (in particular, VEGF-A) triggers the formation of new blood vessels in tumor tissues (Kerbel 2008). VEGFR-2 is the major mediator of angiogenesis. The role of VEGFR-1 is controversial: it has been described as both a positive and a negative regulator of VEGFR-2 signaling (Olsson et al. 2006). VEGFR-3, which is expressed by lymphatic endothelial cells, promotes tumor lymphangiogenesis and tumor spread through lymphatic vessels (Laakkonen et al. 2007). Platelet-derived growth factors and their receptors (PDGFR-α and PDGFR-β) are crucial for the maturation and stability of the new vascular vessels and for the induction of lymphangiogenesis (Cao 2013). It is important to recall that VEGFRs and PDGFRs are also expressed by the neoplastic cells themselves, where they promote tumor growth and tumor motility through a VEGF- and PDGF-related autocrine loops (Jechlinger et al. 2006; Kerbel 2008).

Cell lines as well as tumor tissues and serum from patients with thyroid cancer of follicular cell origin are characterized by high expression of proangiogenic factors – including VEGF, PDGF, and their receptors – which is strongly correlated with the tumor phenotype (Klein et al. 2001; Lennard et al. 2001; Marotta et al. 2015; Vieira et al. 2005; Yu et al. 2005). VEGF overexpression in patients with papillary thyroid cancer has been associated with a higher risk of metastasis and recurrence and decreased disease-free survival (Marotta et al. 2015). Proangiogenic gene expression patterns have also been confirmed in medullary thyroid cancer (MTC) (Mancikova et al. 2014; Verrienti et al. 2016), where it seems to correlate with a more aggressive phenotype, as suggested by the overexpression of VEGFR-2 in MTC metastases (Rodriguez-Antona et al. 2010). Notably, there is also some evidence supporting correlation between proangiogenic pathway activation and thyroid tumor genotype (Mancikova et al. 2014; Verrienti et al. 2016; Vieira et al. 2005). These features make thyroid cancers an excellent target for drugs that inhibit proangiogenic signaling by preventing phosphorylation of the tyrosine kinase receptors that trigger it. The vast majority of tyrosine kinase inhibitors (TKIs) have multiple targets. In general, those that inhibit the kinases involved in proangiogenic signaling also exert direct anti-proliferative effects mediated by their inhibition of molecules involved in other intracellular pathways (Marotta et al. 2015). The latter effects may contribute to the efficacy of these drugs in the treatment of cancer, particularly those of the thyroid, since the mutations in *RET* and *BRAF* genes known to drive many of these tumors induce neoplastic transformation and progression by activating kinases implicated in the MAP kinase and/or the PI3K/Akt/mTOR signaling cascades (Cancer Genome Atlas Research Network 2014; Marotta et al. 2015).

Antiangiogenic Tyrosine Kinase Inhibitors Currently Approved for Treatment of Advanced Thyroid Cancer

Four orally available VEGFR-targeting TKIs with antiangiogenic properties are currently approved in the United States and the European Union for treatment of advanced thyroid cancer. The targets of these drugs are shown in Table 1. Features of

the phase III trials that supported their approval for this indication are summarized in Table 2 and discussed below.

VEGFR-Directed TKI Therapy for Advanced, RAI-Resistant DTC

Radioactive iodine (RAI) therapy plays a major role in the treatment of DTCs, including advanced stage tumors. However, in approximately two-thirds of patients with distant metastases from DTCs, the tumor exhibits inherent or acquired insensitivity to radioiodine (Durante et al. 2006). The loss of this treatment option negatively impacts the prognosis (Spitzweg et al. 2014) since conventional cytotoxic chemotherapy offers limited efficacy in these cases, as well as considerable toxicity (Sherman 2010). This therapeutic void is currently being filled by VEGFR-targeting TKIs, which are recommended for progressive, symptomatic, RAI-refractory forms of metastatic DTC by both the American Thyroid Association (Haugen et al. 2016) and the National Comprehensive Cancer Network (Tuttle et al. 2014). The importance of angiogenesis in DTC is well-documented (Harris and Bible 2011; Haugen et al. 2016; Ishiwata et al. 1998; Klein et al. 2001; Lennard et al. 2001; Soh and Clark 1996; Soh et al. 2000; Spitzweg et al. 2014; Yu et al. 2005), and numerous TKIs that inhibit this process have been tested in the treatment of advanced DTC. As shown in Table 2, sorafenib and lenvatinib are the only two currently approved for this indication by regulatory authorities in both the United States and the European Union.

Sorafenib

Sorafenib was approved by the US Federal Drug Administration (FDA) in 2013 for the treatment of advanced DTC; European Medicines Agency (EMA) authorization followed shortly thereafter. The decisions were based largely on the results of a randomized, placebo-controlled, phase III trial conducted in 417 patients with locally advanced or metastatic RAI-refractory DTC that had progressed within the past 14 months (DECISION study) (Brose et al. 2014). The median progression-free survival (PFS, the primary end point) was almost twice as long in the sorafenib group (10.8 months versus 5.8 months in the placebo arm, hazard ratio [HR] 0.59, 95% confidence interval [CI] 0.45–0.76; $P < 0.0001$). Moreover, 12.2% of the patients treated with sorafenib had objective responses (all partial). Overall survival was prolonged in the sorafenib cohort, as compared with the cohort receiving placebo, although this difference was not statistically significant (HR 0.80, 95% CI 0.54–1.19; $p = 0.14$). The latter finding is related at least in part to the design of the trial, which allowed placebo-treated patients to cross over to sorafenib treatment when tumor progression occurred.

Despite these encouraging findings, quality of life appeared to be slightly worse in the sorafenib-treated arm (Schlumberger et al. 2013). Adverse events (generally grades 1–2) occurred in 204 of 207 (98.6%) patients receiving double-blind treatment with sorafenib and 183 of 209 (87.6%) of those receiving placebo. Three out of four patients in the sorafenib group developed hand-foot skin reactions (76.3%) although very few of these events led to drug discontinuation. Diarrhea (68.6%), alopecia (67.1%), and rash or desquamation (50.2%) were also common (Brose et al. 2014).

Overall, the group treated with sorafenib had a rate of drug withdrawals caused by adverse effects that was over four times higher than that of the placebo arm and an adverse event-related mortality rate that was almost twice as high (Table 2).

Lenvatinib

Lenvatinib targets VEGFRs 1–3, FGFRs 1–4, PDGFRα, RET, and the stem cell growth factor receptor KIT. The FDA approved lenvatinib for treatment for advanced DTC in 2015, following publication of data from the phase III placebo-controlled trial, SELECT (Schlumberger et al. 2015). As shown in Table 2, the median PFS in the lenvatinib arm of the study exceeded that of the placebo arm by 14.7 months (HR 0.21, 95% CI 0.14–0.31; $p < 0.001$). This was a substantially greater gain relative to placebo than that recorded in other placebo-controlled trials, and the improvement was thought to be partly related to lenvatinib's unique target profile and in particular its effects on FGFRs (St Bernard et al. 2005). The benefit over placebo benefit was observed across all DTC histological types (including poorly differentiated tumors), and it was unrelated to the *BRAF* or *RAS* mutation status of the tumor. Importantly, it was also significant in patients who had already received one TKI treatment. Responses (including four that were complete) were observed in almost two-thirds of the lenvatinib group and 1.5% in the placebo arm ($P < 0.001$). Interestingly, progression of existing bone metastases (a problem that is frequently intractable) was also less common in the lenvatinib group (23.7% vs 59.0% in the placebo arm). The median overall survival was not reached in either group. Most patients in the placebo arm ultimately crossed over to lenvatinib. Overall survival in the study group was appreciably albeit nonsignificantly prolonged (adjusted HR 0.62, 95% CI 0.40-1-00). Subsequent prespecified subgroup analysis demonstrated improved overall survival in patients over 65 years old when treated with lenvatinib compared with placebo (HR 0.53, 95% CI 0.31–0.91) (Brose et al. 2017).

Treatment-related adverse effects were common in the lenvatinib group and included hypertension (67.8% of the patients), diarrhea (in 59.4%), fatigue or asthenia (in 59.0%), decreased appetite (in 50.2%), decreased weight (in 46.4%), and nausea (in 41.0%). Adverse events that led to discontinuations of the study drug occurred in 37 patients (14.2%) vs 3 patients who received placebo (2.3%). Notably, 6 of the 20 deaths that occurred during lenvatinib treatment were classified as drug-related.

VEGFR-Directed TKI Therapy for Advanced MTC

The two TKIs currently approved for the treatment of progressive, locally advanced or metastatic MTC, vandetanib and cabozantinib, are both potent inhibitors of RET kinase, which is constitutively activated in almost all hereditary MTCs and roughly half of those that are sporadic. The RET-suppressing activity of these two drugs was in fact the major reason they were originally proposed for the treatment of MTC. Since then, however, relatively little evidence has emerged to indicate that this activity is essential to their antitumoral effects in MTC (Sherman 2013). Vandetanib and cabozantinib are also strong inhibitors of key angiogenic pathway components (Table 1), and phase III trial data suggest that vandetanib and cabozantinib are also beneficial in MTCs with wild-type RET mutation.

Vandetanib

Vandetanib's major targets are VEGFRs 2 and 3, epidermal growth factor (EGF) receptor, and RET. Its potent inhibition of RET was the original basis for its proposed use in the treatment of MTC. It was approved for the treatment of advanced MTC based on the results of a phase III, multicenter, double-blind, placebo-controlled trial that enrolled 331 adults with metastatic (95%) or locally advanced MTC, 90% of which were sporadic (ZETA study). PFS (the primary end point) was significantly prolonged in the vandetanib-treated patients, as compared with the placebo arm (30.5 vs 19.3 months HR 0.46, 95% CI 0.31–0.69; $p < 0.001$), and this benefit was accompanied by significantly higher objective response, biochemical response, and disease control rates ($p < 0.001$ for all). As in the DECISION and SELECT trials, unmasking and crossover were allowed at progression. For this reason, overall survival was not a primary end point, and no significant change in this parameter was observed at the end of the study (Wells et al. 2012).

The most common grade 3–4 adverse events observed in ZETA were those typical of TKIs in general, and all were significantly more common in the study drug group (Table 2). QTc interval prolongation was observed in 8% of the patients in the vandetanib arm (although none had torsade de pointes) (Wells et al. 2012). In the United States, the FDA has issued boxed warnings on this risk and restricted prescription to providers who have undergone training in Risk Evaluation and Mitigation Strategy (REMS). A recent meta-analysis of phase II/III trial data on FDA-approved TKI treatments (any FDA-approved drug) revealed a significantly increased risk for QTc prolongation only for sunitinib and vandetanib. The risk was independent of the duration of vandetanib treatment, but it did increase with the dose administered (Ghatalia et al. 2015). A phase IV trial is currently being conducted to assess the efficacy of lower-dose therapy (150 mg/day vs 300 mg/day) in MTC patients (AstraZeneca to Compare the Effects of Two Doses of Vandetanib in Patients with Advanced Medullary Thyroid Cancer. (Accessed February 27, 2015: http://www.clinicaltrials.gov/ct2/show/NCT01496313.).

Vandetanib is currently approved only for adult patients with MTC, but it has also been tested in a phase I/II trial that included 16 children/adolescents with locally advanced or metastatic MEN2B-related MTCs. Patients were started on an initial dose of 100 mg/m^2/day (equivalent to the adult dose of 180 mg/day). All 15 with M918T RET germline mutations experienced tumor shrinkage, and objective responses were observed in 7 (44%). The remaining patient, who harbored a RET polymorphism (G691S, S836S), progressed after 2 months of treatment with the drug (Fox et al. 2013). Diarrhea was the most common dose-limiting adverse reaction. There were no cases of dose-limiting prolongation of QTc.

Cabozantinib

Cabozantinib inhibits the VEGF2R, as well as the hepatocyte growth factor receptor (MET) and RET. The efficacy and safety of cabozantinib (140 mg per day) were assessed in 330 patients with metastatic MTC (sporadic or hereditary) associated with radiographically documented progression at entry (Elisei et al. 2013) (EXAM) (Table 2). Therefore, the disease in question was on the whole more aggressive than

that examined in the phase III trial of vandetanib, where enrollment was not limited to patients with progressive disease. The estimated PFS in the cabozantinib arm was almost three times that of the placebo group (11.2 months 4.0 months; HR 0.28, 95% CI 0.19–0.40; $P < 0.001$), and this improvement was unrelated to age, prior TKI exposure, or RET mutation status (hereditary or sporadic). Objective responses – all partial – were seen exclusively in the cabozantinib arm (28%) and were also unrelated to the *RET* mutation status.

Although crossover was not allowed in the EXAM trial, the planned interim analysis (mid-June 2011) revealed no significant difference in overall survival (Elisei et al. 2013). Updated data reported in 2015 also showed an estimated median survival in the cabozantinib arm that was not significantly longer than that of placebo-treated patients (26.6 months versus 21.1 months in the placebo group; HR 0.85, 95% CI 0.64–1.12; $p = 0.241$). However, subgroup analysis of the patients harboring *RET* M918T mutation found an overall survival rate among those receiving cabozantinib that was over twice as high as that in the placebo arm (44.3 vs 18.9 months, HR 0.60, 95% CI 0.38–0.95; $p = 0.026$).

The adverse effects most commonly seen in the cabozantinib arm included diarrhea, palmar-plantar erythrodysesthesia, weight loss, decreased appetite, nausea, and fatigue. In 79% of the patients, adverse events resulted in dose reductions in 79%, and in 16% treatment had to be discontinued. The latter included some cases of life-threatening adverse events that have been seen in other VEGF inhibitor trials, including gastrointestinal perforations, fistula formation, and hemorrhage (Blevins et al. 2014; Lamartina et al. 2016). Notably, clinically relevant QTc prolongation lasting over 500 ms was not observed in any of the cabozantinib-treated patients (Elisei et al. 2013).

VEGFR-Directed TKI Therapy for Anaplastic Thyroid Cancer

Attempts to treat anaplastic thyroid carcinoma (ATC) with antiangiogenic drugs have been largely unsuccessful. However, promising preliminary results have emerged from an ongoing phase II study of lenvatinib in Japanese patients with various forms of advanced thyroid cancer, including ATC. With median follow-ups of approximately 1 year, objective responses were observed in 3 (27.3%) of the 11 patients with ATCs. Five patients have received more than 6 months of treatment with lenvatinib. Treatment-emergent grade 3 or above adverse events were quite rare in the ATC subgroup, and the single death that occurred was considered unrelated to treatment (Takahashi et al. 2016).

Choosing the Right Antiangiogenic Drug

On the basis of the available data, it is not possible to conclude that sorafenib or lenvatinib is the first-line antiangiogenic drug of choice for advanced, RAI-refractory DTC; the same applies to the use of vandetanib versus cabozantinib for advanced MTC. For both types of thyroid cancer, the designs of the trials assessing the two drugs differed considerably in several important respects, and direct comparison of the two sets of results must therefore be undertaken with caution.

A look at the trials conducted in populations with DTC (Table 2) shows, for example, that tumor progression at study entry was assessed locally, at participating centers, in the DECISION study of sorafenib and centrally, by blinded independent review, in the lenvatinib trial (SELECT). The methods used to assess responses to treatment in these trials also differed. The Response Evaluation Criteria in Solid Tumor (RECIST), version 1.0, was used in the DECISION study, while version 1.1 was used in the SELECT study. The main difference lies in the number of target lesions to be followed: up to ten target lesions in total, up to five per organ in DECISION, and up to five target lesions in total, up to two per organ in SELECT. Finally, patients with prior TKI exposure were eligible for enrollment in the SELECT study, while those included in the DECISION trial had to be TKI-naïve.

The populations studied in the ZETA and EXAM trials were also significantly different. As noted above, in the vandetanib study, enrollment was open to patients with locally advanced or metastatic MTCs, whereas in the cabozantinib trial, patients had to have a documented progression of locoregional or metastatic disease. The impact of this emerges clearly from the markedly shorter PFS of the placebo arm in the EXAM trials (4 months compared with 19 months in the vandetanib trial) (Table 2).

Without data from comparative studies, discussion of the relative effectiveness of these drugs is largely speculative, and the choice of a first-line antiangiogenic TKI for DTC or for MTC has to be made on an individual basis. The toxicity profile of each drug (Table 2) will naturally play an important role in such decisions.

Future work will be aimed at identifying molecular biomarkers to predict tumor responses to these drugs. The genetic signature of a tumor may be helpful for identifying subsets of patients potentially responsive to the antiangiogenic drugs. Thus far, the four trials reviewed above have failed to identify a specific thyroid cancer genotype that associated with objective responses. The in vivo efficacy of cabozantinib has, however, been compared in patients with RET-mutant and RAS-mutant MTCs. Response rates to the drug were similar in the two groups, and there were no statistically intergroup differences in PFS. Nonetheless, PFS among patients with RET-mutant tumors was appreciably longer (60 weeks vs 47 weeks in the RASmut group) (Sherman et al. 2016).

Despite their partially overlapping mechanisms of action, cross-resistance between various TKIs appears to be limited. An alternative TKIs may thus be considered if progression occurs with first-line TKI therapy. Lenvatinib and cabozantinib in particular have proved to be effective in both first- and second-line settings (Elisei et al. 2013; Schlumberger et al. 2015).

Therapies Targeting MAPK Pathway Signaling

Rationale for Targeting Mitogen-Activated Protein Kinase (MAPK) Signaling in Thyroid Cancers

Activated signaling through the MAPK pathway is a hallmark of most thyroid carcinomas, both those derived from follicular epithelium and the neuroendocrine MTC. For the most common, PTC, activating mutations of BRAF (particularly a

valine-to-glutamic acid mutation in codon 600) **account for nearly 60%**, whereas RAS mutations and activating rearrangements of receptor tyrosine kinases such as RET and TRK account for nearly 30% (Cancer Genome Atlas Research Network 2014; Fagin and Wells 2016). MTC is associated with activating point mutations of RET kinase in at least half of cases (including germline mutations in nearly all patients with the inherited multiple endocrine neoplasia type 2 and somatic mutations in up to half of those with sporadic MTC) and RAS mutations in another 25%. Although associated with considerably more genomic heterogeneity and instability, anaplastic carcinomas also harbor high frequencies of BRAF and RAS mutations (Landa et al. 2016). Follicular carcinomas, in contrast, appear less dependent upon activated signaling, with fewer than 20% bearing RAS mutations but other abnormalities including those affecting phosphatidylinositol 3-kinase/Akt signaling being more prominent.

The functional impacts of these mutations in various MAPK signaling intermediates are overlapping but clinically relevant. Mutations in BRAF, RAS, and RET are clearly oncogenic and are highly likely to represent tumor-initiating events; this is most evident in the case of multiple endocrine neoplasia type 2, in which germline inheritance of a single mutant RET allele can lead to near-complete penetrance of the malignancy (Krampitz and Norton 2014). All of these initiating events lead to enhanced cellular proliferation. However, BRAF mutations are associated with greater degrees of functional loss of differentiation, including extensive gene expression alterations leading to loss of radioiodine avidity (Cancer Genome Atlas Research Network 2014). Consequently, BRAF mutations in PTC have been associated with greater degrees of tumor invasiveness and metastasis, higher rates of tumor recurrence, and potentially greater risk for mortality (Xing et al. 2013, 2015). Later secondary events that can lead to increased tumor aggressiveness include mutations (PI3K/PTEN, Akt, p53, and TERT promoter), gene amplifications (EGFR, VEGFR), or epigenetic silencing (Landa et al. 2016; Liu and Xing 2016; Ricarte-Filho et al. 2009).

BRAF-Directed Therapy for Advanced Thyroid Cancers

Given the more advanced disease associated with BRAF mutations, considerable interest exists in the application of BRAF inhibitor therapy, analogous to the use of this approach successfully in other BRAF-mutant malignancies pioneered in melanoma (Halilovic and Solit 2008). Initial support came from in vitro studies, documenting successful inhibition of downstream MAPK signaling in treated BRAF-mutant cell lines (Ouyang et al. 2006). The so-called "type 1" inhibitors, vemurafenib and dabrafenib, which preferentially bind to the active conformation of the ^{V600E}BRAF-mutant kinase domain, have been approved for treatment of advanced BRAF-mutant melanoma, and they demonstrate greater selectivity for the mutations most commonly found in PTC (Cabanillas et al. 2015). Several other BRAF inhibitors remain in phase I trials, without available data yet regarding activity in thyroid cancer.

Vemurafenib

Vemurafenib is a potent kinase inhibitor of ^{V600E}BRAF with IC_{50} of 31 nM, whereas potency against wild-type BRAF kinase is threefold less (Sala et al. 2008). In the initial phase I trial of vemurafenib, three RAI-refractory PTC patients were treated with the investigational drug; one had a confirmed partial response, and the median time to progression was nearly 12 months (Kim et al. 2013). A subsequent multicenter phase II study of vemurafenib starting at 960 mg twice daily was conducted in 26 previously VEGFR-directed TKI-naïve patients and 25 previously treated with a VEGFR-directed TKI (Brose et al. 2016). In the TKI-naïve cohort, the partial response rate was 38.5% (95% CI 20.2–59.4), median duration of response was 16.5 months (95% CI 5.7–not estimable), and median progression-free survival was 18.2 months (95% CI 15.5–29.3). In the cohort of patients previously treated with a VEGFR-directed TKI, typically sorafenib, vemurafenib appeared somewhat less effective, with a partial response rate of 27.3% (95% CI 10.7–50.2), median duration of response of 7.4 months (95% CI 3.7 – not estimable), and median progression-free survival of 8.9 months (95% CI 5.5 – not estimable). The most commonly reported adverse events included rash (in 69% of patients), fatigue (in 67%), weight loss (in 51%), decreased appetite (in 45%), alopecia (in 41%), and arthralgia (in 41%), which were similar to those noted in melanoma patients treated in the pivotal phase III trial (Chapman et al. 2011); elevated creatinine levels were seen in 37% of patients as well. Of note, higher frequencies of adverse events were seen in patients treated longer with the drug, and about one-quarter discontinued therapy due to side effects of the drug. As seen in melanoma patients, cutaneous squamous cell carcinoma was reported in 22% of patients treated with vemurafenib. Finally, two patients were described as developing non-cutaneous squamous malignancies of the head and neck, which were suspected to have represented squamous dedifferentiation of metastatic thyroid carcinoma as was also seen in the original phase I experience (Kim et al. 2013). Similar efficacy has also been reported in a cohort of patients with RAI-refractory BRAF-mutant PTC treated "off label" with vemurafenib (Dadu et al. 2015). These studies provide supportive evidence that vemurafenib is likely an effective first- or second-line therapy in these patients, particularly if use of VEGFR-directed TKI is relatively contraindicated.

Recently, a small trial reported that neoadjuvant vemurafenib, administered for 8 weeks prior to anticipated surgery for bulky invasive BRAF-mutant PTC, led to greater than 50% postoperative complete response, suggesting that preoperative therapy in selected patients may be beneficial (Cabanillas et al. 2017).

Several case reports have also been published describing transient responses to monotherapy with vemurafenib for patients with BRAF-mutant anaplastic thyroid carcinoma. One patient was reported to have a near-complete response but eventually died of progressive disease after 61 weeks of therapy (Prager et al. 2016).

Dabrafenib

Binding to both the kinase hinge region and the ATP binding site, dabrafenib inhibits several of the codon 600 variants of BRAF, including V600E (IC_{50} 0.5 nM), V600 K

(0.6 nM), and V600D (1.9 nM) (Rheault et al. 2013). In the initial phase I trial of dabrafenib, three of nine evaluable patients with BRAF-mutant thyroid cancer experienced a partial response (Falchook et al. 2012). In a subsequent report of the full thyroid expansion cohort from that phase I trial, 14 patients with progressive, RAI-refractory, BRAF-mutant thyroid cancer were treated with dabrafenib, 150 mg twice daily (Falchook et al. 2015). The partial response rate was 29% (95% CI 8–58%), median duration of response had not been not reached, and median progression survival was 11.3 months (95% CI 2.1 – not estimable). The most commonly reported adverse events were cutaneous papilloma (in 57% of patients), hyperkeratosis (in 36%), alopecia (in 29%), arthralgia (in 14%), hair texture changes (in 14%), pyrexia (in 14%), seborrheic keratosis (in 14%), and skin hypertrophy (in 14%), similar to the original report of patients primarily with melanoma (Falchook et al. 2012).

In the thyroid expansion cohort of the phase I trial, there was one patient with BRAF-mutant anaplastic carcinoma who had a mixed response to dabrafenib, experiencing shrinkage of his target lesions but growth of one new lesion that qualified as a progression event. Subsequently, two patients with metastatic ATC were reported with clinically significant though transient responses to dabrafenib therapy, lasting 3 months and 11 weeks, respectively, before the patients succumbed to fatal progression (Lim et al. 2016).

Mechanisms of primary and acquired resistance to BRAF-directed therapy have been explored, primarily focusing on mutations in RAS as well as upregulation of feedback pathways that reduce the effectiveness of the treatment. For example, one study has demonstrated rapid upregulation of HER3 expression and autocrine stimulation of HER3 kinase and MAPK signaling through secretion of neuregulin when PTC cells are exposed to a BRAF inhibitor, an effect that is blocked by concurrent treatment with the HER kinase inhibitor lapatinib (Montero-Conde et al. 2013). This observation is the basis for recently initiated clinical trials of combined BRAF- and HER-directed therapies.

MEK-Directed Therapy for Advanced DTC

As MEK is the common downstream effector of activated signaling from either RAS or RAF, it has been an attractive potential therapeutic target for many solid tumors dependent upon the MAPK signaling pathway, particularly those with BRAF mutations. MEK-directed therapies, however, have yet not advanced successfully as monotherapy to induce tumor shrinkage in thyroid cancer.

Selumetinib preferentially inhibits MEK at a low concentration (IC_{50} 12 nM) but has not been approved yet for treatment of any malignancy (Yeh et al. 2007). Inhibition of MEK by selumetinib, leading to marked reduction in phosphorylated ERK, only led to cytostatic inhibition of tumor growth in a BRAF-mutant PTC xenograft model (Ball et al. 2007). A phase II trial was performed in 39 patients with progressive, RAI-refractory PTC, with a starting selumetinib dose of 100 mg twice daily (Hayes et al. 2012). A confirmed partial response was reported in only one patient (3%), and an unconfirmed partial response was described in one other;

median progression-free survival was 32 weeks. Although the analysis was limited by small numbers, the progression-free survival was 33 weeks in patients with tumors bearing *BRAF* mutation, compared with only 11 weeks in BRAF wild type ($P = 0.3$). Significant adverse events included rash, fatigue, diarrhea, and peripheral edema. There are no data available regarding monotherapy for thyroid cancer using the two MEK inhibitors, trametinib and cobimetinib, that are FDA approved for treatment of BRAF-mutant malignancies.

Combined BRAF- and MEK-Directed Therapy for Advanced Thyroid Cancers

Simultaneous inhibition of BRAF and MEK may provide a more effective approach to blocking activated MAPK signaling with possibly reduced toxicity, as has been demonstrated in melanoma (Flaherty et al. 2012). Based on this hypothesis, a randomized phase II study has recently been completed, comparing 53 RAI-refractory BRAF-mutant PTC patients treated with either dabrafenib alone (150 mg twice daily) or dabrafenib plus trametinib (150 mg twice daily +2 mg daily) (Shah et al. 2017). The partial response rates were 45.5% for dabrafenib alone, compared with 37.5% for the combination, which did not significantly differ; including minor responses (reduction of sum of tumor diameters between 20% and 29.9%), the predefined objective response rates were 50% and 54%, respectively. Median durations of response were 15.6 months (95% CI 4.2 – not estimable) and 13.3 months (95% CI 9.7 – not estimable), and median progression-free survivals were 11.4 months (95% CI 3.8 – not estimable) and 15.1 months (95% CI 11.7 – not estimable), respectively. Both regimens were similarly tolerated as well, and thus both could be considered possible therapeutic options for further exploration. Prolonged tolerance of a modified regimen of combination therapy, 5 weeks on followed by 3 weeks off medication in each 8 week cycle, has been reported with maintenance of efficacy in two PTC patients, suggesting further exploration of optimal dosing regimens may be appropriate (White et al. 2017).

In contrast with the limited transient efficacy seen anecdotally with BRAF-directed monotherapy in ATC, combination of BRAF- and MEK-directed treatment may be particularly effective. In a small cohort of five patients with BRAF-mutant ATC treated with dabrafenib and trametinib, three were reported with partial response to the combination, and two others had prolonged stable disease (Iyer et al. 2016). In a larger, phase 2 basket trial evaluating dabrafenib plus trametinib in multiple rare tumors, 16 patients with BRAF-mutant ATC were treated at starting doses of 150 mg twice daily and 2 mg daily, respectively (Subbiah et al. 2017). The overall response rate was 69% (95% CI 47–87%). After 12 months of therapy, 90% of responses persisted, and the 12-month progression-free and overall survivals were 79% and 80%, respectively. Although not a randomized trial, these outcomes far exceeded historical comparators. Across the basket trial, the most common adverse events were fatigue (in 38%), pyrexia (37%), and nausea (in 35%), whereas hyponatremia (in 19%), pneumonia (in 13%), and anemia (in 13%) were the most common grade 3 or 4 in the ATC cohort.

Redifferentiation with BRAF- and MEK-Directed Therapy

For many years, researchers have attempted to identify disease mechanisms in DTC that lead to loss of radioiodine responsiveness and restore or "redifferentiate" such tumors pharmacologically, albeit with little evidence of objective response (Haugen 2004). Given extensive evidence that PTC tumors with activated MAPK signaling are less likely to be radioiodine avid and demonstrate lower expression of genes coding for differentiated thyroid proteins such as thyroglobulin and sodium-iodine symporter, it was hypothesized that therapy directed against BRAF or MEK might restore these differentiated functions. Support for this hypothesis was provided in an elegant transgenic mouse model, in which treatment with either a BRAF-directed or MEK-directed drug could restore radioiodine uptake and retention that had been lost following doxycycline-inducible ^{V600E}BRAF expression (Chakravarty et al. 2011). Subsequently, a pilot study of short-term selumetinib treatment was performed in 20 evaluable patients with RAI-refractory thyroid carcinoma to determine if the potential for objective response following RAI therapy could be restored (Ho et al. 2013). Before and after a 4 week treatment course with selumetinib, 75 mg twice daily, 12 patients (60%) experienced increased uptake documented on ^{124}I-PET scans, of whom 7 subsequently received therapeutic ^{131}I treatment and 5 had partial responses; thus, the intent-to-treat objective response rate was 25%. Serum thyroglobulin levels declined by >90% in the ^{131}I-treated patients. No difference was observed in response between BRAF-mutant and wild-type tumors, but all of the patients with NRAS mutations reacquired uptake. Although no high-grade toxicities were attributed to the selumetinib, one patient who had previously received nearly 1000 mCi of ^{131}I for thyroid cancer and pelvic irradiation for prostate cancer subsequently developed myelodysplastic syndrome 51 weeks after RAI treatment.

Dabrafenib was studied similarly in ten patients with RAI-refractory BRAF-mutant PTC (Rothenberg et al. 2014). After 25 days of therapy with 150 mg twice daily, standard radioiodine diagnostic scanning demonstrated restoration of uptake in six patients; this subset subsequently received another 17 days of dabrafenib followed by therapeutic radioiodine administration. Overall, two patients had partial response of their metastatic disease, for an intent-to-treat response rate of 20%.

A recent report described the results of treatment with RAI in 13 patients who had already been on long-term BRAF- and/or MEK-directed therapy for treatment of their progressive, RAI-refractory metastatic DTC (Jaber et al. 2017). Nine patients with a BRAF mutation had been treated with a BRAF inhibitor, and three patients with a RAS mutation and one without documented oncogenic mutation had been treated with a MEK inhibitor. Following a median duration of about 14 months of kinase inhibitor therapy, nine had sufficient restoration of RAI uptake to allow treatment with a median of 204 mCi ^{131}I, followed by immediate discontinuation of BRAF- or MEK-directed therapy. Of these, seven remained progression-free after median follow-up of 8.3 months, without re-initiation of systemic therapy. Radiation pneumonitis was seen in two patients after RAI therapy.

Multiple studies are now ongoing to evaluate prospectively the role of inhibitors of BRAF and/or MEK to enhance RAI responsiveness at various stages of the

disease process, from initial adjuvant therapy in high-risk patients to treatment of RAI-refractory metastatic disease. Given the evidence discussed previously of the role of autocrine activation of HER3 signaling following BRAF inhibition to restore activated MAPK signaling, studies are also focusing on the use of HER inhibitors in combination with MAPK pathway inhibitors for greater redifferentiation effect (Cheng et al. 2017).

Therapies Targeting Phosphatidylinositol 3-Kinase/AKT Pathway Signaling

Mammalian Target of Rapamycin (mTOR)-Directed Therapy

Many advanced thyroid malignancies demonstrate activation of signaling through the PI3K/AKT pathway, either due to enhanced upstream signaling from RAS, diminished expression of PTEN or mutation, and/or amplification or overexpression of either PI3K or AKT (Saji and Ringel 2010). As activation of PI3K signaling through mTOR results in enhanced tumor proliferation, migration, and survival, mTOR-directed inhibitor therapy has been attempted in several small clinical trials.

A phase II study was performed that included 28 patients with progressive, RAI-refractory DTC, treated with everolimus at a starting dose of 10 mg daily (Schneider et al. 2017). With a median duration of treatment of 11 months, there were no objective responses observed, whereas 39% had progressive disease as the best observed response. Median progression-free and overall survivals were 9 months and 18 months, respectively. A planned subgroup analysis examined the relationship between blood everolimus concentrations and efficacy, but there were no significant differences in duration of stable disease, progression-free survival, or overall survival in patients whose concentrations were in the upper 50th percentile compared with the lower 50th percentile. Treatment-related adverse events included anemia (in 64%), cough (in 64%), stomatitis (in 61%), and hyperglycemia (in 61%). Preliminary data have been presented from a similar phase II trial in 33 patients with progressive, RAI-refractory DTC treated with everolimus starting at 10 mg daily (Lorch et al. 2013). Only one partial response was observed, and the median progression-free survival was 16 months (95% CI 10–not estimable). Adverse events were similar to those previously reported with the drug. An earlier multicenter trial included 24 patients with RAI-refractory DTC, treated with everolimus starting at 10 mg daily (Lim et al. 2013). Partial response was seen in only two patients, lasting 21 and 24 weeks each, and the median progression-free survival for the DTC cohort was 43 weeks.

Each of the trials described also included separate cohorts with MTC patients, based upon known efficacy of everolimus in other neuroendocrine malignancies. In one study, none of seven MTC patients experienced an objective response, and the median progression-free survival was only 33 weeks (95% CI 8–56) (Schneider et al. 2015). In the second study, one of ten MTC patients had a partial response, and the others remained stable for at least 6 months (Lorch et al. 2013). In the third study, no

partial responses were reported in nine MTC patients, and the 12-month progression-free survival was about 70% (Lim et al. 2013).

Combining mTOR-Directed Therapy with VEGFR-Directed TKI

With evidence of possible synergism between inhibition of mTOR and MAPK signaling, several trials have examined the effectiveness of treatment with an mTOR-directed inhibitor combined with other agents in patients with advanced thyroid cancers.

A phase II study of the mTOR inhibitor temsirolimus combined with sorafenib was recently completed, having evaluated 36 patients with progressive, RAI-refractory carcinoma derived from thyroid follicular epithelium (including 2 with anaplastic carcinoma) (Sherman et al. 2017). Fifty-six percent had been previously treated, including cytotoxic chemotherapy in 25% and a VEGFR-directed therapy in 44%. Starting doses of sorafenib were 200 mg twice daily and of temsirolimus were 25 mg weekly. The partial response rate for the entire cohort was 22%, though slightly higher response rates were seen in patients with a BRAF mutation (30%) or no prior systemic treatment (38%). Interestingly, one patient who had previously progressed rapidly on single agent sorafenib had a marked tumor reduction on combination; subsequent tumor mutational profiling demonstrated a PTEN mutation that may have sensitized to temsirolimus therapy. One year progression-free survival was 31%, and median overall survival was 24.6 months. The most common grade 3–5 adverse events were hyperglycemia (in 19%), fatigue (in 14%), anemia (in 11%), and oral mucositis (in 8%); no information was provided about lower-grade adverse events. Given that the response rate to single agent sorafenib in previously untreated patients in the DECISION trial was only 12%, it was suggested that the combination of sorafenib and temsirolimus was appropriate for further study (Brose et al. 2014).

Higher response rates were preliminarily reported using the combination of everolimus and sorafenib in a phase II trial of patients not previously exposed to either drug (Sherman et al. 2015). Of 28 patients with RAI-refractory DTC treated with starting doses of sorafenib 400 mg twice daily and everolimus 5 mg daily, 61% had partial response, and there was a 40% partial response rate in the 10 MTC patients treated in the same trial. Of various subgroups, seven of nine (78%) patients with Hurthle cell carcinoma had partial responses, and the median duration on treatment for this subgroup was about 1.5 years. In contrast, in a phase II trial that evaluated 33 RAI-refractory DTC patients who previously progressed on sorafenib monotherapy, sorafenib was dose escalated up from 200 mg per day below their previous sorafenib dose, after adding everolimus 10 mg daily (Brose et al. 2015). Median progression-free survival was 13.7 months, despite having previously progressed on sorafenib alone, but only one patient had a partial response on the combination.

In summary, although monotherapy with mTOR-directed therapy does not appear to be particularly beneficial in thyroid cancer, adding either everolimus or

temsirolimus to sorafenib may be considerably more effective than sorafenib alone. Whether these agents contribute to higher efficacy if added to other TKIs, such as lenvatinib, or whether the combination with sorafenib is in fact superior to single agent lenvatinib remains to be determined.

Selectively Targeting RET or TRK Kinase Mutations

Mutated kinases that are oncogenic in thyroid carcinoma are also oncogenic in many other tumor types and therefore have been valued targets for therapy across oncology. The recent recognition that activating mutations of the RET kinase, particularly fusion rearrangements and amplifications, are common in tumors, such as non-small cell lung and breast carcinomas, has led to development of drugs that selectively target RET kinase (Kato et al. 2017; Subbiah and Roszik 2017). Given that many of the toxicities associated with VEGF-directed therapies that also inhibit RET are thought to derive from VEGFR or PDGFR inhibition, the greater selectivity for RET of these newer agents may enhance their tolerability and therefore efficacy. In developing RET-directed therapies, effort has also been made to create drugs that might prevent secondary resistance by ensuring effective inhibition of gatekeeper mutations such as ^{V804M}RET. Three such selective agents are currently in phase I trials, without available efficacy data yet in RET-driven thyroid cancers: RXDX-105 (with IC_{50} nearly 800-fold lower for RET than VEGFR,but also effectively inhibits BRAF), LOXO-292 (IC_{50} at least 100-fold lower for RET), and BLU-667 (IC_{50} at least 70-fold lower for RET) (Brandhuber et al. 2016; Li et al. 2017; Rahal et al. 2016).

A similar strategy was employed to develop drugs that selectively target activating fusion mutations in TRK kinases, which are seen infrequently in common malignancies such as colon and lung carcinoma but commonly in rare malignancies such as salivary carcinomas and infantile fibrosarcoma (Khotskaya et al. 2017). In a collected series of phase I trials, the novel TRK-directed agent larotrectinib was recently reported to yield an objective response rate of 78% across a broad range of tumor histologies, including partial responses in four of five patients with TRK-rearranged PTC (Hyman et al. 2017). Median duration of response had not been reached at the time of this initial report. The most common adverse events included fatigue (in 30%), dizziness (in 28%), and nausea (in 28%), but only 11% of patients required dose reductions due drug toxicity.

Immune Checkpoint-Directed Therapy

Therapy directed against immune checkpoints, to target activated T cell regulatory pathways that are suppressing anticancer immunologic responses, has provided an important new treatment for many malignancies such as melanoma and non-small cell lung and bladder carcinomas (Sharma and Allison 2015). Drugs that inhibit immune checkpoints such as CTLA-4, PD-1, and PD-L1 have proven generally most

effective in tumors that carry high levels of neoantigens capable of stimulating antitumor immune responses, and these are typically found in malignancies with higher rates of nonsynonymous mutations (Colli et al. 2016). One might expect, therefore, that DTC would not be an attractive candidate for immune checkpoint-directed therapy, given the low rates of mutations typically reported (Cancer Genome Atlas Research Network 2014; Colli et al. 2016). Conversely, cancers that demonstrate higher levels of expression of immune checkpoints, such as PD-L1 on tumor cells, may be more likely to respond to such targeted therapies, particularly if in combination with other treatments that enhance the expression of immune checkpoint proteins themselves (Cunha et al. 2017; French et al. 2017).

Following this latter rationale, a cohort of 22 patients with RAI-refractory DTC were included in a large basket trial of the PD-1 inhibitor pembrolizumab, dosed intravenously at 10 mg/kg every 2 weeks (Mehnert et al. 2016). All patients had tumors that demonstrated PD-L1 expression on at least 1% of cells, similar to cutoffs recommended for other malignancies treated successfully with pembolizumab. The partial response rate was 9.1% (95% CI 1.1–29.2%), median duration of response was not reached, and 6-month progression-free survival was 58.7%. Most common adverse events were diarrhea (in 32%) and fatigue (in 18%), and one patient experienced grade 3 colitis. Multiple other clinical trials involving checkpoint inhibitors are ongoing in both DTC and MTC.

Anaplastic carcinoma, on the other hand, has a markedly high mutation rate, and evidence suggests that ATC may be a particularly “hot” immunogenic environment appropriate for immunotherapy (Bastman et al. 2016; Dadu et al. 2016; Landa et al. 2016). Anecdotal cases have been reported of ATC patients as exceptional responders to anti-PD-1 therapy, and trials have been initiated both for monotherapy as well as combination of checkpoint inhibitors with either oncogene-directed therapy or radiation therapy (Kollipara et al. 2017; Tang et al. 2017; Verschraegen et al. 2017).

References

Akslen LA, Livolsi VA. Increased angiogenesis in papillary thyroid carcinoma but lack of prognostic importance. Hum Pathol. 2000;31(4):439–42.

Ball DW, Jin N, Rosen DM, Dackiw A, Sidransky D, Xing M, et al. Selective growth inhibition in BRAF mutant thyroid cancer by the mitogen-activated protein kinase kinase 1/2 inhibitor AZD6244. J Clin Endocrinol Metab. 2007;92(12):4712–8.

Bastman JJ, Serracino HS, Zhu Y, Koenig MR, Mateescu V, Sams SB, et al. Tumor-infiltrating T cells and the PD-1 checkpoint pathway in advanced differentiated and anaplastic thyroid cancer. J Clin Endocrinol Metab. 2016;101(7):2863–73.

Blevins DP, Dadu R, Hu M, Baik C, Balachandran D, Ross W, et al. Aerodigestive fistula formation as a rare side effect of antiangiogenic tyrosine kinase inhibitor therapy for thyroid cancer. Thyroid. 2014;24(5):918–22.

Brandhuber B, Haas J, Tuch B, Ebata K, Bouhana K, McFaddin E, et al. The development of a potent, KDR/VEGFR2-sparing RET kinase inhibitor for treating patients with RET-dependent cancers. Eur J Cancer. 2016;69:S144.

Brose MS, Nutting CM, Jarzab B, Elisei R, Siena S, Bastholt L, et al. Sorafenib in radioactive iodine-refractory, locally advanced or metastatic differentiated thyroid cancer: a randomised, double-blind, phase 3 trial. Lancet. 2014;384(9940):319–28.

Brose MS, Troxel AB, Yarchoan M, Cohen AB, Harlacker K, Dyanick NA, et al. A phase II study of everolimus (E) and sorafenib (S) in patients (PTS) with metastatic differentiated thyroid cancer who have progressed on sorafenib alone. J Clin Oncol. 2015;33(15):6072.

Brose MS, Cabanillas ME, Cohen EE, Wirth LJ, Riehl T, Yue H, et al. Vemurafenib in patients with BRAF(V600E)-positive metastatic or unresectable papillary thyroid cancer refractory to radioactive iodine: a non-randomised, multicentre, open-label, phase 2 trial. Lancet Oncol. 2016;17(9):1272–82.

Brose MS, Worden FP, Newbold KL, Guo M, Hurria A. Effect of age on the efficacy and safety of lenvatinib in radioiodine-refractory differentiated thyroid cancer in the phase III SELECT trial. J Clin Oncol. 2017;35(23):2692–9.

Cabanillas ME, Patel A, Danysh BP, Dadu R, Kopetz S, Falchook G. BRAF inhibitors: experience in thyroid cancer and general review of toxicity. Horm Cancer. 2015;6(1):21–36.

Cabanillas ME, Busaidy NL, Zafereo M, Waguespack SG, Hu MI, Hofmann MC, et al. Neo-adjuvant vemurafenib in patients with locally advanced papillary thyroid cancer (PTC). Eur Thyroid J. 2017;6(Suppl 1):38.

Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. Cell. 2014;159(3):676–90.

Cao Y. Multifarious functions of PDGFs and PDGFRs in tumor growth and metastasis. Trends Mol Med. 2013;19(8):460–73.

Carlomagno F, Vitagliano D, Guida T, Ciardiello F, Tortora G, Vecchio G, et al. ZD6474, an orally available inhibitor of KDR tyrosine kinase activity, efficiently blocks oncogenic RET kinases. Cancer Res. 2002;62:7284–90.

Carlomagno F, Anaganti S, Guida T, Salvatore G, Troncone G, Wilhelm SM, et al. BAY 43-9006 inhibition of oncogenic RET mutants. J Natl Cancer Inst. 2006;98(5):326–34.

Chakravarty D, Santos E, Ryder M, Knauf JA, Liao XH, West BL, et al. Small-molecule MAPK inhibitors restore radioiodine incorporation in mouse thyroid cancers with conditional BRAF activation. J Clin Invest. 2011;121(12):4700–11.

Chapman PB, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med. 2011;364 (26):2507–16.

Cheng L, Jin Y, Liu M, Ruan M, Chen L. HER inhibitor promotes BRAF/MEK inhibitor-induced redifferentiation in papillary thyroid cancer harboring BRAFV600E. Oncotarget. 2017;8(12): 19843–54.

Colli LM, Machiela MJ, Myers TA, Jessop L, Yu K, Chanock SJ. Burden of nonsynonymous mutations among TCGA cancers and candidate immune checkpoint inhibitor responses. Cancer Res. 2016;76(13):3767–72.

Cunha LL, Marcello MA, Rocha-Santos V, Ward LS. Immunotherapy against endocrine malignancies: immune checkpoint inhibitors lead the way. Endocr-Relat Cancer. 2017;24(12):T261–81.

Dadu R, Shah K, Busaidy NL, Waguespack SG, Habra MA, Ying AK, et al. Efficacy and tolerability of vemurafenib in patients with BRAF(V600E) -positive papillary thyroid cancer: M.D. Anderson cancer center off label experience. J Clin Endocrinol Metab. 2015;100(1): E77–81.

Dadu R, Vilalabos PA, Para Cuentas ER, Rodriguez Canales J, Wistuba II, Zhou S, et al. Anaplastic thyroid cancer (ATC) is a hot immunogenic environment: immunoprofiling of a large cohort of ATC tumors. Thyroid. 2016;26(S1):OR-12.

Dhar DK, Kubota H, Kotoh T, Tabara H, Watanabe R, Tachibana M, et al. Tumor vascularity predicts recurrence in differentiated thyroid carcinoma. Am J Surg. 1998;176(5):442–7.

Durante C, Haddy N, Baudin E, Leboulleux S, Hartl D, Travagli JP, et al. Long-term outcome of 444 patients with distant metastases from papillary and follicular thyroid carcinoma: benefits and limits of radioiodine therapy. J Clin Endocrinol Metab. 2006;91(8):2892–9.

Durante C, Tallini G, Puxeddu E, Sponziello M, Moretti S, Ligorio C, et al. BRAFV600E mutation and expression of proangiogenic molecular markers in papillary thyroid carcinomas. Eur J Endocrinol. 2011;165(3):455–63.

Elisei R, Schlumberger MJ, Muller SP, Schoffski P, Brose MS, Shah MH, et al. Cabozantinib in progressive medullary thyroid cancer. J Clin Oncol. 2013;31(29):3639–46.

Fagin JA, Wells Jr SA. Biologic and clinical perspectives on thyroid cancer. N Engl J Med. 2016;375(11):1054–67.

Falchook GS, Long GV, Kurzrock R, Kim KB, Arkenau TH, Brown MP, et al. Dabrafenib in patients with melanoma, untreated brain metastases, and other solid tumours: a phase 1 dose-escalation trial. Lancet. 2012;379(9829):1893–901.

Falchook GS, Millward M, Hong D, Naing A, Piha-Paul S, Waguespack SG, et al. BRAF inhibitor dabrafenib in patients with metastatic BRAF-mutant thyroid cancer. Thyroid. 2015;25(1):71–7.

Fallahi P, Mazzi V, Vita R, Ferrari SM, Materazzi G, Galleri D, et al. New therapies for dedifferentiated papillary thyroid cancer. Int J Mol Sci. 2015;16(3):6153–82.

Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, Sosman J, et al. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. N Engl J Med. 2012;367(18):1694–703.

Fox E, Widemann BC, Chuk MK, Marcus L, Aikin A, Whitcomb PO, et al. Vandetanib in children and adolescents with multiple endocrine neoplasia type 2B associated medullary thyroid carcinoma. Clin Cancer Res. 2013;19(15):4239–48.

French JD, Bible K, Spitzweg C, Haugen BR, Ryder M. Leveraging the immune system to treat advanced thyroid cancers. Lancet Diabetes Endocrinol. 2017;5(6):469–81.

Ghatalia P, Je Y, Kaymakcalan MD, Sonpavde G, Choueiri TK. QTc interval prolongation with vascular endothelial growth factor receptor tyrosine kinase inhibitors. Br J Cancer. 2015;112 (2):296–305.

Halilovic E, Solit DB. Therapeutic strategies for inhibiting oncogenic BRAF signaling. Curr Opin Pharmacol. 2008;8(4):419–26.

Harris PJ, Bible KC. Emerging therapeutics for advanced thyroid malignancies: rationale and targeted approaches. Expert Opin Investig Drugs. 2011;20(10):1357–75.

Haugen BR. Redifferentiation therapy in advanced thyroid cancer. Curr Drug Targets Immune Endocr Metabol Disord. 2004;4(3):175–80.

Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American Thyroid Association Management Guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association Guidelines task force on thyroid nodules and differentiated thyroid cancer. Thyroid. 2016;26(1):1–133.

Hayes DN, Lucas AS, Tanvetyanon T, Krzyzanowska MK, Chung CH, Murphy B, et al. Phase II efficacy and pharmacogenomic study of selumetinib (AZD6244; ARRY-142886) in iodine-131 refractory papillary thyroid carcinoma (IRPTC) with or without follicular elements. Clin Cancer Res. 2012;18(7):2056–65.

Ho AL, Grewal RK, Leboeuf R, Sherman EJ, Pfister DG, Deandreis D, et al. Selumetinib-enhanced radioiodine uptake in advanced thyroid cancer. N Engl J Med. 2013;368(7):623–32.

Hyman DM, Laetsch TW, Kummar S, DuBois SG, Farago AF, Pappo AS, et al. The efficacy of larotrectinib (LOXO-101), a selective tropomyosin receptor kinase (TRK) inhibitor, in adult and pediatric TRK fusion cancers. J Clin Oncol. 2017; 35(18_suppl):LBA2501-LBA.

Ishiwata T, Iino Y, Takei H, Oyama T, Morishita Y. Tumor angiogenesis as an independent prognostic indicator in human papillary thyroid carcinoma. Oncol Rep. 1998;5(6):1343–8.

Iyer P, Dadu R, Busaidy N, Ferrarotto R, Gule-Monroe M, Lu C, et al. Harvesting high-hanging fruit: Targeted therapy for BRAF mutant (BRAFm) and BRAF wild-type (BRAFwt) anaplastic thyroid cancer (ATC). Thyroid. 2016;26(Suppl 1):A64–A5.

Jaber T, Waguespack SG, Cabanillas ME, Vu T, Santos EB, Dadu R, et al. Efficacy of targeted therapy in resensitization to radioactive iodine (RAI) in advanced thyroid cancer: The MD Anderson experience. Endocr Rev. 2017;38(3 Supplement):OR39–6.

Jebreel A, England J, Bedford K, Murphy J, Karsai L, Atkin S. Vascular endothelial growth factor (VEGF), VEGF receptors expression and microvascular density in benign and malignant thyroid diseases. Int J Exp Pathol. 2007;88(4):271–7.

Jechlinger M, Sommer A, Moriggl R, Seither P, Kraut N, Capodiecci P, et al. Autocrine PDGFR signaling promotes mammary cancer metastasis. J Clin Invest. 2006;116(6):1561–70.
Kato S, Subbiah V, Marchlik E, Elkin SK, Carter JL, Kurzrock R. RET aberrations in diverse cancers: next-generation sequencing of 4871 patients. Clin Cancer Res. 2017;23(8):1988–97.
Kerbel RS. Tumor angiogenesis. N Engl J Med. 2008;358(19):2039–49.
Khotskaya YB, Holla VR, Farago AF, Mills Shaw KR, Meric-Bernstam F, Hong DS. Targeting TRK family proteins in cancer. Pharmacol Ther. 2017;173:58–66.
Kim KB, Cabanillas ME, Lazar AJ, Williams MD, Sanders DL, Ilagan JL, et al. Clinical responses to vemurafenib in patients with metastatic papillary thyroid cancer harboring BRAF(V600E) mutation. Thyroid. 2013;23(10):1277–83.
Klein M, Vignaud JM, Hennequin V, Toussaint B, Bresler L, Plenat F, et al. Increased expression of the vascular endothelial growth factor is a pejorative prognosis marker in papillary thyroid carcinoma. J Clin Endocrinol Metab. 2001;86(2):656–8.
Kollipara R, Schneider B, Radovich M, Babu S, Kiel PJ. Exceptional response with immunotherapy in a patient with anaplastic thyroid cancer. Oncologist. 2017;22(10):1149–51.
Krampitz GW, Norton JA. RET gene mutations (genotype and phenotype) of multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma. Cancer. 2014;120(13):1920–31.
Laakkonen P, Waltari M, Holopainen T, Takahashi T, Pytowski B, Steiner P, et al. Vascular endothelial growth factor receptor 3 is involved in tumor angiogenesis and growth. Cancer Res. 2007;67(2):593–9.
Lamartina L, Ippolito S, Danis M, Bidault F, Borget I, Berdelou A, et al. Antiangiogenic tyrosine kinase inhibitors: occurrence and risk factors of hemoptysis in refractory thyroid cancer. J Clin Endocrinol Metab. 2016;101(7):2733–41.
Landa I, Ibrahimpasic T, Boucai L, Sinha R, Knauf JA, Shah RH, et al. Genomic and transcriptomic hallmarks of poorly differentiated and anaplastic thyroid cancers. J Clin Invest. 2016;126(3):1052–66.
Lennard CM, Patel A, Wilson J, Reinhardt B, Tuman C, Fenton C, et al. Intensity of vascular endothelial growth factor expression is associated with increased risk of recurrence and decreased disease-free survival in papillary thyroid cancer. Surgery. 2001;129:552–8.
Li GG, Somwar R, Joseph J, Smith RS, Hayashi T, Martin L, et al. Antitumor activity of RXDX-105 in multiple cancer types with RET rearrangements or mutations. Clin Cancer Res. 2017;23(12):2981–90.
Lim SM, Chang H, Yoon MJ, Hong YK, Kim H, Chung WY, et al. A multicenter, phase II trial of everolimus in locally advanced or metastatic thyroid cancer of all histologic subtypes. Ann Oncol. 2013;24(12):3089–94.
Lim AM, Taylor GR, Fellowes A, Cameron L, Lee B, Hicks RJ, et al. BRAF inhibition in BRAFV600E-positive anaplastic thyroid carcinoma. J Natl Compr Canc Netw. 2016;14(3):249–54.
Liu R, Xing M. TERT promoter mutations in thyroid cancer. Endocr-Relat Cancer. 2016;23(3):R143–55.
Lorch JH, Busaidy N, Ruan DT, Janne PA, Limaye SA, Wirth LJ, et al. A phase II study of everolimus in patients with aggressive RAI refractory (RAIR) thyroid cancer (TC). J Clin Oncol. 2013;31(15):6023.
Mancikova V, Inglada-Perez L, Curras-Freixes M, de Cubas AA, Gomez A, Leton R, et al. VEGF, VEGFR3, and PDGFRB protein expression is influenced by RAS mutations in medullary thyroid carcinoma. Thyroid. 2014;24(8):1251–5.
Marotta V, Sciammarella C, Vitale M, Colao A, Faggiano A. The evolving field of kinase inhibitors in thyroid cancer. Crit Rev Oncol Hematol. 2015;93(1):60–73.
Matsui J, Yamamoto Y, Funahashi Y, Tsuruoka A, Watanabe T, Wakabayashi T, et al. E7080, a novel inhibitor that targets multiple kinases, has potent antitumor activities against stem cell factor producing human small cell lung cancer H146, based on angiogenesis inhibition. Int J Cancer. 2008;122(3):664–71.
Mehnert JM, Varga A, Brose M, Aggarwal RR, Lin C-C, Prawira A, et al. Pembrolizumab for advanced papillary or follicular thyroid cancer: preliminary results from the phase 1b KEYNOTE-028 study. J Clin Oncol. 2016;34(15_suppl):6091.

Montero-Conde C, Ruiz-Llorente S, Dominguez JM, Knauf JA, Viale A, Sherman EJ, et al. Relief of feedback inhibition of HER3 transcription by RAF and MEK inhibitors attenuates their antitumor effects in BRAF mutant thyroid carcinomas. Cancer Discov. 2013;3(5):520–33.

Okamoto K, Kodama K, Takase K, Sugi NH, Yamamoto Y, Iwata M, et al. Antitumor activities of the targeted multi-tyrosine kinase inhibitor lenvatinib (E7080) against RET gene fusion-driven tumor models. Cancer Lett. 2013;340(1):97–103.

Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling - in control of vascular function. Nat Rev Mol Cell Biol. 2006;7(5):359–71.

Ouyang B, Knauf JA, Smith EP, Zhang L, Ramsey T, Yusuff N, et al. Inhibitors of Raf kinase activity block growth of thyroid cancer cells with RET/PTC or BRAF mutations in vitro and in vivo. Clin Cancer Res. 2006;12(6):1785–93.

Prager GW, Koperek O, Mayerhoefer ME, Muellauer L, Wrba F, Niederle B, et al. Sustained response to vemurafenib in a BRAFV600E-mutated anaplastic thyroid carcinoma patient. Thyroid. 2016;26(10):1515–6.

Rahal R, Evans EK, Hu W, Maynard M, Fleming P, DiPietro L, et al. The development of potent, selective RET inhibitors that target both wild-type RET and prospectively identified resistance mutations to multi-kinase inhibitors. Cancer Res. 2016;76(14 Supplement):2641.

Rheault TR, Stellwagen JC, Adjabeng GM, Hornberger KR, Petrov KG, Waterson AG, et al. Discovery of dabrafenib: a selective inhibitor of Raf Kinases with antitumor activity against B-Raf-driven tumors. ACS Med Chem Lett. 2013;4(3):358–62.

Ricarte-Filho JC, Ryder M, Chitale DA, Rivera M, Heguy A, Ladanyi M, et al. Mutational profile of advanced primary and metastatic radioactive iodine-refractory thyroid cancers reveals distinct pathogenetic roles for BRAF, PIK3CA, and AKT1. Cancer Res. 2009;69(11):4885–93.

Rodriguez-Antona C, Pallares J, Montero-Conde C, Inglada-Perez L, Castelblanco E, Landa I, et al. Overexpression and activation of EGFR and VEGFR2 in medullary thyroid carcinomas is related to metastasis. Endocr Relat Cancer. 2010;17(1):7–16.

Rothenberg SM, McFadden DG, Palmer EL, Daniels GH, Wirth LJ. Redifferentiation of iodine-refractory BRAF V600E-mutant metastatic papillary thyroid cancer with dabrafenib. Clin Cancer Res. 2015;21(5):1028–35.

Saji M, Ringel MD. The PI3K-Akt-mTOR pathway in initiation and progression of thyroid tumors. Mol Cell Endocrinol. 2010;321:20–8.

Sala E, Mologni L, Truffa S, Gaetano C, Bollag GE, Gambacorti-Passerini C. BRAF silencing by short hairpin RNA or chemical blockade by PLX4032 leads to different responses in melanoma and thyroid carcinoma cells. Mol Cancer Res. 2008;6(5):751–9.

Schlumberger M, Jarzab B, Elisei R, Siena S, Bastholt L. de la Fouchardiere C. Phase III randomized, double-blinded, placebo-controlled trial of sorafenib in locally advanced or metastatic patients with radioactive iodine (RAI)-refractory differentiated thyroid cancer (DTC)-exploratory analyses of patient-reported outcomes. Thyroid. 2013;23(Suppl. 1):A-49.

Schlumberger M, Tahara M, Wirth LJ, Robinson B, Brose MS, Elisei R, et al. Lenvatinib versus placebo in radioiodine-refractory thyroid cancer. N Engl J Med. 2015;372(7):621–30.

Schneider TC, de Wit D, Links TP, van Erp NP, van der Hoeven JJM, Gelderblom H, et al. Beneficial effects of the mTOR inhibitor everolimus in patients with advanced medullary thyroid carcinoma: subgroup results of a phase II trial. Int J Endocrinol. 2015;2015:348124.

Schneider TC, de Wit D, Links TP, van Erp NP, van der Hoeven JJM, Gelderblom H, et al. Everolimus in patients with advanced follicular-derived thyroid cancer: results of a phase II clinical trial. J Clin Endocr Metab. 2017;102(2):698–707.

Shah MH, Wei L, Wirth LJ, Daniels GA, Souza JAD, Timmers CD, et al. Results of randomized phase II trial of dabrafenib versus dabrafenib plus trametinib in BRAF-mutated papillary thyroid carcinoma. J Clin Oncol. 2017;35(15_suppl):6022.

Sharma P, Allison JP. The future of immune checkpoint therapy. Science (New York, NY). 2015;348(6230):56–61.

Sherman SI. Cytotoxic chemotherapy for differentiated thyroid carcinoma. Clin Oncol (R Coll Radiol). 2010;22:464–8.

Sherman SI. Lessons learned and questions unanswered from use of multitargeted kinase inhibitors in medullary thyroid cancer. Oral Oncol. 2013;49(7):707–10.
Sherman EJ, Ho AL, Fury MG, Baxi SS, Dunn L, Lee JS, et al. Combination of everolimus and sorafenib in the treatment of thyroid cancer: update on phase II study. J Clin Oncol. 2015;33(15):6069.
Sherman SI, Clary DO, Elisei R, Schlumberger MJ, Cohen EE, Schoffski P, et al. Correlative analyses of RET and RAS mutations in a phase 3 trial of cabozantinib in patients with progressive, metastatic medullary thyroid cancer. Cancer. 2016;122(24):3856–64.
Sherman EJ, Dunn LA, Ho AL, Baxi SS, Ghossein RA, Fury MG, et al. Phase 2 study evaluating the combination of sorafenib and temsirolimus in the treatment of radioactive iodine-refractory thyroid cancer. Cancer. 2017;123(21):4114–21.
Soh EY, Clark OH. Surgical considerations and approach to thyroid cancer. Endocrinol Metab Clin N Amer. 1996;25:115–39.
Soh EY, Eigelberger MS, Kim KJ, Wong MG, Young DM, Clark OH, et al. Neutralizing vascular endothelial growth factor activity inhibits thyroid cancer growth in vivo. Surgery. 2000;128 (6):1059–65; discussion 65–6.
Spitzweg C, Bible KC, Hofbauer LC, Morris JC. Advanced radioiodine-refractory differentiated thyroid cancer: the sodium iodide symporter and other emerging therapeutic targets. Lancet Diabetes Endocrinol. 2014;2(10):830–42.
St Bernard R, Zheng L, Liu W, Winer D, Asa SL, Ezzat S. Fibroblast growth factor receptors as molecular targets in thyroid carcinoma. Endocrinology. 2005;146(3):1145–53.
Subbiah V, Roszik J. Towards precision oncology in RET-aberrant cancers. Cell Cycle. 2017;16(9): 813–4.
Subbiah V, Kreitman RJ, Wainberg ZA, Cho JY, Schellens JHM, Soria J-C, et al. Efficacy of dabrafenib (D) and trametinib (T) in patients (pts) with BRAF V600E–mutated anaplastic thyroid cancer (ATC). J Clin Oncol. 2017;35(15_suppl):6023.
Takahashi S, Kiyota N, Yamazaki T, Chayahara N, Nakano K, Inagaki L, et al. Phase II study of lenvatinib in patients with differentiated, medullary, and anaplastic thyroid cancer: final analysis results. J Clin Oncol. 2016;34:6088.
Tang C, Welsh JW, de Groot P, Massarelli E, Chang JY, Hess KR, et al. Ipilimumab with stereotactic ablative radiation therapy: phase I results and immunologic correlates from peripheral T cells. Clin Cancer Res. 2017;23(6):1388–96.
Tuttle RM, Haddad RI, Ball DW, Byrd D, Dickson P, Duh QY, et al. Thyroid carcinoma, version 2.2014. J Natl Compr Canc Netw. 2014;12(12):1671–80; quiz 80.
Verrienti A, Tallini G, Colato C, Boichard A, Checquolo S, Pecce V, et al. RET mutation and increased angiogenesis in medullary thyroid carcinomas. Endocr-Relat Cancer. 2016;23(8):665–76.
Verschraegen CF, Rehman H, Pulluri B, DeKay J, Barry M, Folefac E, et al. When clinical trial participation is not an option in the era of personalized medicine: the case of two patients. Oncology. 2017;92(1):55–60.
Vieira JM, Santos SCR, Espadinha C, Correia I, Vag T, Casalou C, et al. Expression of vascular endothelial growth factor (VEGF) and its receptors in thyroid carcinomas of follicular origin: a potential autocrine loop. Eur J Endocrinol. 2005;153(5):701–9.
Wedge SR, Ogilvie DJ, Dukes M, Kendrew J, Chester R, Jackson JA, et al. ZD6474 inhibits vascular endothelial growth factor signaling, angiogenesis, and tumor growth following oral administration. Cancer Res. 2002;62(16):4645–55.
Wells Jr SA, Robinson BG, Gagel RF, Dralle H, Fagin JA, Santoro M, et al. Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. J Clin Oncol. 2012;30(2):134–41.
White PS, Pudusseri A, Lee SL, Eton O. Intermittent dosing of dabrafenib and trametinib in metastatic BRAFV600E mutated papillary thyroid cancer: two case reports. Thyroid. 2017; 27(9):1201–5.
Wilhelm SM, Carter C, Tang L, Wilkie D, McNabola A, Rong H, et al. BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis. Cancer Res. 2004;64(19):7099–109.

Xing M, Alzahrani AS, Carson KA, Viola D, Elisei R, Bendlova B, et al. Association between BRAF V600E mutation and mortality in patients with papillary thyroid cancer. JAMA. 2013;309(14):1493–501.

Xing M, Alzahrani AS, Carson KA, Shong YK, Kim TY, Viola D, et al. Association between BRAF V600E mutation and recurrence of papillary thyroid cancer. J Clin Oncol. 2015;33(1):42–50.

Yakes FM, Chen J, Tan J, Yamaguchi K, Shi Y, Yu P, et al. Cabozantinib (XL184), a novel MET and VEGFR2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth. Mol Cancer Ther. 2011;10(12):2298–308.

Yeh TC, Marsh V, Bernat BA, Ballard J, Colwell H, Evans RJ, et al. Biological characterization of ARRY-142886 (AZD6244), a potent, highly selective mitogen-activated protein kinase kinase 1/2 inhibitor. Clin Cancer Res. 2007;13(5):1576–83.

Yu XM, Lo CY, Chan WF, Lam KY, Leung P, Luk JM. Increased expression of vascular endothelial growth factor C in papillary thyroid carcinoma correlates with cervical lymph node metastases. Clin Cancer Res. 2005;11(22):8063–9.

Part VII

Other Conditions Influencing Thyroid Function or Inducing Thyroid Dysfunction

Thyroid Physiology and Thyroid Diseases in Pregnancy

23

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Abstract

Thyroid disorders are common in pregnancy and can result in serious complications for the pregnant woman and her child. It is well recognized that optimum treatment of both overt hypothyroidism and hyperthyroidism reduces the complications, and pregnant women with these conditions should be treated. However, because of limited evidence for the benefit from intervention studies, it remains controversial whether subclinical thyroid diseases, such as subclinical hypothyroidism, maternal hypothyroxinemia, and euthyroid autoimmune thyroid disease should be treated and whether all pregnant women should be screened for these conditions. Management of many thyroid disorders (for example, Graves' disease) in pregnant women is complex and requires a multidisciplinary approach for optimal outcome. This chapter summarizes current knowledge of thyroid physiology and management of thyroid disorders in pregnancy.

Keywords

Graves' disease · Hypothyroidism · Human chorionic gonadotrophin · Iodine · Pregnancy · TPO antibodies · TSH receptor antibodies · Thyroxine · Hypothyroxinemia

Abbreviations

Ab	Antibodies
ART	Assisted reproductive technology
FNAC	Fine needle aspiration cytology
hCG	Human chorionic gonadotrophin
HLA	Human leukocyte antigen
HPT	Hypothalamic-pituitary-thyroid
IQ	Intelligence quotient
ICCIDD	International Council for the Control of Iodine Deficiency Disorders
T3	Triiodothyronine
T4	Thyroxine
TBG	Thyroxine binding globulin
Tg	Thyroglobulin
Th1	Type 1 helper T cell
Th2	Type 2 helper T cell
TPO	Thyroid peroxidase
TR	Thyroid hormone receptor
T_{REG}	Regulatory CD4 + CD25 + T cells
TSH	Thyrotrophin
UIC	Urinary iodine concentration
WHO	World Health Organisation

Introduction

Thyroid disorders are common amongst women of the reproductive age. Due to the physiological changes of pregnancy, preexisting thyroid disorders may get exacerbated or go into remission during pregnancy. New onset thyroid disease may also arise during pregnancy or a thyroid condition that predated pregnancy may only get diagnosed when a young woman seeks antenatal care. Thus, it is important that clinicians are aware of the potential effects of pregnancy on thyroid conditions and vice versa and adopt a multidisciplinary approach for effective management.

There is good understanding of the normal physiological changes within the thyroid axis during pregnancy and also well-documented historical evidence of improved pregnancy outcomes with treatment of overt thyroid disease. However, there is much variation in clinical practice with regards the precise management of thyroid disorders in pregnancy and many aspects remain controversial. Moreover, managing women in pregnancy requires the consideration of several additional dimensions: an understanding of the dynamic physiological changes occurring across the duration of pregnancy, the concept of time-limited gestational windows of development, as well as an appreciation that the interests of both mother and fetus need to be considered, and sometimes their interests are conflicting. This chapter will provide a framework comprising current knowledge of thyroid physiology and disease in pregnancy to which new knowledge can be easily incorporated in this rapidly progressing field.

Changes in Maternal Thyroid Physiology in Pregnancy

Following fertilization, the blastocyst starts releasing endocrine signals that bring about a cascade of physiological changes in the pregnant women even before she is aware she is pregnant. One of the earliest signals from the conceptus is the release of human chorionic gonadotrophin (hCG), which shares the same alpha subunit as thyrotropin (TSH) but has a beta subunit which differs in the terminal 30 amino acids. The peak in hCG level occurs around 10 weeks' gestation and begins to decline from 14 weeks to a stable low level from 20 weeks until delivery. The high homology to TSH makes hCG mildly thyrotrophic and able to stimulate the thyroid gland to increase thyroxine production from the start of pregnancy (Fig. 1). This predates the estrogen-stimulated rise in thyroxine binding globulin (TBG) occurring from 6–8 weeks gestation. Increased TBG concentrations result in a corresponding increase in total circulating thyroxine by 50% in the first trimester which is sustained until the end of pregnancy (Chan and Mandel 2007).

A normally functioning thyroid gland would be able to meet this increased thyroid demand. The resultant effect is a significant decline in circulating TSH concentrations over the course of the first trimester, reaching a nadir around 10 weeks' gestation when the hCG level is at its peak (Glinoer et al. 1990, 1993). In about 3% of normal pregnancies, almost complete TSH suppression occurs. Clear ethnic variation is observed with a greater gestational suppression of TSH occurring

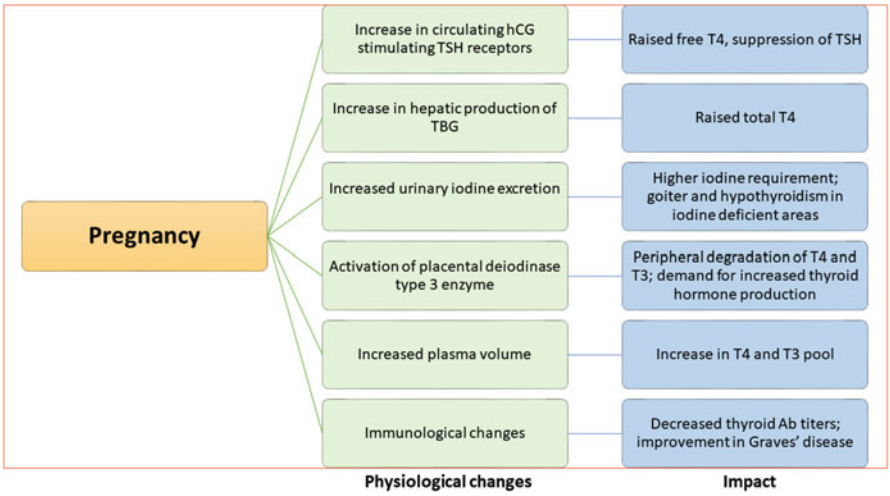

Fig. 1 Physiological changes in pregnancy and their impact on the thyroid (Abbreviations: Ab, antibodies; hCG, human chorionic gonadotrophin; TBG, thyroxine binding globulin; TSH, thyrotrophin; T4, thyroxine; T3 triiodothyronine)

in Asians and Blacks compared with Caucasians (Price et al. 2001; Benhadi et al. 2007). TSH then stabilizes at a level slightly lower than preconception concentrations in the second trimester before rising again during the third trimester towards preconception concentrations (Dashe et al. 2005). On the other hand, free thyroxine (T4) concentrations would rise in the first trimester then gradually decline over the course of pregnancy. Mostly the rise of free T4 in the first trimester is only slight but in a small proportion of cases significantly elevated free T4 concentrations similar to those seen in thyrotoxicosis may occur (see below, gestational transient thyrotoxicosis). The degree of decline in later pregnancy is largely dependent on the assay platform used for measurement (as they are differentially affected by the protein milieu of pregnancy) and is typically around 20% to 50% from the first trimester to the end of pregnancy (Sapin et al. 2004). Some have argued that the hypothalamic-pituitary-thyroid (HPT) axis dynamically changes its negative feedback set point centrally across gestation to maintain appropriate levels of thyroid hormone at each stage of pregnancy for optimal fetal development (Brent 1997).

Increased thyroid hormone demand and changes in thyroid function during pregnancy are also due to several other factors (Fig. 1). There is a gradual increase in maternal blood volume starting from the first trimester and rising by as much as 40% by the second trimester of pregnancy. The placenta is rich in deiodinase type 3 enzymes which metabolize thyroid hormones and regulate the trans-placental passage of thyroid hormones from the mother to the fetus (Chan et al. 2009). In iodine sufficient regions of the world, a physiological increase in the size of the thyroid gland by an average of 10% to 20% contributes to the maintenance of thyroid homeostasis during pregnancy (Rasmussen et al. 1989; Glinoer et al. 1990).

Iodine is an essential constituent of thyroid hormones. Renal excretion of iodine increases from the first trimester requiring a higher daily intake of iodine to ensure the increased thyroid hormone production can be sustained. With mild to moderate iodine deficiency, some further diffuse enlargement of the thyroid gland may occur, so the normal physiological changes in thyroid function may not be affected significantly (Zimmermann and Delange 2004), but in more severe iodine deficiency, pregnancy can trigger significant hypothyroidism or hypothyroxinemia, particularly in the third trimester (Zimmermann 2009).

During pregnancy, the mother's immune system undergoes several physiological changes to maintain tolerance of her fetus. Of these changes, an expansion of the specific regulatory CD4+ CD25+ T (T_{REG}) cells is the most important as these cells play a crucial role in maintaining the immune tolerance (Weetman 2010). This expansion starts in early pregnancy, the number of T_{REG} cells increasing rapidly and peaking in the second trimester. They accumulate mostly in the decidua and to a less extent in the maternal peripheral circulation. T_{REG} cells are capable of suppressing both type 1 helper T cell (Th1) and type 2 helper T cell (Th2) immune response against fetal alloantigens. Because of this physiological state of suppressed immune responsiveness, levels of autoantibodies (including TSH receptor antibodies) tend to decline during pregnancy, leading to amelioration of several autoimmune disorders, including Graves' disease. After delivery, the number of T_{REG} cells rapidly falls to prepregnancy level, which can often lead to postpartum exacerbation of thyroid autoimmunity (for example, relapse of Graves' disease). In addition to the expansion of T_{REG} cells, immunomodulatory effects of hormones (for example, reduced B cell activity in response to increased progesterone level), a shift from Th1 to Th2 cytokines profile and the expression of immune regulatory molecules (for example, HLA G) in the trophoblast also contribute to the immune tolerance in pregnancy.

Regulation of Fetal Thyroid Function and Thyroid Hormone Action

The fetal thyroid gland is formed and begins accumulating iodine from 10–12 weeks of gestation but only starts to endogenously release an appreciable amount of thyroid hormones from 16–18 weeks of gestation following the secretion of pituitary TSH (Thorpe-Beeston et al. 1991). However, a fully mature HPT axis with appropriate negative feedback is not attained until postnatal life. Consequently, during in utero life both TSH and thyroid hormones rise concurrently and different physiological mechanisms are employed during fetal development to regulate biological activity at a tissue level. Also, the fetal thyroid is especially sensitive to excessive iodine which can suppress fetal thyroid activity (Williams et al. 2017).

Transplacental passage of maternal thyroid hormones, primarily thyroxine, from the mother to the fetus occurs throughout gestation. Circulating thyroid hormones in the fetus before 16 weeks' gestation are of maternal origin only and becomes of dual origin following endogenous fetal production. In athyroidal fetuses, transplacental

maternal supply is capable of restoring about 50% of normal fetal thyroid hormone levels by the end of pregnancy (Vulsma et al. 1989). In normal healthy pregnancies, there is a positive correlation between maternal third trimester free T4 levels and fetal free T4 levels in cord blood at birth, with every 10% increase in maternal free T4 leading to a 0.18 pmol/L increase in fetal cord blood free T4 (Shields et al. 2011). These observations indicate that maternal thyroid hormones contribute to the thyroid hormone pool of the fetus throughout its in utero life, underlining the importance of maintaining normal maternal thyroid function from early gestation right up to the end of gestation. Premature neonates suffer an abrupt termination of maternal thyroid supply resulting in significantly lower circulating thyroxine concentrations compared with in utero fetuses of the same gestational age; however, iodine and thyroxine supplementation in this context remains controversial (Williams et al. 2006, 2014; van Wassenaer-Leemhuis et al. 2014).

The fetus and placenta themselves require thyroid hormones for optimal development, even before endogenous fetal thyroid hormone production. Classical understanding of thyroid hormone action is that circulating T4 is activated by local tissue conversion to the active thyroid hormone ligand, triiodothyronine (T3), by deiodinase type 2 enzyme. It is clear from animal models that thyroid hormones are important for normal fetal brain development from very early pregnancy (de Escobar et al. 2008), and human data demonstrate that the fetal brain could be thyroid-responsive from the early first trimester (Chan et al. 2002). In later pregnancy, thyroid hormone also play a role in the maturation of the auditory apparatus; pulmonary, cardiovascular, hepatic, and adipose tissues; skeletal muscles; bones; and the autonomic nervous system in preparation for extrauterine life (Fowden and Forhead 2013). Thyroid hormones also directly regulate placental development (Barber et al. 2005; Oki et al. 2004; Vasilopoulou et al. 2010) and decidual cell function (Vasilopoulou et al. 2014) and can influence pregnancy outcome.

In utero thyroid hormone-dependent development is both spatially and temporally regulated (Chan et al. 2009). Orchestrating such complex requirements demand multiple levels of regulation, at systemic as well as at individual tissue levels. Excessive or inadequate, premature or delayed thyroid hormone action could all potentially harm the course of normal development. Firstly, the transfer of thyroid hormones across the placenta is controlled by ontogenic changes in the expression and activity of multiple plasma membrane thyroid hormone transporters, deiodinase enzymes (especially deiodinase type 3 whose activity declines with gestation), other enzymes that metabolize thyroid hormone, and secretion of thyroid hormone binding proteins (e.g., transthyretin) at the maternal-trophoblastic interface (Chan 2010). Similarly, the local fetal tissue action of thyroid hormones is regulated by developmental changes in transporter, enzyme, and receptor activities. Secondly, fetal production of systemic thyroid hormone binding proteins is initially low to allow the small amount of transplacentally derived thyroid hormone to attain physiologically relevant free T4 concentrations for biological action (Calvo et al. 2002). Binding proteins then increases as thyroid hormone availability rises. Thirdly, unlike adults, where circulating thyroid hormones comprise predominantly T4 and T3, circulating fetal thyroid hormones are found in wide ranging forms including reverse

T3, sulfated and glucuronidated iodothyronines, and other metabolites such as iodothyronamines, which may all have separate biological roles as demonstrated in animal models (Wu et al. 2005; Farwell et al. 2006). However, the main mechanism of action of TH in the fetus is still that mediated by classical thyroid hormone receptors (TRs) and the active ligand, T3. Nonetheless, the availability of thyroid hormones in multiple other forms, which do not directly bind TRs, enables timely tissue-specific T3 activity since only specific tissues expressing the relevant transporters and enzymes can utilize particular forms of thyroid hormones. In this way, tissues can exercise local control of thyroid hormone action by regulating its own timely expression of the required proteins for action, while other tissues are protected from undue thyroid hormone exposure (Visser 1996).

Assessment of Thyroid Function in Pregnancy

The dynamic physiological changes involving the thyroid axis at different stages of pregnancy impacts on the assessment of thyroid function in pregnant women in several ways. Firstly, the reference ranges of thyroid function tests for pregnant women are different from those of nonpregnant general population. There are also wide variations in the reference ranges across different countries (in part explained by population iodine status), ethnicity, and assay platforms. Secondly, the thyroid stimulating action of placental hCG and the varying levels of circulating hCG in different trimesters mean trimester-specific reference ranges of TSH and free thyroid hormones are necessary to assess thyroid function in pregnancy. Thirdly, the measurement of free thyroid hormones (free T4 and free T3) when carried out using an automated immunoassay is affected by changes in TBG and albumin levels in pregnancy leading to a substantial decrease in free T4 levels in the third trimester. However, this problem is assay dependent, with the effect more pronounced with some assays than the others. These issues are less relevant when free thyroid hormones are analyzed with methods using direct measurement (for example, assays using equilibrium dialysis). However, assays based on direct measurement are labor intensive, expensive, and not readily available for routine clinical practice. The establishment and the use of assay specific reference ranges can ameliorate the problem to some extent. Finally, because of the above-mentioned problems associated with measurement of free thyroid hormones, some experts recommend the use of total thyroid hormones (total T4 and total T3) levels in pregnancy. However, because of the increase in TBG level during pregnancy, total thyroid hormone levels are higher than in nonpregnant state. If total T4 measured in pregnancy, the reference range should be adjusted depending upon the gestational age at the time of the test (gestational age: <7 weeks – no adjustment, 7–16 weeks – increase upper limit of the nonpregnant reference range by 5% per week starting at week 7, >16 weeks – 50% higher than the prepregnancy reference range) (Alexander et al. 2017). Total thyroid hormone assays are no longer in routine use in many countries.

Although ideally each laboratory should determine and use assay and trimester specific reference ranges for the thyroid function tests in pregnancy, these are not widely available. In the absence of assay specific and trimester specific reference

ranges, the guidelines from the European Thyroid Association recommend the upper limit of TSH reference range as 2.5 mIU/L in the first trimester, 3.0 mIU/L in the second trimester, and 3.5 mIU/L in the third trimester (Lazarus et al. 2014). However, the recent guidelines from the American Thyroid Association recommend the upper limit of the TSH reference range in the first trimester as 4.0 mIU/L or 0.5 mIU/L lower than the upper limit of the nonpregnant reference range, and lesser adjustments from the nonpregnant reference ranges in the second and third trimesters (Alexander et al. 2017), highlighting the ongoing controversy surrounding the TSH reference ranges in pregnancy.

Iodine Deficiency and Its Consequences

Iodine deficiency is the leading cause of congenital mental impairment worldwide. In 2011, a survey of 115 countries representing 96.1% of the global population estimated that 29.8% of all school-aged children worldwide continue to have insufficient iodine intake despite major strides made in global salt-iodization programs since the turn of the century (Andersson et al. 2012). Adequate iodine intake is most pertinent during pregnancy and lactation given the increased iodine requirements and the importance of iodine to early brain development.

The iodine status of a population can be assessed by measuring median urinary iodine concentration (UIC) (WHO et al. 2007). In a general population, median UIC of 100 μg/L or higher is suggestive of iodine sufficiency, while median UIC of 50–99 μg/L is classified as mild iodine deficiency, 20–49 μg/L as moderate iodine deficiency, and <20 μg/L as severe iodine deficiency. For a population of pregnant women, a median UIC of 150–249 μg/L suggests an optimum iodine status (Table 1) (WHO and UNICEF 2007). As urinary iodine excretion fluctuates widely depending upon the recent iodine intake, UIC is only suitable for the assessment of iodine status at a population level and not for diagnosing iodine deficiency in an individual (Zimmermann 2009).

With severe iodine deficiency, iodine supplementation in pregnancy improves maternal T4 concentrations (Zimmermann 2009) with correspondingly decreased offspring cretinism rates (Pharoah et al. 1971) and increased offspring intelligence quotient (IQ) by about 12 points (Qian et al. 2005). Severe iodine deficiency is also associated with increased perinatal mortality, early infant death, and miscarriage with good evidence that supplementation can also significantly reduce these risks (Chaouki and Benmiloud 1994; DeLong et al. 1997).

With mild-to-moderate maternal iodine deficiency, a milder neurodevelopmental phenotype has been described. A dose-dependent delay in verbal IQ and reading comprehension, and lower IQ scores in offspring at aged 8–9 years have been reported (Bath et al. 2013), but there has been no clear evidence reported of adverse obstetric outcomes. With iodine supplementation in this context, however, maternal and new-born thyroid function remained unchanged (Zimmermann 2009), and available data from the few nonrandomized prospective intervention studies with relatively small sample sizes have shown conflicting results with respect to offspring neurocognition (Berbel et al. 2009; Velasco et al. 2009; Murcia et al. 2011). Systematic reviews of

Table 1 The criteria for iodine status of a population of pregnant women

Iodine status	Median urinary iodine concentration
Insufficient	<150 μg/L
Adequate	150–249 μg/L
Above requirements	250–499 μg/L
Excessive	≥ 500 μg/L

Table 2 The WHO recommended daily iodine intake for different population groups

Populations	Daily intake of iodine
Women of reproductive age (15–49 years)	150 μg
Pregnant women	250 μg
Lactating women	250 μg

iodine supplementation in mild-moderately deficient pregnant populations could not draw definitive conclusions, and in the absence of high-quality evidence from placebo-controlled clinical trials the benefits of iodine supplementation remains debatable (Taylor et al. 2013; Zhou et al. 2013). It is possible that iodine may not only have a positive effect on brain development through improvements in thyroid function and may also have other thyroid-independent effects on fetal development (Yang et al. 2007).

The World Health Organisation (WHO), UNICEF, and International Council for the Control of Iodine Deficiency Disorders (ICCIDD) recommend that all pregnant and lactating women should have a total daily iodine intake of 250 μg (Table 2) (WHO and UNICEF 2007). Dietary sources of iodine include milk and dairy products, fish, shellfish, egg, meat, and iodized salt. WHO and UNICEF recommend routine iodine supplements in pregnancy in a population where less than 90% of households use iodized salt and where the median urinary iodine in school children is consistent with iodine deficiency (WHO and UNICEF 2007). Meanwhile professional bodies such as the Endocrine Society and the American Thyroid Association recommend routine oral iodine supplementation of 150 μg (a dose found in many common antenatal multivitamin nutritional supplements) in all pregnant and lactating women (De Groot et al. 2012; Alexander et al. 2017) regardless of the iodine status of the population since iodine deficiency is prevalent during pregnancy even in an ordinarily iodine-replete population. A cost-effectiveness study using conservative models that limited the benefits of iodine supplementation and overestimated its potential harms still found that universal iodine supplementation in pregnancy was cost saving in a mild to moderate iodine-deficient population in the absence of a population-based iodine supplementation program such as universal salt iodization (Monahan et al. 2015). In iodine-replete countries, excess iodine intake during pregnancy can be a potential hazard as well, since excessive iodine intake (UIC ≥500 μg/L) has been shown to be associated with a twofold increase in the risk of subclinical hypothyroidism (Shi et al. 2015). An increased awareness of the optimal iodine requirement in pregnancy as well as the knowledge of different sources of iodine is necessary to improve the compliance and achieve an optimum iodine intake in pregnant women.

Hypothyroidism

Disruption in the hypothalamic-pituitary-thyroid axis leading to any decreased thyroid function can be considered in three broad categories: (a) overt hypothyroidism (defined as elevated serum TSH concentration with below normal free T4 concentration), (b) subclinical hypothyroidism (elevated serum TSH concentration with normal free T4 concentration), and (c) isolated hypothyroxinemia (normal TSH concentration with below normal free T4 concentration). A distinction can also be made between thyroid function abnormalities that predate pregnancy or those that are triggered by the physiological "thyroid-stress test of pregnancy".

Overt Hypothyroidism

Overt hypothyroidism affects 0.3–0.5% of pregnant women. Chronic autoimmune thyroiditis is the commonest cause of hypothyroidism in iodine sufficient countries. This is usually associated with the presence of thyroid peroxidase (TPO) or thyroglobulin (Tg) autoantibodies. However, rarely, hypothyroidism is caused by the presence of blocking TSH receptor antibodies (Konishi et al. 1985). Iatrogenic hypothyroidism following radioactive iodine treatment or thyroidectomy for thyrotoxicosis is also a common cause. In many parts of the world, iodine deficiency remains the most important cause (Table 3).

Pregnant women with overt hypothyroidism may present with classical symptoms and signs, including tiredness, cold intolerance, and weight gain. However, the symptoms of hypothyroidism are nonspecific and often confused with those of pregnancy leading to a delay in diagnosis. Therefore, a high index of suspicion is necessary for new diagnosis of hypothyroidism in pregnancy.

As thyroid hormone is important for neurological development of the fetus and the fetus relies on maternal thyroid hormone for its development, particularly during early pregnancy, untreated or inadequately treated maternal hypothyroidism during pregnancy may impair neuropsychological development of the offspring. This is most evident in the case of severe iodine deficiency causing hypothyroidism in both the mother and the fetus, where the child suffers from severe neurological deficit (cretinism). More recent studies, however, indicate that even milder forms of maternal hypothyroidism in pregnancy may affect

Table 3 Causes of hypothyroidism in pregnancy

Chronic autoimmune thyroiditis
Iodine deficiency
Post-thyroidectomy
Post-radioiodine treatment
Drugs (for example, antithyroid drugs, amiodarone, lithium)
Subacute or painless thyroiditis
Postpartum thyroiditis
Hypopituitarism (secondary hypothyroidism)

Table 4 Adverse outcomes associated untreated or inadequately treated maternal overt hypothyroidism in pregnancy

Maternal adverse effects	Fetal adverse effects
Gestational hypertension	Fetal loss (miscarriage and still birth)
Preeclampsia and eclampsia	Premature birth
Postpartum hemorrhage	Placental abruption
	Intrauterine growth retardation
	Low birth weight
	Impaired neuropsychological development
	Increased neonatal respiratory distress

offspring neuropsychological development, including a reduction of IQ (Haddow et al. 1999). In addition, untreated maternal hypothyroidism is also associated with several obstetric outcomes, including spontaneous miscarriage, still birth, preterm delivery, preeclampsia, placental abruption, and postpartum hemorrhage (Table 4).

Management of Pregnant Women with a New Diagnosis of Overt Hypothyroidism

All pregnant women with a new diagnosis of overt hypothyroidism should be treated with levothyroxine replacement as soon as possible. To achieve euthyroidism promptly, full replacement dose of levothyroxine (2 μg/kg body weight) should be started. The dose of levothyroxine should then be adjusted based on thyroid function tests every 4–6 weeks throughout the pregnancy with an aim to keep TSH within the trimester specific reference range or below 2.5 mu/L. However, care must be taken to avoid over-replacement of levothyroxine as it has been shown that high maternal serum free T4 level (as well low free T4 level) during pregnancy may have an adverse impact on the offspring's neurodevelopment (Korevaar et al. 2016).

Management of Pregnant Women with Preexisting Hypothyroidism

In contrast to healthy pregnant women, hypothyroid women are less able to increase endogenous production of thyroid hormone in response to the stimulation of placental hCG during pregnancy. Therefore, most women with preexisting hypothyroidism need an increased dose of levothyroxine during pregnancy to maintain euthyroidism, and this increase in thyroid hormone requirement occurs as early as 4 weeks of gestation (Alexander et al. 2004). Indeed, a failure to optimize levothyroxine dose in pregnant women with hypothyroidism can result in raised serum TSH levels indicating an inadequate control of hypothyroidism and leading to adverse pregnancy outcomes, such as miscarriages (Taylor et al. 2014). The extent of the dose increment required to maintain euthyroidism in pregnancy depends upon the etiology and the preconception control of hypothyroidism. Women with hypothyroidism due to Hashimoto's thyroiditis tend to require a smaller dose increment than women who had total thyroidectomy or ablative dose of radioactive iodine. Likewise, women with good control of hypothyroidism, as indicated by serum TSH

level towards the lower end of reference range, in the preconception period require a smaller or sometimes no increment in the levothyroxine dose during pregnancy.

Women with preexisting hypothyroidism should ideally optimize the levothyroxine dose before conception to keep serum TSH between lower end of the reference range and 2.5mIU/L. They should increase the dose of levothyroxine by 25–30% as soon as pregnancy is confirmed. A pragmatic approach is to advise the women to double their dose of levothyroxine on 2 days per week after a positive pregnancy test (Yassa et al. 2010). The levothyroxine dose should be further adjusted with thyroid function tests every 4–6 weeks during the pregnancy to keep serum TSH within the trimester specific reference range or below 2.5 mu/L. It is important to inform the women that some drugs commonly taken during pregnancy, for example, iron tablets, may interfere with the absorption of levothyroxine if taken together. Following delivery, the women should reduce the dose of levothyroxine to prepregnancy dose, and repeat thyroid function tests in 6–8 weeks postdelivery. Women on levothyroxine can breast feed without causing any adverse effect on the baby's thyroid function.

Subclinical Hypothyroidism

Subclinical hypothyroidism, also known as mild hypothyroidism, affects about 2–3% of pregnant women. However, the prevalence is widely variable in different populations, depending upon the iodine status of the population, ethnicity and TSH cut-offs used for the definition of subclinical hypothyroidism. Indeed, some studies using the first trimester TSH cut-off values of 2.5 mIU/L, as recommended by the previous guidelines from the American Thyroid Association and the Endocrine Society (Stagnaro-Green et al. 2011; De Groot et al. 2012), have reported the prevalence of subclinical hypothyroidism in pregnancy as high as 15% (Blatt et al. 2012).

Due to the changes in the thyroidal physiology and the increased demand for thyroid hormone production in pregnancy, some women who are euthyroid in the preconception period may develop subclinical hypothyroidism when they become pregnant. This risk is particularly high for women living in iodine deficient regions. However, in iodine-replete countries, excessive iodine intake is also a recognized risk factor for subclinical hypothyroidism in pregnancy (Shi et al. 2015). Another important risk factor for subclinical hypothyroidism is thyroid autoimmunity, which may be associated with a reduced thyroid reserve. It has been shown that the stimulation of thyroid hormone secretion in response to placental hCG in early pregnancy is blunted in women with TPO antibodies (Korevaar et al. 2017). Finally, several common genetic variants are known to influence serum TSH and thyroid hormone levels, and one of these (variants of the Phosphodiesterase-8B gene) has been shown to be associated with subclinical hypothyroidism in pregnancy (Shields et al. 2009).

Most pregnant women with subclinical hypothyroidism do not present with symptoms of hypothyroidism, and the diagnosis is usually made following a thyroid function test carried out as a screening or as a part of case-finding strategy. Although

studies assessing association between subclinical hypothyroidism and pregnancy outcomes have not shown consistent results, there is evidence to support that pregnant women with subclinical hypothyroidism have an increased risk of several adverse pregnancy outcomes, including fetal loss, placental abruption, premature rupture of membranes, premature birth, and neonatal death (van den Boogaard et al. 2011; Chan and Boelaert 2015; Maraka et al. 2016). There is also weak evidence to support for an association between subclinical hypothyroidism and impaired neuropsychological development in offspring (Fan and Wu 2016). Whether levothyroxine treatment can prevent the adverse obstetric outcomes remains uncertain; however, there is some evidence for the benefit in women with positive TPO antibodies (Negro et al. 2006; Reid et al. 2013; Nazarpour et al. 2017). In contrast, two large randomized controlled trials have failed to show a benefit of levothyroxine treatment for maternal subclinical hypothyroidism in pregnancy to improve neuropsychological outcomes in offspring (Lazarus et al. 2012; Casey et al. 2017). However, a major limitation of both studies is that levothyroxine treatment began rather late in the first or early second trimesters of pregnancy when the most critical time for thyroid hormone dependent brain development has passed. It remains unknown whether commencing treatment earlier may still be beneficial.

The treatment of levothyroxine for subclinical hypothyroidism in pregnancy to improve obstetric outcomes is controversial due to limited evidence for the benefit from randomized controlled trials. Furthermore, in a large retrospective study, although levothyroxine treatment for subclinical hypothyroidism in pregnancy was associated with a reduction in the incidence of pregnancy loss, there was an increased incidence of preterm delivery, gestational diabetes, and preeclampsia in the women who were treated (Maraka et al. 2017). However, it is worth noting that neither of the two previously mentioned randomized controlled trials (Lazarus et al. 2012; Casey et al. 2017), reported as secondary outcomes, showed differences in obstetric complications between the intervention and control arms. Because of the potential benefit of levothyroxine in reducing pregnancy loss, the current guidelines from the American Thyroid Association recommend treatment of pregnant women with subclinical hypothyroidism in the presence of TPO antibodies (Alexander et al. 2017). However, it is reasonable to consider levothyroxine treatment for a TPO antibodies negative pregnant woman whose serum TSH is above 4.5 mIU/L (or above the trimester-specific reference range) or in those with a history of pregnancy loss, after discussing uncertainties surrounding the treatment and its potential benefits and risks.

If the decision is made to treat a pregnant woman with newly diagnosed subclinical hypothyroidism, a smaller initiating dose of levothyroxine (for example, 50–75 μg daily) should be used. The dose of levothyroxine should be adjusted, as in women with overt hypothyroidism, with regular thyroid function tests. Most women who develop subclinical hypothyroidism in pregnancy are euthyroid outside of the pregnancy (Shields et al. 2013). Therefore, women who started levothyroxine for a new diagnosis of subclinical hypothyroidism in pregnancy should be reassessed for the need to continue levothyroxine following delivery by rechecking thyroid function test after a short period of time off the drug.

Maternal Hypothyroxinemia

About 2% of pregnant women have isolated hypothyroxinemia; however, the prevalence rates in different populations vary depending upon the iodine status of the population, the assays used for thyroid function tests, and the free T4 cut-off levels to define the condition. The etiology of maternal hypothyroxinemia remains largely uncertain, although iodine deficiency, environmental pollutants, and obesity have been implicated as potential causes (Dosiou and Medici 2017). Thyroid autoimmunity is not associated with maternal hypothyroxinemia.

Like subclinical hypothyroidism, most women with maternal hypothyroxinemia do not report classical symptoms of hypothyroidism, and the diagnosis is made after a thyroid function test carried out as a screening or for case-finding. Although there is lack of consistency regarding the free T4 cutoff levels used in different studies for the diagnosis of maternal hypothyroxinemia, the recent American Thyroid Association guidelines define the condition as a free T4 level in the lower 2.5th–5th percentile of the population together with a normal serum TSH level (Alexander et al. 2017).

It remains uncertain whether maternal hypothyroxinemia increases the risk of adverse obstetric outcomes as studies have shown inconsistent results (Casey et al. 2007; Cleary-Goldman et al. 2008; Chan and Boelaert 2015). However, there is some evidence to suggest that women with maternal hypothyroxinemia have an increased risk of placental abruption, gestational diabetes, and adverse metabolic profile (Cleary-Goldman et al. 2008; Chan and Boelaert 2015; Knight et al. 2016). There is no randomized controlled trial designed primarily to assess if levothyroxine treatment for maternal hypothyroxinemia improves obstetric outcomes. In contrast to obstetric outcomes, there is stronger evidence for an association between maternal hypothyroxinemia and neurodevelopmental delay and learning difficulties in offspring (Pop et al. 1999; Henrichs et al., 2010; Finken et al. 2013; Noten et al. 2015), although some studies have failed to confirm the association (Oken et al. 2009; Craig et al. 2012). Two randomized controlled trials found no benefit of levothyroxine treatment in maternal hypothyroxinemia to improve neuropsychological outcomes in offspring (Lazarus et al. 2012; Casey et al. 2017). As secondary outcomes, no differences in obstetric outcomes were found in these trials either. In the absence of evidence from randomized controlled trials for the benefit, levothyroxine treatment is not recommended for maternal hypothyroxinemia.

Screening for Hypothyroidism in Pregnancy

It is controversial whether all pregnant women should be screened for hypothyroidism. Although several observational studies have shown associations between mild maternal thyroid hormone insufficiency (including subclinical hypothyroidism and hypothyroxinemia) during pregnancy and impaired neuropsychological development in offspring and other obstetric complications, randomized controlled trials so far have failed to show a benefit of screening and levothyroxine treatment

to reduce these adverse outcomes (Negro et al. 2010; Lazarus et al. 2012; Casey et al. 2017). The guideline from the European Thyroid Association does not endorse screening all pregnant women for subclinical hypothyroidism, although the majority of authors of the guideline recommend screening pregnant women for undiagnosed overt hypothyroidism (Lazarus et al. 2014). The current guideline from the American Thyroid Association also does not support universal screening of thyroid function in pregnancy but recommend targeted case-finding in pregnant women who are at high risk for thyroid dysfunction (Alexander et al. 2017). The high risk group includes women with personal or family history of thyroid disease, personal history of autoimmune disorders such as type 1 diabetes, symptoms of thyroid disease or a goiter, known thyroid autoimmunity, history of head or neck irradiation, and history of pregnancy loss and premature birth. However, such case-finding approach is not only inherently difficult to implement in the routine clinical practice (Vaidya et al. 2002) but also has been shown to miss a significant proportion of pregnant women with hypothyroidism (Vaidya et al. 2007). More studies are needed to resolve the controversy of screening thyroid function in pregnancy.

Euthyroid Autoimmune Thyroid Disease

About 10% of pregnant women have antibodies against TPO or Tg. Although most of these women have normal thyroid hormone levels, they are at an increased risk of developing hypothyroidism during pregnancy. Furthermore, these women also show a higher incidence of obstetric complications, particularly miscarriage and premature birth (Thangaratinam et al. 2011). The underlying mechanism for this higher rate of obstetric complications in euthyroid women with thyroid autoimmunity remains uncertain. It may be due to decreased thyroid hormone production in response to stimulation by placental hCG in pregnant women with thyroid autoimmunity (Korevaar et al. 2017). Alternatively, positive thyroid autoantibodies are merely a marker of dysregulated systemic immune responses, which in turn is the cause of the obstetric complications.

One study has shown that levothyroxine treatment in euthyroid pregnant women with thyroid autoimmunity reduces the incidence of miscarriages and premature birth (Negro et al. 2006); however, this is yet to be confirmed by further studies. Therefore, currently a routine treatment with levothyroxine for such women is not recommended although their thyroid function should be monitored regularly during pregnancy. The use of selenium supplement in autoimmune thyroid disease has been shown to reduce circulating thyroid antibody levels; however, currently there is lack of evidence from interventional studies that this improves pregnancy outcomes in women with autoimmune thyroid disease (Winther et al. 2017). After delivery, these women are at high risk of developing postpartum thyroiditis, and the diagnosis should be considered in the presence of suggestive symptoms. A randomized controlled trial of selenium supplement (selenomethionine 200 μg/day) in euthyroid pregnant women with positive TPO antibodies showed a

reduction in the incidence of postpartum thyroiditis in the treated group, as compared the placebo group (Negro et al. 2007). However, this observation is yet to be replicated. Further, an association between selenium supplementation and development of type 2 diabetes in nonpregnant populations call for caution in supplementing pregnant women who are already in a more insulin resistant state (Rayman and Stranges 2013). Therefore, the routine use of selenium supplement for euthyroid pregnant women with autoimmune thyroid disease is not recommended.

Thyroid Function in Female Subfertility and Women Undergoing Assisted Reproductive Technology

Overt hypothyroidism is associated with menstrual irregularities, ovulatory dysfunction, and subfertility. The association between subclinical hypothyroidism and subfertility remains uncertain because of the limited number of studies using appropriate controls and the use of inconsistent definitions of subclinical hypothyroidism in different studies. However, thyroid autoimmunity (defined by the presence of thyroid antibodies, particularly TPO antibodies) has been shown to be associated with an increased risk of subfertility by 1.5- to 2-folds (Poppe et al. 2007; van den Boogaard et al. 2011). This association is more evident in women with endometriosis and ovarian causes of subfertility (for example, polycystic ovary syndrome). There is inadequate data from interventional studies whether levothyroxine treatment in women with subclinical hypothyroidism or euthyroid autoimmune thyroid disease improves fertility.

Women failing to conceive after conventional fertility therapies may be offered treatments based on assisted reproductive technology (ART), which involves manipulation of sperm and eggs. The two most frequently used treatments based on ART include the in vitro fertilization (IVF), and the intracytoplasmic sperm injection (ICSI). ART procedures are associated with a rapid rise in plasma estradiol level, which may put an additional stress on the HPT axis. There is some evidence to suggest that maternal subclinical hypothyroidism affects the success rate of ART (Baker et al. 2006). Likewise, although thyroid autoimmunity is not associated with reduced rates of fertilization and clinical pregnancy following ART, thyroid autoimmunity carries an increased risk of miscarriages (Toulis et al. 2010; Busnelli et al. 2016). Furthermore, there is evidence to suggest that levothyroxine treatment in women with both subclinical hypothyroidism and thyroid autoimmunity improves live birth rates after ART (Velkeniers et al. 2013). The most recent guidelines from the European Thyroid Association and the American Thyroid Association thus recommends that women with subclinical hypothyroidism undergoing ART should be treated with levothyroxine with an aim to keep TSH below 2.5 mIU/L (Alexander et al. 2017). Levothyroxine treatment should also be considered in euthyroid women with TPO antibodies undergoing ART, although the treatment is controversial because of the lack of good-quality evidence for benefit from interventional studies.

Thyrotoxicosis

Thyrotoxicosis is a relatively uncommon condition in pregnancy, with a new diagnosis in about 0.05% of all pregnant women (Cooper and Laurberg 2013). In addition, as thyrotoxicosis is common in women of reproductive age, many women with previously diagnosed thyrotoxicosis become pregnant. The two most common causes of thyrotoxicosis in pregnancy are: (a) gestational transient thyrotoxicosis and (b) Graves' disease (Table 5). Toxic multinodular goiter, toxic thyroid nodule, thyroiditis, and drugs are rare causes of thyrotoxicosis in pregnancy, while trophoblastic disorders (hydatidiform mole and choriocarcinoma), struma ovarii, nonautoimmune hyperthyroidism due to activating TSH receptor mutation and TSH-secreting pituitary adenoma are extremely rare.

Gestational Transient Thyrotoxicosis

Gestational transient thyrotoxicosis is caused by an excessive stimulation of thyroid follicular cells by placental hCG (Table 5), and affects 1–3% of pregnancies. It is more common in pregnancies associated with high levels of serum hCG, such as

Table 5 Causes and pathogenesis of thyrotoxicosis in pregnancy

Causes	Pathogenesis
Common causes	
Gestational transient thyrotoxicosis	Excessive TSH receptor stimulation by hCG
Graves' disease	Excessive TSH receptor stimulation by autoantibodies
Rare causes	
Toxic multinodular goiter	Autonomous thyroid hormone secretion
Toxic thyroid nodule	Autonomous thyroid hormone secretion
Thyroiditis (subacute and painless)	Release of preformed thyroid hormone due to inflammation of thyroid follicular cells
Overtreatment of hypothyroidism or factitious use of thyroid hormone	Ingestion of levothyroxine, liothyronine, or desiccated thyroid extract
Drugs (for example, amiodarone, lithium, and interferon)	Inflammation of thyroid follicles with release of thyroid hormones (thyroiditis) or autoimmune stimulation of TSH receptor
Extremely rare causes	
Trophoblastic disorders (hydatidiform mole and choriocarcinoma)	Excessive TSH receptor stimulation by hCG
Struma ovarii	Ovarian teratoma containing thyroid tissue and secreting thyroid hormone
Nonautoimmune hyperthyroidism due to activating TSH receptor mutation	Constitutively active TSH receptor due to mutation in the TSH receptor gene
TSH secreting pituitary adenoma	Excessive pituitary secretion of TSH stimulating thyroid follicular cells

hCG human chorionic gonadotrophin, *TSH* thyrotrophin

hyperemesis gravidarum and multiple pregnancies, and its severity correlates with the levels of serum hCG. Some cases of gestational transient thyrotoxicosis are associated with isoforms of hCG displaying more potent thyroid stimulatory activity (Kimura et al. 1993). Rarely, it is caused by mutations in the TSH receptor gene resulting in an increased sensitivity of the receptor to the stimulatory action of hCG (Rodien et al. 1998; Coulon et al. 2016).

Gestational transient thyrotoxicosis is usually mild, with only a minority of affected women presenting with symptoms of thyrotoxicosis, and the diagnosis often goes unrecognized in women with mild symptoms. Some women present with symptoms and signs of the associated condition of hyperemesis gravidarum, including nausea, vomiting, dehydration, electrolyte disturbance, and weight loss. About 40–60% of women with hyperemesis gravidarum display biochemical thyrotoxicosis. Gestational transient thyrotoxicosis can often be difficult to distinguish from an initial presentation of Graves' disease in the first trimester. However, in contrast to Graves' disease, it is not associated with the presence of goiter, thyroid eye disease, or thyroid autoantibodies (in particular, TSH receptor antibodies) (Table 6). Women with gestational transient thyrotoxicosis usually have milder thyroid dysfunction with lower serum free T4, free T3, and free T3:T4 ratio than those with active Graves' disease (Yoshihara et al. 2015; Ide et al. 2017). However, there is a big overlap in the thyroid hormone levels, making these unsuitable for distinguishing between the gestational transient thyrotoxicosis and Graves' disease. Likewise, although serum hCG levels tend to be higher in women with gestational transient thyrotoxicosis, there is a considerable overlap with the levels seen in Graves' disease and therefore serum hCG levels are not useful in distinguishing between the two conditions (Yoshihara et al. 2015).

Gestational transient thyrotoxicosis resolves spontaneously by 18–20 weeks of gestation as serum hCG levels decrease (Fig. 2) and is not associated with adverse obstetric outcomes (Kinomoto-Kondo et al. 2017). Antithyroid drugs do not alter clinical outcomes and are not recommended in this condition. In symptomatic

Table 6 Comparison between gestational transient thyrotoxicosis and Graves' disease

Features	Gestational transient thyrotoxicosis	Graves' disease
Symptoms of thyrotoxicosis	Often absent or mild	Present
Goiter	Absent	Often present
Thyroid eye disease	Absent	May be present
TSH receptor antibodies	Absent	Present
Resolution of thyrotoxicosis	Remits in the second half of the pregnancy	Tends to improve in later pregnancy
Adverse obstetric outcomes	No association	Associated in case of suboptimal control of thyrotoxicosis
Antithyroid drugs	Not indicated	Often indicated

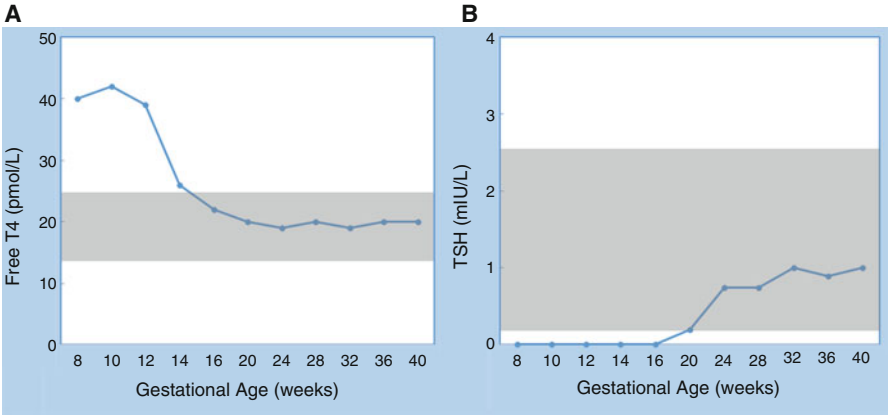

Fig. 2 Thyroid function tests in a woman with transient gestational thyrotoxicosis. Serum free T4 spontaneously normalized by gestational week 16 (***Panel A***) although serum TSH remained suppressed until gestational week 20 (***Panel B***). The shaded areas represent normal ranges of free T4 and TSH, respectively

women, a short course of a β-blocker may be used for symptom control. Women with associated hyperemesis gravidarum may require treatment with intravenous fluid, electrolytes replacement, and antiemetic drugs.

Graves' Disease

Graves' disease is autoimmune hyperthyroidism resulting from stimulating TSH receptor antibodies. The activation of the TSH receptors by the autoantibodies leads to excess thyroid hormone production and diffuse enlargement of the thyroid gland (Smith and Hegedüs 2016). Aside from gestational transient thyrotoxicosis, it is the commonest cause of clinically overt hyperthyroidism in pregnancy, affecting about 1 in 500 pregnant women. Graves' disease may be first diagnosed during pregnancy or women with preexisting Graves' disease may become pregnant. In pregnant women with preexisting Graves' disease (either actively thyrotoxic or under remission following a course of antithyroid drugs), thyrotoxicosis may worsen in the first trimester but tends to improve during the latter part of the pregnancy, associated with a decrease in the levels of TSH receptor antibodies.

Diagnosis of Graves' Disease

The common symptoms of Graves' disease include heat intolerance, sweating, nervousness, palpitations, tremor, and weight loss. As several of these symptoms mimic those of pregnancy, the diagnosis may be delayed. Women with Graves' disease often present with a diffuse goiter and occasionally have signs of thyroid eye disease. Rarely, dermopathy (also called pretibial myxedema) and thyroid acropachy (clubbed nails) may be present (Smith and Hegedüs 2016). The diagnosis of Graves'

Table 7 Adverse outcomes associated with untreated or inadequately treated Graves' disease

Maternal adverse effects	Fetal adverse effects
Gestational hypertension	Fetal loss (miscarriage and still birth)
Preeclampsia and eclampsia	Premature birth
Cardiac arrhythmias	Placental abruption
Cardiac failure	Intrauterine growth retardation
Thyroid storm	Accelerated bone maturation
	Fetal and neonatal thyrotoxicosis
	Central congenital hypothyroidism

disease is made based on biochemical thyrotoxicosis (elevated serum free T4 or free T3 with a suppressed serum TSH), associated with thyroid autoimmunity (positive TSH receptor antibodies), thyroid eye disease, or dermopathy. In women presenting with thyrotoxicosis in the first trimester, Graves' disease must be distinguished from gestational transient thyrotoxicosis (Table 6).

Complications of Graves' Disease in Pregnancy

Untreated or inadequately treated Graves' disease in pregnancy is associated with an increased risk of severe adverse effects for both mother and her fetus (Table 7). Maternal complications include gestational hypertension, preeclampsia, arrhythmias, heart failure, and rarely thyroid storm. Poorly controlled maternal hyperthyroidism is also associated with fetal loss (miscarriage and still birth), premature birth, and low birth weight (Millar et al. 1994). In addition, fetal and neonatal thyrotoxicosis is more common in uncontrolled maternal Graves' disease (see below, fetal and neonatal thyrotoxicosis). Rarely, neonates born to women with uncontrolled thyrotoxicosis in late pregnancy may develop central congenital hypothyroidism resulting from transplacental passage of excess maternal thyroid hormones to the fetus suppressing secretion of TSH from its pituitary gland (Matsuura et al. 1997). This is a transient disorder, with thyroid function of the affected neonate normalizing within a few weeks.

Management Options for Graves' Disease in Pregnancy

Optimal treatment of maternal Graves' disease is important to prevent pregnancy complications. ***Antithyroid drugs*** are the mainstay of treatment for Graves' disease in pregnancy. A short course of ***β-blockers*** (for example, propranolol) may be used for symptom control in case of severe thyrotoxicosis until the full effect of antithyroid drug has taken place. β-blockers are associated with mild fetal growth restriction, and their prolonged use should be avoided in pregnancy. ***Thyroidectomy*** in pregnant women carries higher risk of complications than in nonpregnant adults and is generally reserved for those pregnant women in whom antithyroid drugs fail to control thyrotoxicosis despite high doses or who develop side-effects. When thyroidectomy is necessary, it is best carried out in the second trimester. Most women undergoing thyroidectomy in pregnancy will require preoperative treatment with potassium iodide to control thyrotoxicosis before surgery. ***Radioiodine treatment*** is

contraindicated in pregnancy as it exposes the fetus to the radiation. Furthermore, if the treatment is given after 12 weeks of gestation (when fetal thyroid starts functioning), it ablates the fetal thyroid gland resulting in fetal hypothyroidism (Bonnema and Hegedüs 2012). Indeed, all women of reproductive potential must have a negative pregnancy test before radioiodine treatment and should be advised not to conceive for at least 6 months after treatment (Weetman 2007).

Antithyroid Drugs for Graves' Disease in Pregnancy

Thionamide antithyroid drugs, propylthiouracil and methimazole (as well as its precursor carbimazole, which is available for use instead of methimazole in some countries), are widely used to treat thyrotoxicosis in pregnancy. These drugs inhibit the synthesis of thyroid hormones by blocking the action of thyroid peroxidase enzyme, which catalyzes iodination of tyrosine residue of thyroglobulin and coupling of monoiodothyronine and diiodothyronine molecules in the thyroid hormone synthesis pathway. In addition, propylthiouracil also suppresses conversion of T4 to active T3 in the peripheral tissues. Both propylthiouracil and methimazole/carbimazole readily cross the placenta (Mortimer et al. 1997). Therefore, treatment of maternal Graves' disease with these antithyroid drugs also reduces the risk of fetal thyrotoxicosis (see Fetal and Neonatal Thyrotoxicosis); however, overtreatment can result in fetal hypothyroidism and goiter.

Although there are no randomized controlled trials comparing different antithyroid drugs in pregnancy, both propylthiouracil and methimazole/carbimazole are considered equally effective in controlling thyrotoxicosis in pregnancy (Wing et al. 1994). Therefore, the choice of antithyroid drugs in pregnancy depends upon their side-effect profiles. The prevalence of skin rash, itching, upper gastrointestinal disturbance, arthralgia, and rare agranulocytosis are similar with propylthiouracil and methimazole/carbimazole. However, propylthiouracil is associated with rare but severe life-threatening fulminant hepatic failure both in nonpregnant population and in pregnant women (Cooper and Rivkees 2009; Taylor and Vaidya 2012). Methimazole/carbimazole can also cause liver toxicity, but it is usually mild with cholestatic pattern of liver injury, in contrast to hepatocellular pattern associated with propylthiouracil.

The use of both methimazole/carbimazole and propylthiouracil in early pregnancy is associated with birth defects in offspring; however, the patterns of associated birth defects are different (Table 8) (Yoshihara et al. 2012; Andersen et al. 2013). Methimazole/carbimazole is associated with a characteristic severe embryopathy, including choanal atresia, tracheoesophageal fistula, patent vitelline intestinal duct, nipple abnormalities, aplasia cutis, dysmorphic facies, and cardiovascular malformations (Fig. 3). Birth defects associated with propylthiouracil appear to be less severe than those associated with methimazole/carbimazole (Andersen et al. 2014). The critical period when maternal use of antithyroid drugs increases the risk of birth defects in offspring is 5–10 weeks of gestation (Laurberg and Andersen 2014).

The differential side-effect profiles of propylthiouracil and methimazole/carbimazole in relation to hepatotoxicity and birth defects means the choice of

Table 8 Birth defects associated with carbimazole/methimazole and propylthiouracil (Bowman et al. 2012; Yoshihara et al. 2012; Andersen et al. 2013, 2014)

Methimazole/Carbimazole	Propylthiouracil
Choanal atresia	Defects in the face and neck region (e.g., periauricular sinus, branchial cleft sinus)
Tracheoesophageal fistula	Renal tract defects (e.g., congenital hydronephrosis, renal cyst, unilateral renal agenesis)
Esophageal atresia	Cardiovascular defects (e.g., cardiac outflow obstruction)
Patent vitellointestinal duct	–
Omphalocele	–
Cardiovascular defects (e.g., ventricular septal defect, situs inversus with dextrocardia)	–
Aplasia cutis	–
Nipple abnormalities	–
Facial dysmorphism (e.g., broad nasal bridge, upward slanting palpebral aperture, thin upper lip, hypoplastic nasal alae)	–

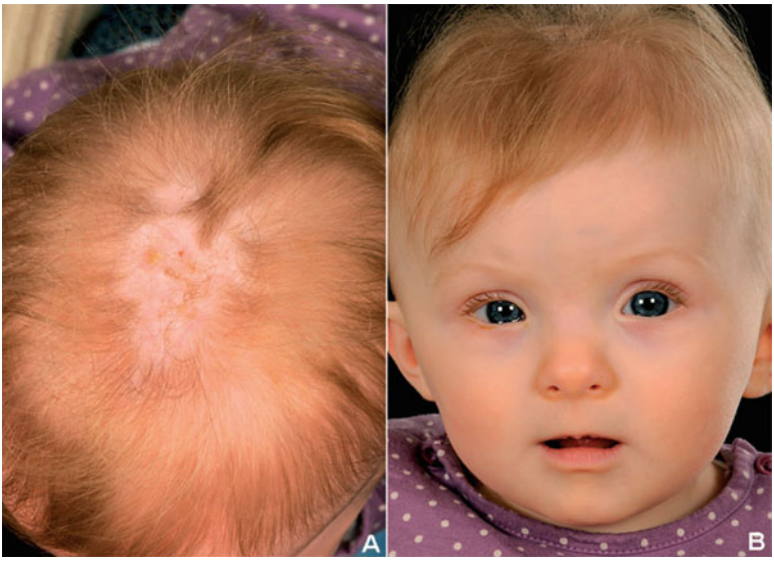

Fig. 3 Carbimazole embryopathy in a girl exposed to Carbimazole in utero showing aplasia cutis of the scalp (***Panel A***), and mild facial dysmorphism (wide nasal bridge, upward slanting palpebral aperture, thin upper lip, and hypoplastic nasal alae) (***Panel B***). She also had laryngomalacia, patent vitellointestinal tract, and right nasolacrimal atresia. (Reproduced with permission from: Bowman et al. 2012)

antithyroid drugs in pregnancy demands careful consideration taking an account of severity of thyrotoxicosis, gestational age, and the patient's view. For example, as the risk of obstetric complications with subclinical hyperthyroidism is minimal (Casey et al. 2006), pregnant women with subclinical hyperthyroidism due to Graves' disease do not need treatment with antithyroid drugs. In addition, if a woman with stable Graves' disease who is euthyroid on a small dose of antithyroid drug becomes pregnant, she should be assessed whether it is safe for her to avoid antithyroid drugs in the critical period of 5–10 weeks gestation (Laurberg and Andersen 2014). If an antithyroid drug is considered necessary, in view of the association between methimazole/carbimazole use in early pregnancy and severe embryopathy, propylthiouracil has been recommended as the preferred antithyroid drug in the first trimester. Even though propylthiouracil has also been itself associated with risk of congenital anomalies with a different profile to methimazole/carbimazole (Table 8), the overall risk is still thought to be less (Andersen et al. 2013). In contrast, the risk of fulminant hepatotoxicity with propylthiouracil means that methimazole/carbimazole is preferable for women needing antithyroid drugs after the first trimester. However, women who have stable euthyroidism on propylthiouracil in early gestation may wish to continue the drug after the first trimester as swapping the drug to methimazole/carbimazole may require dose titrations.

When antithyroid drugs are needed in pregnancy, the lowest possible dose should be used to avoid fetal hypothyroidism and goiter. The "block and replace" regime (combination of high-dose antithyroid drug and levothyroxine) should not be used in pregnancy as antithyroid drugs pass through placenta more readily than levothyroxine resulting in fetal hypothyroidism. Thyroid function should be monitored closely (every 4 weeks) to adjust the dose of antithyroid drugs with an aim to keep maternal free T4 level towards the upper end of the reference range. TSH concentrations should not be used to guide dose adjustments. This is because, in pregnant women taking antithyroid drugs, maternal free T4 at mid or lower end of reference range (even with suppressed TSH) is associated with a significant risk of fetal hypothyroidism (Momotani et al. 1986). It is usually possible to decrease the dose of antithyroid drug in the later part of the pregnancy, and indeed a significant proportion will be able to stop the drug altogether by the mid-second or third trimesters. However, the risk of recurrence of thyrotoxicosis after delivery is high in these women.

Breastfeeding and Antithyroid Drugs

Antithyroid drugs can be used to treat thyrotoxicosis in lactating mothers. Both carbimazole/methimazole and propylthiouracil are secreted in small amounts in milk, and methimazole doses up to 20 mg daily (or propylthiouracil doses up to 300 mg daily) during breastfeeding is thought not to affect infant's thyroid function significantly (Karras and Krassas 2012). A case-controlled study found that infants breast fed by thyrotoxic mothers with methimazole up to the daily dose of 20–30 mg have no impaired physical or neuropsychological development at age 48 to 86 months (Azizi et al. 2003). In view of the association of liver toxicity with propylthiouracil, carbimazole/methimazole is the preferred antithyroid drug for

lactating women. The drug should be taken in smaller divided doses, and just after breastfeeding. If high doses of antithyroid drug are required to control thyrotoxicosis in a lactating mother, the infant's thyroid function should be monitored.

Thyroid Storm in Pregnancy

Thyroid storm is a rare but life-threatening complication of Graves' disease in pregnancy, which may be fatal without prompt treatment. It occurs in women with uncontrolled thyrotoxicosis and often precipitated by sudden stoppage of antithyroid drugs, infection, surgery, or labor. Clinical features of thyroid storm include fever, nausea, vomiting, diarrhea, tachycardia, arrhythmias, heart failure, cardiogenic shock, hepatic dysfunction, restlessness, confusion, stupor, and coma (Klubo-Gwiezdzinska and Wartofsky 2012). Thyroid storm is ideally managed in an intensive care unit and require general supportive treatment with intravenous fluid, electrolytes, and oxygen. Specific treatments include antithyroid drug, potassium iodide, and sometimes glucocorticoid for thyrotoxicosis; β-blocker to control tachycardia; and paracetamol and cooling blankets for pyrexia. Propylthiouracil is the preferable antithyroid drug in thyroid storm because of its additional effect inhibiting peripheral conversion of T4 to T3.

Fetal and Neonatal Thyrotoxicosis

Fetal or neonatal thyrotoxicosis affects 4–5% of offspring of women with Graves' disease due to transplacental passage of maternal TSH receptor antibodies stimulating the fetal thyroid gland (Polak et al. 2004; Levy-Shraga et al. 2014). Offspring of women with levels of TSH receptor antibodies three times above the upper limit of the reference range are particularly at risk (Abeillon-du Payrat et al. 2014). Antithyroid drugs taken by pregnant women with Graves' disease also pass through placenta reducing the risk of fetal thyrotoxicosis. Notably, women with previous Graves' hyperthyroidism treated with thyroidectomy or radioactive iodine therapy may still carry high levels of TSH receptor antibodies even if their current thyroid function is normal, and therefore, their babies remain at risk of developing fetal and neonatal thyrotoxicosis. There are stimulatory and inhibitory TSH receptor antibodies, and very rarely, transplacental passage of maternal antibodies inhibiting TSH receptor may cause transient neonatal hypothyroidism (Evans et al. 2011). Rarely, women with Hashimoto's thyroiditis may also carry stimulating TSH receptor antibodies, which in pregnancy may pass through placenta causing fetal and neonatal thyrotoxicosis (Kiefer et al. 2017).

As fetal thyroid gland becomes sensitive to TSH receptor antibodies from about 20 weeks of gestation, fetal thyrotoxicosis typically manifests in the second half of pregnancy. It is associated with fetal goiter, fetal tachycardia, intrauterine growth retardation, oligohydramnios or hydrops, premature bone ossification, microcephaly, and sometimes fetal death. In a pregnant woman with Graves' disease taking antithyroid drug, fetal goiter may be either a sign of fetal hypothyroidism (due to excessive dose of antithyroid drug) or fetal thyrotoxicosis (resulting from maternal TSH receptor antibodies). Serial fetal ultrasound and doppler studies by an experienced ultrasonographer can discriminate between these conditions (Luton et al. 2005). Rarely,

fetal thyroid function test after a percutaneous umbilical cord blood sampling (cordocentesis) is necessary for the diagnosis; however, the procedure is associated with a significant risk of fetal complications and should only be undertaken by a clinician well experienced in the procedure. Treatment of fetal thyrotoxicosis includes starting or increasing the dose of antithyroid drug for the mother. As fetal thyrotoxicosis is usually diagnosed in the third trimester, methimazole/carbimazole is preferable to propylthiouracil. The dose of antithyroid drug is adjusted by monitoring fetal heart rate, fetal growth, and fetal goiter size. In case of development of maternal hypothyroidism due to antithyroid drug, treatment of levothyroxine (in addition to antithyroid drug) may be necessary (Bucci et al. 2017).

Clinical manifestations of neonatal thyrotoxicosis may be evident at birth although in babies born to women requiring antithyroid drug in the late pregnancy, symptoms of thyrotoxicosis often develop 7–10 days after birth when the effect of maternal antithyroid drug ceases. Symptoms and signs of neonatal thyrotoxicosis include restlessness, irritability, diarrhea, failure to thrive, tachycardia, and goiter (van der Kaay et al. 2016). Many affected infants have eye signs, including lid retraction and proptosis. Hepatosplenomegaly may be present. Complications of neonatal thyrotoxicosis include cardiac failure, cardiac arrhythmia, advanced bone age, craniosynostosis, and microcephaly. Neonatal thyrotoxicosis is treated with antithyroid drug and propranolol. In view of the association between propylthiouracil and severe hepatotoxicity, methimazole/carbimazole is the preferable antithyroid drug, and the infant's thyroid function should be monitored carefully to adjust the dose. Neonatal thyrotoxicosis usually resolves by 1–3 months after birth as maternal TSH receptor antibodies are cleared from the infant's circulation, and the treatment can be stopped (van der Kaay et al. 2016).

As TSH receptor antibodies help to predict fetal and neonatal thyrotoxicosis, these should be checked in all pregnant women with active or previous history of Graves' disease. In women with high levels of TSH receptor antibodies (three times above the upper limit of the reference range), their babies should be monitored carefully for fetal and neonatal thyrotoxicosis (Abeillon-du Payrat et al. 2014). A specialist multidisciplinary care involving endocrinologists, obstetricians, midwives, and neonatologists is necessary to ensure a good pregnancy outcome for these women.

Preconception Counselling for Women with Graves' Disease Planning Pregnancy

It is important that all women of reproductive age diagnosed with Graves' disease are given information about issues relating to the management of the condition in pregnancy, including the association between the use of antithyroid drugs in early pregnancy and birth defects in offspring (Lazarus 2012). Women with overt thyrotoxicosis should be advised to delay conception until thyrotoxicosis is controlled. Pros and cons of different treatment modalities including antithyroid drugs, radioiodine treatment, and thyroidectomy should be discussed so that the woman can make an informed choice. If radioiodine therapy is chosen, conception must be delayed for at least 6 months (Weetman 2007). Both radioiodine and thyroidectomy lead to hypothyroidism, and thyroid function must be stable on levothyroxine replacement before conception. If the woman chooses antithyroid drugs to control thyrotoxicosis before

pregnancy, propylthiouracil is the preferable antithyroid drug. If the woman becomes pregnant on antithyroid drug, thyroid function must be checked as soon as pregnancy is confirmed to ascertain if she needs to continue the drug or adjust the dose.

Thyroid Nodules and Thyroid Cancer

Thyroid Nodules

Thyroid nodules are common in pregnancy, with the prevalence over 25% in some populations (Kung et al. 2002; Sahin et al. 2014). The prevalence rate increases with increasing maternal age and parity. Women with a preexisting thyroid nodule may notice enlargement of the nodule during pregnancy. Pregnant women may also present with new thyroid nodules. As in the nonpregnant adult population, most of the thyroid nodules encountered in pregnancy are benign and are not associated with excessive autonomous thyroid hormone production.

Thyroid nodules first discovered during pregnancy should be assessed in a similar way to those found in the nonpregnant adults with a careful clinical history (including exposure to ionizing radiation in the past and a family history of thyroid cancer or a syndrome associated with thyroid cancers, such as multiple endocrine neoplasia type 2 or Cowden's syndrome) and physical examination (Hegedüs 2004). Serum TSH should be checked to exclude thyroid dysfunction. Thyroid ultrasonography is the key investigation for assessment of thyroid nodules, and nodules with highly suspicious features in ultrasound examination should undergo fine needle aspiration cytology (FNAC). FNAC is not associated with additional complications in pregnancy and can be performed at any trimester. The routine calcitonin measurement in thyroid nodules remains controversial; however, it should be checked if there is a suspicion of medullary thyroid carcinoma (for example, in presence of family history of medullary thyroid carcinoma or multiple endocrine neoplasia type 2). Radionuclide thyroid uptake scan is contraindicated in pregnancy.

The efficacy and safety of levothyroxine treatment suppressing serum TSH to decrease the size of thyroid nodules in pregnancy is unknown, and the treatment should not be used (Hegedüs 2004; Gharib et al. 2016). Thyroid nodules with malignancy on FNAC diagnosed in early pregnancy should undergo surgery in the second trimester, especially if there is evidence of rapid growth or involvement of lymph nodes. If the malignant cytology is detected in the third trimester, the surgery could be delayed until after the delivery in the absence of evidence for aggressive thyroid cancer (for example, rapidly increasing size).

Thyroid Cancer

Thyroid cancer can present as a new diagnosis in pregnancy or a woman with a preexisting diagnosis of thyroid cancer can become pregnant. Surgery is the

treatment of choice for thyroid cancer diagnosed in pregnancy; however, the timing of surgery depends upon the type of cancer, gestational age, and patient choice. For most women diagnosed with differentiated thyroid carcinoma, the surgery can be delayed until after the delivery because surgery during pregnancy carries a higher risk of complications than in nonpregnant adults, and such an approach is not associated with a worse prognosis. These women, however, should be monitored carefully with serial ultrasonography. Surgery should be considered during pregnancy in case of evidence for aggressive form of differentiated thyroid carcinoma (as evidenced by rapidly enlarging tumor or involvement of cervical lymph nodes) or if medullary thyroid carcinoma or anaplastic thyroid carcinoma is suspected. Second trimester is the safest period for thyroidectomy in pregnancy (Perros et al. 2014). In case of aggressive differentiated thyroid carcinoma undergoing thyroidectomy, TSH suppression with levothyroxine therapy is necessary. Radioiodine ablation is contraindicated in pregnancy.

In women with successfully treated differentiated thyroid cancer, pregnancy is not associated with an increased risk of relapse, and therefore additional monitoring to detect relapse is unnecessary. However, these women should have regular thyroid function tests during pregnancy so that the dose of levothyroxine can be adjusted to maintain TSH suppression at the prepregnancy level. Most women will need to increase the dose of levothyroxine by 30–50% during pregnancy to achieve this. However, care must be taken to avoid excessive treatment since highly elevated levels of T4 may affect fetal neurodevelopment (Korevaar et al. 2016). Pregnant women with residual thyroid carcinoma or those suspected of having a recurrence should be monitored with ultrasonography and serum thyroglobulin in each trimester.

Conclusion

In the last 25 years, there have been significant advances in our understanding of pregnancy-associated physiological changes affecting the thyroid, the role of thyroid hormones in fetal development (particularly neurodevelopment), the assessment of thyroid function in pregnancy, and the impact of maternal thyroid dysfunction on obstetric outcomes and offspring development. However, new questions and uncertainties surrounding the optimum management of thyroid disorders in pregnancy have emerged. For example, the recent observations of the associations between antithyroid drugs and birth defects in the offspring as well as liver toxicity have increased the complexity of managing Graves' disease in pregnancy. The optimum management of subclinical thyroid disease remains uncertain as work to generate clinical evidence to support practice has been challenging as large sample sizes are required to demonstrate potential efficacy. It also remains highly controversial whether all pregnant women should be screened for thyroid dysfunction. Currently ongoing and future studies will clarify many of these uncertainties in the coming years.

Summary

- Due to pregnancy-associated physiological changes in the thyroidal axis, trimester and assay specific reference ranges for thyroid function tests are necessary for assessment of thyroid function in pregnant women.
- Adequate iodine intake is important in pregnancy as even mild iodine deficiency may impair neurodevelopment of the offspring. The WHO recommends a total daily iodine intake of 250 μg for pregnant and lactating women.
- Untreated and inadequately treated hypothyroidism in pregnancy is associated with impaired neuropsychological development of the offspring and several adverse obstetric effects, including fetal loss and premature birth. Women with preexisting hypothyroidism need an increased dose of levothyroxine in pregnancy to maintain euthyroidism.
- Pregnant women with a new diagnosis of subclinical hypothyroidism should be tested for thyroid peroxidase (TPO) antibodies. The women with positive TPO antibodies should be treated with levothyroxine. Treatment of pregnant women with subclinical hypothyroidism in the absence of TPO antibodies is controversial.
- Currently there is inadequate evidence to support treatment of pregnant women with maternal hypothyroxinemia and euthyroid autoimmune thyroid disease.
- Uncontrolled Graves' disease in pregnancy is associated with adverse obstetric outcomes; however, both methimazole/carbimazole and propylthiouracil is associated with birth defects in offspring when used in early pregnancy. Therefore, management of Graves' disease in pregnancy requires careful consideration of severity of thyrotoxicosis, gestational age, and the woman's view.
- When treating Graves' disease with antithyroid drugs in pregnancy, use the smallest dose required to keep free T4 at or just above the upper end of the reference range. "Block & replace" regime (combination of high dose of antithyroid drugs and levothyroxine) should not be used in pregnancy.
- Pregnant women with an active or a previous history of Graves' disease should be tested for TSH receptor antibodies. If the antibodies are positive, careful surveillance for fetal and neonatal thyrotoxicosis is necessary.
- Pregnancy is not associated with an increased risk of relapse of successfully treated differentiated thyroid cancer, and extra monitoring to detect relapse during pregnancy is unnecessary. However, most of these women will need adjustment of levothyroxine dose to maintain TSH suppression at the prepregnancy level.

Cross-References

► Diagnosis and Treatment of Hypothyroidism
► Graves' Disease
► Hashimoto's Thyroiditis
► Postpartum Thyroiditis and Silent Thyroiditis
► Thyroid Autoantibodies

► Thyroid Nodule
► Treatment of Graves' Disease

References

Abeillon-du Payrat J, Chikh K, Bossard N, Bretones P, Gaucherand P, Claris O, Charrié A, Raverot V, Orgiazzi J, Borson-Chazot F, Bournaud C. Predictive value of maternal second-generation thyroid-binding inhibitory immunoglobulin assay for neonatal autoimmune hyperthyroidism. Eur J Endocrinol. 2014;171(4):451–60.

Alexander EK, Marqusee E, Lawrence J, Jarolim P, Fischer GA, Larsen PR. N Engl J Med. 2004;351:241–9.

Alexander EK, Pearce EN, Brent GA, Brown RS, Chen H, Dosiou C, Grobman WA, Laurberg P, Lazarus JH, Mandel SJ, Peeters RP, Sullivan S. 2017 guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and the postpartum. Thyroid. 2017;27(3):315–89.

Andersen SL, Olsen J, CS W, Laurberg P. Birth defects after early pregnancy use of antithyroid drugs: a Danish nationwide study. J Clin Endocrinol Metab. 2013;98(11):4373–81.

Andersen SL, Olsen J, CS W, Laurberg P. Severity of birth defects after propylthiouracil exposure in early pregnancy. Thyroid. 2014;24(10):1533–40.

Andersson M, Karumbunathan V, Zimmermann MB. Global iodine status in 2011 and trends over the past decade. J Nutr. 2012;142(4):744–50.

Azizi F, Bahrainian M, Khamseh ME, Khoshniat M. Intellectual development and thyroid function in children who were breast-fed by thyrotoxic mothers taking methimazole. J Pediatr Endocrinol Metab. 2003;16(9):1239–43.

Baker VL, Rone HM, Pasta DJ, Nelson HP, Gvakharia M, Adamson GD. Correlation of thyroid stimulating hormone (TSH) level with pregnancy outcome in women undergoing in vitro fertilization. Am J Obstet Gynecol. 2006;194(6):1668–74; discussion 1674–5.

Barber KJ, Franklyn JA, McCabe CJ, Khanim FL, Bulmer JN, Whitley GS, Kilby MD. The in vitro effects of triiodothyronine on epidermal growth factor-induced trophoblast function. J Clin Endocrinol Metab. 2005;90(3):1655–61.

Bath SC, Steer CD, Golding J, Emmett P, Rayman MP. Effect of inadequate iodine status in UK pregnant women on cognitive outcomes in their children: results from the Avon Longitudinal Study of Parents and Children (ALSPAC). Lancet. 2013;382(9889):331–7.

Benhadi N, Wiersinga WM, Reitsma JB, Vrijkotte TG, van der Wal MF, Bonsel GJ. Ethnic differences in TSH but not in free T4 concentrations or TPO antibodies during pregnancy. Clin Endocrinol (Oxf). 2007;66(6):765–70.

Berbel P, Mestre JL, Santamaría A, Palazón I, Franco A, Graells M, González-Torga A, de Escobar GM. Delayed neurobehavioral development in children born to pregnant women with mild hypothyroxinemia during the first month of gestation: the importance of early iodine supplementation. Thyroid. 2009;19(5):511–9.

Blatt AJ, Nakamoto JM, Kaufman HW. National status of testing for hypothyroidism during pregnancy and postpartum. J Clin Endocrinol Metab. 2012;97(3):777–84.

Bonnema SJ, Hegedüs L. Radioiodine therapy in benign thyroid diseases: effects, side effects, and factors affecting therapeutic outcome. Endocr Rev. 2012;33(6):920–80.

Bowman P, Osborne NJ, Sturley R, Vaidya B. Carbimazole embryopathy: implications for the choice of antithyroid drugs in pregnancy. QJM. 2012;105(2):189–93.

Brent GA. Maternal thyroid function: interpretation of thyroid function tests in pregnancy. Clin Obstet Gynecol. 1997;40(1):3–15.

Bucci I, Giuliani C, Napolitano G. Thyroid-stimulating hormone receptor antibodies in pregnancy: clinical relevance. Front Endocrinol (Lausanne). 2017;8:137.

Busnelli A, Paffoni A, Fedele L, Somigliana E. The impact of thyroid autoimmunity on IVF/ICSI outcome: a systematic review and meta-analysis. Hum Reprod Update. 2016;22(6):775–90.
Calvo RM, Jauniaux E, Gulbis B, Asunción M, Gervy C, Contempré B, Morreale de Escobar G. Fetal tissues are exposed to biologically relevant free thyroxine concentrations during early phases of development. J Clin Endocrinol Metab. 2002;87(4):1768–77.
Casey BM, Dashe JS, Wells CE, McIntire DD, Leveno KJ, Cunningham FG. Subclinical hyperthyroidism and pregnancy outcomes. Obstet Gynecol. 2006;107(2 Pt 1):337–41.
Casey BM, Dashe JS, Spong CY, McIntire DD, Leveno KJ, Cunningham GF. Perinatal significance of isolated maternal hypothyroxinemia identified in the first half of pregnancy. Obstet Gynecol. 2007;109(5):1129–35.
Casey BM, Thom EA, Peaceman AM, Varner MW, Sorokin Y, Hirtz DG, Reddy UM, Wapner RJ, Thorp Jr JM, Saade G, Tita AT, Rouse DJ, Sibai B, Iams JD, Mercer BM, Tolosa J, Caritis SN, VanDorsten JP, Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal–Fetal Medicine Units Network. Treatment of subclinical hypothyroidism or hypothyroxinemia in pregnancy. N Engl J Med. 2017;376(9):815–25.
Chan SY. Thyroid gland: early adjustment of levothyroxine treatment in pregnancy. Nat Rev Endocrinol. 2010;6(10):537–8.
Chan S, Boelaert K. Optimal management of hypothyroidism, hypothyroxinaemia and euthyroid TPO antibody positivity preconception and in pregnancy. Clin Endocrinol (Oxf). 2015;82(3):313–26.
Chan GW, Mandel SJ. Therapy insight: management of Graves' disease during pregnancy. Nat Clin Pract Endocrinol Metab. 2007;3(6):470–8.
Chan S, Kachilele S, McCabe CJ, Tannahill LA, Boelaert K, Gittoes NJ, Visser TJ, Franklyn JA, Kilby MD. Early expression of thyroid hormone deiodinases and receptors in human fetal cerebral cortex. Brain Res Dev Brain Res. 2002;138(2):109–16.
Chan SY, Vasilopoulou E, Kilby MD. The role of the placenta in thyroid hormone delivery to the fetus. Nat Clin Pract Endocrinol Metab. 2009;5(1):45–54.
Chaouki ML, Benmiloud M. Prevention of iodine deficiency disorders by oral administration of lipiodol during pregnancy. Eur J Endocrinol. 1994;130(6):547–51.
Cleary-Goldman J, Malone FD, Lambert-Messerlian G, Sullivan L, Canick J, Porter TF, Luthy D, Gross S, Bianchi DW, D'Alton ME. Maternal thyroid hypofunction and pregnancy outcome. Obstet Gynecol. 2008;112(1):85–92.
Cooper DS, Laurberg P. Hyperthyroidism in pregnancy. Lancet Diabetes Endocrinol. 2013;1(3):238–49.
Cooper DS, Rivkees SA. Putting propylthiouracil in perspective. J Clin Endocrinol Metab. 2009;94(6):1881–2.
Coulon AL, Savagner F, Briet C, Vernin M, Munier M, Chabre O, Rodien P. Prolonged and severe gestational thyrotoxicosis due to enhanced hCG sensitivity of a mutant thyrotropin receptor. J Clin Endocrinol Metab. 2016;101(1):10–1.
Craig WY, Allan WC, Kloza EM, Pulkkinen AJ, Waisbren S, Spratt DI, Palomaki GE, Neveux LM, Haddow JE. Mid-gestational maternal free thyroxine concentration and offspring neurocognitive development at age two years. J Clin Endocrinol Metab. 2012;97(1):E22–8.
Dashe JS, Casey BM, Wells CE, McIntire DD, Byrd EW, Leveno KJ, Cunningham FG. Thyroid-stimulating hormone in singleton and twin pregnancy: importance of gestational age-specific reference ranges. Obstet Gynecol. 2005;106(4):753–7.
de Escobar GM, Ares S, Berbel P, Obregón MJ, del Rey FE. The changing role of maternal thyroid hormone in fetal brain development. Semin Perinatol. 2008;32(6):380–6.
De Groot L, Abalovich M, Alexander EK, Amino N, Barbour L, Cobin RH, Eastman CJ, Lazarus JH, Luton D, Mandel SJ, Mestman J, Rovet J, Sullivan S. Management of thyroid dysfunction during pregnancy and postpartum: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2012;97(8):2543–65.
DeLong GR, Leslie PW, Wang SH, Jiang XM, Zhang ML, Rakeman M, Jiang JY, Ma T, Cao XY. Effect on infant mortality of iodination of irrigation water in a severely iodine-deficient area of China. Lancet. 1997;350(9080):771–3.

Dosiou C, Medici M. Management of endocrine disease: isolated maternal hypothyroxinemia during pregnancy: knowns and unknowns. Eur J Endocrinol. 2017;176(1):R21–38.

Evans C, Gregory JW, Barton J, Bidder C, Gibbs J, Pryce R, Al-Muzaffar I, Ludgate M, Warner J, John R, Moat SJ. Transient congenital hypothyroidism due to thyroid-stimulating hormone receptor blocking antibodies: a case series. Ann Clin Biochem. 2011;48(Pt 4):386–90.

Fan X, Wu L. The impact of thyroid abnormalities during pregnancy on subsequent neuropsychological development of the offspring: a meta-analysis. J Matern Fetal Neonatal Med. 2016;29(24):3971–6.

Farwell AP, Dubord-Tomasetti SA, Pietrzykowski AZ, Leonard JL. Dynamic nongenomic actions of thyroid hormone in the developing rat brain. Endocrinology. 2006;147(5):2567–74.

Finken MJ, van Eijsden M, Loomans EM, Vrijkotte TG, Rotteveel J. Maternal hypothyroxinemia in early pregnancy predicts reduced performance in reaction time tests in 5- to 6-year-old offspring. J Clin Endocrinol Metab. 2013;98(4):1417–26.

Fowden AL, Forhead AJ. Endocrine interactions in the control of fetal growth. Nestle Nutr Inst Workshop Ser. 2013;74:91–102.

Gharib H, Papini E, Garber JR, Duick DS, Harrell RM, Hegedüs L, Paschke R, Valcavi R, Vitti P, AACE/ACE/AME Task Force on Thyroid Nodules. American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi medical guidelines for clinical practice for the diagnosis and management of thyroid nodules – 2016 update. Endocr Pract. 2016;22(5):622–39.

Glinoer D, de Nayer P, Bourdoux P, Lemone M, Robyn C, van Steirteghem A, Kinthaert J, Lejeune B. Regulation of maternal thyroid during pregnancy. J Clin Endocrinol Metab. 1990;71(2):276–87.

Glinoer D, De Nayer P, Robyn C, Lejeune B, Kinthaert J, Meuris S. Serum levels of intact human chorionic gonadotropin (HCG) and its free alpha and beta subunits, in relation to maternal thyroid stimulation during normal pregnancy. J Endocrinol Invest. 1993;16(11):881–8.

Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, O'Heir CE, Mitchell ML, Hermos RJ, Waisbren SE, Faix JD, Klein RZ. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med. 1999;341(8):549–55.

Hegedüs L. Clinical practice. The thyroid nodule. N Engl J Med. 2004;351(17):1764–71.

Henrichs J, Bongers-Schokking JJ, Schenk JJ, Ghassabian A, Schmidt HG, Visser TJ, Hooijkaas H, de Muinck Keizer-Schrama SM, Hofman A, Jaddoe VV, Visser W, Steegers EA, Verhulst FC, de Rijke YB, Tiemeier H. Maternal thyroid function during early pregnancy and cognitive functioning in early childhood: the generation R study. J Clin Endocrinol Metab. 2010;95(9):4227–34.

Ide A, Amino N, Kudo T, Yoshioka W, Hisakado M, Nishihara E, Ito M, Fukata S, Nakamura H, Miyauchi A. Comparative frequency of four different types of pregnancy-associated thyrotoxicosis in a single thyroid centre. Thyroid Res. 2017;10:4.

Karras S, Krassas GE. Breastfeeding and antithyroid drugs: a view from within. Eur Thyroid J. 2012;1(1):30–3.

Kiefer FW, Klebermass-Schrehof K, Steiner M, Worda C, Kasprian G, Diana T, Kahaly GJ, Gessl A. Fetal/neonatal thyrotoxicosis in a newborn from a hypothyroid woman with Hashimoto thyroiditis. J Clin Endocrinol Metab. 2017;102(1):6–9.

Kimura M, Amino N, Tamaki H, Ito E, Mitsuda N, Miyai K, Tanizawa O. Gestational thyrotoxicosis and hyperemesis gravidarum: possible role of hCG with higher stimulating activity. Clin Endocrinol (Oxf). 1993;38(4):345–50.

Kinomoto-Kondo S, Umehara N, Sato S, Ogawa K, Fujiwara T, Arata N, Sago H. The effects of gestational transient thyrotoxicosis on the perinatal outcomes: a case-control study. Arch Gynecol Obstet. 2017;295(1):87–93.

Klubo-Gwiezdzinska J, Wartofsky L. Thyroid emergencies. Med Clin North Am. 2012;96(2):385–403.

Knight BA, Shields BM, Hattersley AT, Vaidya B. Maternal hypothyroxinaemia in pregnancy is associated with obesity and adverse maternal metabolic parameters. Eur J Endocrinol. 2016;174(1):51–7.

Konishi J, Iida Y, Kasagi K, Misaki T, Nakashima T, Endo K, Mori T, Shinpo S, Nohara Y, Matsuura N, et al. Primary myxedema with thyrotrophin-binding inhibitor immunoglobulins. Clinical and laboratory findings in 15 patients. Ann Intern Med. 1985;103(1):26–31.

Korevaar TI, Muetzel R, Medici M, Chaker L, Jaddoe VW, de Rijke YB, Steegers EA, Visser TJ, White T, Tiemeier H, Peeters RP. Association of maternal thyroid function during early pregnancy with offspring IQ and brain morphology in childhood: a population-based prospective cohort study. Lancet Diabetes Endocrinol. 2016;4(1):35–43.

Korevaar TI, Steegers EA, Pop VJ, Broeren MA, Chaker L, de Rijke YB, Jaddoe VW, Medici M, Visser TJ, Tiemeier H, Peeters RP. Thyroid autoimmunity impairs the thyroidal response to human chorionic gonadotropin: two population-based prospective cohort studies. J Clin Endocrinol Metab. 2017;102(1):69–77.

Kung AW, Chau MT, Lao TT, Tam SC, Low LC. The effect of pregnancy on thyroid nodule formation. J Clin Endocrinol Metab. 2002;87(3):1010–4.

Laulund AS, Nybo M, Brix TH, Abrahamsen B, Jørgensen HL, Hegedüs L. Duration of thyroid dysfunction correlates with all-cause mortality. The OPENTHYRO Register Cohort. PLoS One. 2014;9(10):e110437.

Laurberg P, Andersen SL. Therapy of endocrine disease: antithyroid drug use in early pregnancy and birth defects: time windows of relative safety and high risk? Eur J Endocrinol. 2014;171(1): R13–20.

Lazarus JH. Pre-conception counselling in graves' disease. Eur Thyroid J. 2012;1(1):24–9.

Lazarus JH, Bestwick JP, Channon S, Paradice R, Maina A, Rees R, Chiusano E, John R, Guaraldo V, George LM, Perona M, Dall'Amico D, Parkes AB, Joomun M, Wald NJ. Antenatal thyroid screening and childhood cognitive function. N Engl J Med. 2012;366(6):493–501.

Lazarus J, Brown RS, Daumerie C, Hubalewska-Dydejczyk A, Negro R, Vaidya B. 2014 European thyroid association guidelines for the management of subclinical hypothyroidism in pregnancy and in children. Eur Thyroid J. 2014;3(2):76–94.

Levy-Shraga Y, Tamir-Hostovsky L, Boyko V, Lerner-Geva L, Pinhas-Hamiel O. Follow-up of newborns of mothers with Graves' disease. Thyroid. 2014;24(6):1032–9.

Luton D, Le Gac I, Vuillard E, Castanet M, Guibourdenche J, Noel M, Toubert ME, Léger J, Boissinot C, Schlageter MH, Garel C, Tébeka B, Oury JF, Czernichow P, Polak M. Management of Graves' disease during pregnancy: the key role of fetal thyroid gland monitoring. J Clin Endocrinol Metab. 2005;90(11):6093–8.

Maraka S, Ospina NM, O'Keeffe DT, Espinosa De Ycaza AE, Gionfriddo MR, Erwin PJ, Coddington 3rd CC, Stan MN, Murad MH, Montori VM. Subclinical hypothyroidism in pregnancy: a systematic review and meta-analysis. Thyroid. 2016;26(4):580–90.

Maraka S, Mwangi R, McCoy RG, Yao X, Sangaralingham LR, Singh Ospina NM, O'Keeffe DT, De Ycaza AE, Rodriguez-Gutierrez R, Coddington 3rd CC, Stan MN, Brito JP, Montori VM. Thyroid hormone treatment among pregnant women with subclinical hypothyroidism: US national assessment. BMJ. 2017;356:i6865.

Matsuura N, Harada S, Ohyama Y, Shibayama K, Fukushi M, Ishikawa N, Yuri K, Nakanishi M, Yokota Y, Kazahari K, Oguchi H. The mechanisms of transient hypothyroxinemia in infants born to mothers with Graves' disease. Pediatr Res. 1997;42(2):214–8.

Millar LK, Wing DA, Leung AS, Koonings PP, Montoro MN, Mestman JH. Low birth weight and preeclampsia in pregnancies complicated by hyperthyroidism. Obstet Gynecol. 1994;84(6):946–9.

Momotani N, Noh J, Oyanagi H, Ishikawa N, Ito K. Antithyroid drug therapy for Graves' disease during pregnancy. Optimal regimen for fetal thyroid status. N Engl J Med. 1986;315(1):24–8.

Monahan M, Boelaert K, Jolly K, Chan S, Barton P, Roberts TE. Costs and benefits of iodine supplementation for pregnant women in a mildly to moderately iodine-deficient population: a modelling analysis. Lancet Diabetes Endocrinol. 2015;3(9):715–22.

Mortimer RH, Cannell GR, Addison RS, Johnson LP, Roberts MS, Bernus I. Methimazole and propylthiouracil equally cross the perfused human term placental lobule. J Clin Endocrinol Metab. 1997;82(9):3099–102.

Murcia M, Rebagliato M, Iñiguez C, Lopez-Espinosa MJ, Estarlich M, Plaza B, Barona-Vilar C, Espada M, Vioque J, Ballester F. Effect of iodine supplementation during pregnancy on infant neurodevelopment at 1 year of age. Am J Epidemiol. 2011;173(7):804–12.

Nazarpour S, Ramezani Tehrani F, Simbar M, Tohidi M, Alavi Majd H, Azizi F. Effects of levothyroxine treatment on pregnancy outcomes in pregnant women with autoimmune thyroid disease. Eur J Endocrinol. 2017;176(2):253–65.

Negro R, Formoso G, Mangieri T, Pezzarossa A, Dazzi D, Hassan H. Levothyroxine treatment in euthyroid pregnant women with autoimmune thyroid disease: effects on obstetrical complications. J Clin Endocrinol Metab. 2006;91(7):2587–91.

Negro R, Greco G, Mangieri T, Pezzarossa A, Dazzi D, Hassan H. The influence of selenium supplementation on postpartum thyroid status in pregnant women with thyroid peroxidase autoantibodies. J Clin Endocrinol Metab. 2007;92(4):1263–8.

Negro R, Schwartz A, Gismondi R, Tinelli A, Mangieri T, Stagnaro-Green A. Universal screening versus case finding for detection and treatment of thyroid hormonal dysfunction during pregnancy. J Clin Endocrinol Metab. 2010;95(4):1699–707.

Noten AM, Loomans EM, Vrijkotte TG, van de Ven PM, van Trotsenburg AS, Rotteveel J, van Eijsden M, Finken MJ. Maternal hypothyroxinaemia in early pregnancy and school performance in 5-year-old offspring. Eur J Endocrinol. 2015;173(5):563–71.

Oken E, Braverman LE, Platek D, Mitchell ML, Lee SL, Pearce EN. Neonatal thyroxine, maternal thyroid function, and child cognition. J Clin Endocrinol Metab. 2009;94(2):497–503.

Oki N, Matsuo H, Nakago S, Murakoshi H, Laoag-Fernandez JB, Maruo T. Effects of 3,5,3′-triiodothyronine on the invasive potential and the expression of integrins and matrix metalloproteinases in cultured early placental extravillous trophoblasts. J Clin Endocrinol Metab. 2004;89(10):5213–21.

Perros P, Boelaert K, Colley S, Evans C, Evans RM, Gerrard Ba G, Gilbert J, Harrison B, Johnson SJ, Giles TE, Moss L, Lewington V, Newbold K, Taylor J, Thakker RV, Watkinson J, Williams GR, British Thyroid Association. Guidelines for the management of thyroid cancer. Clin Endocrinol (Oxf). 2014;81(Suppl 1):1–122.

Pharoah PO, Buttfield IH, Hetzel BS. Neurological damage to the fetus resulting from severe iodine deficiency during pregnancy. Lancet. 1971;1(7694):308–10.

Polak M, Le Gac I, Vuillard E, Guibourdenche J, Leger J, Toubert ME, Madec AM, Oury JF, Czernichow P, Luton D. Fetal and neonatal thyroid function in relation to maternal Graves' disease. Best Pract Res Clin Endocrinol Metab. 2004;18(2):289–302.

Pop VJ, Kuijpens JL, van Baar AL, Verkerk G, van Son MM, de Vijlder JJ, Vulsma T, Wiersinga WM, Drexhage HA, Vader HL. Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy. Clin Endocrinol (Oxf). 1999;50(2):149–55.

Poppe K, Velkeniers B, Glinoer D. Thyroid disease and female reproduction. Clin Endocrinol (Oxf). 2007;66(3):309–21.

Price A, Obel O, Cresswell J, Catch I, Rutter S, Barik S, Heller SR, Weetman AP. Comparison of thyroid function in pregnant and non-pregnant Asian and western Caucasian women. Clin Chim Acta. 2001;308(1–2):91–8.

Qian M, Wang D, Watkins WE, Gebski V, Yan YQ, Li M, Chen ZP. The effects of iodine on intelligence in children: a meta-analysis of studies conducted in China. Asia Pac J Clin Nutr. 2005;14(1):32–42.

Rasmussen NG, Hornnes PJ, Hegedüs L. Ultrasonographically determined thyroid size in pregnancy and post partum: the goitrogenic effect of pregnancy. Am J Obstet Gynecol. 1989;160(5 Pt 1):1216–20.

Rayman MP, Stranges S. Epidemiology of selenium and type 2 diabetes: can we make sense of it? Free Radic Biol Med. 2013;65:1557–64.

Reid SM, Middleton P, Cossich MC, Crowther CA, Bain E. Interventions for clinical and subclinical hypothyroidism pre-pregnancy and during pregnancy. Cochrane Database Syst Rev. 2013;5:CD007752.

Rodien P, Brémont C, Sanson ML, Parma J, Van Sande J, Costagliola S, Luton JP, Vassart G, Duprez L. Familial gestational hyperthyroidism caused by a mutant thyrotropin receptor hypersensitive to human chorionic gonadotropin. N Engl J Med. 1998;339(25):1823–6.

Sahin SB, Ogullar S, Ural UM, Ilkkilic K, Metin Y, Ayaz T. Alterations of thyroid volume and nodular size during and after pregnancy in a severe iodine-deficient area. Clin Endocrinol (Oxf). 2014;81(5):762–8.

Sapin R, D'Herbomez M, Schlienger JL. Free thyroxine measured with equilibrium dialysis and nine immunoassays decreases in late pregnancy. Clin Lab. 2004;50(9–10):581–4.

Shi X, Han C, Li C, Mao J, Wang W, Xie X, Li C, Xu B, Meng T, Du J, Zhang S, Gao Z, Zhang X, Fan C, Shan Z, Teng W. Optimal and safe upper limits of iodine intake for early pregnancy in iodine-sufficient regions: a cross-sectional study of 7190 pregnant women in China. J Clin Endocrinol Metab. 2015;100(4):1630–8.

Shields BM, Freathy RM, Knight BA, Hill A, Weedon MN, Frayling TM, Hattersley AT, Vaidya B. Phosphodiesterase 8B gene polymorphism is associated with subclinical hypothyroidism in pregnancy. J Clin Endocrinol Metab. 2009;94(11):4608–12.

Shields BM, Knight BA, Hill A, Hattersley AT, Vaidya B. Fetal thyroid hormone level at birth is associated with fetal growth. J Clin Endocrinol Metab. 2011;96(6):E934–8.

Shields BM, Knight BA, Hill AV, Hattersley AT, Vaidya B. Five-year follow-up for women with subclinical hypothyroidism in pregnancy. J Clin Endocrinol Metab. 2013;98(12):E1941–5.

Smith TJ, Hegedüs L. Graves' disease. N Engl J Med. 2016;375(16):1552–65.

Stagnaro-Green A, Abalovich M, Alexander E, Azizi F, Mestman J, Negro R, Nixon A, Pearce EN, Soldin OP, Sullivan S, Wiersinga W, American Thyroid Association Taskforce on Thyroid Disease During Pregnancy and Postpartum. Guidelines of the American Thyroid Association for the diagnosis and management of thyroid disease during pregnancy and postpartum. Thyroid. 2011;21(10):1081–125.

Taylor PN, Vaidya B. Side effects of anti-thyroid drugs and their impact on the choice of treatment for thyrotoxicosis in pregnancy. Eur Thyroid J. 2012;1(3):176–85.

Taylor PN, Okosieme OE, Dayan CM, Lazarus JH. Therapy of endocrine disease: impact of iodine supplementation in mild-to-moderate iodine deficiency: systematic review and meta-analysis. Eur J Endocrinol. 2013;170(1):R1–R15.

Taylor PN, Minassian C, Rehman A, Iqbal A, Draman MS, Hamilton W, Dunlop D, Robinson A, Vaidya B, Lazarus JH, Thomas S, Dayan CM, Okosieme OE. TSH levels and risk of miscarriage in women on long-term levothyroxine: a community-based study. J Clin Endocrinol Metab. 2014;99(10):3895–902.

Thangaratinam S, Tan A, Knox E, Kilby MD, Franklyn J, Coomarasamy A. Association between thyroid autoantibodies and miscarriage and preterm birth: meta-analysis of evidence. The BMJ. 2011;342:d2616.

Thorpe-Beeston JG, Nicolaides KH, Felton CV, Butler J, McGregor AM. Maturation of the secretion of thyroid hormone and thyroid-stimulating hormone in the fetus. N Engl J Med. 1991;324(8):532–6.

Toulis KA, Goulis DG, Venetis CA, Kolibianakis EM, Negro R, Tarlatzis BC, Papadimas I. Risk of spontaneous miscarriage in euthyroid women with thyroid autoimmunity undergoing IVF: a meta-analysis. Eur J Endocrinol. 2010;162(4):643–52.

Vaidya B, Bilous M, Hutchinson RS, Connolly V, Jones S, Kelly WF, Bilous RW. Screening for thyroid disease in pregnancy: an audit. Clin Med (Lond). 2002;2(6):599–600.

Vaidya B, Anthony S, Bilous M, Shields B, Drury J, Hutchison S, Bilous R. Detection of thyroid dysfunction in early pregnancy: universal screening or targeted high-risk case finding? J Clin Endocrinol Metab. 2007;92(1):203–7.

van den Boogaard E, Vissenberg R, Land JA, van Wely M, van der Post JA, Goddijn M, Bisschop PH. Significance of (sub)clinical thyroid dysfunction and thyroid autoimmunity before conception and in early pregnancy: a systematic review. Hum Reprod Update. 2011;17(5):605–19.

van der Kaay DC, Wasserman JD, Palmert MR. Management of neonates born to mothers with Graves' disease. Pediatrics. 2016;137(4).

van Wassenaer-Leemhuis A, Ares S, Golombek S, Kok J, Paneth N, Kase J, LaGamma EF. Thyroid hormone supplementation in preterm infants born before 28 weeks gestational age and neurodevelopmental outcome at age 36 months. Thyroid. 2014;24(7):1162–9.

Vasilopoulou E, Loubière LS, Martín-Santos A, McCabe CJ, Franklyn JA, Kilby MD, Chan SY. Differential triiodothyronine responsiveness and transport by human cytotrophoblasts from normal and growth-restricted pregnancies. J Clin Endocrinol Metab. 2010;95(10):4762–70.

Vasilopoulou E, Loubière LS, Lash GE, Ohizua O, McCabe CJ, Franklyn JA, Kilby MD, Chan SY. Triiodothyronine regulates angiogenic growth factor and cytokine secretion by isolated human decidual cells in a cell-type specific and gestational age-dependent manner. Hum Reprod. 2014;29(6):1161–72.

Velasco I, Carreira M, Santiago P, Muela JA, García-Fuentes E, Sánchez-Muñoz B, Garriga MJ, González-Fernández MC, Rodríguez A, Caballero FF, Machado A, González-Romero S, Anarte MT, Soriguer F. Effect of iodine prophylaxis during pregnancy on neurocognitive development of children during the first two years of life. J Clin Endocrinol Metab. 2009;94(9):3234–41.

Velkeniers B, Van Meerhaeghe A, Poppe K, Unuane D, Tournaye H, Haentjens P. Levothyroxine treatment and pregnancy outcome in women with subclinical hypothyroidism undergoing assisted reproduction technologies: systematic review and meta-analysis of RCTs. Hum Reprod Update. 2013;19(3):251–8.

Visser TJ. Pathways of thyroid hormone metabolism. Acta Med Austriaca. 1996;23(1–2):10–6.

Vulsma T, Gons MH, de Vijlder JJ. Maternal-fetal transfer of thyroxine in congenital hypothyroidism due to a total organification defect or thyroid agenesis. N Engl J Med. 1989;321(1):13–6.

Weetman AP. Radioiodine treatment for benign thyroid diseases. Clin Endocrinol (Oxf). 2007;66(6):757–64.

Weetman AP. Immunity, thyroid function and pregnancy: molecular mechanisms. Nat Rev Endocrinol. 2010;6(6):311–8.

WHO, UNICEF. Reaching optimal iodine nutrition in pregnant and lactating women and young children. 2007. Available from: http://www.who.int/nutrition/publications/micronutrients/WHOStatement_IDD_pregnancy.pdf. Accessed 9 Aug 2017.

WHO, UNICEF, ICCIDD. Assessment of iodine deficiency disorders and monitoring their elimination. 3rd ed. World Health Organisation: Geneva; 2007.

Williams FL, Visser TJ, Hume R. Transient hypothyroxinaemia in preterm infants. Early Hum Dev. 2006;82(12):797–802.

Williams F, Hume R, Ogston S, Brocklehurst P, Morgan K, Juszczak E, I2S2 team. A summary of the iodine supplementation study protocol (I2S2): a UK multicentre randomised controlled trial in preterm infants. Neonatology. 2014;105(4):282–9.

Williams FL, Watson J, Day C, Soe A, Somisetty SK, Jackson L, Velten E, Boelen A. Thyroid dysfunction in preterm neonates exposed to iodine. J Perinat Med. 2017;45(1):135–43.

Wing DA, Millar LK, Koonings PP, Montoro MN, Mestman JH. A comparison of propylthiouracil versus methimazole in the treatment of hyperthyroidism in pregnancy. Am J Obstet Gynecol. 1994;170(1 Pt 1):90–5.

Winther KH, Bonnema S, Hegedüs L. Is selenium supplementation in autoimmune thyroid diseases justified? Curr Opin Endocrinol Diabetes Obes. 2017. https://doi.org/10.1097/MED.

Wu SY, Green WL, Huang WS, Hays MT, Chopra IJ. Alternate pathways of thyroid hormone metabolism. Thyroid. 2005;15(8):943–58.

Yang XF, Xu J, Guo HL, Hou XH, Hao LP, Liu LG, Sun XF. Effect of excessive iodine exposure on the placental deiodinase activities and Hoxc8 expression during mouse embryogenesis. Br J Nutr. 2007;98(1):116–22.

Yassa L, Marqusee E, Fawcett R, Alexander EK. Thyroid hormone early adjustment in pregnancy (the THERAPY) trial. J Clin Endocrinol Metab. 2010;95(7):3234–41.

Yoshihara A, Noh J, Yamaguchi T, Ohye H, Sato S, Sekiya K, Kosuga Y, Suzuki M, Matsumoto M, Kunii Y, Watanabe N, Mukasa K, Ito K, Ito K. Treatment of Graves' disease with antithyroid drugs in the first trimester of pregnancy and the prevalence of congenital malformation. J Clin Endocrinol Metab. 2012;97(7):2396–403.

Yoshihara A, Noh JY, Mukasa K, Suzuki M, Ohye H, Matsumoto M, Kunii Y, Watanabe N, Suzuki N, Kameda T, Sugino K, Ito K. Serum human chorionic gonadotropin levels and thyroid hormone levels in gestational transient thyrotoxicosis: is the serum hCG level useful for differentiating between active Graves' disease and GTT? Endocr J. 2015;62(6):557–60.

Zhou SJ, Anderson AJ, Gibson RA, Makrides M. Effect of iodine supplementation in pregnancy on child development and other clinical outcomes: a systematic review of randomized controlled trials. Am J Clin Nutr. 2013;98:1241–54.

Zimmermann MB. Iodine deficiency. Endocr Rev. 2009;30(4):376–408.

Zimmermann M, Delange F. Iodine supplementation of pregnant women in Europe: a review and recommendations. Eur J Clin Nutr. 2004;58(7):979–84.

Non-thyroidal Illness

24

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Abstract

The non-thyroidal illness syndrome (NTIS) is a term used to describe alterations in thyroid function tests observed in critically ill patients in the absence of intrinsic thyroid disease. Several studies have demonstrated that it has a high prevalence among hospitalized patients and it is significantly associated with the severity and the outcome of the disease. In the last decades there has been a shift in our view of the pathogenetic mechanisms underlying the syndrome. It has been increasingly recognized that alterations in the hypothalamus and the pituitary play a predominant role in the pathogenesis of NTIS, whereas the contribution of peripheral pathways, such as deiodinase activity, does not seem to be as significant as considered in the past. The majority of studies agree that treatment with thyroid hormone (TH) is not beneficial. However, TH may be reserved as an option for high-risk patients with very low TH levels and protracted disease, in whom some degree of hypothyroidism may be present.

Keywords

Non-thyroidal illness syndrome · Low T_3 syndrome · Euthyroid sick syndrome · Deiodinases · Critical illness

Introduction

The terms non-thyroidal illness syndrome (NTIS), low T_3 syndrome, and euthyroid sick syndrome have been interchangeably used to describe a state of low serum total T_3 levels associated with various illnesses and starvation. NTIS can be associated with any illness and occurs very rapidly after the onset of acute stress; its prevalence is very high among hospitalized patients and it is considered a predictor of clinical outcome (Alevizaki et al. 2007; Plikat et al. 2007). It is conceivable that NTIS represents a physiological response to serve homeostasis and reduce energy expenditure.

Two major mechanisms contribute to the alterations in thyroid function tests (TFTs) in NTIS: a central component with impaired feedback of the hypothalamus-pituitary-thyroid (HPT) axis and changes in peripheral TH metabolism and action.

In this chapter the most recent literature on NTIS is summarized with an overview of basic facts on thyroid hormone (TH) metabolism, key points in the diagnosis of NTIS, and evidence associating the syndrome with disease severity and prognosis. The plausible pathophysiologic mechanisms underlying NTIS are discussed in detail, and data on the management of patients with NTIS are reviewed.

Thyroid Hormone Metabolism and Action

The secretion of TH is tightly regulated by the HPT axis. The release of TRH from the hypothalamus stimulates the synthesis and release of TSH from the anterior pituitary leading to the secretion of TH from the thyroid gland. TSH is normally under strong genetic control, as shown in studies of healthy twins (Hansen et al. 2004). The individual reference range is much narrower than that in the population (Andersen et al. 2002). The major secretory product is T_4, while T_3 is released in much smaller amounts. T_3 is the most metabolically active TH; 80% of T_3 is derived from extrathyroidal tissue by T_4 conversion to T_3. The classic genomic action of T_3 is exerted through binding to the thyroid hormone receptors (TR) TRA1, TRB1, and TRB2, which form heterodimers with retinoid X receptor (RXR) (Brent 2012). This results in conformational changes, dissociation of corepressors, recruitment of coactivators, and recognition of thyroid hormone response elements (THRE) in promoters of target genes to initiate transcription and eventually protein synthesis (Oetting and Yen 2007). T_3 exerts an inhibitory action on TRH and TSH synthesis and secretion via THR signaling and completes a negative feedback loop (Fig. 1).

In target organs, thyroid hormone availability and cellular action is regulated by deiodinases, which belong to a selenocysteine containing enzyme family. T_4 is converted to T_3 by type 1 and type 2 iodothyronine deiodinase (D1 and D2, respectively), whereas the role of type 3 deiodinase (D3) is mainly the conversion of T_4 to the metabolically inactive reverse T_3 (rT_3) (Fig. 2) (Arrojo and Bianco 2011).

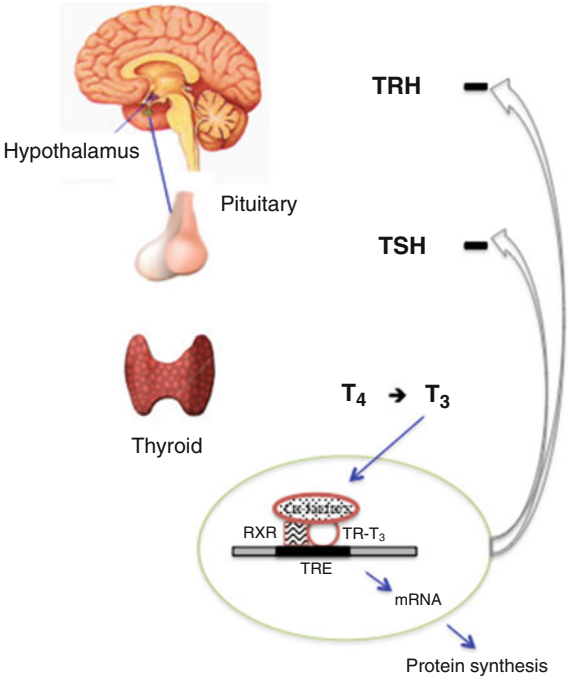

Fig. 1 Overview of the hypothalamus-pituitary-thyroid axis and the genomic action of thyroid hormones. *RXR* retinoid X receptor, *TR* thyroid hormone receptor, *TRE* thyroid response element

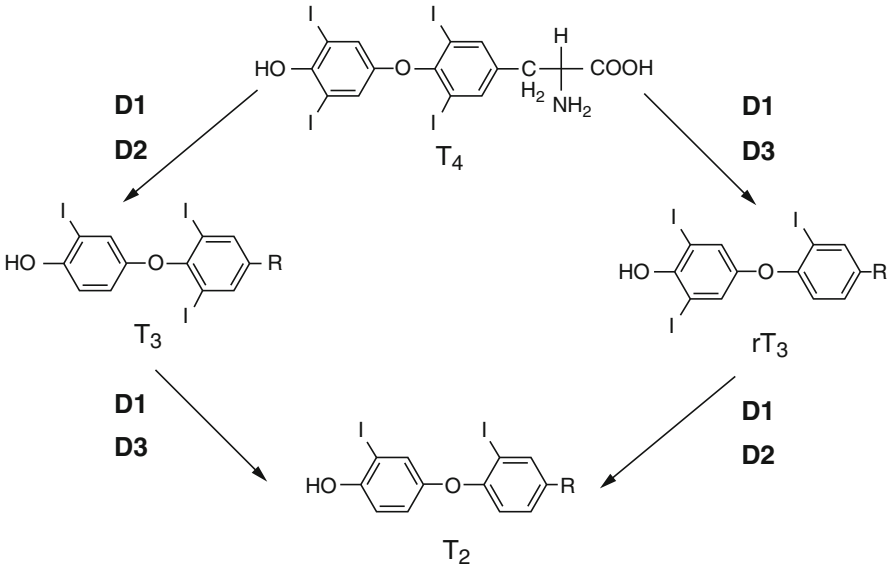

Fig. 2 Summary of the action of the deiodinases on thyroid hormone metabolism. *D1* deiodinase 1, *D2* deiodinase 2, *D3* deiodinase 3, rT_3 reverse T_3, T_2 3,3′-diiodo-I-thyronine

Definition of NTIS

The term non-thyroidal illness syndrome is used to describe an ensemble of thyroid function abnormalities in the absence of intrinsic thyroid disease. These abnormalities typically include isolated hypotriiodothyroninemia, whereas in more severe or prolonged cases, low T_4 levels may also be found. Serum TSH concentration remains low or normal in most cases, and TSH elevation may be observed during the recovery period.

The decrease of thyroid hormone levels during acute illness and fasting has been long documented in the seminal studies of Burger et al. (Burger et al. 1976) and Harris et al. (Harris et al. 1978). NTIS has a high prevalence, up to 50% among hospitalized patients, and it has been most commonly reported in relation to myocardial infarction, coronary artery bypass grafting (CABG), infectious disease, sepsis, trauma, brain injury, chronic obstructive pulmonary disease, gastrointestinal disease, burns, malignancy, surgery, and hospitalization in the intensive care unit (ICU) (Pappa et al. 2011).

Association of NTIS Severity with Outcome

When the illness is mild or acute, the only manifestation is usually low total T_3 levels, whereas when the NTIS is more severe or prolonged, total T_4 levels may also drop, while TSH remains normal or paradoxically low for the decreased TH levels (Table 1).

Table 1 Course of thyroid function tests (serum hormone levels) in the evolution of the non-thyroidal illness syndrome. *NL* within normal range

	Mild	Moderate	Severe	Recovery
Total T_4	NL	NL/ Low NL	↓/ ↓↓	↓/ NL
Total T_3	Low NL/↓	↓/↓↓	↓↓	↓/ NL
TSH	NL	NL/ High NL	NL/ High NL	↑/ NL
Free T_4	NL	NL	Low NL/↓	Low NL/ NL
Reverse T_3	High NL/↑	↑/↑↑	↑↑	↑/ NL

Several studies have correlated the degree of decrease in TH levels with the severity and prognosis of the underlying disease and demonstrated that the lower the serum T_3 (and, in some cases, serum T_4), the higher the mortality risk in these patients (Alevizaki et al. 2007; Plikat et al. 2007). In studies performed in the ICU setting, nonsurvivors had significantly lower T_3, T_4, and TSH levels and higher rT_3 compared to survivors (Peeters et al. 2005; Rothwell and Lawler 1995). The scale of the decline in T_3 and T_4 levels and the increase in rT_3 have been found to reflect the severity of the underlying illness, predict mortality risk, and correlate with several cardiopulmonary and functional parameters, such as the Acute Physiology and Chronic Health Evaluation (APACHE) II score and the degree of disability after acute stroke (Alevizaki et al. 2007) (Fig. 3). Interestingly, an association was found with T_3 and rT_3 concentrations and the activity of liver D1 and skeletal muscle D3 activities in postmortem tissues. In patients who died of cardiovascular collapse and of brain damage liver D1 activity was lowest (Peeters et al. 2003).

Although studies of NTIS in pediatric populations are limited, there are a few studies on infants and children with heart disease or undergoing cardiac surgery, which show a strong correlation between the severity of TFTs alterations and clinical outcome. Children with severe NTIS (lowest serum T_3 and T_4 concentrations) have prolonged hospitalization and increased requirement of mechanical ventilation and pediatric ICU stay (Marks 2009).

There is also evidence that elevated total rT_3 level is associated with mortality even in the independently living elderly population, suggesting that this may serve as a marker of declining health (Forestier et al. 2009).

Diagnosis

It is crucial to correctly and timely differentiate NTIS from other causes of altered TFTs, in which specific treatment is warranted, although this may frequently be challenging. The hallmark of NTIS is low circulating total T_3 levels with

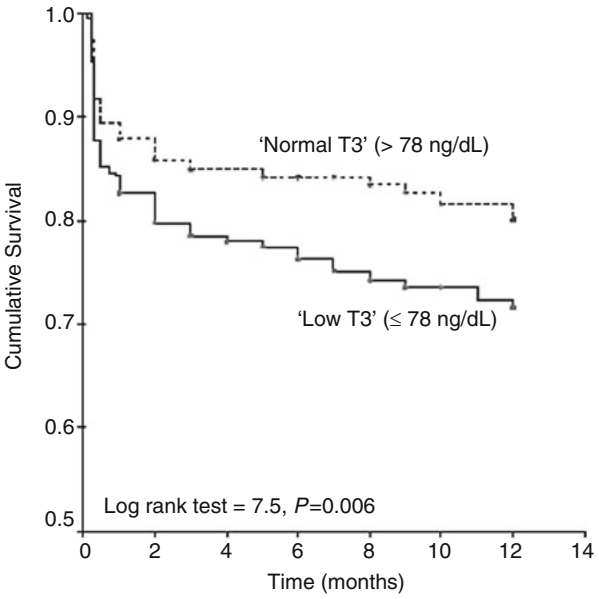

Fig. 3 Kaplan–Meier survival curve of patients with "low T_3" compared to patients with "normal T_3" over the first 12 months after the acute stroke (Reproduced from Alevizaki et al. 2007)

inappropriately normal TSH, accompanied by elevated total rT_3 levels. In severe or protracted diseases, total T_4 levels may decline and a decrease in TSH may be noted.

The patients' clinical history and physical examination are useful in the differential diagnosis between NTIS and intrinsic thyroid disease. An elevated TSH concentration is the key alteration of TFTs in primary hypothyroidism. However, TSH levels may decrease in the acutely ill patients, especially those receiving dopamine or corticosteroids (Haugen 2009). The presence of elevated anti-thyroperoxidase or thyroglobulin antibody levels and/or classical hypoechogenicity at thyroid ultrasound also supports the diagnosis of primary hypothyroidism (Jonklaas et al. 2014). The combination of high TSH with low T_4 levels is suggestive of primary hypothyroidism, although this thyroid profile may also be observed during recovery from NTIS. Therefore, in some cases diagnosis may be delayed until after recovery from the acute illness and hence it is recommended to observe the course of TFTs. Regarding the differential diagnosis of NTIS from secondary hypothyroidism, testing the function of other pituitary axes, such as cortisol, prolactin, and gonadotrophin levels, may provide important clues to the diagnosis (Alexopoulou et al. 2004).

Of special note, the effect of several drugs on thyroid function tests should be taken into consideration (Table 2) (Haugen 2009). Dopamine, a frequently used medication in critically ill patients, is known to suppress TSH and significantly decrease T_4 and T_3 levels to the level of hypothyroidism. Besides dopamine and dopamine agonists (e.g., bromocryptine), other medications, such as glucocorticoids, somatostatin analogues, and bexarotene, an RXR agonist, are associated with substantial TSH suppression (Brabant et al. 1989; Samuels et al. 1992; Ohzeki et al. 1993). The role of certain antiepileptic medication (carbamazepine, oxcarbamazepine, valproic acid) and the biguanide metformin in inhibiting TSH secretion has been reported in some studies (Miller and Carney

Table 2 Summary of drugs interfering with thyroid function tests. *TH* thyroid hormones, *TBG* thyroid hormone-binding globulin (Adapted from Haugen 2009)

Thyroid function alterations	Medication
TSH suppression	Dopamine and dopamine agonists Glucocorticoids Somatostatin analogues Bexarotene (RXR agonists) Metformin Valproic acid Carbamazepine
Inhibition of TH secretion	Iodide Amiodarone Lithium Aminoglutethimide
TH displacement from TBG	Salicylate acid Furosemide (high doses) Heparin Probenecid
Increased TH hepatic metabolism	Phenytoin

2006; Vigersky et al. 2006), but lacks confirmation and, therefore, remains unclarified. In addition, the clinician should be aware of drugs that inhibit the secretion of T_4 and T_3, such as lithium, iodide, amiodarone, and aminoglutethimide. Further, salicylates and high doses of furosemide inhibit binding of T_4 and T_3 to thyroxine-binding globulin (TBG) leading to transient increases in free T_4, whereas phenytoin increases the hepatic metabolism of thyroid hormones (Haugen 2009; Larsen 1972).

It is important to correctly identify patients with severe hypothyroidism presenting with myxedema coma, a diagnosis associated with high mortality rate (Hampton 2013). Of note, in case of co-occurring NTIS, TSH concentration in these patients may not be as markedly increased. Characteristic features in the clinical presentation that guide the diagnosis include hypothermia and altered mental status. The key points in the management of these patients include supportive care, treatment of the precipitating illness (usually myocardial infarction, infection, or cerebrovascular accident), and administration of stress dose corticosteroids followed by judicious replacement with TH.

Pathogenetic Mechanisms

The traditional view of NTIS was that the decline of TH levels is the result of reduced activity of D1 in the liver, leading to decreased conversion of T_4 to T_3 in the periphery, and enhanced D3 activity, resulting in high levels of the inactive metabolite rT_3. However, in the last decades, there is strong evidence that the pathogenesis of NTIS does not merely involve impaired deiodination of thyroid hormones in the liver, but rather includes two major components: (1) a peripheral one involving changes in TH metabolism and action in target tissues (alterations in deiodinase

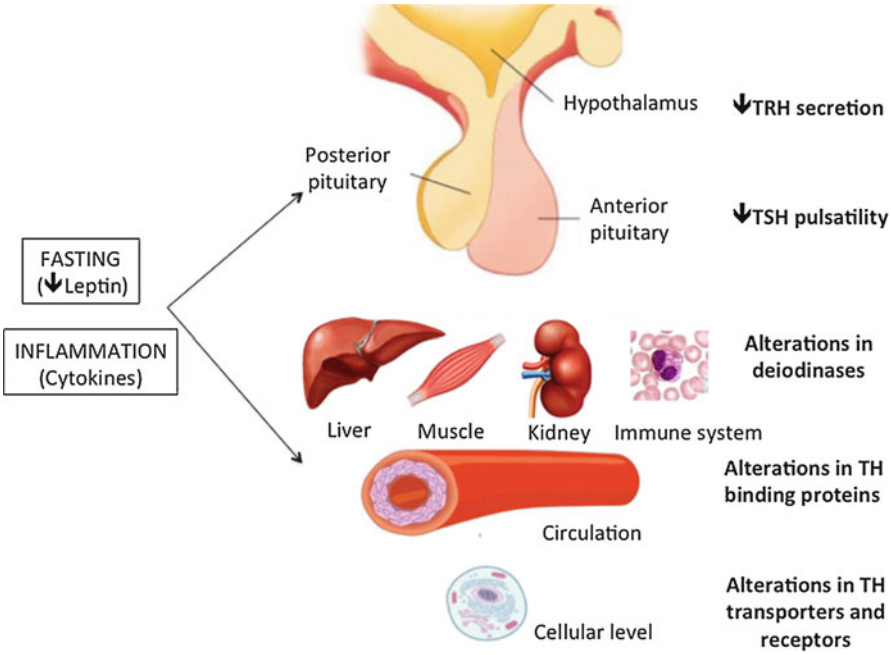

Fig. 4 Schematic representation of the main elements underlying the pathophysiology of non-thyroidal illness syndrome. *TH* thyroid hormones

activity, expression of thyroid hormone receptors, transporters, and binding proteins) (Peeters et al. 2003; Mebis et al. 2009a) and (2) a central one with alterations in the HPT axis and impaired negative feedback in the hypothalamus and the pituitary (decreased TRH secretion and TSH pulsatility) (Fig. 4) (Fliers et al. 1997; de Vries et al. 2015). Also, it appears that these alterations are dependent on the timing, nature, and severity of the underlying illness.

Deiodinases

The majority of literature is in agreement with NTIS being an adaptive, beneficial response to illness in order to reduce the availability of the active T_3 and, thus, decrease energy expenditure and limit catabolism (Everts et al. 1996). This is partly mediated by alterations in the activity of deiodinases, in specific suppression of D1, which decreases the conversion of T_4 to T_3, and induction of D3 resulting in inactivation of TH and rise in total rT_3 (Peeters et al. 2003). It appears that the role of deiodinases may be less significant than previously recognized, whereas the changes occurring at the level of the hypothalamus and the pituitary (discussed below) are of greater importance.

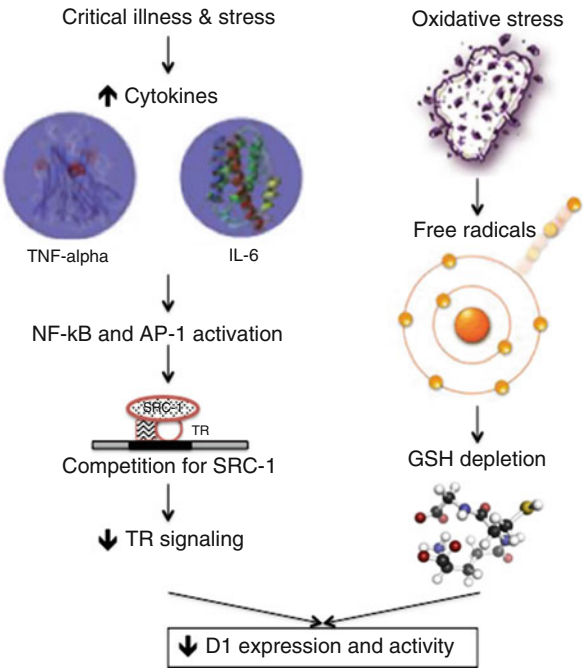

Fig. 5 Possible mechanisms explaining D1 suppression in the non-thyroidal illness syndrome. *NF-kB* nuclear factor kappa-light-chain-enhancer of activated B cell, *AP-1* activator protein-1, *SRC-1* steroid receptor coactivator-1, *TR* thyroid hormone receptor, *GSH* glutathione

Deiodinase 1

The pattern of D1 expression in NTIS has been extensively studied. D1 is localized in the plasma membrane and is expressed mainly in the liver, kidney, thyroid, and pituitary. Several animal models of NTIS and studies in patients with critical illness have demonstrated a significant decrease in liver D1 expression and activity and a correlation with low liver T_3 concentration (Peeters et al. 2003).

Two elegant hypotheses have been proposed to explain the suppressed D1 activity in NTIS: Critical illness and stress result in high cytokine levels that activate the nuclear factor kappa-light-chain-enhancer of activated B cell (NF-kB) and the activator protein-1 pathways. These inflammatory signaling pathways are thought to compete with THR for limiting amounts of steroid receptor coactivator-1 (SRC-1), which in turn results in reduced THR activation and, subsequently, downregulation of D1 expression (Yu and Koenig 2000, 2006) (Fig. 5). In the same study, administration of SRC-1 prevented the decrease of TH levels following lipopolysaccharide (LPS) injection and restored D1 expression in the liver. However, another study demonstrated that THRB knockout mice had similar decreases in TH and D1 levels after LPS treatment as wild type mice, suggesting that THRB does not play an important role in regulating D1 expression and activity (Kwakkel et al. 2008).

Another scenario is that oxidative stress and release of reactive oxygen species lead to depletion of glutathione stores, required for D1 catalytic activity to ensue.

This was further supported by the finding that addition of N-acetylcysteine, an antioxidant that restores intracellular glutathione levels, prevented the interleukin-6 (IL-6)-induced D1 suppression (Wajner et al. 2011).

The concept of decreased D1 activity as the causal factor for low T_3 levels in NTIS has been challenged by studies in D1-deficient mice in that these have normal T_3 levels, suggesting that D1 suppression may in fact be the consequence rather than the cause of low T_3 concentration (Schneider et al. 2006). In addition, it was demonstrated that the decrease of T_3 levels in wild type mice precedes the decrease in liver D1 expression, further supporting the idea that D1 inhibition is not causally related with the TFTs alterations in NTI (Schneider et al. 2006).

Deiodinase 2

Deiodinase 2 is a major enzyme involved in local TH metabolism and tissue T_3 production (Bianco and Kim 2006). D2 is localized in the endoplasmic reticulum and is tightly regulated by TH levels. Specifically, T_3 downregulates D2 mRNA expression, and T_4 and rT_3 (which are substrates for D2) increase its ubiquitination and proteosomal degradation (Bianco and Kim 2006). Skeletal muscle D2 is considered a major source of T_3 under basal conditions. Studies using animal models of NTIS as well as studies in ICU patients have demonstrated that D2 expression in skeletal muscle is increased and thought to be the result of increased cAMP response element-binding protein (CREB) signaling, which functions as a transcriptional antagonist of the thyroid hormone receptor (Mebis et al. 2007). However, in studies performed in septic patients, muscle D2 expression was decreased, and this was attributed to the effect of fasting and caloric deprivation (Rodriguez-Perez et al. 2008).

The increasingly recognized role of D2 in alterations of the central component of NTIS will be discussed in detail below in the relevant section of the chapter.

Deiodinase 3

The role of D3, the major TH inactivating enzyme, in the pathogenesis of NTIS has also been studied. D3 is localized in the plasma membrane and is expressed in brain neurons, liver, and the innate immune system. The effect of illness on D3 expression is dependent on the timing and type of illness. It has been shown that fasting and prolonged illness result in marked increases in D3 expression and activity in the liver and also muscle (Fig. 6) (de Vries et al. 2015). These changes are considered to serve thyroid economy and reduce total energy expenditure in the catabolic setting of illness. Low leptin levels have been hypothesized to explain the high D3 activity in the liver during fasting, whereas the upregulation of D3 in muscle may be the result of hypoxia and decreased tissue perfusion and represent an adaptive mechanism to acute stress (Simonides et al. 2008; Boelen et al. 2012). In support of the latter, it was recently shown that D3 induction during myocardial infarction is associated with expression of several microRNAs that promote the proliferative capacity of cardiomyocytes (Janssen et al. 2016).

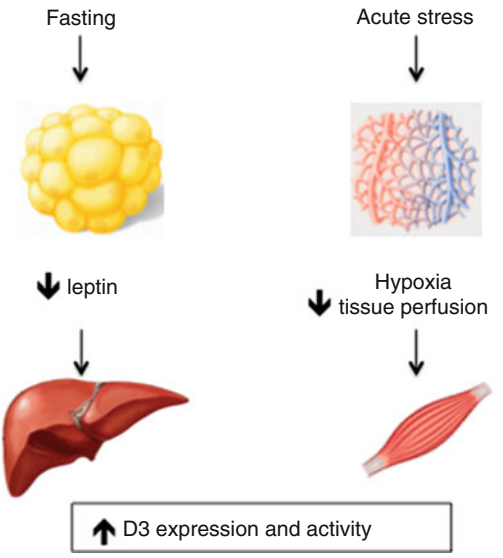

Fig. 6 Pathways underlying D3 induction in the non-thyroidal illness syndrome

Interestingly, D3 activity was increased in granulocytes, a component of the innate immune system, during illness. Myeloperoxidase (MPO) is an important enzyme abundantly expressed in granulocytes, critical for their antimicrobial activity. In their study, Boelen et al. suggested that the increased D3 activity observed in NTIS is a means to provide MPO with iodide and thus augment the bactericidal machinery and immune defense (Boelen et al. 2008).

There is convincing data supporting that D3 expression is increased in various tissues in NTIS. However, and contrary to previous considerations, the alterations in D3 activity in NTIS do not seem to be involved in changes in serum TH levels, since D3 knockout and wild type mice have similar serum TH concentrations during inflammation (Boelen et al. 2011).

Thyroid Hormone-Binding Proteins

One of the earliest explanations for the etiology of NTIS involved changes in TH binding (Chopra et al. 1985). This was supported by findings of reduced concentrations of albumin and other TH-binding proteins and of their reduced affinity to thyroid hormones. Also, it was found that serum total TH levels were significantly decreased in NTIS, whereas free TH levels dropped only modestly, further supporting the role of altered TH binding (Chopra 1998).

Prolonged illness and malnutrition are states of high catabolism and associated with lower albumin and transthyretin levels, whereas the acute phase response results in a decrease in TBG and, thus, total TH concentrations (den Brinker et al. 2005). Serine protein inhibitors, such as serpins, are activated at sites of inflammation and inactivate TBG. It has been demonstrated that in acute stress, such as during CABG, TBG is

rapidly degraded by protease cleavage, leading to an instant drop in total T_3 levels in serum (Afandi et al. 2000). Another hypothesis supports the presence of TBG-binding inhibitors. Reducing the affinity with their main transporter in serum, thyroid hormones are released from the binding proteins and their clearance is increased (Jirasakuldech et al. 2000). Elevated levels of bilirubin and unsaturated nonesterified fatty acids (NEFAs) may impair TH binding, transport, and peripheral conversion of T_4 to T_3 (Lim et al. 1993). As an example, heparin, which is frequently used in critically ill patients, induces the generation of NEFAs, whereas drugs, such as high dose furosemide, antiepileptics, and salicylates, decrease the binding of T_4 to TBG (Bayer 1983). In addition, it has been postulated that alternative pathways, such as sulfation, glucuronidation, and ether link cleavage may accelerate TH degradation and clearance, further reducing total TH levels in serum (Wu et al. 2005). The contribution of these pathways has not been well studied.

On the other hand, the notion of impaired TH binding as a causal factor in NTIS has been disputed (Brent and Hershman 1986). In this study, patients with severe NTIS were treated with LT_4, which quickly restored serum T_4 levels. Finding that the TH pool could easily be replenished with LT_4 argues against a significant role of TBG deficiency or TH-binding inhibitors in the pathophysiology of NTIS.

Cytokines

Cytokines, such as tumor necrosis factor alpha (TNF-alpha), interleukin 1 and 6 (IL-1 and IL-6, respectively), are major mediators of the acute phase response and regulate the release of acute phase proteins and stress hormones (Moshage 1997). Their downstream pathway involves activation of nuclear factor kappa-light-chain-enhancer of activated B cell (NF-kB), a major transcription factor in stress and inflammation. There is a plethora of data from studies in animal models of NTIS and in man, on the role of cytokines in the illness induced decrease of TH levels, yet these data are often contradictory and the role of cytokines in the pathogenesis of NTIS is not straightforward (Fig. 7). IL-6 levels were negatively correlated with serum T_3 in hospitalized patients. Furthermore, infusion of TNF-alpha, IL-1, and IL-6 resulted in a decrease of TH levels mimicking NTIS, although administration of neutralizing antibodies did not reverse the alterations in thyroid function (van der Poll et al. 1995).

Several studies have shown that pro-inflammatory cytokines result in changes in the expression of genes involved in TH production and metabolism. In specific, IL-1 infusion in human and rat cell culture impaired thyroperoxidase (TPO) mRNA expression and protein content, as well as the sodium iodide symporter (NIS) mediated iodide uptake under basal conditions and upon TSH stimulation (Gerard et al. 2006). Interferon-gamma (IFN-gamma) impaired TSH-induced TH and thyroglobulin (Tg) secretion and mRNA expression, as well as TSH-induced TPO and NIS expression and iodide uptake (de Vries et al. 2015). Moreover, administration of TNF-alpha inhibited the TSH-induced cAMP response, Tg secretion, and NIS expression (Tang et al. 1995). Cytokines were also found to inhibit D1

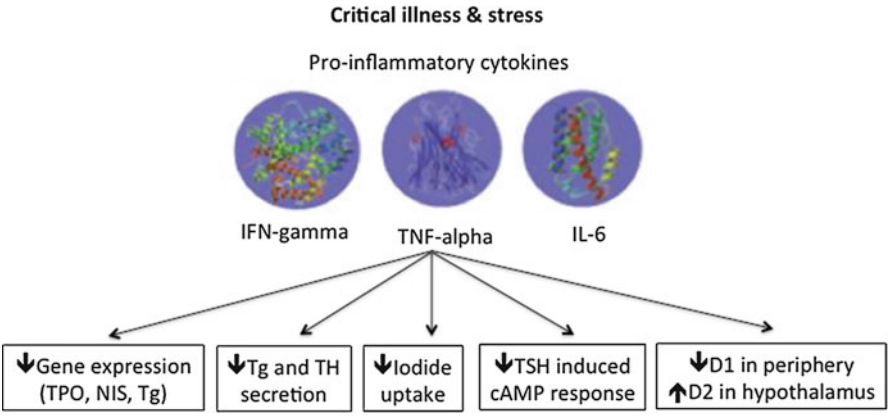

Fig. 7 Summary of the role of pro-inflammatory cytokines in non-thyroidal illness syndrome. *TPO* thyroid peroxidase, *NIS* sodium iodide symporter, *Tg* thyroglobulin, *TH* thyroid hormones

expression and activity and play a major role in central D2 upregulation in the hypothalamus (mechanism described in detail below) (de Vries et al. 2015; Fekete et al. 2004, 2005).

In aggregate, the literature is in agreement on cytokines being key partners in the pathogenesis of NTIS. This concerns interference with various parts of the synthesis pathway of TH in the thyroid, from iodide uptake to TH secretion, as well as the central component of the HPT axis. It seems that it is not a single cytokine, but rather a network of pro-inflammatory molecules, components of the acute phase response, that are implicated (de Vries et al. 2015).

Thyroid Hormone Transporters and Receptors

The main feature of NTIS is the low T_3 levels, observed not only in serum but also at the tissue level (liver, kidneys, brain, lungs). This is considered an adaptive mechanism in response to catabolic states, in order to reduce energy requirements.

The transport of thyroid hormones in the cells is subject to the energy state intracellularly. When T_4 transport in the liver is inhibited, this is rate limiting for the total plasma T_3 production, because there is decreased substrate available for conversion to T_3. In the study of Kaptein et al., T*4* tissue transport in NTIS was decreased by 50% and T*3* production by 70%. The authors suggested hepatic ATP depletion as an underlying mechanism, since NTIS patients are in a negative energy balance, whereas the acidic cellular environment in acute illness and the presence of high concentrations of NEFAs might also explain the decreased T_4 uptake in the liver (Kaptein et al. 1982).

Regarding TR signaling, there is data from mouse models of NTIS after LPS administration, which evokes a systemic inflammatory response, showing decreased

liver TR expression. Along the same line, patients with severe NTIS suffering from septic shock had very low mRNA levels of TRs (both TRA1 and TRB1), RXR, and the thyroid hormone specific transporter MCT8 in their muscle and adipose tissue (Mebis et al. 2009a).

Taken together, these data indicate that NTIS reflects a systemic stress response and most likely serves as a mechanism to combat critical illness by suppressing energy demands.

Alterations in the Central Component of the HPT Axis

The hypothesis that has increasingly gained ground over the last decades is that the etiology of NTIS is mainly a disturbed negative feedback regulation at the level of the hypothalamus and the pituitary, and this is particularly relevant in prolonged illness. It is now believed that in prolonged illness reduced hypothalamic stimulation of the pituitary thyrotrophs and, subsequently, the thyroid gland leads to reduced TH secretion (de Vries et al. 2015; Warner and Beckett 2010; Van den Berghe 2014). Another supporting argument is that recovery from NTIS is usually heralded by a rise in TSH levels (Table 1).

Hypothalamus

Hypothalamic TRH neurons play an important role in the set point of thyroid hormone homeostasis. The main feature of the altered HPT axis in NTIS is the suppression of TRH expression. TRH expression was much lower in the hypothalamic paraventricular (PVN) nuclei in nonsurvivors with chronic illness compared to those with acute illness, and, additionally, a positive correlation was observed between TRH mRNA in the PVN and antemortem tissue T*3* and TSH (Fliers et al. 1997). Also, in a pioneer study of Van den Berghe et al., infusion of TRH resulted in increased T_4 and T_3 concentrations. This intervention led to an increase in rT_3 as well, which was overcome by combining TRH with a GH-secretagogue, which prevented the rise of rT_3 and induced an anabolic response (Van den Berghe et al. 2002).

Several factors have been proposed leading to TRH suppression, including the neurohormonal agents endogenous dopamine and cortisol (Haugen 2009; Alkemade et al. 2005). However, there is accumulating evidence for local induction of D2 inhibiting TRH secretion in the PVN (Fig. 8). The inflammatory response in critical illness can increase D2 and decrease D3 expression in the hypothalamus, leading to a local elevation of T_3 levels altering the negative feedback (Mebis et al. 2009b).

T_3 may be taken up by TRH neurons by either diffusion from the cerebrospinal fluid or by axonal terminals of the TRH neurons present in the median eminence. Another proposed mechanism is that T_3 is released in the arcuate nucleus influencing the neurons projecting in the PVN (Fekete and Lechan 2007). Fekete et al. elegantly demonstrated a marked increase in D2 mRNA expression in tanycytes, specialized cells lining the wall of the third ventricle, in a rat model of NTIS after LPS administration (Fekete et al. 2004).

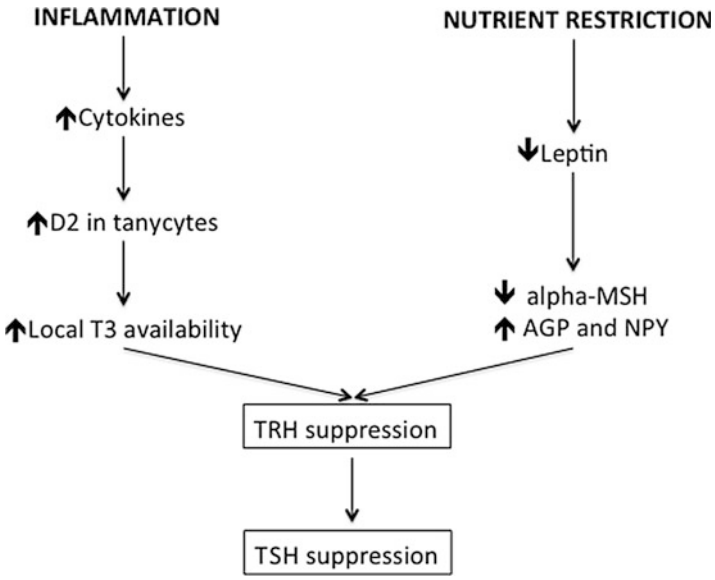

Fig. 8 Proposed central mechanisms of the non-thyroidal illness syndrome. *Alpha-MSH* alpha melanocyte stimulating hormone, *AGP* agouti-related protein, *NPY* neuropeptide Y

The induction of local D2 leads to increased T_4 to T_3 conversion, which can influence adjacent neurons in a paracrine fashion and lower TRH mRNA expression.

Possible triggers for the increase of local D2 expression are: inflammation-induced NF-kB and its associated downstream pathway and a decrease in leptin concentration (Kwakkel et al. 2009). Regarding the latter, it is known that during fasting leptin levels decrease accompanied by a decrease in the production of alpha-melanocyte stimulating hormone (alpha-MSH). Moreover, agouti-related protein (AGP) and neuropeptide Y (NPY) attenuate CREB phosphorylation in TRH neurons (Vella et al. 2011). As a result, the set point for feedback inhibition of the *TRH* gene by thyroid hormones is lowered. Interestingly, the upregulation of D2 in the hypothalamus appears to be independent of the decrease in TH levels, contrary to D2 expression in the pituitary or other areas in the brain, such as the cortex (Escobar-Morreale et al. 1997). An alternative hypothesis could involve D2 induction in the pituitary, which suppresses local TSH mRNA, but this has not been validated in animal models of prolonged NTIS (Beck-Peccoz and Mariotti 2000).

Pituitary

Patients with NTIS have inappropriately normal or low levels of TSH in the presence of low T_3 (Table 3). The inappropriately low TSH levels in the presence of low T_3 and the absence of TSH pulsatility are prominent in prolonged NTIS.

Table 3 Causal factors of low TSH levels in non-thyroidal illness syndrome

Factors explaining low TSH levels in NTIS
↓ TRH secretion
Impaired TRH metabolism
↓ TSH pulsatility
↓ Response to TRH
Loss of TSH nocturnal surge
↓ Leptin levels (?) (causing ↓ TRH and TSH secretion)

This could be the result of suppressed leptin, mediating changes similar to those induced by fasting. Leptin acts via the hypothalamic arcuate nucleus, induces pro-opiomelanocortin and alpha-MSH production, and activates melanocortin 4 receptor (MC4R) (Walley et al. 2009). Studies in mice have shown that fasting results in decreased leptin, suppressed MC4R activation and, thus, decreased TRH and TSH secretion, whereas leptin administration prevents the fasting-associated TSH decrease (Boelen et al. 2006). This mechanism is considered to be energy saving, although, paradoxically, in studies of humans with NTIS leptin levels are either normal or elevated (Bornstein et al. 1997).

As discussed previously, critical illness stimulates D2 expression in the mediobasal hypothalamus, thus increasing local T_3 availability and suppressing TRH, which subsequently inhibits TSH expression. Alternatively, the locally produced T_3 can be transported from the hypothalamus to the pituitary via the portal capillaries and directly inhibit TSH expression.

Furthermore, illness and prolonged restriction of macronutrients result in a reduction of peripheral TH uptake and of THR activation, and the feedback loop leads to a decrease in TSH expression. The TSH decrease following LPS administration is blunted in THRB knockout compared to the wild type mice, suggesting that the NTIS-associated decrease in TSH expression is dependent on THRB signaling (Fekete and Lechan 2014).

There is much evidence that in NTIS the diurnal rhythm and nocturnal surge of TSH may be lost and TSH response to TRH blunted. There is also data supporting that TSH biological activity is reduced due to impaired glycosylation, possibly mediated by cytokines or glucocorticoids (Rothwell and Lawler 1995). In a mouse model of acute NTIS after LPS injection, inflammation induced by cytokines resulted in increased D1 and D2 activities in the pituitary along with decreased TSH expression (Boelen et al. 2004).

Overall, in NTIS, it appears that cytokine activation elicited by inflammation and fasting are the two core elements affecting various pathways in the hypothalamus

and the pituitary. Especially with regard to decreased nutritional intake, the aim is clearly to decrease T_3 in favor of thyroid economy, reduce the metabolic rate, and prevent muscle breakdown in order to promote survival. This way NTIS becomes a protective response towards critical illness.

Acute and Prolonged NTIS

A major difference between acute and protracted illness is that in prolonged NTIS peripheral tissues respond to increase rather than to decrease the availability of thyroid hormones, in an attempt to limit catabolism. Compensation for low tissue T_3 is achieved through upregulation of D2 expression and activity (in skeletal muscle) (Mebis et al. 2007), increased expression of thyroid hormone receptors (contrary to the acute illness, in which their expression is decreased), and increased expression of TH transporters. In a rabbit model with prolonged NTIS, expression of transporters MCT8 and MCT10 increased in liver and muscle, respectively, which was reversed after treatment with T_4 and T_3 (Mebis et al. 2009a).

Clinical Management

The role of treatment of NTIS patients with thyroid hormone has been fiercely debated over the last decades, but currently most experts seem to agree that the majority of critically ill patients do not clearly benefit from TH treatment. Surprisingly, only few randomized controlled trials (RCT) have been performed to properly address this issue. In most studies, pharmacologic doses of either T_4 or T_3 were used and none showed that treatment results in improved patient outcome (Brent and Hershman 1986). A RCT in patients with acute renal failure even showed increased mortality in the group receiving thyroxine therapy (Acker et al. 2000). There are several studies evaluating the effect of T*3* supplementation in heart disease patients, the majority of which showed improvement in cardiac parameters but no significant benefit as regards survival and prognosis (Spratt et al. 2007; Bennett-Guerrero et al. 1996; Pingitore et al. 2008). The limited number of studies with small patient numbers and poor statistical power are limitations that do not allow rejecting, confidently, a beneficial role of treatment of NTIS with T_4 and/or T_3. In addition, only few studies have used mortality and morbidity rates as primary endpoints and most have evaluated the effect of TH therapy on indirect indices, such as cardiopulmonary and functional parameters. There has also been significant variability in the age composition of the study populations and the severity of the underlying illnesses. Lastly, even in patients receiving TH treatment, the intervention did not normalize the tissue levels of TH (Peeters et al. 2005).

Overwhelming evidence indicates that NTIS is an adaptive response to critical illness, and at least part of the TH alterations accompanying critical illness can be explained by fasting (Van den Berghe 2014). Yet, there is a subset of patients with protracted disease, who are well nourished and have TFTs compatible with NTIS. Signs and symptoms of hypothyroidism may be found in these patients, which could be

central given the background of suppressed TRH expression. In the presence of true hypothyroidism, recovery from the underlying illness can be delayed and the evolution of the hospital stay complicated (Schulman and Mechanick 2012). Additionally, an important consideration for patients who have known hypothyroidism and are admitted to the ICU is that treatment with levothyroxine should be considered during their stay in the ICU, although continuation of chronic care is not a primary focus in the ICU.

Although there is currently no compelling evidence to advocate use of TH in critically ill patients, individuals with very low TH levels (T_4 below 4 ug/dl- or 51 nmol/L) who have high mortality rates represent a small subgroup that might benefit from treatment with TH (De Groot 2006). If therapy was to be given in these selected cases, and in the absence of contraindications such as cardiac decompensation and arrhythmias, one could consider initially giving higher LT_3 doses to rapidly restore the TH pool, followed by lower replacement doses and coadministration of LT_4. Adjustment of the dosing schedule should follow serum T_4 and T_3 values targeting low normal TH levels. As deiodination increases, LT_3 doses can gradually be tapered and LT_4 increased (De Groot 2006). It has been argued that patients with NTIS have selenium deficiency, which may contribute to the decreased deiodinase activity. In line with that seen in elderly without serious disease (Winther et al. 2015), selenium supplementation in ICU patients had only a modest effect on TH levels, and therefore selenium is currently not considered to have a role in the treatment of NTIS (Berger et al. 2001).

Alternative Therapies

An alternative approach, which focuses on the pathophysiology of reduced TRH expression in prolonged illness, would be infusion of hypothalamic releasing factors. The aim is to reactivate the HPT axis and induce an anabolic response, which is required to augment the recovery from prolonged illness. Van den Berghe et al. introduced the approach of combination therapy with TRH and GH-releasing peptide-2, intravenously, and showed encouraging results in terms of restoring TH concentrations, TSH pulsatility, as well as overall metabolic indices (bone markers and anabolic variables) (Van den Berghe et al. 1999). This pioneer study highlighted that patients with NTIS have a multifaceted illness that requires management of multiple hormonal deficits and correction of their catabolic status. However, the study included only a limited number of patients. Large, well-designed and adequately powered RCTs are needed to properly investigate the effect of treatment of NTIS with pituitary secretagogues.

Specific Populations

The management of NTIS in premature infants deserves specific attention. Such infants, without exception, present with some degree of hypothyroxinemia. They also often have concurrent illness, such as respiratory distress and infection, which

further aggravates NTIS. It is well established that untreated congenital hypothyroxinemia in neonates has a deleterious impact on brain development. Taken together, it seems reasonable to consider lowering the threshold of TFTs alterations when treating premature infants. This is also supported by a study showing a lower mortality rate in premature infants receiving prophylactic combined T_4 and T_3 treatment compared to untreated ones (Schonberger et al. 1979). Complicating the interpretation, a meta-analysis demonstrated failure of TH treatment in reducing mortality, improving severity of underlying disease or improving neurodevelopmental outcome (Osborn and Hunt 2007). A recent study also failed to show a significant benefit of T_4 supplementation on the growth pattern and neurodevelopmental outcome in very low birth weight infants (Uchiyama et al. 2015).

There is limited data on NTIS in pediatric populations, and most studies have been performed in children undergoing cardiac surgery. Only few prospective studies have assessed the effect of intravenous T_3 treatment in infants and children. These either found no significant difference in outcome measures of illness severity or showed only modest improvement in ventilation requirements and hospital stay, yet with minimal adverse events (Marks 2009).

Regarding treatment of NTIS in subjects undergoing CABG, there is abundant data from studies using animal models demonstrating improvement in cardiac contractility and left ventricular function with T_3 replacement, as well as a decrease in systemic vascular resistance. Patients were found to require less inotropic support and improve their hemodynamic parameters. This was also demonstrated in the pediatric population, but with no clear benefit in terms of survival (Portman et al. 2010).

A limited number of small studies in patients with congestive heart failure have found that LT_3 therapy decreased the systemic vascular resistance and improved the cardiac output and the neurohumoral profile. A decrease in serum norepinephrine, N-terminal pro-B type natriuretic peptide, and aldosterone was noted along with an increase in the left ventricle end-diastolic volume. However, compelling evidence of improved survival is currently lacking (Sacca 2009).

Another specific population to be mentioned is that of brain dead heart donors. Studies in animals as well as man have shown that intravenous T_3 therapy restored hemodynamic and biochemical abnormalities, decreased inotropic support, and preserved the cardiac function prior to transplantation (Novitzky et al. 2014). Again, confirmation by RCTs is awaited. Because T_3 therapy was not deleterious and might even be beneficial, treatment of brain dead heart donors with T_3 has been advocated by many (McKeown et al. 2012).

Summary

The prevalence of the non-thyroidal illness syndrome (NTIS) is high among hospitalized and critically ill patients. NTIS is independently associated with the severity of the underlying disease and serves as a marker of prognosis. Over the last decades, our knowledge concerning the pathogenetic mechanisms behind NTIS has expanded. The syndrome has two major components: one that involves altered

feedback regulation at the level of the hypothalamus and the pituitary, and a peripheral one interfering with the production, transport, and action of thyroid hormones. The bulk of the literature is in agreement with NTIS being a physiologic, adaptive response to environmental factors (nutrient availability and inflammatory and stressful stimuli) and an energy conserving mechanism. Most evidence suggests that replacement therapy with TH is not required in NTIS. It could be considered as an option in carefully selected individuals with severe NTIS, in whom some degree of tissue hypothyroidism may be present.

NTIS reflects the severity of the underlying disease and is not causally linked to the primary illness. Therefore, efforts should focus on improving the management of the disease itself, rather than supplementing the patients with thyroid hormone to improve their thyroid function tests.

References

Acker CG, Singh AR, Flick RP, Bernardini J, Greenberg A, Johnson JP. A trial of thyroxine in acute renal failure. Kidney Int. 2000;57(1):293–8.

Afandi B, Schussler GC, Arafeh AH, Boutros A, Yap MG, Finkelstein A. Selective consumption of thyroxine-binding globulin during cardiac bypass surgery. Metabolism. 2000;49(2):270–4.

Alevizaki M, Synetou M, Xynos K, Pappa T, Vemmos KN. Low triiodothyronine: a strong predictor of outcome in acute stroke patients. Eur J Clin Investig. 2007;37(8):651–7.

Alexopoulou O, Beguin C, De Nayer P, Maiter D. Clinical and hormonal characteristics of central hypothyroidism at diagnosis and during follow-up in adult patients. Eur J Endocrinol. 2004;150(1):1–8.

Alkemade A, Unmehopa UA, Wiersinga WM, Swaab DF, Fliers E. Glucocorticoids decrease thyrotropin-releasing hormone messenger ribonucleic acid expression in the paraventricular nucleus of the human hypothalamus. J Clin Endocrinol Metab. 2005;90(1):323–7.

Andersen S, Pedersen KM, Bruun NH, Laurberg P. Narrow individual variations in serum T(4) and T(3) in normal subjects: a clue to the understanding of subclinical thyroid disease. J Clin Endocrinol Metab. 2002;87(3):1068–72.

Arrojo EDR, Bianco AC. Type 2 deiodinase at the crossroads of thyroid hormone action. Int J Biochem Cell Biol. 2011;43(10):1432–41.

Bayer MF. Effect of heparin on serum free thyroxine linked to post-heparin lipolytic activity. Clin Endocrinol. 1983;19(5):591–6.

Beck-Peccoz P, Mariotti S. In: De Groot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, et al., editors. Physiology of the hypothalamic-pituitary-thyroid axis. South Dartmouth: Endotext; 2000.

Bennett-Guerrero E, Jimenez JL, White WD, D'Amico EB, Baldwin BI, Schwinn DA. Cardiovascular effects of intravenous triiodothyronine in patients undergoing coronary artery bypass graft surgery. A randomized, double-blind, placebo- controlled trial. Duke T3 study group. JAMA. 1996;275(9):687–92.

Berger MM, Reymond MJ, Shenkin A, Rey F, Wardle C, Cayeux C, et al. Influence of selenium supplements on the post-traumatic alterations of the thyroid axis: a placebo-controlled trial. Intensive Care Med. 2001;27(1):91–100.

Bianco AC, Kim BW. Deiodinases: implications of the local control of thyroid hormone action. J Clin Invest. 2006;116(10):2571–9.

Boelen A, Kwakkel J, Thijssen-Timmer DC, Alkemade A, Fliers E, Wiersinga WM. Simultaneous changes in central and peripheral components of the hypothalamus-pituitary-thyroid axis in lipopolysaccharide-induced acute illness in mice. J Endocrinol. 2004;182(2):315–23.

Boelen A, Kwakkel J, Vos XG, Wiersinga WM, Fliers E. Differential effects of leptin and refeeding on the fasting-induced decrease of pituitary type 2 deiodinase and thyroid hormone receptor beta2 mRNA expression in mice. J Endocrinol. 2006;190(2):537–44.

Boelen A, Boorsma J, Kwakkel J, Wieland CW, Renckens R, Visser TJ, et al. Type 3 deiodinase is highly expressed in infiltrating neutrophilic granulocytes in response to acute bacterial infection. Thyroid. 2008;18(10):1095–103.

Boelen A, Kwakkel J, Fliers E. Beyond low plasma T3: local thyroid hormone metabolism during inflammation and infection. Endocr Rev. 2011;32(5):670–93.

Boelen A, van Beeren M, Vos X, Surovtseva O, Belegri E, Saaltink DJ, et al. Leptin administration restores the fasting-induced increase of hepatic type 3 deiodinase expression in mice. Thyroid. 2012;22(2):192–9.

Bornstein SR, Torpy DJ, Chrousos GP, Licinio J, Engelmann L. Leptin levels are elevated despite low thyroid hormone levels in the "euthyroid sick" syndrome. J Clin Endocrinol Metab. 1997;82(12): 4278–9.

Brabant A, Brabant G, Schuermeyer T, Ranft U, Schmidt FW, Hesch RD, et al. The role of glucocorticoids in the regulation of thyrotropin. Acta Endocrinol. 1989;121(1):95–100.

Brent GA. Mechanisms of thyroid hormone action. J Clin Invest. 2012;122(9):3035–43.

Brent GA, Hershman JM. Thyroxine therapy in patients with severe nonthyroidal illnesses and low serum thyroxine concentration. J Clin Endocrinol Metab. 1986;63(1):1–8.

Burger A, Nicod P, Suter P, Vallotton MB, Vagenakis P, Braverman L. Reduced active thyroid hormone levels in acute illness. Lancet. 1976;1(7961):653–5.

Chopra IJ. Simultaneous measurement of free thyroxine and free 3,5,3′-triiodothyronine in undiluted serum by direct equilibrium dialysis/radioimmunoassay: evidence that free triiodothyronine and free thyroxine are normal in many patients with the low triiodothyronine syndrome. Thyroid. 1998;8(3):249–57.

Chopra IJ, Teco GN, Mead JF, Huang TS, Beredo A, Solomon DH. Relationship between serum free fatty acids and thyroid hormone binding inhibitor in nonthyroid illnesses. J Clin Endocrinol Metab. 1985;60(5):980–4.

den Brinker M, Joosten KF, Visser TJ, Hop WC, de Rijke YB, Hazelzet JA, et al. Euthyroid sick syndrome in meningococcal sepsis: the impact of peripheral thyroid hormone metabolism and binding proteins. J Clin Endocrinol Metab. 2005;90(10):5613–20.

De Groot LJ. Non-thyroidal illness syndrome is a manifestation of hypothalamic-pituitary dysfunction, and in view of current evidence, should be treated with appropriate replacement therapies. Crit Care Clin. 2006;22(1):57–86.vi.

de Vries EM, Fliers E, Boelen A. The molecular basis of the non-thyroidal illness syndrome. J Endocrinol. 2015;225(3):R67–81.

Escobar-Morreale HF, Obregon MJ, Hernandez A, Escobar del Rey F, Morreale de Escobar G. Regulation of iodothyronine deiodinase activity as studied in thyroidectomized rats infused with thyroxine or triiodothyronine. Endocrinology. 1997;138(6):2559–68.

Everts ME, de Jong M, Lim CF, Docter R, Krenning EP, Visser TJ, et al. Different regulation of thyroid hormone transport in liver and pituitary: its possible role in the maintenance of low T3 production during nonthyroidal illness and fasting in man. Thyroid. 1996;6(4):359–68.

Fekete C, Lechan RM. Negative feedback regulation of hypophysiotropic thyrotropin-releasing hormone (TRH) synthesizing neurons: role of neuronal afferents and type 2 deiodinase. Front Neuroendocrinol. 2007;28(2–3):97–114.

Fekete C, Lechan RM. Central regulation of hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions. Endocr Rev. 2014;35(2):159–94.

Fekete C, Gereben B, Doleschall M, Harney JW, Dora JM, Bianco AC, et al. Lipopolysaccharide induces type 2 iodothyronine deiodinase in the mediobasal hypothalamus: implications for the nonthyroidal illness syndrome. Endocrinology. 2004;145(4):1649–55.

Fekete C, Sarkar S, Christoffolete MA, Emerson CH, Bianco AC, Lechan RM. Bacterial lipopolysaccharide (LPS)-induced type 2 iodothyronine deiodinase (D2) activation in the mediobasal hypothalamus (MBH) is independent of the LPS-induced fall in serum thyroid hormone levels. Brain Res. 2005;1056(1):97–9.

Fliers E, Guldenaar SE, Wiersinga WM, Swaab DF. Decreased hypothalamic thyrotropin-releasing hormone gene expression in patients with nonthyroidal illness. J Clin Endocrinol Metab. 1997;82(12):4032–6.

Forestier E, Vinzio S, Sapin R, Schlienger JL, Goichot B. Increased reverse triiodothyronine is associated with shorter survival in independently-living elderly: the Alsanut study. Eur J Endocrinol. 2009;160(2):207–14.

Gerard AC, Boucquey M, van den Hove MF, Colin IM. Expression of TPO and ThOXs in human thyrocytes is downregulated by IL-1alpha/IFN-gamma, an effect partially mediated by nitric oxide. Am J Physiol Endocrinol Metab. 2006;291(2):E242–53.

Hampton J. Thyroid gland disorder emergencies: thyroid storm and myxedema coma. AACN Adv Crit Care. 2013;24(3):325–32.

Hansen PS, Brix TH, Sorensen TI, Kyvik KO, Hegedus L. Major genetic influence on the regulation of the pituitary-thyroid axis: a study of healthy Danish twins. J Clin Endocrinol Metab. 2004;89(3):1181–7.

Harris AR, Fang SL, Azizi F, Lipworth L, Vagenakis AG, Barverman LE. Effect of starvation on hypothalamic-pituitary-thyroid function in the rat. Metabolism. 1978;27(9):1074–83.

Haugen BR. Drugs that suppress TSH or cause central hypothyroidism. Best Pract Res Clin Endocrinol Metab. 2009;23(6):793–800.

Janssen R, Zuidwijk MJ, Muller A, van Mil A, Dirkx E, Oudejans CB, et al. MicroRNA 214 is a potential regulator of thyroid hormone levels in the mouse heart following myocardial infarction, by targeting the thyroid-hormone-inactivating enzyme deiodinase type III. Front Endocrinol (Lausanne). 2016;7:22.

Jirasakuldech B, Schussler GC, Yap MG, Drew H, Josephson A, Michl J. A characteristic serpin cleavage product of thyroxine-binding globulin appears in sepsis sera. J Clin Endocrinol Metab. 2000;85(11):3996–9.

Jonklaas J, Bianco AC, Bauer AJ, Burman KD, Cappola AR, Celi FS, et al. Guidelines for the treatment of hypothyroidism: prepared by the American Thyroid Association Task Force on thyroid hormone replacement. Thyroid. 2014;24(12):1670–751.

Kaptein EM, Robinson WJ, Grieb DA, Nicoloff JT. Peripheral serum thyroxine, triiodothyronine and reverse triiodothyronine kinetics in the low thyroxine state of acute nonthyroidal illnesses. A noncompartmental analysis. J Clin Invest. 1982;69(3):526–35.

Kwakkel J, Chassande O, van Beeren HC, Wiersinga WM, Boelen A. Lacking thyroid hormone receptor beta gene does not influence alterations in peripheral thyroid hormone metabolism during acute illness. J Endocrinol. 2008;197(1):151–8.

Kwakkel J, van Beeren HC, Ackermans MT, Platvoet-Ter Schiphorst MC, Fliers E, Wiersinga WM, et al. Skeletal muscle deiodinase type 2 regulation during illness in mice. J Endocrinol. 2009;203(2):263–70.

Larsen PR. Salicylate-induced increases in free triiodothyronine in human serum. Evidence of inhibition of triiodothyronine binding to thyroxine-binding globulin and thyroxine-binding prealbumin. J Clin Invest. 1972;51(5):1125–34.

Lim CF, Docter R, Visser TJ, Krenning EP, Bernard B, van Toor H, et al. Inhibition of thyroxine transport into cultured rat hepatocytes by serum of nonuremic critically ill patients: effects of bilirubin and nonesterified fatty acids. J Clin Endocrinol Metab. 1993;76(5):1165–72.

Marks SD. Nonthyroidal illness syndrome in children. Endocrine. 2009;36(3):355–67.

McKeown DW, Bonser RS, Kellum JA. Management of the heartbeating brain-dead organ donor. Br J Anaesth. 2012;108(Suppl 1):i96–107.

Mebis L, Langouche L, Visser TJ, Van den Berghe G. The type II iodothyronine deiodinase is up-regulated in skeletal muscle during prolonged critical illness. J Clin Endocrinol Metab. 2007;92(8):3330–3.

Mebis L, Paletta D, Debaveye Y, Ellger B, Langouche L, D'Hoore A, et al. Expression of thyroid hormone transporters during critical illness. Eur J Endocrinol. 2009a;161(2):243–50.

Mebis L, Debaveye Y, Ellger B, Derde S, Ververs EJ, Langouche L, et al. Changes in the central component of the hypothalamus-pituitary-thyroid axis in a rabbit model of prolonged critical illness. Crit Care. 2009b;13(5):R147.

Miller J, Carney P. Central hypothyroidism with oxcarbazepine therapy. Pediatr Neurol. 2006;34(3):242–4.
Moshage H. Cytokines and the hepatic acute phase response. J Pathol. 1997;181(3):257–66.
Novitzky D, Mi Z, Sun Q, Collins JF, Cooper DK. Thyroid hormone therapy in the management of 63,593 brain-dead organ donors: a retrospective analysis. Transplantation. 2014;98(10):1119–27.
Oetting A, Yen PM. New insights into thyroid hormone action. Best Pract Res Clin Endocrinol Metab. 2007;21(2):193–208.
Ohzeki T, Hanaki K, Motozumi H, Ohtahara H, Ishitani N, Urashima H, et al. Efficacy of bromocriptine administration for selective pituitary resistance to thyroid hormone. Horm Res. 1993;39(5–6):229–34.
Osborn DA, Hunt RW. Prophylactic postnatal thyroid hormones for prevention of morbidity and mortality in preterm infants. Cochrane Database Syst Rev. 2007;1:CD005948.
Pappa TA, Vagenakis AG, Alevizaki M. The nonthyroidal illness syndrome in the non-critically ill patient. Eur J Clin Investig. 2011;41(2):212–20.
Peeters RP, Wouters PJ, Kaptein E, van Toor H, Visser TJ, Van den Berghe G. Reduced activation and increased inactivation of thyroid hormone in tissues of critically ill patients. J Clin Endocrinol Metab. 2003;88(7):3202–11.
Peeters RP, van der Geyten S, Wouters PJ, Darras VM, van Toor H, Kaptein E, et al. Tissue thyroid hormone levels in critical illness. J Clin Endocrinol Metab. 2005;90(12):6498–507.
Pingitore A, Galli E, Barison A, Iervasi A, Scarlattini M, Nucci D, et al. Acute effects of triiodothyronine (T3) replacement therapy in patients with chronic heart failure and low-T3 syndrome: a randomized, placebo-controlled study. J Clin Endocrinol Metab. 2008;93(4):1351–8.
Plikat K, Langgartner J, Buettner R, Bollheimer LC, Woenckhaus U, Scholmerich J, et al. Frequency and outcome of patients with nonthyroidal illness syndrome in a medical intensive care unit. Metabolism. 2007;56(2):239–44.
van der Poll T, Van Zee KJ, Endert E, Coyle SM, Stiles DM, Pribble JP, et al. Interleukin-1 receptor blockade does not affect endotoxin-induced changes in plasma thyroid hormone and thyrotropin concentrations in man. J Clin Endocrinol Metab. 1995;80(4):1341–6.
Portman MA, Slee A, Olson AK, Cohen G, Karl T, Tong E, et al. Triiodothyronine Supplementation in Infants and Children Undergoing Cardiopulmonary Bypass (TRICC): a multicenter placebo-controlled randomized trial: age analysis. Circulation. 2010;122(11 Suppl):S224–33.
Rodriguez-Perez A, Palos-Paz F, Kaptein E, Visser TJ, Dominguez-Gerpe L, Alvarez-Escudero J, et al. Identification of molecular mechanisms related to nonthyroidal illness syndrome in skeletal muscle and adipose tissue from patients with septic shock. Clin Endocrinol. 2008; 68(5):821–7.
Rothwell PM, Lawler PG. Prediction of outcome in intensive care patients using endocrine parameters. Crit Care Med. 1995;23(1):78–83.
Sacca L. Heart failure as a multiple hormonal deficiency syndrome. Circ Heart Fail. 2009;2(2):151–6.
Samuels MH, Henry P, Ridgway EC. Effects of dopamine and somatostatin on pulsatile pituitary glycoprotein secretion. J Clin Endocrinol Metab. 1992;74(1):217–22.
Schneider MJ, Fiering SN, Thai B, Wu SY, St Germain E, Parlow AF, et al. Targeted disruption of the type 1 selenodeiodinase gene (Dio1) results in marked changes in thyroid hormone economy in mice. Endocrinology. 2006;147(1):580–9.
Schonberger W, Grimm W, Emmrich P, Gempp W. Thyroid administration lowers mortality in premature infants. Lancet. 1979;2(8153):1181.
Schulman RC, Mechanick JI. Metabolic and nutrition support in the chronic critical illness syndrome. Respir Care. 2012;57(6):958–77. discussion 77-8.
Simonides WS, Mulcahey MA, Redout EM, Muller A, Zuidwijk MJ, Visser TJ, et al. Hypoxia-inducible factor induces local thyroid hormone inactivation during hypoxic-ischemic disease in rats. J Clin Invest. 2008;118(3):975–83.
Spratt DI, Frohnauer M, Cyr-Alves H, Kramer RS, Lucas FL, Morton JR, et al. Physiological effects of nonthyroidal illness syndrome in patients after cardiac surgery. Am J Physiol Endocrinol Metab. 2007;293(1):E310–5.

Tang KT, Braverman LE, DeVito WJ. Tumor necrosis factor-alpha and interferon-gamma modulate gene expression of type I 5′-deiodinase, thyroid peroxidase, and thyroglobulin in FRTL-5 rat thyroid cells. Endocrinology. 1995;136(3):881–8.

Uchiyama A, Kushima R, Watanabe T, Kusuda S. Effect of l-thyroxine supplementation on infants with transient hypothyroxinemia of prematurity at 18 months of corrected age: randomized clinical trial. J Pediatr Endocrinol Metab. 2015;28(1–2):177–82.

Van den Berghe G. Non-thyroidal illness in the ICU: a syndrome with different faces. Thyroid. 2014;24(10):1456–65.

Van den Berghe G, Wouters P, Weekers F, Mohan S, Baxter RC, Veldhuis JD, et al. Reactivation of pituitary hormone release and metabolic improvement by infusion of growth hormone-releasing peptide and thyrotropin-releasing hormone in patients with protracted critical illness. J Clin Endocrinol Metab. 1999;84(4):1311–23.

Van den Berghe G, Baxter RC, Weekers F, Wouters P, Bowers CY, Iranmanesh A, et al. The combined administration of GH-releasing peptide-2 (GHRP-2), TRH and GnRH to men with prolonged critical illness evokes superior endocrine and metabolic effects compared to treatment with GHRP-2 alone. Clin Endocrinol. 2002;56(5):655–69.

Vella KR, Ramadoss P, Lam FS, Harris JC, Ye FD, Same PD, et al. NPY and MC4R signaling regulate thyroid hormone levels during fasting through both central and peripheral pathways. Cell Metab. 2011;14(6):780–90.

Vigersky RA, Filmore-Nassar A, Glass AR. Thyrotropin suppression by metformin. J Clin Endocrinol Metab. 2006;91(1):225–7.

Wajner SM, Goemann IM, Bueno AL, Larsen PR, Maia AL. IL-6 promotes nonthyroidal illness syndrome by blocking thyroxine activation while promoting thyroid hormone inactivation in human cells. J Clin Invest. 2011;121(5):1834–45.

Walley AJ, Asher JE, Froguel P. The genetic contribution to non-syndromic human obesity. Nat Rev Genet. 2009;10(7):431–42.

Warner MH, Beckett GJ. Mechanisms behind the non-thyroidal illness syndrome: an update. J Endocrinol. 2010;205(1):1–13.

Winther KH, Bonnema SJ, Cold F, Debrabant B, Nybo M, Cold S, et al. Does selenium supplementation affect thyroid function? Results from a randomized, controlled, double-blinded trial in a Danish population. Eur J Endocrinol. 2015;172(6):657–67.

Wu SY, Green WL, Huang WS, Hays MT, Chopra IJ. Alternate pathways of thyroid hormone metabolism. Thyroid. 2005;15(8):943–58.

Yu J, Koenig RJ. Regulation of hepatocyte thyroxine 5′-deiodinase by T3 and nuclear receptor coactivators as a model of the sick euthyroid syndrome. J Biol Chem. 2000;275(49):38296–301.

Yu J, Koenig RJ. Induction of type 1 iodothyronine deiodinase to prevent the nonthyroidal illness syndrome in mice. Endocrinology. 2006;147(7):3580–5.

Drugs and Other Substances Interfering with Thyroid Function

25

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Abstract

Several drugs and supplements may interfere, at different levels, with regulation of thyroid function. Some drugs may also cause thyroid autoimmunity. This chapter reviews drugs that significantly affect thyroid function. Glucocorticoids, dopamine agonists, somatostatin analogs, and retinoids inhibit TSH secretion. Lithium, tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRIs), antiepileptics, rifampin, metformin, and amiodarone mainly affect directly thyroid function. Interferon-α and antiretroviral drugs may have several effects, including inducing thyroid autoimmunity. The heterogeneous adverse effects induced by antineoplastic agents (cytotoxic and novel anticancer agents, tyrosine kinase inhibitors, bexarotene- and iodine-based cancer therapies, and radioimmunotherapies) will be highlighted. Immunoregulatory drugs (IL-2, denileukin diftitox, thalidomide and lenalidomide, IFN-α, alemtuzumab) and immune checkpoint inhibitors (anti-CTLA4 and anti-PD-1 monoclonal antibodies) mainly promote thyroid autoimmunity. The effects of endocrine disruptors and nutraceuticals and over-the-counter products (selenium, L-carnitine, thyroid hormones, iodine, and biotin) will also be discussed. The last part of the chapter concerns drugs that interfere with levothyroxine (LT4) absorption.

Keywords

Alemtuzumab · Amiodarone · Anti-CTLA4 monoclonal antibodies · Anti-PD-1 monoclonal antibodies · Bexarotene · Cytotoxic agents · Denileukin diftitox · Dopamine · Endocrine disruptors · Glucocorticoids · IFN-α · IL-2 · Immune

checkpoint inhibitors · Inositol · Interferons · L-carnitine · Lenalidomide · Levothyroxine sodium · Lithium · Metformin · Nutraceuticals · Rexinoids · Rifampin · Selective serotonin reuptake inhibitors · Selenium · Somatostatin · Thalidomide · Tricyclic antidepressants · Tyrosine kinase inhibitors

Drugs that Interfere with Hypothalamic-Pituitary-Thyroid Regulation

Glucocorticoids

Corticosteroids are involved in the regulation of diurnal variation of TSH secretion, and high levels of glucocorticoids inhibit TSH secretion (Haugen 2009). The decrease of TSH secretion is due to TRH inhibition in the paraventricular nucleus of the hypothalamus. A single low dose (0.5 mg) of dexamethasone is sufficient to alter TSH levels, while long-term high-dose glucocorticoids (30 mg prednisone/day for 1 week) or endogenous hypercortisolism (Cushing's syndrome) does not result in central hypothyroidism (Brabant et al. 1989; Haugen 2009). Large doses of glucocorticoids, for example, 4 mg of dexamethasone per day, cause a 30 percent decrease in serum T3 concentrations within several days due to inhibition of type 1 deiodinase (Surks and Sievert 1995) (Table 1) (Fig. 1A, C).

Dopamine, Dopamine Agonists, and Dopamine Antagonists

Dopamine is a major regulator of the HPT axis. This is achieved by activating D2 receptors, thereby slightly stimulating hypothalamic TRH secretion and potently inhibiting TSH secretion (Haugen 2009). Dopamine administered intravenously at doses of $\geq$1 μg/kg/min and dopamine agonists administered orally, such as bromocriptine and L-dopa, inhibit TSH secretion. Conversely, the dopamine antagonist metoclopramide increases TSH secretion (Sarne 2016). However, prolonged treatment with bromocriptine does not induce central hypothyroidism (Haugen 2009) (Fig. 1A, B).

Somatostatin

Somatostatinergic pathways are involved in the regulation of pituitary function. Administration of somatostatin decreases both pulse amplitude and pulse frequency of TSH secretion by exerting a direct inhibition on TSH secretion and blunting TRH-stimulated TSH levels in healthy volunteers (Haugen 2009). Somatostatin and long-acting analogs bind to five different extracellular receptors in the pituitary and, via adenylate cyclase signaling, inhibit hormone secretion (Haugen 2009), though not sustainedly. Depending on dose, somatostatin and its analogs may induce a transient subclinical central hypothyroidism (Fig. 1A).

Table 1 Drugs and other substances interfering with thyroid function

1. Drugs interfering with hypothalamic-pituitary-thyroid function
(a) *Glucocorticoids*
(b) *Dopamine, dopamine agonists and dopamine antagonists*
(c) *Somatostatin and somatostatinanalogues*
(d) *Retinoids*
2. Drugs interfering with thyroid function
(a) *Lithium*
(b) *Tricyclic antidepressants and selective serotonin reuptake inhibitors (SSRIs)*
(c) *Antiepileptics*
(d) *Rifampin*
(e) *Metformin*
(f) *Amiodarone*
3. Interferon and other anti-viral drugs
(a) *Interferon-α*
(b) *Highly active antiretroviral therapy (HAART)*
4. Antineoplastic agents
(a) *Cytotoxic and novel anticancer agents*
(b) *Tyrosine kinase inhibitors*
(c) *Bexarotene*
(d) *Iodine-based cancer therapies and radioimmunotherapies*
5. **Immunoregulatory drugs**
(a) *IL-2*
(b) *Denileukindiftitox*
(c) *Thalidomide and lenalidomide*
(d) *IFN-α*
(e) *Alemtuzumab*
(e) *Immune checkpoint inhibitors*
I Anti-CTLA4 monoclonal antibodies
II Anti-PD-1 monoclonal antibodies
6. **Other substances interfering with thyroid function**
(a) *Endocrine disruptors*
(b) *Nutraceuticals and over-the-counter products.*
(1) Selenium and inositol
(2) L-carnitine
(3) Thyroid hormones and iodine
(4) Biotin
7. **Comorbidities and drugs interfering with L-thyroxine absorption** (Table 6)

Retinoids

Rexinoids are a group of derivatives of vitamin A (retinol) that regulate complex gene networks involved in vision and in cell differentiation, proliferation, and apoptosis (Haugen 2009). The retinoid X receptor (RXR) is a member of the nuclear receptor superfamily, which forms heterodimers with other nuclear receptors, such as peroxisome proliferator-activated receptor (PPAR), liver X receptor, and farnesoid X receptor (Lefebvre et al. 2010). Rexinoids are selective agonists of nuclear hormone receptors that enable the formation of the heterodimers to regulate gene expression and inhibit proliferation (Wagner et al. 2017). In normal rats, vitamin A deficiency

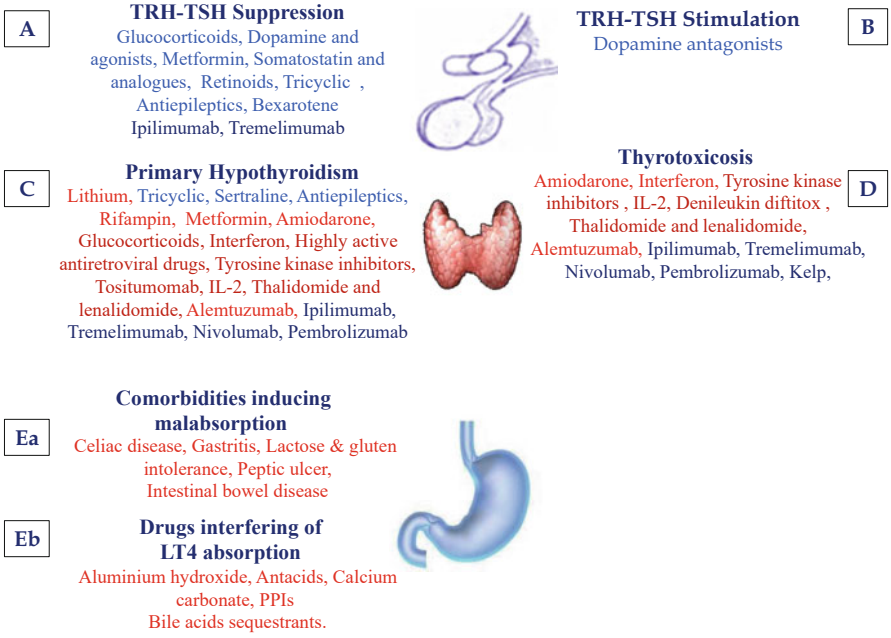

Fig. 1 (**A, B, C, D, Ea, Eb**) Drugs and conditions that affect thyroid hormone function or affect the intestinal absorption or orally taken thyroid hormone

induces central hyperthyroidism by increasing TSHβ mRNA in the pituitary and consequently increasing serum T4 and T3 levels; treatment with retinoic acid normalizes TSHβ mRNA levels (Graeppi-Dulac et al. 2014). Accordingly, retinoids can induce central hypothyroidism by suppressing TSH at the pituitary or the hypothalamic level. Thyroid effects of bexarotene are reported in the section on Antineoplastic Agents (Fig. 1A).

Drugs that Interfere with Thyroid Function

Lithium

Lithium is an effective treatment for bipolar disorder, but long-term lithium treatment has been associated with hypothyroidism (approximately 20%) and goiter (approximately 40%) (Fig. 1C).

In rats lithium decreases the release of thyroid iodine without affecting its uptake, resulting in an increase of intrathyroidal iodine (Berens et al. 1970). It also inhibits thyroid hormone release in both euthyroid and hyperthyroid patients (Spaulding et al. 1972). However, T4 levels do not change as an effect of the prolonged half-life of T4 due to the adaptive reduction of type 1 5′ deiodinase activity (Berens et al. 1970).

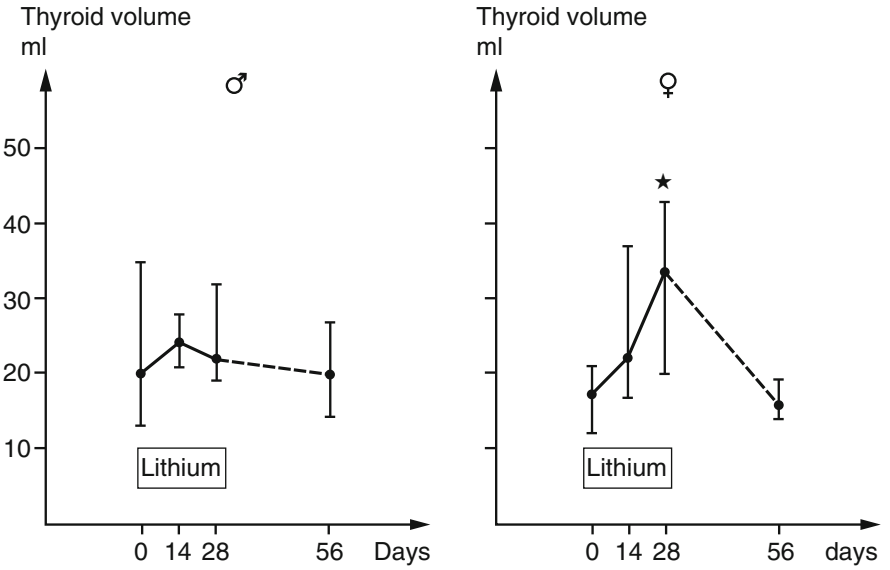

Fig. 2 Thyroid volume (median and range) before, during (day 14 and 28) and after (day 56) treatment with lithium carbonate in 8 males and 8 females. *$P<0.01$ denotes significant difference between day 0 and 28 (paired *t*-test) (From Perrild et al. 1984)

Goiter is the most common thyroidal side effect of lithium therapy, with a highly variable incidence (0–60%), depending on gender, geographical area, methods for diagnosing goiter, and duration of therapy (Fig. 2) (Perrild et al. 1984; Lazarus (2009). Goiter is probably due to the inhibition of thyroid hormone secretion rate and the consequent rise in TSH levels and a likely direct proliferative effect. There is no evidence that thyroid enlargement is associated with an increased incidence of thyroid nodules (Bocchetta and Loviselli 2006). A small study reported that LT4 treatment for hypothyroidism or goiter can prevent further lithium-induced enlargement of the goiter (Bauer et al. 2007).

The incidence of lithium-induced hypothyroidism is variable, depending on the population under evaluation, gender, and the methods for detection. In one study, the annual rate of developing hypothyroidism during lithium therapy was 1.5%, with 6.4% in thyroid antibody-positive and 0.8% in thyroid antibody-negative individuals (Bocchetta et al. 2007), although lithium treatment has not been associated with an increase in thyroid autoimmunity (Lazarus et al. 1986). Lithium-induced hypothyroidism requires prompt treatment with LT4, taking into account that hypothyroidism can worsen symptoms of depression, but does not require withdrawal of the drug.

Thyrotoxicosis is less commonly observed during treatment with lithium. Although some cases of autoimmune hyperthyroidism have been reported, thyrotoxicosis due to destructive thyroiditis is more common. Such cases are self-limiting and the symptoms can be treated with β-blockers. As a consequence of its action on thyroid hormone release, lithium has been used in the treatment of primary hyperthyroidism, although

with less efficacy than that of thionamide therapy (Lazarus 2009). Lithium has been shown, by some authors, to increase the efficacy of 131I treatment for hyperthyroidism and to reduce the increase in thyroid hormone concentrations occurring after treatment (Bogazzi et al. 1999, 2002), but others have failed to demonstrate this effect (Bal et al. 2002). Lithium alters thyroglobulin structure, inhibits iodotyrosine coupling, and inhibits thyroid hormone secretion (Lazarus 2009). In a retrospective cross-sectional study, conducted to determine the risk factors associated with development of thyroid disease in patients receiving lithium, women younger than 60 years were at greatest risk (Kirov et al. 2005). Indeed, women were more prone than men to develop overt or subclinical hypothyroidism (25.8% vs. 8.7%), with prevalence among females exceeding 50% by the age of 65 years (Lazarus 2009). If overt hypothyroidism appears, LT4 therapy should be started, but lithium therapy may be continued (Shine et al. 2015).

Tricyclic and Selective Serotonin Reuptake Inhibitors (SSRIs)

Tricyclic antidepressants may variably interfere with the hypothalamic-pituitary-thyroid axis. The classical antipsychotics, phenothiazines, may alter iodine uptake, though it is unknown whether they can inhibit the sodium/iodide symporter (NIS). Tricyclic antidepressants can decrease TSH response to TRH via the noradrenergic or serotonergic systems (Sauvage et al. 1998). In addition, tricyclic antidepressants may promote autoimmunity, and favor appearance of thyroid autoantibodies, by enhancing the expression of major histocompatibility complex antigens (Sauvage et al. 1998). In a randomized controlled study, fluoxetine or sertraline induced a significant reduction of both serum T3 and T4 levels, albeit within their normal ranges and only in patients without preexisting thyroid disease (de Carvalho et al. 2009). On the other hand, in an open-label study of 62 patients with major depression, running over a period of 11 weeks, and treated with reboxetine, sertraline, or venlafaxine, the reboxetine group showed a significant reduction in serum TSH and increase in T4 (Eker et al. 2008), whereas the sertraline group had increased serum TSH and decreased T4 levels. Moreover, in the sertraline group, baseline TSH levels correlated with response to treatment. These observations support the safety of treatment with SSRIs, as they induce only minor changes in thyroid hormone economy. The different mechanisms of action of the various antidepressants might explain the variability of their actions on the HPT (Fig. 1A, C).

Antiepileptics

Antiepileptic drugs (AEDs) are a heterogeneous group of compounds, widely used in children and adults (Yılmaz et al. 2014). Their use has been associated with various adverse effects, some of which concern the thyroid (Verrotti et al. 2008). Alterations in thyroid hormone serum levels and occurrence of subclinical hypothyroidism have been reported particularly for phenytoin, valproate, and carbamazepine (Verrotti et al. 2008). Recent data from both cross-sectional and prospective studies have

documented subclinical hypothyroidism and reduced T4, T3, FT4, FT3, and thyroid-binding globulin (TBG) concentrations with phenobarbital, phenytoin, carbamazepine, valproic acid, and oxcarbazepine, but not with the more recently developed lamotrigine, levetiracetam, tiagabine, and vigabatrin (Hamed 2015). Of note, carbamazepine, oxcarbemazepine, and valproic acid increase metabolism of thyroid hormones through the hepatic P450 system but may also influence the pituitary feedback and thereby induce central hypothyroidism (Hamed 2015). Carbamazepine and phenytoin have been also associated with an increased frequency of goiter. The increased hepatic degradation of thyroid hormones could in part be the explanation for the goitrogenic effect, as a compensatory mechanism (Hegedüs et al. 1985) (Fig. 1C).

Rifampin

Rifampin, a rifamycin antibiotic possessing germicide and bacteriostatic properties, induced hypothyroidism in three out of 25 Hashimoto's thyroiditis patients and in none of 42 patients with no autoimmune thyroid disease. When rifampin was discontinued, the hypothyroidism resolved (Takasu et al. 2006). Rifampin enhances T4 clearance, via increased hepatic T4 metabolism along with biliary excretion of iodothyronine conjugates. Meanwhile, studies in healthy individuals have suggested that rifampin appears to diminish circulating thyroid hormone levels without changing TSH levels while inducing hypothyroidism in Hashimoto's thyroiditis (Takasu et al. 2005). After administering rifampin, an increased LT4 dose was required for 50% of patients in the TSH suppression group for thyroid cancer and 26% of patients in the replacement group for hypothyroidism (Kim et al. 2017). Treatment with rifampin can also induce a rapid increase of thyroid size (Christensen et al. 1989) (Fig. 1C).

Metformin

Metformin is widely used in the treatment of type 2 diabetes mellitus and insulin resistance. Recently, it was reported that treatment with metformin suppresses TSH in patients with hypothyroidism, subclinical hypothyroidism, and diabetes but not in normal individuals (Cappelli et al. 2014; Lupoli et al. 2014). TSH suppression is not accompanied by altered T4 or T3 levels. In a study evaluating thyroxine absorption, when L-T4 was concomitantly ingested with metformin, L-T4 absorption remained unchanged, suggesting that other mechanisms may be involved in the TSH suppression (Al-Alusi et al. 2015). Treatment with metformin in euthyroid patients with type 2 diabetes did not induce goiter (Díez and Iglesias 2014) (Fig. 1C).

Amiodarone

Amiodarone is an iodine-rich drug used for the treatment of tachyarrhythmias and other cardiac conditions. Since its structure resembles that of thyroid hormones, it

may interact with thyroid hormone receptors, inducing some antagonist effects. Its half-life is 40–60 days (Holt et al. 1983). After deiodination a daily dose of 300 mg of amiodarone provides 11 mg of inorganic iodine, which is 30–100 times the required daily dose (Rao et al. 1986). In addition, amiodarone inhibits the activity of type 1 deiodinase, inducing low T3 levels, high T4 levels, and high reverse T3 levels (Amico et al. 1984). TSH levels usually increase during the early months of treatment (Martino et al. 2001). Clinically significant thyroid dysfunction (thyrotoxicosis and hypothyroidism) occurs in a minority of patients treated with amiodarone. Amiodarone-induced thyrotoxicosis (AIT) and amiodarone-induced hypothyroidism (AIH) can develop in normal thyroids or in glands with preexisting disease, with a prevalence of 2–10% and 5–22%, respectively (Martino et al. 2001) (Fig. 1C, D). AIT has been reported to be more frequent in iodine-deficient areas, while AIH is more common in iodine-sufficient areas (Martino et al. 2001). AIT can develop from a few weeks until many years after treatment and even many months after its withdrawal (Martino et al. 1987a). Two forms of AIT have been described: type 1 AIT ensues in preexisting thyroid disease (pretoxic multinodular goiter or Graves' disease) and type 2 AIT develops in apparently normal thyroid glands (Table 2). The possible mechanism underlying type 1 AIT is the excess synthesis of thyroid hormone induced by the iodine load. This is supported by the finding of low to normal iodine uptake, pointing toward failure of the thyroid gland to adapt normally to the iodine load. Conversely, in type 2 AIT, iodine uptake is very low, indicating the presence of thyroid destruction. Diagnosing the type of AIT may be cumbersome and mixed forms are common (Martino et al. 2001). Flow by color Doppler sonography is a useful tool in differentiating the two forms of AIT, vascularity being increased in type 1 and absent in type 2 (Bogazzi et al. 1997). The main therapy of type 1 AIT consists of large doses of thionamides and perchlorate, while in type 2 AIT, glucocorticoids are effective. Mixed forms require the combination of the three drugs. In patients resistant to medical therapy, thyroidectomy is indicated, while radioiodine therapy is only effective when uptake is adequate. Withdrawal of amiodarone is usually recommended but is not mandatory (Barbesino 2010; Eskes

Table 2 Clinical and pathogenetic features of the two main forms of AIT

	Type 1 AIT	Type 2 AIT
Preexisting thyroid disease	Yes	No
	(latent Graves' disease, single or multinodulargoiter)	
FT4/FT3 ratio	Often> 4	<4
Spontaneous remission	No	Possible
Thyroid CFDS	Increased vascularity	Absent vascularity
Thyroidal RAIU	Low-to-normal uptake	Low-to-absent uptake
Thyroid autoantibodies	Sometimes present	Usually absent

Abbreviations: *CFDS* color flow Doppler Sonography, *RAIU* radioactive iodine uptake
Modified from Bogazzi et al. (2014)

and Wiersinga 2009). AIH is more common in females and in subjects with thyroid autoimmunity. Patients with elevated TPOAb levels have a 7.3 times increased risk of developing autoimmune thyroid disease as compared to individuals without these antibodies (Trip et al. 1991). The inability of patients with Hashimoto's thyroiditis to escape from the Wolff-Chaikoff effect (i.e., the inability to restore the normal thyroid hormone production during an iodine load) is the likely cause of AIH. Withdrawal of amiodarone is followed by restoration of euthyroidism, with the exception of patients with thyroid antibodies in whom it could be permanent (Martino et al. 1987b). AIH can easily be treated with LT4 replacement when the discontinuation of amiodarone is not feasible because of the underlying cardiac disease (Martino et al. 2001). Patients can be treated with perchlorate to accelerate resolution of AIH (Bogazzi et al. 2008).

Interferon and Other Antiviral Drugs

Interferon-α

Interferons (IFN) are a family (α, β, γ) of small molecules that are produced by cells in response to viral infections and other synthetic and biological inducers. They reduce tumor growth and modulate immune response and are employed in the treatment of viral, autoimmune, and neoplastic diseases. For a long period, the mainstay in the treatment of hepatitis C (HCV) has been the combination of IFN-α (in the form or recombinant IFN-α1 or long-acting pegylated IFN-α) with ribavirin (RBV), a synthetic analog of guanoside. HCV affects nearly 3% of the global population, leads to chronic disease in more than 60% of the affected subjects, and results in cirrhosis in 16% of patients after 20 years (Nair Kesavachandran et al. 2013). In the early studies, treatment with IFN-α and RBV was associated with thyroid diseases in the form of positive thyroid autoantibodies, autoimmune hypothyroidism, destructive thyroiditis, and Graves' disease (Prummel and Laurberg 2003). The frequency of thyroid dysfunction and thyroid autoantibodies following IFN-α therapy varies considerably in available reports. Reasons include heterogeneity in the definition of thyroid disorders and differences in the control populations (Nair Kesavachandran et al. 2013). Several aspects of the correlation between IFN-α plus RBV treatment for HCV and thyroid side effects have been discussed for some time, and many remain unsettled. The first point is whether HCV infection per se increases the incidence of thyroid diseases. While some epidemiological studies report a higher incidence of thyroid dysfunction in untreated HCV patients, as compared to the normal population or patients with hepatitis B, others do not (Antonelli et al. 2004; Huang et al. 1999; Ganne-Carrie et al. 2000; Loviselli et al. 1999; Fontaine et al. 2001). Several mechanisms have been proposed to explain the induction of thyroid autoimmunity in HCV infection: (i) a generalized autoimmunity induced by the infection with increased production of endogenous IFN-γ and consequent expression of HLA-DR antigens on thyrocytes, (ii) the infection of thyrocytes by HCV with production of IFN-γ and expression of HLA-DR antigens,

and (iii) the binding of HCV E2 proteins to CD81 molecules on thyroid cells, followed by activation of interleukin-8 (Menconi et al. 2011).

As for the effect of treatment of HCV on thyroid dysfunction, monotherapy with IFN-α has been shown to induce thyroid autoantibodies in 20.6% and thyroid dysfunction in 2.7% of patients, while the combined treatment (IFN-α plus RBV) caused development of thyroid autoantibodies in 5.0% and thyroid dysfunction in 12.8% of patients (Nair Kesavachandran et al. 2013). Fifty percent of patients, with positive TPOAb before treatment, developed thyroid dysfunction in comparison with 5.4% of autoantibody-negative patients (Koh et al. 1997). Other predisposing factors for thyroid dysfunction are gender (female to male ratio, 4.4:1) ethnicity (the importance of Asian origin is debated), and genetic background (HLA-A2 and DRB1*11 alleles are associated with IFN-α-induced thyroid dysfunction). IFN-α interacts with specific cell surface receptors and activates several signaling pathways that lead to the expression of adhesion molecule and cytokine genes, such as IL-6, that are associated with autoimmune thyroiditis and upregulate the expression of MHC class I proteins, which again lead to the activation of cytotoxic T-cells. In addition, IFN-α induces the switching of the immune response to the Th1 pattern and the activation of several cells, including lymphocytes, macrophages, and dendritic cells. In conclusion, IFN-α triggers an autoimmune response in genetically predisposed individuals. In addition, direct effects of IFN-α on thyrocytes, with an initial increase in the levels of TSH, TPO, Tg, and sodium/iodide symporter, and subsequently apoptosis have been reported (Menconi et al. 2011).

The most common abnormality is the induction of thyroid autoantibodies in the absence of thyroid dysfunction, which occurs in about 10–40% of subjects. Thyroid autoantibodies remain positive after the end of treatment in the majority of patients and can be associated with subsequent development of autoimmune hypothyroidism (Fig. 1C). Subclinical or overt hypothyroidism is seen in 2.4–19.0% of patients (Carella et al. 2004). Hypothyroidism can be the expression of an autoimmune disease or a destructive process. In the latter case, it is usually preceded by a transient (and sometimes overlooked) phase of thyrotoxicosis and is not associated with the development of thyroid autoantibodies (Fig. 1D). Thyrotoxicosis is usually a destructive process, presents with a mild or subclinical course, and is transient, lasting just a few weeks or months. With rare exceptions, Graves' disease is reported to be an uncommon phenotype in IFN-α-induced thyrotoxicosis (Menconi et al. 2011).

Because of the high frequency of thyroid disorders, clinical examination, detection of TPOAb and TgAb, and measurement of serum TSH are recommended. Clinical disorders can occur after a few or many months of treatment. Hypothyroidism can easily be corrected with levothyroxine treatment and does not require IFN-α withdrawal. After discontinuation of IFN-α, hypothyroidism may remit or persist. The latter occurs particularly in patients who initially had positive thyroid autoantibodies. Destructive thyroiditis occurs in 5% of patients treated with IFN-α and can lead to permanent hypothyroidism. Low radioactive iodine uptake (RAIU) and negative TSH-R autoantibodies (TRAb) are its characteristic features. This thyroiditis can be treated with beta-blockers, while corticosteroids are contraindicated in HCV. Graves' hyperthyroidism is characterized by positive TRAb and high RAIU

and can be treated with antithyroid drugs and, when severe, with radioiodine or thyroidectomy (Smith and Hegedüs 2016). Serum TSH should be measured every 2–3 months during IFN-α treatment and 6 months after its discontinuation. Patients with positive thyroid autoantibodies are prone to develop permanent thyroid dysfunction (Carella et al. 2004; Menconi et al. 2011).

The recently introduced direct-acting antiviral drugs have significantly changed the treatment of HCV infection. They have replaced regimens based on IFN-α, while RBV is still employed in selected patients (D'Ambrosio et al. 2017).

Highly Active Antiretroviral Therapy (HAART)

Autoimmune phenomena, including AITD, after immune recovery due to HAART have been reported. Patients infected with HIV have a higher prevalence of thyroid dysfunction when compared with the general population, with euthyroid sick syndrome, Graves' disease, and subclinical hypothyroidism being the most common (Abelleira et al. 2014) (Fig. 1C, D). Several studies have suggested an association between hypothyroidism and treatment with nucleoside reverse transcriptase inhibitors, particularly stavudine and non-nucleoside reverse transcriptase inhibitors such as efavirenz (Abelleira et al. 2014). Vos et al. described three patients who developed Graves' disease after starting HAART. They also refer to 13 patients reported in the literature (Vos et al. 2006). In a Brazilian study of 153 ambulatory HIV-infected women (Carvalho et al. 2013), the frequency of thyroid disorders was 7.8% (12/153 patients), and all were on HAART at the time of diagnosis, yielding a prevalence of 9.3% in patients receiving HAART compared with 0% in patients not on HAART. AITD, hyperthyroidism, and hypothyroidism were detected in 4.6%, 3.1%, and 4.1% of HAART patients, compared to none in untreated patients.

Antineoplastic Agents

Cytotoxic and Novel Anticancer Agents

Traditional cytotoxic agents rarely induce thyroid abnormalities but can sensitize the thyroid to the effects of radiation therapy, thereby increasing the risk of hypothyroidism. However, few studies have evaluated, prospectively, thyroid function in adult patients receiving cytotoxic agents. Mitotane, an agent used against adrenocortical cancer, induces a reduction in serum FT4 but not in FT3 and TSH levels (Daffara et al. 2008).

Thyroid abnormalities are more commonly observed in patients treated with novel antineoplastic agents, namely, targeted therapies and immunotherapies. Data from such studies are often discordant because of heterogeneity in the definition of thyroid abnormalities and whether clinical and subclinical abnormalities or only clinical dysfunction is included. Some publications suggest that patients experiencing thyroid dysfunction have an increased likelihood of response to therapy (Hamnvik et al. 2011) (Table 3).

Table 3 Antineoplastic agents interfering with thyroid function

Drug	Effect	Mechanism of action
Cytotoxic and novel anticancer agents		
All	*Hypothyroidism*	Sensitization to radiotherapy
Mitotane	↓ *FT4*	–
Tyrosine kinase inhibitors	*Thyrotoxicosis*	Destructive thyroiditis
	Hypothyroidism	Inhibition of VEGF receptor
		Impaired iodine uptake
	↑ *LT4 requirement*	Modification in LT4 metabolism
		Interference with TSH clearance,
		Hypothalamic pituitary loopand
		Thyroid hormone metabolism
		Inhibition of MCT8
Bexarotene	*Central hypothyroidism*	Selective agonist of RXR
Iodine based cancer therapies and radioimmunotherapies	*Hypothyroidism*	Destructive thyroiditis

Tyrosine Kinase Inhibitors

Tyrosine kinase inhibitors (TKIs) are employed for the treatment of several tumors, including renal cell carcinomas, gastrointestinal stromal tumors, medullary and differentiated follicular thyroid tumors, pancreatic endocrine tumors, and non-small cell lung cancers. TKIs are small molecules that directly block the ATP-binding site of tyrosine kinase and thereby interfere with cell proliferation, angiogenesis, and the potential to metastasize.

These molecules may induce transient thyrotoxicosis and primary hypothyroidism in patients with normal thyroid function and may increase the required dose in hypothyroid patients on LT4 therapy (Fig. 1C, D). While thyroid destruction has been involved in thyrotoxicosis (Grossmann et al. 2008), several mechanisms have been proposed for the onset of hypothyroidism in euthyroid subjects starting on TKIs. One possible mechanism is capillary dysfunction due to inhibition of VEGF receptors (Makita and Iiri 2013). Indeed, the thyroid gland has the highest blood flow rates per weight unit of any tissue in man (Wang et al. 1998). This mechanism is relevant for TKI inhibitors such as sunitinib, which specifically target VEGF receptors. In addition, capillary dysfunction may directly lead to thyroid destruction (Makita and Iiri 2013). An impaired iodine uptake has been reported to play a role in sunitinib-induced hypothyroidism in vivo but not in vitro (Mannavola et al. 2007; Salem et al. 2008). The role of autoimmunity in inducing hypothyroidism has been

excluded by some authors (Mannavola et al. 2007), while the appearance of TPOAb following a longer period of treatment was recently reported (Pani et al. 2015).

Many evidences suggest that TKIs may interfere with metabolism of thyroid hormones and their feedback. The fact that TKI treatment increases LT4 requirement in athyreotic patients can be related to a modification in LT4 metabolism or to the interference with TSH clearance (Verloop et al. 2013) or the hypothalamic-pituitary loop (Makita and Iiri 2013). In addition, TKIs inhibit, non-competitively, thyroid hormone membrane transport by MCT8 (Braun et al. 2012). Influence of TKIs on thyroid hormone metabolism is suggested by data in humans (increased serum TSH) and rats (decreased serum T3 and T4 and increased activity of hepatic type 3 deiodinase) (Kappers et al. 2011).

Hypothyroidism is commonly observed during treatment with sorafenib, sunitinib, and imatinib, but less frequently with vandetanib, axitinib, and cabozantinib. In clinical trials with sunitinib, the incidence of thyroid dysfunction ranges between 7% and 85%, and about 90% of patients start on LT4 treatment. Serum TSH levels rise during the on-periods and decrease during the off-periods (Illouz et al. 2014).

Before starting TKI treatment, evaluation of thyroid function and thyroid morphology by ultrasound is recommended. Careful monitoring of thyroid function is advised 4–6 weeks after starting treatment and – in the absence of thyroid dysfunction – approximately every three drug cycles hereafter (Illouz et al. 2014). During treatment with sunitinib, it has been proposed to postpone levothyroxine therapy until a rise in TSH levels during the treatment with TKI is confirmed at the end of an off-phase. This approach aims at avoiding thyrotoxicosis (Wolter et al. 2008; Illouz et al. 2014).

Bexarotene

Bexarotene is the only rexinoid currently approved for clinical use, primarily as second-line treatment for early- and late-stage refractory cutaneous T-cell lymphomas (Willemze et al. 2005). Because of its action as selective agonist of the RXR, it induces central hypothyroidism (low levels of serum FT4 with low-normal TSH) in 4–8 h in 40–100% of patients (Torino et al. 2013). Replacement L-T4 therapy and regular monitoring of FT4 are required during bexarotene treatment (Sherman 2003). The effect of bexarotene reverts in a few days after its discontinuation. In thyroidectomized patients who start bexarotene, a dramatic fall in FT4 levels without an appropriate rise in TSH levels has been observed. Most probably this is an effect on peripheral thyroid metabolism, via non-deiodinase mechanisms (Smit et al. 2007) (Fig. 1A).

Iodine-Based Cancer Therapies and Radioimmunotherapies

Several antineoplastic agents act by delivering ^{131}I to target cells. Tositumomab is a cluster of anti-CD20 antibodies combined with ^{131}I, which is approved for treatment of non-Hodgkin lymphoma (Hamnvik et al. 2011) (Fig. 1C). ^{131}Imetaiodobenzylguanidine

and ^{131}I iodobenguane target ^{131}I into neuroendocrine tissue. Thyroid cells concentrate ^{131}I from these agents leading to hypothyroidism in 10–65% of patients (Hamnvik et al. 2011). A saturated solution of potassium iodide (SSKI) (four drops three times daily) or Lugol's solution (24 drops three times daily), starting 24 h before and ending 2 weeks after administration of the medication, can prevent hypothyroidism (Hamnvik et al. 2011).

Immunoregulatory Drugs

IL-2

IL-2 is a cytokine that activates natural killer cells and antigen-specific T-cells and is approved for treatment of advanced melanoma and renal cell cancer. Thyroid diseases have been reported in 10–50% of patients treated with IL-2, alone or in combination with other immunotherapies (Atkins et al. 1988; Weijl et al. 1993; Krouse et al. 1995). Hypothyroidism, thyrotoxicosis, and hypothyroidism after a phase of thyrotoxicosis have been reported. Hypothyroidism is more common in patients with preexisting thyroid autoantibodies and may remit after discontinuation of IL-2. Activation of autoreactive T lymphocytes is the likely mechanism involved in thyroid toxicity (Fig. 1C, D).

Denileukin Diftitox

In denileukin diftitox, the ligand-binding domain of IL-2 is fused to diphtheria toxin. It binds to IL-2 receptors on lymphocytes and macrophages, leading to their death. It is approved in cutaneous T-cell lymphoma and graft-versus-host disease after allogenic stem cell transplantation. It can induce thyrotoxicosis (Ghori et al. 2006) (Fig. 1D). Destructive thyroiditis or triggering of autoimmunity in predisposed individuals is the proposed mechanism (Hamnvik et al. 2011).

Thalidomide and Lenalidomide

Thalidomide and its derivative lenalidomide have many immunoregulatory actions, including stimulation and proliferation of T-cells and increasing the number and function of natural killer cells. These drugs also have antiangiogenic activity. Both drugs are approved for treatment of multiple myeloma, lenalidomide also for 5q myelodysplastic syndrome. Subclinical hypothyroidism, occurring 1–6 months after initiation of therapy, has been reported in 20% of patients treated with thalidomide (Badros et al. 2002). The reported rate of hypothyroidism after lenalidomide is 5–10% (List et al. 2006; Dispenzieri et al. 2007). Both hypothyroidism and thyrotoxicosis have been reported in another study (Figaro et al. 2011). Interference with thyroid hormone secretion, reduction of iodine uptake, destructive thyroiditis by

ischemia, or immune-mediated mechanisms have been proposed as the potential causes of thyroid dysfunction induced by thalidomide and lenalidomide (Torino et al. 2013) (Fig. 1C, D).

IFN-α

IFN-α is approved for malignant melanoma, renal cell carcinoma, AIDS-related Kaposi's sarcoma, and some hematologic malignancies. It is extensively discussed in the section "Interferon and Other Antiviral Drugs" (Fig. 1C).

Alemtuzumab

Alemtuzumab is a humanized monoclonal antibody that induces profound lymphopenia by binding to the CD20 receptors on lymphocytes and monocytes. It is used in B-cell chronic lymphocytic leukemia, stem cell transplants, graft-versus-host disease after allogeneic cell transplant, and in multiple sclerosis. Alemtuzumab causes thyroid dysfunction in 30% of patients, with onset ranging from 6 to 61 month, with the greatest risk 12–36 months after the first infusion (Coles et al. 2012; Cossburn et al. 2011). About half of the cases have been Graves' disease with or without ophthalmopathy (Willis and Robertson 2014). Of patients with overt Graves' hyperthyroidism, 23% spontaneously became euthyroid and an additional 15% spontaneously developed hypothyroidism (Daniels et al. 2014). The annual incidence of a first episode of thyroid dysfunction increased each year through year 3 and then decreased each subsequent year (Daniels et al. 2014). Management of alemtuzumab-induced Graves' disease is similar to the management of classic Graves' disease (Smith and Hegedüs 2016) (Fig. 1C, D).

Immune Checkpoint Inhibitors

Blocking of immune checkpoints, such as cytotoxic T-lymphocyte antigen-4 (CTLA4) and programmed death-1 (PD1), two co-inhibitor receptors that are expressed on activated T-cells, has emerged as an option for treatment of cancer. By activating T-cells, these drugs alter immune tolerance, inducing the control of neoplastic cells but also the breaking of immune tolerance, inducing "immune-related adverse effects" (IRAEs). Thyroid and other endocrine glands are often involved (González-Rodríguez and Rodríguez-Abreu 2016).

Anti-CTLA4 Monoclonal Antibodies

Ipilimumab and tremelimumab are mAbs directed against CTLA4. Ipilimumab is approved for use in advanced cutaneous malignant melanoma and tremelimumab for metastatic prostate cancer. Hypophysitis (which can cause central hypothyroidism) is the most severe and dose-limiting endocrine adverse effect, observed in 0–17.4%

of patients treated with ipilimumab and 2.6% of those treated with tremelimumab (Torino et al. 2013; González-Rodríguez and Rodríguez-Abreu 2016). Thyroid disorders have been reported in 0%–7.4% of patients treated with ipilimumab, with an incidence of hypothyroidism of 0%–9% and of hyperthyroidism of 0%–2.8%. Thyroid disorders occur in 0.5–5.2% of patients treated with tremelimumab (Fig. 1C, D). The onset of thyroid dysfunction occurs after two to four infusions of anti-CTLA4 mAbs. Most cases are subclinical and transient; others evolve into permanent hypothyroidism (Di Giacomo et al. 2010). It is known that different CTLA4 polymorphisms have been associated with Graves' orbitopathy. Of note, some patients developed euthyroid Graves' orbitopathy following treatment with anti-CTLA4 mAbs (Min et al. 2011; McElnea et al. 2014).

Anti-PD-1 Monoclonal Antibodies

PD-1 is a negative regulatory receptor expressed on T and B lymphocytes and natural killer cells which limits their response. Nivolumab is an anti-PD-1 mAb approved for treatment of advanced malignant melanomas, renal cell carcinomas, and non-small cell lung cancer, while pembrolizumab is approved for treatment of advanced malignant melanoma and non-small cell lung cancer. Compared to standard treatment, these drugs showed a lower risk of adverse effects. Thyroid dysfunction has been observed in 9% of treated patients, with 3% developing hyperthyroidism and 6.5% developing hypothyroidism (Costa et al. 2017; González-Rodríguez and Rodríguez-Abreu 2016) (Fig. 1C, D) (Table 4).

Table 4 Immunoregulatory drugs interfering with thyroid function

Drug	Effect	Mechanism of action
IL-2	*Hypothyroidism/ thyrotoxicosis*	Activation of autoreactive T lymphocytes
Denileukindiftitox	*Thyrotoxicosis*	Destructive thyroiditis, triggering of autoimmune thyroid disease
Thalidomide and lenalidomide	*Hypothyroidism*	Interference with thyroid hormone secretion reduction of iodine uptake,
	Thyrotoxicosis	Destructivethyroiditis, immune mediated mechanisms
IFN-α	↑ *AbTPO, AbTg, TSH*	Triggering of autoimmune thyroid disease
Alemtuzumab	*Graves' disease*	Triggering of autoimmune thyroid disease
Immune checkpoint inhibitors		
I. anti-CTLA4 mAbs (ipilimumab, tremelimumab)	*Central hypothyroidism Hypothyroidism Euthyroid Graves' orbithopathy*	Hypophysitis
II. anti-PD-1mAbs (nivolumab, pembrolizumab)	*Hypothyroidism Hyperthyroidism*	Triggering of autoimmune thyroid disease

Other Substances Interfering with Thyroid Function

Endocrine Disruptors

Thyroid disruption can derive from occupational or environmental exposure (Diamanti-Kandarakis et al. 2009; Leung et al. 2014; Marini et al. 2012). A recent article (Benvenga et al. 2015) reviewed the studies investigating the onset of Hashimoto's thyroiditis and/or thyroid nodules following occupational or environmental exposure to polluting substances. Among these pollutants, there are the polychlorinated biphenyls (PCB), polybrominated biphenyls (PBB), pesticides (the most studied being organochlorines, including dichlorodiphenyltrichloroethane [DDT], aldrin, heptachlor, chlordane, and lindane), and heavy metals.

Two of the biggest Chinese cities, Beijing and Guangzhou, are heavily polluted. Beijing recently recorded the world's highest level of sulfur dioxide, the third highest level of nitrogen dioxide, and one of the highest levels of particulates (Benvenga et al. 2015). In urban areas of Beijing, the incidence of differentiated thyroid cancer (DTC) has increased (or risen) sevenfold over the years 1995–2010, with an increase of 539%. Guangzhou has recently recorded a higher level of particulates in the air (Benvenga et al. 2015). Similar to Beijing, in the urban area of Guangzhou, the incidence of DTC increased three times over the time period 2000–2011 (Benvenga et al. 2015). Concerning occupational exposure, working as a mechanic and metal worker and having contact with solvents were identified as risk factors for developing thyroid cancer. Also, following the September 11, 2001, World Trade Center disaster and the subsequent release of toxic substances into the environment, thyroid cancer increased 2.3-fold over the years 2002–2012 in police officers who were in service on that day (Benvenga et al. 2015).

Turning to thyroid autoimmunity, smoke (and the related passive smoking), PCB, solvents, metals, and other anthropogenic compounds (see below) have been implicated in thyroid inflammation and autoimmunity. They might act by disrupting the immune tolerance with the subsequent triggering of AITD. Alternatively, thyroid disruptors can alter thyroglobulin structure by post-transductional modifications, thus increasing their immunogenicity (Benvenga et al. 2015). The rate of chronic lymphocytic thyroiditis at FNAC is higher in subjects living in the area of a large petrochemical complex (located in southeastern Sicily) compared with subjects from a control area located just 15 km away (32% vs 23%) (Arena et al. 2015). These two areas are environmentally distinct, because the concentrations in the atmosphere of four heavy metals (nickel, vanadium, chromium, and mercury) were higher in the petrochemical complex area compared with the control area. Also, the rate of suspiciously malignant or overtly malignant cytologies from thyroid nodules was twofold increased in patients living in the petrochemical complex area as in the control patients (Arena et al. 2015).

Slovakian workers who were exposed to PCB are an example of AITD resulting from occupational exposure. These workers had higher thyroid volume and higher frequency of thyroid antibody positivity compared with controls. Furthermore, the more these workers had worked in contact with PCB, the higher the prevalence of clinical and/or laboratory signs of thyroid diseases (Benvenga et al. 2015).

Food, in particular fish, is another important means of exposure. The west and south coasts of Newfoundland (Canada) are in contact with the Gulf of St. Lawrence, the outlet of St. Lawrence river. This river, its estuary, and its gulf are one of the most polluted water sources in the world. Hence, seafood consumed by the coastal communities of Newfoundland is contaminated with thyroid-disrupting chemicals. Indeed, the rate of hypothyroidism in the west and on the south coast is twice that of the east coast (Benvenga et al. 2015). The type of seafood consumed influences both the positivity rate and the serum levels of thyroid autoantibodies throughout pregnancy and postpartum (Benvenga et al. 2016 i). Indeed, the group of women who consumed swordfish, a top predator fish that concentrates pollutants (mainly, mercury), as the sole or predominant seafood, had the highest positivity rate of thyroid autoantibodies (25% at the first trimester and 12.5% at day 4 postpartum).

On the other hand, blood measurements for all metals and assessment of thyroid function showed that mercury was associated with reductions in T3 and T4 and cadmium was linked to decreased TSH (Duntas 2015). It is worth noting that endocrine disruptors (EDs) frequently exert nonlinear effects, i.e., acting in a U-shaped or inverted-U manner; thus, seemingly paradoxically, a minimal dose of EDs can cause more abnormalities than higher doses. The fact that even small amounts of EDs are capable of causing adverse effects, which however cannot be predicted by their effects at much higher doses, reintroduced what was first determined in the 1990s, namely, the "low-dose hypothesis," together with the concept of non-monotonic dose response curves describing a nonlinear relationship between dose and effect (Köhrle 2008).

A variety of benzophenone UV screens (BP2), when applied for 5 days in adult ovariectomized rats, led to a significant reduction of T4 and T3 plasma levels. The suggested mechanism was the inhibition of thyroid peroxidase (Jarry et al. 2004). It is important to be aware that UV screens, besides being potent estrogen disruptors, may exert potential thyroid-disrupting activity within just a few days.

Nutraceuticals and Over-the-Counter Products

Selenium and Inositol

The effects of selenium on thyroid and other targets were recently reviewed (Duntas and Benvenga 2015; Winther et al. 2017). A large cross-sectional study in China showed a higher prevalence of Hashimoto's thyroiditis and goiter in a low selenium area (median serum selenium concentration of 57.4 mcg/L) compared to an adequate selenium area (median serum selenium concentration of 103.6 mcg/L) (Wu et al. 2015). In an Italian study (Negro et al. 2007), 77 TPOAb-positive pregnant women were supplemented with 200 μg selenomethionine from the first trimester of gestation through month 12 postpartum. The average reduction in TPOAb, compared to baseline, was 62% during pregnancy and 48% during the 12 months postpartum. In the 74 TPOAb-positive pregnant women treated with placebo, the reduction in pregnancy and postpartum was significantly lower (44% and just 1%, respectively). The rate of thyroid hypoechogenicity was significantly lower in the

selenomethionine-treated group compared to the placebo group. Postpartum thyroid dysfunction and permanent hypothyroidism were lower in the treated group compared with the placebo group (Negro et al. 2007).Other studies confirmed the reduction of TPOAb and TgAb in patients with chronic Hashimoto's thyroiditis but no changes in quality of life and in thyroid function (Winther et al. 2017). Some small studies demonstrated the effectiveness of selenium supplementation in Graves' disease and Graves' orbitopathy. Some ongoing trials are investigating larger groups of patients. The available data do not support the routine use of selenium supplementation in patients with AITD with the exception of a 6-month trial of selenium in patients suffering from mild Graves's orbitopathy suggested by the European Thyroid Association (Bartalena et al. 2016).

It is known that insulin resistance is correlated with serum TSH, that insulin enhances the effects of the inositols, and that inositols are involved in TSH signaling (Benvenga and Antonelli 2016). For these reasons, one study investigated the effect of myoinositol supplementation on subclinical hypothyroidism and thyroid autoantibody levels (Nordio and Pajalich 2013). The study was based on co-administration of myoinositol with selenomethionine in 48 women with subclinical autoimmune hypothyroidism and evaluated restoration of normal TSH levels, reduction of serum TPOAb and TgAb, and improvement of thyroid hypoechogenicity. Patients were randomized into two groups, one receiving orally 83 μg selenomethionine/day in a soft gel capsule and another a combined treatment, namely, 600 mg myoinositol contained in a 83 μg selenomethionine soft gel capsule. TSH concentrations decreased in the second but not in the first group, while TPOAb and TgAb significantly decreased in both groups. Changes in ultrasound echogenicity were more common in the second than in the first group (Nordio and Pajalich 2013). The association of myoinositol with selenomethionine was recently demonstrated to be more effective than each of them in reducing the H_2O_2-induced oxidative stress on peripheral mononuclear cells in vitro in both control and HT women (Benvenga et al. 2017).

L-Carnitine

The naturally occurring quaternary amine L-carnitine is characterized as a modulator of thyroid hormone action in peripheral tissues, with a prevalent inhibitory effect of nuclear uptake (Benvenga et al. 2000). No modification of thyroid hormone or TSH levels have been observed. Studies in patients with spontaneous and iatrogenic thyrotoxicosis, including the most severe form (thyroid storm), showed that treatment with carnitine ameliorates symptoms of thyrotoxicosis (Benvenga et al. 2004, 2003).

Thyroid Hormones and Iodine

Thyrotoxicosis factitia from over-the-counter products containing one or both thyroid hormones or iodine is common (Hoang et al. 2013). Complementary medication and herbal medicine are commonly used, especially for purposes of weight loss, and an increasing number of patients consume herbal medicine without reporting their use to physicians (Johnston 1997). Use of kelp, large seaweeds belonging to the brown algae (Phaeophyceae) in the order Laminariales, has been associated with thyrotoxicosis (Müssig et al. 2006) (Fig. 1D).

Table 5 Other substances interfering with thyroid function

Drug	Effect	Mechanism of action
Endocrine Disruptors		
Polychlorinated biphenyls (PCB)	*Thyroid cancer*	
Polybrominated biphenyls (PBB)	*Thyroid nodules*	
Pesticides [dichlorodiphenyltrichloroethane (DDT), aldrin, heptachlor, chlordane, lindane]	*Induction of autoimmune thyroid disease*	Disruption of immune tolerance
Heavy metals [nickel, vanadium, chromium, mercury]	*Hypothyroidism*	
Smoke		
Benzophenone UV-screens (BP2)	*Reduction of T4 and T3*	Inhibition of TPO
Nutraceuticals and over-the-counter products		
Selenium and inositol	*prevention of thyroid autoimmunity*	
L-carnitine	*modulation of thyroid hormone action*	
Thyroid hormones and iodine (kelps)	*thyrotoxicosis*	
Biotin	*T4,T3, TSH alterations*	interference with FT4, FT3, TSH measurement

Biotin

A low to medium dose of biotin (vitamin B7) is commonly present in multivitamin preparations, while high doses of biotin (10,000 times the recommended daily intake of approximately 30 μg) have been reported to improve clinical outcome and quality of life in patients with progressive multiple sclerosis (Elston et al. 2016). Many current immunoassays for determination of thyroid and other endocrine variables contain biotin, since they use a biotin-streptavidin detection system (Elston et al. 2016). Depending on the biotin assay used, thyroid hormone results can be falsely high or low. Temporary discontinuation of biotin treatment results in complete resolution of the biochemical abnormalities (Barbesino 2016) (Table 5).

Comorbidities and Drugs Interfering with LT4 Absorption

Levothyroxine sodium is among the most frequently prescribed drugs worldwide and continuously increases in tandem with the increasing incidence of thyroid disease over the last few decades. Based on community data of prescriptions for thyroid hormone in England, the amount of levothyroxine prescribed from 1998 to 2007 has nearly tripled, from 7 to almost 19 million prescriptions, while the duration of prescriptions has fallen by 25% over the same time (Mitchell et al. 2009). About 9% of patients of six general practitioner practices in Germany were taking thyroid

hormones (Viniol et al. 2013). Levothyroxine tablets are most commonly taken in the fasting state, once a day, and lifelong for hypothyroidism. The safety of long-term administration of levothyroxine is well-known, although regular routine laboratory controls are required to ascertain that the proper dose has been prescribed. The absorption of levothyroxine compounds may be reduced by gastrointestinal comorbidities, such as gastritis, peptic ulcer, celiac disease, and irritable bowel disease, while in addition many other drugs can interfere with its absorption (Table 6) (Fig. 1Ea, Eb). Commonly prescribed drugs, such as anticoagulants, nonsteroidal anti-inflammatory drugs, antiepileptics, selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants, iodine-containing antiarrhythmics (amiodarone), β-blockers, also antibiotics, cytokines, and tyrosine kinase inhibitors (TKIs), among many more, interact with the absorption or peripheral metabolism of thyroxine or have an impact on the hypothalamic-pituitary-thyroid (HPT) axis, all of which potentially induce thyroid dysfunction (Liwanpo and Hershman 2009; Barbesino 2010; Colucci et al. 2013; Benvenga 2013; Haugen 2009; Trifirò et al.

Table 6 Factors and conditions that impair the intestinal absorption of thyroxine

A. Inappropriate modality of storing L-T4 tablets
B. Inappropriate modality of ingestion of the L-T4 tablet
Involuntary noncompliance
Voluntary noncompliance (pseudomalabsorption)
Non-empty stomach (insufficient time elapsed between food and ingestion of the L-T4 tablet)
L-T4 taken while eating or less than 60 min after having eaten
Fiber-rich food (bread, bran, cereals, papaya)
Improper liquid for taking the L-T4 tablet
Coffee, grapefruit juice
C. Diseases or problems of the digestive system
Lactose intolerance
Gastritis (not necessarily associated with *Helicobacter pylori*)
Celiac disease
Duodenitis, Enteritis, irritable bowel disease
Intestinal parasitoses
Interventions of bariatric surgery
Chronic liver disease
Pancreatic insufficiency
Medications
Non absorbable antacids
Absorbable antacids (proton-pump inhibitors, etc . . .)
Iron salts
Calcium salts
Phosphate binders
Bile acid sequestrants
Ion resin exchangers
Orlistat

2015) (Fig. 1). Novel formulations (soft gel capsules, oral liquid solution) of LT4 may enhance the absorption of LT4 (Vita et al. 2014; Virili et al. 2016) and reduce the frequency of TSH measurement (Ferrara et al. 2017).

References

Abelleira E, De Cross GA, Pitoia F. Thyroid dysfunction in adults infected by human immunodeficiency virus. Medicina (B Aires). 2014;74:315–20.

Al-Alusi MA, Du L, Li N, Yeh MW, He X, Braverman LE, Leung AM. Metformin does not suppress serum thyrotropin by increasing levothyroxine absorption. Thyroid. 2015;25:1080–4.

Amico JA, Richardson V, Alpert B, Klein I. Clinical and chemical assessment of thyroid function during therapy with amiodarone. Arch Intern Med. 1984;144(3):487–90.

Antonelli A, Ferri C, Pampana A, Fallahi P, Nesti C, Pasquini M, Marchi S, Ferrannini E. Thyroid disorders in chronic hepatitis C. Am J Med. 2004;117(1):10–3.

Arena S, Latina A, Baratta R, Burgio G, Gullo D, Benvenga S. Chronic lymphocytic thyroiditis: could it be influenced by a petrochemical complex? Data from a cytological study in South-Eastern Sicily. Eur J Endocrinol. 2015;172:383–9.

Atkins MB, Mier JW, Parkinson DR, Gould JA, Berkman EM, Kaplan MM. Hypothyroidism after treatment with interleukin-2 and lymphokine-activated killer cells. N Engl J Med. 1988;318(24):1557–63.

Badros AZ, Siegel E, Bodenner D, Zangari M, Zeldis J, Barlogie B, Tricot G. Hypothyroidism in patients with multiple myeloma following treatment with thalidomide. Am J Med. 2002;112(5):412–3.

Bal CS, Kumar A, Pandey RM. A randomized controlled trial to evaluate the adjuvant effect of lithium on radioiodine treatment of hyperthyroidism. Thyroid. 2002;12(5):399–405.

Barbesino G. Drugs affecting thyroid function. Thyroid. 2010;20(7):763–70.

Barbesino G. Misdiagnosis of graves' disease with apparent severe hyperthyroidism in a patient taking biotin megadoses. Thyroid. 2016;26(6):860–3.

Bartalena L, Baldeschi L, Boboridis K, Eckstein A, Kahaly GJ, Marcocci C, Perros P, Salvi M, Wiersinga WM, European Group on Graves' Orbitopathy (EUGOGO). The 2016 European thyroid association/European group on graves' orbitopathy guidelines for the management of graves' orbitopathy. Eur Thyroid J. 2016;5(1):9–26.

Bauer M, BlumentrittH FR, Schlattmann P, Adli M, Baethge C, Bschor T, Müller-Oerlinghausen B, Berghöfer A. Using ultrasonography to determine thyroid size and prevalence of goiter in lithium-treated patients with affective disorders. J Affect Disord. 2007;104:45–51.

Benvenga S. When thyroid hormone replacement is ineffective? Curr Opin Endocrinol Diabetes Obes. 2013;20:467–77.

Benvenga S, Antonelli A. Inositol(s) in thyroid function, growth and autoimmunity. Rev Endocr Metab Disord. 2016;17(4):471–84.

Benvenga S, Lakshmanan M, Trimarchi F. Carnitine is a naturally occurring inhibitor of thyroid hormone nuclear uptake. Thyroid. 2000;10:1043–50.

Benvenga S, Lapa D, Cannavò S, Trimarchi F. Successive thyroid storms treated with L-carnitine and low doses of methimazole. Am J Med. 2003;115:417–8.

Benvenga S, Amato A, Calvani M, Trimarchi F. Effects of carnitine on thyroid hormone action. Ann N Y Acad Sci. 2004;1033:158–67.

Benvenga S, Antonelli A, Thyroid VR. nodules and thyroid autoimmunity in the context of environmental pollution. Rev Endocr Metab Disord. 2015;16:319–40.

Benvenga S, Vigo MT, Metro D, Granese R, Vita R, Le Donne M. Type of fish consumed and thyroid autoimmunity in pregnancy and postpartum. Endocrine. 2016;52:120–9.

Benvenga S, Vicchio T, Di Bari F, Vita R, Fallahi P, Ferrari SM, Catania S, Costa C, Antonelli A. Favorable effects of myo-inositol, selenomethionine or their combination on the hydrogen

peroxide-induced oxidative stress of peripheral mononuclear cells from patients with Hashimoto's thyroiditis: preliminary in vitro studies. Eur Rev Med Pharmacol Sci. 2017;21(2 Suppl):89–101.

Berens SC, Wolff J, Murphy DL. Lithium concentration by the thyroid. Endocrinology. 1970;87(5):1085–7.

Bocchetta A, Loviselli A. Lithium treatment and thyroid abnormalities. Clin Pract Epidemiol Ment Health. 2006;2:23.

Bocchetta A, Cocco F, Velluzzi F, Del Zompo M, Mariotti S, Loviselli A. Fifteen-year follow-up of thyroid function in lithium patients. J Endocrinol Investig. 2007;30(5):363–6.

Bogazzi F, Bartalena L, Brogioni S, Mazzeo S, Vitti P, Burelli A, Bartolozzi C, Martino E. Color flow Doppler sonography rapidly differentiates type I and type II amiodarone-induced thyrotoxicosis. Thyroid. 1997;7(4):541–5.

Bogazzi F, Bartalena L, Brogioni S, Scarcello G, Burelli A, Campomori A, Manetti L, Rossi G, Pinchera A, Martino E. Comparison of radioiodine with radioiodine plus lithium in the treatment of Graves' hyperthyroidism. J Clin Endocrinol Metab. 1999;84(2):499–503.

Bogazzi F, Bartalena L, Campomori A, Brogioni S, Traino C, De Martino F, Rossi G, Lippi F, Pinchera A, Martino E. Treatment with lithium prevents serum thyroid hormone increase after thionamide withdrawal and radioiodine therapy in patients with Graves' disease. J Clin Endocrinol Metab. 2002;87(10):4490–5.

Bogazzi F, Bartalena L, Tomisti L, Dell'Unto E, Cosci C, Sardella C, Tanda ML, Lai A, Gasperi M, Aghini-Lombardi F, Martino E. Potassium perchlorate only temporarily restores euthyroidism in patients with amiodarone-induced hypothyroidism who continue amiodarone therapy. J Endocrinol Investig. 2008;31(6):515–9.

Bogazzi F, Tomisti L, Bartalena L, Martino E. Amiodarone-induced thyrotoxicosis. In: Matfin G, editor. Endocrine and metabolic medical emergencies. Washington, DC: Endocrine Press; 2014.

Brabant A, Brabant G, Schuermeyer T, Ranft U, Schmidt FW, Hesch RD, von Zur Muhlen A. The role of glucocorticoids in the regulation of thyrotropin. Acta Endocrinol. 1989;121:95–100.

Braun D, Kim TD, le Coutre P, Köhrle J, Hershman JM, Schweizer U. Tyrosine kinase inhibitors noncompetitively inhibit MCT8-mediated iodothyronine transport. J Clin Endocrinol Metab. 2012;97(1):E100–5.

Cappelli C, Rotondi M, Pirola I, Agosti B, Formenti AM, De Cata P, Salvetti M, Chiovato L, Castellano M. Metformin-induced thyrotropin suppression is not associated with cardiac effects. Hormones (Athens). 2014;13:252–8.

Carella C, Mazziotti G, Amato G, Braverman LE, Roti E. Clinical review 169: Interferon-alpha-related thyroid disease: pathophysiological, epidemiological, and clinical aspects. J Clin Endocrinol Metab. 2004;89(8):3656–61. Review.

Carvalho LG, TeixeiraPde F, Panico AL, Cohen MV, Pinheiro MF, Barroso PF, Vaisman M. Evaluation of thyroid function and autoimmunity in HIV-infected women. Arq Bras Endocrinol Metabol. 2013;57:450–6.

Christensen HR, Simonsen K, Hegedüs L, Hansen BM, Døssing M, Kampmamn JP, Hansen JM. Influence of rifampicin on thyroid gland volume, thyroid hormones, and antipyrine metabolism. Acta Endocrinol. 1989;121(3):406–10.

Coles AJ, Fox E, Vladic A, Gazda SK, Brinar V, Selmaj KW, Skoromets A, Stolyarov I, Bass A, Sullivan H, Margolin DH, Lake SL, Moran S, Palmer J, Smith MS, Compston DA. Alemtuzumab more effective than interferon β-1a at 5-year follow-up of CAMMS223 clinical trial. Neurology. 2012;78(14):1069–78.

Colucci PH, Seng Yue C, Ducharme M, Benvenga S. A review of the pharmacokinetics of levothyroxine for the treatment of hypothyroidism. Eur Endocrinol. 2013;9:40–7.

Cossburn M, Pace AA, Jones J, Ali R, Ingram G, Baker K, Hirst C, Zajicek J, Scolding N, Boggild M, Pickersgill T, Ben-Shlomo Y, Coles A, Robertson NP. Autoimmune disease after alemtuzumab treatment for multiple sclerosis in a multicenter cohort. Neurology. 2011 Aug 9;77(6):573–9.

Costa R, Carneiro BA, Agulnik M, Rademaker AW, Pai SG, Villaflor VM, Cristofanilli M, Sosman JA, Giles FJ. Toxicity profile of approved anti-PD-1 monoclonal antibodies in solid tumors: a systematic review and meta-analysis of randomized clinical trials. Oncotarget. 2017;8(5):8910–20.

D'Ambrosio R, Degasperi E, Colombo M, Aghemo A. Direct-acting antivirals: the endgame for hepatitis C? Curr Opin Virol. 2017;24:31–7. Review.

Daffara F, De Francia S, Reimondo G, Zaggia B, Aroasio E, Porpiglia F, Volante M, Termine A, Di Carlo F, Dogliotti L, Angeli A, Berruti A, Terzolo M. Prospective evaluation of mitotane toxicity in adrenocortical cancer patients treated adjuvantly. Endocr Relat Cancer. 2008;15(4):1043–53.

Daniels GH, Vladic A, Brinar V, Zavalishin I, Valente W, Oyuela P, Palmer J, Margolin DH, Hollenstein J. Alemtuzumab-related thyroid dysfunction in a phase 2 trial of patients with relapsing-remitting multiple sclerosis. J Clin Endocrinol Metab. 2014;99:80–9.

de Carvalho GA, Bahls SC, Boeving A, Graf H. Effects of selective serotonin reuptake inhibitors on thyroid function in depressed patients with primary hypothyroidism or normal thyroid function. Thyroid. 2009;19:691–7.

Di Giacomo AM, Biagioli M, Maio M. The emerging toxicity profiles of anti-CTLA-4 antibodies across clinical indications. Semin Oncol. 2010;37(5):499–507.

Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, SotoAM ZRT, Gore AC. Endocrine-disrupting chemicals: an endocrine society scientific statement. Endocr Rev. 2009;30:293–42.

Díez JJ, Iglesias P. An analysis of the relative risk for goiter in euthyroid patients with type 2 diabetes. Clin Endocrinol. 2014;80(3):356–61.

Dispenzieri A, Lacy MQ, Zeldenrust SR, Hayman SR, Kumar SK, Geyer SM, Lust JA, Allred JB, Witzig TE, Rajkumar SV, Greipp PR, Russell SJ, Kabat B, Gertz MA. The activity of lenalidomide with or without dexamethasone in patients with primary systemic amyloidosis. Blood. 2007;109(2):465–70.

Duntas LH. Chemical contamination and the thyroid. Endocrine. 2015;48(1):53–64.

Duntas LH, Benvenga S. Selenium: an element for life. Endocrine. 2015;48:756–75.

Eker SS, Akkaya C, Sarandol A, Cangur S, Sarandol E, Kirli S. Effects of various antidepressants on serum thyroid hormone levels in patients with major depressive disorder. Prog Neuro-Psychopharmacol Biol Psychiatry. 2008;15(32):955–61.

Elston MS, Sehgal S, Du Toit S, Yarndley T, Conaglen JV. Factitious graves' disease due to biotin immunoassay interference – A case and review of the literature. J Clin Endocrinol Metab. 2016;101:3251–5.

Eskes SA, Wiersinga WM. Amiodarone and thyroid. Best Pract Res Clin Endocrinol Metab. 2009;23(6):735–51.

Ferrara R, Ientile V, Arcoraci V, Ferrajolo C, Piccinni C, Fontana A, Benvenga S, Trifirò G. Treatment pattern and frequency of serum TSH measurement in users of different levothyroxine formulations: a population-based study during the years 2009–2015. Endocrine. 2017;58(1):143–52.

Figaro MK, Clayton Jr W, Usoh C, Brown K, Kassim A, Lakhani VT, Jagasia S. Thyroid abnormalities in patients treated with lenalidomide for hematological malignancies: results of a retrospective case review. Am J Hematol. 2011;86(6):467–70.

Fontaine H, Nalpas B, Poulet B, Carnot F, Zylberberg H, Brechot C, Pol S. Hepatitis activity index is a key factor in determining the natural history of chronichepatitis C. Hum Pathol. 2001;32(9):904–9.

Ganne-Carrie N, Medini A, Coderc E, Seror O, Christidis C, Grimbert S, Trinchet JC, Beaugrand M. Latent autoimmune thyroiditis in untreated patients with HCV chronic hepatitis: a case-control study. J Autoimmun. 2000;14(2):189–93.

Ghori F, Polder KD, Pinter-Brown LC, Hoff AO, Gagel RF, Sherman SI, Duvic M. Thyrotoxicosis after denileukin diftitox therapy in patients with mycosis fungoides. J Clin Endocrinol Metab. 2006;91(6):2205–8.

González-Rodríguez E, Rodríguez-Abreu D. Spanish group for cancer immuno-biotherapy (GETICA) immune checkpoint inhibitors: review and management of endocrine adverse events. Oncologist. 2016;21(7):804–16.

Graeppi-Dulac J, Vlaeminck-Guillem V, Perier-Muzet M, Dalle S, Orgiazzi J. The impact of retinoids on the thyroid axis. Eur J Endocrinol. 2014;170:R253–62.

Grossmann M, Premaratne E, Desai J, Davis ID. Thyrotoxicosis during sunitinib treatment for renal cell carcinoma. Clin Endocrinol. 2008;69(4):669–72.

Hamed SA. The effect of antiepileptic drugs on thyroid hormonal function: causes and implications. Expert Rev Clin Pharmacol. 2015;8:74150.

Hamnvik OP, Larsen PR, Marqusee E. Thyroid dysfunction from antineoplastic agents. J Natl Cancer Inst. 2011;103(21):1572–87.

Haugen BR. Drugs that suppress TSH or cause central hypothyroidism. Best Pract Res Clin Endocrinol Metab. 2009;23:793–800.

Hegedüs L, Hansen JM, Lühdorf K, Perrild H, Feldt-Rasmussen U, Kampmann JP. Increased frequency of goiter in epileptic patients on long-term phenytoin or carbamazepine treatment. ClinEndocrinol (Oxf). 1985;23(4):423–9.

Hoang TD, Mai VQ, Clyde PW, Shakir MK. Over-the-counter-drug-induced thyroid disorders. Endocr Pract. 2013;19:268–74.

Holt DW, Tucker GT, Jackson PR, Storey GC. Amiodarone pharmacokinetics. Am Heart J. 1983;106(4 Pt 2):840–7.

Huang MJ, Tsai SL, Huang BY, Sheen IS, Yeh CT, Liaw YF. Prevalence and significance of thyroid autoantibodies in patients with chronic hepatitis C virus infection: a prospective controlled study. Clin Endocrinol. 1999;50(4):503–9.

Illouz F, Braun D, Briet C, Schweizer U, Rodien P. Endocrine side-effects of anti-cancer drugs: thyroid effects of tyrosine kinase inhibitors. Eur J Endocrinol. 2014;171(3):R91–9.

Jarry H, Christoffel J, Rimoldi G, Koch L, Wuttke W. Multi-organic endocrine disrupting activity of the UV screen benzophenone 2 (BP2) in ovariectomized adult rats after 5 days treatment. Toxicology 2004;205(1-2):87–93.

Johnston BA. One-third of nation's adults use herbal remedies: market estimated at 3.2 billion. Herbal Gram. 1997;(40):49. American Botanical Council.

Kappers MH, van Esch JH, Smedts FM, de Krijger RR, Eechoute K, Mathijssen RH, Sleijfer S, Leijten F, Danser AH, van den Meiracker AH, Visser TJ. Sunitinib-induced hypothyroidism is due to induction of type 3 deiodinase activity and thyroidal capillary regression. J Clin Endocrinol Metab. 2011;96(10):3087–94.

Kim HI, Kim TH, Kim H, Kim YN, Jang HW, Chung JH, Moon SM, Jhun BW, Lee H, Koh WJ, Kim SW. Effect of rifampin on thyroid function test in patients on levothyroxine medication. PLoS One. 2017;12(1):e0169775.

Kirov G, Tredget J, John R, Owen MJ, Lazarus JH. A cross-sectional and a prospective study of thyroid disorders in lithium-treated patients. J Affect Disord. 2005;87:313–7.

Koh LK, Greenspan FS, Yeo PP. Interferon-alpha induced thyroid dysfunction: three clinical presentations and a review of the literature. Thyroid. 1997;7(6):891–6.

Köhrle J. Environment and endocrinology: the case of thyroidology. Ann Endocrinol (Paris). 2008;69:116–22.

Krouse RS, Royal RE, Heywood G, Weintraub BD, White DE, Steinberg SM, Rosenberg SA, Schwartzentruber DJ. Thyroid dysfunction in 281 patients with metastatic melanoma or renal carcinoma treated with interleukin-2 alone. J Immunother Emphasis Tumor Immunol. 1995;18 (4):272–8.

Lazarus JH. Lithium and thyroid. Best Pract Res Clin Endocrinol Metab. 2009;23(6):723–33.

Lazarus JH, McGregor AM, Ludgate M, Darke C, Creagh FM, Kingswood CJ. Effect of lithium carbonate therapy on thyroid immune status in manic depressive patients: a prospective study. J Affect Disord. 1986;11(2):155–60.

Lefebvre P, Benomar Y, Staels B. Retinoid X receptors: common heterodimerization partners with distinct functions. Trends Endocrinol Metab. 2010;21:676–83.

Leung AM, Pearce EN, Braverman LE. Environmental perchlorate exposure: potential adverse thyroid effects. Curr Opin Endocrinol Diabetes Obes. 2014;21:372–6.
List A, Dewald G, Bennett J, Giagounidis A, Raza A, Feldman E, Powell B, Greenberg P, Thomas D, Stone R, Reeder C, Wride K, Patin J, Schmidt M, Zeldis J, Knight R. Myelodysplastic syndrome-003 study investigators. Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion. N Engl J Med. 2006;355(14):1456–65.
Liwanpo L, Hershman JM. Conditions and drugs interfering with thyroxine absorption. Best Pract Res Clin Endocrinol Metab. 2009;23:781–92.
Loviselli A, Oppo A, Velluzzi F, Atzeni F, Mastinu GL, Farci P, Orgiana G, Balestrieri A, Cocco PL, Mariotti S. Independent expression of serological markers of thyroid autoimmunity and hepatitis virus C infection in the general population: results of a community-based study in north-western Sardinia. J Endocrinol Investig. 1999;22(9):660–5.
Lupoli R, Di Minno A, Tortora A, Ambrosino P, Lupoli GA, Di Minno MN. Effects of treatment with metformin on TSH levels: a meta-analysis of literature studies. J Clin Endocrinol Metab. 2014;99:E143–8.
Makita N, Iiri T. Tyrosine kinase inhibitor-induced thyroid disorders: a review and hypothesis. Thyroid. 2013;23(2):151–9.
Mannavola D, Coco P, Vannucchi G, Bertuelli R, Carletto M, Casali PG, Beck-Peccoz P, Fugazzola L. A novel tyrosine-kinase selective inhibitor, sunitinib, induces transient hypothyroidism by blocking iodine uptake. J Clin Endocrinol Metab. 2007;92(9):3531–4.
Marini H, Polito F, Adamo EB, Bitto A, Squadrito F, Benvenga S. Update on genistein and thyroid: an overall message of safety. Front Endocrinol (Lausanne). 2012;3:94.
Martino E, Aghini-Lombardi F, Mariotti S, Bartalena L, Braverman L, Pinchera A. Amiodarone: a common source of iodine-induced thyrotoxicosis. Horm Res 1987a;26(1–4):158–171; i
Martino E, Aghini-Lombardi F, Mariotti S, Bartalena L, Lenziardi M, Ceccarelli C, Bambini G, Safran M, Braverman LE, Pinchera A. Amiodarone iodine-induced hypothyroidism: risk factors and follow-up in 28 cases. Clin Endocrinol. 1987b;26(2):227–37.
Martino E, Bartalena L, Bogazzi F, Braverman LE. The effects of amiodarone on the thyroid. Endocr Rev. 2001;22(2):240–54.
McElnea E, Ní Mhéalóid A, Moran S, Kelly R, Fulcher T. Thyroid-like ophthalmopathy in a euthyroid patient receiving Ipilimumab. Orbit. 2014;33(6):424–7.
Menconi F, Hasham A, Tomer Y. Environmental triggers of thyroiditis: hepatitis C and interferon-α. J Endocrinol Investig. 2011;34(1):78–84.
Min L, Vaidya A, Becker C. Thyroid autoimmunity and ophthalmopathy related to melanoma biological therapy. Eur J Endocrinol. 2011;164(2):303–7.
Mitchell AL, Hickey B, Hickey JL, Pearce SHS. Trends in thyroid hormone prescribing and consumption in the UK. BMC Health. 2009;9:132–41.
Müssig K, Thamer C, Bares R, Lipp HP, Häring HU, Gallwitz B. Iodine-induced thyrotoxicosis after ingestion of kelp-containing tea. J Gen Intern Med. 2006;21:C11–4.
Nair Kesavachandran C, Haamann F, Nienhaus A. Frequency of thyroid dysfunctions during interferon alpha treatment of single and combination therapy in hepatitis C virus-infected patients: a systematic review based analysis. PLoS One. 2013;8(2):e55364.
Negro R, Greek G, Mangieri T, Pezzarossa A, Dazzi D, Hassan H. The influence of selenium supplementation on postpartum thyroid status in pregnant women with thyroid peroxidase autoantibodies. J Clin Endocrinol Metab. 2007;92:1263–8.
Nordio M, Pajalich R. Combined treatment with myo-inositol and selenium ensures euthyroidism in subclinical hypothyroidism patients with autoimmune thyroiditis. J Thyr Research. 2013;2013:424163.
Pani F, Atzori F, Baghino G, Boi F, Tanca L, Ionta MT, Mariotti S. Thyroid dysfunction in patients with metastatic carcinoma treated with sunitinib: is thyroid autoimmunity involved? Thyroid. 2015;25(11):1255–61.
Perrild H, Hegedüs L, Arnung K. Sex related goitrogenic effect of lithium carbonate in healthy young subjects. ActaEndocrinol (Copenh). 1984;106(2):203–8.

Prummel MF, Laurberg P. Interferon-alpha and autoimmune thyroid disease. Thyroid. 2003;13(6):547–51.
Rao RH, McCready VR, Spathis GS. Iodine kinetic studies during amiodarone treatment. J Clin Endocrinol Metab. 1986;62(3):563–8.
Salem AK, Fenton MS, Marion KM, Hershman JM. Effect of sunitinib on growth and function of FRTL-5 thyroid cells. Thyroid. 2008;18(6):631–5.
Sarne D. Effects of the environment, chemicals and drugs on thyroid function. In: De Groot LJ, Beck-Peccoz P, Chrousos G, Dungan K, Grossman A, Hershman JM, Koch C, Mc Lachlan R, New M, Rebar R, Singer F, Vinik A, Weickert MO, editors. Endotext [Internet]. South Dartmouth (MA): MD Text.com, Inc; 2016. p. 2000–10.
Sauvage MF, Marquet P, Rousseau A, Raby C, Buxeraud J, Lachâtre G. Relationship between psychotropic drugs and thyroid function: a review. Toxicol Appl Pharmacol. 1998;149:127–35.
Sherman SI. Etiology, diagnosis, and treatment recommendations for central hypothyroidism associated with bexarotene therapy for cutaneous T-cell lymphoma. Clin Lymphoma. 2003;3:249–52.
Shine B, McKnight RF, Leaver L, Geddes JR. Long-term effects of lithium on renal, thyroid, and parathyroid function: a retrospective analysis of laboratory data. Lancet. 2015;386:461–8.
Smit JW, Stokkel MP, Pereira AM, Romijn JA, Visser TJ. Bexarotene-induced hypothyroidism: bexarotene stimulates the peripheral metabolism of thyroid hormones. J Clin Endocrinol Metab. 2007;92(7):2496–9.
Smith TJ, Hegedüs L. Graves' disease. N Engl J Med. 2016;375:1552–65.
Spaulding SW, Burrow GN, Bermudez F, Himmelhoch JM. The inhibitory effect of lithium on thyroid hormone release in both euthyroid and thyrotoxic patients. J Clin Endocrinol Metab. 1972;35(6):905–11.
Surks MI, Sievert R. Drugs and thyroid function. N Engl J Med. 1995;333(25):1688–94.
Takasu N, Takara M, Komiya I. Rifampin-induced hypothyroidism in patients with Hashimoto's thyroiditis. N Engl J Med. 2005;352:518–9.
Takasu N, Kinjou Y, Kouki T, Takara M, Ohshiro Y, Komiya I. Rifampin-induced hypothyroidism. J Endocrinol Investig. 2006;29(7):645–9.
Torino F, Barnabei A, Paragliola R, Baldelli R, Appetecchia M, Corsello SM. Thyroid dysfunction as an unintended side effect of anticancer drugs. Thyroid. 2013;23(11):1345–66.
Trifirò G, Parrino F, Sultana J, Giorgianni F, Ferrajolo C, Bianchini E, Medea G, Benvenga S, Cricelli I, Cricelli C, Lapi F. Drug interactions with levothyroxine therapy in patients with hypothyroidism: observational study in general practice. Clin Drug Investig. 2015;35(3):187–95.
Trip MD, Wiersinga W, Plomp TA. Incidence, predictability, and pathogenesis of amiodarone-induced thyrotoxicosis and hypothyroidism. Am J Med. 1991;91(5):507–11.
Verloop H, Smit JW, Dekkers OM. Sorafenib therapy decreases the clearance of thyrotropin. Eur J Endocrinol. 2013;168(2):163–7.
Verrotti A, Scardapane A, Manco R, Chiarelli F. Antiepileptic drugs and thyroid function. J Pediatr Endocrinol Metab. 2008;21:401–8.
Viniol A, Bösner S, Baum E, Donner-Banzhoff N. Forgotten drugs: long-term prescriptions of thyroid hormones – a cross-sectional study. Int J Gen Med. 2013;26(6):329–34.
Virili C, Trimboli P, Romanelli F, Centanni M. Liquid and softgel levothyroxine use in clinical practice: state of the art. Endocrine. 2016;54(1):3–14.
Vita R, Fallahi P, Antonelli A, Benvenga S. The administration of L-thyroxine as soft gel capsule or liquid solution. Expert Opin Drug Deliv. 2014;11:1103–11.
Vos F, Pieters G, Keuter M, van der Ven A. Graves' disease during immune reconstitution in HIV-infected patients treated with HAART. Scand J Infect Dis. 2006;38:124–6.
Wagner CE, Jurutka PW, Marshall PA, Heck MC. Retinoid X receptor selective agonists and their synthetic methods. Curr Top Med Chem. 2017;17(6):742–67.
Wang JF, Milosveski V, Schramek C, Fong GH, Becks GP, Hill DJ. Presence and possible role of vascular endothelial growth factor in thyroid cell growth and function. J Endocrinol. 1998;157(1):5–12.

Weijl NI, Van der Harst D, Brand A, Kooy Y, Van Luxemburg S, Schroder J, Lentjes E, Van Rood JJ, Cleton FJ, Osanto S. Hypothyroidism during immunotherapy with interleukin-2 is associated with antithyroid antibodies and response to treatment. J Clin Oncol. 1993;11(7):1376–83.

Willemze R, Jaffe ES, Burg G, Cerroni L, Berti E, Swerdlow SH, Ralfkiaer E, Chimenti S, Diaz-Perez JL, Duncan LM, et al. WHO-EORTC classification for cutaneous lymphomas. Blood. 2005;105:3768–85.

Willis M, Robertson NP. Drug safety evaluation of alemtuzumab for multiple sclerosis. Expert Opin Drug Saf. 2014;13:1115–24.

Winther KH, Bonnema S, Hegedüs L. Is selenium supplementation in autoimmune thyroid diseases justified? Curr Opin Endocrinol Diabetes Obes. 2017;24(5):348–55.

Wolter P, Stefan C, Decallonne B, Dumez H, Bex M, Carmeliet P, Schöffski P. The clinical implications of sunitinib-induced hypothyroidism: a prospective evaluation. Br J Cancer. 2008;99(3):448–54.

Wu Q, Rayman MP, Lv H, Schomburg L, Cui B, Gao C, Chen P, Zhuang G, Zhang Z, Peng X, Li H, Zhao Y, He X, Zeng G, Qin F, Hou P, Shi B. Low population selenium status is associated with increased prevalence of thyroid disease. J Clin Endocrinol Metab. 2015;100(11):4037–47.

Yılmaz U, Yılmaz TS, Akıncı G, Korkmaz HA, Tekgül H. The effect of antiepileptic drugs on thyroid function in children. Seizure. 2014;23:29–35.

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