Medullary Thyroid Cancer

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Editors
Tracy S. Wang, MD, MPH
Associate Professor of Surgery; Chief, Section of Endocrine Surgery
Medical College of Wisconsin
Milwaukee, WI
USA

Douglas B. Evans, MD
Donald C. Ausman Family Foundation Professor in Surgery and Chair
Medical College of Wisconsin
Milwaukee, WI
USA

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Preface

Medullary thyroid cancer (MTC) is a unique form of thyroid cancer and distinct from the more common differentiated variants (papillary and follicular). As a neuroendocrine tumor, MTC is derived from the parafollicular cells, or C cells, of the thyroid; the secretion of calcitonin and carcinoembryonic antigen (CEA) enables these serum peptides to be utilized as pre- and postoperative tumor markers, which correlate with the extent of disease and tumor burden. This is in contrast to papillary and follicular thyroid cancer where the use of thyroglobulin as a measure of disease extent is much less sensitive and specific. Serum levels of calcitonin and CEA can be utilized to guide the extent of preoperative imaging, the results of which influence the extent of initial surgery. MTC which is localized to the neck remains largely a surgical disease as there is no effective adjuvant therapy currently available to minimize disease recurrence. In addition, while the majority of patients have sporadic disease, approximately one-fourth of patients with newly diagnosed MTC will have an inherited form, secondary to multiple endocrine neoplasia (MEN) type 2A or 2B, or the related syndrome of familial MTC.

These unique biologic features can make the management of patients with MTC quite challenging and require the multidisciplinary expertise of internists, pediatricians, endocrinologists, genetic counselors, medical oncologists, pathologists, and thyroid surgeons. This text is not meant to replace the current American Thyroid Association guidelines, last published in 2015, which serves as the point of reference for the management of patients with MTC; but rather, we hope to provide a more in-depth discussion of some of the clinical challenges which physicians and patients face.

We would like to extend our deepest thanks to each of the contributors to this text for their clinical expertise and generosity of time and talent. We would also like to thank the editors, Maria Smilios and Samantha Lonuzzi, for their patience and assistance in the production of this book. Most importantly, we are grateful for the courageous patients and families who have formed the basis of our experience and reinforce the importance of multimodality care, clinical trials, and translational research.

Milwaukee, WI, USA

Tracy S. Wang
Douglas B. Evans

Contents

Contributors

Sylvia L. Asa, MD Laboratory Medicine Program, Department of Pathology, University Health Network, Toronto, ON, Canada; Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada

Rozita Bagheri-Yarmand, MD Department of Endocrine Neoplasia and Hormonal Disorders, MD Anderson Cancer Center, The University of Texas, Houston, TX, USA

Naira Baregamian, MD Division of Surgical Oncology and Endocrine Surgery, Department of Surgery, Vanderbilt University Medical Center, Nashville, TN, USA

James Brierley, MD Department of Radiation Oncology, Princess Margaret Cancer Centre, University of Toronto, Toronto, ON, Canada

Naifa L. Busaidy, MD Department of Endocrine Neoplasia and Hormonal Disorders, MD Anderson Cancer Center, The University of Texas, Houston, TX, USA

Glenda G. Callender, MD Department of Surgery, Yale University School of Medicine, New Haven, CT, USA

Azadeh A. Carr Section of Endocrine Surgery, Division of Surgical Oncology, Department of Surgery, Medical College of Wisconsin, Milwaukee, WI, USA

Scott Coman Department of Otolaryngology, Princess Alexandra Hospital, Brisbane, QLD, Australia

Gilbert J. Cote, PhD Department of Endocrine Neoplasia and Hormonal Disorders, MD Anderson Cancer Center, The University of Texas, Houston, TX, USA

Nazanene H. Esfandiari, MD Division of Metabolism, Endocrinology, & Diabetes, University of Michigan, Ann Arbor, MI, USA

Douglas B. Evans, MD Section of Endocrine Surgery, Division of Surgical Oncology, Department of Surgery, Medical College of Wisconsin, Milwaukee, WI, USA

Gilbert G. Fareau, MD Division of Endocrinology, Department of Medicine, Medical College of Wisconsin, Milwaukee, WI, USA

Abbey Fingeret, MD Division of General and Gastrointestinal Surgery, Massachusetts General Hospital, Boston, MA, USA

Robert F. Gagel, MD Department of Endocrine Neoplasia and Hormonal Disorders, MD Anderson Cancer Center, The University of Texas, Houston, TX, USA

Jennifer L. Geurts, MS Division of Surgical Oncology, Department of Surgery, Medical College of Wisconsin, Milwaukee, WI, USA

Meredith Elana Giuliani, MD Princess Margaret Cancer Centre, Radiation Medicine Program, Toronto, ON, Canada

Jason A. Glenn, MD Section of Endocrine Surgery, Division of Surgical Oncology, Department of Surgery, Medical College of Wisconsin, Milwaukee, WI, USA

Elizabeth G. Grubbs, MD, MPH Department of Surgical Oncology, MD Anderson Cancer Center, The University of Texas, Houston, TX, USA

Raefe Gundelach, MD Department of Otolaryngology, Princess Alexandra Hospital, Brisbane, QLD, Australia

Adrian Harvey, MD Faculty of Medicine, Department of Surgery and Oncology, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada

Megan R. Haymart, MD Division of Metabolism, Endocrinology, & Diabetes, University of Michigan Health System, Ann Arbor, MI, USA; Division of Hematology/Oncology, University of Michigan Health System, Ann Arbor, MI, USA

Marie-Claude Hofmann, MD Department of Endocrine Neoplasia and Hormonal Disorders, MD Anderson Cancer Center, The University of Texas, Houston, TX, USA

Jacqueline Jonklaas, MD Division of Endocrinology, Georgetown University, Washington, DC, USA

Lily J. Kwatampora, MD Endocrinology Diabetes and Metabolism, Baylor College of Medicine, Houston, TX, USA

Terry C. Lairmore, MD Division of Surgical Oncology, Department of Surgery, Baylor Scott & White Health, Texas A&M University Health Science Center, Temple, TX, USA

Carrie C. Lubitz, MD, MPH Harvard Medical School, Massachusetts General Hospital, Boston, MA, USA

Ozgur Mete, MD Laboratory Medicine Program, Department of Pathology, University Health Network, Toronto, ON, Canada; Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada

Mary M. Mrdutt, MD Baylor Scott and White Health, Temple, TX, USA

Janice L. Pasieka, MD Faculty of Medicine, Department of Surgery and Oncology, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada

Sanziana A. Roman, MD Section of Endocrine Surgery, Duke University School of Medicine, Durham, NC, USA

Randall P. Scheri, MD Section of Endocrine Surgery, Duke University School of Medicine, Durham, NC, USA

Carmen C. Solórzano, MD Division of Surgical Oncology and Endocrine Surgery, Department of Surgery, Vanderbilt University Medical Center, Nashville, TN, USA

Julie Ann Sosa, MD, MA Section of Endocrine Surgery, Duke University School of Medicine, Durham, NC, USA

Steven G. Waguespack, MD Department of Endocrine Neoplasia and Hormonal Disorders, MD Anderson Cancer Center, The University of Texas, Houston, TX, USA

Tracy S. Wang, MD, MPH Section of Endocrine Surgery, Division of Surgical Oncology, Department of Surgery, Medical College of Wisconsin, Milwaukee, WI, USA

Linwah Yip, MD Division of Endocrine Surgery, Department of Surgery, University of Pittsburgh, Pittsburgh, PA, USA

Jessica E. Zwiener, MD Department of Endocrine Neoplasia and Hormonal Disorders, MD Anderson Cancer Center, The University of Texas, Houston, TX, USA

History and Epidemiology

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Mary M. Mrdutt and Terry C. Lairmore

Medullary thyroid carcinoma (MTC) was not recognized as a distinct pathologic type of thyroid cancer until relatively recently. The correct classification of this thyroid neoplasm followed earlier histopathologic studies that identified a separate population of parafollicular or C-cells, the recognition that these cells had an embryologic origin from the neural crest, and the discovery that this cell population was responsible for the production of the peptide hormone calcitonin. Subsequent clinical studies established the strong familial patterns for the development of MTC and its association with specific hereditary cancer syndromes. The unique features of MTC include its intermediate level of biologic aggressiveness within the spectrum of thyroid cancer types, the production of a specific hormone product that can serve as a sensitive tumor marker for occult or recurrent disease (calcitonin), and its lack of susceptibility to radioiodine, making complete surgical removal of all cancer and lymphatic metastases the primary treatment goal. In addition, there have been great advances in the understanding of the molecular pathogenesis of MTC with the identification of germline-activating mutations in the *RET* proto-oncogene that are responsible for its development in association with the multiple endocrine neoplasia type 2 (MEN2) syndromes, as well as similar somatic mutations that underlie a significant proportion of sporadic tumors. Importantly, direct genetic testing allows for presymptomatic identification of patients who have inherited a disease-associated *RET* mutation and are at essentially 100 % risk of developing thyroid cancer during their lifetime. This ability to make a definitive genetic diagnosis prior to the detection of clinical, sonographic, or biochemical evidence of neoplasia allows the unique opportunity to perform an early thyroidectomy to remove the end organ at risk before invasive malignancy develops. Early prophylactic thyroidectomy for patients with MEN2 was one of the first and is perhaps still one of the best examples of a surgical intervention based on genetic testing that is intended to completely prevent subsequent cancer development in patients with an inherited cancer susceptibility.

History

Description of the Thyroid C-Cells

The story of MTC begins with the discovery of a separate population of cells dispersed in very small numbers within the thyroid parenchyma

M.M. Mrdutt
Baylor Scott and White Health, Temple, TX, USA

T.C. Lairmore (✉)
Division of Surgical Oncology, Department of Surgery, Baylor Scott & White Health, Texas A&M University Health Science Center, MS-01-730C, 2401 S. 31st Street, Temple, TX 76508, USA
e-mail: terry.lairmore@bswhealth.org

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that are embryologically, histologically, and functionally distinct from the thyroid follicular cell. In his communication to the Royal Society of London on January 27, 1876, entitled "Contributions to the Minute Anatomy of the Thyroid Gland of the Dog," E. Cresswell Baber first described this morphologically distinct population of cells [1]. Baber was studying the lymphatics of the gland and aptly declared during his preamble that the thyroid "is one of those organs, commonly known by the name of ductless or blood glands, about which our knowledge is still in a very unsatisfactory condition." Baber termed the separate population *parenchymatous cells*. These non-follicular cells were subsequently referred to descriptively as *parafollicular cells*, as proposed by Nonidez in 1932 [2]. This term is not entirely accurate, however, as the cells occur in both parafollicular and intrafollicular locations. The early studies of this cellular population were performed on animal thyroid glands, which have more prominent and larger numbers of "parafollicular" cells when compared to their human counterparts. José Fernandez Nonidez, an anatomist at Cornell Medical College in New York, performed histochemical studies as part of his work on the innervation of the thyroid gland of the dog. He utilized the reduced silver nitrate method of Cajal to characterize these cells based on their argyrophilic cytoplasmic granules. He recognized that these cells differed from the follicular cells in the thyroid glands of young puppies, in that they were larger and contained clear vesicular nuclei [3, 4]. Nonidez rediscovered Baber's "parenchymatous cells," and in discussing his elegant studies, he correctly proposed that the argyrophil granules represented "the antecedent of an endocrine secretion poured directly into the vessels." As reviewed by Roediger [5] and Hazard [6], these cells were recognized variously in subsequent studies of the mammalian thyroid as protoplasm-rich cells [7], ovoid cells [8], interfollicular cell [9, 10], neurohormonal cell [11], giant-light cell [12], mitochondrion-rich cell [13, 14], macrothyrocytes [15, 16], argyrophilic cell [17], gray cell [18], and stem cell [19]. The descriptive terms above were offered during precise early anatomic studies that focused on the morphology and histologic location of the cells within the thyroid gland. Pearse in 1966 [20] proposed the term C-cell, accurately indicating their function in the secretion of calcitonin.

The reasons for the late identification of this distinct cellular population in the human thyroid gland include their presence in very small numbers and non-specific appearance on routine histology. In the human thyroid, the C-cells exist singly or in small groups and are not easily discernible from the surrounding follicular cells unless special techniques are employed. On standard hematoxylin and eosin staining, the cells are slightly paler than the surrounding follicular cells but not sufficiently to provide positive identification. The most important historical staining method to identify the C-cells is the Grimelius silver nitrate argyrophil method [21], which results in characteristic, dark granular staining of the numerous secretory granules in the cytoplasm. Specific immunoperoxidase staining based on the presence of the hormone calcitonin was used in subsequent detailed studies of the distribution of C-cells in the human thyroid [22–24]. Ultrastructurally, C-cells are characterized by numerous round secretory granules that fill the cytoplasm, a well-developed Golgi complex, and moderate numbers of mitochondria. Electron microscopy studies have demonstrated that these cells are found in close proximity to follicular cells and within the follicular basement membrane. Thus, the "parafollicular cells" may occur in both a parafollicular and intrafollicular location.

The demonstration that these non-follicular cells synthesized, stored, and secreted calcitonin was the next major advance in the understanding of the thyroid gland's cellular structure and function. Copp and colleagues [25] in 1962 demonstrated the presence of calcitonin in canine thyroid–parathyroid preparations, but interpreted the source of the hormone to be the parathyroid glands. Foster [13] found that these "mitochondrion-rich" cells in the canine thyroid were responsive to hypercalcemia and proposed the name thyrocalcitonin to distinguish the "enzyme levels" from the hormone believed to be

produced by the parathyroid glands in the earlier studies by Copp. Bussolati and Pearse [26] subsequently demonstrated calcitonin in the C-cells of the porcine thyroid by direct immunofluorescent studies. Pearse [20] introduced the term "C-cells" to indicate the role of these cells in the production of calcitonin, which is most appropriate to describe their function. The C-cells belong to a dispersed family of peptide hormone-producing cells that Pearse [27] included under the descriptive term amine precursor uptake and decarboxylation (APUD) cells, based on common cytochemical and ultrastructural features, as well as his hypothesis that they shared a common embryological origin from the neural crest.

Dr. Anthony "Tony" G.E. Pearse was a pioneering pathologist and histochemist born in Kent, England, in 1916. He served as Surgeon Lieutenant during the Second World War and held an academic post in the Department of Morbid Anatomy (Histopathology) at the Hammersmith Hospital and the Royal Postgraduate Medical School of London, where he was appointed to Chair and first Professor of Histochemistry. Although subsequent studies demonstrated that some of the cells in the group did not derive from neural crest or even neuroectoderm, his pioneering work in expanding field of histochemistry, enzyme localization, and experimental embryology inspired a large number of scientists and contributed immeasurably to insight into the origins of the diffuse endocrine system and the biochemistry of peptide hormone-producing cells. He died in South Molton, Devon, on May 24, 2003.

The thyroid gland has a dual embryologic origin, originating from both the primitive pharynx and the neural crest. The main thyroid primordium develops as a midline thickening or proliferation of endodermal epithelial cells on the surface of the developing pharyngeal floor between the first and second pouches, in a location termed the foramen cecum. This proliferation begins as a hollow structure and later becomes solid and bilobed. The stem remains as the thyroglossal duct that does not descend into the lateral lobes. The rudimentary lateral thyroid anlage has origins from the pharyngeal endoderm and neural crest cells. The early ultimobranchial body arises from the pharyngeal endoderm and is subsequently populated by neural crest cells. The resulting ultimobranchial body gives rise to the C-cells. The derivation of parafollicular or C-cells from the neural crest was established by the experiments [28] with grafts of neural rhombencephalic primordium from 6 to 10 somite quail embryos implanted into the developing chick. The neural crest cells of quail have easily identifiable nuclear characteristics. Transplantation of these cells into non-quail neural crest was performed with the subsequent demonstration of cells with quail nuclei populating the ultimobranchial body in chick embryos, supporting the conclusion that C-cells arise from the neural crest and migrate during embryologic development. These cells associate embryologically with the ultimobranchial body, which is a ventral derivative of the fourth (or fifth) pharyngeal pouch. Some additional more recent studies, however, have suggested that revision of the concept of origin of the C-cells in mammals entirely from neuroectoderm may be necessary [29].

Anatomic studies have detailed the distribution of C-cells within the human thyroid gland. The C-cells have been shown to have an unequal distribution within different regions of the thyroid parenchyma. An early study of goitrous thyroid glands by Englund and coworkers [30] using argyrophil and fluorescent microscopy found that the extreme periphery of the lateral lobes just beneath the capsule and the isthmus was largely devoid of C-cells. In 1974, Wolfe et al. [24] and Tashjian et al. [22] mapped the distribution of C-cells in nonpathologic adult thyroid glands with detailed study of serial sections using immunoperoxidase localization and correlation of the histologic findings with radioimmunoassay for calcitonin in adjacent paired sections. These investigators concluded that the C-cells represent less than 0.1 % of the epithelial mass of the gland. The cells were shown to be primarily parafollicular, but also occurred in an intrafollicular location adjacent to the basement membrane and covered by a thin cytoplasmic

layer of the adjoining follicular cells which provided separation from the colloid within the follicle. These studies also demonstrated that C-cells are predominantly located within the deep portions of the middle and upper thirds of the thyroid lobe and are essentially absent in the isthmus. Wolfe et al. [23] performed a detailed study of six neonatal thyroid glands obtained immediately postmortem from normocalcemic subjects without associated endocrine disorders. They employed an immunoperoxidase localization technique with correlation by bioassay and immunoassay for calcitonin. In their study of neonatal thyroid glands, the C-cells were also distributed primarily in the middle to upper thirds of the lateral lobes. These cells were more numerous in the neonate than in adult (up to 75 C-cells per low power field) and more frequently intrafollicular in contrast to the findings from prior studies of the adult thyroid gland. The reason for the finding of increased numbers of C-cells in the neonatal thyroid was not clearly revealed in these studies, although it was speculated that the increased proportion might reflect the relative fraction in relation to the smaller gland volume, or alternatively that calcitonin might have an important role for the developing fetus. The anatomic distribution of C-cells within upper to middle thirds of the thyroid parenchyma corresponds to the most frequent location for the clinical development of medullary carcinoma within the gland.

Medullary Thyroid Carcinoma Arises from the Thyroid C-Cells

In his comprehensive contemporary 1977 review article "The C-Cells (Parafollicular Cells) of the Thyroid Gland and Medullary Thyroid Carcinoma" [6], Hazard attributes the first record of MTC as a specific pathologic diagnosis for a thyroid tumor to his unpublished observations in 1947. The solid cellular arrangement of the tumor type had previously resulted in its inclusion in the group of undifferentiated thyroid carcinomas, which were characterized by a highly aggressive malignant growth pattern. Dr. Hazard recognized a select subset of tumors with a characteristic "uniformity of cell type, a moderate number of mitoses as compared with the undifferentiated or anaplastic carcinoma, a uniform arrangement of cell sheets separated by stroma, and especially, the presence of amyloid in the tumor features that identified it as a distinctive histologic type."

Descriptions of malignant thyroid tumors containing amyloid have been found in the German literature from the early twentieth century, as cited by Ljungberg [31]. Horn in 1951 [32] published the findings from a series of 7 cases of a distinct variant of thyroid carcinoma with a moderate grade of malignancy and the gross and histologic features of medullary carcinoma, without description of the associated amyloid-containing stroma. A case report by Brandenberg in 1954 [33] characterized a thyroid tumor as "metastasizing amyloid struma," and Laskowski in 1957 [34] proposed the term "carcinoma thyroideum hyalinicum."

In 1959, a landmark paper by Hazard, Hawk, and Crile [35] from the Cleveland Clinic provided the first complete description of medullary (solid) carcinoma of the thyroid and identified this type of thyroid cancer as a distinct clinicopathologic entity. They reviewed the pathologic material from 600 carcinomas of the thyroid removed at the Cleveland Clinic Hospital between 1926 and 1957 and identified 21 cases with unique features warranting classification as a separate type of thyroid carcinoma. This report distinguished medullary carcinoma by its solid non-follicular histologic pattern, the presence of amyloid in the stroma, and a high incidence of lymph node metastasis. They noted that these tumors demonstrated an intermediate grade of malignancy, in distinction to the aggressive undifferentiated thyroid carcinomas with which they had been previously grouped.

John Beach Hazard was born on January 7, 1905, in White Horse, Pennsylvania. After completing his training at the Mallory Institute of Pathology, Boston City Hospital, he was a consultant in Pathology at the Robert Bent Brigham Hospital (1937–1946) and then ultimately chairman of the Division of Pathology at the

Cleveland Clinic Foundation (1958–1970). His many important contributions included the definitive first description of MTC and the original description of the presence of amyloid in the tumor. Known to his friends as "Beach," he had lifelong interest in horses and passion for horse racing and was regularly seen in attendance at the Kentucky Derby with his wife Mae. Dr. Hazard died in Key Biscayne, Florida, on September 13, 1994.

The origin of MTC from the C-cell was proposed by Williams [36] in 1966, and he suggested that the neoplasm was the source of a product with hypocalcemic activity. Meyer and coworkers provided support for this proposal through ultrastructural studies [37, 38] demonstrating the similarities of the cytoplasmic secretory granules of the tumor cells and C-cells, as well as the thyrocalcitonin-like activity exhibited by the tumor in tissue culture studies [38]. Subsequent additional studies [39, 40] confirmed the production of calcitonin by MTC tumor cells, and specific demonstration of calcitonin in the parafollicular C-cells was shown with the use of fluorescein-labeled antibody to calcitonin [41, 42]. The presence of an amorphous material in the stroma was a characteristic feature of the early descriptions of MTC, and this material was identified as amyloid. Albores-Saavedra et al. [43] identified amyloid fibrils by electron microscopy and confirmed that the amyloid material was a product of the MTC cells by tissue culture studies. Ultrastructural studies [37, 38] further characterized the amyloid fibrils and revealed that the formation of amyloid was predominately extracellular, but suggested that the secretory granules in MTC cells were involved in production of the material. Calcitonin was subsequently directly identified within the amyloid stroma by immunohistochemistry and immunofluorescence [44, 45]. Sequence analysis of the isolated amyloid medullary carcinoma of thyroid (AMCT) protein confirmed the presence of calcitonin, but with a higher molecular weight. This finding leads to the conclusion that an alternatively processed prohormone of calcitonin was a component of the amyloid material [45]. More recently, elegant mass spectrometry studies of the amyloid in MTC by Khurana et al. concluded that the full-length calcitonin was present [46], and mass spectrometry-based proteonomic analysis identified the peptides calcitonin and katacalcin [47]. Katacalcin is a member of the calcitonin gene family and flanks calcitonin in the human calcitonin precursor which is subsequently cleaved into calcitonin and katacalcin.

Peptide Hormones and Tumor Markers

Hirsch et al. [48] in 1964 purified a factor from extracts of porcine thyroid glands that was demonstrated to lower serum calcium and inorganic phosphate in rats. This agent, termed thyrocalcitonin, appeared to be a polypeptide that was distinct from thyroxine and triiodothyronine. The peptide hormone, later identified as calcitonin, assumed great importance as a tumor marker for MTC. Calcitonin plays a role in calcium homeostasis especially in lower animals, with a less prominent influence in humans. In common with other cells belonging to the dispersed neuroendocrine system derived from neural crest and the APUD cell concept of Pearse, the C-cells are capable of producing a variety of peptides, as well as biogenic amines, prostaglandins [49], and ectopic hormones such as ACTH [50], as reviewed in [51]. Baylin et al. [52, 53] demonstrated increased histaminase activity in the serum of patients with MTC and further reported that high levels of histaminase are present only in malignant C-cells suggesting that this activity might serve as a marker to distinguish microscopic carcinoma from C-cell hyperplasia. Elevated plasma levels of carcinoembryonic antigen (CEA) in patients with MTC were reported by Ishikawa and Hamada [54]. Wells et al. [55] studied 37 patients with MTC and demonstrated that serum CEA levels were elevated in 62 %, whereas serum calcitonin levels were elevated in 72 % of patients.

The finding that calcitonin is elaborated by normal C-cells and MTC tumor cells represented the next major advance in the diagnosis and clinical characterization of patients with this specific tumor type. The production of calcitonin

by C-cells and MTC tumor cells provides the ability to utilize circulating levels of this hormone as a sensitive tumor marker and proved to be a sensitive method to detect even small amounts of subclinical disease. Tashjian and Melvin [56] in 1968 reported their findings of a hypocalcemic agent, thyrocalcitonin, in studies of plasma and tumor extracts from two patients with medullary thyroid carcinoma. In the same year, they presented a case [40] of a thyrocalcitonin-secreting thyroid carcinoma with resultant hypocalcemia and "secondary hyperparathyroidism" from increased levels of circulating thyrocalcitonin. Further work described the clinical measurement of calcitonin and studies in patients, including the early diagnosis by means of the calcitonin immunoassay [57, 58].

The discovery that calcitonin secretion was stimulated by pentagastrin [59] and calcium [60] provided a sensitive clinical test to detect the presence of increased numbers of C-cells or occult carcinoma, even with very small numbers of neoplastic cells. Measurement of calcitonin response to secretagogues such as calcium and pentagastrin combined [61, 62] was employed clinically for the early diagnosis of MTC as well as to detect microscopic residual disease or follow patients for recurrence.

Familial Occurrence of Medullary Thyroid Carcinoma and Association with Specific Hereditary Endocrine Neoplasia Syndromes

The initial observation that eventually led to the recognition of the autosomal dominant inheritance pattern of hereditary cases of MTC, and its association with the specific MEN2 familial endocrine cancer syndromes, was the recognition that thyroid cancer occurred more frequently in patients with pheochromocytoma than would be expected by chance. Decourcy and Decourcy in 1952 [63] and Sipple in 1961 [64] noted an increased incidence of thyroid cancer in patients with pheochromocytoma. Sugg [65] recorded the interesting historical aspects Dr. Sipple's original observations based on direct personal interviews. Dr. John H. Sipple carefully documented the clinical findings in a case report of a 33-year-old index patient with both pheochromocytoma and carcinoma of the thyroid that he saw in a third-year medical student and later attended the patient's autopsy. Through an extensive literature review, he made the important observation that among 537 reported cases of pheochromocytoma, there were 5 additional cases also associated with thyroid cancer. Dr. Sipple was not an endocrinologist or a surgeon, but rather a pulmonologist. He graduated from Cornell University Medical College in 1955 and was a resident in internal medicine at State University of New York (SUNY) in Syracuse. After completing a fellowship in Pulmonary Disease at Johns Hopkins Hospital in Baltimore, he returned to SUNY where he was appointed Clinical Professor of Medicine in 1977. Sipple's observation of the association of pheochromocytoma with carcinoma of the thyroid gland is an early recognition of the dominant features of a specific genetic syndrome which eventually became Sipple's syndrome, or MEN2A.

Cushman subsequently recognized the association of pheochromocytoma, MTC, and parathyroid adenoma as a distinct clinical entity, in his 1962 report of a three-generation family with these tumors [66]. He noted that the specific type of thyroid carcinoma was medullary and correctly concluded that the syndrome was inherited in an autosomal dominant fashion. In 1965, Schimke and Hartmann described two families with inherited MTC, one with MTC only and one with MTC and pheochromocytoma, and identified amyloid-producing medullary carcinoma as the specific pathologic type of thyroid cancer with a strong familial association [67]. In that same year, Williams [68] reported the occurrence of thyroid carcinoma with pheochromocytoma in 17 patients, including 2 personal patients and 15 from the literature. In his report, Williams observed that MTC is the specific type of thyroid cancer associated with pheochromocytoma, the adrenal tumors may be bilateral, and that there is a frequent familial pattern of inheritance of these tumors. His report also recognized a few cases that involved

parathyroid tumors, raising the possibility of this pattern was related to a "multiple endocrine adenoma" syndrome. He emphasized, however, that this combination of endocrine tumors was distinct from the previously described syndrome of MEN1. A subset of Williams' cases also included multiple neuromas, and based on the neural crest origin of pheochromocytomas, he correctly surmised the possible neural origin of MTC. The complete description of the association and familial occurrence of MTC, pheochromocytoma, and parathyroid tumors as a distinct clinical entity was provided by Steiner et al. [69] in 1968, designating this constellation of findings MEN2. In this same year, a related syndrome including MTC, bilateral pheochromocytomas, and mucosal neuromas was described [70, 71], which was later recognized as MEN2B [72].

Genetic Basis for the Multiple Endocrine Neoplasia Type 2 Syndromes

A familial cancer trait is characterized by the occurrence of an unusual combination of otherwise rare endocrine tumors in a single patient, which is inherited from generation to generation. This pattern of clinical findings is ultimately the result of a disease-associated mutation in a specific human gene. In the 1980s, almost all of the human genetic sequence was uncharted territory and only a handful of human disease gene mutations had been identified. Prior to the human genome project, the only specific gene mutations that had been associated with human diseases had been identified either by first isolating the abnormal protein responsible, or in unusual circumstances where an obvious chromosomal abnormality gave a clue to the gene location (e.g., Duchenne muscular dystrophy and retinoblastoma). For researchers at the time, it was a formidable problem to search for and identify a disease mutation within the vast genome sequence, when the protein or gene location was not known. Genetic linkage analysis provided a way to follow the inheritance of markers within families and "map" a disease trait within the genome and to a specific chromosome region. The earliest human disease genes discovered were successfully identified through a laborious process termed positional cloning or "reverse genetics" [73], which involved searching for markers that are consistently inherited with the disease trait and using these signposts as a starting point to create a physical map of the disease gene region. "Chromosome walking" and "chromosome jumping" were strategies to cover associated areas [74, 75], but ultimately an exhaustive search of the nearby genes to uncover base-pair changes that only occurred in patients with disease was required. By 1990, only a few disease gene mutations had been identified, including genes for chronic granulomatous disease [76], Duchenne muscular dystrophy [77], cystic fibrosis [78, 79], retinoblastoma [80], and neurofibromatosis type 1 [81]. Identification of the specific gene mutations associated with familial adenomatous polyposis (FAP)/inherited colon cancer [82, 83], and inherited breast and ovarian cancer [84, 85] followed in the early 1990s.

The MEN2A disease gene was mapped by linkage analysis to the pericentromeric region of human chromosome 10 in 1987 [86, 87]. The three clinically distinct syndromes of MEN2A, MEN2B, and familial medullary thyroid carcinoma (FMTC) were shown to map to the same locus [88, 89]. Subsequent physical and genetic mapping studies focused the candidate interval to an approximately 500-kb region of chromosome 10q11.2 [90–92]. The MEN2 syndromes were ultimately shown to be associated with germline mutations in the *RET* proto-oncogene by two groups in 1993 [93, 94], thereby successfully identifying the disease gene. The rearranged during transfection (*RET*) proto-oncogene encodes a transmembrane receptor tyrosine kinase. *RET* mapped to the interval of interest on chromosome 10 and was known to be expressed in diverse tissue types including endocrine tissues, but allelic deletions or large chromosome aberrations (translocations, deletions, rearrangements) were not detected in tumor DNA from patients with *MEN 2*. The missense germline mutations associated with MEN2A were ulti-

mately identified by PCR amplification from *RET* genomic or cDNA and detection of sequence variants by single-strand conformational analysis or chemical cleavage mismatch procedure. The majority of these missense mutations occurred within one of several conserved cysteine residues in the extracellular domain immediately adjacent to the transmembrane portion. A single missense mutation in codon 918 within the intracellular catalytic domain of *RET* was shown to be associated with MEN2B [95, 96]. The occurrence of *RET* proto-oncogene mutations associated with the MEN2 syndromes was therefore within the earliest group of human disease genes identified by positional cloning.

Prophylactic Thyroidectomy for MEN2 Based on Genetic Testing

The ability to determine whether a patient has inherited a disease-associated germline *RET* mutation by direct DNA mutational testing provides the opportunity to intervene to completely remove the organ at risk for cancer development prior to its development. Early, prophylactic thyroidectomy can be performed based on genetic testing alone, before the development of biochemical or clinical evidence of MTC with the intent of eliminating the risk of subsequent neoplastic transformation and metastasis. Considering the three principle features of the MEN2 syndromes (MTC, pheochromocytoma, parathyroid tumors), MTC is the dominant clinical feature that is expected to develop in essentially 100 % of patients who inherit the mutation if followed for sufficient time. MTC is also the only consistently malignant component that accounts for almost all of the disease-related mortality. Because the end organ at risk for malignant transformation in MEN2, namely the thyroid gland, can be essentially completely extirpated at an early age by a meticulous and safe surgical procedure, this disease represents one of the first and perhaps still one of the best examples of a preventative surgical procedure performed for an inherited cancer susceptibility on the basis of a genetic test. Other notable examples include total proctocolectomy for FAP and bilateral prophylactic mastectomy/oophorectomy for patients with a *BRCA* mutation.

Skinner et al. [97] reported the long-term results of early prophylactic thyroidectomy in 50 children with a *RET* mutation identified through prospective genetic screening, with 100 % follow-up. A standard operation including total thyroidectomy, central cervical lymphadenectomy, and heterotopic parathyroid autotransplantation was performed in all patients by experienced pediatric and endocrine surgeons. All patients were evaluated patients for persistent or recurrent disease with basal and stimulated serum calcitonin levels. At 5 or more years postoperatively, 44 (88 %) of 50 patients had no evidence of disease based on this rigorous criteria. The rate of postoperative hypoparathyroidism was 6 %. Current guidelines include the performance of prophylactic thyroidectomy at approximately age 5 for patients with MEN2A and within the first years of life for patients with the more aggressive MEN2B syndrome (reviewed in [98]). For patients with mutations in one of the more infrequent, lower risk *RET* codons, thyroidectomy may be recommended at a later age.

Epidemiology

Thyroid cancer is the most common endocrine malignancy, representing roughly 96 % of endocrine cancer diagnoses and 3.8 % of all malignancies. There were approximately 62,450 new thyroid cancer cases and 1,950 deaths in 2015 [99–102]. Data from the Surveillance, Epidemiology, and End Results (SEER) program demonstrate that the overall incidence of thyroid cancer has been increasing, with the detection of new cases nearly tripling between 1975 and 2009, from 4.9 to 14.3 per 100,000 individuals [103, 104]. Using data from the National Vital Statistics System of the Centers for Disease Control and Prevention (CDC), it has been argued [104] that during the past nearly 35 years, the mortality rate from thyroid cancer has remained relatively stable (0.5 deaths per

100,000), leading to the conclusion that most of the increases in diagnoses represent small, subclinical papillary thyroid carcinomas with little clinical significance or impact on survival. However, others have argued that based on mortality data available from the SEER program, mortality from thyroid cancer actually increased approximately 1.2 % per year from 2001 to 2010 [99]. Notwithstanding a recent increase in thyroid cancer deaths, the incidence of follicular, medullary, and anaplastic thyroid carcinomas has remained stable during this time period [104, 105]. These less common thyroid cancer types are very infrequently detected as microcarcinomas. A major contribution to the observed marked increase in new thyroid cancer diagnoses over more than three past decades appears to be routine medical surveillance and improved detection of more subclinical tumors through widespread use of ultrasonography and fine needle aspiration of even small thyroid nodules [102, 106, 107]. However, there has also been an increase in the detection of large thyroid cancers (>5 cm) based on the SEER data from 1980 to 2005 [105]. Increased detection of small, clinically insignificant papillary cancers therefore likely does not explain all of the observed increase in thyroid cancer incidence. Stratification of the data by race [108] reveals that whites are experiencing the largest increase in overall age-adjusted thyroid cancer incidence (5.6 % per year), followed by blacks (4.8 % per year), American Indian/Alaskan natives (3.2 % per year), and Asians/Pacific Islanders (2.3 % per year). Non-Hispanics experienced a significantly greater increased incidence (5.5 % per year), compared with Hispanics (3.3 % per year).

Medullary thyroid cancer (MTC) comprises a small percentage of all thyroid cancer cases; the frequency in published reports ranges from 1 to 9 % over the last few decades [102, 107]. Despite the overall increase in thyroid cancer, the absolute incidence of MTC is likely relatively stable [107, 109]. A stable incidence of MTC with increased total thyroid malignancy (i.e., small papillary thyroid carcinomas) incidence may explain a relative trend toward a decreasing percentage of medullary cancers [106, 107, 109, 110].

Age

There are numerous factors that may contribute to the risk of developing MTC. These include age, gender, race, and genetic predisposition. In addition, the age at presentation, associated clinical characteristics, and timing of diagnosis and intervention influence prognosis and outcome in patients with MTC. The overall incidence of thyroid cancer (principally represented by differentiated papillary and follicular carcinomas) is known to have a midlife peak. MTC incidence, however, generally increases steadily with age and then tapers off somewhat in the extreme elderly. This age-related incidence pattern for MTC, stratified by gender, is illustrated in Fig. 1.1 (reproduced from [111]). Patients with hereditary MTC may be diagnosed in the first or second decade of life based on a program of biochemical screening in individuals at known risk, with an earlier distribution than for sporadic tumors. In a study by Kebebew [112], patients identified through genetic testing and/or prospective biochemical screening had a lower incidence of cervical lymph node metastases ($P < 0.05$) and were more likely to be cured at last follow-up when compared to patients with sporadic MTC not detected by screening (94.7 % vs. 49.4 %, $P < 0.0001$). Patients that underwent a total thyroidectomy and cervical lymphadenectomy were also more likely to be cured. In univariant analysis, age, gender, clinical presentation, TNM stage, sporadic/hereditary MTC, distant metastases, and extent of thyroidectomy were significant prognostic factors in patients with MTC. However, only age and stage remained independent prognostic factors in multivariant analysis.

Gender and Race

In keeping with other pathologic types of thyroid cancer, there is a gender predominance of females affected with MTC. However, this gender disparity is smaller in magnitude compared with differentiated thyroid cancers and continues to decrease with age [111]. There are modest

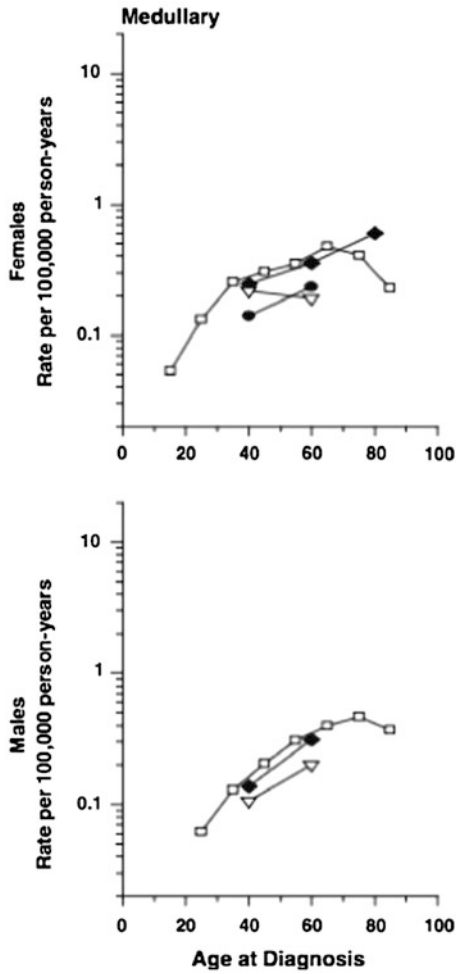

Fig. 1.1 Adapted and reproduced from Aschebrook-Kilfoy, 2011 [111]. Age-specific incidence of medullary thyroid cancer stratified by racial/ethnic group, SEER-13. Whites-*square* (□), Hispanics-*black diamond* (◆), Asians-*white down-pointing triangle* (▽), Blacks-*black circle* (●)

differences in the incidence of MTC according to race/ethnic background. In general, whites and Hispanics are more commonly affected than Asian/Pacific Islanders and blacks. Based on SEER data between 1992–2006, the overall incidence of thyroid cancer was 7.7 per 100,000 person-years. The highest incidence of MTC was in Hispanic and white women (0.21 and 0.22 per 100,000 woman-years). Among men, Hispanics had the highest rates (0.18 per 100,000 man-years) [99, 111] of MTC. Careful analysis of the SEER program data demonstrates uniformity of occurrence patterns between different geographic regions, arguing against socioeconomic disparities and access to health care as factors in the observed differences in incidence of MTC according to race and ethnicity [101, 111].

Sporadic Versus Hereditary MTC

MTC presents as both sporadic and hereditary disease. The majority (75 %) of cases are sporadic, while hereditary MTC occurs in approximately 25 % of diagnoses [111, 113]. Sporadic MTC is caused by somatic mutations in the *RET* proto-oncogene. Sporadic MTC commonly occurs in the fifth and sixth decades and demonstrates a slight gender preference toward female patients (1:1.13) [114–116].

Hereditary MTC is associated with the autosomal dominant inheritance of germline mutations in the *RET* proto-oncogene that are associated with *MEN 2* syndromes, with each child of an affected parent having 50 % risk of inheriting the gene mutation [109, 117–119]. Owing to the autosomal dominant inheritance pattern, males and females are affected equally with familial forms of MTC, in contrast to sporadic MTC. The inheritance of a disease-associated germline *RET* mutation confers essentially a 100 % risk of the subsequent development of thyroid C-cell malignancy if patients are followed long enough. Hereditary disease can be divided into clinically well-defined syndromes, reflecting the specific genotypic mutations responsible for the disorder. These clinical entities consist of *MEN 2A*, *MEN 2B*, and FMTC. The combined prevalence of the MEN2 syndromes is estimated to be between 1 in 30,000 and 35,000, with the majority of cases being represented by *MEN 2A*. In the USA, just under 500 cases of MEN2-related MTC are diagnosed annually [116].

MTC is the principle feature (greater than 95 % patients) of MEN2A and the consistently malignant component of the MEN2A syndrome. Hereditary MTC occurring in association with the MEN2A syndrome has an intermediate

Table 1.1 Classification and clinical features of medullary thyroid carcinoma

Variety of MTC and Incidence		Age at clinical diagnosis	Associated endocrinopathies
Sporadic—75 %		5th decade	None
Hereditary—25 %	MEN2A (~23 %)	3rd decade	Pheochromocytoma, parathyroid adenoma, cutaneous lichen amyloidosis
	MEN2B (~2 %)	1st decade	Pheochromocytoma, mucosal neuromas
	FMTC (rare)	Varied	None

Adapted from Friedhelm Raue and Karin Frank-Raue. Epidemiology and clinical presentation of medullary thyroid carcinoma [102]

aggressiveness, similar to that seen with sporadic MTC. The MTC that occurs in association with the rarer MEN2B syndrome (MTC, pheochromocytomas, marfanoid habitus, and multiple neuromas) is associated with a more aggressive biologic behavior conferred by the specific codon 918 *RET* mutation associated with this syndrome. Patients with MEN2B present with invasive MTC an early age, and late recognition of the syndrome and diagnosis of MTC results in a high frequency of metastatic disease in many patients.

FMTC is characterized by the autosomal dominant inheritance of MTC, without associated pheochromocytomas or parathyroid tumors [120]. The clinical characteristics of FMTC include the absence of pheochromocytoma or primary hyperparathyroidism and typically a later age of onset (after age 50 years) of MTC in multiple affected family members.

In summary, review of the available data on the epidemiology of MTC reveals that this distinct pathologic subtype represents a small percentage of thyroid cancer diagnoses. Overall, its incidence increases with age, and there is a slight female predominance. A summary of the clinical presentation of MTC is depicted in Table 1.1. The level of MTC cancer risk, age-of-onset, associated clinical manifestations, and recommendations for the extent of thyroid surgery and lymphadenectomy vary according to the specific *RET* mutation responsible for the MTC in a given patient [98]. The relationship of the underlying germline *RET* mutation to the aggressiveness of the MTC and the frequency of associated endocrinopathies is depicted in Table 1.2, as summarized in the revised American Thyroid Association Guidelines for the Management of Medullary Thyroid Carcinoma, 2015 [107]. It is estimated that up to 15 % of MTC is diagnosed post-resection on final surgical pathology, which may result in missed detection of pheochromocytoma and hyperparathyroidism and recognition of the familial inheritance risk, as well as an inadequate oncologic resection in some patients. These findings highlight the importance of heightened awareness for the early diagnosis and appropriate management of MTC [110].

Table 1.2 Relationship of common *RET* mutations to the risk of aggressive MTC in *MEN 2A* and *MEN 2B* and to the incidence of PHEO, HPTH, CLA, and HD in MEN2A (Reproduced from the Revised American Thyroid Associated Guidelines for the Management of Medullary Thyroid Carcinoma, 2015) [107]

RET mutation[a]	Exon	MTC risk levef[b]	Incidence of PHEO[c]	Incidence of HPTH[c]	CLA[d]	HD[d]
G533C	8	MOD	+	−	N	N
C609F/G/R/S/Y	10	MOD	+/++	+	N	Y
C611F/G/S/Y/W	10	MOD	+/++	+	N	Y
C618F/R/S	10	MOD	+/++	+	N	Y
C620F/R/S	10	MOD	+/++	+	N	Y
C630R/Y	11	MOD	+/++	+	N	N
D631Y	11	MOD	+++	−	N	N
C634F/G/R/S/W/Y	11	H	+++	++	Y	N
K666E	11	MOD	+	−	N	N
E768D	13	MOD	−	−	N	N
L790F	13	MOD	+	−	N	N
V804L	14	MOD	+	+	N	N
V804M	14	MOD	+	+	Y	N
A883F	15	H	+++	−	N	N
S891A	15	MOD	+	+	N	N
R912P	16	MOD	−	−	N	N
M918T	16	HST	+++	−	N	N

[a]The references for each of *RET* mutations can be found in the supplementary information, where all reported *RET* mutations in MTC are listed
[b]Risk of aggressive MTC: *MOD* moderate; *H* high; *HST* highest
[c]Incidence of PHEO and HPTH: + = ∼10 %; ++ = ∼20–30 %; +++ = ∼50 %
[d]*Y* positive occurrence; *N* negative occurrence

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Initial Evaluation and Diagnosis of Medullary Thyroid Carcinoma

2

Abbey Fingeret and Carrie C. Lubitz

Medullary Thyroid Carcinoma (MTC) is a neuroendocrine tumor arising from the neural crest parafollicular, or C-cells, of the thyroid. MTC is much less common than differentiated thyroid cancer (DTC), representing less than 5 % of thyroid malignancies diagnosed and has not had the same increase in incidence noted for DTC over the past decade. The prevalence of occult MTC, identified by autopsy series and incidental findings on pathological examination after thyroidectomy for other indications, ranges from 0.13 to 0.69 % [1–7]. While the incidence of MTC is comparatively lower than DTC, disease-specific survival is significantly worse; 5-year survival for MTC is approximately 50 %, compared to approximately 98 % for DTC [8]. Early diagnosis of MTC is paramount in optimizing outcomes for patients, underscored by the significant decline in survival with regional and metastatic disease. Furthermore, preoperative knowledge of MTC has important implications for extent of surgery. While the extent of prophylactic cervical lymphadenectomy in the lateral compartment for localized MTC is a matter of debate, there is a consensus that a complete level VI dissection should be performed in all patients with MTC [9].

MTC can be inherited or sporadic. Three-quarters of patients have sporadic disease, and most often present in the 5th–6th decade of life. Screening for thyroid cancer is not practiced in patients without a family history and, therefore, most cases of sporadic disease are identified during the evaluation for a palpable thyroid nodule or nodule found incidentally on imaging for other indications. Sporadic MTC is thought to be more aggressive with frequent central (level VI), ipsilateral lateral (levels II–V), and contralateral lateral (40 %) cervical lymph node metastases at presentation. Somatic mutations of the REarranged-during-Transfection (*RET*) proto-oncogene are seen within the C-cells of sporadic tumors in approximately 50 % of cases, the majority of which (79 %) are a point mutation in codon 918 of exon 16. This mutation is associated with larger size of tumor at presentation, increased incidence of nodal disease, and decreased survival [9–11]. When compared to patients with RET^{-} tumors, sporadic RET^{+} patients had higher incidence of nodal disease, distant metastases, and recurrences [10]. Between 18 and 50 % of RET^{-} tumors have been found to have *RAS* mutations [12, 13].

The remainder of MTC patients have hereditary disease, with nearly all patients carrying one of a number of gain of function germline

A. Fingeret (✉)
Division of General and Gastrointestinal Surgery, Massachusetts General Hospital, 15 Parkman Street, Wang ACC 460, Boston, MA 02114, USA
e-mail: afingeret@mgh.harvard.edu

C.C. Lubitz (✉)
Harvard Medical School, Massachusetts General Hospital, 55 Fruit Street, Yawkey 7B, Boston, MA 02114, USA
e-mail: clubitz@partners.org

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mutations in the *RET* gene. In recent years, a growing body of evidence supports a strong genotype–phenotype relationship for age of presentation, penetrance of disease, and associated features [14]. Knowledge of specific mutation can help guide treatment and surveillance. This is a rare disease with incidence of approximately 1 out of 30,000 individuals. These autosomal dominant mutations lead to the multiple endocrine neoplasia (MEN) syndromes—MEN2A (Sipple's syndrome), MEN2B, and familial MTC (FMTC). MEN2A is four times more common than MEN2B, with 85 % possessing a single-point mutation in codon 634 cysteine for arginine [15]. When patients present with a known family history of MTC, it is crucial that patients be screened for multicentric MTC (90 %), pheochromocytoma (50 %), parathyroid hyperplasia (15 %), and be tested for a germline *RET* mutation. Some consider sending a *RET* mutational analysis in any case of MTC, particularly at a young age, or C-cell hyperplasia. FMTC is a variant of MEN2A without associated pheochromocytoma or parathyroid disease. FMTC families have a lower penetrance of MTC and typically present at an older age than MEN2A families [16]. Hirschsprung's disease and cutaneous lichen amyloidosis of the upper back are associated with germline *RET* mutations, and presentation should prompt mutational analysis.

On average, patients with MEN2A develop MTC at 3–5 years of age, although the age at presentation is correlated with the specific *RET* mutation. Given that nearly all patients are noted to have C-cell hyperplasia on pathology, recommendations for prophylactic thyroidectomy are dependent upon risk stratification. Recently, the revised MTC guidelines from the American Thyroid Association (ATA) categorized the risk of MTC aggressiveness as moderate (MOD), high (H), or highest (HST) based on the specific codon mutation [17]. Children with a M918T mutation are in the highest risk category and should undergo thyroidectomy within the first year of life. Children with MEN2A and *RET* codon 634 mutations are at high risk for developing MTC during the first years of life and are recommended to begin annual screening with ultrasound and serum calcitonin levels beginning at age 3 years, with prophylactic thyroidectomy at or before age 5 years, based on the results of the screening studies. Children in the moderate risk category should undergo screening beginning at age of 5 years with thyroidectomy when the serum calcitonin level becomes elevated [17].

MTC associated with MEN2B is less common but more aggressive; however, this is thought to be secondary to the more advanced stage at presentation than aggressiveness of the MTC per se. There is 100 % penetration of MTC in MEN2B families, and metastases within the first year of life have been described [18]. While nearly all cases of MEN2A patients have a positive family history of the disease, over 50 % of MEN2B patients are de novo mutations [19]. This makes diagnosis at an early stage particularly challenging. Like MEN2A, the incidence of pheochromocytoma is 50 % in affected families. MEN2B patients carry a singular phenotype with mucosal ganglioneuromas of lips, tongue, eyelids, and a marfanoid body habitus. Because of the early onset of disease, it is paramount that family members of affected individuals are screened as soon as the diagnosis is made in the proband. Commonly, without screening, patients present with multifocal, bilateral MTC.

Role of Routine Serum Calcitonin Levels in Patients with Thyroid Nodules

In both inherited and sporadic MTC, disease recurrence and survival is highly correlated with the extent of disease at presentation. This is underscored by the significant decrease in 10-year survival associated with Stage III disease [20]. Unfortunately, half of patients have regional nodal metastases at the time of presentation. Moreover, lower preoperative serum calcitonin levels are correlated with the absence of lymph node metastases and with normalization of postoperative calcitonin levels [21]. Given that

early diagnosis and surgery gives patients the best chance of cure, screening for MTC with routine serum calcitonin levels has been debated.

MTC can be diagnosed as a result of screening (i.e., inherited syndromes in patients with family history) or during the workup of a thyroid nodule. There is a clear association with early detection of MTC and outcomes—smaller tumors are correlated with a decreased incidence of metastases, and the majority of patients presenting with a palpable nodule already have nodal metastases [22]. There is no current consensus on the use of screening serum calcitonin levels in patients undergoing initial evaluation of a thyroid nodule, given the low prevalence of disease (MTC). The ATA guidelines on the management of thyroid nodules do not recommend for or against the routine use; however, the consensus statement by the European Thyroid Cancer Task Force recommends calcitonin screening in all patients undergoing evaluation of a thyroid nodule [9, 23]. Arguments for the use (primarily in Europe) include improved accuracy of serum calcitonin levels in the diagnosis of MTC, compared to cytological results from fine needle aspiration biopsy (FNA) and improved preoperative planning, dependent on the extent of elevation of serum calcitonin levels. Elisei et al. [24] showed that routine serum calcitonin screening, which identified 44 cases of MTC in a group of 10,000 patients with thyroid nodules, resulted in a cure rate of 59 % in the calcitonin-screened group, as compared to a cure rate of 2.7 % in historical controls; those in the calcitonin-screened group also had improved 10-year survival rates. Moreover, they and others have found that serum calcitonin was more accurate than FNA in diagnosing MTC [25]. Hahm et al. [2] found that 56 of 1448 total nodular thyroid disease patients had a basal serum calcitonin >10 pg/mL, 10 of whom went on to have pathologically confirmed MTC (46 did not). While thought to be more sensitive than FNA, specificity is of concern given the potential for unnecessary surgery [7, 26]. This has led to other groups to use a higher positivity criterion for basal serum calcitonin levels to be considered diagnostic for MTC. In a study from Denmark that used a threshold of 100 pg/mL in 959 patients with nodular thyroid disease, the positive predictive value (PPV) was only 15.4 %; using the same cutoff, Costante et al. reported a PPV of 100 % [5, 26]. Other large series using a basal serum calcitonin threshold of 10 pg/mL had sensitivities of 100 %, specificities >96 %, but PPV of 6–39 % [3, 27–29]. This led to the ATA's recommendation for a threshold of basal or stimulated serum calcitonin of >100 pg/mL "if obtained" to be considered diagnostic for MTC [17].

Those arguing against routine serum calcitonin screening in all patients with thyroid nodules cite the low prevalence of disease (<1 % of thyroid nodules), risk of unnecessary surgery, and increased costs [24]. A cost-effectiveness study assessing the addition of serum calcitonin in all patients with thyroid nodules in the USA to the 2009 ATA recommendations for evaluation of patients with thyroid nodules found it cost-effective at accepted willingness to pay thresholds of $12,000 per life-year saved, at a cost increase of 5.3 %, comparable to the cost-efficacy of colonoscopy or mammography screening [30]. Critics of this analysis cite the inclusion of patients with C-cell hyperplasia and medullary microcarcinoma in the prevalence estimates of MTC that has uncertain clinical significance [23]. An additional argument in favor of routine serum calcitonin screening is the use to monitor for recurrence, with some recommending follow-up starting three months postoperatively then annually if undetectable. A lower preoperative serum calcitonin of 10–49 pg/mL was predictive of postoperative normalization for 44/45 patients in a French cohort of 226 with MTC [31].

Challenges regarding the implementation of routine serum calcitonin screening for patients with thyroid nodules in the USA include establishment of uniform threshold values, gender-specific thresholds, and applicability of stimulatory testing. Current European consensus guidelines recommend routine measurement of serum calcitonin in the initial diagnostic evaluation of all patients with thyroid nodules with concurrent evaluation for comorbid conditions which may cause false positive elevation of serum calcitonin levels: renal failure, ectopic calcitonin production from non-thyroidal neuroendocrine tumors, hypergastrinemia, Hashimoto's thyroiditis,

and heterophilic antibodies; however, no threshold value for screening is suggested [9]. In addition to these benign conditions that may falsely elevate serum calcitonin levels, a patient's sex must be taken into consideration when determining normative values, with males having higher baseline serum calcitonin levels [5, 32]. Studies to determine ideal serum calcitonin thresholds by patient sex have demonstrated thresholds of 14–26 pg/mL for females and 32–68 pg/mL for males [6, 33, 34].

Higher threshold for screening serum calcitonin will yield a higher PPV of the test, as shown by Costante et al.; increasing the threshold from >20 pg/mL to >100 pg/mL improved PPV from 23.1 to 100 % [5]. Similarly in a sample of >20,000 patients, Rink et al. demonstrated that increasing the upper limit from 10 to 15 pg/mL provided 100 % sensitivity while decreasing false positive cases [34]. As expected, increasing the threshold value improved the PPV and specificity of the test, but also impacted the negative predictive value; using a cutoff of 30 pg/mL in 7276 patients, Iacobone et al. [4] demonstrated 100 % PPV but a negative predictive value of only 63 %.

Calcitonin stimulation with a secretagogue, such as pentagastrin, is not currently approved in the USA. However, pentagastrin stimulation is still used in other countries, particularly in Europe, to evaluate mild elevations in baseline serum calcitonin levels and to differentiate possible MTC from other etiologies of an elevated serum calcitonin level, such as other neuroendocrine tumors, small cell lung cancer, and chronic renal failure. Use of pentagastrin stimulation for patients with baseline serum calcitonin levels of 10–100 pg/mL improved the PPV to 25–40 % [2–5, 29, 35, 36]. Testing involves an overnight fast, injection of pentagastrin, and serial blood draws over 10 min. This test is not currently used in the USA, secondary to a poor side effect profile and the lack of additional diagnostic information for the management of patients with elevated basal calcitonin levels. Furthermore, improved basal calcitonin assays have greatly improved sensitivity of the assay.

When utilizing serum calcitonin levels for initial diagnosis of and screening for MTC, clinicians must be aware of the potential for false positive and false negative results. The hook effect may cause false depression of serum calcitonin levels. This occurs when a high antigen concentration binds with signal antibodies and is discarded with the liquid phase of the testing process [7]. Heterophilic antibodies may also falsely lower serum calcitonin levels. Additionally, some patients with MTC may be nonsecretors of calcitonin. In a cohort of 839 patients with sporadic MTC patients from 2 tertiary referral centers, 7 (0.83 %) patients had normal serum calcitonin and carcinoembryonic antigen (CEA) levels despite advanced tumor stage [37]. Conditions that may lead to elevation of serum calcitonin levels include, as previously discussed, renal failure, ectopic production from neuroendocrine tumors, hypergastrinemia, thyroiditis, or use of certain medications such as omeprazole [38]. In a cohort of 1,425 patients with nodular thyroid disease, the baseline serum calcitonin level was elevated in 23 (1.6 %) patients, including 9 (0.63 %) patients with MTC [27]. In patients with renal failure, despite clearance of calcitonin by dialysis, serum calcitonin levels may be elevated both pre- and post-dialysis [39].

Other polypeptide hormones have been suggested as alternatives to serum calcitonin for MTC screening, including vasoactive intestinal peptide, serotonin, somatostatin, CEA, and procalcitonin. Elevations in serum CEA in patients with MTC have been associated with a poorer prognosis, especially in those with a decreased or stable serum calcitonin level, suggesting tumor dedifferentiation. CEA has also been correlated with positive margins [21]. CEA should be interpreted with caution though as it may be elevated from other malignancies of gastrointestinal, lung, prostatic, breast, or ovarian origin as well as benign conditions of the gastrointestinal and respiratory systems. Procalcitonin, a 116 amino acid peptide produced in parafollicular C-cells, shows promise as a biomarker for screening due to its stability at room temperature and concentration-independent half-life

of 20–24 h. Though currently used as a clinical marker for sepsis, procalcitonin has reliable commercial assays yielding similar results [40]. Several studies have demonstrated concordance and similar diagnostic accuracy for MTC between calcitonin and procalcitonin [41–43].

Evaluation of a Thyroid Nodule

The workup of a palpable or incidentally discovered thyroid nodule should proceed with the measurement of serum thyroid stimulating hormone (TSH) and diagnostic imaging with ultrasonography of the thyroid gland and cervical lymph nodes [14]. Ultrasound provides confirmation of physical examination findings or alternate imaging modality including the size, location, and features of a nodule, the presence of additional nodules or lymphadenopathy. Ultrasonographic findings in MTC are variable, with 66–72 % demonstrating characteristic suspicious features including height greater than width, speculation, hypoechogenicity, calcifications, extrathyroidal extension, lymphadenopathy, or extranodal extension [44–46]. While not universally present, suspicious ultrasound features confer a 450 % increased risk of advanced stage MTC, especially metastatic lymphadenopathy or extrathyroidal extension [46]. Use of quantitative elastography for MTC has also been evaluated with equivocal results; in a small sample of 18 patients with MTC, 55.6 % had low-to-intermediate elastography scores of one or two [47]. Once a diagnosis of MTC has been established cytopathologically following neck ultrasound, no further diagnostic imaging is usually necessary prior to operative intervention for locoregional disease, although this is dependent on the extent of elevation of serum calcitonin levels. Fluorodeoxyglucose positron emission tomography is not recommended for the routine preoperative evaluation of patients with MTC but may be used to assess for recurrent disease [48]. If metastatic MTC is suspected preoperatively by patient signs or symptoms of distant metastases, the presence of extensive neck disease, or calcitonin greater than 500 pg/mL further diagnostic imaging, is indicated. Computed tomography (CT) should be used to detect lung and mediastinal lymph node metastases, contrast enhanced CT or magnetic resonance imaging (MRI) to evaluate for liver metastases, as well as MRI and bone scintigraphy for bone metastases [49].

The diagnostic evaluation of a patient with a thyroid nodule on ultrasonography should proceed with FNA, depending on patient's risk factor profile, biochemical results, and sonographic features [23]. The detection rate of MTC by FNA is lower than that for DTC, varying from 12 to 88 % with 59 to 86 % sensitivity compared to serum calcitonin measurement [2, 27, 50–52]. This comparatively low detection rate and sensitivity is due to the variable appearance of MTC on aspiration cytology which may show spindle-shaped, plasmacytoid or epithelioid cells. Amyloid—characteristically associated with MTC—can also be found in follicular lesions and systemic amyloidosis. MTC may be misdiagnosed as a follicular neoplasm or desmoid tumor. In a study of 91 patients with proven MTC, the best distinguishing cytological features were dispersed triangular cells with coarse granular chromatin and cytoplasmic granularity [53]. To verify the diagnosis, immunohistochemistry for the presence of calcitonin, chromogranin, CEA, and the absence of thyroglobulin may be performed on aspirates [50, 53, 54]. Results of studies examining calcitonin measurement in aspiration needle washout are heterogeneous but despite small sample sizes demonstrate excellent sensitivity and specificity for diagnosis of MTC [54, 55]. Calcitonin measurement of FNA aspirate as an adjunct to serum calcitonin measurement may differentiate patients with MTC from those with false positive elevations of serum calcitonin as FNA calcitonin values in MTC are greater than seventy times higher than serum [56]. The use of calcitonin measurement of fine needle aspirate may improve diagnostic accuracy for MTC especially in cases of borderline serum calcitonin elevation.

Because 1–7 % of patients with presumed sporadic MTC actually have hereditary disease, all patients with pathologically confirmed MTC should be referred for genetic counseling and DNA analysis for a *RET* mutation [57, 58]. Clinicians must be aware of the duty to warn

potentially affected family members if a mutation is detected and should discuss disclosure with the patient prior to initiating testing. First-degree relatives of patients found to have a *RET* germ line mutation should also be offered genetic counseling and testing. If hereditary MTC is found, a clinical evaluation for pheochromocytoma and hyperparathyroidism is indicated with surgical management of pheochromocytoma taking precedent over thyroidectomy if identified.

Conclusion

The initial evaluation and diagnosis of Medullary Thyroid Carcinoma begins with the workup of a palpable or incidentally detected thyroid nodule by history and physical examination, measurement of thyroid-stimulating hormone, and neck ultrasound including the thyroid and cervical lymph nodes, and fine needle aspiration. There is no consensus regarding the routine use of serum calcitonin screening in patients with thyroid nodules in the USA, however if screened, patients with levels >100 pg/mL should be considered for total thyroidectomy with central lymphadenectomy. For patients with serum calcitonin, 10–100 pg/mL stimulatory testing or calcitonin assay of fine needle aspirate may improve specificity and positive predictive value for the diagnosis of MTC. No further radiographic evaluation is indicated unless distant metastatic disease is suspected by extensive locoregional disease, serum calcitonin >500 pg/mL, or signs or symptoms indicative of metastases. All patients with pathologically confirmed MTC should be referred for genetic counseling and RET mutation analysis. If a RET mutation is identified, patients should be evaluated for pheochromocytoma and hyperparathyroidism prior to thyroidectomy.

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3 The Physiology of Serum Calcitonin and Carcinoembryonic Antigen in Medullary Thyroid Cancer

Naira Baregamian and Carmen C. Solórzano

Medullary thyroid carcinoma (MTC) is a malignant tumor of the parafollicular C-cells of the thyroid gland. The parafollicular C-cells are derived from the neural crest and predominantly secrete calcitonin, as well as several other hormones and amines, including carcinoembryonic antigen (CEA), adrenocorticotropic hormone (ACTH), β-melanocyte stimulating hormone, chromogranin, histaminase, neurotensin, and somatostatin [1–6]. Calcitonin is expressed by almost all MTCs; hence, it represents both a valuable tumor-specific marker and a potential therapeutic target [7]. Both serum calcitonin and CEA are considered important tumor markers in patients with MTC, as their serum concentrations directly correlate to the C-cell mass. Below we will discuss in detail the origin of these markers, expected and altered serum levels, reference ranges, doubling times and the utility of these biomarkers in the perioperative and long-term management of adult MTC.

N. Baregamian · C.C. Solórzano (✉)
Division of Surgical Oncology and Endocrine Surgery, Department of Surgery, Vanderbilt University Medical Center, 2220 Pierce Ave, 597 Preston Research Building, Nashville, TN 37232, USA
e-mail: carmen.solorzano@vanderbilt.edu

N. Baregamian
e-mail: naira.baregamian@vanderbilt.edu

Calcitonin

Calcitonin is a linear 32-amino acid monomeric polypeptide that is involved in calcium homeostasis and is primary metabolized by the kidney via proteolysis. Calcitonin protein synthesis begins with the proteolytic cleavage of the 141 amino acid precursor protein preprocalcitonin to the calcitonin precursor procalcitonin. Procalcitonin is composed of 116 amino acids and is also produced by the neuroendocrine cells of the lung and intestine. Calcitonin is a product of the CALC1 gene located on the short arm of the chromosome 11p15.2, which encodes for both calcitonin and calcitonin gene-related peptide (CGRP). The CALC1 gene belongs to a superfamily of related protein hormone precursors, including islet amyloid precursor protein (IAPP, or amylin) and preproadrenomedullin. The products of these precursors (amyloid beta, adrenomedullin, and CGRP) play a critical role in various disease processes involving amyloid formation in diabetes, plaque formation in Alzheimer's disease, and potent vasodilator effects in pheochromocytoma (adrenomedullin) and migraine (CGRP).

Calcitonin is present in large amounts only in the C-cells of the thyroid gland. Enteric and pulmonary neuroendocrine tumors, pheochromocytoma, paraganglioma, small cell and large cell lung cancers, and prostate cancer have all been shown to secrete calcitonin ectopically. Importantly, in the majority of paraneoplastic

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syndromes resulting from neuroendocrine tumors, the basal elevation of serum calcitonin >50 pg/ml and/or the five- to ten-fold calcitonin elevations following pentagastrin or calcium stimulation, which are commonly seen in MTC, are not typically observed [8–12].

Calcitonin secretion from C-cells is strongly stimulated by an increase in serum-ionized calcium concentration ([$Ca2^+$]), gastrin and pentagastrin, β-adrenergics, glucagon, glucocorticoids, CGRP, and alcohol. Calcitonin acts to reduce serum calcium levels, opposing the effects of parathyroid hormone (PTH) [13, 14]. Provocative testing is the clinical administration of potent secretagogues, such as intravenous calcium or pentagastrin, and is thought to increase the sensitivity of serum calcitonin testing. The utility of provocative testing is limited to a specific subset of patients such as the pediatric population with inherited mutated RET allele, in the evaluation of patients for persistent or recurrent MTC following thyroidectomy, and for the patients with nodular goiter being screened for MTC [15, 16]. With the recent emergence of more sensitive immunochemilluminometric assays (ICMA) for the measurement of basal serum calcitonin levels, there has been a sharp decline in utilization of overall provocative testing by many clinicians. When provocative testing is used, the interpretation of stimulated calcitonin levels remains difficult due to the lack of established, clear reference guidelines for abnormal stimulated calcitonin levels.

Carcinoembryonic Antigen

CEA is a glycoprotein produced by the gastrointestinal tract cells predominantly during embryonic development. Serum levels of CEA are relatively low after birth. Increased serum concentrations of CEA are observed in MTC and gastrointestinal malignancies. In contrast to serum calcitonin, CEA is not thought to be a specific biomarker for MTC and is not useful in early diagnosis of MTC [17]. Measurement of serum CEA is less sensitive than serum calcitonin for detecting the presence of tumor; however, when compared to serum calcitonin, CEA has less minute-to-minute variability and serves as a useful indicator of tumor mass. CEA is considered useful for evaluating disease progression in patients with clinically evident MTC and for postsurgical monitoring of recurrent disease [18].

Basal levels of serum calcitonin and CEA are typically measured together and marked elevation in serum CEA levels out of proportion to lower serum calcitonin levels indicates an advanced MTC. The most aggressive MTC tumors exhibit intense CEA staining, suggesting that CEA is a marker for early epithelial differentiation and therefore retained, whereas calcitonin is thought to be a late marker for terminal differentiation and is consequently lost. Therefore, measurement of serum CEA complements basal serum calcitonin levels in the biochemical workup and follow-up of MTC.

Interpretation of Calcitonin and Carcinoembryonic Antigen Serum Levels

False elevations or low serum calcitonin and CEA levels can occur physiologically or with a variety of clinical diseases, and clinicians should be aware of these scenarios. Normal physiologic variations in serum levels are associated with the age and sex of the patient. Markedly elevated physiologic serum calcitonin levels are observed in infants under six months of age (as high as 75 pg/mL), and such levels approach normal adult range by three years of age. The reference range for serum calcitonin level is higher in males when compared to females due to a presumed larger C-cell mass in the thyroid gland [19, 20]. Current American Thyroid Association (ATA) revised guidelines for MTC do not specify reference ranges for basal or stimulated serum calcitonin levels, allowing for individual laboratories to set their own reference ranges

[17]; however, it is accepted that the upper limit of normal for basal or stimulated serum calcitonin should be 10 pg/mL. Because there may be variability in calcitonin measurements among commercial assays, it is recommended that serum calcitonin levels be evaluated with the same assays before and after thyroidectomy and during follow-up.

Elevated serum calcitonin levels can also be observed in patients with many benign or malignant conditions [21]. Serum calcitonin levels can be elevated in patients with enteric and pulmonary neuroendocrine tumors, small cell and large cell lung cancers, and prostate cancer [9–12, 22–25], as these tumors can secrete calcitonin. Similarly, elevated serum CEA levels are observed in patients with gastrointestinal tract inflammatory disease, benign lung disease, various gastrointestinal malignancies, and in patients who smoke tobacco. Interestingly, in patients with sepsis and other general inflammatory conditions, tissue elevated levels of procalcitonin are present, but they do not affect calcitonin gene transcription and serum calcitonin levels [26, 27]. However, a high serum procalcitonin to calcitonin ratio in patients with MTC has been shown to correlate with an increased risk of progressive disease and shortened progression-free survival [28, 29].

False elevations of serum calcitonin and CEA levels may be observed with the use of heterophilic human antibodies which can display broad reactivity with antibodies of other animal species [21]. Falsely low levels of serum calcitonin have been previously detected by the older generation immunoradiometric assays (IRA) resulting from very high serum levels of calcitonin, and this phenomenon has been described as the "hook effect." In patients with widely disseminated MTC, the binding capacity of the antibody becomes saturated, hence, leading to falsely lower serum levels of calcitonin [30]. With the use of newer ICMAs, the likelihood of the "hook effect" has been significantly reduced. However, clinicians should remain vigilant when a patient with large MTC tumor burden shows a surprisingly low serum calcitonin level [17].

Calcitonin and CEA Preoperative Levels to Help in Operative Planning and as Predictors of MTC Disease Extent

Initial surgical management of patients with MTC is planned using an array of diagnostic modalities. These include fine-needle aspiration biopsy (FNA) of the suspicious thyroid nodule, serum calcitonin and CEA levels, ultrasound (US) assessment of the central and lateral neck lymph node compartments, and genetic analysis for a *RET* germline mutation. Due to the wide spectrum of cytomorphologic variability and growth patterns of MTC, initial FNA assessment may present a diagnostic challenge. When the cytopathologic features yield inconclusive results, the specimen should be evaluated by immunohistochemical staining (IHC) for calcitonin, chromogranin, CEA, thyroglobulin, and when possible, by measuring calcitonin levels in the FNA washout fluid. Presence of amyloid is a variable cytomorphologic feature and can be accounted in nearly half of MTC cases [31]. When a *RET* germline mutation is detected, additional testing for pheochromocytoma and primary hyperparathyroidism (pHPT) must be undertaken to rule out multiple endocrine neoplastic (MEN) syndrome. Pheochromocytoma should be resected prior to MTC treatment, whereas pHPT (when present) can be treated concurrently with the thyroidectomy for MTC.

According to the most recent ATA guidelines for the management of the MTC, a serum calcitonin level >500 pg/mL warrants additional imaging procedures to evaluate for metastatic disease [17, 32]. Similarly, the National Comprehensive Cancer Network (NCCN) guidelines identify a serum calcitonin level ≥400 pg/mL and/or the presence of node-positive (N1) disease as triggers to obtain additional imaging. The recommended imaging studies include computed tomography (CT) of the chest and triple-phase contrast-enhanced multi-detector liver CT and/or contrast-enhanced magnetic resonance imaging (MRI). These imaging studies are the most sensitive modalities for

detecting lung and mediastinal lymph node and liver metastasis, respectively. Axial MRI and bone scintigraphy are the most sensitive imaging modalities used to detect bone metastasis. Regardless of the serum calcitonin level, patients who have extensive neck disease and/or signs and symptoms of distant metastasis should undergo preoperative imaging to look for systemic disease. With preoperative serum calcitonin levels <400–500 pg/mL, total thyroidectomy with central compartment neck dissection should be planned at minimum, with appropriate extent of lateral compartment lymph node dissection depending on preoperative US and intraoperative findings.

The preoperative basal serum calcitonin level can be useful in gauging the extent of lymph node involvement. One can expect to find a correlative relationship between basal serum calcitonin levels and the degree of the lymph node compartment involvement. As basal serum calcitonin levels approach 500 pg/mL, the likelihood of ipsilateral central, ipsilateral lateral, contralateral central or even contralateral lateral compartment lymph node involvement will increase [32]. A central compartment neck dissection is almost always performed in patients with MTC. Surgical treatment option(s) for the lateral neck compartments in patients with basal serum calcitonin levels of up to 1000 pg/mL or bulky central neck disease without obvious involvement of the lateral neck compartments by US remain controversial. Some clinicians advocate an ipsilateral, or in some cases, a bilateral prophylactic/elective lateral neck dissection in order to maximize the likelihood of biochemical cure [32, 33]. Because extensive lateral neck dissections can be associated with unwanted morbidity, and prophylactic dissection of lateral neck compartments may not improve biochemical cure or survival, many clinicians will only dissect the lateral compartments when palpable or US-detectable lymph node disease is present. The benefit of more extensive lymph node surgery in patients with serum calcitonin levels >10,000 pg/mL appears to be lost [32].

The preoperative serum CEA levels can also be helpful in determining the extent of lymph node involvement and tumor burden and for preoperative planning. Successive increases in serum CEA levels (>4.7, 4.7–10, 10.1–30, 30.1–100, and >100 ng/mL) are associated with the involvement of lymph node compartments from ipsilateral central and lateral neck to contralateral compartments [34]. This relationship mirrors similar findings of preoperative basal calcitonin levels predicting the extent of neck lymph node compartments. Therefore, both calcitonin and CEA are valuable predictors for disease extension preoperatively and play an important role in operative planning for patients with MTC.

Calcitonin and CEA Levels After Intended Curative Resection of MTC

Postoperative undetectable basal or stimulated serum calcitonin levels (or levels just below 10 pg/mL) indicate curative resection [35]. Others have proposed stimulated serum calcitonin levels <10 pg/mL after thyroidectomy as curative. Complete removal of the thyroid gland and involved lymph nodes should demonstrate undetectable serum calcitonin levels, unless residual thyroid tissue remains, or when ectopic secretion of calcitonin from a non-thyroid malignancy is detected. There is much controversy about the acceptable time frame required for normalization of serum calcitonin levels postoperatively, and several investigators have proposed that the optimal time to determine the nadir of serum calcitonin is within three months [36, 37]. Normalization of serum calcitonin levels is central to attaining biochemical cure postoperatively, and it is thought to be associated with better outcomes, whereas progressive postoperative increases in serum calcitonin levels indicate tumor recurrence [38]. Due to prolonged half-life, serum CEA levels take even longer to reach nadir; hence, postoperative CEA levels are considered less useful in the initial assessment of curative thyroidectomy.

Revised ATA guidelines for management of MTC have put forth recommendations to measure serum calcitonin and CEA levels three months postoperatively. If these levels are

undetectable or found to be within normal range, then they should be repeated in 6–12 months, and yearly thereafter [17]. These recommendations are also echoed in the NCCN guidelines for postoperative MTC management. Therefore, postoperative serum calcitonin and CEA levels are central in planning long-term follow-up of patients with MTC and in predicting outcome.

Postoperative Serum Calcitonin and CEA Levels and Their Utility in Detecting Residual Disease and Progression of MTC

A normalized or undetectable serum calcitonin level following intended curative thyroidectomy for MTC indicates biochemical cure and warrants a baseline physical exam and neck US. If these tests are normal, such patients can be evaluated every six months for the first year, and then annually. If serum calcitonin levels are found to be detectable but <150 pg/mL, and there is no evidence of persistent or recurrent disease on physical exam or neck US, patients can be followed with physical exam and US every six months. Further, serum calcitonin and CEA levels should be measured at three- to six-month intervals to determine doubling times. If serum calcitonin levels are >150 pg/mL or continue to rise, additional imaging studies are necessary to detect persistent or recurrent disease.

Multiple imaging modalities can be used to detect persistent or recurrent MTC such as neck and chest CT, triple-phase contrast-enhanced CT, contrast-enhanced MRI and US of the liver, bone scintigraphy, MRI of the spine and pelvis, positron emission tomography (PET), and [18F]-fluorodeoxyglucose (FDG)-PET/CT, and Fluorodopa (F-DOPA)-PET/CT [39–45]. Surgical resection for regional disease is advocated, whereas progressive systemic disease should be treated with systemic therapy, and preferably in a clinical trial setting. Measuring serum calcitonin and CEA levels can complement sequential imaging studies and help estimate the rate of disease progression by determining the doubling rates for each marker. Simultaneous elevations of serum CEA and calcitonin levels suggest disease progression. As previously mentioned, rising levels of serum CEA, in association with stable or declining levels of serum calcitonin, suggest poorly differentiated and progressive MTC [17, 46].

Measuring serum calcitonin or CEA levels over multiple time points (at least four data points over a minimum of two years) can help determine the rate at which each marker doubles [47–50]. Serum calcitonin doubling time has been shown to be a strong independent predictor of survival, and a better predictor of survival than the CEA doubling time [47]. Rapid doubling times are deemed unfavorable. Serum calcitonin doubling times >24 months impart significant survival benefit, whereas doubling times of <six months indicate poor prognosis. Serum calcitonin and CEA doubling times are more often concordant than not, with similar survival benefit observed with longer doubling times of these markers. However, when a discordant trend is observed with either of these markers' doubling time, one must suspect further progression of the disease and implement additional imaging studies [48]. The latest ATA guidelines for the management of MTC support the practice of determining doubling times of both markers and provide clinicians with a calculator to determine doubling times of serial serum calcitonin and CEA measurements (www.thyroid.org/thyroid-physicians-professionals/calculators/thyroid-cancer-carcinoma).

In conclusion, serum calcitonin and CEA serve as essential tumor markers of MTC and play an important role in determining tumor extent, and in preoperative planning. These markers are also central to the long-term postoperative follow-up of patients with MTC. The prognostic impact of calcitonin and CEA doubling times on patient survival is instrumental in navigating the complex landscape of postoperative surveillance to assess for recurrent or progressive MTC.

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Cytology and Pathology: Pitfalls and Challenges

4

Sylvia L. Asa and Ozgur Mete

Introduction

Medullary thyroid carcinoma (MTC) is defined as a tumor derived from parafollicular C-cells of the thyroid. These cells are members of the dispersed neuroendocrine system and are responsible for the synthesis and secretion of calcitonin. C-cells, initially described by Karl Hürthle in 1904 [1], constitute less than 1 % of the thyroid epithelium; they are large cells with abundant clear cytoplasm that are scattered singly or in small clusters under the basement membrane of follicles at the junction of the upper third and lower two-thirds of each lateral thyroid lobe. In normal situations, they are randomly scattered in this region, near the ultimobranchial body remnants, and there are no more than 5–7 cells associated with any individual cluster or follicle. While Hürthle described these cells, their function was not known until the discovery of the hormone calcitonin and the pathological description of MTC [2].

S.L. Asa (✉) · O. Mete
Laboratory Medicine Program, Department of Pathology, University Health Network, 200 Elizabeth Street, 11th Floor, Toronto, ON M5G 2C4, Canada
e-mail: dr.sylvia.asa@uhn.ca

O. Mete
e-mail: ozgur.mete2@uhn.ca

S.L. Asa · O. Mete
Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON M5G 2C4, Canada

The morphological spectrum of parafollicular C-cell proliferations includes C-cell hyperplasia, MTC, and mixed or composite medullary and follicular epithelial carcinomas. With the advent of immunohistochemistry, C-cell proliferations can now reliably be identified by localizing their markers of neuroendocrine differentiation, synaptophysin and chromogranin, and their specific peptide products, calcitonin and carcinoembryonic antigen (CEA). However, MTC can be mistaken for follicular neoplasms both on cytologic evaluation of fine needle aspiration (FNA) biopsies and even on histologic examination [3, 4]. It is therefore important to be aware of the differential diagnosis of this important disease.

Diagnostic Considerations and Differential Diagnosis

The most important component of making the diagnosis of MTC is to consider it in the differential diagnosis. Since in many places the preoperative measurement of serum calcitonin is not routinely performed in the evaluation of thyroid nodules, this biochemical marker is not used to predict the likelihood of this disorder in patients with thyroid nodules, and often, the pathologist is the first to raise the possibility of the diagnosis of MTC.

MTC has distinctive cytologic and histologic features, but variants of this lesion exist that can

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be misdiagnosed [5, 6]. These neoplasms can be mistaken for follicular epithelial-derived neoplasms for the following reasons: (i) Trapping of thyroid follicles gives rise to a pseudofollicular variant in some cases, while in others, the presence of true follicular structures mimics follicular neoplasms. To complicate the quandary, there are rare examples of composite medullary and follicular epithelial carcinomas; (ii) MTC can display pseudopapillary and papillary growth that can be mistaken for papillary thyroid carcinoma; and (iii) the architecture of these lesions also can be seen in the hyalinizing trabecular tumor that is largely accepted as a variant of papillary thyroid carcinoma and, in poorly differentiated carcinoma of follicular cells, the so-called insular carcinoma, and these are important considerations to exclude.

When recognized as neuroendocrine tumors on cytology, the differential diagnosis includes a variety of intrathyroidal neuroendocrine proliferations, including parathyroid proliferations (hyperplasia, adenoma, and carcinoma) [7], intrathyroidal thymic neuroendocrine tumors [8], thyroid paragangliomas that are often associated with benign behavior [9–11], and metastatic neuroendocrine carcinomas from other sites [12, 13].

Plasmacytoid and small cell cytomorphology associated with MTC mimic hematologic malignancies and neuroblastoma [14], and some variants composed of large spindle cells or with occasional giant cells, may raise the possibility of anaplastic (undifferentiated) thyroid carcinoma. In all of these situations, the application of immunohistochemistry will allow the correct classification of any individual tumor.

Pitfalls in the Cytologic Assessment of Fine Needle Aspiration Biopsies

The diagnosis of MTC can be made on cytologic examination of aspirates [15–17]. MTC has distinctive cytologic features (Fig. 4.1), but variants of this neoplasm can pose diagnostic challenges. The most important aspect of making the diagnosis of MTC is to consider it, especially when the biopsy is from a lymph node rather than the thyroid. Unfortunately, the literature suggests that the diagnosis is only made in approximately half of the cases [18, 19], a finding that suggests inadequacy in the process used by cytopathologists who do not consider the diagnosis of MTC.

The weakly cohesive or dyscohesive nature of tumor cells is one of the most important features on cytology that should alert the diagnostician to the possibility of a neuroendocrine tumor. Like in other neuroendocrine neoplasms, the tumor cells are mainly epithelioid, but usually there are scattered spindle cells as well. Their nuclei exhibit the classical granularity of chromatin known as the "salt-and-pepper" appearance that is found in most neuroendocrine tumors; nucleoli are usually prominent. Binucleate cells and occasional nuclear pseudoinclusions mimicking those of papillary thyroid carcinoma can be seen, but giant cells and bizarre nuclei are unusual. The cytoplasm contains perinuclear azurophilic granules that are best seen on air-dried slides stained with the May–Grünwald–Giemsa stain. However, the diagnosis can be more challenging when the tumors are composed of populations of epithelioid, oncocytic or clear cells, plasmacytoid or small cells, and squamoid or spindle cells [5, 20].

Amyloid is a hallmark of MTC and, when present, usually heralds the diagnosis; it can be confused with colloid in cytologic preparations due to its translucence and round, smooth edges, but unlike colloid, it does not usually crack, and it has characteristic apple–green birefringence on polarized light even without the application of the Congo red stain. It is important to note, however, that not all amyloids in thyroid aspirates are associated with MTC, and other causes must also be considered, including amyloid goiter and lymphoma [21–24].

In cases where the diagnosis of MTC is suspected but not definitive, the application of immunohistochemistry (see below) can be used to confirm the diagnosis. There are also reports of the biochemical measurement of calcitonin in FNA washouts as a method of confirmation [25], but this is not the practice in most centers.

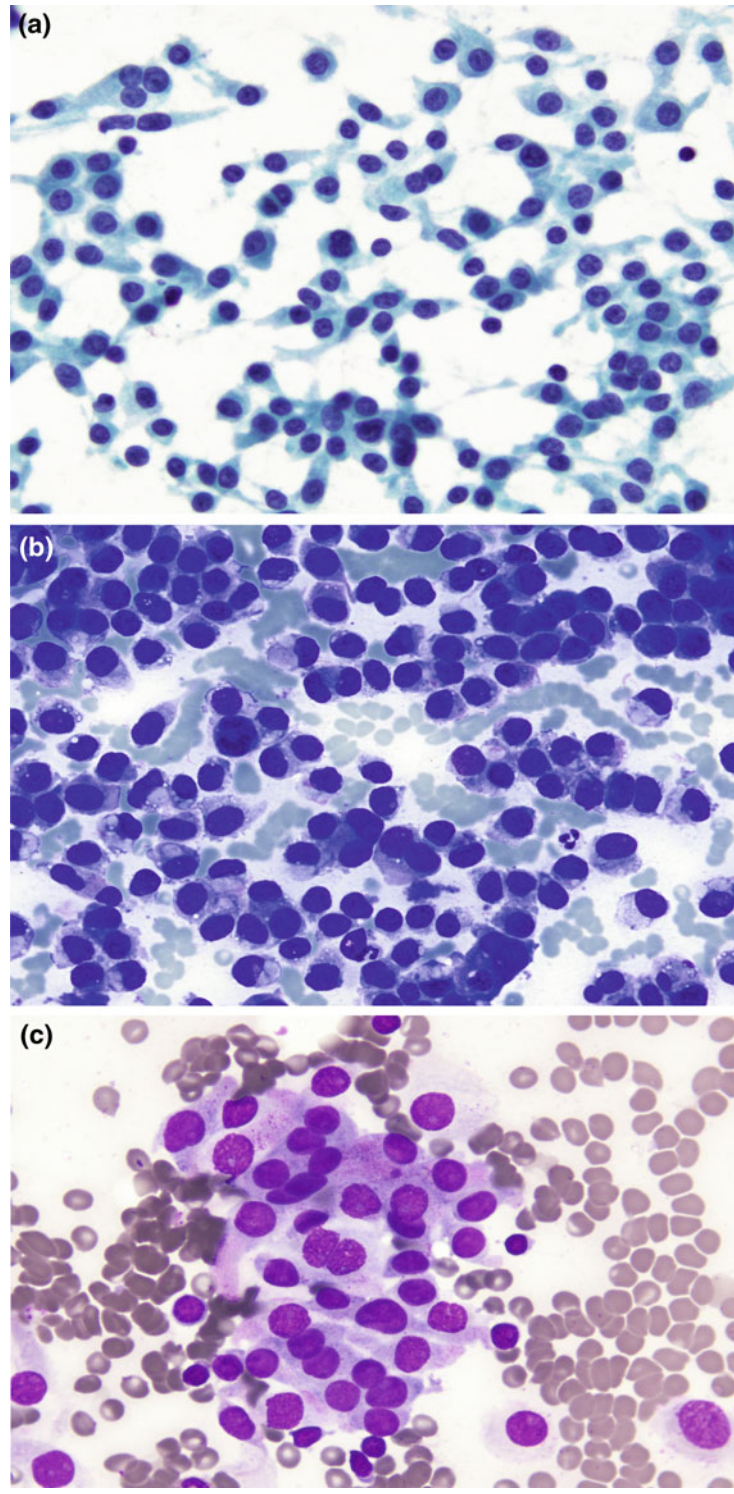

Fig. 4.1 Cytologic features of medullary thyroid carcinoma. The weakly cohesive or dyscohesive nature of tumor cells is one of the characteristic features of medullary thyroid carcinoma on cytology (**a**–**c**). The tumor cells can have spindle, plasmacytoid, or epithelioid appearance. Their nuclei exhibit the classical granularity of chromatin known as the "salt-and-pepper" appearance. The cytoplasm often contains perinuclear calcitonin-containing azurophilic granules that are best seen on air-dried slides stained with the May–Grünwald–Giemsa stain **c**. (**a** Papanicolaou stain, **b** Diff-Quik stain, **c** May–Grünwald–Giemsa stain)

Pitfalls in the Histopathologic Examination of C-cell Proliferations

The morphological spectrum of C-cell disease encompasses C-cell hyperplasia, MTC, and mixed medullary and follicular epithelial-derived thyroid carcinomas.

Medullary Thyroid Carcinoma

The diagnosis of MTC is often rendered with no difficulty on histologic examination of core biopsies or thyroidectomy specimens [3]. The majority of these tumors have a classical appearance, being composed of solid nests and sheets of round to polygonal and elongated cells with poorly defined cell borders (Fig. 4.2a). The stroma can be vascular and fibrotic, and approximately half contain prominent amyloid with its characteristic apple–green birefringence under polarized light that is seen even without Congo red staining (Fig. 4.2b). Some tumors are completely devoid of amyloid, and in some, amyloid is present only as intracytoplasmic hyaline material that can be easily missed. As in

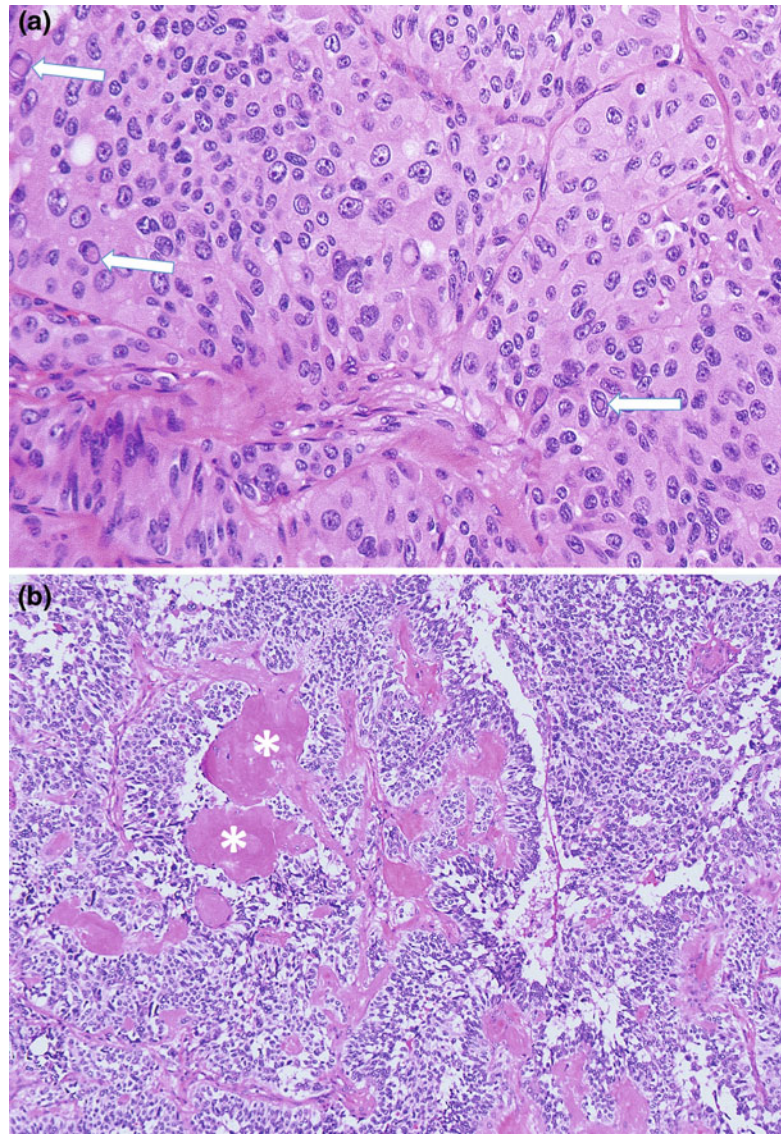

Fig. 4.2 Histopathologic features of medullary thyroid carcinoma. The vast majority of these neoplasms are composed of solid nests and sheets of round to polygonal and elongated cells with ill-defined cell borders (**a**). Similar to papillary thyroid carcinoma, medullary thyroid carcinoma can also have intranuclear pseudoinclusions (**a** *arrows*). Amyloid deposition can be identified without Congo red straining (**b** *asterisks*). (**a**, **b**: Hematoxylin and eosin stains)

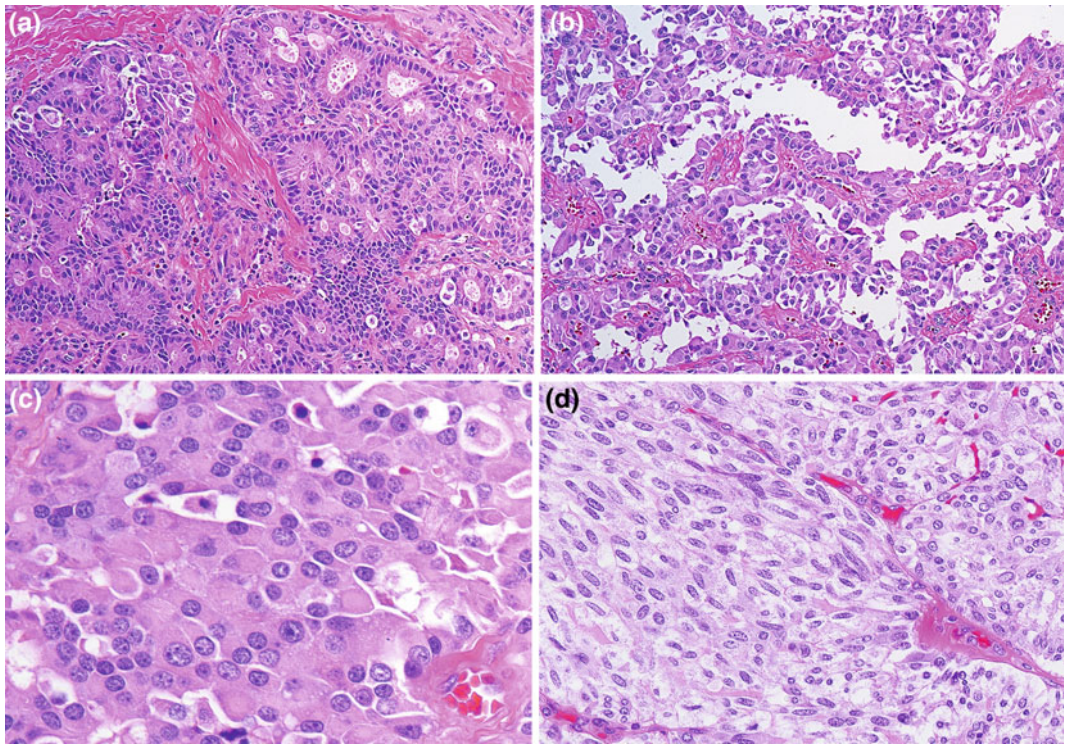

Fig. 4.3 The many faces of medullary thyroid carcinoma. Some histologic variants of medullary carcinoma are illustrated in this composite photomicrograph: These are the follicular or glandular variant (**a**), the pseudopapillary variant (**b**), the oncocytic variant (**c**), and a spindle cell variant (**d**). (**a**–**d**: Hematoxylin and eosin stains)

cytology, the identification of amyloid does not confirm the diagnosis of MTC, since it can also be found in other thyroid carcinomas and in amyloid goiter [21–23]. Foreign body giant cells and calcification can occasionally be found associated with amyloid deposits, and even psammoma bodies have been reported in these tumors.

Many histologic variants of MTC have been described including the following: follicular (glandular) (Fig. 4.3a) and pseudofollicular, papillary, pseudopapillary (Fig. 4.3b) and cystic [26, 27], encapsulated [28], oncocytic (Fig. 4.3c) [29], clear cell, spindle cell (Fig. 4.3d), squamous cell, small cell, giant cell, melanin-producing (pigmented or melanotic variants) [30], mucin-producing (mucinous) [31], hyalinizing trabecular adenoma-like [32], angiosarcoma-like [33], neuroblastoma-like, and paraganglioma-like variants [3, 4].

Since MTC cells surround and trap thyroid follicles, the resulting pseudofollicular appearance can mimic a follicular lesion. In addition, some rare tumors form true fibrovascular cores (papillary variant of MTC), or tumor dehiscence and fixation artifact can create pseudopapillae (pseudopapillary variant of MTC) (Fig. 4.3b), and cystic changes (cystic variant of MTC); these variants can be mistaken for papillary thyroid carcinoma [26, 27]. Intranuclear pseudoinclusions (Fig. 4.2a) and nuclear grooves, although extremely rare, can further complicate this distinction. The formation of glandular structures raises the possibility of an adenocarcinoma, and occasional mucinous variants have been described [3, 31].

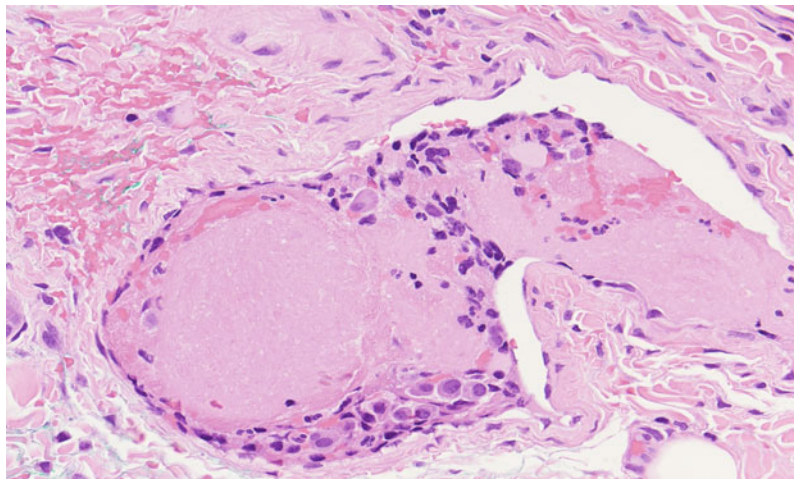

Fig. 4.4 Angioinvasive medullary thyroid carcinoma. The identification of angioinvasion characterized by tumor cells invading through a vessel wall and intravascular tumor cells admixed with thrombus is an important feature that has been shown to be the most reliable predictor of disease recurrence and death. (Hematoxylin and eosin stain)

Oncocytic change makes the tumor cells resemble follicular epithelium and not infrequently results in a misdiagnosis of a "Hürthle cell tumor," a nomenclature that has mistakenly come to signify follicular differentiation [29, 34]. The identification of amphophilic or basophilic cytoplasmic granularity rather than bright eosinophilic appearance argues against thyroid follicular epithelial origin (Fig. 4.3c). It is important to emphasize that paragangliomas also share morphological features of MTC; this distinction can only be made by using immunohistochemistry. Predominance of monomorphic plasmacytoid cells can lead to the misdiagnosis of plasmacytoma [35], and as these tumors dedifferentiate, the cells develop a small cell morphology that can be mistaken for lymphoma, neuroblastoma, or metastatic small cell neuroendocrine carcinoma. Binucleate cells, bizarre nuclei and occasional giant cells, especially around amyloid deposits are rare features that can mimic anaplastic (undifferentiated) thyroid carcinoma or papillary thyroid carcinoma with a focal spindle and giant cell component.

Staging of these lesions should follow the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) guidelines. In addition, accurate identification of angioinvasion characterized by tumor cells invading through a vessel wall and intravascular tumor cells admixed with thrombus is an important feature that has been shown to be the most reliable predictor of disease recurrence and death (Fig. 4.4) [36].

Mixed Medullary and Follicular Epithelial-Derived Thyroid Carcinoma

Mixed (composite) medullary and follicular epithelial-derived thyroid carcinomas are extremely rare. The diagnosis of a mixed follicular–parafollicular cell carcinoma composed of a monomorphous population of cells showing dual differentiation is controversial [37–39]. In contrast, there are many reports of composite tumors composed of intermixed populations of thyroglobulin-positive follicular cells and calcitonin- and CEA-reactive C-cells; the follicular component usually exhibits the cytologic features of papillary thyroid carcinoma (Fig. 4.5) [40–42]. These likely are coincidental collision tumors. While several hypotheses have been introduced to explain their pathogenesis, the differential patterns of *RET* proto-oncogene mutation profile, LOH, and X-chromosomal inactivation in the two different

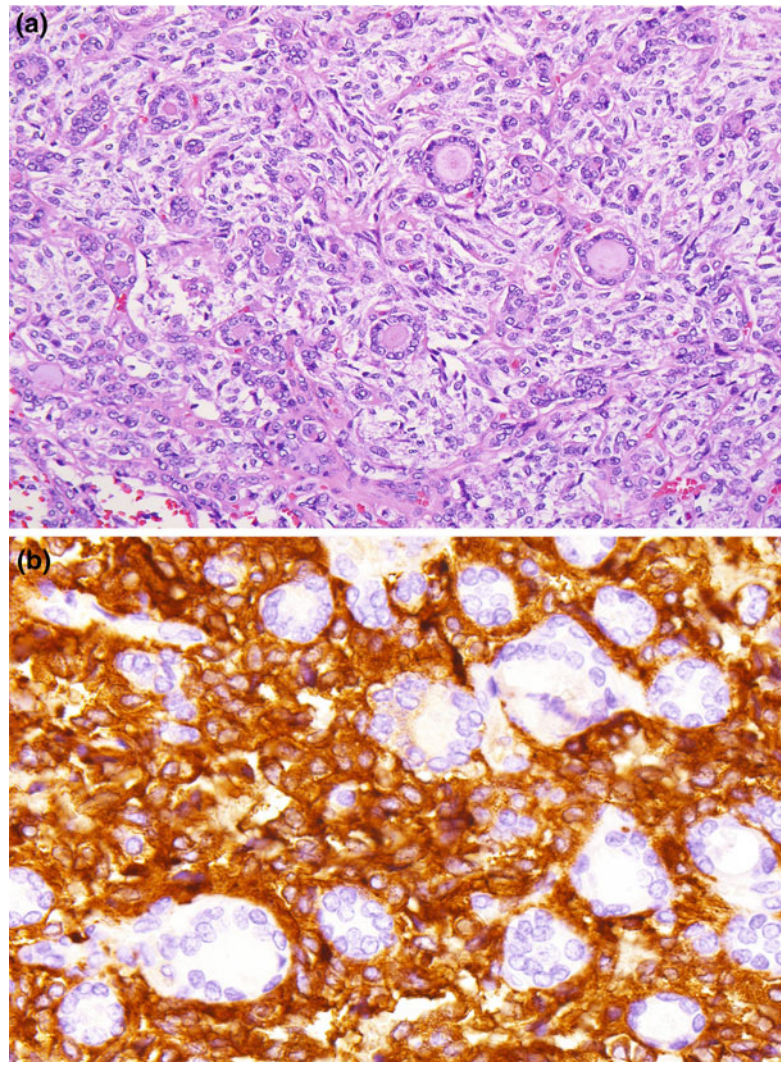

Fig. 4.5 Mixed medullary and follicular epithelial-derived carcinoma. Mixed (composite) medullary and follicular variant papillary thyroid carcinoma (**a**) is illustrated in this figure. Follicular structures display nuclear membrane irregularities in enlarged nuclei with chromatin margination (optically clear nuclei), consistent with papillary thyroid carcinoma. Calcitonin is negative in the papillary carcinoma component, whereas the medullary thyroid carcinoma component is positive for calcitonin (**b**)

cell lineages argue that the two tumors have independent pathogenesis [43].

At the morphological level, parafollicular C-cells often constitute the majority of these combined tumors. The follicular epithelial cells are typically intermingled with the C-cell component. The diagnosis of a mixed medullary and follicular thyroid carcinoma is controversial if the follicular epithelial cell component does not show invasive growth. On the other hand, the diagnosis of a mixed medullary and papillary carcinoma is relatively less problematic; however, the distinction of reactive cytologic atypia in entrapped follicular epithelial cells from follicular variant papillary thyroid carcinoma can be challenging [42]. In problematic cases, positivity for the markers of malignancy of follicular epithelial cells (HBME-1, CK19, galectin-3) may be useful in the absence of documented evidence of a metastatic tumor deposit showing both C-cell and follicular epithelial cell components [42]. However, metastatic spread to a lymph node confirms malignancy of the follicular component, when in doubt, and the two neoplasms occurring independently in the thyroid gland can present with a combined metastasis within a lymph node.

C-cell Hyperplasia

In patients with a genetic predisposition to develop MTC due to germline *RET* mutations (see Chap. 5), the morphological findings are usually distinctive and diagnostic. In advanced cases, the thyroid exhibits multifocal bilateral disease, usually most prominent in the upper- to mid-lateral lobes; in cases detected early, there may be a single dominant lesion associated with C-cell hyperplasia, C-cell hyperplasia only, or multifocal microinvasive medullary microcarcinoma arising from precursor nodular C-cell hyperplasia [44, 45].

C-cell hyperplasia is defined as increased numbers of C-cells with >7 cells per cluster, and there are often complete follicles surrounded by C-cells; in addition, the finding of C-cells outside the normal location, such as in the lower pole of the lateral lobes or in the isthmus, should prompt the investigation of C-cell hyperplasia [46, 47]. It is also important to emphasize that when dealing with a solitary MTC, the distinction between microscopic tumor spread and C-cell hyperplasia cannot be evaluated reliably in the anatomic site of the tumor; therefore, C-cell mapping is typically performed on the contralateral lobe [46].

C-cell hyperplasia has been reported to have two growth patterns. In some cases, proliferating C-cells line the follicular structures, known as "linear C-cell hyperplasia" (Fig. 4.6a); in others, they obliterate the lumen of the follicle and expand into the surrounding parenchyma, known as "nodular C-cell hyperplasia" (Fig. 4.6b). However, most patients have mixed patterns. It has been recommended that the predominant

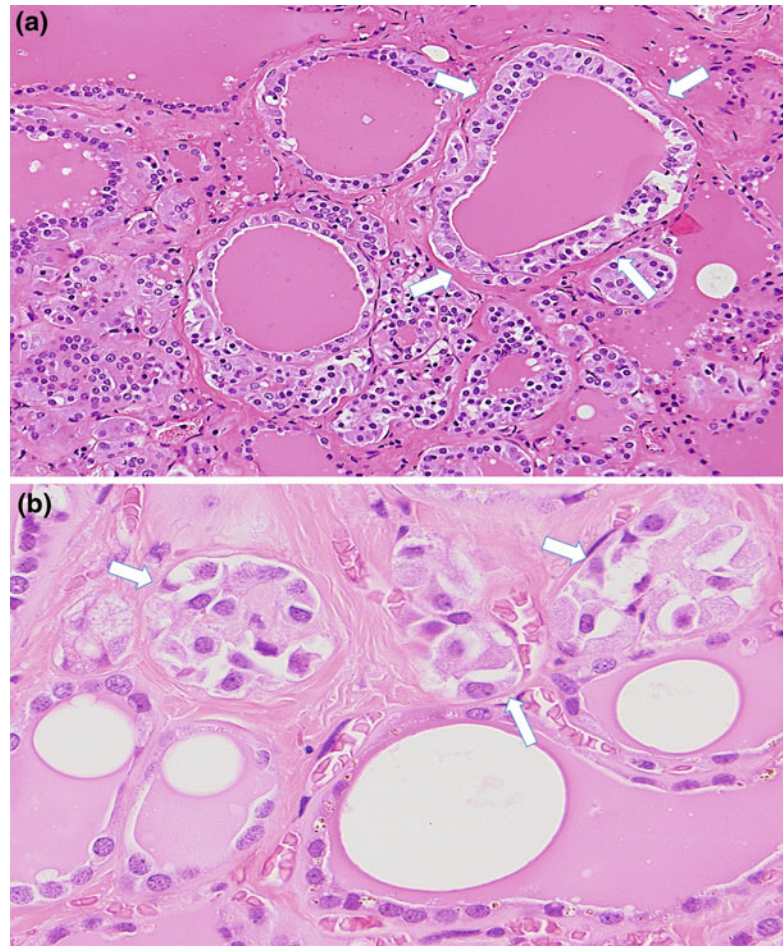

Fig. 4.6 C-cell hyperplasia. Proliferating C-cells can have a predominant linear growth pattern around follicles (**a** *arrows*) and/or nodular growth (**b** *arrows*). (**a**, **b**: Hematoxylin and eosin stain)

growth pattern be reported. Nodular C-cell hyperplasia and bilateral C-cell hyperplasia should be considered an indication for genetic testing, but occasional patients will not have germline mutations of the *RET* proto-oncogene since reactive or "secondary" C-cell hyperplasia can also be found with aging and in individuals with underlying thyroid follicular epithelial proliferations, chronic hypercalcemia, hypergastrinemia, and thyroiditis, and in association with certain medications [48–50]. However, these disorders usually cause linear C-cell hyperplasia instead of the characteristic predominant nodular form of C-cell hyperplasia seen in with MEN2 (including familial medullary thyroid carcinoma [FMTC]) syndromes. Patients with PTEN hamartoma tumor syndromes (PHTS) have a diffuse form of C-cell hyperplasia associated with multiple adenomatous follicular nodules [51]; again, this is not typically associated with MTC. Drugs implicated in this disorder include anti-diabetic incretins, especially glucagon-like peptide-1 (GLP-1) analogs (e.g., exenatide, liraglutide, taspoglutide) that have been shown to cause C-cell hyperplasia and MTC in rats and mice [52, 53]. While GLP-1 receptors are typically present in normal rodent C-cells, receptors for both GLP-1 and gastric inhibitory peptide (GIP; another incretin also known as glucose-dependent insulinotropic polypeptide) are not expressed in normal human C-cells [54]. However, evidence suggests that approximately 90 and 30 % of human medullary thyroid carcinomas express GIP and GLP-1 receptors in high density, respectively. These observations have raised a link between incretin receptor expression and C-cell disease, leading to recommendations to monitor patients with calcitonin measurements prior to and during the administration of GLP-1 analogs [54].

MTC measuring ≤ 1.0 cm is classified as medullary microcarcinoma. The distinction between nodular C-cell hyperplasia and microinvasive medullary microcarcinoma can be challenging in some cases. The diagnosis of medullary microcarcinoma is rendered when the C-cell proliferation breaches the basal membrane and invades into the surrounding parenchyma [46, 55]. The presence of a desmoplastic tissue reaction surrounding an irregular nodular C-cell growth suggests medullary microcarcinoma. The use of type IV collagen immunohistochemistry is also useful in the distinction of invasive growth in problematic cases.

Ancillary Tools

Immunohistochemistry

The diagnosis can and should be verified by immunohistochemistry. The most specific markers are chromogranin, calcitonin (Fig. 4.7a), and CEA (Fig. 4.7b). Chromogranin alone only confirms the diagnosis of a neuroendocrine tumor and is not specific to MTC; the most specific marker is calcitonin. Calcitonin staining can be strong and diffuse, but may show only focal expression in dedifferentiated tumors. Even the amyloid present in MTC will stain positive for calcitonin, since it is composed of a precursor of the calcitonin molecule. Expression of CEA alone is not specific but may have prognostic significance, since focal or weak calcitonin positivity and abundant CEA expression are thought to reflect dedifferentiation and to predict a worse prognosis [56, 57]. CEA is not expressed by follicular thyroid epithelium; therefore, the identification of positivity excludes a follicular cell-derived tumor and should raise the possibility of MTC or other lesions, including thymic carcinomas that occur in the neck [58], or a metastasis from a non-thyroid malignancy. It is important, however, to ensure that the CEA stain is performed with a highly specific monoclonal antibody, since polyclonal antisera can result in CEA positivity due to non-specific cross-reactivity [59].

TTF-1 is expressed in up to 75 % of MTC (Fig. 4.7c). Of note, TTF-1 and calcitonin can also be expressed in pulmonary neuroendocrine tumors; in that situation, the use of monoclonal CEA can be used to distinguish a metastatic pulmonary neuroendocrine tumor from MTC that would be strongly positive.

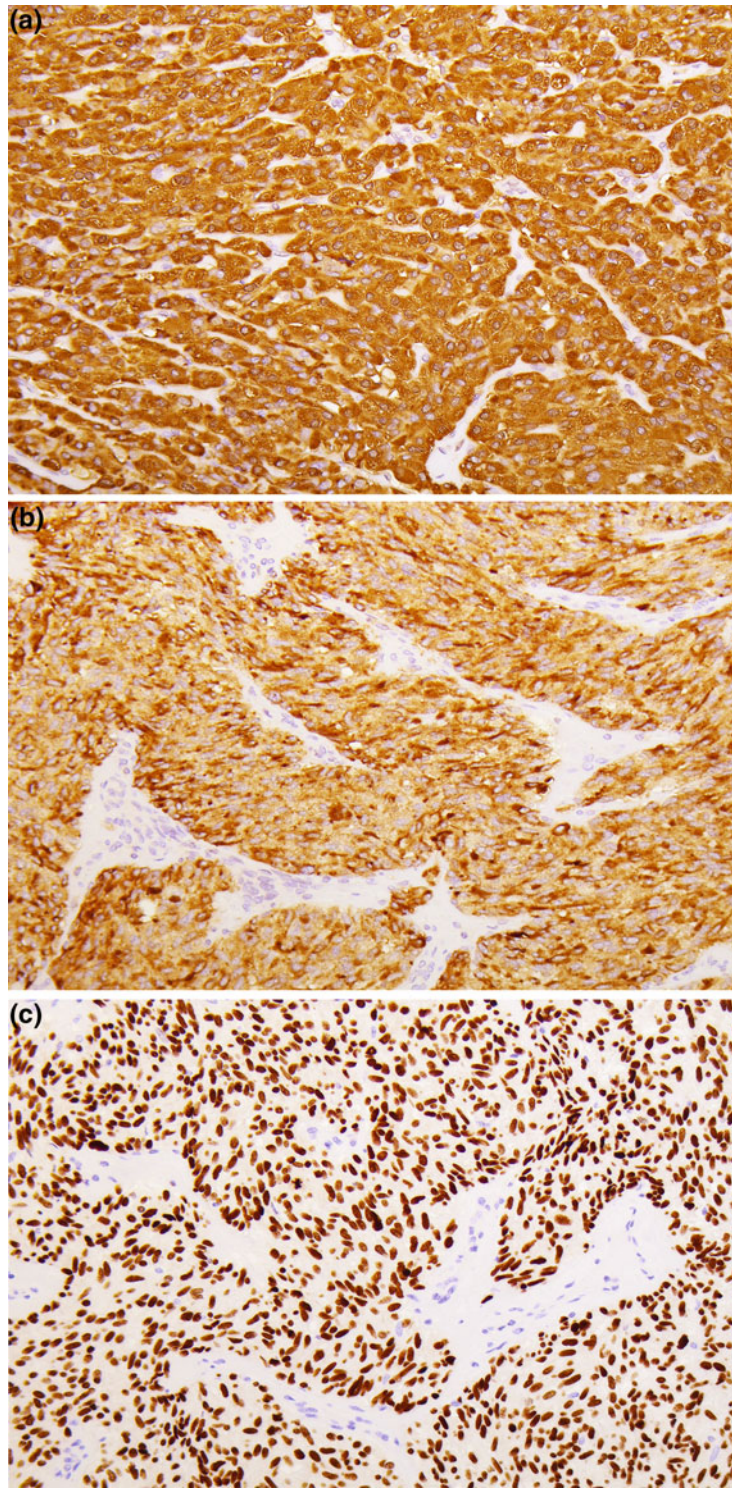

Fig. 4.7 Immunohistochemical features of medullary thyroid carcinoma. C-cell-derived proliferations like this medullary thyroid carcinoma are typically positive for calcitonin (**a**) and CEA (**b**) and can also express TTF-1 (**c**).

When the differential diagnosis includes plasmacytoma or lymphoma, the application of cytokeratin stains can be helpful, since MTC is positive for pankeratins, CK7 and CK18 [60]. Tumors that stain for chromogranin may also represent other neuroendocrine neoplasms. The possibility of a primary parathyroid lesion should prompt staining for parathyroid hormone. A tumor that is negative for cytokeratins and TTF-1 but positive for chromogranin tumor should be investigated for the possibility of paraganglioma using tyrosine hydroxylase. In both of these situations, these lesions are often associated with benign behavior, and therefore, the distinction is important [5].

While calcitonin is the main hormonal product of MTC, these tumors also produce other peptides, including calcitonin gene-related peptide (CGRP), somatostatin, and serotonin. The value of these markers is evidenced by rare medullary thyroid carcinomas that do not produce calcitonin but express CGRP [61]. Occasional tumors produce derivatives of the proopiomelanocortin (POMC) molecule including ACTH, MSH, ß-endorphin, and enkephalin and can give rise to ectopic Cushing syndrome. Tumors have also been reported to express, glucagon, gastrin, cholecystokinin, VIP, bombesin, and α-HCG [62].

Several potentially important biomarkers may represent targets for novel therapies including COX-1/2, Bcl-2a, Gst-pi, Gli-1, Gli-2, Gli-3, and Bmi-1; the immunohistochemical profile of somatostatin receptors may also be of value in determining the feasibility of peptide receptor radionuclide treatment. [36].

Electron Microscopy

Electron microscopy is not usually required for the diagnosis of MTC, but if performed, it identifies features of neuroendocrine differentiation, and amyloid can be recognized in some tumors.

Molecular Diagnostics

Approximately 25 % of MTC are associated with familial disease. Molecular analysis of *RET* should not be performed on the tumor tissue to determine genetic predisposition; instead, the testing should be performed on white blood cells. This is because 40 to 50 % of sporadic MTC may harbor somatic *RET* mutations [63]; they most frequently involve codon 918 and may have prognostic significance [63, 64]. *RAS* mutations are also identified in sporadic tumors [65–67]. There seems to be no association between the presence of *RAS* mutations, tumor size, and other epidemiological and pathological features. A single patient with a novel *RET* fusion has been identified as a sporadic MTC [68]. Telomerase activation occurs in about half of these tumors, and the alternative lengthening of telomeres (ALT) mechanism is implicated in some; these features are associated with more aggressive disease and may provide additional prognostic information [69]. Dysregulated microRNAs, including downregulated miR-10a, miR-200b/-200c, miR-7, and miR-29c and upregulated miR-130a, miR-138, miR-193a-3p, miR-373, and miR-498, target E-cadherin, transforming growth factor β (TGFβ)-2, and TGFβ-1 and appear to predict metastatic behavior in MTC [70].

Implications of Diagnosis

The diagnosis of MTC is critically important. Not only is the prognosis different, but the management considerations are radically different from follicular thyroid lesions; since MTC does not take up iodine, therapy with radioactive iodine is not indicated. Instead, MTCs express somatostatin receptors [71] providing novel diagnostic [72, 73] and potential therapeutic approaches [74, 75]. In addition, the fact that MTC is frequently hereditary has implications for both the patient and members of the family [76].

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Molecular Mechanisms of Disease: The RET Proto-oncogene

5

Gilbert J. Cote, Rozita Bagheri-Yarmand, Marie-Claude Hofmann and Robert F. Gagel

Introduction

This chapter focuses on the role of the *RET* proto-oncogene and its mutations in the development of medullary thyroid carcinoma (MTC), a tumor derived from the thyroid parafollicular or C-cell. A hallmark of the thyroid C-cell is the expression and secretion of calcitonin, a peptide that serves a valuable role in monitoring the progression of MTC. C cells comprise a minor population of the thyroid, representing approximately 2–4 % of the organ's cells. The discovery that activating mutations of *RET* initiate and drive the oncogenic transformation of the C-cell in hereditary MTC defined its important role more than 20 years ago [1–3], but the specific mechanism by which this mutated receptor drives transformation is largely unknown. What is certain is that a germline *RET* mutation predicts the eventual presence of MTC development, permitting removal of the thyroid gland in most cases prior to metastasis. Activating *RET* mutations are found and are thought to play an initiating role in ~40 % of sporadic MTCs [4]. In both cases, there exists a need to further understand *RET*'s mechanism of action and to uncover other mediators of C-cell transformation and tumor progression. It is important to recognize that *RET*-independent mechanisms appear to account for the majority of sporadic MTC. For example, activating mutations in *HRAS* and *KRAS* are associated with ~15 % of sporadic MTC, and there have been a handful of tumors identified with *BRAF* mutations and *ALK* rearrangements [5]. Detailed discussion of their role in MTC can be found in several recent reviews [6, 7]. Continued expansion of our understanding of the biology of the normal C-cell and of MTC will aid in the future treatment of this malignancy by refining the timing of resection of the thyroid in hereditary cases and allowing further development of novel, molecular-targeted therapies in advanced cases.

RET Structure and Function

Receptor Tyrosine Kinase and Intracellular Signaling

The *RET* proto-oncogene is localized on human chromosome 10q11.2 and encodes the RET (Receptor Tyrosine Kinase), a single-pass transmembrane protein [8]. RET is the receptor for glial cell line-derived neurotrophic factor (GDNF), neurturin (NRTN), persephin (PSPN), and artemin (ARTN). In order to stimulate RET tyrosine kinase activity, these growth factors need first to form a complex with a

G.J. Cote (✉) · R. Bagheri-Yarmand · M.-C. Hofmann · R.F. Gagel
Department of Endocrine Neoplasia and Hormonal Disorders, MD Anderson Cancer Center, The University of Texas, 1515 Holcombe Blvd., Unit 1461, Houston, TX 77030, USA
e-mail: gcote@mdandserson.org

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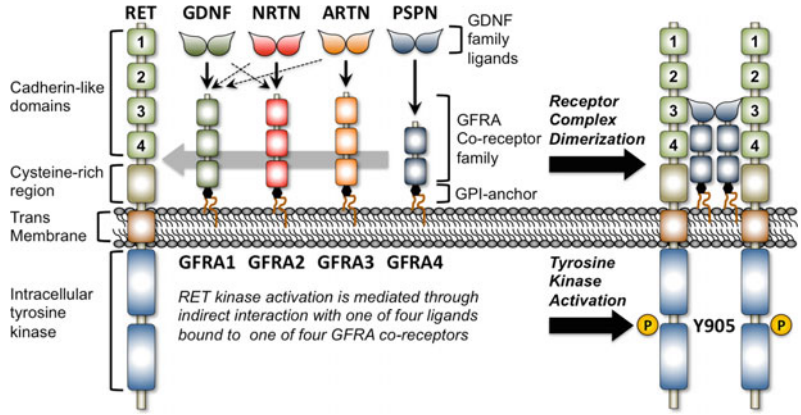

Fig. 5.1 RET receptor activation. Shown is a schematic figure outlining the basic structural organization of the transmembrane receptor tyrosine kinase RET and the pathway to its activation. One of four GDNF family ligands is recruited to the receptor upon binding to its cognate coreceptor. *Solid arrows* indicate the primary ligand/coreceptor interactions. *Dotted arrows* show secondary binding interactions. Generation of the heterotrimeric complex allows for receptor dimerization causing kinase activation and autophosphorylation (P). The predominant receptor complex observed in MTC cells is comprised of PSPN and GFRA4

glycosylphosphatidylinositol (GPI)-anchored coreceptor family member, GDNF receptor-α1-4 (GFRA1–4) (Fig. 5.1). This complex then recruits RET, which promotes receptor dimerization and its activation by trans-autophosphorylation. Within its intracellular domain, the RET receptor contains multiple tyrosine sites that, when phosphorylated, will mediate differential downstream signaling [9, 10]. The Y905 site plays a key role in RET receptor kinase activation because its phosphorylation serves as the initiating event for additional phosphorylations [11]. Tyrosine 1062 (Y1062) performs a primary role in downstream signaling because this residue is a multidocking site for proteins containing a phosphotyrosine-binding (PTB) domain [12]. Once recruited, these docking proteins will activate the RAS-MAPK and the PI3K-AKT pathways (Fig. 5.2). The nearby Y1096 site, which exists only on the long isoform of RET, also couples to the RAS-MAPK and the PI3K-AKT pathways. Other tyrosine residues are involved in targeting different downstream signaling pathways. For example, Y752, Y981, and Y1015 are coupled to STAT3, SRC, and PLC-γ, respectively (not shown in Fig. 5.1) [13–16]. These RET-activated signaling pathways have the ability to modulate several key mechanisms controlling cell proliferation (Fig. 5.2). For example, in developing spermatogonial stem cells, the RET-mediated PI3K-AKT pathway causes MYCN upregulation, while MAPK pathway activation leads to *FOS* and *cyclin gene family* expression [17–20]. Both MAPK and PI3K pathways are crucial for normal kidney development [21, 22]. In oncogenic processes, *MYCN* upregulation has been demonstrated in RET-dependent neuroblastoma and MEN2A [23, 24].

Among the four ligands known to activate RET, GDNF-mediated activation is perhaps the best studied. The signaling pathway was originally established as part of the juxtacrine loop of glial cells providing support to neurons [25–27]. It is now established that GDNF/RET signaling also mediates the upregulation of the Ets-family transcription factor ETV5, which is critical for kidney, urinary tract, and testis development [28–30]. The same axis also regulates expression levels of the stem cell factors ID4, BCL6B, POU3F1, and Brachyury in spermatogonial stem cells [31, 32]. Finally of specific relevance to this chapter, normal human C cells and adrenal chromaffin cells selectively coexpress GFRA4 and RET. Therefore, it has been inferred that in

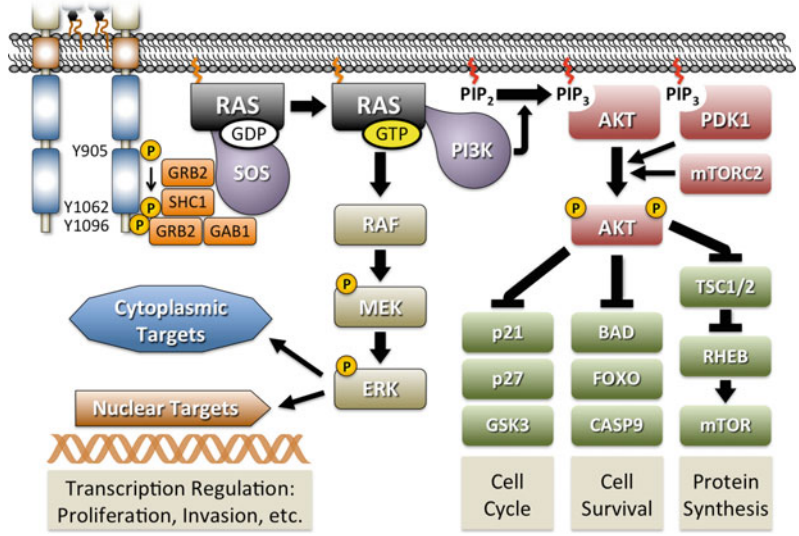

Fig. 5.2 The RET signal transduction network. Tyrosine phosphorylation results in the recruitment of adaptor proteins (*orange boxes*) that lead to the activation of the RAS/MAPK pathway. Activation of RAS initiated through interaction with phosphorylated RET. Signal transduction is primarily mediated by tyrosines 1062 and 1096 through adaptor proteins GRB2, SHC1, and GAB1. The guanine nucleotide exchange factor SOS is responsible for initiating the formation of active GTP-bound RAS. Active RAS is capable of both activation of MAPK signaling through RAF or PI3K activation leading to AKT signaling. Major components of both pathways are shown with their specific outcome of regulating cellular functions indicated

these cells, RET autophosphorylation is activated by PSPN, the ligand for GFRA4, through the PSPN-GFRA4 complex [33]. For a more extensive discussion of RET signaling and its role in disease, the reader is referred to the following excellent recent reviews [15, 16].

Novel Cytoplasmic and Nuclear Functions

In addition to cell membrane-associated signaling, RTKs can be trafficked to a variety of intracellular organelles such as the Golgi apparatus, the endoplasmic reticulum, mitochondria, and the nucleus [34–37]. Depending on the individual receptor and the site of trafficking, activated RTKs can have functions beyond signal transduction. Most unexpectedly, data from several laboratories have demonstrated nuclear localization of RTKs in both normal and transformed cells and, in some cases, found ligand-mediated receptor activation occurring within the nucleus [38–41]. It is particularly notable that changes in the nuclear expression levels of RTKs are associated with cancer progression and correlate with poor overall survival of patients with different types of malignancies [38–42]. It is therefore not surprising that nuclear RTKs are directly involved in regulation of gene expression, DNA replication, and DNA repair, which in turn lead to increased cell proliferation, survival, and resistance to therapeutic agents [39–41, 43]. We have recently reported that the full length RET receptor, similar to other RTKs, can localize to the nucleus and that nuclear localization is enhanced in MTC cells expressing activated RET, can be induced by ligand activation, and is not observed in the presence of a kinase-dead RET receptor [44].

These studies have uncovered the central role of a critical mediator of stress-induced apoptosis, activating transcription factor 4 (ATF4), in the regulation of C-cell survival. The enhanced nuclear localization of RET seen in MTC is associated with downregulation of ATF4

(Fig. 5.3). Experimentally, downregulation of ATF4 is blocked by tyrosine kinase inhibition or in models using a kinase-dead RET, defining a direct relationship between nuclear localization of RET and ATF4. Interestingly, activation of RET causes phosphorylation of ATF4 at both tyrosine and threonine residues leading to its enhanced degradation; this effect is inhibited by mutation of specific threonines. The reduced expression of ATF4 causes downregulation of several genes that promote apoptosis including *NOXA* and *PUMA*. Furthermore, our data suggest that RET acts in two ways to inhibit ATF4 function, through regulation of its stability and through blocking its binding to the promoter regions of *NOXA* and *PUMA* (Fig. 5.3).

As a central regulator of stress-induced apoptosis, ATF4 induces cell death in times of nutrient deprivation or ER stress. Thus, this novel threonine kinase activity of RET, which inhibits ATF4 function, is beneficial to tumor cell survival and proliferation. Since activation and nuclear translocation of RET are involved in the prevention of cell death and therapeutic resistance, blocking the nuclear translocation of RET or its nuclear functions may prove useful to improve therapeutic efficacy or to overcome therapeutic resistance of MTC. Unfortunately, the use of tyrosine kinase inhibitors targeting RET only leads to modest increases in ATF4 in MTC cells in vitro [44]. This is likely due to the lack of stressors under normal culture conditions. However, treatment with the stress inducer eeyarestatin results in increased occupancy of ATF4 at the promoter of *NOXA*, a target proapoptotic gene, stimulating expression of this gene. Therefore, treatments that combine RET kinase inhibition with activation of cellular stress or specific induction and stabilization of ATF4 may prove effective in the treatment of MTC.

The identification of the central role of ATF4 may have importance in the genesis of MTC. In hereditary MTC, there is an accumulation of C cells within the thyroid gland, collectively known as C-cell hyperplasia. This histologic abnormality

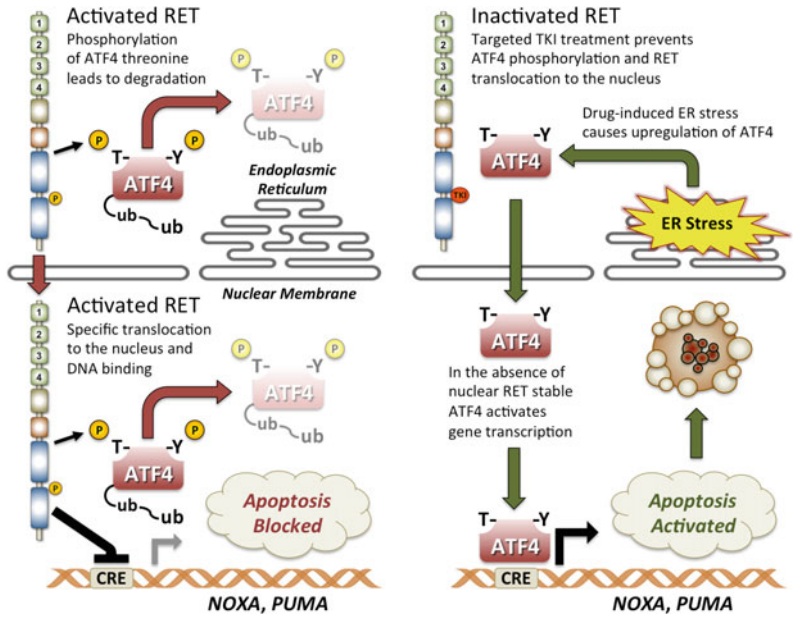

Fig. 5.3 A novel RET pathway. This figure shows the regulation of ATF4 function by RET. In MTC cells activated RET phosphorylates cytoplasmic or nuclear ATF4 on threonine residues to induce its degradation. Nuclear RET is also capable of DNA binding of the cAMP response element to block transcription of genes associated with apoptosis. In cells treated with tyrosine kinase inhibitors, there is activation of apoptosis resulting from the absence of nuclear RET and upregulation of ATF4

precedes the development of MTC and may exist for years or decades prior to its development. The recognition that activation of RET or inactivation of ATF4 can inhibit C-cell death through its effects on *NOXA* and *PUMA* provides a plausible mechanism for the accumulation of C cells seen in children with hereditary MTC.

Physiological Roles of RET

Developmental Roles

Mouse models lacking global RET function die before birth, demonstrating that RET signaling is crucial for normal mammalian embryonic development. These mutant embryos exhibit abnormal kidney development and aberrant ureter, ovary, and intestine morphology [45]. A recent review details the role of RET signaling in kidney and lower urinary tract development [21]. RET signaling is also essential for the development and survival of lumbar spinal cord motor neurons; alterations in its phosphorylation pattern have been linked to amyotrophic lateral sclerosis [46, 47]. In addition, RET activation is required for the development and migration of neural crest-derived lineages, in particular the enteric nervous system, adrenal medulla chromaffin cells, and thyroid C-cells [16, 48, 49]. Therefore, loss of RET function in migratory enteric neurons precursors, together with other gene mutations, leads to the absence of enteric ganglia and is responsible for approximately 10 % of hereditary Hirschsprung's disease [46].

In addition, congenital central hypoventilation syndrome (Ondine's curse), a life-threatening abnormality of the autonomic neurons of the ventral medulla of the brain stem, is caused by RET or GDNF mutations [50]. Also in mice, loss of RET function in neural crest cells is associated with a 37 % reduction of C-cells in the thyroid gland [51]. Importantly, this role of RET is reproduced in both knockout [45] and Hirschsprung's/MTC knockin mouse models (RET C620R) [52, 53]. However, loss of GFRA4 does not impair C-cell numbers despite a decrease in calcitonin production [51, 54]. Thus, RET function is required in the development of a number of tissues.

Recently, a role of RET in regulation of the number of pituitary stem cells has been uncovered [55]. In this organ, RET limits somatotrophs numbers by regulating apoptosis [56]. In the mouse retina, specific ablation of Nrtn-mediated RET activation leads to reduced scotopic and photopic responses [57]. In addition, RET drives hematopoietic stem cell survival, expansion, and function, and its ligands are produced in the bone marrow environment [58]. Finally, GDNF/RET signaling has a clearly established role in driving spermatogonial stem cell self-renewal in the testis and is therefore essential to male fertility [17, 59–61]. Thus, in therapies targeting RET activity, a potential impact on maintenance of specific adult tissues and on stem cells driving several regenerative processes must be considered.

Functional Roles in Neuroendocrine and Nervous System Cells

Although its specific roles in normal neuroendocrine cells remain to be elucidated, RET has been extensively studied in the nervous system. Most notably, within the central nervous system, GDNF/RET signaling is critical for the maintenance of the nigrostriatal pathway by preventing dopaminergic neuron death and onset of Parkinson's disease [25, 26, 62]. For this reason, neurobiologists are looking for tools that specifically activate RET signaling rather than inhibiting it. Because of the limited proliferation capacity of neurons, the role of RET is predominantly to maintain their survival and inhibit apoptosis. Thus, cancer therapies seeking to reduce RET activity may potentially cause neuronal cell death, though Parkinsonian symptoms have not been associated with long-term treatments to date.

Much of our understanding of RET function in neuroendocrine systems derives from its study in transformed cells; as a result, little is known about its function in differentiated cell types. RET is expressed in both thyroid parafollicular

C-cells and follicular cells [63], but its role in adult C-cell function or proliferation is not known. The impact of targeted knockout of RET in adult mouse C-cells has not been examined. It is noteworthy however that at least at the mRNA level, no changes in RET expression are observed during cellular regeneration following partial thyroidectomy in mice, a process marked by a C-cell hyperplasia [64]. This observation is in opposition to studies reporting elevated RET immunoreactivity associated with C-cell hyperplasia in humans [65]. RET is weakly expressed in the normal adrenal medulla that exhibits neuronal cytological features including vesicular nuclei with prominent nucleoli [66]. RET expression increases after the onset of chromaffin cell proliferation in diffuse adrenal medullary hyperplasia associated with multiple endocrine neoplasia (MEN) 2A [66]. Finally, RET expression is found in normal parathyroid tissues and adenomas without detection of any mutation, suggesting that RET plays a minor role in proliferation of the parathyroid cells relative to its substantial role in C-cell growth [67].

RET in C-cell Transformation

Dysregulation of growth factor receptor signaling, both directly and indirectly through alterations of intracellular pathway components, is considered a hallmark feature of carcinogenesis [68]. Growth factors are instrumental for progenitor cell survival, normal C-cell development, migration from the neural crest, and finally regulation of C-cell function and proliferation. The pathways they trigger are normally tightly regulated. Imbalance of these factors and mutations of their pathway components is therefore associated with the development of MTC. The RET receptor is perhaps the most common target of dysregulation associated with MTC and thus the focus of this section. However, other components of downstream signaling pathways such as RAS or RAF can also be mutated or dysregulated. For a more extensive discussion of other mechanisms of C-cell oncogenesis, the reader is referred to the following excellent recent reviews [6, 7].

Activating RET Mutations Associated with Hereditary and Sporadic MTC

The *RET* proto-oncogene can cause or contribute to oncogenesis by several different mechanisms. Activating mutations, caused either by single nucleotide substitutions commonly found in MTC or rearrangements found in papillary thyroid, lung, or colorectal cancer, are the most commonly noted abnormalities of RET. In other neoplasms such as breast cancer, RET may be overexpressed. Finally, increased expression of the growth factors (GDNF, PSP, ARTM, and NTN) or GFRA1-4 may result in greater activation of the receptor, or a RET polymorphism such as codon 691 may result in greater receptor affinity for ligand [69–76]. Germline-activating mutations of *RET* are the cause of MEN2. A detailed discussion of specific mutations associated with MEN2 appears in Chap. 8 of this book, and a continually updated ARUP database is maintained online [77]. Activating mutations of the *RET* receptor gene fall into two major classes, which affect either the extracellular or the intracellular domain of the protein (Fig. 5.4). The extracellular cysteine-rich domain of RET is most frequently mutated at cysteine 634 (85 % of the extracellular domain mutations), but mutations also occur distributed among all extracellular cysteines (C609, C611, C618, C620, and C630) [78]. Intracellular RET mutations are observed in the RET kinase domain and with the exception of codon 918, which is specifically associated with MEN2B, have a variable impact on RET activity. Since the initial association of *RET* mutations with MEN2A, the frequency of intracellular mutations has increased from 2 to 52 % [79–82]. The major factor in this increase appears to be the routine application of germline genetic testing in patients with apparent sporadic MTC. Previously, these patients were classified as sporadic MTC, because “noisy” manifestations of MEN2A such as pheochromocytoma or hyperparathyroidism are less common with intracellular domain mutations. As the field has adopted routine germline testing for all patients with sporadic MTC, these mutations have revealed themselves.

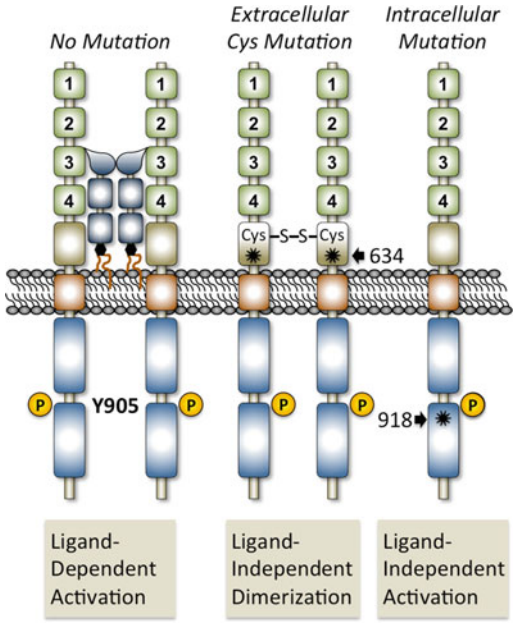

Fig. 5.4 Mechanism of oncogenic RET activation. Shown is a schematic figure outlining the normal ligand-dependent activation of RET compared with ligand-independent mechanisms resulting from receptor mutation. The *asterisks* show the general location of two common activating RET mutations. In all cases, RET activation is associated with initial phosphorylation (P) of tyrosine (Y) at codon 905 located within the intracellular domain

Somatic *RET* mutations are observed in approximately 40 % of sporadic MTC [4]. A comparison between databases reporting germline (ARUP) and somatic (COSMIC) mutations finds that approximately 20 % of codons targeted by germline mutations are also targeted in sporadic MTC [4, 77]. Thus, it is important to consider the possibility that mutations detected in tumors may represent hereditary MTC. Additionally, the distribution of somatic *RET* mutations differs greatly from germline mutations. In sporadic MTC, the M918T mutation is most frequently observed (~70 % of *RET* mutations), distantly followed by mutations involving codon 634 (~10 %) or deletions targeting extracellular cysteines (6 %) [4]. Several other *RET* mutations are reported at greatly reduced frequency. The high frequency of the M918T mutation defines it as the most prevalent RET-mediated route to C-cell oncogenesis.

The molecular mechanisms by which the major *RET* mutations trigger transformation have been partially elucidated [48, 83, 84]. As discussed, the normal route to activation involves binding of a GFRA1-4/ligand complex to RET, which promotes dimerization and in turn autophosphorylation of the receptor. Mutations present in the extracellular domain which targets the highly conserved cysteine residues affect receptor dimerization. Because these residues are normally involved in intramolecular disulfide bond formation, mutation generates a free sulfhydryl group. Mutant RET receptors in close proximity will then form intermolecular disulfide bonds, which function to stabilize dimerization even in the absence of the ligand (Fig. 5.4). This will therefore constitutively activate autophosphorylation and the intracellular pathways downstream of the receptor [83, 85, 86].

In contrast, mutations in the intracellular tyrosine kinase domain of RET target regions associated with the ATP-binding pocket, activation loop, and substrate binding pocket [83, 87, 88]. Depending on their specific location, they also induce structural changes that alter RET substrate specificity. Thus, these changes could result in increased ATP binding, decreased autoinhibition, and increased kinase activity. Therefore, the mutated receptor no longer needs to dimerize and can induce signal as a monomer, or in some cases, the receptor can be synergistically activated through ligand-mediated dimerization. Activated RET is then linked to cellular pathways known to contribute to enhanced proliferation, anti-apoptosis, and invasive behavior of the tumor cells. To date, few consistent mutations other than those in RET have been uncovered in tumors from MEN2 patients with defined germline mutations [89, 90]. Thus, mutation of RET appears to sufficient for transformation, although the long period of latency (1–20 years) suggests there must be other as yet unrecognized events occurring.

Animal Models of RET-Driven MTC

The discovery that *RET* mutations cause MEN2 began the work to generate genetic mouse models to better understand the pathogenesis of this disease. Early transgenic mice used the CT/CGRP promoter to target the thyroid C cell, focusing on the common RET mutations C634R (MEN2A) and M918T (MEN2B) [91–93]. A single *RET* C634R (MEN2A) transgenic line has been created using nonspecific Moloney murine leukemia virus LTR [94]. Direct comparison of transgenic mouse models is difficult given that in addition to different promoters, one must account for a variable number of integrated gene copies in each founder line and the use of long or short RET isoforms as transgenes. Despite these variations, all mice studied to date have been reported to develop C-cell hyperplasia as a precursor to MTC (Table 5.1), but, unlike humans, the overall penetrance of MTC is low in most models. Furthermore, the progression of MTC in mice expressing *RET* M918T appeared less aggressive than that observed in C634R models though this may be related to transgene expression levels. Genetic knockin models, which provide a more accurate representation of human disease, actually fail to develop MTC [52, 53, 95]. The *RET* M919T mouse model (in mice, *RET* M919T is equivalent to the human MEN2B M918T) develops early onset of C-cell hyperplasia and pheochromocytoma. It is also the only RET model described to develop pheochromocytoma. MEN2A models bearing a *RET* C620R mutation display a colonic pathology consistent with Hirschsprung's disease and a low incidence of C-cell hyperplasia in older age (Table 5.1). Finally, it should be noted that similar to humans, RET-independent MTC formation has been observed in animal models [7, 96].

RET Interactions with Other Mediators of Tumorigenesis

The differential ages of onset of MTC in humans, a phenomenon also shown in mouse models, argue for additional events beyond RET activation in C-cell oncogenesis. Prospective screening studies from the 1970s through the early 1990s document a spectrum of ages, ranging from 5 to 25 years, for the development of serum calcitonin abnormalities in kindred with an identical mutation [97]. Two mouse studies provide direct support for the hypothesis that RET-mediated oncogenesis requires a cooperative genetic background. First, Cranston et al. demonstrated that the development of MTC was highly strain-specific. Using mice with a single *RET* C634R insertion driven from the CT/CGRP promoter, tumor production varied from zero in FVB/n mice to nearly 100 % in CBA/ca mice (Table 5.1) [98]. Of note, the RET MEN2B knockin mouse model was established in an FVB/n mixed background, which might explain its failure to develop MTC. The strain-specific genetic modifiers responsible for differential MTC development remain to be identified. The second study is an extensive comparison of *RET* M918T-mediated tumorigenesis in mice with copy number variations of the cyclin-dependent kinase inhibitor genes *p18* (*CDKN2C*) and *p27* (*CDKN1B*) [99]. This work demonstrates clear synergism between aberrant RET activation and deregulation of cell cycle progression (Table 5.1). In humans, hemizygous loss of *CDKN2C* is observed in approximately 40 % of sporadic MTC tumors as demonstrated by chromosome 1p deletions, while *CDKN1B* loss appears to be a rare event (less than 5 %) [100, 101]. Furthermore, a more aggressive phenotype is observed in patients with coincident somatic *RET* M918T mutation and *CDKN2C* loss, suggesting a synergism similar to that observed in mice (unpublished observations).

Other potential mediators of RET function have been proposed based on the identification of genetic changes in MTC cell lines and tumors. In patient tumors, *RET* mutation is associated with nonspecific increases in both copy number variants and somatic mutations genes other than *RET* [89, 100, 101]. The suggestion is that aberrant activation of RET destabilizes the genome. It was the frequent loss of 22q (~40 %) in MTC

Table 5.1 RET mouse models of MTC

Genetic target	C cell phenotype	Reference
Transgenic models		
Tg-RET C634R short isoform Rat CT/CGRP promoter Human RET C634R	MTC seen in 3 of 4 founder lines CCH or MTC in 93 % of mice MTC seen at 8, 13, 14 months in each founder	[92]
Tg-RET C634R long isoform Human CT/CGRP promoter Human RET C634R	MTC only seen in 1 of 3 founder lines (2A-3) MTC/PTC seen in 1 of 3 founder lines (2A-1) MTC in 35 % of 2A-3 at 3–4 months MTC in 70 % of 2A-3 at 12 or older months	[93]
Tg-RET M918T short isoform Human CT/CGRP promoter Human RET M918T	MTC seen in 3 founders, highest in line 42 CCH in 77 % of line 42 mice at 8 months MTC in 13 % of line 42 mice at 11 months	[91]
Tg-RET C634R long isoform MoMuLV LTR promoter Human RET C634R	Two founder lines only 1 with MTC Line 121 (22 copies) MTC in 100 % of mice at 9 months	[94]
KnockIn models		
MEN2B M919T knockin $ret^{MEN2B/+}$ $ret^{MEN2B/MEN2B}$	Nodular CCH in 14 % at 8–12 months Nodular CCH in 60 % at 6–10 months	[95]
MEN2A C620R knockin $ret^{C620R/+}$ $ret^{C620R/+}$ $ret^{C620R/C620R}$	No thyroid phenotype out to 24 months CCH in 48.4 % compared to 22.8 % in control mice at 20–30 months Homozygous is a neonatal lethal	[52] [53] [52]
Role of modifier genes		
Tg-RET C634R long isoform Human CT/CGRP promoter Human RET C634R CT-2A-3 (1 copy)	MTC in 0 % FVB/n mice at 43 weeks MTC in 14 % of BALB/c mice at 43 weeks MTC in 64 % of C57BL/6J 9 mice at 43 weeks MTC in 98 % of CBA/ca mice at 43 weeks	[93, 98]
Tg-RET M918T with: p18+/+; p27+/+ p18+/-; p27 +/+ p18−/−; p27+/+ p18+/+; p27+/- p18+/-; p27 +/- p18−/−; p27+/-	MTC in 0 % of mice at 12 months MTC in 26 % of mice at 12 months MTC in 43 % of mice at 12 months MTC in 4 % of mice at 12 months MTC in 27 % of mice at 12 months MTC in 100 % of mice at 9 months	[99]

tumors [100, 101] that led to the identification of ATF4 as a mediator of RET anti-apoptotic function [44]. Unfortunately, exome sequencing studies have yet to uncover genes or even pathways targeted at a similar high frequency in MTC tumors with activating *RET* mutations [89, 90]. Therefore, roles for mediators of RET oncogenic function beyond CDKN2C and ATF4 remain to be identified.

RET as a Therapeutic Target

It was the discovery that activating *RET* mutations caused MEN2 and the subsequent finding that they exist at high frequency in sporadic MTC tumors which led to its consideration as a therapeutic target. The first experimental demonstration that MEN2-mutated RET had

clear transforming activity was demonstrated by transfection of NIH/3T3 cells [83]. Both the common RET C634R MEN2A mutation and the RET M918T MEN2B mutation demonstrated transforming activities through dimer-dependent and dimer-independent mechanisms, respectively. Mouse modeling further confirmed a role of mutated *RET* in the initiation of C-cell oncogenesis but was unable to address whether continuous activation of RET was required for tumorigenesis [92]. The first evidence that RET is critical for C-cell tumor progression was the demonstration of acquired *RET* mutations in MTC tumors found in *Rb*+/-; *p53*+/- mice [102]. These findings showed a clear role of activated RET in tumor progression following initiation, which were subsequently supported by subsequent mouse modeling [99]. Together, these data established that oncogenic growth of thyroid C cells is driven by aberrantly activated RET, a mechanism that has become known as "oncogene addiction." It was therefore reasonable to postulate that inhibition of RET could inhibit tumor cell proliferation.

Inhibition of RET in Cell Line Models

It is difficult to attempt to pinpoint the earliest demonstrations of RET inhibition in MTC as rat and human MTC cell line models actually predate the discovery of RET as a key driver. However, one of the earliest examples of specific targeting of RET to inhibit transformation is studies using ribozymes [103, 104]. These RNA-based enzymes, which predate RNAi, provided the first molecular interventional approach for genetic knockdown through the targeting of expressed RNA. Hammerhead ribozymes targeting mutant RET mRNA efficiently inhibited RET-mediated transformation of NIH/3T3 cells [103]. Studies that followed demonstrated that targeting RET with either ribozymes [104] or expressing its dominant-negative form [105] was capable of inhibiting growth of the human MTC cell line TT. While both studies clearly demonstrated RET as a viable therapeutic target, the requirement for viral delivery of these macromolecules impeded their clinical application. However, the development of the first small molecule tyrosine kinase inhibitor STI-571 (Imatinib, Gleevec) against ABL [106], the subsequent demonstration of its activity against the KIT receptor tyrosine kinase [107], and its rapid FDA approval for use in the treatment of cancer [108] signaled a change in how treatment of MTC could be approached [109]. Several groups quickly tested to see whether STI-571 functioned to inhibit MTC cell growth through inhibition of RET [110, 111]. The initial excitement of demonstrating growth inhibition mediated through RET was lessened by the discovery that inhibition was not achieved at clinically relevant doses [112]. Ultimately, other drugs where discovered to effectively inhibit RET activity [113, 114]. The fact that one of these compounds, ZD6474 (vandetanib, Caprelsa®), had already completed Phase I patient studies immediately brought this drug to the forefront. The cell line studies demonstrating efficacy of ZD6474, and subsequently XL184 (cabozantinib, Cometriq®), were not published until well after clinical trials were initiated [115, 116].

Inhibition of RET in Animal Models

Evidence that RET inhibition reduced cellular proliferation in culture led to testing in animal models. The first demonstration that targeted inhibition of RET altered tumor growth was done through adenovirus delivery of dominant-negative RET into TT cells [117]. Treatment of TT cells prior to nude mouse injection completely inhibited tumor formation, while a single intratumoral injection of adenovirus into established tumors increased the median survival of animals threefold. Adenoviral transfer of dominant-negative RET reduced endogenous tumor size in the RET C634R transgenic mouse model [118] and clearly demonstrated the potential value of RET inhibition in established MTC. Pharmacological inhibition of RET was first reported with the tyrosine kinase inhibitor CEP-751 and its prodrug,

CEP-2563, which slowed TT xenograft growth by approximately 50 % [114]. While these studies clearly established the effectiveness of targeting MTC tumors in mice, preclinical testing of ZD6474 took a slightly different route. Instead, a mutant *Drosophila* containing equivalent MEN2A and MEN2B *RET* mutations was fed ZD6474 [119]. The studies demonstrated that ZD6474 was able to reverse a RET-dependent eye phenotype and further was not toxic to flies. These are the only published animal studies pre-dating vandetanib clinical trials. The first report of ZD6474 inhibition of MTC xenograft grown in nude mice was not published until after the completion of the Phase II trial conducted in MTC patients [120, 121]. Demonstration of the effectiveness of XL184 in treating human MTC in mice has taken a similar route. The original studies demonstrating XL184 treatment that slows MTC cell xenograft growth remain unpublished (except in abstract form) though a recent study completed after the 2012 Food and Drug Administration (FDA) approval of this drug exists [122].

Early Promise of RET Inhibition in Patients with Metastatic MTC

At the time of the writing of this chapter, two FDA-approved drugs were available for the treatment of MTC patients with metastatic progressing disease, ZD6474/vandetanib and XL184/cabozantinib. The use of these drugs in patient care, as well as other therapies targeting RET and its pathway, is discussed in other chapters of this book. The goal of this section was simply to recount the strategy by which RET targeting moved from "bench to bedside." Vandetanib was developed as an anti-angiogenic drug specifically designed to target VEGF receptor family members. While the results of vandetanib Phase I trial demonstrated patient safety, initial tumor responses were not as robust as hoped [123]. It was the discovery that vandetanib-targeted tyrosine kinase receptors beyond VEGF, importantly RET, allowed for a repurposing of this drug. An open label, two-stage, Phase II study to evaluate vandetanib in the treatment of advanced MTC enrolled its first patient in November of 2004 [124]. Just a year later, positive objective remissions and stabilization of tumor growth were reported forever changing concepts related to systemic treatment of MTC [125]. While these results prompted the immediate consideration of other drugs, not all tyrosine kinase inhibitors showed the same level of response [126, 127]. It was not until a few years later that the Phase I studies testing the highly potent RET inhibitor cabozantinib (in vitro IC50 1.8 nM) demonstrated a second drug with consistent objective responses in patients with metastatic MTC [128]. Ultimately, the Phase III clinical trials for vandetanib and cabozantinib demonstrated that targeting of RET is beneficial for a subset of MTC patients [129, 130]. However, the actual role that RET targeting plays in the treatment of patients with these drugs remains to be fully addressed as *RET* mutation status was not fully monitored in these trials. Finally, it is important to consider that RET plays fundamental roles in normal cells beyond the thyroid C cell that might be impacted by targeted treatment (see Sect. 4.3). Thus, a clearer understanding of the biology of the treatment response for these and related drugs will help identify those patients that will benefit most from RET targeting, ultimately providing better patient outcomes.

Summary

The RET receptor tyrosine kinase, similar to other RTKs, plays a fundamental role in transducing extracellular growth factor signals, primarily through activation of the RAS/MAPK or PI3K pathways. However, like other RTKs, the actions of RET are not limited to the cell surface and complementary actions are now known to occur within the nucleus. Fundamental roles of RET in the formation of several tissues including the enteric nervous system, kidneys, and male gonads have been established through the development of animal models and the genetic association of RET deficiency with Hirschsprung's disease [131, 132].

However, it was the identification of aberrantly activated RET as a driver of MTC that drove understanding of RET and how inhibition of RET could be used to treat this tumor. Asymptomatic individuals with RET germline mutations can be treated with prophylactic thyroidectomy. When metastatic MTC is present, RET again serves to direct treatment as targeted small molecule inhibitors provide reduction and stabilization of tumor growth. Targeted treatment is based on the observation that inactivation of RET in MTC cell culture models causes a marked reduction in tumor cell growth, supporting a mechanism of oncogene addiction [115, 133]. Unfortunately, little is known about the mechanisms behind the C cell's unique sensitivity to RET-mediated oncogenesis or pathways beyond RET that play critical roles in tumor progression. Genetic modeling in mice is beginning to help gain insight into both processes and is leading to a more in-depth examination of the molecular biology of MTC. Indeed, new studies suggest that targeting the nuclear functions of RET may be critical in enhancing treatment responses [44]. Currently, only surgery provides a long-term cure for MTC, and the timing of surgery is critically tied to RET mutation analysis. In sporadic MTC, knowledge of RET activation plays a role in targeted inhibitor treatment, but the mechanisms surrounding response remain unclear. A greater understanding of the function of RET and other genes that contribute to C-cell oncogenesis is still needed to guide future treatment strategies.

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Genetic Evaluation of the Patient with Medullary Thyroid Cancer

6

Jennifer L. Geurts

Introduction

The hereditary form of medullary thyroid cancer (MTC) is termed multiple endocrine neoplasia type 2 (MEN2). The *RET* gene is the only gene known to cause MEN2 syndrome, and up to 98 % of patients with MEN2 have an identifiable pathogenic mutation in this gene. The *RE*arranged during *T*ransfection (*RET*) proto-oncogene is located on chromosome 10q11.21, comprised of 21 exons, approximately 11,000 amino acids, and spans over 55 kb of genomic sequence. MEN2 is thought to have a prevalence of 1 in 35,000 people [1]. The condition is autosomal-dominant; offspring have a 50 % of inheriting the mutation, regardless of gender.

Approximately 70–75 % of MTC cases are considered sporadic, due to acquired somatic mutations rather than a hereditary cause. The remaining 25–30 % are hereditary, attributed to a germline pathogenic mutation in the *RET* gene, causing MEN2. MTC in the setting of MEN2 typically presents at a younger age than in sporadic MTC. C-cell hyperplasia along with multifocal disease is more common in MEN2-associated MTC. All patients with MTC or C-cell hyperplasia should have *RET* gene testing regardless of age at diagnosis or family history. Early identification of patients with *RET* gene mutations allows for pre-clinical disease to be detected in family members, resulting in a higher cure rate of affected patients and a better prognosis.

History of MEN2

Pentagastrin stimulation testing historically had been used as an index of C-cell hyperfunction before the *RET* gene had been cloned. In 1993, once the MTC susceptibility locus had been identified, *RET* gene testing could be utilized for the identification of at-risk family members [2, 3]. The genotype–phenotype management implications were appreciated in the years to follow as mutations in additional *RET* codons became identified [4–7]. The *RET* gene testing confirms or rules out a diagnosis MEN2 before there is any biochemical or clinical evidence of disease, and for this reason, DNA testing is superior to biochemical screening. The approach of stimulating secretion of calcitonin by either pentagastrin or calcium infusion is no longer recommended, since elevated calcitonin is not a specific or sensitive enough marker for MTC [8].

Subtypes of MEN2

MEN 2 is comprised of two distinct subtypes (Table 6.1):

J.L. Geurts (✉)
Division of Surgical Oncology, Department of Surgery, Medical College of Wisconsin, 8701 Watertown Plank Rd, Milwaukee, WI 53226, USA
e-mail: jgeurts@mcw.edu

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Table 6.1 Genotype–phenotype correlations in MEN2

RET gene mutation protein change	Exon	MEN2 subtype	MTC risk level[a]	Incidence of PCC[b]	Incidence of pHPT[b]	CLA[c]	HD[c]
G533C	8	MEN2A	MOD	+	–	N	N
C609F/G/R/S/Y	10	MEN2A	MOD	+/++	+	N	Y
C611F/G/S/Y/W	10	MEN2A	MOD	+/++	+	N	Y
C618F/R/S	10	MEN2A	MOD	+/++	+	N	Y
C620F/R/S	10	MEN2A	MOD	+/++	+	N	Y
C630R/Y	11	MEN2A	MOD	+/++	+	N	N
D631Y	11	MEN2A	MOD	+++	–	N	N
C634F/G/R/S/W/Y	11	MEN2A	H	+++	++	Y	N
K666E	11	MEN2A	MOD	+	–	N	N
E768D	13	MEN2A	MOD	–	–	N	N
L790F	13	MEN2A	MOD	+	–	N	N
V804L	14	MEN2A	MOD	+	+	N	N
V804M	14	MEN2A	MOD	+	+	Y	N
A883F	15	MEN2B	H	+++	–	N	N
S891A	15	MEN2A	MOD	+	+	N	N
R912P	16	MEN2A	MOD	–	–	N	N
M918T	16	MEN2B	HST	+++	–	N	N

Adapted from 2015 ATA Guidelines [27]
[a]Risk of aggressive MTC: MOD, moderate; H, high; HST, highest
[b]Incidence of PCC and pHPT: + = ~10 %; ++ = ~20–30 %; +++ = ~50 %
[c]Y, positive occurrence; N, negative occurrence

- MEN2A, which is suspected in individuals with one or more specific endocrine tumors: MTC, pheochromocytoma, or parathyroid adenoma/hyperplasia.
- MEN2B, which is suspected in individuals with MTC and characteristic dysmorphology described in detail below.

MEN2A was previously named Sipple syndrome, after the physician who first described an association between MTC, parathyroid adenoma, and pheochromocytoma in 1961 [9]. MTC is typically the first manifestation of MEN2A, with presentation prior to age 35 in most cases. In patients with MEN2A, primary hyperparathyroidism (pHPT) is rarely the first manifestation. The average age at diagnosis of pHPT is approximately 34 years; however, it has been reported in children as young as 2 years of age, and multigland parathyroid hyperplasia is frequently observed [10–13]. The pathogenic variants in the *RET* codon 634 are associated with the highest risk of pHPT (up to 30 %) [14].

Pheochromocytoma (PCC) risk in MEN2A is approximately 50 %, with many individuals developing bilateral pheochromocytomas. The risk of PCC development is variable and dependent on the specific pathogenic *RET* gene mutation (see Table 6.1). *RET* gene mutations in codon 634 represent some of the highest penetrance for PCC, with one study reporting an 88 % risk by 77 years of age [15]. Patients with exon 10 *RET* gene mutations have reduced penetrance, 4–26 % risk, depending on the specific codon altered [16]. Presentation of PCC typically occurs after MTC or at the time of initial MTC diagnosis and subsequent workup. However, in up to 27 % of individuals with MEN2A, the PCC will be the first recognized manifestation [17–19]. MEN2-related PCC is often diagnosed at a younger age than in sporadic PCC, and

patients typically have subtle symptoms or may be asymptomatic [20, 21]. For patients with initially a unilateral PCC, a contralateral PCC will usually develop within 10 years [22]. Extra-adrenal paraganglioma occurrence has rarely been reported, and malignant transformation of PCC has been observed in approximately 4 % of cases [19, 23]. Failure to recognize PCC can have a lethal effect due intractable hypertension or anesthesia-induced hypertensive crises. The importance of genetic identification of MEN2 is highlighted by the fact that historically (prior to DNA analysis), the most common cause of death in MEN2 was complications from PCC not metastatic MTC [24].

The disease spectrum of MEN2A includes patients with cutaneous lichen amyloidosis (CLA) and Hirschsprung disease (HD). CLA presents as intense pruritus with hyperpigmented lesions that subsequently develop from scratching. The symptoms may present at a young age, prior to clinically evident MTC, and thus can serve as an early clinical indicator of MEN2 [25, 26]. CLA is not pathognomonic for MEN2A, as it can also appear sporadically or in a hereditary pattern independent of MEN2. Pathogenic *RET* gene mutations in exon 10 are associated with HD, which occurs due to abnormal development of enteric nerves during embryogenesis. Symptoms of HD include chronic constipation or diarrhea, abdominal swelling and may result in delayed growth. Indications typically present after birth, but may not be apparent until later in life.

Familial medullary thyroid cancer (FMTC) was historically regarded as a separate syndrome from MEN2A. FMTC was only considered when four or more family members across a wide range of ages had isolated MTC, with no other features of MEN2A. Currently, FMTC is thought of as a phenotypic variant with reduced penetrance of PCC and pHPT, a part of the syndrome that manifests at the milder end of the MEN2A clinical spectrum. In 2015, the American Thyroid Association (ATA) Task Force recommended that the MEN2 subtypes consist of only MEN2A and MEN2B [27]. Families with *RET* gene mutations have a history of MTC but no PCC or should be classified as a phenotypic variant of MEN2A. Families with the presence of MTC only should not be precluded from screening for PCC and pHPT.

MEN2B was historically referred to as mucosal neuroma syndrome and Wagenmann-Froboese syndrome [28]. The MTC in MEN2B typically presents in infancy or early childhood and is highly aggressive, leading to a higher morbidity and mortality than in MEN2A [27]. The distinct, recognizable facial features include mucosal neuromas on tongue, palate and lips, cutaneous neuromas, thickened and everted eyelids, and mild ptosis (Fig. 6.1). Approximately 75 % of MEN2B patients display a Marfanoid habitus with skeletal deformation, joint laxity, and decreased subcutaneous fat; however, they are not necessarily taller than average. Diffuse intestinal ganglioneuromas are seen in about 40 % of patients with MEN2B. Symptomatology includes abdominal distension, megacolon, constipation, or diarrhea. These bowel issues in early childhood and infancy may represent first symptom of MEN2B. The lifetime risk of PCC development is approximately 50 % in MEN2B with a high incidence of bilateral disease.

Genotype–phenotype Correlations

The genotype–phenotype correlations in MEN2 are well described and summarized in Table 6.1. Importantly, these observations dictate the

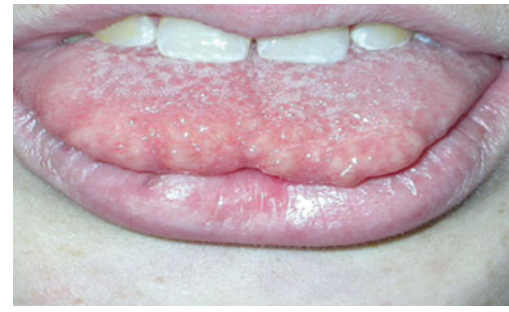

Fig. 6.1 Mucosal neuromas on the tongue of a patient with MEN2B

medical management and onset of interventions in the treatment of MEN2, the details of which are described in Chaps. 8, 9, and 10. The cysteine codons 609, 611, 618, and 620 in exon 10 of the *RET* gene are associated with MEN2A, HD, low transforming activity, and a ATA moderate MTC risk level [27, 29].

RET gene codon 634 pathogenic mutations are seen with a higher incidence of PCC and pHPT along with an ATA high MTC risk level [27, 30–32]. This codon location is also associated with the development of cutaneous lichen amyloidosis [33]. The V804M pathogenic mutation displays high intrafamilial variability, with some affected individuals diagnosed with MTC by 5 years of age, whereas their relative with the same pathogenic variant had no clinical evidence of MTC by 86 years of age [34, 35].

The pathogenic mutation M918T in the *RET* gene is associated with MEN2B and the ATA highest MTC risk level [27]. Approximately 95 % of patients with MEN2B have the exon 16 mutation, whereas fewer than 5 % have the exon 15 mutation (A883F) [36–39]. There have been a few reports of a rare group of patients with atypical MEN2B with a later onset. These patients have been identified as having 2 pathogenic *RET* mutations which are in cis (on the same allele) involving codon V804M and either Y806C, S904C, E805K, or Q781R [40–43]. In addition, there have been observations of rare variants that are thought to modify the phenotype of MEN2 when inherited in combination with a pathogenic variant [44, 45].

Allelic Disorders

In contrast to the gain of function mutations that cause MEN2, loss of function mutations in the *RET* gene causes familial HD. These loss of function mutations do not predispose to MTC, PCC, or pHPT. However, patients diagnosed with HD who have an exon 10 *RET* mutation should be evaluated for MEN2A, due to the phenotypic overlap of these syndromes.

Genetic Evaluation

Both the ATA and American Society of Clinical Oncology (ASCO) recommend all patients with C-cell hyperplasia, MTC, or with a clinical diagnosis MEN2 be offered germline *RET* gene testing and genetic counseling [27, 46]. Notably, up to 7 % of individuals with suspected sporadic MTC will be found to have a germline pathogenic mutation in the *RET* gene [47–50]. Identification of these previously unrecognized kindreds is imperative for early detection and disease prevention in affected individuals.

The majority of pathogenic *RET* gene mutations occur in exons 10, 11, and 13–16, although recently mutations in exons 5 and 8 also have been reported. Most *RET* mutations can be detected through sequence analysis of select exons. In the case of a distinct phenotype, the genotype can be evaluated in a tiered approach. For example, since 95 % of MEN2B patients have the M918T mutation, it may be most appropriate to assess that codon first and then, if negative, continue to A883F mutation testing. If the mutation is not identified in these first two tiers, one should proceed with sequencing of the entire *RET* gene coding region.

The *RET* gene is comprised of 21 exons, and full gene sequencing of all the exons is another testing approach; however, it may be more costly than select exon testing as only 9 of the exons are known to harbor pathogenic mutations. Therefore, *RET* full gene sequence is typically considered as a second tier test if no pathogenic variant is found by select exon testing. *RET* gene testing is a very accurate test for the diagnosis of MEN2. The analytical sensitivity and specificity of *RET* gene sequencing reaches 98 %. In general, the clinical sensitivity and specificity is 97–98 %; however, it is strongly dependent on multiple factors including phenotype, age at MTC diagnosis, and family history [51].

The *RET* gene mutations associated with MEN2 occur through a gain of function mechanism (typically missense variants). Therefore, testing for large gene deletions or duplications in

a patient with MEN2 is not indicated, as structural alterations typically result in a loss of function. The Genetic Testing Registry (GTR) provides a central location to identify clinical laboratories that offer *RET* gene testing (http://www.ncbi.nlm.nih.gov/gtr/).

All of the MEN2 subtypes are inherited in an autosomal-dominant manner, in which there is a 50 % chance to pass the condition on to each of the offspring, independent of gender. MEN2 displays variable expressivity and age-related penetrance, which is influenced by the genotype. It is important to recognize that a large number of patients with MEN2 will have no prior family history of the disease. In MEN2B, approximately 50–75 % of cases are due to a de novo germline *RET* gene pathogenic mutation [27, 52, 53]. In contrast, only 5–9 % of MEN2A cases are thought to be de novo [54]. The majority of de novo mutations in the *RET* gene originate from the paternal allele [53]. There has been one case of post-zygotic mosaicism reported in the literature [55]. Mosaicism is defined as when an individual or tissue is composed of cells of more than one genotype. This type of mutation would be acquired during embryogenesis rather than inherited from a parent. However, if the mutation is present in the affected individual's reproductive organs, it can be passed on to the next generation, which has important genetic counseling implications.

When an individual with MEN2A is newly diagnosed, even if family history is not suggestive of a hereditary disease, it is still appropriate to evaluate the parents as reduced penetrance may be the cause rather than a de novo mutation. Since there is a high de novo rate with MEN2, it is often assumed this is the mechanism when parent neither has the pathogenic variant nor has clinical evidence of the disorder. However, other plausible explanations should be addressed such as alternate paternity, undisclosed assisted reproduction (non-maternity due to egg donor), or adoption.

Gene Variant Classification

Most pathogenic *RET* gene variants identified in MEN2 are well-described mutations and occur independently in multiple unrelated families. However, some variants identified through *RET* gene sequencing may be novel, not previously reported in the medical literature. In 2015, the American College of Medical Genetics and Genetics (ACMG) along with the Associations for Molecular Pathology (AMP) published guidelines for the interpretation of sequence variants [56]. These standards are based on the consideration of multiple lines of evidence for pathogenicity or benign impact including population data, computational and predictive data, functional data, segregation data, de novo data, allelic data, phenotypic data, and reputable databases. The recommended classifications are as follows:

- Pathogenic: Collective evidence strongly supports that variant has damaging effect on protein structure or function.
- Likely pathogenic: Greater than 90 % certainty, the variant is disease-causing, high likelihood.
- Uncertain significance: Conflicting evidence regarding the functionality of this variant, often variant in this category has not been previously reported in the published literature, so there is not sufficient information to assess function. Clinical decision making should not be determined by this variant result, and management should be based on personal and family history.
- Likely benign: Greater than 90 % certainty, the variant is not disease-causing, high likelihood of benign.
- Benign: Collective evidence strongly supports that variant has no damaging effect on protein structure or function.

As the genetic knowledge regarding variation evolves, reclassification of unknown variants may occur based on emerging data. As population data have become more readily available

through public databases, many variants of uncertain significance have been reclassified as benign. For this reason, it is important that patients who receive an uncertain genetic test result maintain follow-up communications with their genetics provider as variant reclassification may impact their future care. Likely benign and benign variants are typically not included on a clinical genetic testing report, as they do not have implications for medical management.

A comprehensive database of all known *RET* gene variants is maintained by the University of Utah and ARUP Laboratories [44]. This database curates a record of *RET* gene mutations relevant to MEN2 along with associated clinical information, pertinent literature references, and genotype–phenotype correlations. There are over 100 germline single nucleotide variants, duplications, insertions, or deletions in the *RET* gene which have been identified in patients with MEN2. The most common pathogenic mutations are listed in Table 6.1, along with associated phenotypic information.

Genetic Counseling

Genetic testing for MEN2 has immense clinical utility as *RET* gene mutations are highly penetrant, and testing informs clinical decision making and facilitates the prevention or early detection of disease which leads to improved health outcomes. Considering the medical, social, ethical, and legal implications of genetic testing, informed consent, patient education, and genetic counseling are imperative. The National Comprehensive Cancer Network (NCCN) and ASCO have highlighted the importance of both pretest and posttest genetic counseling provided by a healthcare practitioner with education and experience in hereditary cancer syndromes, such as a genetic counselor [46, 57]. Pretest counseling is the process of educating patients regarding their medical options and the impact that genetic test results may have on them and their family members. Posttest counseling includes interpretation of test results, discussion of follow-up, and facilitation of communicating results to family members. Importantly, the patient must properly understand the meaning of a negative test result, interpretation of a variant of uncertain significance, and implications of a positive test result. The concept of a "false negative" result is possibly the most important message for the patient to comprehend. When a patient has a clinical diagnosis of MEN2 and gene sequencing fails to identify the causative *RET* mutation, the patient still maintains a diagnosis of MEN2 and appropriate medical management should follow. In families where the *RET* gene mutation is unidentified, the genetic testing is uninformative for at-risk family members, and biochemical screening and imaging screening protocols must be applied to all.

The elements of genetic counseling and informed consent in cancer susceptibility testing are summarized in Table 6.2. As genetic test results may have significant medical and psychological impact, adequate education and counseling is a necessity. An initial component of the informed consent process is educating the patient regarding the genetic test being performed, along with the medical management implications of the results for both the patient and possible family members. They should also understand that genetic testing is highly accurate and essential to disease risk estimation, acknowledging that other methods for detection are inadequate (i.e., pentagastrin stimulation). The most plausible risks with genetic testing include emotional distress, ambiguous test results, or misinterpretation of test results.

In the rare circumstance where genetic testing is not feasible for an individual with MEN2 or if *RET* gene sequencing fails to identify a causative mutation, DNA banking may be offered. This option provides long-term storage of extracted DNA for future genetic testing use. As the testing methodology and our knowledge of genetics improve, it may be valuable to retest a sample in the future.

Reproductive decision making and family planning is an essential component of genetic counseling. Prenatal testing is available to individuals with MEN2, through either invasive diagnostic testing (chorionic villus sampling in

Table 6.2 Elements of genetic counseling and informed consent

1. Information regarding the specific test being performed
2. Implications of a positive, uncertain, or negative result
3. Possibility that the test will not be informative
4. Options for risk estimation and disease detection without genetic testing
5. Inheritance pattern and risk of passing disease on to children
6. Technical accuracy and detection rate of the test
7. Psychological implications of test results (benefits and risks)
8. Genetic discrimination
9. Confidentiality and privacy
10. Medical management implications and prevention options following testing
11. Relevance of sharing genetic test results with at-risk relatives
12. Plans for disclosing test results and follow-up

the first trimester or amniocentesis in the second trimester) or noninvasive prenatal screening on cell-free fetal DNA obtained from maternal blood serum [58–60]. Preimplantation genetic diagnosis (PGD) is available to couples prior to conceiving a fetus if they desire to have children that are not affected with MEN2 [61]. PGD utilizes in vitro fertilization techniques that allow for single-site *RET* gene mutation testing in the early embryonic stage of a blastocyst. Embryos that do not contain the *RET* gene mutation are then implanted into the uterus. This method has led to the successful delivery of an unaffected child and may be an option for families who wish to eliminate the disease in successive generations [62]. The availability of PGD in the setting of MEN2 is not without controversy. Since there are interventions for MEN2 that lead to prevention and early detection of disease, morbidity and mortality can be reduced, particularly in MEN2A. Regardless, some patients may not desire to subject their future children to the same medicalization that they themselves went through. As these issues are ethically, legally, and socially complex, it is important for patients to be referred to a genetic counselor for a discussion regarding the technologies available and reproductive options. Clinicians should also provide clear medical documentation of the options that were discussed and patient decision making.

Both federal law and local state statutes provide levels of protection against genetic discrimination by employers and health insurers. The Genetic Information Nondiscrimination Act (GINA), signed to law in 2008, includes the following safeguards [63]:

- Prohibits health insurance carriers from using genetic information to determine eligibility or premiums and denying coverage based on genetic test results, and in addition, they cannot request or require genetic testing.
- Prohibits employers from using genetic information to influence employment decisions and forbids requesting, requiring, or purchasing genetic information about employees or their family members.

Patients should be informed that GINA does not cover life, disability, or long-term-care insurance and does not apply to members of the military. GINA applies to all employers with 15 or more employees, regardless if it is a not-for-profit organization or a corporation. The limitations of GINA are particularly relevant for pretest counseling of at-risk relatives who have yet to be diagnosed with cancer. Importantly, GINA has significant authority if insurers or employers test the effectiveness of the law, as the regulations are enforced by the Department of Health and Human Services, the Department of Labor, the Department of Treasury, and the Equal Opportunity Employment Commission. Remedies for violations include correction action

and monetary penalties against the discriminating agency. Additional federal protections that cover certain aspects of genetic information include the Americans with Disabilities Act of 1990 (ADA) and Health Insurance Portability and Accountability Act of 1996 (HIPAA).

There is some contradiction in the courts regarding "duty to warn" and genetic testing. ASCO exerts the provider's obligations to at-risk relatives that are fulfilled by communicating the familial risk to the patient undergoing testing and by emphasizing the importance of sharing this information with relatives [57]. Traditionally, a provider's primary responsibility is to their patient and protecting privacy. Both ethical and legal dilemmas can arise if patient confidentiality is breached to warn at-risk relatives. In addition, at-risk relatives may have their autonomy violated if their desire is not to know about genetic risks in the family. For these reasons, even before testing is initiated, it is important to broach the topic of disclosure to family members and importance of dissemination of information to at-risk relatives. Even when covering the issue of "duty to warn" during pretest informed consent, unforeseen difficulties may arise, management of which should include consultation by clinical ethicists, social workers, and legal counsel, and in addition, the ATA's Ethics Committee may provide guidance.

Genetic Evaluation of Relatives at Risk

Once a pathogenic *RET* gene mutation has been identified in a family, genetic counseling and testing should be offered to all at-risk relatives [27, 46]. Early identification of patients with *RET* gene mutations allows for preclinical disease to be detected, resulting in a higher cure rate of affected patients with better prognosis, improved quality of life, and prolonged life expectancy [63, 64]. When the familial mutation is known, subsequent family members who have a negative test can be reassured and can avoid unnecessary thyroidectomy and require no further biochemical screening. In addition, a normal gene test result in the setting of a family with a known pathogenic mutation may provide significant emotional relief for family members determined to be at average risk.

Due to the early interventions required for cancer prevention, testing children before the age of 5 is recommended in MEN2A families and shortly after birth in MEN2B families. This is in contrast to other hereditary cancer syndromes with adult onset disease where it is typically advised that minors wait until adulthood before pursuing gene testing. Since there are available and effective preventive strategies during childhood and a positive gene test result confers a high risk of cancer with onset in childhood, testing minors is accepted on clinical grounds in the case of MEN2. Genetic counseling should address the timing of results disclosure to children; typically, it is recommended that parents consider at what age the child has sufficient cognitive and emotional maturity to comprehend the results. As the child approaches adulthood, they will require this information for medical decision making and to inform their future healthcare providers. In addition, there are implications on reproductive decision making and unplanned pregnancy.

When a patient with a clinical diagnosis of MEN2 has an undetectable pathogenic *RET* gene mutation, the at-risk family members should undergo screening for MTC, PCC, and pHPT [27]. In this case, further genetic testing of unaffected family members would not be fruitful, and DNA banking for the affected individual should be considered.

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Preoperative Evaluation of the Patient with Medullary Thyroid Cancer

7

Glenda G. Callender and Tracy S. Wang

Introduction

Medullary thyroid carcinoma (MTC) represents only 1–2 % of all thyroid cancers in the USA [1]. Although MTC is relatively rare, optimal management requires the clinician to be familiar with the details of genetics, pathology, endocrinology, surgery, and radiology that specifically pertain to MTC. The purpose of this chapter is to summarize the current standard-of-care preoperative evaluation of the patient with MTC.

Preoperative History and Physical Exam

The evaluation of a patient with known or suspected MTC begins with a thorough history and physical examination. The clinician should ask about symptoms of mass effect, such as globus sensation, difficulty swallowing, pressure in the neck when turning the head or lying supine, difficulty breathing, and subjective voice changes, including hoarseness. MTC develops from C-cells, which are predominately located posteriorly in the upper portions of the thyroid lobes. This is clinically relevant: the posterior and superior location means that even small cancers can involve the esophagus, trachea, or recurrent laryngeal nerve. Approximately 15 % of patients present with dysphagia, dyspnea, or hoarseness, reflecting a locally advanced cancer [2]. In 5–10 % of patients with MTC, distant metastatic disease is present at the time of presentation [2, 3], which may cause symptoms such as flushing and diarrhea due to high serum levels of calcitonin (hypercalcitoninemia) or local pain from bone metastasis. Patients should be asked specifically about any prior history of surgery or radiation to the head or neck, with special attention paid to eliciting details about procedures in the central and lateral neck, including previous thyroidectomy, parathyroidectomy, carotid endarterectomy, cervical spine procedures, and thoracic procedures.

Up to 25 % of cases of MTC are hereditary, and thus, patients should be asked about symptoms of the component tumors that can arise alongside MTC in patients with an inherited endocrinopathy, such as multiple endocrine neoplasia (MEN), types 2A and 2B: primary hyperparathyroidism, and pheochromocytoma. This includes a personal history of kidney stones,

G.G. Callender (✉)
Department of Surgery, Yale University School of Medicine, New Haven, CT, USA
e-mail: glenda.callender@yale.edu

T.S. Wang
Section of Endocrine Surgery, Division of Surgical Oncology, Department of Surgery, Medical College of Wisconsin, Milwaukee, WI, USA
e-mail: tswang@mcw.edu

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pancreatitis, ulcers, osteoporosis, anxiety, tremor, panic attacks, headaches, difficult-to-control hypertension, and "spells." The family history is also essential, and care should be taken that appropriate broad-based questions are asked. For example, a patient may not be familiar with the medical details, but may recall that a family member had a scar on their neck. A family member with kidney stones, pancreatitis, ulcers, or osteoporosis may have had primary hyperparathyroidism. Likewise, a family member who had significant hypertension or who died of an unknown cause, especially at a young age, may have had a pheochromocytoma.

Physical examination should include a general assessment of the patient and focused evaluation of the neck, with particular attention to the characteristics of palpable thyroid nodules. A cancer is often hard and irregular, and an advanced, locally invasive cancer may be fixed to neck structures and immobile on palpation. More than 50 % of patients with MTC have central or lateral neck lymph node metastasis at the time of diagnosis; [2, 3] careful palpation of the cervical lymph node basins may detect lymph nodes that are grossly involved. It is important to auscultate over the carotid arteries to evaluate for a carotid bruit; thyroidectomy requires lateral retraction of the carotid arteries, which can lead to intraoperative stroke in a patient with carotid atherosclerotic disease. The skin overlying the upper back should be examined to evaluate for cutaneous lichen amyloidosis, a rare skin condition leading to a pruritic plaque over the upper back and scapula region that can be associated with MEN2A (Fig. 7.1). It is also important to note any physical feature characteristic of MEN2B, in particular a tall, thin (Marfanoid) body habitus and neuromas of the eyelids and anterior third of the tongue (Fig. 7.2).

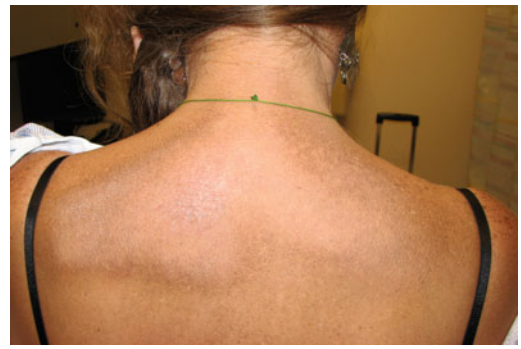

Fig. 7.1 Cutaneous lichen amyloidosis in a patient with MEN2A. From Callender et al. [29]

Preoperative vocal cord evaluation may be performed using direct (flexible fiberoptic or rigid) or indirect (mirror) laryngoscopy. Preoperative laryngoscopy is essential in a patient with hoarseness or voice changes, or in a patient who has previously undergone neck or mediastinal surgery that could have resulted in recurrent laryngeal nerve injury [4]. However, in a patient with a normal voice, and who has not undergone a prior procedure that could have led to an immobile vocal cord, laryngoscopy is unlikely to reveal an abnormality [5]. Nonetheless, preoperative

Fig. 7.2 Neuromas of the anterior tongue and eyelids in a patient with MEN2A. From Callender et al. [29]

laryngoscopy adds little time to the physical examination, creates minimal discomfort and risk to the patient, and has the potential to provide useful information; thus, it has become routine in many high-volume practices [6–8].

Biochemical Evaluation

The first step in the evaluation of a patient with a new, histologically confirmed diagnosis of MTC is the determination of whether the disease is sporadic or hereditary in nature. The management of sporadic and hereditary MTC differs to some degree, and failure to identify inherited MTC means that the patient may not be appropriately treated, and members of the patient's family may remain undiagnosed. Thus, according to the current (2015) American Thyroid Association (ATA) guidelines, every patient with a new diagnosis of MTC should undergo direct DNA analysis of peripheral blood cells in order to evaluate for the presence of a germline *RET* proto-oncogene mutation (U.S. Preventive Services Task Force, Agency for Healthcare Research and Quality, Grade B recommendation: based on fair evidence that the service or intervention can improve important health outcomes; Fig. 7.3) [1]. Although the incidence of a germline *RET* mutation in a patient with apparently sporadic MTC is only as high as 7 %, failure to identify hereditary disease could result in potentially severe consequences, and therefore, testing is justified and indicated in every individual diagnosed with MTC [9]. Although bilateral MTC is much more common in hereditary disease, bilateral MTC is present in up to 9 % of patients with sporadic MTC and a documented absence of a *RET* mutation; therefore a patient with bilateral MTC should not necessarily be assumed to have hereditary disease and should still undergo formal genetic counseling and testing [10–12].

In addition to germline *RET* testing, current ATA guidelines recommend that a patient with sporadic MTC or index-case hereditary MTC should undergo the measurement of serum calcitonin and carcinoembryonic antigen (CEA) levels to serve as baseline tumor markers and to indicate the extent of disease and guide decisions regarding the need for additional staging studies to identify distant metastatic disease (Grade B recommendation; Fig. 7.3) [1]. Occasionally, ultrasound and fine-needle aspiration (FNA) biopsy are insufficient to definitively confirm the presence of MTC; in such cases, serum calcitonin measurement may be necessary in order to make the diagnosis. A serum calcitonin level ≥100 pg/ml has a 100 % positive predictive value for MTC [13]. The usual ratio of calcitonin:CEA in MTC is approximately 10:1 [14]. If CEA is elevated out of proportion to calcitonin, the patient may require evaluation for the presence of another type of CEA-producing cancer (e.g., with colonoscopy). Serum calcitonin and CEA levels should also be measured in any patient who is an asymptomatic carrier of a *RET* mutation associated with MEN2 and for whom prophylactic thyroidectomy is planned.

Finally, according to current ATA guidelines, a patient with sporadic MTC or index-case hereditary MTC should undergo biochemical testing to evaluate for the presence of primary hyperparathyroidism and pheochromocytoma (Grade B recommendation; Fig. 7.3) [1]. Screening for primary hyperparathyroidism is performed by the measurement of serum calcium and intact parathyroid hormone (PTH) levels and should be performed preoperatively so that primary hyperparathyroidism, if present, can be surgically addressed at the same time as the initial thyroidectomy. Biochemical screening for pheochromocytoma is performed by the measurement of plasma-free metanephrine or 24-h urinary catecholamine levels. Elevated levels should prompt dedicated imaging of the abdomen with adrenal protocol computed tomography (CT) or magnetic resonance imaging (MRI). It is critical that pheochromocytoma be diagnosed preoperatively if it is present, so that the patient may be appropriately medically blocked prior to the administration of an anesthetic, and the potentially catastrophic cardiovascular consequences associated with massive catecholamine release can be avoided. When pheochromocytoma is present in a patient with newly diagnosed

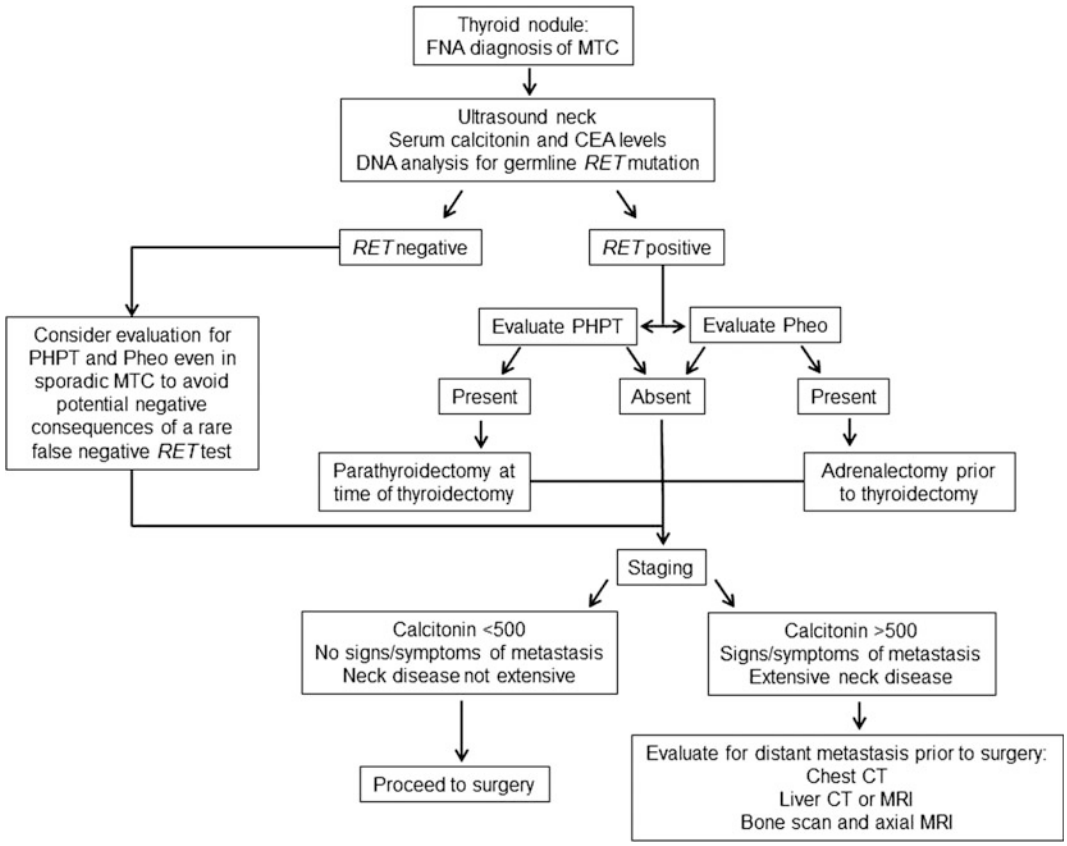

Fig. 7.3 Algorithm for the preoperative evaluation of the patient with newly diagnosed medullary thyroid carcinoma. Adapted from Wells et al. [1]

MTC, adrenalectomy should be performed prior to thyroidectomy [1]. Current ATA guidelines specifically recommend biochemical evaluation of primary hyperparathyroidism and pheochromocytoma in patients with documented or suspected hereditary MTC, but this recommendation is not specifically made for patients with sporadic MTC [1]. However, the consequences of missing pheochromocytoma or primary hyperparathyroidism in a patient scheduled to undergo thyroidectomy for MTC are potentially serious, and false-negative *RET* testing is theoretically possible (although rare) [15]. Thus, it makes intuitive sense to test all patients with MTC for the presence of these two disorders, rather than relying on *RET* mutational analysis alone.

Lifelong surveillance for the development of primary hyperparathyroidism and pheochromocytoma is critical; the age at which screening should begin is dependent on the specific codon mutation, given the genotype–phenotype relationship associated with MEN2 [16]. If the patient's specific *RET* mutation places them at risk to develop primary hyperparathyroidism and pheochromocytoma, these should be excluded biochemically starting at age 11 for the ATA "highest-risk" and "high-risk" categories (*RET* mutation M918T and *RET* mutations C634 and A883F, respectively), and starting at age 16 for the ATA "moderate-risk" category (*RET* mutations other than M918T, C634, and A883F) [1]. Most known carriers of RET mutations will have

already undergone prophylactic thyroidectomy by the age at which screening for primary hyperparathyroidism and pheochromocytoma is recommended to begin. A recent case report documented a 6-cm pheochromocytoma in an 8-year-old patient with MEN2A and a C634Y *RET* mutation; [17] therefore, a low threshold for biochemical evaluation should be maintained if there exists any clinical suspicion, even in a young child.

Imaging Evaluation

Staging for Locoregional Metastases

According to current ATA guidelines, a patient with a new diagnosis of MTC (sporadic MTC or index-case hereditary MTC) should undergo ultrasound examination of the neck for locoregional staging of disease (U.S. Preventive Services Task Force, Agency for Healthcare Research and Quality, Grade C recommendation: based on expert opinion; Fig. 7.3) [1]. The neck ultrasound should specifically examine the thyroid, central compartment (level VI) lymph nodes and bilateral lateral compartment (levels II–V) lymph nodes. Although preoperative high-resolution neck ultrasound only detects central compartment lymph node metastasis in approximately half of patients who are ultimately confirmed to have histopathology-proven involved lymph nodes, the sensitivity of high-resolution ultrasound to detect metastatic thyroid cancer in lateral compartment lymph nodes is 90–100 % [18–21]. Abnormal lymph nodes should be evaluated by FNA biopsy; biopsy-proven disease in the lateral compartment is an indication for a compartment-oriented lateral neck dissection.

A patient who is an asymptomatic carrier of a *RET* mutation associated with MEN2 should also undergo thorough cervical ultrasound prior to surgery (prophylactic thyroidectomy). Thyroid nodules meeting criteria for biopsy, based on consensus guidelines from the ATA and/or National Comprehensive Cancer Network, and any abnormal lymph nodes should be evaluated with FNA biopsy prior to surgery [22, 23].

Staging for Distant Metastases

A patient with a new diagnosis of MTC (sporadic MTC or index-case hereditary MTC) who appears to have disease limited to the neck requires no additional preoperative imaging studies beyond a neck ultrasound for locoregional staging. However, the presence of metastatic disease, especially if extensive, may alter the aggressiveness of planned neck surgery, and thus, preoperative evaluation of distant metastatic disease is important in the appropriate clinical setting. According to current ATA guidelines, indications for additional imaging to detect distant metastatic disease include extensive neck disease, signs or symptoms of distant metastasis, and a serum calcitonin level >500 pg/mL (Grade C recommendation; Fig. 7.3) [1]. Although evaluation for distant metastatic disease is recommended in the setting of extensive neck disease or signs/symptoms indicating possible distant metastasis even if the serum calcitonin level is <500 pg/mL, a recent study evaluating 300 patients with MTC revealed that no distant metastases were detected on imaging studies if the baseline serum calcitonin level was <500 pg/mL [24].

Appropriate imaging studies to evaluate for distant metastases include chest CT to evaluate for metastases to the lung and mediastinal lymph nodes; 3-phase contrast-enhanced multidetector liver CT or contrast-enhanced MRI to evaluate for liver metastases; and both bone scintigraphy and axial MRI to detect bone metastases [25]. According to current ATA guidelines, neither 2-[^{18}F] –fluoro-2-deoxy-D-glucose positron emission tomography/CT (FDG-PET/CT) nor ^{18}F-dihydroxyphenyl-alanine F-DOPA-PET/CT are recommended in this patient population, as they are less sensitive than the other imaging procedures (Grade C recommendation) [1, 25].

Preoperative Evaluation of Patients Requiring Reoperation

In a patient with persistent or recurrent MTC, reoperation has the potential to offer cure if disease is limited to the neck. Preoperative evaluation in this setting is similar to evaluation prior to initial neck surgery for MTC. Prior operative notes and pathology reports must be obtained and carefully examined for documentation of recurrent laryngeal nerve anatomy, location of in situ or auto-transplanted parathyroid glands, boundaries of prior lymph node dissection, etc. Physical examination must include laryngoscopy to evaluate for potentially occult recurrent laryngeal nerve injury at the time of the prior neck operation. Measurement of serum calcitonin and CEA levels should be performed to document new baselines and to give an indication of extent of disease. High-resolution ultrasound of the neck, including the thyroid bed, central compartment lymph nodes, and bilateral lateral compartment lymph nodes, should be performed, with FNA biopsy of any abnormal findings. A CT of the neck and upper mediastinum should be considered: although no data are available for patients with MTC, CT neck has been shown to provide additional anatomic detail over neck ultrasound in patients undergoing reoperation for papillary thyroid cancer (e.g., suggestion of tracheal invasion and more extensive lymph node involvement than indicated by neck ultrasound, especially in the central compartment) [26].

Reoperative neck surgery carries additional risks over initial neck surgery; therefore, a distant metastatic evaluation with the imaging studies described in the previous section is typically performed prior to subjecting the patient to reoperative neck surgery, particularly in the setting of substantial hypercalcitoninemia. Although FDG-PET/CT and DOPA-PET/CT are less sensitive for the evaluation of metastatic MTC than are neck ultrasonography, chest CT, abdominal CT or MRI, or bone scan in combination with MRI of the axial skeleton, they can occasionally detect lesions not revealed by the aforementioned imaging studies and thus can be useful in the reoperative setting if routine imaging is negative but a very high serum calcitonin level suggests metastases [25, 27]. Liver metastases in MTC can be miliary in nature and thus very difficult to detect on standard imaging. A study from 1995 evaluated open or laparoscopic liver biopsy as well as selective venous sampling with the measurement of hepatic and peripheral vein stimulated calcitonin levels as methods to detect liver metastases; open or laparoscopic liver biopsy identified metastatic disease in 20 % of patients with negative imaging, whereas selective venous sampling was not useful [28]. Based on this data, current ATA guidelines recommend that consideration be given to laparoscopic or open liver biopsy in order to detect radiographically occult liver metastases in a patient with substantial hypercalcitoninemia prior to subjecting the patient to a "long and arduous" repeat neck operation that would presumably be performed for curative intent (Grade C recommendation) [1].

Conclusion

MTC remains a challenging disease entity. Although an individual clinician may only encounter it rarely, optimal management requires very specific understanding of a variety of disciplines. A methodical approach to preoperative evaluation, as summarized in this chapter, will ensure that important components of patient care are addressed appropriately so that ideal surgical outcomes are ultimately possible.

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8 Inherited Medullary Thyroid Carcinoma: Indications and Technique of Early Thyroidectomy

Elizabeth G. Grubbs and Steven G. Waguespack

Introduction to Inherited Medullary Thyroid Carcinoma

When diagnosed during childhood and early adulthood, medullary thyroid carcinoma (MTC) most commonly results from a dominantly inherited or de novo gain-of-function mutation in the *RE*arranged during *T*ransfection (*RET*) proto-oncogene [1–4] that is associated with either multiple endocrine neoplasia (MEN) syndrome type 2A or type 2B (Fig. 8.1). Previously, there was a third distinct category of familial MTC, which is now considered a phenotypic variant of MEN2A with decreased penetrance and/or delayed onset of the other MEN2A-defining manifestations [5, 6]. MTC diagnosed in older adulthood, while more likely to be sporadic in origin, still has the potential to be secondary to an inherited *RET* mutation, even in the absence of a family history [6–8]. This observation has led to guideline recommendations for all individuals diagnosed with MTC, regardless of age, to undergo germline *RET* testing, preferably in the setting of formal genetic counseling [6, 9, 10]. If a mutation is found, first-degree relatives should be counseled to undergo testing as well, likely resulting in the diagnosis of additional family members with a *RET* mutation who may not exhibit any clinical or biochemical evidence of MTC or other MEN2-associated diseases. These individuals are called asymptomatic carriers and in this chapter, we will discuss their management in the context of their risk for developing clinically relevant MTC.

The original description of MEN2A is credited to Sipple, who reported the case of a 33-year-old man who died of intracranial hemorrhage and was found on a postmortem examination to have bilateral MTC, bilateral pheochromocytomas, and possible parathyroid hyperplasia [11]. Cushman subsequently proposed a relationship between these endocrine tumors [12] and this distinct clinical syndrome was thereafter named MEN2 by Steiner in 1968 [13]. Successively, several large kindreds of MEN2A were identified, which led to linkage analysis studies and the localization of the putative gene to chromosome 10 [14, 15]. In due course, mutations in the gene, *RET* (originally cloned as an oncogene [16]), were reported in 1993 to cause MEN2A [1, 2]. The MEN2B phenotype was originally characterized in the English literature in 1966 by Williams and Pollock [17], and MEN2B was further discerned as a variant of hereditary MTC with a mucosal neuroma phenotype [18]. In 1994, MEN2B was also identified to be caused by a germline-activating *RET* mutation [3, 4].

E.G. Grubbs
Department of Surgical Oncology, MD Anderson Cancer Center, The University of Texas, 1400 Pressler Street, Unit 1484, Houston, TX 77030, USA

S.G. Waguespack (✉)
Department of Endocrine Neoplasia and Hormonal Disorders, MD Anderson Cancer Center, The University of Texas, 1400 Pressler Street, Unit 1461, Houston, TX 77030, USA
e-mail: swagues@mdanderson.org

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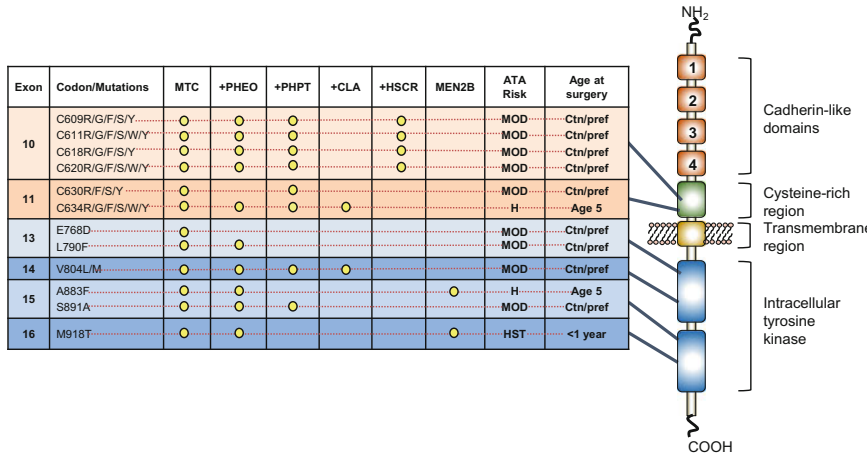

Exon	Codon/Mutations	MTC	+PHEO	+PHPT	+CLA	+HSCR	MEN2B	ATA Risk	Age at surgery
10	C609R/G/F/S/Y	○	○	○		○		MOD	Ctn/pref
	C611R/G/F/S/W/Y	○	○	○		○		MOD	Ctn/pref
	C618R/G/F/S/Y	○	○	○		○		MOD	Ctn/pref
	C620R/G/F/S/W/Y	○	○	○		○		MOD	Ctn/pref
11	C630R/F/S/Y	○		○				MOD	Ctn/pref
	C634R/G/F/S/W/Y	○	○	○	○			H	Age 5
13	E768D	○						MOD	Ctn/pref
	L790F	○	○					MOD	Ctn/pref
14	V804L/M	○	○	○	○			MOD	Ctn/pref
15	A883F	○	○				○	H	Age 5
	S891A	○	○	○				MOD	Ctn/pref
16	M918T	○	○				○	HST	<1 year

Fig. 8.1 The RET receptor (illustration adapted from Ref. [28], with permission) and the commonly mutated codons and associated phenotypes in the MEN2 syndromes, including the 2015 ATA risk stratification (from Ref. [6]). Age at surgery denotes the age at which thyroidectomy is recommended with "Ctn/pref" denoting that timing can be determined by calcitonin (Ctn) and parent/patient preference in moderate-risk (MOD) patients. Abbreviations: MTC, medullary thyroid carcinoma; PHEO, pheochromocytoma; PHPT, primary hyperparathyroidism; CLA, cutaneous lichen amyloidosis; HSCR, Hirschsprung's disease; ATA, American Thyroid Association; MOD, moderate risk; H, high risk; HST, highest risk; and Ctn/pref, calcitonin/preference

Because of the initial collaborative efforts of the International *RET* Mutation Consortium, it became quickly apparent that MEN2 was associated with a limited number of mutations and that convincing genotype–phenotype correlations were present (Fig. 8.1) [19]. The high penetrance of hereditary MTC and evolving knowledge regarding the age of MTC onset in MEN2 led to the rapid integration of genetic testing into management algorithms for patients with or at risk for MTC and this, in turn, marshaled in the era of performing total prophylactic thyroidectomy for asymptomatic *RET* carriers [20]. In the contemporary era, in which genetic testing is routinely incorporated into clinical care, children and young adults with hereditary MTC rarely present with clinical disease, outside of the rare sporadic case, newly identified MEN2A kindred or MTC associated with MEN2B. Thus, in the twenty-first century, the presymptomatic identification of a positive *RET* mutation is the predominate route to an MEN2A diagnosis in young patients. Additionally, an increasing number of asymptomatic adult carriers have been identified as testing for *RET* mutations has become more widespread [21]. The utilization of genetic testing in the management of MEN2 has become increasingly complex due to greater recognition of the capricious aggressiveness of hereditable MTC, even among members of a kindred with the same *RET* mutation [22, 23]. Furthermore, the mutational landscape continues to evolve as novel and uncommon *RET* DNA variants are identified but remain incompletely characterized as pathogenic mutations and are thus deemed "variants of unknown significance" [8, 21, 24–27]. These factors, plus the utilization of routine calcitonin screening and ultrasonography in asymptomatic *RET* carriers [6, 28, 29], have led to the present quandary of determining the optimal timing of early thyroidectomy, with the goal of diminishing potential medical and surgical morbidity while simultaneously ensuring an excellent oncologic outcomes by removing the thyroid before MTC metastasis occurs.

MTC is usually the first clinical manifestation of MEN2, and it is most commonly multifocal, bilateral, and located in the middle to upper regions of the thyroid lobes, an area where the calcitonin-positive C-cells are the most highly concentrated [20, 30–32]. There is a well-described, age-related progression of

malignant disease with lymph node and distant metastases usually occurring years after the onset of tumorigenesis [33]. C-cell hyperplasia represents the initial stage in the oncologic evolution to microscopic, noninvasive MTC and ultimately to frankly invasive carcinoma that gives rise to metastatic disease [28, 30, 32, 33]. The cervical and mediastinal lymph nodes are the most common sites of metastatic disease; typical distant sites for hematogenous spread include the lungs, liver, and bone/bone marrow.

In MTC, older age at diagnosis, larger tumor size, positive lymph node disease, and the presence of distant metastases predict lower disease-free survival and higher mortality [34–40]. Patients with MTC arising as part of the MEN2 syndromes have a better prognosis than patients with sporadic MTC [38, 39], and individuals with MEN2B have the worst outcome [38]. However, it remains unclear that MTC arising in the context of MEN2B is inherently more biologically aggressive [41], as overall survival may be more impacted by the extremely early onset of MTC in MEN2B coupled with its frequently delayed diagnosis [42, 43].

MTC in MEN2A

MEN2A accounts for around 95 % of MEN2 cases and is characterized by the variable development of MTC in >90 % of *RET* mutation carriers during the course of their lifetimes [6, 19, 21, 26, 28, 44–46] (Fig. 8.2). Mutations associated with MEN2A are primarily located in the extracellular cysteine-rich domain of the *RET* proto-oncogene, usually in exon 10 (codons 609, 611, 618 or 620) or exon 11 (codon 634) but can also be found in the intracellular tyrosine kinase domain in exon 13 (codons 768 or 790), exon 14 (codon 804), or exon 15 (codon 891) [6, 8, 19, 47]. Whereas in past decades mutations in codon 634 (exon 11) accounted for the vast majority of MEN2A cases, more recently the prevalence of other mutations has increased [8, 48]. Individuals with a *RET* codon 634 mutation represent the prototypical MEN2A patient and have the greatest risk of developing early MTC followed by those with mutations in codons 609, 611, 618, 620, or 630, whereas mutations in codons 768, 790, 804, or 891 impart the lowest risk for clinically aggressive MTC [6, 21, 28, 45]. Although genotype–phenotype correlations are typically strong in MEN2A, it is important to remember that the age of MTC onset and disease aggressiveness may not always hold true to the predicted phenotype [22, 23].

MTC in MEN2B

MTC occurring in the clinical context of MEN2B is a completely penetrant disease with a uniformly early age of onset, even within the first few months of life [49]. The tempo of disease progression is accelerated compared with MEN2A, and metastatic lymph node disease has been documented within the first twelve months of life [50]. The average age of onset of MTC is in the second decade of life, about 10 years earlier than that seen in individuals with MEN2A [37, 43, 51–53]. MEN2B is due to a de novo *RET* mutation in >90 % of cases [8, 49], and thus, the diagnosis is usually delayed, leading to the likelihood that MTC will already have metastasized and become incurable. However, recent data would suggest that, even in de novo cases, surgery may still be curative if performed before age 4 years [49]. The M918T *RET* mutation is identified in >95 % of MEN2B patients. Rarer MEN2B mutations include double RET mutations involving codon 804 and the A883F mutation, which may be associated with a less aggressive MTC phenotype [54].

Early Thyroidectomy

After it metastasizes beyond the thyroid gland, MTC is most often an incurable disease and one that may ultimately lead to the death of the patient. However, given our ability to identify *RET* mutation carriers before they develop clinically relevant disease, hereditary MTC has become one of the few malignancies that can be prevented (via prophylactic thyroidectomy) or

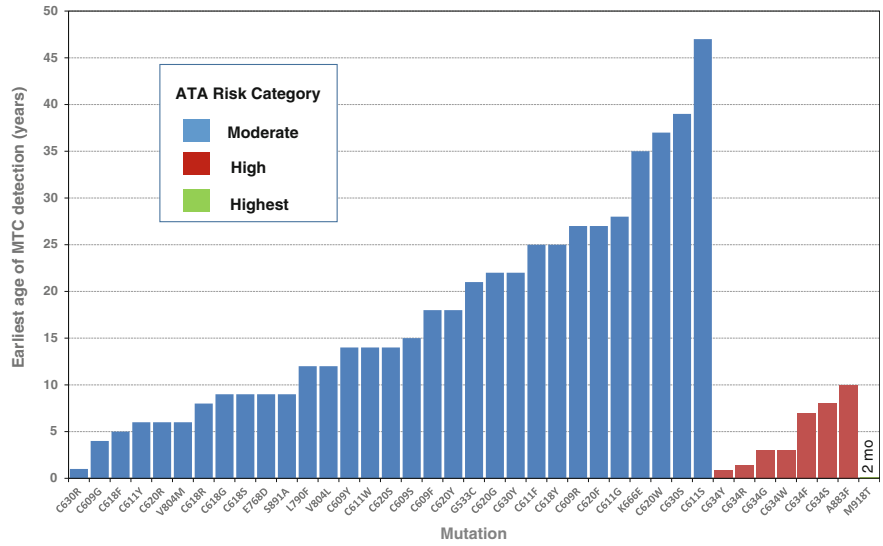

Fig. 8.2 Earliest age of medullary thyroid carcinoma (MTC) onset varies depending on the specific *RET* mutation present. The age of onset of MTC is decreasing for mutations in most codons over time probably because of more widespread genetic testing and earlier surgical intervention. Thus, a well-defined age determination for early thyroidectomy is becoming less clear based upon genotype alone. The mutations are color-coded by the ATA risk stratification Ref. [6]. Data compiled from the ARUP MEN2 *RET* online database [26] and an exhaustive literature review

cured (via early thyroidectomy) before it becomes clinically apparent. In the hands of experienced surgeons, children with MEN2 who have a total thyroidectomy performed prior to the onset of metastatic disease have an excellent chance of remaining disease-free with minimal morbidity [6, 28, 36, 37, 49, 55–59].

Commonly accepted is the notion that early thyroidectomy is curative for MTC and ultimately will become necessary for most individuals with a *RET* mutation, but debate remains as to the optimal timing of surgery for those MEN2 patients with mutations in codons other than 918 and 634, particularly as rarer and less virulent *RET* mutations are becoming more prevalent in the genetic testing era. In general, the primary oncologic goal of early intervention is to render the patient free of MTC and the potential risk of death from metastatic disease. A true prophylactic thyroidectomy will prevent malignancy from occurring in the first place, but most important is to remove the thyroid before metastasis occurs (early thyroidectomy). As more children undergo thyroidectomy earlier than in the past, it is anticipated that the earliest age of diagnosis of MTC for any given *RET* mutation will continue to drop (Fig. 8.2), but the diagnosis of isolated cases of microscopic non-metastatic disease at an extremely young age should not justify a broad prescription for similar at-risk patients to have surgery at that age.

Before the identification of *RET* and the incorporation of routine genetic testing into the management of kindreds with MTC/MEN2, the calcitonin response to the intravenous administration of a calcitonin secretagogue (calcium, pentagastrin) [60] was used to identify individuals at risk for MTC and to help establish the timing of surgery. This practice was supplanted by genetic testing, and the first recommendations regarding the appropriate age for thyroidectomy were generated on the basis of the specific *RET* mutation present and the earliest age at which MTC had been diagnosed for that particular mutation [20, 51].

Evolving from the 7th International Workshop on MEN in 1999, a consensus statement issued in 2001 was the first to categorize *RET*

proto-oncogene mutations into one of three separate risk levels (Levels 1–3, level 3 being the highest risk) [51]. Subsequently, the American Thyroid Association (ATA) published guidelines in 2009 that further developed the risk categories, assimilating updated data on *RET* mutations and phenotypes and placing codon 634 mutations within a separate risk level [61]. The 2009 ATA guidelines stratified all known *RET* mutations into one of four risk levels (ATA risk levels A–D, level D being the highest risk), and the idea of safely deferring early thyroidectomy while offering careful, expectant monitoring in individuals with lower risk *RET* mutations was introduced. This new concept of monitoring children in whom the chance of identifying MTC is low (those with normal calcitonin levels, normal neck ultrasonography, and a less aggressive MTC family history) was shown to be a reasonable approach to the care of low-risk MEN2 patients [56, 62]. However, a subsequent study demonstrated the low sensitivity of ultrasonography in predicting microscopic MTC in the asymptomatic MEN2A carrier [29]. In 2015, the ATA guidelines were further refined to simplify the risk levels into "highest risk" (the previous level D, which includes MEN2B patients with a *RET* codon M918T mutation), "high risk" (includes patients with *RET* codon C634 mutations, the previous level C, and the *RET* codon A883F mutation, formerly level D), and "moderate risk" (previous levels A and B) [6] (Figs. 8.1 and 8.2).

The latest guidelines suggest performing a total thyroidectomy in the first year of life in asymptomatic carriers with the highest risk mutation and at or before age 5 for those with a high-risk mutation. With all other *RET* mutations, the timing of surgery can be determined by the detection of an elevated serum calcitonin level, recognizing that the ultimate decision should be made by the multidisciplinary team in consultation with the child's parents or guardian, who may opt for an earlier intervention. Although largely unstudied, the approach of cautious surveillance may even be appropriate in select children with high-risk mutations, who may still be cured of their disease even if surgery is not undertaken at the currently prescribed ages [36, 37, 41, 43, 49, 54, 56, 58, 59, 62–65].

Children over the age of 36 months who have basal serum calcitonin levels <30–40 pg/ml and thyroid nodules <0.5 cm on a high-quality ultrasound are unlikely to have metastatic MTC [37, 61, 62, 66]. Thus, in MEN2 patients who have a normal basal serum calcitonin level and a normal thyroid ultrasound and therefore very little chance of having MTC, the benefits of postponing surgical intervention most likely outweigh the associated risks of early thyroidectomy, particularly if access to a high-volume multidisciplinary care center is unavailable. Nonetheless, the use of calcitonin monitoring must be undertaken with knowledge regarding normal serum calcitonin levels in children, which are highest in infancy and decline to adult levels after age 3 years [67, 68], and understanding that large studies validating normal calcitonin ranges in young children are not available for all commercial assays. Moreover, an elevated serum calcitonin level does not always indicate the presence of malignant C-cell disease [37, 66] and can also be found in non-neoplastic conditions, most notably chronic kidney disease, autoimmune thyroiditis, and hyperparathyroidism [6]. Conversely, MTC can also be present pathologically even in the presence of a normal serum calcitonin level [29].

It is essential for clinicians who treat children with MEN2 to distinguish those who clearly require early thyroidectomy to prevent MTC morbidity and mortality and not to overtreat those *RET* mutation carriers who are unlikely to develop clinically relevant disease over the short term. Although surgical intervention in a child by a high-volume surgeon should be as safe as it is in an adult, the regrettable truth is that many children with MEN2 do not have access to such experts [35, 69], and their complication rates may be higher for that reason [69, 70]. Treating permanent hypoparathyroidism in a child is quite challenging, in addition to the lifelong impact it has on the patient and family. Parental guilt and psychological distress may also occur after the identification of a child with a *RET* mutation

[71], and reassuring parents that their child can be safely monitored instead of steering him or her swiftly to surgery may have positive effects on the family. Additionally, early surgical and medical intervention may also negatively impact the child, stressing the need for initial and ongoing psychological and genetic counseling support [28, 51, 61, 71]. Finally, it is apparent that many children with MEN2 have inadequate thyroid hormone replacement on follow-up [57, 72, 73], and the potential sequelae of iatrogenic hypothyroidism remain poorly studied in this population. Thus, a frank discussion regarding the need to adhere to lifelong levothyroxine therapy should also have when the timing of early thyroidectomy is discussed.

Although the timing of surgery is most often considered in the context of the pediatric age group, adult asymptomatic carriers are becoming more common as *RET* testing expands in at-risk cohorts. Considerations for surgical intervention in this population are similar to their younger counterpart with the exceptions that the risk of hypocalcemia is less and the patients are able to make well-informed decisions for themselves.

Surgical Management

Surgery remains the primary treatment and only curative intervention for MTC [6, 9, 10]. In MEN2A and MEN2B, early thyroidectomy, performed either in a prophylactic or therapeutic fashion, can alter the natural history of disease and improve long-term oncologic outcomes [36, 37, 55–59]. When discussing early thyroidectomy in asymptomatic *RET* mutation carriers, notable is the fact that surgical complication rates are higher in children compared with adults [70]. Therefore, as with any rare disease and to lessen the likelihood of iatrogenic injury, children requiring surgery should be operated on by a high-volume thyroid surgeon familiar with the MEN2 syndromes [6, 28]. Optimizing outcomes by utilizing high-volume surgeons necessitates a multidisciplinary approach and advocacy by parents, pediatricians, endocrinologists, surgeons, and third-party payors [69].

In addition to the decision of when to perform an early thyroidectomy, the surgeon must also understand the patient's genotype and clinical data and incorporate this information into the decision-making process to determine the approach to lymph node and parathyroid gland management. For surgical planning, a thorough preoperative cervical ultrasound to identify nodular thyroid disease and lymph node metastases is essential. The goal of thyroidectomy in the MEN2 syndromes is the complete and safe removal of all thyroid tissue, including the posterior capsule (i.e., not a near-total or subtotal thyroidectomy) [74]. A central compartment (Level VI) neck dissection [75] is not required in the setting of an early thyroidectomy without clinical evidence of MTC, as lymph node metastases are exceedingly rare in that setting [6, 33, 36, 37, 47, 62, 66, 74]. However, in a child with MEN2B, central compartment neck dissection should be considered if the parathyroid glands can be readily identified and safely preserved or if previously unidentified lymph node metastases are identified intraoperatively. If the surgical intervention is therapeutic for a clinically evident tumor (i.e., evidence of lymph node metastases, serum calcitonin level >40 pg/ml), total thyroidectomy and a concomitant central compartment neck dissection should be performed [6]. Compartment-oriented dissection of the lateral cervical lymph node compartments (levels IIA–V) is indicated in cases in which there is clear evidence of lateral neck involvement and can be considered in MEN2B patients who have clinically apparent disease and significantly elevated serum calcitonin levels. In the rare presence of a high burden of distant metastatic disease, less aggressive neck surgical intervention may be appropriate [6].

Though highly unlikely, if primary hyperparathyroidism is diagnosed at the time of early thyroidectomy in patients with MEN2A, concomitant parathyroidectomy of the affected gland(s) should be performed [6, 10]. In the absence of primary hyperparathyroidism, normal parathyroid glands should be left in situ to offer

the greatest chance at maintaining function, though there is some controversy in the literature [6, 74, 76, 77]. In the event that a normal parathyroid gland is devascularized during the course of the operation, the gland should be autotransplanted into either the sternocleidomastoid muscle or non-dominant forearm, depending on the specific *RET* mutation present and the inherent risk for the future development of primary hyperparathyroidism [6, 28]. *RET* mutations associated with primary hyperparathyroidism include those located on Exon 10, 11, 14, and 15.

Conclusions

Hereditary MTC and the MEN2 syndromes are rare endocrine disorders that are increasingly being managed during an asymptomatic phase secondary to the increased utilization of genetic testing in patients with MTC and at-risk family members. Despite the discovery of *RET* and its role in MTC, remarkable advances in our understanding and clinical care have occurred. It remains important to recognize the well-established MEN2 genotype–phenotype correlations while also acknowledging our limitations in predicting the appropriate timing of surgery in the asymptomatic, low-risk MEN2 patient. Future research should focus on the long-term oncologic and quality-of-life outcomes after early thyroidectomy, better delineation of genotype–phenotype correlations (especially as more *RET* DNA variants are characterized), and how to predict more accurately which individuals would benefit from timely thyroidectomy based upon clinical data and which patients can be safely monitored without early intervention.

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Surgical Management of Inherited MTC: Therapeutic Thyroidectomy

9

Linwah Yip

Introduction

Inherited medullary thyroid cancer (MTC) occurs in ~25 % of cases as part of an autosomal dominant syndrome caused by germline mutations in the *RET* proto-oncogene [1]. The three characterized syndromes are distinguished by location and type of *RET* mutation, associated manifestations, and degree of MTC aggressiveness [2]. In contemporary management, MTC is the primary source of syndrome-related morbidity and mortality in *RET* mutation carriers. Overall survival for MTC associated with distant metastasis is 26 % and 14 % at 5 and 10 years, respectively [3]. Early recognition of premalignant disease allows for prophylactic thyroidectomy, the performance of which has been shown to improve outcomes [4]. However, for patients who present with MTC and de novo germline *RET* mutations or for gene carriers who have not had systematic screening, thyroidectomy after MTC has already manifested is still often needed.

Preoperative Evaluation

MTC can be diagnosed preoperatively either by fine needle aspiration (FNA) biopsy of thyroid nodules or by an elevation in serum calcitonin levels. Cytology for MTC classically demonstrates a dispersed cell pattern of triangular, polygonal, or spindled cells. Characteristic features include eccentric nuclei and the coarse granular (i.e., salt-and-pepper) chromatin often seen with neuroendocrine tumors [5]. Amyloid usually appears slightly pink to translucent on Papanicolaou stain, and its presence is highly suggestive of MTC, yet can also occasionally be identified in benign goiter. Some MTC variants have a microfollicular-type pattern that could lead to the cytologic diagnosis of a follicular neoplasm [5]. Calcitonin is the most helpful immunocytochemical marker for MTC especially when thyroglobulin staining is absent, and calcitonin levels can also be measured in FNA biopsy wash, which improves preoperative diagnostic accuracy [6, 7].

Elevated serum calcitonin levels can be diagnostic for MTC and are a useful marker for both the presence and the extent of disease. Basal serum calcitonin levels that are normal (<20 pg/mL) or clearly abnormal ($\geq$ 100 pg/mL) are accurate at predicting the absence or presence of MTC [2]. False-positive serum calcitonin elevations can occur when levels are between 20 and 100 pg/mL, and the etiology includes renal failure, proton pump inhibitors, chronic lymphocytic thyroiditis,

L. Yip (✉)
Division of Endocrine Surgery, Department of Surgery, University of Pittsburgh, Suite 101-3471 Fifth Avenue, Pittsburgh, PA, USA
e-mail: yipl@upmc.edu

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pancreatitis, or other extrathyroidal malignancies such as breast, small cell lung, and neuroendocrine tumors [8]. Pediatric patients also have higher basal serum calcitonin levels [9]. However, in patients who are *RET* mutation positive, basal elevations in serum calcitonin levels should be considered a marker of either premalignant C-cell hyperplasia or MTC. In a study by Elisei et al., 84 patients with germline *RET* mutations were studied who underwent screening for MTC with ultrasounds and basal and pentagastrin-stimulated serum calcitonin levels [10]. In 32 patients with normal basal and stimulated serum calcitonin levels, only 2 ultimately had thyroidectomy; 1 had C-cell hyperplasia and 1 had benign histology. In 20 patients with basal serum calcitonin level >10 pg/mL, all had histologic MTC after thyroidectomy. Stimulated serum calcitonin levels >10 pg/mL were the indication for surgery in an additional 31 patients; of these, 25 had MTC, while 6 had C-cell hyperplasia [10]. Although the study results suggested that using pentagastrin stimulation may help better identify *RET* mutation carriers who have MTC, pentagastrin is not available in the USA. Calcium-stimulated calcitonin may be an alternative but was not specifically studied.

More importantly, the results by Elisei et al. also demonstrated that isolated intrathyroidal disease without lymph node metastasis was identified in all *RET* mutation carriers who had a basal serum calcitonin <60 pg/mL and all remained biochemically disease-free after long-term follow-up [10]. In another series of 170 young (age <21 years) *RET* mutation carriers from France, a preoperative basal serum calcitonin level <30 pg/mL was associated with both N0 disease and disease-free status at last follow-up. Preoperative basal serum calcitonin levels were accurate at predicting extent of disease in all *RET* mutation risk categories [11]. Therefore, even in *RET* mutation carriers, preoperative basal serum calcitonin levels are an accurate indicator of MTC and can be used to help guide timing and extent of initial surgery.

Serum carcinoembryonic antigen (CEA) levels may be useful as a secondary marker of disease, especially during surveillance following thyroidectomy, and basal preoperative levels should be obtained [2, 12]. Postoperatively, rising serum CEA levels with stable serum calcitonin levels can be a marker of aggressive dedifferentiated disease. However, CEA lacks the specificity and sensitivity required to be useful in the initial diagnosis of MTC. MTC may also secrete adrenocorticotrophic hormone (ACTH) and occasionally somatostatin, and these can be additional disease markers to evaluate preoperatively if symptoms are present. Biochemical testing specifically for *RET* mutation-positive patients should include preoperative evaluation for pheochromocytoma with free plasma or 24-hour fractionated urinary metanephrines and primary hyperparathyroidism with albumin-corrected serum calcium and parathyroid hormone. If pheochromocytoma is suspected biochemically, it should always be surgically treated prior to thyroidectomy [2, 13, 14].

Preoperative neck ultrasound (US) is essential for all patients with a biochemical and/or cytologic diagnosis of MTC and can provide important anatomic information that may alter the surgical approach. Malignant nodules located on the posterior surface of the thyroid are more likely to be in close proximity to the ipsilateral recurrent laryngeal nerve and alter preoperative counseling regarding the possibility of injury and postoperative voice quality. Any concern for extrathyroidal extension into the trachea and esophagus could also require bronchoscopy and/or esophagoscopy to assess transmural involvement and potentially the need for adjacent organ resection. Cervical US is particularly helpful for assessment of lymph node involvement and can identify nonclinically evident nodal disease in up to 49 % of MTC patients at initial or reoperative procedures [15]. On US, suspicious lymph nodes are sonographically rounded with an absent fatty hilum, are markedly hypoechogenic, and may have calcifications [2]. FNA biopsy with calcitonin measurement of the aspiration fluid should be performed for confirmation of disease. Contrast-enhanced neck computed tomography (CT) should be strongly considered for patients with large primary tumors, significant nodal disease, and/or concern

for mediastinal lymph node involvement especially if extensive neck disease is evident [2]. Because metastatic MTC is more infiltrative than differentiated thyroid cancers, mobilization of mediastinal disease into the cervical incision is often difficult and concurrent partial or full sternotomy should be considered in patients with bulky mediastinal disease.

Preoperative assessment of voice function should also occur at initial examination. In patients who have voice changes or who have prior neck or chest surgery, including open heart bypass or cervical spine fusion via anterior approach, additional testing of vocal fold function should be obtained using either direct fiberoptic or indirect laryngoscopy [2]. Patients with MTC and extensive central compartment disease should also have preoperative vocal cord assessment, regardless of clinical examination. Transcutaneous laryngeal ultrasound has also been used to accurately assess vocal cord function and is noninvasive [16–18].

Extent of Thyroidectomy

In *RET* mutation carriers, the initial extent of thyroid surgery should always be total thyroidectomy (TT) due to the high rate of multicentric and bilateral disease [2, 19]. C-cells concentrate in the upper poles of the thyroid, and specific attention should be made to ensure that both upper poles are entirely resected. The operative goal is to resect all gross and microscopic disease while avoiding the morbidity of surgery and reoperation.

TT is typically performed under general anesthesia with either same day or next day discharge. After induction, the neck is gently extended which is facilitated by the placement of a shoulder roll. Somatosensory evoked potential (SSEP) monitoring may help reduce the risk of positional spinal cord compression in patients who already have mild symptomatic radiculopathy without indications for preoperative cervical spine surgery. A transverse cervical incision is then made at least 1–2 cm below the cricoid cartilage following Langer's lines. Subplatysmal flaps may be developed to help facilitate visualization through a small incision. The strap muscles are divided along the median raphe to expose the thyroid isthmus and are then dissected off the thyroid to expose the superior pole vessels. Any strap muscle attached to the malignant nodule should be resected en bloc with the thyroid. It is unusual to otherwise require division of the strap muscles unless the thyroid and/or thyroid malignancy is sizable and immobile.

The superior pole vessels can be divided either between surgical ties or with an energy device such as the LigaSure or Harmonic [20]. The vessels should be taken close to the thyroid capsule to avoid injury to the superior parathyroid gland and external branch of the superior laryngeal nerve. As discussed earlier, the C-cells are embryonically concentrated in the upper poles, and thus, care should be taken to make sure that no upper pole thyroid tissue is retained. The lower pole vessels are then isolated and divided in a similar fashion to the upper pole with careful preservation of the inferior parathyroid gland. Early division of the middle vein can also assist mobilization.

The recurrent laryngeal nerve should be specifically visualized which reduces the risk of its injury during both the thyroidectomy and central compartment lymphadenectomy [21]. A nonrecurrent right laryngeal nerve is present in up to 1.5 % of cases and is associated with an aberrant right subclavian artery (i.e., arteria lusoria) [22]. Much less commonly, a nonrecurrent nerve can occur on the left (<0.1 %) and is associated with a right aortic arch in addition to an anatomic variant such as dextrocardia or the absence of an arterial ligament on the left [23]. Although the nerve can have a variable course, it inserts in a typical location under the inferior constrictor muscle at the junction of the cricothyroid muscle and trachea. The nerve's insertion is reliably lateral and dorsal to the ligament of Berry. If needed, division of the isthmus and mobilization of the medial aspect of the lobe can facilitate dissection at the ligament especially if the malignant nodule is located near

the insertion of the recurrent laryngeal nerve. The contralateral lobe is then approached in a similar manner. Postoperative drain placement is not routinely necessary after TT and does not prevent reoperation or wound complications, which are both very rare complications following thyroidectomy [24].

Intraoperative recurrent laryngeal nerve monitoring is an adjunct that can be used to help identify the nerve, but has not been shown to reduce the incidence of nerve injury. In an analysis of the Nationwide Inpatient Sample between 2008 and 2011, <7 % of thyroidectomy cases were performed with nerve monitoring, and its use was associated with a higher rate of vocal cord paralysis [25]. The authors analyzed a number of accessible potential confounding variables including hospital volume, coding practices, and use of laryngoscopy which did not seem to affect the results. However, utilization of nerve monitoring was much lower than previously reported, and the database used did not capture thyroidectomies performed in ambulatory settings, which may be a significant bias of the study [25]. In another meta-analysis of recurrent laryngeal nerve monitoring inclusive of 20 articles, there were no differences in nerve palsy or paralysis rates when nerve monitoring was used compared to when the nerve was visualized without nerve monitoring [21]. Thus, the use of nerve monitoring does not appear to reduce the risk of recurrent laryngeal nerve injury.

If the recurrent laryngeal nerve is transected intraoperatively, immediate reinnervation should be strongly considered [26]. Reinnervation can help maintain thyroarytenoid muscle tone and vocal fold bulk, which improves voice outcomes after any necessary laryngoplasty. Options for a tension-free repair include either primary neurorrhaphy or anastomosis between the recurrent laryngeal and ansa cervicalis nerves. After removing any nonviable tissue, the 2 nerve ends can be approximated by placing 2–3 interrupted epineural stitches using a fine (9-0 or 8-0) monofilament nonabsorbable suture.

All *RET* mutation-positive patients should be screened preoperatively for primary hyperparathyroidism. Patients with codon 634 mutations have the highest incidence of primary hyperparathyroidism followed by patients with exon 10 mutations [2]. If primary hyperparathyroidism is diagnosed, then intraoperative parathyroid hormone (PTH) monitoring should be considered at the time of thyroidectomy to guide extent of parathyroidectomy. Typically, the enlarged parathyroid(s) are resected with preservation of normal parathyroid glands in situ on their vascular pedicles. If primary hyperparathyroidism has been excluded, based on the patient's *RET* codon mutation, then devascularized or nonviable glands should be autotransplanted into either the ipsilateral sternocleidomastoid muscle or the brachioradialis muscle in the forearm [27–29]. Using preoperative serum calcitonin levels to direct whether central compartment neck dissection is needed may also reduce the risk of permanent hypoparathyroidism.

Postoperatively, patients are monitored for hematoma, airway issues, voice changes, and hypocalcemia. The incidence of hematoma is 0.3–4 % and can lead to life-threatening airway compromise [30]. The presence of new onset hoarseness and/or postoperative stridor is consistent with either unilateral or bilateral recurrent laryngeal nerve injury. Airway protection should be the primary concern followed by immediate postoperative assessment with fiberoptic laryngoscopy. Perioral or peripheral tingling can be an early symptom of hypocalcemia which can progress to tetany if not recognized and treated. All patients should be started postoperatively on replacement L-thyroxine at a dose of 1.5–1.7 mcg/kg, and thyroid stimulating hormone levels should be checked in 6–8 weeks with the goal of maintaining levels within normal range (see Chap. 13) [2].

Extent of Lymphadenectomy

The majority of studies on patterns of lymph node metastasis in MTC have included both hereditary and nonhereditary patients. Although bilateral tumors are more common in hereditary MTC patients, the pattern and frequency of lymph node metastases appear to be similar in

both groups [31]. When lymph node metastases are clinically evident either on preoperative neck US or intraoperatively, a compartment-oriented lymph node dissection should always be performed. Prior to initial surgery, FNA biopsy of sonographically concerning level VI lymph nodes is not necessary, but FNA biopsy may be helpful to confirm lateral compartment (levels II–V) metastatic disease. Since MTC is commonly located at the central to upper lobes, the upper third of the lateral compartment (levels IIB and III) is more commonly involved [32]. Lateral compartment lymph node metastasis (LLNM) can be found without central compartment lymph node metastasis (CLNM), or so-called skip metastasis, in up to 10 % of MTC patients [32]. Thus, careful preoperative US assessment of all cervical lymph node basins is warranted.

In the absence of clinically evident lymph node involvement, serum calcitonin levels can be used to guide extent of concurrent lymphadenectomy. The likelihood of lymph node disease is exceedingly low when preoperative serum calcitonin levels are <40 pg/mL and lymphadenectomy may not be needed [12, 33]. However, for many *RET* mutation carriers with a preoperative diagnosis of MTC, determined either biochemically or by FNA biopsy, the preoperative serum calcitonin is likely $\geq$ 40 pg/mL, and at a minimum, concurrent central compartment lymph node dissection is indicated. In a systematic evaluation of 300 patients with MTC, the likelihood of central compartment lymph node metastases when the basal serum calcitonin level was <50 pg/mL was 9 % and increased with rising basal serum calcitonin levels [12]. In 73 hereditary and nonhereditary MTC patients who presented with a palpable nodule, 79 % had central compartment lymph node metastases [34]. Furthermore, intraoperative assessment by an experienced surgeon identified positive lymph node in only 64 % of patients with positive lymph nodes. Thus, a compartment-oriented central compartment lymph node dissection is recommended for all patients with clinically evident MTC.

When lateral compartment neck disease is not evident on preoperative imaging, LLNM may still be likely especially if CLNM are present. In a study by Machens et al., when even one central compartment lymph node was positive for metastatic MTC, the likelihood of LLNM was 71 % [32]. However, unless clinically apparent, CLNM is often diagnosed on histology. It remains controversial if prophylactic dissection of the ipsilateral lateral compartment should be routinely performed for all patients who have a central compartment neck dissection, or if extent of lateral neck dissection should be guided by ultrasound findings [2]. When basal serum calcitonin levels are $\geq$ 200 pg/mL, contralateral lateral compartment neck dissection can also be considered, even if imaging is negative [2, 12]. Preoperative CEA levels can also be useful to predict volume of disease.

Following surgery, the likelihood of biochemical cure is ~30 % in patients with node-positive disease and this is inversely proportional to the number of involved lymph nodes [35]. The likelihood of calcitonin normalization in MTC patients with >10 LNM was only 4 %, strongly suggesting that microscopic distant metastasis was present at the time of diagnosis [31]. Although this may temper enthusiasm for aggressive surgical management, extent of surgery may impact survival in patients with MTC and distant metastasis. In a study of 2,968 MTC patients from the National Cancer Data Base, extent of surgery (e.g., lobectomy versus total thyroidectomy versus total thyroidectomy with lymph node resection) did not affect survival in patients with MTC <2 cm and without distant metastasis. However, aggressive surgery including lymphadenectomy did improve survival for patients with distant metastasis suggesting that control of cervical disease is of some benefit [35].

The central compartment (Level VI) is bounded by the hyoid superiorly, the carotid arteries laterally, and the innominate artery on the right with the corresponding plane on the left inferiorly. The anterior border is the superficial layer of the deep cervical fascia, and the posterior border is the deep layer of the deep cervical fascia [36]. The recurrent laryngeal nerve is initially identified and skeletonized. The superior parathyroid gland should be identified and can often be preserved in situ. However, the inferior

gland frequently requires autotransplantation as it is usually located within the resected specimen. A central compartment lymph node dissection can result in a higher rate of temporary hypoparathyroidism. But with experienced surgeons, the rates of recurrent laryngeal nerve paralysis and permanent hypoparathyroidism do not appear to be significantly higher [37, 38].

Whenever necessary, lateral compartment neck dissection should always be an anatomic resection of the fibrofatty tissue within the compartment to avoid the need for preoperative surgery. The lateral neck is bordered medially by the carotid artery, superiorly by the posterior belly of the digastric muscle, posterolaterally by the spinal accessory nerve, and inferior by the thoracic inlet [39]. The sternocleidomastoid muscle, internal jugular vein, carotid artery, and vagus nerve are preserved unless grossly involved by tumor. Dissection at the left thoracic inlet around the internal jugular vein can injure the thoracic duct and result in a high-output lymphatic leak that causes hypoproteinemia and hyponatremia, in severe cases. If recognized intraoperatively, ligation of the duct is preferred with or without tissue flap coverage, while a delayed repair may require thoracoscopic ligation of the duct.

Low-volume lymphatic fistulas occur in 2–8 % of lateral compartment neck dissections and can usually be treated nonoperatively with a medium-chain triglyceride diet, total parental nutrition, and/or octreotide therapy [40, 41]. Closed suction drain placement may help identify and manage this complication, but its routine use is controversial. Other complications include spinal accessory nerve paresis and/or shoulder dysfunction, which has been reported in up to 50 % of patients who have undergone a lateral compartment neck dissection [42]. Prompt recognition of this complication and physiotherapy can help optimize trapezius muscle compensation. Phrenic nerve injury can cause respiratory compromise and is especially debilitating if a bilateral nerve injury occurs. Injury to the sympathetic chain resulting in an ipsilateral Horner's syndrome can occur in <1 % of patients [39].

In summary, advances in genetic testing have led to preclinical identification of many at-risk patients; however, MTC can still often be diagnosed after manifestation of disease in de novo *RET* mutation carriers and in susceptible patients who have not pursued genetic or biochemical screening. After exclusion of pheochromocytoma, TT with central compartment lymph node dissection remains the optimal initial surgical procedure in *RET* mutation carriers with known MTC, while ultrasound findings and preoperative basal serum calcitonin levels can be used to guide need and extent of lateral compartment lymphadenectomy.

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10 Surgical Management of Parathyroid and Adrenal Glands in Inherited Medullary Thyroid Carcinoma

Azadeh A. Carr and Douglas B. Evans

Hereditary medullary thyroid cancer (MTC) is a component of the type 2 multiple endocrine neoplasia syndromes (MEN). The American Thyroid Association (ATA) guidelines recommend that there should be two MEN2 syndromes: MEN2A and MEN2B which are due to mutations in the RET oncogene located on chromosome 10q11.2 [1]. MEN2A accounts for 95 % of MEN2 cases and should be divided into four variants: classical MEN2A with the presence of MTC and less frequent occurrence of pheochromocytoma and/or primary hyperparathyroidism (PHPT), MEN2A with cutaneous lichen amyloidosis, MEN2A with Hirschsprung's Disease, and familial MTC (FMTC) for patients with *RET* germline mutations who have MTC but neither pheochromocytoma or PHPT in the family. This chapter will focus on management of the adrenal glands and parathyroid glands in classical MEN2A and MEN2B.

For classical MEN2A, the frequency/penetrance of pheochromocytoma and/or PHPT varies depending on the specific *RET* mutation. Pheochromocytoma occurs in 40–50 % of MEN2A patients, with higher penetrance in patients with *RET* codon 634 mutations, a lower penetrance in patients with exon 10 *RET* codon mutations (609, 611, 618, 620), and is rare in patients with exon 15 mutations (codon 791, 804) [2–6]. PHPT in MEN2A occurs in approximately 20–30 % of patients and is generally mild and may involve one to four parathyroid glands (a supernumerary fifth gland is also possible) at presentation [3]. Once again, there is a higher penetrance in patients with *RET* codon 634 mutations than in patients with *RET* mutations in codons 609, 611, 618, and 620 [7–9]. Frequency of PHPT and pheochromocytoma in patients with cutaneous lichen amyloidosis and hirschsprung's disease is thought to occur at the same frequency, while patients with familial medullary thyroid carcinoma, by definition, do not develop pheochromocytomas or PHPT [10, 11].

A large number of MEN2B patients (75 %) are sporadic due to de novo *RET* mutations with the majority of mutations, approximately 95 %, occurring in exon 16 (codon 918T) [12, 13]. In MEN2B, pheochromocytoma will develop in approximately 50 % of patients [1, 3]. Other features of the syndrome include ganglioneuromatosis; marfanoid habitus; enlarged lips; and mucosal neuromas in the tongue, lips, and eyelids.

To aid in screening and decision making for the inherited MTC related syndromes, the ATA has created risk categories of *RET* mutations based on aggressiveness of MTC: highest risk, high risk, and moderate risk [1]. In general,

A.A. Carr (✉) · D.B. Evans
Section of Endocrine Surgery, Division of Surgical Oncology, Department of Surgery, Medical College of Wisconsin, 9200 West Wisconsin Ave, Milwaukee, WI 53226, USA
e-mail: acarr@mcw.edu

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higher-risk *RET* mutations for MTC also have higher risk of associated other tumors.

Pheochromocytomas

Pheochromocytomas in MEN2 are usually benign, multicentric, bilateral, and confined to the adrenal gland [3]. There is histopathologic evidence that the resected adrenal medullae in patients with MEN2 have diffuse or nodular hyperplasia as a precursor to often multicentric pheochromocytomas [14–16]. Adrenal medullary hyperplasia has not been directly associated with catecholamine excess but should be monitored closely.

The penetrance of pheochromocytoma varies with specific *RET* mutations as discussed earlier [17]. Generally, MTC will be the presenting tumor in MEN2; however, pheochromocytoma has been found to occur first in up to 15–25 % of patients (2–15 years before MTC) and concurrent with MTC in 30–40 % of patients [18–20]. Pheochromocytomas in MEN2 tend to be diagnosed at a younger age than are sporadic tumors; MEN2A at approximately 35 years (average age of onset), and MEN2B at approximately 25 years [19, 21–24] (Table 10.1). Diagnosis at a young age (in comparison with sporadic pheochromocytoma) is facilitated by annual screening and MEN2 patients are often asymptomatic at the time of diagnosis [25]. Screening should start at age 11 years for high- (exon 11 C634 and exon 15 A833) and highest-risk patients (exon 16 M918), and 16 years for moderate-risk patients [1]. We use measurement of plasma-free metanephrines and normetanephrines as the preferred screening method; urinary metanephrines and normetanephrines can also be assessed. Pheochromocytoma should always be excluded prior to surgery for MTC (either prophylactic or therapeutic) [1]. MEN2 patients who plan to become pregnant should also be screened for pheochromocytoma.

Pheochromocytoma should be confirmed by adrenal CT or MRI in patients with positive biochemical results. CT is preferable due to better resolution and more detailed anatomic representation although MRI is also useful as it avoids exposure to ionizing radiation which may be relevant especially in children and pregnant women [26]. A physiologically significant pheochromocytoma will be visible on cross-sectional imaging; a normal adrenal gland on CT/MRI excludes the presence of a physiologically significant pheochromocytoma (and operation for MTC or a routine pregnancy can proceed). On CT imaging, pheochromocytoma will appear heterogeneous and hypervascular with slow contrast washout. Synchronous bilateral pheochromocytomas occur in 30–60 % of patients, and a metachronous contralateral pheochromocytoma will develop in an

Table 10.1 Demographics and disease characteristics of pheochromocytoma in MEN2 patients

	Thosani, et al [19]		Grubbs et al. [20]		Castinetti et al. [17]
	MEN2a	MEN2b	MEN2A	MEN2B	MEN2 (2b ~6 %)
n	70	15	66	12	563
Age at pheochromocytoma diagnosis, median (range)	34(17–60)	25(18–40)	33(17–60)	24(18–40)	36(12–89)
Pheochromocytoma initial diagnosis	–	–	13 (20 %)	0	81 (15 %)
Tumor size, cm, median (range)	3.8(1–14)	2.5(1–6.4)	3.5(1–14)	2(1–3.4)	–
Pheochromocytoma diagnosis secondary to screening	48 (69 %)	9 (60 %)	–	–	175 (31 %)
Follow-up, months, median (range)	119(0–527)	57(1–251)	105(0–142)		156 (unavailable)
Bilateral pheochromocytoma, synchronous	43 (61 %)	7 (47 %)	36 (55 %)	5 (42 %)	250 (44 %)
Bilateral pheochromocytoma, metachronous	8 (11 %)	3 (20 %)	–	–	95 (17 %)

additional 10–20 % of patients who underwent prior unilateral adrenalectomy [19, 21, 22, 24]. Extra-adrenal pheochromocytoma (paraganglioma) and metastatic disease is very rare in MEN2 (malignancy <4 % and many experts in the field have never seen a patient with a malignant MEN2-associated pheochromocytoma) [18, 27]. Functional imaging is generally not indicated but may be considered in very large tumors.

Surgical Approach

Surgical approach to pheochromocytoma in MEN2 should be aimed at removing the secreting lesion while trying to preserve adrenal function to avoid corticosteroid dependence with associated risks of acute adrenal insufficiency, which may be fatal. Cortical-sparing adrenalectomy was developed based on the clinical observation that metastatic pheochromocytoma in MEN2 is very rare (otherwise stated, pheochromocytoma in MEN2 is benign). By preserving the adrenal cortex, one can avoid glucocorticoid dependence and this technique has become the recommended approach to resection of pheochromocytoma in MEN2. Cortical-sparing adrenalectomy has been shown to be safe, with the majority of patients not requiring daily corticosteroid supplementation [14, 19, 27–29]. The safety and benefit of avoiding corticosteroid dependence outweigh the risk of recurrence and the possible need for repeat resection in the future [19, 22].

For patients with unilateral pheochromocytoma and a macroscopically normal contralateral gland, a unilateral total adrenalectomy has been recommended, preferably by a minimally invasive method (laparoscopic transabdominal or retroperitoneoscopic approach) (Fig. 10.1) [14, 30–32]. With the advent of the posterior retroperitoneoscopic approach to the adrenal gland, resection of a recurrence (after an anterior transabdominal cortical sparing adrenalectomy) provides a new opportunity to consider cortical-sparing in all adrenal glands that harbor an MEN2-associated pheochromocytoma (Fig. 10.2). A cortical-sparing operation is only possible if the anatomy of the gland is acceptable, allowing a limb of the gland to be preserved in situ and well vascularized (Fig. 10.3) [22, 33, 34]. This is usually not possible if the pheochromocytoma is very large and has completely replaced the adrenal gland. Although there is a risk of recurrent pheochromocytoma with cortical-sparing adrenalectomy, the recurrence can be removed through the posterior approach in most patients, in contrast to the complexity of a reoperative anterior approach in a patient who previously underwent a laparoscopic transabdominal cortical-sparing adrenalectomy. The reoperative anterior approach is unlikely to be successfully performed laparoscopically while the retroperitoneoscopic approach can regularly be completed with three trocars. An additional reason to spare cortex in all adrenal glands, at all operations (whenever possible), includes the reality that a metachronous contralateral pheochromocytoma which develops years after a prior unilateral complete adrenalectomy, may be associated with anatomic characteristics which prevent a successful cortical-sparing procedure such as tumor size, location, or multiplicity of tumors. Prophylactic adrenalectomy of an unaffected gland is not recommended [31, 35].

For patients who present with MEN2-associated bilateral pheochromocytoma, surgical options include bilateral total adrenalectomy versus unilateral total adrenalectomy and cortical-sparing adrenalectomy of other/contralateral gland, or bilateral cortical-sparing adrenalectomy. Patients who undergo bilateral total adrenalectomy will be completely glucocorticoid and mineralocorticoid dependent with a 20–30 % risk of addisonian crisis, which is associated with significant morbidity and potential mortality [14, 22, 36, 37]. In patients who undergo cortical-sparing adrenalectomy in at least one gland, corticosteroid dependence may be avoided in 57–90 % of patients [19, 22, 29]. Recurrence of pheochromocytoma, either ipsilateral or contralateral, has been reported in 5–52 % of patients [14, 19, 22]. Patients who undergo cortical-sparing adrenalectomy should be monitored closely for persistent and recurrent disease as should all MEN2 patients [38].

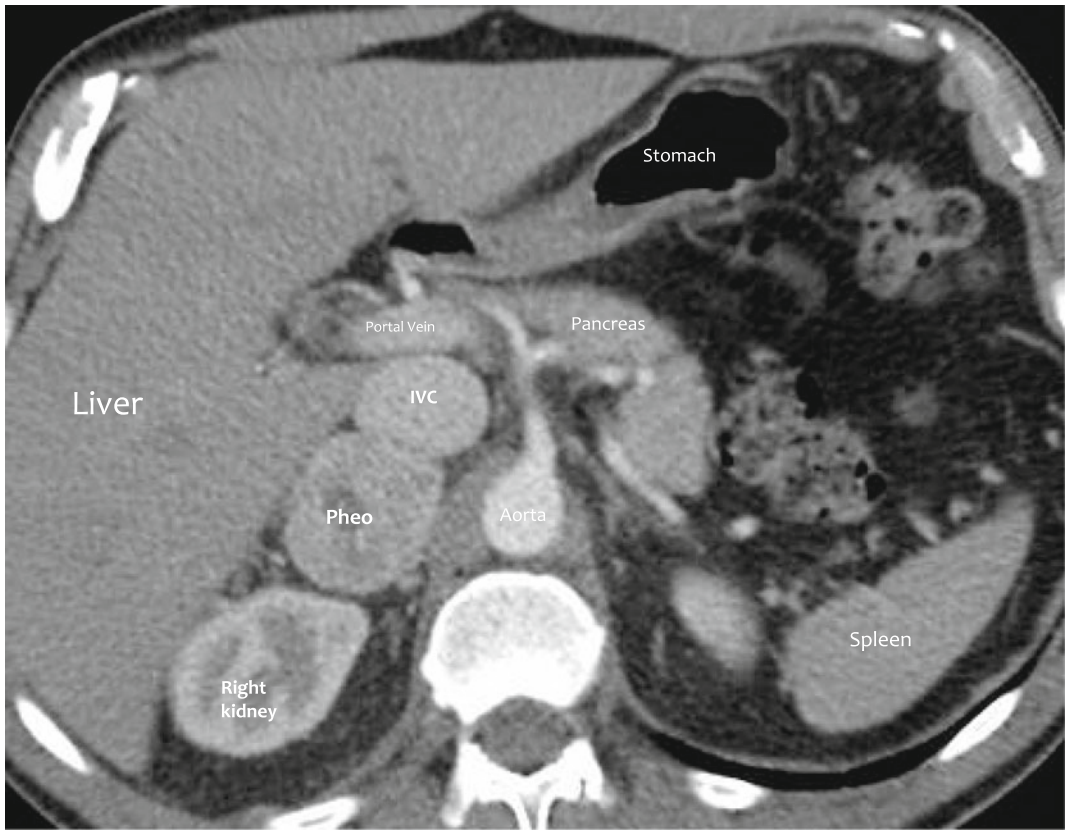

Fig. 10.1 Axial CT image of a right adrenal pheochromocytoma, which was removed through a right anterior laparoscopic approach as it is on the upper range of size to be removed through posterior retroperitoneoscopic approach. Abbreviations: *IVC* inferior vena cava; *Pheo* pheochromocytoma

Generally, preserving the cortex on at least one side (both adrenal glands if possible) is recommended to avoid the risk of corticosteroid dependence. Originally, cortical-sparing adrenalectomy was only performed as an open procedure through a midline anterior incision. With increasing experience in minimally invasive procedures, institutions have reported success with laparoscopic transabdominal or most recently, retroperitoneoscopic approaches [22, 29, 39]. The availability of the two approaches allows one to perform the initial operation for an MEN2-associated pheochromocytoma through a laparoscopic transabdominal approach and in the event of recurrence, reoperation through the posterior retroperitoneoscopic approach will avoid prior scar tissue (Fig. 10.4). This supports performing cortical-sparing adrenalectomy in all initial adrenal operations performed for MEN2-associated pheochromocytomas.

The success of cortical-sparing adrenalectomy depends on having adequate arterial supply and venous return to the remnant gland. The adrenal gland is known to have multiple arterial branches that originate from the aorta as well as inferior phrenic and renal arteries [40, 41]. The risk of ischemia with this vast arterial supply is low as long as the gland is not completely mobilized to allow preservation of a vascular network [42]. The venous return from the adrenal gland is more uniform although variation can exist [43, 44]. While some studies have recommended preservation of the main adrenal vein, others have shown that preservation of adrenal function is not dependent on adrenal vein preservation [4, 45–47].

Fig. 10.2 Operative table used for the posterior retroperitoneoscopic adrenalectomy. The patient is placed in the prone jackknife position

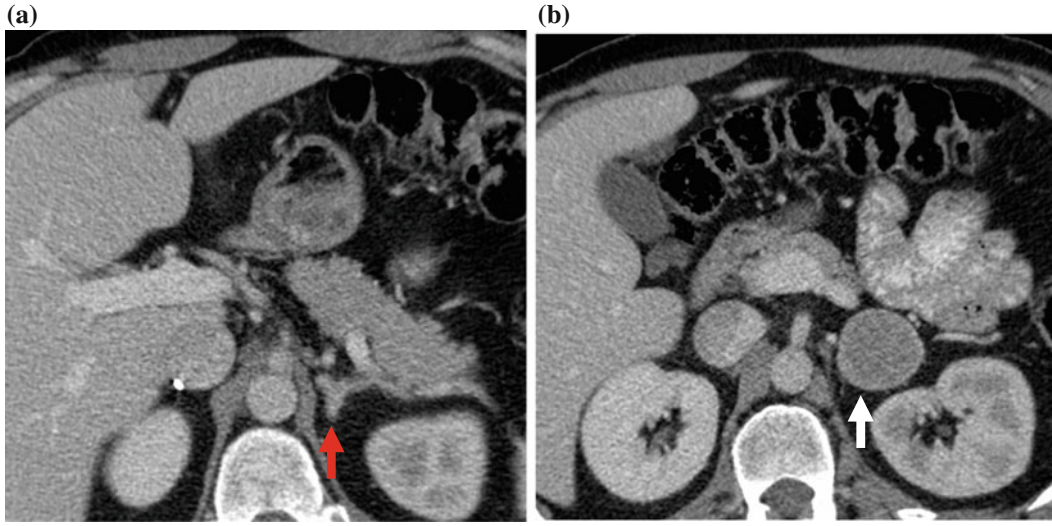

Fig. 10.3 Axial CT image of a left adrenal gland in an MEN2A patient who had undergone prior right complete adrenalectomy in the past and was referred for cortical-sparing left adrenalectomy. **a** Normal appearing adrenal limb at the superior extent of the left-sided pheochromocytoma (*red arrow*); **b** Pheochromocytoma (*white arrow*)

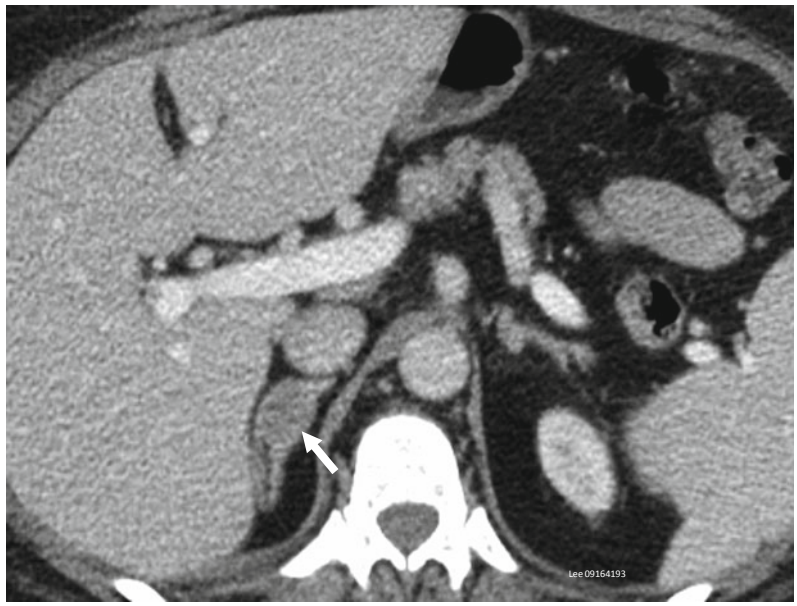

Fig. 10.4 Axial CT image of a right adrenal pheochromocytoma (*white arrow*), which could be removed with a cortical sparing procedure done either through a right anterior laparoscopic approach or through the posterior retroperitoneoscopic approach. We would prefer the anterior approach, thereby saving the posterior route for the future—in the event of recurrent pheochromocytoma on this same right side

The optimal amount of adrenal tissue that should be left in place is difficult to determine. It has been suggested that 15–30 % of adrenal tissue left in situ is adequate; however, usually the anatomy of the pheochromocytoma determines the amount of cortex left in situ [29, 33, 48]. If a single contralateral adrenal gland is present, due to prior total unilateral adrenalectomy, it may be of benefit to be more liberal with the amount of residual adrenal tissue left in situ. Intraoperative ultrasound has been advocated by some surgeons for laparoscopic cases to better delineate the borders of a pheochromocytoma [22, 49]. Reoperative subtotal adrenalectomy has also been reported for very selected patients with recurrent pheochromocytoma although this is not possible in most patients with recurrence [27, 34].

Primary Hyperparathyroidism

Primary hyperparathyroidism (PHPT) is reported to develop in 20–30 % of patients with MEN2A [50–52]. Screening should begin at age 11 years for at-risk patients in the ATA high-risk category and at age 16 for patients in the ATA moderate-risk category. Screening should include albumin-corrected calcium or ionized serum calcium annually with or without serum intact parathyroid hormone (PTH) levels. Diagnostic criteria and indications for surgery are similar to sporadic PHPT [50, 51, 53–55]. PHPT occurs most frequently in patients who have exon 11 *RET* codon mutations, most often with the codon 634 mutation [9]. Unlike MEN1, PHPT associated with MEN2A can be mild and asymptomatic [54, 56–58]. It may be diagnosed synchronously with MTC or more likely, after thyroidectomy, but it is rarely the presenting diagnosis for a patient with MEN2A [8]. In addition, PHPT may be due to a single enlarged parathyroid gland although multigland hyperplasia does occur [59, 60].

Surgical Approach

The challenge for most surgeons caring for patients with MEN2A-associated PHPT is that

they may have undergone previous total thyroidectomy with or without central lymph node dissection with the inherent risks of devascularization of the non-enlarged parathyroid glands [8]. Most often, at the time of thyroidectomy for MEN2A-associated MTC/C-cell hyperplasia, either prophylactic or therapeutic, PHPT has not developed although an enlarged parathyroid gland(s) may be found even in patients with preoperative normocalcemia [50, 51]. In order to preserve parathyroid function, prophylactic parathyroidectomy is not recommended and care should be taken maintain parathyroids in situ on their vascular pedicles. If devascularization inadvertently occurs, then the parathyroid gland should be autografted. For patients with MEN2A or an MEN2A-associated mutation, we prefer to autograft in a heterotopic muscle bed, such as the brachioradialis muscle of the forearm, rather than the sternocleidomastoid muscle of the neck [61].

Management of MEN2A-associated PHPT is complex and such patients may present with one of the following scenarios: (1) normal serum calcium and either a planned prophylactic thyroidectomy (with a low likelihood of harboring invasive MTC) or a planned therapeutic thyroidectomy for known invasive MTC. When invasive MTC is present, there is, by definition, a risk for metachronous local recurrence (of MTC) which may require reoperation; (2) PHPT and planned prophylactic or therapeutic thyroidectomy; and (3) PHPT diagnosed after the patient has had a thyroidectomy (PHPT may be a new diagnosis or recurrent) which is the most common scenario (Table 10.2) [60].

In the first scenario, namely a normal calcium and planned prophylactic thyroidectomy when invasive MTC is not suspected and therefore, central neck (level VI) dissection is not indicated, parathyroid gland preservation is critically important [1, 32]. Parathyroid glands which appear normal in size and shape should not be removed prophylactically as the patient may never develop PHPT [62]. If one or more parathyroid gland looks abnormal, then it should be removed as long as the patient will have one or more normal parathyroid glands remaining in situ and well vascularized at the end of the operation. If a normal appearing gland becomes devascularized, autografting should be performed into a heterotopic muscle bed. We would not autograft a parathyroid gland from an MEN2A patient into the sternocleidomastoid muscle of the neck. For patients scheduled to undergo therapeutic thyroidectomy in the setting of a normal serum calcium level, a central neck (level VI) dissection is needed and a devascularized parathyroid gland should be autografted into the forearm. Autografting is especially important in this situation as the patient is at risk for recurrent MTC and the need for reoperation on the central neck. Reoperation in the central neck is associated with an increased risk of hypoparathyroidism as in situ glands may be devascularized and/or not found within the surgical specimen, and therefore not autografted. Performing a parathyroid autograft at the first operation, therapeutic thyroidectomy, is critically important to prevent hypoparathyroidism associated with a reoperative neck procedure.

When PHPT is present prior to prophylactic or therapeutic thyroidectomy, intraoperative PTH (IOPTH) monitoring should be used to guide the extent of resection and provide further data to the surgeon in addition to the gross appearance of the parathyroid glands. Subtotal parathyroidectomy may be needed based on the IOPTH data and the size and appearance of the parathyroid glands. Previous studies have recommended total parathyroidectomy with heterotopic autotransplantation; however, this is no longer recommended for the initial operation in MEN2A patients [8, 57]. Forearm autografting should be performed if there is concern regarding the viability of the remaining in situ parathyroid gland(s) or in the setting of advanced MTC and the concern that reoperation on the central neck may occur in the future.

When MEN2A-associated PHPT is diagnosed after prior thyroidectomy (with or without concomitant parathyroidectomy at the first neck operation), the indications for surgery should be similar to patients with sporadic PHPT [54, 58]. Preoperative localization studies should be performed (combination of sestamibi, ultrasound, and/or CT) [55, 63]. In surgical planning, it is important to be aware of the risk that one or more

Table 10.2 Operative management of parathyroid glands in MEN2A

Preoperative diagnosis		Planned procedure	Parathyroid gland management	Management of devascularized parathyroid glands
Thyroid	Parathyroid			
First neck operation: no evidence of MTC (normal ultrasound and serum calcitonin level)	Calcium normal: no evidence of PHPT	Prophylactic thyroidectomy, no central (level VI) neck dissection	– Maintain normal appearing parathyroid glands in situ – Remove abnormal appearing glands – If there is any concern over the viability of the in situ parathyroid glands (surgeon judgment), use IOPTH to confirm a level of 10 pg/mL or more prior to closing	– Do not autograft an abnormal parathyroid gland if IOPTH is >10 pg/mL and the in situ gland(s) appear well vascularized. – If a normal appearing parathyroid is removed, autograft in heterotopic site (forearm) if IOPTH is <10 pg/mL or there is clinical concern over the vascularity of the in situ glands.
First neck operation: suspected or confirmed MTC	Calcium normal: no evidence PHPT	Therapeutic thyroidectomy with central (level VI) neck dissection	– Maintain normal appearing parathyroid glands in situ – Remove abnormal appearing glands – Inferior parathyroid glands likely devascularized with the central neck dissection and therefore, removed - Use IOPTH to confirm a level of 10 pg/mL or more prior to closing	– Autograft at least one of the normal appearing devascularized parathyroid glands in a heterotopic site (forearm)
First neck operation: no MTC or suspected/confirmed MTC	Calcium elevated: PHPT present	Prophylactic/therapeutic thyroidectomy ± central (level VI) dissection; parathyroidectomy with IOPTH monitoring	– Remove abnormal parathyroid glands. – Use IOPTH to confirm a level of 10 pg/mL or more prior to closing	– If invasive MTC is present autograft at least one gland in heterotopic site (forearm), preferably a normal appearing gland if available. – If MTC is not suspected (prophylactic thyroidectomy), and a normal appearing parathyroid is removed, autograft in heterotopic site (forearm) if IOPTH is <10 pg/mL or if there is clinical concern over the vascularity of the in situ glands.
Prior thyroidectomy (±) central neck dissection	PHPT	Parathyroidectomy with IOPTH monitoring (obtain preoperative imaging studies and prior operative and pathology reports to guide procedure)	– Remove abnormal parathyroid gland(s) – Use IOPTH to confirm a level of 10 pg/mL or more prior to closing	– Autograft into heterotopic site (forearm) if IOPTH <10 pg/mL

Abbreviations: *MTC* medullary thyroid cancer; *PHPT* primary hyperparathyroidism; *IOPTH* intraoperative parathyroid hormone

parathyroid glands may have been removed or devascularized with the initial procedure. It is helpful to obtain prior surgical operative reports as well as pathology reports. In general, the operation is a focused procedure based on preoperative imaging and therefore, only enlarged parathyroid glands should be removed and normal glands are usually left in situ. IOPTH monitoring should be used to determine whether all hyperfunctioning parathyroid tissue has been removed. IOPTH monitoring is also critically important to ensure that there is adequate parathyroid function (IOPTH level $\geq$10 pg/mL) before the operation is terminated. If IOPTH monitoring indicates lack of functioning parathyroid tissue (undetectable PTH level or a level <10 pg/mL), a portion of the removed parathyroid gland should be autografted in a heterotopic site (forearm).

Some institutions have reported success with cryopreservation of parathyroid tissue for subsequent autografting if needed; however, this technique is not widely available and results are varied; delayed autografting even if technically possible is likely not as successful as autografting at the time of initial operation [64, 65]. If only one enlarged parathyroid gland is identified and there is evidence that three parathyroid glands were removed in previous procedures, then one is left with either leaving a portion of the enlarged parathyroid gland in situ with adequate blood supply or autografting a portion of the removed parathyroid to the forearm. Some experts in the field would perform both; then, if needed, and based on the PTH level the morning after surgery, the forearm autograft can be removed.

Conclusions

Hereditary medullary thyroid carcinoma is most commonly diagnosed as part of the MEN2 syndromes. Most frequently, MTC is the presenting diagnosis in both newly diagnosed kindreds and those who are under active screening. However, in treating hereditary MTC, careful evaluation for associated conditions including pheochromocytoma and PHPT is crucial. Thoughtful management of adrenal disease and parathyroid disease can help avoid or delay long-term corticosteroid dependence and hypoparathyroidism.

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11 Surgical Management of Sporadic Medullary Thyroid Cancer (Extent of Thyroid Resection and the Role of Central and Lateral Neck Dissection)

Randall P. Scheri, Sanziana A. Roman and Julie Ann Sosa

Background

Medullary thyroid cancer (MTC) constitutes 1–2 % of all thyroid cancers [1]. Approximately 75 % of MTC is sporadic, with a mean age of presentation in the 5th decade of life, and a slight female preponderance [2]. The stage at presentation has been stable in the USA for several decades [3, 4]. Most patients with sporadic MTC present with a thyroid nodule [5]. For patients with clinically palpable nodules, up to 75 % will have cervical lymph node metastases, and 10 % will have distant metastases [6]. Less frequently, sporadic MTC is detected by screening with measurement of serum calcitonin in patients with thyroid nodules, a practice done routinely in Europe, but not in the USA [7]. Occasionally, MTC can be detected incidentally after thyroidectomy for other pathology.

The mainstay of treatment for patients with sporadic MTC is thyroidectomy and compartment-oriented lymph node dissection. Several studies have evaluated the efficacy of different surgical management strategies for these patients; however, these studies have largely been non-randomized and retrospective. The American Thyroid Association (ATA) published revised guidelines for the management of MTC in 2015 [8]. The guidelines include 66 recommendations; however, insufficient or contradictory data and differing opinions among thought leaders perpetuated areas of debate where the expert panel could recommend neither for nor against a particular treatment. This chapter summarizes the revised 2015 ATA MTC guidelines and the somewhat contradictory evidence regarding surgical management strategies for sporadic MTC.

Preoperative Evaluation

All patients diagnosed with MTC should have a thorough evaluation for symptoms of dysphagia, dyspnea, and/or dysphonia. Any of these symptoms may indicate the presence of locally advanced disease that occurs in up to 15 % of patients with palpable MTC [4]. Diarrhea, flushing, or bone pain are symptoms that should raise concern for distant metastases. Symptoms of pheochromocytoma and hyperparathyroidism also should be sought, because 6–7 % of patients with apparently sporadic MTC may harbor an unsuspected, often de novo, rearranged during transfection (*RET*) germline mutation [9, 10]. A thorough family history should be obtained with attention to thyroid, parathyroid, adrenal

R.P. Scheri · S.A. Roman · J.A. Sosa (✉)
Section of Endocrine Surgery, Duke University School of Medicine, DUMC 2945, Durham, NC 27710, USA
e-mail: Julie.sosa@duke.edu

R.P. Scheri
e-mail: r.scheri@duke.edu

S.A. Roman
e-mail: sanziana.roman@duke.edu

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diseases, hypertension, and sudden cardiac death. Physical examination should determine the size of the primary mass, potential for fixation to local neck structures, and the possible presence and extent of cervical lymph node metastases.

Nearly all MTCs secrete calcitonin and/or carcinogenic embryonic antigen (CEA) [6]. Calcitonin is an excellent tumor marker for MTC, with a sensitivity of 99 % and specificity of 95 % [11]. CEA is not specific for MTC and is often within normal limits for small (<1 cm) tumors [12]. CEA is usually elevated with clinically evident disease, so it is helpful to evaluate for disease progression or for surveillance after surgery [13]. CEA may also be the only marker in calcitonin-negative MTC [14].

Serum calcitonin and CEA levels should be obtained preoperatively and compared to postoperative values two to three months after surgery, as these tumor markers may take several months to reach their nadir [15]. It is important that the same calcitonin assay is used over time given the considerable variability that exists among different assays and laboratories [16, 17]. Preoperative serum calcitonin and CEA levels correlate well with volume and extent of disease [18]. Postoperative values are prognostic of outcome and may predict biochemical cure [19]. All patients with presumed sporadic MTC should be referred for genetic counseling for *RET* proto-oncogene testing. Commercially available testing is accurate, detecting 95 % of known mutations [20]. Patients with an unknown *RET* status should have biochemical testing to rule out pheochromocytoma (plasma fractionated metanephrines or 24-hour urine catecholamines and metanephrines) and primary hyperparathyroidism (serum calcium and intact parathyroid hormone levels).

Preoperative imaging is required to assess for the extent of local and regional disease. Cervical ultrasound (US) is excellent for determining the size and location of the primary tumor and the presence of features concerning for invasion into local structures (Fig. 11.1). Cervical lymph node mapping with US evaluates for lymph node

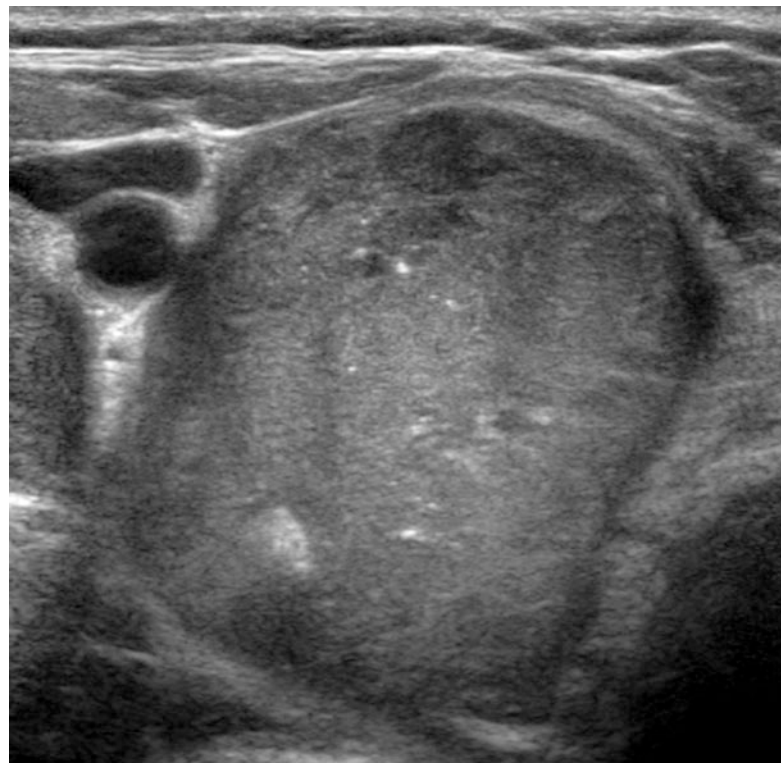

Fig. 11.1 Ultrasound of medullary thyroid cancer with hypoechoic heterogeneous appearance and microcalcifications

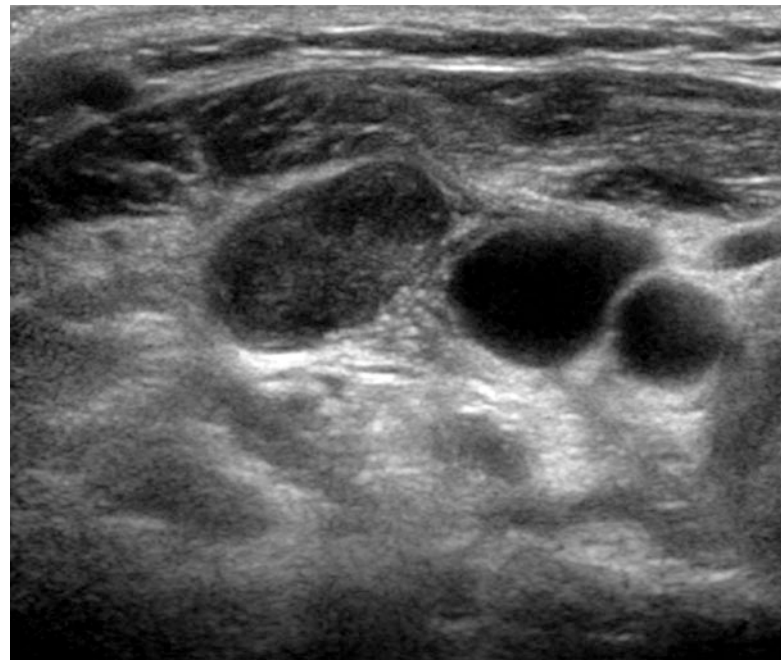

Fig. 11.2 Ultrasound of suspicious enlarged cervical lymph node with hypoechoic appearance and rounded shape

metastases (Fig. 11.2). US-guided fine needle aspiration biopsy of any concerning lymph nodes should be performed to establish the presence or absence of metastases. Immunohistochemistry staining with calcitonin and calcitonin washout of fine needle aspiration biopsy increases sensitivity and should be performed for any biopsy that is indeterminate [21, 22].

Patients with extensive cervical disease and/or signs and symptoms concerning for distant metastases, and all patients with a serum calcitonin level >500 pg/mL require imaging to evaluate for distant metastases [8]. Contrast-enhanced computed tomography (CT) of the neck and the chest assesses for invasion into surrounding structures, including the trachea, esophagus, and major vascular structures, as well as lung and mediastinal lymph node metastases (Fig. 11.3). Three-phase contrast-enhanced liver CT or contrast-enhanced magnetic resonance imaging (MRI) evaluates for liver metastases, and axial MRI or bone scintigraphy assesses for bone metastases. Positron emission tomography with 18-flouro-2-deoxyglucose (FDG-PET) is less sensitive in detecting metastases compared to the above imaging procedures, so it is not recommended [23].

Treatment for Clinically Occult Medullary Thyroid Cancer

Clinically occult sporadic MTC may be detected by screening with serum calcitonin measurements in patients with thyroid nodular disease, thus permitting earlier diagnosis of MTC and possibly downstaging of patients for improved outcomes. Elevated serum calcitonin levels can occur with benign C-cell hyperplasia, renal failure, thyroiditis, follicular neoplasms, neuroendocrine tumors of organs other than the thyroid, and other diseases (Table 11.1). For patients with mildly elevated serum calcitonin levels (10–100 pg/mL), pentagastrin-stimulated calcitonin improves test sensitivity, but is unavailable in many countries, including the USA. In a single-institution prospective study by Elisei et al. of 10,684 patients with thyroid nodular disease, screening with serum calcitonin detected MTC in 44 (0.4 %) patients [7]. MTC was detected at an earlier stage, and serum calcitonin levels were more frequently undetectable after surgery for the patients in the calcitonin-screening group compared to historically matched

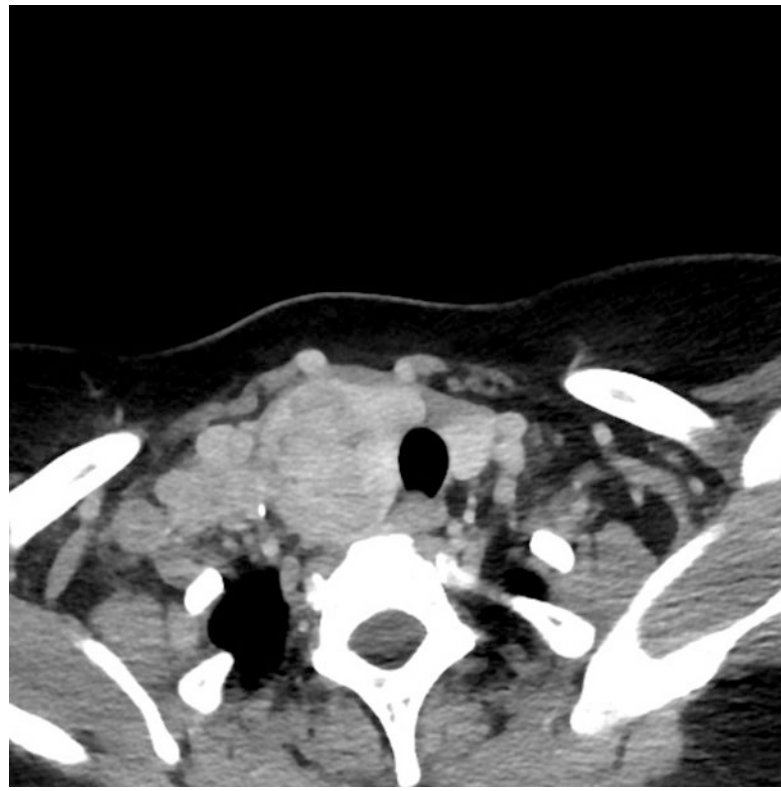

Fig. 11.3 Computed tomography demonstrating significant lateral lymphadenopathy

controls. At the end of follow-up, complete remission was observed in 59 % of the screening group compared to 2.7 % of the control group ($P = 0.0001$).

In a single-institution prospective study by Constante et al., 5,817 patients with thyroid nodules had basal serum calcitonin levels obtained; they were elevated in 282 (4.8 %) patients [24]. Basal serum calcitonin levels were only mildly elevated (10–20 pg/mL; normal <10 pg/mL) in 216 (3.7 %) patients. Of the remaining 66 patients (1.1 %), 9 had basal serum calcitonin levels >100 pg/ml, all of whom had surgery with MTC identified in the surgical specimen. The remaining 57 patients had pentagastrin stimulation testing followed by thyroidectomy and central compartment lymph node dissection if the stimulated serum calcitonin level was >100 pg/mL. Stimulated calcitonin was positive in 4 of 8 patients with basal serum calcitonin levels between 50 and 100 pg/mL; thyroidectomy revealed MTC in 2 patients and C-cell hyperplasia in 2 patients; and in 8 of 49 patients with basal serum calcitonin levels between 20 and 50 pg/mL, thyroidectomy revealed MTC in 4 patients and C-cell

Table 11.1 Alternative causes for elevated calcitonin

C-cell hyperplasia	Renal failure
Follicular neoplasm	Mastocytosis
Autoimmune thyroiditis	Heterophilic antibodies
Hyperparathyroidism	Small-/large-cell lung cancer
Proton pump inhibitors	Neuroendocrine tumors
Smoking	Prostate cancer

hyperplasia in 4 patients. None of the patients with basal serum calcitonin levels <100 pg/mL had cervical lymph node metastases.

In another single-institution prospective study by Iacobone et al., basal serum calcitonin levels were obtained in 7,276 patients with thyroid nodular disease [25]. Patients underwent pentagastrin-stimulated calcitonin testing when the basal serum calcitonin level was >10 pg/mL. Thyroidectomy and central compartment lymph node dissection were performed in 66 patients with either a basal serum calcitonin level >30 pg/mL or a stimulated calcitonin level >100 pg/mL. On final pathology, MTC was present in 45 patients, C-cell hyperplasia in 16, and no abnormality was identified in 5 patients. No patients with a basal serum calcitonin level <30 pg/mL or a stimulated calcitonin <200 pg/mL had lymphatic or distant metastases. These and other similar studies led the German Society for Endocrinology to propose a consensus recommendation for serum calcitonin screening in all patients with nodular thyroid disease [26]. They recommended pentagastrin stimulation testing for basal serum calcitonin levels >10 pg/mL, excluding patients with renal insufficiency or taking proton pump inhibitors. Thyroidectomy was recommended for stimulated calcitonin levels >100 pg/mL because the risk of MTC in these patients was >50 %. Thyroidectomy and lymphadenectomy were recommended for stimulated calcitonin levels >200 pg/mL. In 2006, the European Thyroid Association and Cancer Research Network recommended routine serum calcitonin screening for all patients with thyroid nodular disease [27].

Calcitonin screening has not been similarly embraced in the USA. This is largely due to the lack of pentagastrin in this country, limiting the ability to accurately screen patients with mildly elevated serum calcitonin levels. While the positive predictive value for detecting MTC is 100 % for a basal calcitonin >100 pg/mL, it drops to 25 % for basal serum calcitonin levels between 50 and 100 pg/mL, and it is only 8 % for basal serum calcitonin levels between 20 and 50 pg/mL [24]. If all patients with mildly elevated basal serum calcitonin levels underwent surgery, many would have unnecessary surgery along with the attendant risk of surgical morbidity. Calcium, a widely available, inexpensive calcitonin secretagogue, has been evaluated in small studies as an alternative to pentagastrin. A single-institution prospective study by Columbo et al. compared calcium and pentagastrin infusion for 141 patients (normal volunteers, multinodular goiters, *RET* mutation carriers, and known MTC patients) and revealed that calcium infusion reliably diagnosed MTC with fewer side effects than pentagastrin [28]. The threshold serum calcitonin level discriminating MTC is different with calcium infusion than pentagastrin and is still not clearly defined. In addition to the lack of a reliable secretagogue, there are concerns regarding the cost of screening and subsequent surgery for patients that test positive [29]. A recent decision and cost-effectiveness analysis addressed these concerns about cost in the USA and concluded that serum calcitonin screening in this country would be cost-effective if thyroidectomy was to be performed for calcitonin levels >50 pg/mL [30].

The 2015 revised ATA guidelines for the management of MTC and the 2009 ATA guidelines for thyroid nodules/well-differentiated thyroid cancer recommended "neither for nor against" calcitonin screening, suggesting that physicians should be left to decide whether the technique is useful in the management of patients in their clinic [8, 31]. The 2015 MTC guidelines had no recommendations for the management of patients that are screened with calcitonin. Based on the available data, it is reasonable to assume that patients with a basal serum calcitonin level >100 pg/mL should be considered to harbor MTC and should undergo preoperative cervical lymph node mapping as well as a total thyroidectomy with at least a central compartment lymph node dissection. If metastatic lateral cervical lymph nodes are identified, a lateral compartment lymph node dissection is required. When the serum calcitonin level is mildly elevated (10–100 pg/mL), calcium stimulation can be considered. However, expected calcium-stimulated serum calcitonin thresholds have not been set the way they have been for pentagastrin stimulation; therefore, exact serum calcitonin elevation associations needed to

discriminate MTC from other pathology are not well defined. Clinical and biochemical surveillance is recommended for stimulated calcitonin levels that are <100 pg/mL. If pentagastrin is unavailable and the basal serum calcitonin level is between 50 and 100 pg/mL, a thyroidectomy with central compartment lymph node dissection can be considered on an individual basis after discussion with the patient. Biochemical and clinical surveillance are recommended for a basal serum calcitonin level that is between 10 and 50 pg/mL.

Compartment-Oriented Dissection for Clinically Apparent Medullary Thyroid Cancer

Patients with sporadic MTC usually present with a palpable thyroid nodule [5]. Cervical lymph node metastases are common, but in many patients, they are clinically and radiologically occult [32]. Metastases may occur in the central (Level VI), lateral (Levels II–V), and mediastinal (Level VII) compartments. Metastases involving Level I are exceedingly rare [33]. In a single-institution retrospective study by Scollo et al., 27 (50 %) of 54 patients with sporadic MTC presented with a palpable nodule only, 8 (15 %) with palpable lymph nodes only, and 16 (30 %) with both a palpable nodule and palpable lymph nodes [34]. Lymph node metastases were present in 34 of 54 (63 %) patients in the lymph node dissection specimen. Central, ipsilateral lateral, and contralateral lateral compartment lymph node metastases were present in 50, 57, and 28 % of patients, respectively. The frequency of central and lateral compartment lymph node metastases were dependent on the size of the primary tumor, and they were present in 30 % of patients with tumors <1 cm in size, 50 % of patients with tumors 1–3 cm, and 100 % of patients with tumors >3 cm. A national study by Kazaure et al. of 310 patients with microMTC (mean tumor diameter 5.7 mm) identified cervical lymph node metastases in 65 of 176 (37 %) patients who had any lymph nodes removed and distant metastases in 5 % of patients [35]. A single-institution retrospective study by Moley et al. identified cervical lymph node metastases in more than 75 % of 32 patients with palpable MTC treated with bilateral central and lateral compartment lymph node dissections [5]. Patients with unifocal MTC had central, ipsilateral lateral, and contralateral lateral compartment lymph node metastases in 81, 81, and 44 % of patients, respectively.

The pattern of lymph node metastases from MTC is typically stepwise, from the central compartment lymph nodes to the ipsilateral lateral compartment lymph nodes and lastly to the contralateral lateral compartment lymph nodes [8]. Distant metastases are infrequent without nodal metastases, occurring in only 5–11 % of patients with MTC [32, 34]. Skip metastases directly to the lateral compartment lymph nodes without metastases to the central compartment lymph nodes most frequently occur for tumors located in the upper third of the thyroid gland, which is commonly seen with MTC due to the high concentration of parafollicullar C-cells in this area. Besides the location of the primary tumor, the incidence of lateral compartment nodal metastases also is related to the number of central compartment lymph node metastases. In a single-institution retrospective study by Machens et al. of 195 patients with MTC, the rates of ipsilateral lateral compartment lymph node metastases were 10, 77, and 98 % when 0, 1–3, or ≥ 4 central compartment nodal metastases were present, respectively [36]. The rates of contralateral lateral compartment lymph node metastases were 5, 38, and 77 % when 0, 1–9, or ≥ 10 central compartment lymph node metastases were present, respectively. The extent of lymph node involvement is also predictive of distant metastases. In another single-institution retrospective study by Machens et al. of 105 patients with MTC who underwent bilateral central and lateral compartment lymph node dissections, metastases to the contralateral lateral and mediastinal lymph nodes were predictive of distant metastases [37].

The preoperative basal serum calcitonin level is also predictive of the pattern and extent of

lymph node metastases. In a single-institution retrospective study by Machens et al. of 300 patients with MTC who underwent bilateral central and lateral compartment lymph node dissections, there were no lymph node metastases when the serum calcitonin level was <20 pg/mL (normal <10 pg/mL) [38]. A basal serum calcitonin level of >20, 50, 200, and 500 pg/mL was associated with lymph node metastases in the ipsilateral central and lateral compartments, contralateral central neck, contralateral lateral neck, and upper mediastinum, respectively. Bilateral central and lateral compartment lymph node dissections achieved biochemical cure in 50 % of patients with a basal serum calcitonin level <1000 pg/mL, but not in patients with serum calcitonin levels >10,000 pg/mL. In short, basal serum calcitonin levels can provide important information regarding the extent of lymph node metastases and thereby help guide clinical decision making about the planned extent of surgery.

The decision regarding optimal extent of lymph node dissection should consider the location of the tumor, imaging findings, basal serum calcitonin level, and the presence of distant metastases. It is important to keep in mind that remedial neck dissections have a higher risk of complications, so the best time to dissect the involved lymph node compartments is during the initial surgery. A complete compartment-oriented lymph node dissection always should be performed to excise all nodal tissue; "berry-picking" of clinically involved lymph nodes is strongly discouraged. A central (Level VI) compartment lymphadenectomy encompasses removal of all lymph nodes bound by the hyoid bone superiorly, the innominate artery inferiorly, and the carotid arteries laterally (Fig. 11.4) [39]. A lateral compartment (Levels II–V) lymph node dissection encompasses removal of all lateral compartment lymph nodes, typically with preservation of the internal jugular vein, sternocleidomastoid muscle, and spinal accessory nerve. A mediastinal (Level VII) compartment lymph node dissection encompasses removal of the pretracheal and paratracheal superior mediastinal lymph nodes between the innominate vein and sternal notch. Surgery for patients with MTC can be technically challenging and is associated with a higher risk of complications [40]. Ideally, surgery should be done at a high-volume center by an experienced thyroid surgeon in order to safely and adequately remove all disease with a

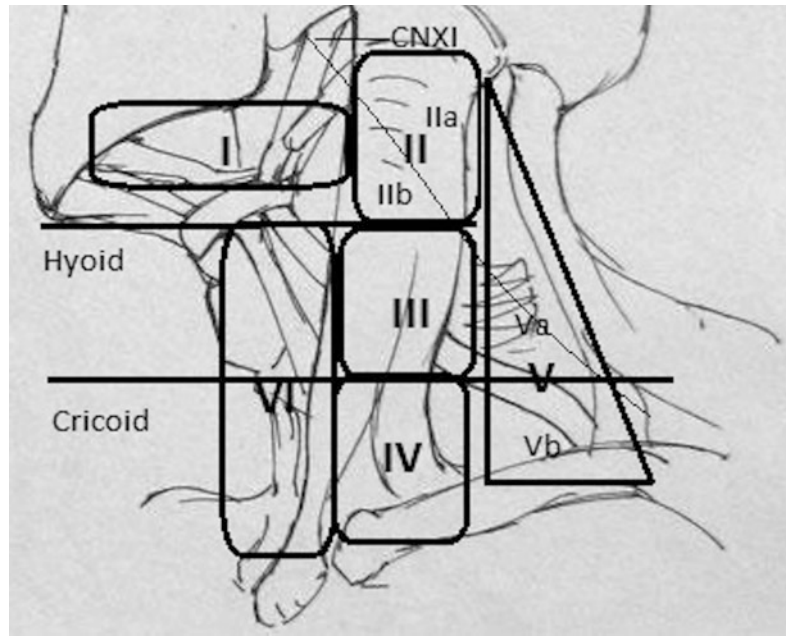

Fig. 11.4 Lymph node compartments of the neck

minimal risk of complications. The recent ATA 2015 MTC guidelines have recommended total thyroidectomy and central compartment lymph node dissection for patients without evidence of lateral lymph node metastases on ultrasound or distant metastases [8]. The extent of lateral compartment lymphadenectomy for patients with MTC is still controversial. Some clinicians feel that lateral compartment lymph node dissections should not be done for patients with lymph nodes that appear normal on ultrasound and without biopsy-proven lymph node metastases. The possible complications of lateral compartment dissection, including injury to the spinal accessory, phrenic, brachial plexus, vagus nerves, bleeding, lymphatic, and thoracic duct leak, should be avoided without proven metastases. However, others feel that prophylactic lateral compartment lymph node dissection should be performed for serum calcitonin levels that are >20 pg/mL, due to the high frequency of occult nodal metastases and improved chance of biochemical cure. The 2015 MTC guidelines "neither recommended for nor against" lateral compartment lymph node dissection in patients with normal appearing lymph nodes on ultrasound and without distant metastases based on basal serum calcitonin levels. The guidelines recommend that all patients with MTC confined to the neck and cervical lymph nodes should have a total thyroidectomy, bilateral central compartment lymph node dissection, and dissection of biopsy-proven lateral lymph node compartments. For patients with biopsy-proven ipsilateral lateral compartment lymph node metastases and negative ultrasound/biopsy in the contralateral lateral compartment, contralateral lymph node dissection may be considered for serum calcitonin levels >200 pg/mL.

Unfortunately, the data guiding management of clinically node-negative MTC patients are inconclusive. For these patients, an individualized approach taking into consideration patient age, comorbidities, and care plan goals may be best. Young and healthy patients without clinically involved lateral compartment lymph nodes may be considered for a more aggressive approach with prophylactic ipsilateral lateral compartment lymphadenectomy for basal serum calcitonin levels >20 pg/mL and contralateral lateral compartment lymph node dissection for basal serum calcitonin levels >200 pg/mL, while observation with serial ultrasound may be appropriate for less healthy, elderly patients, or those unwilling to accept the risk of surgical complications if no metastatic lymph nodes are identified on final pathology (negative lymphadenectomy).

In the presence of distant metastases, the goals of surgery are palliative, with attention focused on preservation of the airway and minimization of surgical complications. Thyroidectomy and lymphadenectomy of clinically evident disease to obtain local control and prevent airway compromise are appropriate in some patients with distant metastases, as MTC may behave in an indolent fashion. Systemic therapy with vandetanib or cabozantinib is recommended for patients with progressive MTC that is locally advanced or associated with distant metastases and therefore not amenable to surgical resection [8]. A prospective randomized multi-institution phase III trial by Wells et al. of 331 patients compared vandetanib versus placebo for symptomatic, progressing locally advanced or metastatic MTC and revealed prolonged progression-free survival in the vandetanib group (median 30.5 vs. 19.3 months, $p < 0.0001$) [41]. Patients treated with vandetanib also experienced less pain and diarrhea and had an improved quality of life. A prospective randomized multi-institution phase III trial by Elisei et al. of 330 patients compared cabozantinib versus placebo for patients with progressive, metastatic, or locally advanced MTC and revealed improved progression-free survival in the cabozantinib group (median 11.2 vs. 4 months, $p < 0.0001$) [42]. For patients with metastatic MTC, the decision for surgery/treatment is complex and is best made on an individual basis in conjunction with the multidisciplinary care team and the patient, who should be presented with the risks and benefits of alternative management approaches.

Unilateral Surgery for Medullary Thyroid Cancer

In patients with sporadic MTC, the incidence of multifocal MTC ranges from 8 to 22 %, and the incidence of bilateral tumors ranges from 5 to 9 % [43, 44]. In a single-institution prospective study by Myauchi et al., 15 patients with sporadic MTC were treated with unilateral thyroid lobectomy, bilateral central compartment lymph node dissection, and ipsilateral lateral compartment lymphadenectomy; 12 of these patients (80 %) achieved biochemical cure with this surgical approach, as defined by a normal stimulated serum calcitonin level in the postoperative setting [45]. These were compared to a historical retrospective cohort of 22 patients from the same institution with sporadic MTC who were treated with total thyroidectomy, bilateral central compartment lymph node dissection, and ipsilateral lateral compartment lymph node dissection; 15 patients (68 %) of this cohort achieved biochemical cure. Large tumor size and the presence of lymph node metastases were associated with persistent disease. The extent of thyroid resection was not related to biochemical cure in this small series. On follow-up (mean, 107 ± 67 months), none of the patients treated with thyroid lobectomy recurred in the residual thyroid lobe. The study suggested that thyroid lobectomy with bilateral central and ipsilateral lateral compartment lymph node dissections may be appropriate for patients with sporadic MTC who have no contralateral disease on physical examination or ultrasonography. Theoretical advantages of unilateral thyroid lobectomy compared to total thyroidectomy include minimizing risk of contralateral recurrent laryngeal or bilateral recurrent laryngeal nerve injury, hypoparathyroidism, and the absolute requirement for lifelong thyroid hormone replacement. This approach of thyroid lobectomy and compartment-oriented central and ipsilateral lateral compartment lymph node dissection has not been evaluated outside of Japan, but likely warrants consideration of a trial, given that long-term biochemical cure rates appear similar to those from studies where total thyroidectomy was done [46, 47]. The 2015 MTC guidelines still recommend total thyroidectomy for sporadic MTC, but do state that complete thyroidectomy for presumed sporadic MTC was detected after thyroid lobectomy is only recommended for patients with a *RET* proto-oncogene mutation, an elevated postoperative serum calcitonin level, or imaging studies indicating residual MTC.

Management of Medullary Thyroid Cancer After Incomplete Thyroidectomy or Lymph Node Dissection

Occasionally, MTC is diagnosed after the patient undergoes a thyroid lobectomy for other reasons. MTC can be misdiagnosed on cytology as a Hürthle cell neoplasm, follicular neoplasm, or papillary thyroid carcinoma [48]. For these patients, serum calcitonin and CEA levels as well as *RET* proto-oncogene testing and cervical ultrasonography should be obtained following surgery. A completion thyroidectomy is recommended for patients with a *RET* mutation, positive tumor margins, C-cell hyperplasia, tumor multifocality, elevated serum calcitonin or CEA levels, and/or imaging studies which indicate that there is likely residual MTC. These data are sparse regarding management of patients with incidental sporadic MTC without evidence of residual disease. In a single-institution retrospective study by Peix et al., 18 patients with occult sporadic MTC (median size 3.6 mm, range 1–11 mm) were identified after thyroid resection; 6 of these were diagnosed after thyroid lobectomy [49]. Only one patient developed an elevated serum calcitonin level on follow-up (mean 44 months, range 18–70 months). Completion thyroidectomy and bilateral central and lateral compartment lymph node dissection were performed, revealing three lymph node metastases (ipsilateral central, ipsilateral lateral, and contralateral lateral nodes). The authors suggested that completion thyroidectomy and a compartment-oriented lymph node dissection should be reserved for patients with elevated serum calcitonin levels due to the low risk of

recurrence and the morbidity associated with reoperation.

By comparison, patients with inadequate initial surgery and residual lymph node metastases are likely to benefit from reoperative compartment-oriented lymph node dissection. In a single-institution retrospective study by Machens et al., re-operative central and lateral compartment lymphadenectomy was performed on 334 patients with persistent MTC, as determined by elevated serum calcitonin levels (1,797 pg/mL, range 885–2,736 pg/mL) after total thyroidectomy [40]. For patients with no lymph node metastases removed at the initial surgery, biochemical cure was achieved with reoperative surgery in 59 (44 %) of 133 patients. Patients with 1–5 or >5 nodal metastases removed at the initial operation attained biochemical cure with reoperative surgery in just 12 (18 %) of 65 patients and 2 (5 %) of 43 patients, respectively. Biochemical cure was achieved with reoperative surgery in only 1 of 76 patients with serum calcitonin levels >1000 pg/mL. In this series, the risk of complications was significantly elevated even when expert endocrine surgeons performed the reoperative surgery. After the reoperative surgery, hypoparathyroidism was observed in 37 (11 %) patients, permanent unilateral recurrent laryngeal nerve palsy occurred in 8 (2.4 %) patients, and bleeding requiring reoperation occurred in 16 (5 %) patients. The complication rates were much higher during initial surgeries performed at the referring outside institutions. The 2015 MTC guidelines recommend that for patients with inadequate lymph node dissection at initial surgery, reoperative surgery should be considered with compartment-oriented lymph node dissection as long as the basal serum calcitonin level is <1000 pg/mL and <5 lymph node metastases were resected at the initial surgery.

Summary/Recommendations

Surgery remains the mainstay of treatment for sporadic MTC, although administration of vandetanib or cabozantinib is indicated for patients with progressive MTC that is locally advanced or associated with distant metastases and therefore not amenable to surgical removal. Lymph node metastases are frequent and occur even with early-stage primary tumors, arguing for aggressive compartment-oriented lymph node dissections in patients without suspected distant metastases. However, extensive surgery, particularly for patients with locally advanced disease, is complex and frequently associated with a higher attendant risk of complications. Decisions regarding the management of these patients, including the extent of lymph node dissection, should incorporate the recommendations of evidence-based guidelines; however, controversy and uncertainty remains for some clinical scenarios due to a scarcity of data. In these cases, management decisions should be individualized, taking into consideration the apparent extent of disease, patient demographic, and clinical factors such as age, comorbidities, and preferences, as well as the availability of local surgical expertise. Optimally, patients with MTC should be referred to high-volume endocrine neoplasia centers with experienced multidisciplinary teams and high-volume thyroid surgeons in order to enhance patient outcomes and minimize complications.

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Surgical Management of Invasive Medullary Thyroid Carcinoma

12

Scott Coman and Raefe Gundelach

The clinical course of medullary thyroid cancer (MTC) is usually more aggressive than differentiated thyroid cancer (DTC). Rates of recurrence and mortality tend to be higher. A significant proportion of patients with sporadic MTC have extrathyroidal disease, with 15 % demonstrating upper aerodigestive tract invasion [1].

Extrathyroidal extension, which is a precursor to invasion, occurs in up to 35 % in some series [2, 3] and has been shown to be a predictor of diminished survival [4–6] in patients with MTC. Incomplete surgical excision has also been shown to be associated with higher mortality [3]. In light of this and the fact that surgery remains the mainstay of treatment for MTC, the appropriate surgical management of disease invading the larynx and trachea is imperative. Failure to adequately control thyroid cancer in the central neck can result in significant morbidity for patients and may be the cause of death in up to half the patients who die of thyroid cancer [7].

There is a paucity of data which specifically addresses aerodigestive tract invasion in MTC. This reflects the rarity of the disease. Some series that deal with invasive thyroid carcinoma have included MTC [8–12] but the numbers of cases tend to be small. As such many of the recommendations regarding invasive MTC are arrived at by simply extrapolating from series that primarily involve DTC. In a large series from the Mayo Clinic, for example, of 296 patients with invasive thyroid carcinoma, 10 (<3 %) had MTC [13]. There are some significant differences, however, between the behavior of DTC and MTC that may influence its surgical approach.

Firstly, disease spread beyond the thyroid tends to occur at an early stage in MTC [14], resulting in over 50 % of patients presenting with a palpable thyroid nodule having regional metastases and up to 5 % having distant disease evident on presentation. Furthermore, regional metastases tend to have a more significant effect on prognosis than in differentiated thyroid cancer.

There is also a more predictable relationship between extent of primary tumor and likelihood of regional and distant disease [15]. In other words, patients presenting with invasive disease will often already be incurable by virtue of extent of their regional, and occult or clinically apparent distant disease. This has important implications for clinical decision-making. Despite this, there may be a role for segmental resection of limited invasive disease in the context of minimal distant

S. Coman (✉) · R. Gundelach
Department of Otolaryngology, Princess Alexandra Hospital, 199 Ipswich Rd, Woolloongabba, Brisbane, QLD 4107, Australia
e-mail: scoman68@gmail.com; dscoman@powerup.com.au

R. Gundelach
e-mail: raefe@greenslopesent.com.au

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metastases as these patients may live for many years.

The decision-making process in patients with invasive disease is complex and must take into account many factors including patient age, comorbidities, anticipated short- and medium-term morbidity (e.g. airway obstruction, pain, dysphagia) patient wishes, morbidity of treatment and likelihood of disease control. These decisions should ideally be made in a multidisciplinary environment. Furthermore, the surgical procedures may be complex and may require referral to a centre with the appropriate expertise in airway resection.

Clinical Presentation

The key to managing invasive disease well is to accurately recognize it preoperatively. The hallmarks of invasive thyroid disease are hoarseness, shortness of breath, dysphagia and haemoptysis. The majority of patients with invasive disease, however, will be asymptomatic, and the clinician should ideally identify those patients at greatest risk of invasive disease based on the findings of the clinical examination. Large size or fixity of a thyroid mass, its location over the laryngeal cartilages or rapid increase in size should prompt more extensive investigation. Fiberoptic examination of the larynx is recommended. This may identify a vocal cord palsy, which may be a cardinal sign of an invasive thyroid malignancy [16]. Hoarseness or a change in voice may alert the surgeon to these changes; however, a not inconsiderable proportion of patients with invasive thyroid cancer and a vocal cord palsy may have a normal voice, due to gradual compensation by the contralateral vocal cord. For this reason, Fiberoptic laryngeal examination is recommended in all patients suspected of having thyroid cancer [17].

Laryngeal examination may also identify a submucosal mass within the larynx or subglottis, or gross intraluminal extension. Office-based flexible bronchoscopy may be available in some centres.

Investigations

Patients with suspicion of MTC or certainly any features that would suggest invasion such as those outlined above should undergo computed tomography (CT) of the neck with intravenous contrast. This enables staging of nodal metastases and planning for surgery and should help elucidate signs and extent of extra-thyroidal extension. Although some series claim good sensitivity of ultrasound in identifying invasion, it is very dependent on the experience of the operator and some series have clearly demonstrated the increased sensitivity of CT in this situation [18].

Furthermore if signs of invasion are detected on CT, we would recommend a magnetic resonance imaging (MRI) examination to further define the extent of invasion. MRI offers good resolution of soft-tissue structures and may help identify the depth of the tracheal, laryngeal or esophageal invasion which has important implications for the type of surgical procedure that may be planned [19] (see Figs. 12.1 and 12.2). Clinical or radiological suspicion of airway invasion should also indicate the need for formal endoscopic assessment in the form of bronchoscopy. A rigid endoscopic examination of the airway under general anesthetic with the patient spontaneously breathing offers an excellent means of assessing for signs of transluminal invasion. Signs of invasion may include localised erythema, a submucosal mass or frank ulceration (see Fig. 12.3). Biopsy may be undertaken to confirm intraluminal invasion. Rigid esophagoscopy may also be undertaken, and these procedures can be done at the same time as the planned resection. If the broncho-esophagoscopic findings have major implications for surgical decision-making, it may be best to undertake a panendoscopy prior to the planned resection. In addition to this, patients with suspicion of invasive

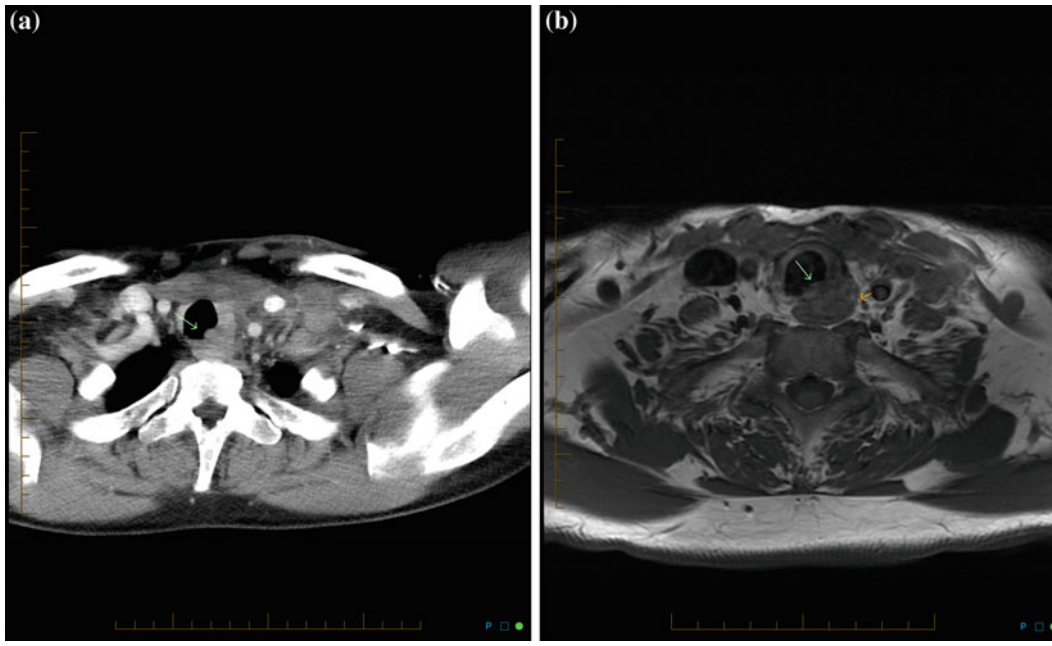

Fig. 12.1 **a** Axial CT scan demonstrating recurrent MTC invading esophagus and trachea. **b** T1 Axial MRI

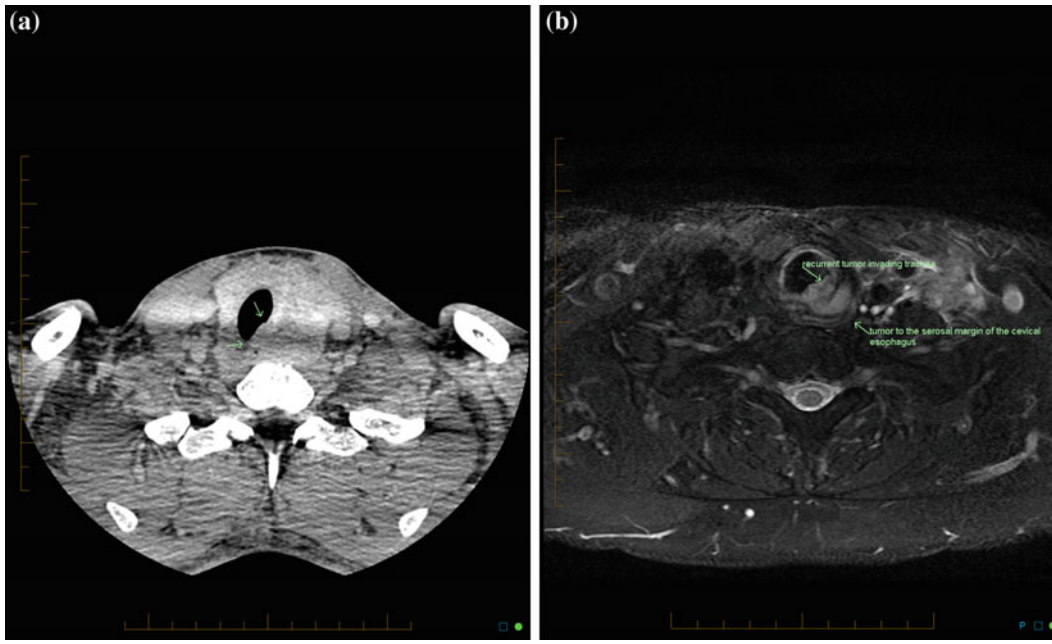

Fig. 12.2 **a** CT Axial scan demonstrating tracheal and oesophageal invasion with MTC in a patient with MEN2B syndrome. **b** T2 MRI scan clearly demonstrating the extent of transmural tracheal involvement with tumour. The MRI images complement the CT scan and more precisely elucidate the extent of visceral invasion

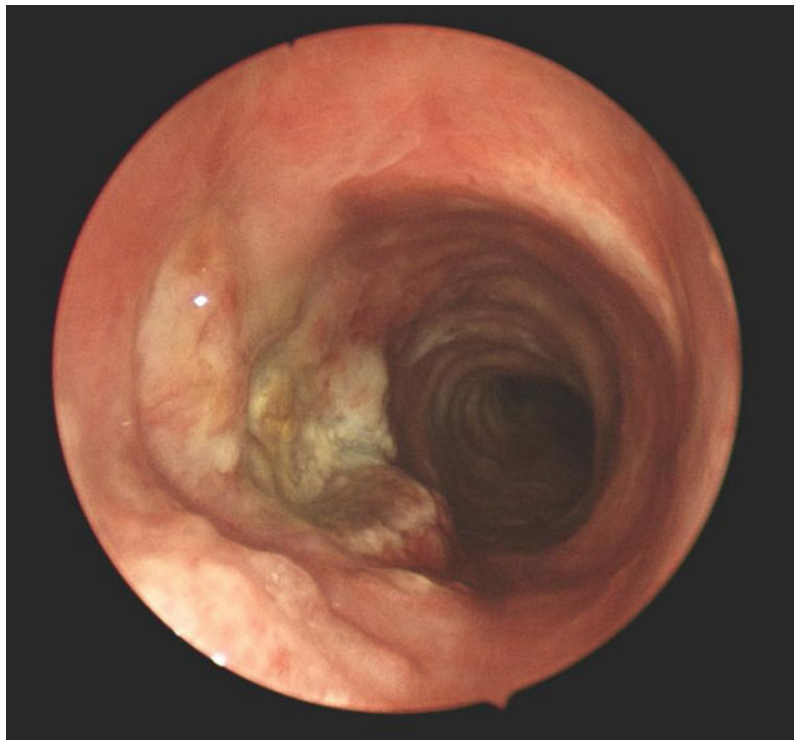

Fig. 12.3 Bronchoscopic view demonstrating transmural tracheal invasion with recurrent MTC. The patient was deemed inoperable due to the extent of thoracic tracheal involvement

MTC should have imaging of the chest and abdomen to assess for distant metastases, particularly if the serum calcitonin measurement is over 500 pg/ml [20].

Surgical Management

The goal of surgical management of invasive thyroid cancer is complete resection of all disease with negative margins, whilst preserving the function of voice, airway and swallowing. This is best achieved with a comprehensive knowledge of the disease extent prior to surgery. In many cases, however, invasive disease may not be anticipated and it is important that the surgeon identifies and confirms invasive disease if encountered intraoperatively and has a surgical strategy for dealing with it.

The surgical approaches for dealing with tracheal invasion vary from 'shave' procedures to window and segmental resections to laryngopharyngectomies. The number of cases of MTC in surgical series of invasive thyroid cancer is low, which not only reflects the rarity of the disease, but probably also the tendency of surgeons to be less aggressive in cases of invasive MTC.

A decision to offer a potentially morbid surgical procedure must be made in the light of the patients' expected outcome. Advanced age, advanced regional disease (especially a large number of metastatic lymph nodes), significantly elevated serum calcitonin levels, and/or radiological evidence of distant metastases, all portend a poor outcome and aggressive resectional approaches are probably best avoided unless the purpose is to achieve palliation of significant or impending symptoms. On the other hand, a younger patient with limited invasion of the trachea and with stable distant disease may benefit from a transmural resection provided it can be done with a low likelihood of long-term morbidity. The objective in this situation is to gain

local disease control and preserve the patient's capacities of voice, airway and swallowing. Prolonged survival may also be attained in this circumstance, although the likelihood of biochemical cure is low.

There is unresolved controversy in the literature regarding the extent of resection for tracheal invasion. The critical issue is that all visible disease is resected as it is universally accepted that leaving gross residual disease on the airway portends a poor prognosis [4, 21]. This may be even more so in MTC where radioactive iodine is not a therapeutic option and external beam radiation therapy has variable results. The extent of resection required depends primarily on the depth of tumour invasion.

Mechanism of Invasion

There are few specific pathological studies that address MTC and its microscopic mode of invasion.

The prerequisite of invasion is extrathyroidal extension, and this is indicative of increased biological aggression. In studies of DTC, the likelihood of extrathyroidal extension and invasion increases with tumor size [22, 23]. Invasion occurs when the tumor extends out from the primary lesion within the thyroid but may occur less commonly with extranodal extension from pretracheal or paratracheal lymph nodes. The central neck structures vulnerable to invasion include the strap muscles and RLN most commonly, the trachea, larynx, and less commonly the esophagus. The esophagus is more vulnerable to invasion by a paratracheal node by virtue of its posterior location [21].

Medullary thyroid cancer arises from the parafollicular C-cells which are located in the posterior upper third of the gland. There does not seem to be any data, however, on the impact of the geographical location of medullary thyroid cancer within the thyroid on the likelihood or otherwise of invasion of proximate structures. The manner by which DTC invades the trachea has been studied [24, 25], and it may be reasonable to make inferences about the behaviour of invasive MTC on this basis.

The posterior capsule of the thyroid and the perichondrium of the trachea have only a thin layer of separation. Once tumour has violated the external perichondrium, extension tends to occur through the dense fibrous tissues between the cartilaginous rings. Through these spaces, the vessels tend to run perpendicularly and may provide a pathway to further spread. Further progression leads to disease involving the lamina propria of the tracheal mucosa where it has a tendency to spread circumferentially. Ozaki [25] has shown that this tendency to circumferentially spread may lead to underestimation of lateral disease extent. Clinically, this disease becomes apparent as a smooth, often erythematous bulge visible on tracheo-bronchoscopy, often with loss of definition of the tracheal rings. The final stage of transmucosal progression results is stippling or 'cobblestoning' of the mucosa or frank ulceration.

Recurrent Laryngeal Nerve (RLN)

The RLN is the nerve most at risk with invasive thyroid carcinoma. Examination of RLN function is vital pre-operatively and may be the only indication that the patient is harbouring invasive disease.

In Randolph's series of 21 patients with invasive thyroid cancer, 70 % had RLN paralysis compared with only 0.3 % of patients with benign or non-invasive thyroid cancer. Voice changes were identified in only 40 % [16].

If the RLN is non-functioning preoperatively, then it may be resected to allow clearance of disease. If preoperative function is normal but the nerve is applied to or encased in tumor, an attempt should be made to separate the tumor from the nerve with sharp dissection, provided there is no visible tumor left behind. In cases of MTC, where radioactive iodine (RAI) treatment cannot be relied upon to treat residual microscopic disease, the surgeon may have a lower threshold to resect an involved RLN providing that the contralateral RLN function is normal. RLN monitoring is particularly useful in this situation to provide the surgeon with reliable

prognostic information about nerve function. Intraoperative neuromonitoring data may inform decision-making about when to sacrifice a nerve. If the RLN is sacrificed, good rehabilitative procedures may be offered to improve vocal outcome, such as vocal cord medialisation. Some surgeons may elect to cable graft a resected RLN or perform an anastomosis between the outer branch of the ansa cervicalis and the distal RLN at the time of resection [26].

Tracheal Invasion

Shin has classified tracheal invasion by papillary thyroid carcinoma (PTC) into 4 categories.

This is a histopathological staging system that recognises the significance of depth of invasion.

Shin classification of airway invasion with PTC [24].

Stage 1	Tumor demonstrates extra-thyroidal extension and abuts perichondrium but does not invade it
Stage 2	Early cartilage invasion but no transmucosal extension
Stage 3	Tumor extends through cartilage to lamina propria of trachea mucosa but does not breach it
Stage 4	Transmucosal extension of tumor into tracheal lumen

Although based on observations of PTC and not MTC, it is unlikely that sufficient data will be amassed to allow a similar system for MTC in view of its infrequent occurrence. Depth of invasion of the trachea is likely to have a bearing on prognosis in MTC, and considering tracheal invasion in these terms may aid surgical decision-making. In this series, patients with Stage 4 disease had a significantly worse prognosis.

The literature that addresses the extent of resection required in tracheal invasion by thyroid carcinoma is somewhat confounding. There is no uniformity of reporting of depth of invasion or type of resection, and indeed, the comparisons between shave resections and full-thickness resections in some series that have compared these procedures may not be completely informative because disease requiring full-thickness resection tends to have a significantly worse prognosis to begin with. In other words, the two groups may not have had comparable degrees of invasion. Depth of invasion has been identified as an important prognostic factor and should determine the extent of resection [24, 27].

There are several series that support conservative 'shave' resections for selected patients with invasive DTC [27–32]. These series have reported good long-term survival (albeit with frequently higher locoregional recurrence rates) and minimal surgically morbidity. The disadvantage of this technique is the inability to confirm complete resection histologically. In MTC, the long survival rates seen in DTC treated with shave resection may not be repeatable due to the more aggressive nature of the disease and the lack of RAI avidity.

Transmural resections in the form of either window resections or segmental or sleeve resections and re-anastomosis can be performed in experienced hands with acceptably low rates of morbidity and mortality [33–35]. Window resections may be performed for limited (<2 cm in length, less than 1/3 circumference) Shin type II or III disease. A full-thickness portion of involved cartilage en bloc with the thyroidectomy can be excised following mobilization of the thyroid. The resulting defect may be repaired with a sternocleidomastoid muscle flap [36]. We have described a technique using a composite nasal septal mucosal and cartilage graft for repairing tracheal window defects in a small series of invasive thyroid cancer cases [37] (see Figs. 12.4, 12.5 and 12.6). This enables repair of the trachea with a graft that is biologically well matched and lined with respiratory mucosa. There is a risk of surgical emphysema which is usually self-limiting and may be avoided with use of non-suction drain. Tracheostomy should be avoided.

Shin type 4 or more extensive vertical or circumferential involvement of the trachea may necessitate a sleeve or segmental resection. Four to five tracheal rings may be excised without the need for additional mobilising techniques, although chin-to-chest immobilisation sutures are

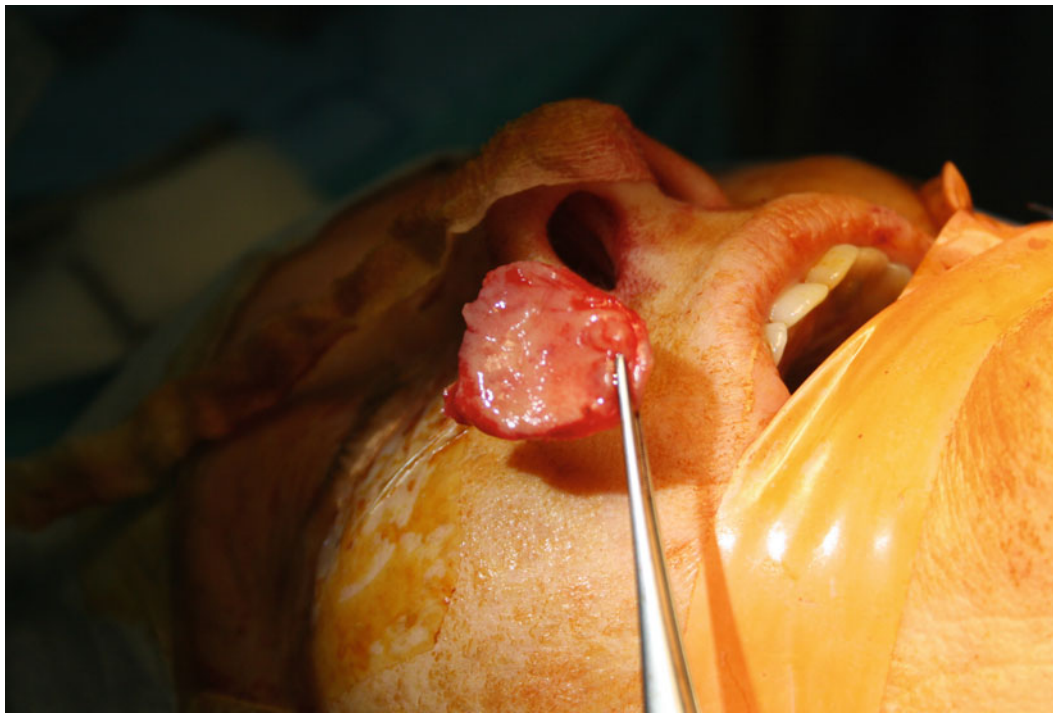

Fig. 12.4 Nasal septal composite graft (septum and mucosa) harvested from septum leaving enough septal structural support

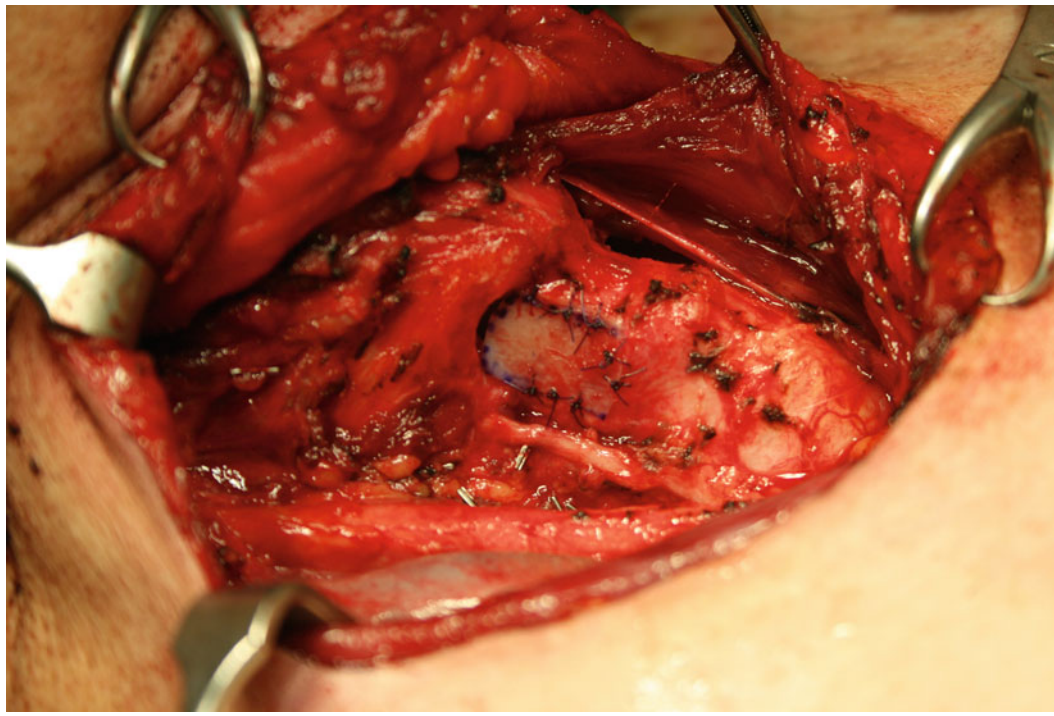

Fig. 12.5 Nasal septal composite graft sutured into window defect in right upper trachea. Mucosal side is intraluminal

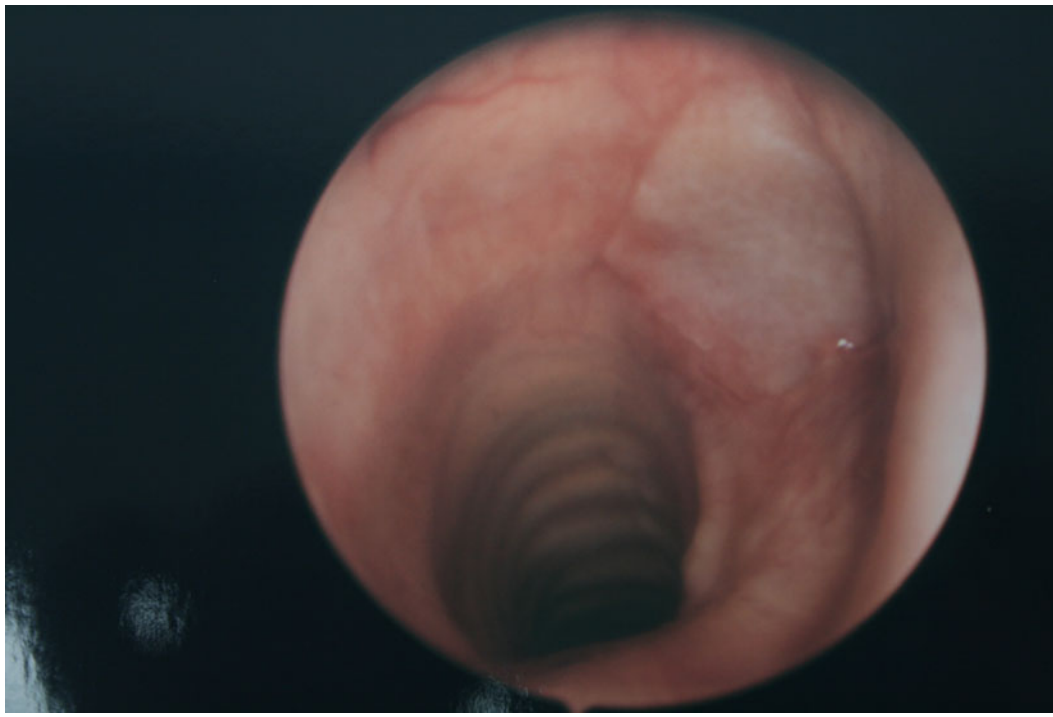

Fig. 12.6 Bronchoscopic view of nasal septal composite graft 8 weeks after surgery demonstrating excellent healing and luminal contour

recommended. If more tracheal length is excised and up to one-third of the total tracheal length may be excised, anterior mobilisation of the mediastinal trachea and infra-hyoid release may be performed. The tracheal anastomosis is performed with 3/0 Prolene sutures, placed submucosally around the complete tracheal ring. The anastomosis must be performed without undue tension. It is critical that at least one RLN is preserved. A tracheostomy is also best avoided, and the patient can be extubated either at the end of the case or following a night ventilated in ICU.

The advantage of this technique is that it enables histological confirmation of complete disease clearance. The potential risks include stenosis, bilateral RLN palsy (and need for tracheostomy), and anastomotic failure which may be fatal.

In some cases, the mortality rates may be as high as 5–9 %. [38] but tend to be lower in high-volume centers. These potential risks must be weighed up against the gain of greater disease control.

Larynx

The larynx may be invaded by MTC to a variable degree. Limited involvement of the thyroid or cricoid cartilages may be amenable to 'shaving' or laminar resection with minimal morbidity. The thyroid cartilages can be resected unilaterally with minimal morbidity. Cricoid cartilage invasion of up to 30 % may be amenable to full-thickness resection of this portion and repair with costal cartilage.

More extensive involvement of the larynx, such as invasion of the paraglottic space, may be amenable to vertical partial laryngectomy. However, laryngectomy or pharyngolaryngectomy is rarely performed in patients with MTC in view of the degree of surgical morbidity and the predictably bleak prognosis. These patients may

be palliated by other means such as a tracheostomy if necessary.

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Importance of Thyroid Hormone Replacement Therapy in Patients with Medullary Thyroid Cancer

13

Jacqueline Jonklaas

Initiation of Thyroid Hormone Postoperatively

Following thyroidectomy performed for the initial treatment of medullary thyroid cancer (MTC) all patients require a full replacement dose of thyroid hormone, given as levothyroxine (LT4), which is the standard of care for treatment for primary hypothyroidism [1]. There is no need to initiate LT4 replacement in a gradual fashion, as patients would be expected to be euthyroid preoperatively. There is also no need for an intervening period of hypothyroidism for diagnostic testing with thyroid-stimulating hormone (TSH)-stimulated tumor marker measurement and iodine scanning or radioiodine therapy, as these procedures are not useful for MTC. In fact, LT4 should be initiated promptly before free T4 levels fall substantially, so that the patient is not subject to an unnecessary period of iatrogenic hypothyroidism. Concerns that exist about starting full-dose LT4 replacement in children with primary hypothyroidism, such as psychological changes, benign intracranial hypertension, and compromised adult height [2], should not apply, as the euthyroid state would be present prior to surgery and should be maintained post-thyroidectomy. The goal of therapy for post-surgical hypothyroidism is to maintain the euthyroid state and prevent the multiple symptoms of thyroid hormone deficiency and the accompanying biochemical abnormalities. LT4 is easily administered, efficacious and inexpensive and, in the vast majority of cases, ameliorates the symptoms of hypothyroidism [1].

LT4 Requirement in Patients with MTC

Patients who have undergone surgery for MTC are athyreotic. However, there is more literature available to guide LT4 replacement in non-surgical hypothyroidism. Replacement doses required by both children and adults can be calculated by various means. LT4 dose requirements are higher in children than in adults. This higher requirement is believed to be due to accelerated LT4 clearance in children compared with adults [3, 4]. For newborns, the LT4 dose proposed is 10 mcg/kg/day [5]. The recommended doses suggested in a 1992 review of pediatric autoimmune hypothyroidism are 4–6 mcg/kg/day for patients 1–3 years, 3–4 mcg/kg/day for patients 3–10 years, and 2–4 mcg/kg/day for patients 10–16 years [5] (Table 13.1). Another review suggests that full replacement doses for acquired hypothyroidism in children are 5 mcg/kg/day for those patients 1–5 years, 4 mcg/kg/day for patients 6–12 years, and 3 mcg/kg/day for

J. Jonklaas (✉)
Division of Endocrinology, Georgetown University, Suite 230, Bldg. D, 4000 Reservoir Road, NW, Washington, DC 20007, USA
e-mail: jonklaaj@georgetown.edu

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adolescents [6]. Similar ranges are suggested in a review of acquired hypothyroidism in childhood and adolescents: 4–6 mcg/kg/day in patients of 1–3 years, 3–5 mcg/kg/day in patients 3–10 years, and 2–4 mcg/kg/day in patients 10–16 years [7] (Table 13.1). It has also been suggested that a replacement dose can be calculated based on body surface area using 100 mcg/m^2/day [8].

The dose requirement of children who are athyreotic as a result of thyroidectomy does not seem to have been well studied. In one 1987 study of athyreotic patients of various ages, the highest dose requirement of 3.4 mcg/kg body weight was in the 6- to 20-year-old age group, compared with 2.8, 2.6, and 2.4 mcg/kg in the 21- to 40-year-, 41- to 60-year-, and >61-year-old groups, respectively [9] (Table 13.1). As this study assessed doses of LT4 that achieved TSH suppression for differentiated thyroid cancer, the reduced requirement with advancing age, but not the absolute requirement would be relevant for patients with MTC. In a more recent small study of 11 children with MTC with a median age of 13 years, a dose of LT4 of 91 mcg/m^2/day was associated with a mean TSH value of 4.37 mIU/L [10] (Table 13.1).

For athyreotic adults, several approaches to estimating LT4 dose have been used. These include weight-based, lean body mass-based, empiric dosing based on weight, body mass index (BMI), age ranges, or various formulas with additional corrections combined with weight. A weight-based dose in athyreotic patients is approximately 1.8 mcg/kg/day [1]. The range of doses that are required to normalize

Table 13.1 LT4 replacement doses (shown in normal font) and suppressive doses (shown in italics) in children

Reference	Additional information	Age Group	Dose (mcg/kg/day)
Bartalena (1987)	Dose for TSH suppression in 180 patients	*6–20 years*	*3.4*
		21–40 years	*2.8*
		41–60 years	*2.6*
		>60 years	*2.4*
Lafranchi (1992)	Review of autoimmune hypothyroidism, dose for normal TSH	0–3 months	10–15
		3–6 months	8–10
		6–12 months	6–8
		1–3 years	4–6
		3–10 years	3–4
		10–15 years	2–4
		>15 years	2–3
Vliet (2012)	Review of acquired hypothyroidism, dose for normal TSH	1–5 years	5
		6–12 years	4
		Adolescents	3
Lodish (2015)	11 patients with MTC, normal TSH	9–17 years	91 mcg/m^2/day
Lafranchi (2015) Jonklaas (2014)	Review of acquired hypothyroidism, dose for normal TSH	1–3 years	4–6
		3–10 years	3–5
		10–16 years	2–4

serum TSH vary from 1.5 to 2.1 mcg/kg/day in various studies [11–16]. There seems to be a tendency for older studies to find that higher doses are required [13, 14]. This could obviously be accounted for by multiple factors including differences in LT4 formulations. Recent studies typically report LT4 dose requirements of 1.7–2.0 mcg/kg/day based on actual body weight [11, 15, 16]. Lean body mass-based doses proposed include 2.75 mcg/kg/day [15] and 2.12 mcg/kg/day for patients weighing less than 60 kg [16]. Examples of empiric, weight-based, BMI-based, and age range-based doses are shown in the upper section of Table 13.2 [16, 17]. Several formulae for estimating the LT4 dose requirement in athyreotic patients that include additional corrective factors combined with weight are also shown in the lower section of Table 13.2 [18, 19].

TSH Goal in Patients with MTC

Medullary thyroid cancer cells are not regulated by ambient TSH concentrations. Therefore, in contrast to the situation with differentiated thyroid cancer, suppression of serum TSH is not beneficial [20]. In individuals with primary hypothyroidism, it has been recommended that the serum TSH target should be in the lower half of the normal range [21]. Certainly, the goal is to keep the patient's serum TSH within an age-adjusted reference interval [1]. Modified, higher TSH targets may thus be appropriate for older age groups if an age-adjusted reference interval is not used [1, 22]. After initiating or changing a LT4 dose, serum TSH can be checked about six weeks later when the steady state has been reached; at that time, the LT4 dose can be titrated up or down if the TSH is above or below the reference interval,

Table 13.2 LT4 replacement doses in adults

Reference	Various estimates of LT4 dose requirements		
	Empiric dosing		
Olubowale et al.	Weight (kg)		LT4 dose (mcg)
	≤ 53		100
	54–86		125
	87–108		150
	>108		175
Di Donna et al.	Age	BMI	LT4 dose (mcg/kg/day)
	≤ 40 years	≤ 23	1.8
		24–26	1.7
		>28	1.6
	41–55 years	≤ 23	1.7
		24–26	1.6
		>28	1.5
	>55 years	≤ 23	1.6
		24–26	1.5
		>28	1.4
	Formula-based dosing		
Mistry et al.	LT4 (mcg) = body weight (kg) − age (years) + 125		
Ojomo et al.	LT4 (mcg/kg/day) = −0.018 × BMI + 2.13		

respectively [1]. Although many practitioners will attempt to achieve a serum TSH within the range of 1–2 mIU/L in order to replicate the values seen in a population free of thyroid disease [23], there is currently little evidence to support that this approach improves patients' symptoms or well-being [24, 25]. Iatrogenic thyroid disease should be avoided in all age groups due to the attendant risks, but several studies suggest that this goal may not be achieved in as many of 40 % of adults [23, 26–28].

Iatrogenic subclinical hyperthyroidism occurs when the serum TSH falls below the lower end of the normal range with the thyroid hormone levels remaining normal. Symptoms of such over-replacement can overlap with symptoms of overt hyperthyroidism and may include anxiety, insomnia, tachyarrhythmias, and weight loss [29]. Particular concerns about over-treatment in children include behavioral dysfunction, altered neurologic development, reduced peak bone mass, and accelerated bone maturation [2]. Iatrogenic hyperthyroidism, presumably unintended, may occur in up to 20 % of patients receiving LT4 therapy [23, 26–28].

An elevated serum TSH indicates reduced thyroid hormone feedback upon the pituitary thyrotropes and is the cardinal feature of inadequately treated hypothyroidism. Patients with modest degrees of under-replacement may have a free T4 level within the normal range and few symptoms of hypothyroidism. In a cross-sectional study of individuals attending a health fair, 1,525 individuals were taking LT4 and 269 of these (17.6 %) had subclinical hypothyroidism [28]. Compiling several studies, iatrogenic subclinical hypothyroidism occurred in about 15 % of those being treated with LT4 [23, 26–28]. Despite the controversy regarding initiating LT4 in those who have a diagnosis of endogenous subclinical hypothyroidism [30], when LT4 is being prescribed in an athyreotic individual every attempt should be made to fully normalize thyroid parameters [1]. Even modest under-replacement of thyroid hormone can potentially have untoward effects in children. These effects could include decreased growth, poor school performance, and delayed puberty [6]. Symptoms of hypothyroidism, seen with TSH elevation in both untreated and inadequately treated patients, include fatigue, weight gain, depression, exercise intolerance, cold intolerance, dry skin, coarse hair, constipation, and impaired mentation [28]. A greater number of symptoms were seen in patients with overt hypothyroidism compared with subclinical hypothyroidism in the same study [28]. Athyreotic individuals have an absolute requirement for thyroid hormone and will eventually inevitably develop decompensated hypothyroidism if they remain unreplaced. Patient education about the lifelong requirement for LT4 is important, as discontinuation of LT4 is among the causes identified for myxedema coma [31].

Decreased Effectiveness of LT4 Therapy

Timing of LT4 Administration

Approximately 75 % of an oral dose of LT4 is absorbed under optimum conditions. Impaired absorption is associated with close proximity to meals, supplements that adsorb the LT4, and medications that decrease gastric acidity, to give a few examples [1]. With respect to meals, a 1-h delay of breakfast after taking LT4 may provide optimum absorption, with bedtime consumption of LT4 being the next best option in terms of absorption [32]. But a lesser degree of absorption that is predictably maintained, as may occur with a different consistent schedule, is preferable to missed doses. For example, it is possible that a bedtime dose of LT4, which provides better absorption than LT4 taken 30 min before breakfast [33], may be an easier schedule to adhere to in a child or adolescent who has a rushed morning schedule. There are certain foods and formulas that, in particular, should not be co-administered

with LT4. One of these is soy formula. More difficulty in normalizing serum TSH levels has been reported in infants fed soy formula than in infants receiving non-soy formula [34, 35].

Medications Affecting LT4 Absorption

Of the many medications and supplements that can diminish LT4 absorption, perhaps one of the most relevant is calcium carbonate, given that hypoparathyroidism may occur in approximately 1.9–6.3 % of patients undergoing thyroidectomy [36–40]. Studies of patients undergoing thyroidectomy find that the rates of hypocalcemia are higher in those undergoing thyroidectomy for differentiated thyroid cancer and MTC than those having surgery for benign disease [41, 42]. Rates are also higher in those undergoing nodal dissection along with thyroidectomy [43]. Furthermore, rates of hypoparathyroidism after thyroidectomy may also be higher in children [20], with 9.3 % of children experiencing hypocalcemia, compared with 5.7 % of adults in one study [44]. In addition to the occurrence of hypoparathyroidism requiring calcium supplementation following thyroidectomy, patients with multiple endocrine neoplasia (MEN) type 2A may also suffer hypocalcemia, and thus be taking calcium supplementation, as a result of parathyroid removal for treatment of parathyroid hyperplasia.

When calcium carbonate was taken with LT4 for several months, the serum TSH of hypothyroid patients increased from a mean of 1.6 to 2.7 mIU/L. The serum TSH returned to 1.4 mIU/L after discontinuation of the calcium [45]. In vitro binding studies suggested adsorption of LT4 to calcium at acidic pH levels [45]. A subsequent absorption study showed that both the maximum serum concentration (C_{max}) and area under the curve (AUC) were reduced when calcium carbonate and LT4 were co-administered [46]. Calcium carbonate, citrate, and acetate all appear to have similar effects, with absorption studies in healthy volunteers showing a decrement in absorption of approximately 20 % [47]. Thus, patients should ideally separate their LT4 from calcium supplements by 4 h [1]. Should this be difficult to adhere to, a consistent schedule with a predictable degree of diminished absorption would be the best approach.

Another possibly relevant group of medications is proton pump inhibitors. Proton pump inhibitors have been associated with impaired LT4 absorption and an increased serum TSH in patients who are taking thyroid hormone replacement [48]. It has been suggested that individuals with primary hyperparathyroidism may have increased susceptibility to peptic ulcer disease [49, 50]. Should this be the case, individuals with MEN2A (who could have both MTC and primary hyperparathyroidism) might be at risk for decreased LT4 absorption while they are also taking proton pump inhibitors. Other pertinent gastrointestinal conditions may also occur in patients with MEN2. Hirshsprung's disease occurs in MEN2A syndrome and patients may be underweight and have symptoms of abdominal pain, distension, and constipation prior to their initial surgical procedure [51]. Intestinal ganglioneuromatosis occurs in patients with MEN2B [51, 52]. Manifestations can include abdominal distention, alternating diarrhea and constipation, and vomiting [52]. If a patient with MEN2 syndrome has difficulty with achieving a therapeutic TSH level or has a fluctuating LT4 requirement, these gastrointestinal manifestations of their disease could be potential culprits. A LT4 preparation in a gel capsule, rather than in traditional tablet form, might potentially be better absorbed with concomitant proton pump inhibitor use [53] and in other cases of malabsorption [54], although this has yet to be tested in a systematic trial.

Medications Affecting Metabolism or Transport

Many medications interfere with the metabolism or transport of LT4 and as a result of these actions may alter, most often increase, the patient's requirement for LT4. These medications include phenobarbital, phenytoin, carbamazepine, rifampin, sertraline, estrogen, and androgens [1]. A class of medications of particular relevance to patients with MTC is tyrosine

kinase inhibitors. In a clinical trial of adults receiving vandetanib for treatment of MTC, 49 % of these athyreotic patients required an increase in their LT4 dose [55]. In another study of a mixed group of patients with differentiated thyroid cancer and MTC, 74 % required an increase in their LT4 dose during vandetanib therapy [56]. In a study specifically examining children and adolescents undergoing vandetanib treatment for MTC, 91 % of children were noted to have increased TSH levels and decreased free thyroxine (FT4) levels, requiring an average increase of 37 % in their LT4 dose [10]. A similar effect is seen with motesanib [57]. This finding may be a class effect due to induction of the type 3 deiodinase resulting in increased LT4 clearance [56, 58].

Special Considerations in Pediatric Patients

Adherence to Thyroid Hormone

It may be imagined that adherence to taking LT4 may be particularly difficult in the pediatric population. Parents may not necessarily understand how critical LT4 is to the health of their children. Patients themselves may also not understand the key role of thyroid hormone for their growth and development and, additionally, may be reliant on parental supervision or assistance. In the case of congenital hypothyroidism, 38 % of children have discontinued their LT4 by 36 months, despite the prevailing recommendation that LT4 should be continued until after retesting has taken place at 3 years [59]. In a retrospective review of pediatric patients who had undergone thyroidectomy (either for differentiated thyroid cancer, MTC, or benign disease), 33 % were found to have elevated TSH values during the first year following thyroidectomy, despite the fact that in a portion of the patients, their physicians were prescribing doses of LT4 intended to maintain TSH suppression [60] (Table 13.3). The recent guidelines for the management of pediatric differentiated thyroid cancer [61] suggest that "medication adherence can be a challenge and frequent assessments of thyroid hormone levels along with education about the benefit of TSH suppression in the long-term management of differentiated thyroid cancer are important" and that "motivational interviewing [62, 63] may be a non-judgmental means by which to improve compliance." A similar concern about the importance of maintaining a normal serum TSH is applicable to pediatric patients with MTC. Patients should receive education that emphasizes their lifelong requirement for LT4.

Identifiable Thyroid Hormone Products

There is some data that switching between identifiable LT4 products, for example between different brand names, or between a branded product and a generic product, may be associated with altered absorption and an altered serum TSH. Although the data are of weak quality in adults, there are stronger data that this is the case in children [1]. In a retrospective study of children being treated for congenital hypothyroidism, there appeared to be a decrease in serum TSH associated with generic LT4 compared with brand name LT4 by one method of analysis, but no TSH differences between groups by another analysis. Frequency of dosage adjustments and clinical outcomes were similar between the groups [64]. On the other hand, a randomized trial of a generic product versus a branded product for treating children with hypothyroidism showed that comparable serum TSH values were not achieved in both groups after 8 weeks of treatment [65]. The difference (higher TSH levels with the generic product) was primarily due to the effects seen in the subset of children with congenital hypothyroidism. Although it is not known whether the same finding applies to euthyroid children being given LT4 replacement after thyroidectomy, it seems prudent to monitor TSH values if a child is provided with a different identifiable LT4 product.

Table 13.3 Medication adherence in children post-thyroidectomy

Reason for thyroidectomy	Number of patients	Out of range TSH values (%)	
		Year 1	Year 2
Medullary thyroid cancer	16	22	17
Differentiated thyroid cancer	39	25	42
Benign disease	19	65	54
Frequency of reported non-adherence	Number of patients	Reasons for non-adherence	
≥ 1 episode associated with out of range TSH values	19	Forgetting or refusing levothyroxine	
		Family issues (e.g., dual residence)	
		Incorrect dose, taking with calcium	
		Inadequate funds/loss of insurance (included 3 patients who completely stopped levothyroxine)	

Data taken directly from Morris et al.

Adjustment of LT4 with Maturation

As already mentioned in the earlier discussion of LT4 requirement, LT4 dose requirements progressively decrease as children age, such that newborns may require 10 mcg/kg/day, but by 10–16 years of age children may require 2–4 mcg/kg/day (see Table 13.1). Given that euthyroidism is critical for normal central nervous system development, normal bone maturation, normal linear growth, normal pubertal development, and normal school performance, careful maintenance of euthyroidism by appropriate adjustments in LT4 dose is necessary [5, 7].

Special Considerations in Young Adult Patients

Possible Financial Difficulties

Because of their absolute requirement for LT4, any financial difficulties that compromise a patient's ability to obtain insurance and purchase their medications could result in inadequately treated hypothyroidism. Inadequate funds and loss of insurance were cited as reasons for stopping LT4 in one study [60]. Patients with a cancer diagnosis were at greater risk for bankruptcy in one study, than those without a cancer diagnosis [66]. Thyroid cancer was one of the malignancies included in this analysis. Financial difficulties could obviously be further compounded if a patient's therapy included the need for medications such as tyrosine kinase inhibitors, which would be a significant expense, particularly if the patient is not participating in a clinical trial [67]. In both children and young adults with progressive or life-threatening MTC, the burden for patients and caregivers is likely to be considerable, based on experiences with other malignancies affecting young individuals [68, 69].

Change in Thyroid Hormone Requirement During Pregnancy

Specific TSH goals are recommended when hypothyroidism is being treated in the pregnant

population [70]. TSH reference intervals are 0.1–2.5 mIU/L during the first trimester, 0.2–3.0 mIU/L during the second trimester, and 0.3–3.0 mIU/L during the third trimester, and generally, treatment to keep the serum TSH within these values is endorsed [70]. Most patients require an increase in their LT4 dose during pregnancy in order to keep their TSH within these reference intervals. Close monitoring of serum TSH is especially important in those who are athyreotic, as they tend to require the greatest magnitude of increase in their LT4 dose [71].

Special Consideration in Older Patients

Avoidance of Iatrogenic Hyperthyroidism

Avoidance of iatrogenic thyroid disease is importance in any patient group, but several studies suggest that this goal may not be achieved in as many of 40 % of patients [23, 26, 28]. Iatrogenic hyperthyroidism may occur in up to 20 % of patients [23, 26, 28], including in those over 65 years of age [27]. It is particularly important in the elderly to prevent any detrimental effects of excessive LT4 treatment on bone health and cardiovascular functioning [30]. Atrial fibrillation is one of the best-described adverse effects of subclinical hyperthyroidism [72].

Modified, Higher TSH Targets

An additional consideration when choosing the TSH target for a particular older patient is that TSH reference intervals are modified by age [73]. The upper end of the reference interval increases with advancing age. Maintenance of higher TSH values is therefore advisable in older individuals [1]. Although clinical laboratories do not generally report age-specific reference intervals, these can be estimated from fairly simple formulae [74].

Thyroid Hormone Therapy During Surgery and Hospitalization

Athyreotic patients will become profoundly hypothyroid after 4–6 weeks of abstinence from LT4 [75]. As mentioned previously, discontinuation of LT4 is one of the causes of myxedema coma [31]. If patients who have received a thyroidectomy for treatment of MTC are hospitalized, it is essential for them to continue their LT4. If there is impaired absorption associated with other medications or tube feedings, intravenous LT4 can be employed [1]. The equivalent intravenous dose is approximately 75 % of the oral dose that normalized the patient's serum TSH [1].

Quality of Life in Athyreotic Patients

A subset of those patients replaced with LT4 preparations continue to have residual symptoms such as fatigue, weight gain, and impaired well-being [1, 76, 77]. These residual symptoms may occur despite the fact that the serum TSH has been normalized. Patients taking LT4 tend to have higher FT4 and lower triiodothyronine (T3) levels than those with their own endogenous thyroid function [78–81]. It is known that the intact thyroid gland itself usually contributes about 20 % of circulating serum T3 levels in humans [82]. Two studies have been performed in rats that showed more successful achievement of normal T4 and T3 levels in serum and tissues during intravenous LT4 and liothyronine (LT3) infusion, compared with LT4 infusion alone [83, 84]. Recent rat studies have also suggested impairment of deiodinase activity during LT4 monotherapy in association with lower serum T3 levels [85]. The thyroidal contribution to T3 production is greater in rats (40 %), than in humans (20 %). Although it is possible that the magnitude of the T3 reduction may be greater in rats than in humans [85], the impact in humans may nevertheless be clinically significant. Based on such evidence, it has been hypothesized the

decreased quality of life in patients treated for hypothyroidism is due to the missing T3 component and that combination therapy with both LT4 and LT3 may be the key to providing greater patient satisfaction.

Combination Therapy in Athyreotic Patients

Despite the attractiveness of this idea, obtaining clear supportive evidence for the link between "sub-therapeutic" T3 levels and patient quality of life has been difficult. Thirteen trials of various regimens of combination therapy have not consistently shown superiority of combination therapy [1, 86–88]. Only some of these trials included athyreotic participants, who could potentially be the ones who might most benefit from combination therapy. A meta-analysis performed in 2006 [88] did not find an association between positive outcomes from combination therapy and the athyreotic state. However, the number of athyreotic patients in the combined trials was relatively small. A secondary analysis of one of the combination trials [89] found that those carrying the Thr92Ala type 2 deiodinase polymorphism responded more favorably to combination therapy than those without the polymorphism [90]. The authors suggested that patients with this polymorphism may have impaired deiodinase functioning, resulting in increased reliance on serum T3 levels to maintain brain levels of T3. However, this finding has not yet been confirmed prospectively. Moreover, this particular polymorphism is not associated with altered circulating serum T3 levels in a population-based cohort [91]. Therefore, if combination therapy is most beneficial in those with this polymorphism, the ability of combination therapy to sustain adequate T3 levels must only be detected in comparison with the inadequate T3 levels achieved with LT4 monotherapy. Some patients in the 13 combined trials expressed a preference for combination therapy with T3 [1]. It remains possible that there is a potentially unrecognized parameter or endpoint affected by the T3-containing combination that leads to this preference.

It should be emphasized that most combination therapy trials have been of short durations of approximately 8–16 weeks. Therefore, long-term risk and benefits have not been assessed, especially in older age groups and men. Other concerns about this body of evidence include heterogeneity of both study design and results, and varying and non-validated endpoints [1]. Trials examining T3 therapy in other forms are limited. A single randomized trial of desiccated thyroid extract compared with LT4 showed an average weight loss of 3 lbs with the extract and preference for the extract in 49 % of participants [92]. A small, randomized crossover trial of three times daily LT4 compared with LT3 in 14 patients, 12 of whom were athyreotic, illustrated the difficulties in adhering to a thrice daily regimen, and demonstrated a modest weight loss and improved LDL cholesterol with the LT3 regimen [93]. A sustained release T3 preparation is eagerly awaited. Whether such a preparation will show improved outcomes in hypothyroid patients, including those who are athyreotic, will be discovered through future research.

Conclusion

While we seek to find better thyroid hormone preparations that will restore all athyreotic patients to full health, it is important to ensure that patients are optimally managed based on our current state of knowledge. This includes providing full replacement that normalizes serum TSH at all times and avoidance of iatrogenic thyroid disease. Patients should be educated about their absolute reliance on exogenous LT4 and about medications that may alter their LT4

requirement. Euthyroidism is importance across the life span: particularly in maturing children, pregnant women, and older adults.

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Medullary Thyroid Cancer: The Role of External Beam Therapy

14

Meredith Elana Giuliani and James Brierley

Introduction

Medullary thyroid carcinoma (MTC) is a rare form of thyroid carcinoma where the primary management is with thyroidectomy and lymphadenectomy. The role of radiotherapy is confined to external beam radiation (EBRT) in the adjuvant setting, for unresectable disease or for palliation. There is no role for ^{131}Iodine.

Indications for Radiotherapy

EBRT has a role in unresectable MTC and adjuvant therapy. In patients with unresectable MTC, or those who have macroscopic residual disease following surgery, radiotherapy can achieve local control in only about 20 % [1], but is still of value to try and prevent the devastating effects of uncontrolled disease in the neck. Surgical resection with negative margins is therefore essential as is early diagnosis to ensure the cancer is resectable, both of which are discussed elsewhere in this text.

The role of adjuvant radiotherapy following compete surgical resection in MTC patients is controversial [1, 2]. There is no role for postoperative radiation in patients with early stage, node-negative MTC that has been completely resected. Even in locally advanced MTC, following surgery there is no evidence that radiotherapy improves overall survival; however, it may have a role in reducing locoregional recurrences [1–5]. In a Surveillance, Epidemiology, and End Results program (SEER) analysis, the addition of EBRT after surgery in patients with metastatic lymph nodes did not show a survival advantage [6]; local recurrence data is not available from SEER. However, improving locoregional control is beneficial due to the eloquent structures affected by locoregionally recurrent disease and the role of EBRT in selected patients is supported by retrospective institutional experiences.

Preventing locoregional recurrence in the head and neck is important for symptomatic control and preventing complications such as tracheostomy, which have a significant impact on a patient's quality of life. Optimal locoregional disease control has important implications for quality of life in patients with MTC, and thus, radiotherapy can have an integral role in management. The pathological extent of disease and postoperative calcitonin levels may influence the use of radiotherapy. Calcitonin, as a tumor marker in MTC [7], may aid in selecting patients for

M.E. Giuliani
Princess Margaret Cancer Centre, Radiation Medicine Program, 610 University Ave, Toronto, ON M5G2M9, Canada
e-mail: Meredith.Giuliani@rmp.uhn.on.ca

J. Brierley (✉)
Department of Radiation Oncology, Princess Margaret Cancer Centre, University of Toronto, 610 University Ave, Toronto, ON M5G2M9, Canada
e-mail: James.Brierley@rmp.uhn.on.ca

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EBRT. However, Fersht et al. reported that in a series of 51 patients with complete resection, no distant metastases, and persistently elevated serum calcitonin levels, after radiation only 1 patient's serum calcitonin level normalized. Importantly, however, there was an improvement in locoregional control in patients who received radiation therapy, compared to those patients who were observed (59 % vs. 29 %). The authors concluded that advanced disease, not the presence of elevated serum calcitonin levels, should guide the use of adjuvant radiation [4]. High-risk features for local recurrence include extrathyroidal extension, multiple involved lymph nodes, and microscopic or gross residual disease [4], soft tissue extension, and mediastinal involvement [2] all of which may be indicators for a role of EBRT.

In an advanced group of 34 MTC patients, stages IVa-c, with the characteristics listed above, EBRT conferred a high (87 %) 5-year locoregional relapse-free survival; despite this, high-risk disease, survival was 56 % at 5 years [2]. In the Mayo Clinic experience, patients receiving adjuvant radiation had no local recurrences, in all 11 patients who received adjuvant or salvage EBRT for advanced-stage disease, only 1 patient, who had salvage EBRT, had locoregional relapse during follow-up [3] and Brierley et al. reported a 10-year local/regional relapse-free rate of 86 % in those who received EBRT and 52 % in those who did not ($P = 0.049$) [1].

It would appear therefore that patients with gross residual disease, microscopic residual disease, gross extrathyroidal extension, and mediastinal involvement may benefit from EBRT, given the increased rates of locoregional recurrence. The use of EBRT in patients with multiple involved lymph nodes, however, is more controversial. In most of the earlier series on the use of EBRT that showed a benefit in patients with extensive nodal involvement, patients did not necessarily undergo the extensive nodal resection that is now the surgical standard of care in patients with clinically evident nodal disease at the time of presentation. Reoperation can achieve normalization of serum calcitonin levels, but if further surgery is not possible, or if residual disease is still evident despite repeated good quality surgical clearance, adjuvant EBRT to the thyroid bed and regional nodal tissue may be of value. The recently revised American Thyroid Association (ATA) guidelines on the management of patients with MTC advised that postoperative adjuvant EBRT to the neck and mediastinum should be considered in patients at high risk for local recurrence (microscopic or macroscopic residual MTC, extrathyroidal extension, or extensive lymph node metastases), and those at risk of airway obstruction, although potential benefits must be weighed against the acute and chronic toxicity associated with the therapy [8]. Similarly, the British Thyroid Association guidelines recommend that EBRT should be considered only once optimal surgery has been performed and if there is significant risk of local recurrence (macroscopic residual disease or microscopic residual disease on the background of large volume). Importantly, these guidelines also comment that EBRT should not be used to consolidate inadequate surgery [9].

The benefit of EBRT in preventing recurrence must be weighed against the toxicity of therapy, as described below, and the risks of yet further surgery if EBRT is not given and the tumor were to recur. It must also not be forgotten that the characteristics of a tumor that predict for recurrence also predict for distant metastases, hence the lack of survival benefit from EBRT. Similarly if after high-risk disease is resected and the serum calcitonin level normalizes, the necessity and benefit of additional EBRT may be questioned, despite the data from Fersht et al. described above. It is therefore essential that the decision to proceed with EBRT is made by an experienced multidisciplinary team after surgery by an experienced MTC surgeon.

Radiation Dose and Treatment Volumes

The optimal dose of radiotherapy in the adjuvant setting is controversial. Institutional series reports a range of dose fractionation regimens

generally from 50 to 70 Gy in 2 Gy per fraction. The Mayo Clinic experienced reported a median dose of 60.8 Gy (55–70 Gy) [3]. The Memorial Sloan Kettering experience reported a median radiation dose of 63 Gy and 54 Gy for microscopic nodal levels at risk. They also described a comprehensive elective nodal coverage including levels II–VI and mediastinal coverage to the carina [5]. Schwartz et al. [2] reported a median radiation dose of 60 Gy using intensity-modulated radiotherapy (IMRT) and the Princess Margaret experience used a median radiation dose of 40 Gy in 20 fractions with a 10 Gy in 5 fractions boost and had a 86 % 10-year local/regional relapse-free rate [1].

The side effects associated with radiation are important factors when considering the use of radiotherapy and when informing patients of their treatment options. Terezakis et al. reported a series of 12 patients with MTC an observed a 25 % local recurrence rate (all in pT4 cases) following adjuvant EBRT. They also reported 18 % of patients had grade 3 mucositis and 32 % of patients had grade 3 dysphagia; other common side effects were fatigue, xerostomia, and hoarseness. In this series, there was a 5 % rate of feeding tube dependency [5].

Intensity-Modulated Radiotherapy

Historically, radiotherapy in the adjuvant setting was challenging due to the curvature of the neck and air in the trachea and their effects on the radiotherapy distribution with techniques such as electrons. However, electron-based treatments have largely been replaced with IMRT approaches. IMRT has been shown in randomized trials in other head and neck cancer populations, such as nasopharyngeal carcinoma, to reduce toxicity and improve disease control [10, 11]. Major organs at risk include the skin, spinal cord, submandibular glands, parotid glands and mandible in MTC patients, and IMRT may provide a more favorable balance between target coverage and sparing organs at risk. Due to the rarity of MTC, there is no specific data in this patient population on the impact of IMRT, but extrapolation from other head and neck cancer sites is reasonable. See Fig. 14.1.

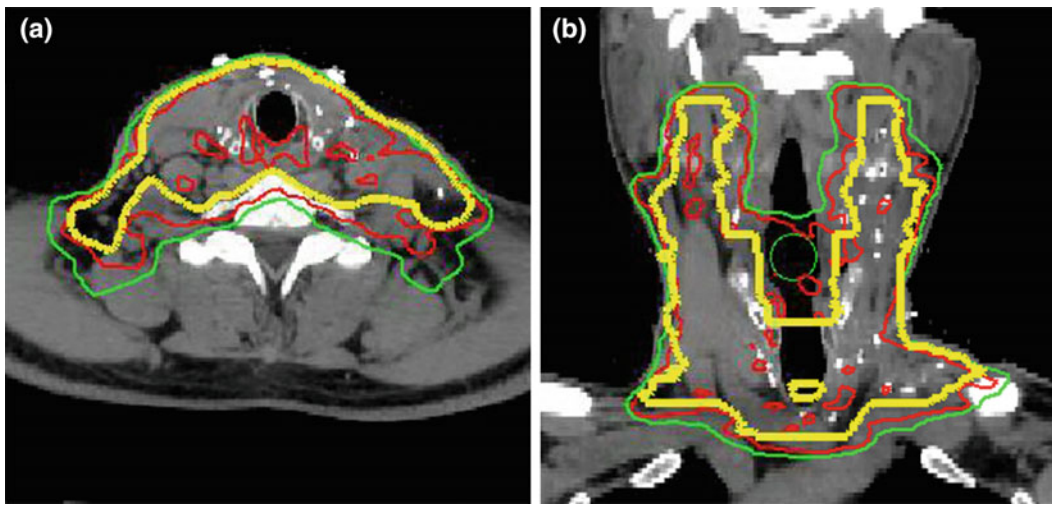

Fig. 14.1 A radiotherapy plan for adjuvant radiotherapy (50 Gy in 25 fractions) for a 54-year-old man with pT1b, N1b medullary thyroid cancer with <1 mm margins and 25 of 49 lymph nodes involved with disease. His preoperative calcitonin was 206.0, and post-surgery, it was 41.8. **a** Axial image **b** Coronal image. The *yellow line* represented the clinical target volume, the *red* represents the 100 % isodose line (or 50 Gy) and the *green line* represents the 95 % isodose line (or 47.5 Gy)

Table 14.1 Results of adjuvant external radiotherapy in high-risk disease medullary thyroid cancer from retrospective studies

	Surgery alone	Surgery and RT
	Local/regional recurrence	Local/regional recurrence (%)
Brierley et al. [1][a]	48 %	14
Fersht et al. [4][a]	59 %	29
Schwartz [2][b]	N/A	13
Nguyen et al. [13][b]	N/A	30
Call [3][c]	N/A	9

[a]at 10 years
[b]at 5 years
[c]not specified

Palliative Radiotherapy

Radiotherapy has an established role in the palliation of metastatic sites from MTC. Bone metastases have a high rate of symptomatic response to radiotherapy [12]. In the setting of limited metastatic disease, higher dose radiotherapy such as 40 Gy in 15 fractions may be used for bone metastases. Otherwise, common palliative regimens include 8 Gy in a single fraction, 20 Gy in 5 fractions, or 30 Gy in 10 fractions.

Radiotherapy may also be used to palliate lung and mediastinal metastases, as well as brain metastases from MTC. In addition, single-fraction palliation, such as 8 Gy, is effective at controlling bleeding (i.e., hemoptysis) related to metastatic disease.

Quality of Life and Survivorship

Due to the rarity of MTC, there are no published data comparing quality of life outcomes in patients who did and did not receive radiotherapy. The side effects of radiotherapy described previously must be balanced against the effects of locoregionally recurrent cancer. Future work to assess quality of life and unmet survivorship needs are needed in this patient population.

Conclusions

External beam radiotherapy should be considered in patients with unresectable disease, gross residual disease after surgery or those with high-risk features following surgery in whom the risk of local recurrence is considered high. The optimal dose of adjuvant radiotherapy is controversial (Table 14.1).

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15 Management of Patients with Metastatic Medullary Thyroid Carcinoma: The Role for Systemic Therapy

Gilbert G. Fareau

Introduction

Medullary thyroid carcinoma (MTC) is an uncommon and frequently aggressive malignancy that comprises between 3 and 5 % of all thyroid cancer cases, but which is responsible for approximately 13 % of thyroid-related deaths [1, 2]. This observation is explained partly by the relatively high propensity for extrathyroidal and particularly extracervical spread of disease: at diagnosis, 7–23 % of patients will have distant metastases, with multiple sites demonstrating multiple foci of disease, particularly the liver (49–62 %), lungs (33–35 %), and bones (40–74 %) [3]. Ten-year survival is 75–95 % in patients with locoregional disease, but falls to 40 % with distant organ involvement [4, 5]. For some individuals tumor activity will follow a relatively indolent pattern of growth characterized by extended periods of quiescence, and in these cases, conservative monitoring is typically advisable and may continue for many years before there is any need for treatment. Eventually, however, even a slow progression of tumor burden, if persistent, will threaten surrounding structures or lead to the onset of new compressive symptoms. Additionally, for some patients, the tempo of disease progression will be brisk from the time of diagnosis and a more immediate decision must be made regarding the role for intervention. Surgery and other localized therapies (e.g., radiation, chemoembolization, and radiofrequency ablation) may be considered when disease progression is isolated to one or two sites. However, for the patient with a disseminated pattern of tumor progression, consideration must be given to the use of systemic therapy.

Patient Selection for Systemic Therapy

In many cases, the need for systemic therapy will be readily apparent. For example, patients with symptoms due to mass effect or hormonal excess (calcitonin-induced diarrhea, adrenocorticotrophic hormone (ACTH)-dependent Cushing's syndrome, etc.) would be eligible candidates to start systemic treatment. However, most patients with metastatic MTC will be asymptomatic at the time of diagnosis with discovery of distant tumor sites following radiological assessment to explain a new or worsening elevation of the serum calcitonin and/or CEA level. Rapid doubling time of these tumor markers (e.g., <6 months) is frequently a good predictor of which patients will eventually require systemic therapy [6, 7]. However, the decision to start systemic therapy should not be

G.G. Fareau (✉)
Division of Endocrinology, Department of Medicine, Medical College of Wisconsin, 9200 W. Wisconsin Avenue, Milwaukee, WI 53226, USA
e-mail: gfareau@mcw.edu

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Table 15.1 Conditions for systemic therapy

Multiple tumors >1–2 cm with evidence of rapid progression (<12 months)
Large tumors >3 cm with evidence of slow progression (>12 months)
Tumors causing compressive symptoms or threatening neighboring structures
Hormonally active tumors (diarrhea, ectopic ACTH syndrome)

based exclusively upon changes in tumor markers. Rather, the overall tumor burden should be appraised and monitored initially with serial imaging using an objective methodology, such as Response Evaluation Criteria in Solid Tumor (RECIST), in order to more accurately define the extent of disease progression [8]. By doing so, an informed recommendation for systemic therapy can then be made based on factors that include tumor site, size, and rate of growth. In general, patients with small tumors (<1 cm) or limited tumor burdens with only a few lesions that have not progressed over 12 months do not require immediate systemic treatment [3, 9, 10]. These patients can be monitored with scheduled imaging every 3–12 months based upon tumor marker doubling times and the rate of radiological progression. Patients with large tumor deposits (>3 cm) should be considered for treatment, especially if any further growth would potentially threaten neighboring structures [3, 9, 10]. Patients with multiple tumors greater than 1–2 cm in size should also be considered for treatment if there is evidence of progression within 12 months (Table 15.1) [3, 9, 10].

Options for Systemic Therapy

Systemic cytotoxic chemotherapy has never been shown to be effective in the management of MTC, since responses are generally infrequent and usually of short duration [11]. Of the cytotoxic agents that have shown some limited benefit, doxorubicin, either alone or in combination with other agents (e.g., cisplatinum), and combination 5-fluorouracil with dacarbazine have both demonstrated response rates in up to 20 % of patients [12]. For this reason, systemic cytotoxics should not be used as first-line agents but may be considered in rare cases when tumor growth is exceptionally rapid and immediately life threatening. Fortunately, the last few decades have witnessed a rapid growth in our understanding of the molecular events that cause neoplastic transformation in many malignancies. This has resulted in a proliferation of small molecule inhibitors that interrupt specific intracellular signaling cascades implicated in tumorigenesis. Several oral tyrosine kinase inhibitors (TKI's), particularly those targeting *RET* and *VEGR*, have been developed that demonstrate efficacy in the treatment of advanced MTC [13]. At the current time, two TKI's, vandetanib and cabozantinib, have been approved by the US Food and Drug Administration (USFDA) and European Medicines Agency (EMA) for the use in progressive MTC. Additionally, several other agents are commercially available and may be used off-label when approved therapies are not tolerated or are otherwise unavailable.

FDA-Approved Therapies

Vandetanib

Vandetanib is a potent inhibitor of several tyrosine kinases, including RET, VEGFR2, VEGFR3, and EGFR. Encouraging results from two phase II trials lead to a prospective randomized phase III trial, in which 331 patients with advanced MTC were randomly assigned in a 2:1 ratio to receive vandetanib or placebo; patients with disease progression on placebo were given the option to cross over to open-label treatment with vandetanib [14–16]. Progression-free survival (PFS) was increased from 19.5 months in the placebo arm, to a predicted median of 30.5 months in the vandetanib arm (hazard ratio = 0.46; $p < 0.0001$) [16]. A partial response rate of 45 % was seen in the treatment arm, with a predicted median duration

of 22 months [16]. In 2011, vandetanib was approved by both the USFDA and EMA for the treatment of progressive inoperable MTC. Study data confirmed a small but significant risk for QT prolongation and potentially lethal arrhythmia with the use of vandetanib, and implementation of a risk evaluation and mitigation strategies (REMS) program was a condition for USFDA approval; prescriptive authority requires enrollment and completion of the REMS program, which can be done through the manufacturer's Web site [17].

Cabozantinib

Cabozantinib is a small molecule inhibitor of RET, VEGFR2, FLT3, KIT, and c-MET. Data from a phase I/II trial confirmed partial response rates in 10 of 35 MTC patients treated, including patients previously treated with at least one other TKI [18]. A phase III trial of 330 MTC patients with documented progression over the preceding 14 months randomly assigned patients in a 2:1 ratio to receive either cabozantinib or placebo; there was no crossover component to the study [19]. An overall response rate was noted in 28 % of treated patients, and the median PFS was improved from 4.0 months in the placebo arm, to 11.2 months in the treatment arm (HR = 0.28; $p < 0.0001$) [19]. Although overall survival was not uniformly improved across all treated patients, a subgroup analysis did show a statistically significant improvement in subjects with the RET M918T mutation (44.3 vs. 18.9 months; HR = 0.60; $p = 0.026$) [20]. Cabozantinib was approved for use in progressive MTC by the USFDA in 2012 and by the EMA in 2014.

Non-FDA-Approved Therapies

Sorafenib

Sorafenib is a multikinase inhibitor that targets RET, VEGFR1-3, FLT3, KIT, PDGFR, FGFR1, and BRAF. Its use has been approved in the management of progressive differentiated follicular epithelial thyroid cancer, but it is not currently approved for use in MTC. However, phase II data have shown partial response and disease stability rates of 6–50 % and 43–80 %, respectively, in MTC patients treated with sorafenib, making it a potential option for patients who do not respond or cannot tolerate the current FDA-approved medications [21–23].

Pazopanib

Pazopanib is an oral inhibitor of VEGFR1-3, KIT, PDGFR, and FGFR that has been shown to have efficacy in treating advanced MTC. A single-arm, open-label phase II trial examined the use of pazopanib in patients with metastatic MTC who had progression confirmed within the preceding six months [24]. Five of 35 patients showed a partial response rate (14.3 %) and another 20 had stable disease (57 %), and the median PFS was 9.4 months [24].

Lenvatinib

Lenvatinib is a small molecule inhibitor of RET, VEGFR1-3, FGFR, PDGFR, and KIT that was approved by the FDA in 2015 for use in radioiodine refractory differentiated thyroid cancer. A multicenter open-label phase II trial evaluated treatment response to lenvatinib in 59 patients with advanced MTC who had documented disease progression within the preceding 12 months [25]. The objective response rate was 36 %, the disease control rate was 80 %, and median PFS was 9.0 months [25].

Sunitinib

Sunitinib is a small molecule inhibitor of several kinases that include RET, VEGFR2, PDGFR, FLT3, KIT, FGFR1, and BRAF. A phase II trial of daily sunitinib in FDG-PET-positive metastatic thyroid cancer enrolled 7 patients with MTC; 3 of 6 patients had confirmed partial response (50 %), and 2 of 7 patients showed overall disease stability, for an overall disease control rate of 71 % [26]. Preliminary data from other phase II studies have demonstrated similar response rates [27, 28]. Sunitinib is currently approved for use in renal cell carcinoma and gastrointestinal stromal tumors and is available as an off-label treatment option in MTC.

Starting Therapy and Monitoring Response

The recommendation to proceed with systemic therapy must always begin with a thoughtful discussion with the patient, with careful consideration of their expectations and preferences. Patients need to be informed that these therapies are not curative and that a realistic treatment goal is for disease stabilization and possible limited tumor reduction. It is important to also clarify that unlike traditional cytotoxic regimens that are typically administered over a finite number of cycles, oral TKI therapy is an open-ended and ongoing treatment that continues until medication intolerance or disease progression. Education regarding the frequent side effects associated with therapy is essential to ensure that the patient is in a position to make an informed decision regarding consent to treatment (Table 15.2). Lastly, whether to start treatment with one of the presently available medications or to seek enrollment in an available clinical trial is an important topic of discussion. There are very limited data to show that overall survival is actually extended with any of the presently available medications and patients should be given the opportunity to explore investigative therapies that may offer potentially superior outcomes.

If a decision is made to proceed with one of the currently available TKI's, the patient should undergo an extensive clinical assessment prior to initiation of therapy (Table 15.3). This assessment should include documentation of a thorough physical examination, complete laboratory evaluation, and comprehensive cross-sectional imaging, particularly if a current set of

Table 15.2 Common TKI-related adverse events

Constitutional	Cardiac	Musculoskeletal
Fatigue	Hypertension	Arthralgia
Asthenia	Congestive heart failure	**Endocrine**
Anorexia/weight loss	QT prolongation	Hypothyroidism
Gastrointestinal	**Dermatologic**	**Vascular**
Nausea	Rash	Bleeding
Diarrhea	Hand-foot syndrome	Thrombosis
Oral pain	Alopecia	
Stomatitis	**Neurologic**	
Abdominal pain	Headache	

Table 15.3 Initial and follow-up diagnostic studies

Hematology	Clinical chemistry	Imaging (CT or MRI)	Other
Hemoglobin/Hematocrit	Electrolytes	Neck	Electrocardiogram
White blood cell count	BUN/creatinine	Chest	±Echocardiogram
Platelet count	Calcium/magnesium	Abdomen/Pelvis	±Pregnancy test
PT/PTT/INR	AST/ALT	±MRI brain[a]	
	Alkaline phosphatase Phosphorous	±MRI skeleton[a]	
	Total bilirubin		
	TSH/free T4		

[a]Based upon clinical suspicion for metastatic involvement

Table 15.4 Starting and modifying TKI doses

Medication	Starting daily dose (mg)	Sequential dose reduction (mg)
Vandetanib	300	200, 100
Cabozantinib	140	100, 60
Sorafenib[a]	800	600, 400, 200
Pazopanib	800	600, 400, 200
Lenvatinib	24	20, 14, 10
Sunitinib[b]	50	37.5, 25, 12.5

[a]Divided into twice daily doses
[b]Dosed four weeks on treatment and two weeks off treatment

radiological studies has not been obtained within one to two months of the anticipated start of treatment. The latter cannot be overemphasized, since accurate determination of treatment response is most reliably accomplished when comparison is made to the most current pre-treatment imaging. As with all cancer care, performance status should be quantified prior to therapy using an established method such as the Eastern Cooperative Oncology Group (ECOG) or Karnofsky scales [29, 30], with appropriate discretion utilized prior to starting therapy if poor functional status is verified. A baseline electrocardiogram should be obtained, especially prior to starting vandetanib, to confirm a normal QT interval. An echocardiogram may also be considered to determine baseline ejection fraction since some agents (e.g., sunitinib) have been infrequently associated with congestive heart failure [31]. Hypertension, if present, should be treated aggressively prior to starting therapy since it is likely to be exacerbated by the use of the current group of TKI's. Electrolyte abnormalities should be identified and corrected. Also, thyroid hormone levels should be optimized prior to starting therapy since metabolism of levothyroxine is frequently altered with the use of the current TKI's [32].

In general, therapy should begin with one of the two current FDA-approved agents (i.e., vandetanib or cabozantinib). At the present time, there are no clear data to support the choice of one therapy over the other, and in many cases it may come down to provider preference and medication availability. Typically, therapy will begin with the standard starting dose, although consideration may be given to the use of a lower starting dose in patients for whom tolerability of the drug is suspect (see Table 15.4). Patients should have frequent follow-up in the initial phase of care to ensure that the medication is tolerated and that any side effects are identified and addressed appropriately. Practice patterns vary among medical centers, but a reasonable schedule of follow-up would include return visits every two weeks for the first three months, then monthly for the next three months, and then every three months thereafter [33]. The patient should be screened for any new symptoms and should undergo repeat physical examination with careful attention to the presence of weight loss or hypertension. Repeat laboratory work should be obtained to monitor for any hematological or biochemical disturbance. Serial radiological assessment should be performed every three months, although this interval may be shortened in cases of very rapidly growing disease [33, 34].

Managing Adverse Events

It is expected that almost all patients started on TKI therapy will experience one or more adverse events (AE's) referable to treatment. In the phase III trial of cabozantinib, 79 % of patients required dose reduction due to adverse events and 16 % of patients discontinued treatment, underscoring the extent of toxicity associated with this class of drug [16]. Although generally better tolerated than cytotoxic chemotherapies, TKI-mediated side effects can be frequently debilitating and sometimes lethal. Since many

Table 15.5 Management of treatment-related toxicity

Treatment-related toxicity[a]	Clinical response
Grade 1	Continue treatment. No dose reduction
Grade 2 (intolerable)	**First occurrence**: Interrupt treatment until resolved to grade 0–1. No dose reduction
	Subsequent occurrence: Interrupt treatment until resolved to grade 0–1. Dose reduction
Grade 3	**Any occurrence**: Interrupt treatment until resolved to grade 0–1. Dose reduction
Grade 4	**Any occurrence**: Discontinue treatment

[a]Grading as per CTCAE (v4.0) [39]

patients will remain on the therapy for long periods of time, even relatively milder symptoms, when persistent, can greatly impact the overall quality of life. For this reason, a successful outcome relies upon collaboration between the patient and the healthcare team to actively monitor and address AE's as they develop. Patients should be instructed to notify their healthcare team immediately in the event of any new symptoms. In some cases, therapy can continue uninterrupted and supportive treatment may be provided to attenuate symptoms; an elaborate outline of the various approaches to supportive care for specific TKI-related AE's is beyond the scope of this chapter, and the reader is directed to several excellent published reviews on the subject [35–38]. When an AE is more severe and cannot be easily addressed with supportive care, it may be necessary to temporarily interrupt treatment to allow resolution. Depending upon the initial severity of any subsequent recurrence, dose reduction may also be required. A valuable resource is The Common Terminology Criteria for Adverse Events (CTCAE) that is published by the National Cancer Institute and which lists common AE's and grades the events from 1 (mild) to 4 (life threatening) [39]. In general, grade 1 AE's can be monitored and therapy may be continued without interruption. Grade 2 AE's that the patient finds tolerable can also be monitored; however, intolerable grade 2 events will require interruption of therapy until the event has resolved to grade 0–1. Treatment can be restarted at the original starting dose, but any subsequent occurrences that require drug interruption should be followed by dose reduction. In the event of a grade 3 AE, therapy should be interrupted until the event has resolved (i.e., grade 0–1) and the dose reduced prior to restarting treatment. Continued dose reductions should be made for any further occurrences (Table 15.5).

Treatment Failure

Individual response to treatment is highly idiosyncratic, and anecdotal experience indicates that some patients may experience prolonged disease control for several years on the same therapy. However in general, the predicted duration of response with the current TKI's is typically between 6 and 24 months, and virtually all patients can be expected to eventually have evidence of disease progression. When focal growth is observed in an isolated tumor site, and an otherwise persistent good response is noted in the remaining lesions, it may be reasonable to continue the current medication and use a localized intervention (e.g., radiation, radiofrequency ablation, and surgery) to address the recalcitrant site of disease. However, if the pattern of progression is more generalized or new lesions arise while on treatment, then consideration will need to be given to transitioning to a second-line agent. Failure of one TKI does not preclude the use of any other drug within the class since the available agents have different but overlapping targets of activity, and where one drug may fail another may be effective [35]. If treatment was started with one of the presently FDA-approved therapies, then it is reasonable to transition to the

other approved medication in the event of disease progression. If persistent tumor growth is observed after sequential exposure to both vandetanib and cabozantinib, the benefit of salvage therapy with any of the remaining TKI's is less well-defined. If continued systemic therapy is deemed worthwhile at this point and the patient remains interested, enrollment in a clinical trial should be considered. Failing this option, the use of any of the remaining TKI's is feasible, although the likelihood for success is uncertain and careful attention will need to be given to supportive measures that ensure that the patient's comfort is maintained. Localized therapies can continue to be utilized as necessary to palliate symptoms as they arise, and if no clear benefit is seen with ongoing systemic therapy, the TKI should be discontinued in order to shift the focus of care to optimizing quality of life.

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Ablative Therapies of the Liver and Bone in Medullary Thyroid Cancer

16

Janice L. Pasieka and Adrian Harvey

Medullary thyroid cancer (MTC) is a rare neuroendocrine tumor derived from the thyroid parafollicular C-cells. Distant metastases are the most common cancer-specific cause of death in patients with MTC [1]. Recent censuses in the USA and Europe suggest that approximately 15 % of MTC patients present with distant metastatic disease [2–5]. Only half of the cases of distant metastases are found at initial presentation, and the other half present themselves following surgical intervention directed at the neck. Metastatic MTC is often multiple, involving multiple organs including liver, lungs, and bone. Lung metastases can be both macro- and micro-nodular, generally diffuse and bilobar (Fig. 16.1). These are best treated with systemic therapy and will be discussed elsewhere in the book. Bone metastases can be either osteolytic or osteoblastic. They can be a source of pain and/or present with orthopaedic or neurological complications. Liver lesions initially present in a small miliary pattern and as such usually undetectable to conventional anatomical imaging (Fig. 16.2). When larger, these lesions are typical of other neuroendocrine tumors (NETs) and tend to be hyperechoic on ultrasound and vascular on computed tomography (CT) (Fig. 16.3). Older series have demonstrated that survival after the discovery of distant metastatic disease is 51 % at 1 year, 26 % at 5 years, and 10 % at 10 years [1, 6–8]. However, with newer targeted therapies demonstrating great promise, these figures will

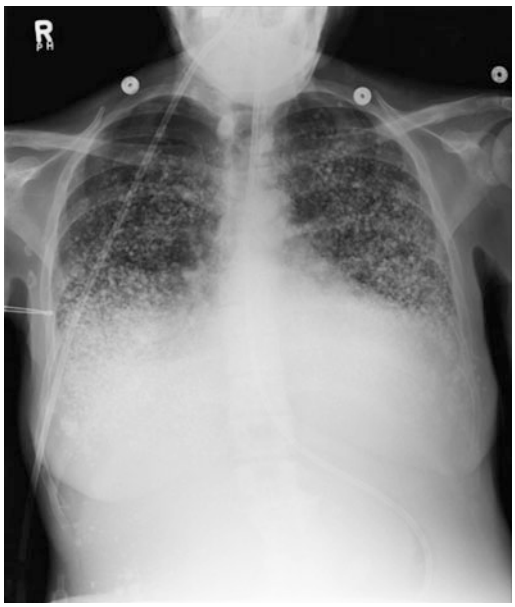

Fig. 16.1 Diffuse lung metastases from MTC on chest X-ray

J.L. Pasieka (✉) · A. Harvey
Faculty of Medicine, Department of Surgery and Oncology, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada
e-mail: Janice.Pasieka@AHS.ca

A. Harvey
e-mail: adrian.harvey@albertahealthservices.ca

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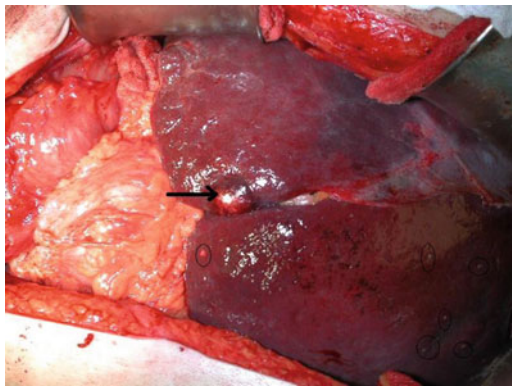

Fig. 16.2 Liver metastases from MTC. *Arrow points* to a known lesion found on preoperative CT scan. The *circles* demonstrate the diffuse miliary pattern of spread not detected on preoperative imaging, typical for MTC

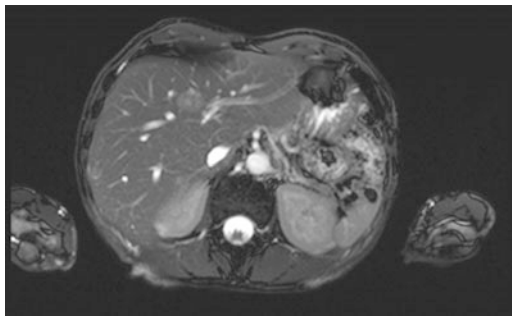

Fig. 16.3 MRI of diffuse liver metastases from MTC demonstrating their hypervascular appearance

likely change in the years to come [2, 9, 10]. The complexity and rarity of Stage IVc MTC requires a multidisciplinary and multimodality approach to therapy. As such, it is difficult to isolate the treatment effect of a single modality in patients with distant metastatic disease. This chapter will focus on the ablative therapies for liver and bone metastases in MTC, while other chapters will discuss the role of targeted and systemic therapies, recognizing that many times ablative therapies are utilized in combination with other treatment modalities.

Liver-Directed Therapies

Surgical Cytoreduction

The management of MTC liver metastases is similar to that of metastatic NETs from the gastrointestinal tract (GI-NET). Much of the evidences for liver-directed therapies are extrapolated from the GI-NET literature. In general, surgical resection of metastatic NET liver disease should be undertaken with a goal of complete R0 resection with curative intent. Unfortunately, for MTC, like GI-NETs, once metastasized to the liver, an R0 resection is rarely, if ever, possible. However, in GI-NETs, when all macroscopic disease is removed leaving only microscopic disease behind (R1), there appears to be a survival benefit compared to patients that did not undergo a resection [11–16]. In functioning GI-NETs, retrospective surgical series have demonstrated that cytoreductive surgery (greater than 70 % of the tumor burden) can provide good palliation with an improvement in symptoms from the endocrinopathy and an improved survival in these highly selected patients [15, 17, 18]. MTC secretes not only calcitonin but may present with an endocrinopathy from other peptides, such as ACTH, gastrin, and VIP [1]. Cytoreduction of the liver disease could be beneficial in providing symptom control; however, most of the time, the liver disease in MTC is diffuse, and resection for this purpose is limited. As such, ablative liver-directed therapies have been utilized.

Radiofrequency and Ethanol Ablation

Ablative therapies including radiofrequency (RFA), microwave, cryotherapy, and ethanol injection are well-established therapies in the treatment of NETs [11, 19, 20]. These therapies are commonly utilized to reduce tumor burden

and functional secretory capacity, either alone or in combination with surgical debulking. In a recent systematic review, Mohan et al. found 8 studies (n = 301) in which patients were treated with RFA for NET liver metastases [19]. Twenty-six of the procedures were percutaneous, and nearly half (48 %) had concomitant liver resections. Symptom control was achieved in 92 %, with a median duration of improvement of 14–27 months. Recurrence was common, as expected, occurring in 63–87 % of the patients. The authors concluded that RFA is a good option for palliative treatment of NETs given its safety profile (morbidity, 10 %, mortality, <1 %); however, there was insufficient evidence to determine whether RFA improved survival in metastatic NETs. RFA can be preformed percutaneously, laparoscopically, or intraoperatively [11, 21]. RFA is most effective in those patients with a low tumor volume, <5 lesions, and lesions <3.5 cm. Large lesions, hilar location, and proximity to the major bile ducts increase the risk of complications, and proximity to the portal vein limits the effectiveness of RFA [11]. Microwave ablation (MWA) can reduce the time to ablate and has the advantage of being able to be utilized near hepatic vasculature when RFA may not be as effective [12, 22]. Similarly, ethanol ablation has been utilized for the treatment of liver tumors [20]. Although some series included cases of MTC, the utilization of these techniques is limited by the diffuse nature of liver metastases and the miliary pattern of spread.

Hepatic Artery Embolization

Hepatic artery embolization (HAE) in NETs is based on the principle that NETs are highly vascular and supplied by the hepatic artery, while the normal liver parenchyma is supplied primarily by the portal vein. The coadministration of cytotoxic chemotherapy such as doxorubicin, cisplatin, or 5-FU with embolization of the hepatic artery (transarterial chemo-embolization [TACE]) can achieve a high concentration of chemotherapy while limiting systemic exposure. In NETs, clinical response rates of over 90 % have been reported with a median progression-free survival of 18 months [12]. There is a limited evidence comparing HAE with TACE in this population of patients. Lorenz et al. reported a small series of 11 MTC patients with calcitonin-induced diarrhea that underwent TACE [23]. All patients reported a symptomatic response; however, only six patients demonstrated a biochemical or radiographic response. In another small series of 12 patients with MTC, Fromigue et al. reported a partial radiographic response in 42 % of patients, stability of disease in 42 % of patients, and progression of liver metastases in 16 % of patients following TACE [24].

Recently, radioembolization using Yttrium 90 microspheres (^{90}Y) delivered into the hepatic artery has been shown to be effective in the treatment of NET hepatic disease. This technology delivers directed radiation to the metastases with a low risk of Grade 3 toxicity [11, 12, 16, 25]. In a recent meta-analysis of ^{90}Y embolization for metastatic NETs, Devcic et al. found 12 quality studies, totaling 436 procedures, which included some patients with MTC. The pooled data demonstrated a random-effects weighted average objective response rate (both complete and partial responses) of 50 % (95 % confidence interval (CI), 38–62 %), a weighted average disease control rate of 86 % (95 % CI, 78–92 %), and improved overall survival in the responders [25].

Radiopeptide Therapy

Somatostatin receptors (SST), predominately type 2, have been shown to be moderately expressed in MTC [26]. This has allowed somatostatin scintigraphy to be utilized both diagnostically and therapeutically. Recently, the development of Gallium-68 DOTA-SST PET/CT imaging with several different compounds (DOTA-TOC, DOTA-NOC, DOTA-TATE) has increased the diagnostic capabilities in patients with metastatic MTC [27–29]. Peptide receptor radionuclide therapy (PRRT) is a promising therapeutic option in NETs with somatostatin

receptor-positive tumors. Several small series of treatment-refractory metastatic thyroid cancer using PRRT have been published [26, 30–33]. The most widely used PRRT compounds are ^{90}Y-DOTA-TOC or lutetium (^{177}Lu)-DOTA-TATE. This therapy is well tolerated with Grade 1–2 toxicity, including nausea, vomiting, and transient anemia, occurring in one-third of patients within the first 24 h [31]. Rarely, renal insufficiency and myleodysplasia (Grade 3 and 4) can occur [26, 31, 32]. In a recent study of 16 treatment-refractory thyroid cancer patients, of which 8 had metastatic MTC, Budiawan et al. found median progression-free survival of 25 months with disease stabilization in 36 % of patients [26]. In a phase II trial of 31 MTC patients treated with ^{90}Y-DOTA-TOC, Iten et al. found a 29 % response rate and demonstrated a survival benefit in those that responded [33]. Although encouraging, these results require confirmation of survival benefit in a randomized phase III clinical trial.

Thirty to forty percent of patients with MTC will take up MIBG (meta-iodobenzylguanidine) on scintigraphy, and treatment with ^{131}I mIBG for palliation has been reported [32, 34]. In a small series of 13 MTC patients, symptom relief from calcitonin-induced diarrhea was achieved in 60 %; 4 patients demonstrated a partial response, and 4 showed stable disease [34]. Although MIBG therapy has been surpassed by ^{90}Y-DOTA-TOC or ^{177}Lu-DOTA-TATE, it may still have a palliative role in refractory cases.

Bone Ablative Therapies

Metastatic disease to the bone may be complicated by pain and neurologic/orthopedic compromise. Data on bone involvement in NETs also suggest that the cause of death may be related to bone disease in 23 % of patients [35]. Involvement of the bone or bone marrow is apparently more common than that is clinically apparent. In a series of 55 patients with elevated calcitonin levels following initial treatment, Giraudet et al. [36] identified bone metastases in 25 (45 %) using MRI, bone scintigraphy, and FDG-PET. When anti-CEA radioimmunoscintigraphy and bone marrow biopsy are added, the incidence of bone metastases in a similar patient population was found to be closer to 75 % [37]. Given that survival in metastatic MTC is typically measured in years, treatment should focus on prolongation of life, palliation of symptoms, and prevention of complications. While first-line therapy for metastatic MTC tends to focus on targeted biologic agents such as tyrosine kinase inhibitors, ablative therapies should be considered for refractory disease [38]. While surgical resection of limited bone disease has demonstrated some benefit in follicular cell-derived thyroid cancers, no data exist to suggest that this may be applied to MTC [39]. Thus, ablative therapies for bone disease are largely confined to external beam radiation and targeted radionuclide therapy.

External Beam Radiation

Evidence regarding the effectiveness of external beam in MTC has largely focused on the treatment of the neck following initial surgical therapy. While large population-based studies have failed to show any survival benefit [40], several series have suggested an impact on local control in high-risk patients [41]. As a result, the American Thyroid Association (ATA) guidelines on the management of patients with MTC have recommended considering ERBT in select patient populations following initial surgery; these include patients with spinal cord compression or those who have bone fractures or impending fractures who are not candidates for surgery [42]. Despite the lack of a survival advantage, these results suggest a degree of radiosensitivity in MTC, which could potentially be exploited in patients with symptomatic bone disease [42].

Radionuclide Therapy

While radiotherapy with I^{131} is a mainstay of treatment in follicular cell-derived thyroid cancer, it is ineffective in MTC as parafollicular cells do

not express the membrane sodium iodide symporter and thus do not concentrate the isotope. Thus, the use of targeted radioimmunotherapy in MTC and other NETs must rely on pairing a β-emitting nuclide with an appropriate ligand that will selectively target the appropriate cell type. Initial efforts in NET metastases have focused on 1^{131} labeled MIBG. This ligand is chemically similar to norepinephrine and is taken up by cells of neural crest origin. Unfortunately, while imaging indicates uptake in a large portion of pheochromocytomas, the sensitivity in MTC in only 25–30 % limits its clinical utility [32].

The expression and secretion of carcinoembryonic antigen (CEA) by MTC cells represents another potential target for radioimmunotherapy. Humanized anti-CEA monoclonal antibodies have been utilized in animal models and human studies for this purpose [43–45]. Radionuclide delivery is accomplished by labeling the monoclonal antibody directly or by following delivery with the infusion of a radiolabelled bivalent hapten targeting the anti-CEA antibody. Chatal et al. compared overall survival in 29 patients with metastatic MTC treated with an I^{131} labeled bivalent hapten to monoclonal anti-CEA with 39 untreated patients of similar prognosis [43]. Subgroup analysis revealed that overall survival was prolonged in high-risk patients, defined as a serum calcitonin doubling time of less than 2 years. Interestingly, bone or bone marrow uptake was associated with longer overall survival on multivariate analysis. Grade 4 bone marrow toxicities were observed in nine patients. In addition, Salaun et al. have shown in a mouse model that the combination of radioimmunotherapy with the anti-angiogenesis drug bevacizumab may have improved efficacy. While promising, anti-CEA-targeted radionuclide therapy is not currently widely used in clinical settings.

As previously described, the expression of SST type 2 receptors in MTC provides an additional therapeutic target. Peptide receptor radionuclide therapy (PRRT) with ^{90}Y- or ^{177}Lu-labeled somatostatin analogs has shown promising results in the treatment of metastatic MTC. However, given that these series typically involve small numbers of patients with considerable heterogeneity with respect to the site of metastatic disease, it is difficult to tease out the specific effect of these treatments on bone metastases. Bodei et al. retrospectively study 21 patients receiving ^{90}Y-DOTA-TOC for non-resectable local or metastatic MTC [46]. Of these, three were reported to have bone metastases on cross-sectional imaging, although previously described research has indicated that occult bone/bone marrow involvement was likely higher [37]. On imaging, stabilization or response was noted in 67 % of patients. Biochemical response or stabilization was observed in 42 %. In the series of 31 patients with metastatic MTC reported by Iten et al., the incidence of bone metastases was not separately reported [31]. However, given the high baseline rate of involvement proposed in this patient population and the 13 % observed rate of hematologic toxicities, it is likely that a significant number of these patients had bone disease. Thus, some of the prolonged survival seen in responders may be related to the treatment of bone disease.

Radiolabeled DOTA-TATE has also shown promise in this patient population. Makis et al. reported two cases of MTC treated with ^{177}Lu-DOTA-TATE. One patient has extensive bone disease and demonstrated stability over 4 treatments [30]. Budiawan et al. reported a series of 16 patients with non-radioiodine avid, metastatic thyroid cancer [26]. Eight patients had MTC, of which four had bone metastases, three of these patients demonstrated stable disease. In addition, PRRT with ^{177}Lu-DOTA-TATE has been evaluated in patients with bone metastases from gastrointestinal and pancreatic NETs [47]. In 41 patients studied, with a median follow-up of 32 months, complete response was noted in 4.8 % and progressive disease in just 11.9 % of patients. Prior to treatment, bone pain was noted in 26 %; all of these patients experienced a complete (55 %) or partial (45 %) improvement in pain.

Summary

Cure of MTC relies on appropriate initial surgery. Despite this, 15 % of patients will develop distant metastatic disease. As multiple organs are often affected and survival is measured in years, care of these patients requires a multidisciplinary team approach. Liver-directed therapies include surgical and ablative cytoreduction, hepatic artery embolization, and radiopeptide therapy. External beam radiation therapy may provide palliation in bone metastases. Radionuclide therapy is the most promising option in these patients. As cure is unlikely, therapies should be carefully selected for symptom control and to prolong overall survival, when possible.

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Emerging Therapies for Progressive Metastatic Medullary Thyroid Cancer

17

Jessica E. Zwiener, Lily J. Kwatampora and Naifa L. Busaidy

Introduction

Targeted therapy in progressive metastatic medullary thyroid cancer (MTC) with current FDA-approved agents, vandetanib and cabozantinib, is associated with improved progression-free survival [1, 2]. In contrast, therapy with cytotoxic chemotherapy is associated with poor response rates of short duration and is not routinely used for the treatment of MTC [3]. Despite these advances in therapy over the years and the use of more directed, targeted therapies with a better and longer duration of response, there have been no reported cures and progression of disease eventually ensues. Development of progression of disease also does not appear to be due to dose reductions nor interruptions [4]. This eventual lack of response in nearly all patients is driven primarily by development of acquired resistance. Although the mechanisms of resistance are not fully understood, there are some hints of which tumors may not respond to target therapy. One example is the presence of mutations in *RET V804M/L*, typically associated with familial and sporadic MTC, which confers primary or acquired resistance to vandetanib and cabozantinib (V804M) [5, 6].

An understanding of the oncogenic pathways and pathways of resistance informs development of trials and appropriate multikinase tyrosine kinase inhibitors (TKIs) or other therapies that target alternative molecular pathways alone, sequentially or in combination, to overcome resistance.

Therapeutic Targets

Although RET activation plays a significant role in mediating C-cell hyperplasia in MTC, alternative genetic mechanisms propagate these actions and contribute to tumor aggressiveness [7]. In sporadic MTC, which accounts for 75 % of all cases, only about 40–50 % have *RET* mutations. Of these, *M918T* mutation is the most prevalent (52–79 %) and portends the worst prognosis. Another 16 % have mutations at codon 634 and generally have less aggressive outcomes. Even less frequently encountered in sporadic disease are mutations in codon 883 [7–9]. A second non-RET oncogenic pathway affecting primarily *HRAS* and *KRAS* has

J.E. Zwiener (✉) · N.L. Busaidy
Department of Endocrine Neoplasia and Hormonal Disorders, MD Anderson Cancer Center, The University of Texas, 1400 Pressler, Unit 1461, Houston, TX 77030, USA
e-mail: jzwiener@gmail.com

N.L. Busaidy
e-mail: nbusaidy@mdanderson.org

L.J. Kwatampora
Endocrinology Diabetes and Metabolism, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA
e-mail: lkwatampora@gmail.com

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been observed in 15 % of sporadic MTC patients and presents a potential target for anti-RAS agents such as tipifarnib [10, 11]. The activation of the mammalian target of rapamycin (mTOR) pathway in association with both *RET* and *RAS* mutations has also been reported in over 90 % of both hereditary and sporadic MTC [12–14]. As such, an upstream or downstream block of the PI3K/AKT/mTOR pathway in combination with anti-RET therapy could be considered in these advanced cases [15, 16].

Additional targets include mutations of the tumor suppressor gene *CDKN2C* that leads to loss of P18 function and contributes to *RET*-induced MTC [17]. Tumors with mutations in this pathway may respond to cyclin D inhibitor therapies. RET-induced inhibition of activating transcription factor-4 (ATF-4) prevents apoptosis and renders MTC cells resistant to TKIs; as such, therapy that targets both RET and ATF-4 could be considered [18]. Recent findings of programed death-ligand 1 (PD-L1) expression in over 25 % of MTC suggest that checkpoint inhibition with anti-PD-L1 therapy could be explored [19]. Still, there exists a role for whole-genome sequencing in the future of personalized medical care to identify other therapeutically targetable mutations. This chapter discusses several emerging therapies that hold promise in progressive metastatic MTC.

Emerging Therapies

Clinical Trials

Given the lack of curative therapies for metastatic MTC, many patients will benefit from enrollment in a clinical trial. This is especially true if they have already shown progression despite treatment with one or both of the FDA-approved TKIs. Ideally, these studies would be prospective, randomized controlled studies comparing multiple MTC therapies head-to-head. It is important for treating clinicians to enroll their patients in various phase clinical trials when appropriate. Phase I trials are the first in-human trials and typically involve small numbers of patients (10–20). The focus of phase I trials was to establish the safety, dosing, and side effect profile of a new drug. Although the goal was not to determine the overall efficacy of a particular treatment, patients (especially those with progressive disease refractory to commercially available TKIs) may still gain therapeutic benefit. Phase II trials are performed in a larger group of patients and evaluate the efficacy of a new treatment, sometimes in comparison with currently available therapies. Phase III trials are larger still and compare a new treatment to the standard of care. They also attempt to generalize the results seen in a successful phase II trial to a larger and more diverse study population. In the rare instance of an especially promising phase I trial, a phase II trial may be bypassed, as was the case with cabozantinib.

Tyrosine Kinase Inhibitors

The *RET* proto-oncogene is mutated in 99 % of familial MTC and about half of sporadic MTC, and as such was initially the primary target for drug development [20]. Vandetanib and cabozantinib, the two TKIs that are FDA-approved for the treatment of progressive MTC (see Chap. 15), both target RET, as well as several other structurally similar tyrosine kinases—vandetanib targets vascular endothelial growth factor receptor (VEGFR)2-3 and endothelial growth factor receptor (EGFR); cabozantinib targets VEGFR2 and c-MET. Multiple other TKIs, targeting some combinations of RET, VEGFR, EGFR, platelet-derived growth factor receptor (PDGFR), KIT, and c-MET, have been shown to have activity against MTC. VEGFR is a promising target for small-molecule TKIs [21]. VEGFR is the main driver of angiogenesis in the thyroid, and VEGF has increased expression in MTC [22]. VEGFR2 expression is higher in MTC metastases compared to the primary tumor [23]. All of the current multikinase inhibitors used to treat MTC target VEGFR in addition to various other targets. In the phase III trials that led to their FDA approval, vandetanib improved progression-free

survival from 19.3 to 30.5 months, and cabozantinib improved progression-free survival from 4.0 to 11.2 months [1, 2]. There were big differences in these studies, including the requirement for progression of disease prior to enrollment in the cabozantinib study and the lack of such requirement in the vandetanib study.

Data regarding the efficacy of these TKIs based on *RET* mutation status are mixed, but overall seems to point to an improved response to therapy in patients with *RET 918* mutation positive tumors. The cabozantinib phase III trial initially noted no difference in response between the *RET* mutation positive and negative cohorts, as all subgroups benefited from the treatment. On expanded analysis, however, investigators found that patients with *RET* mutations had longer progression-free survival than patients with wild-type *RET* (60 vs. 25 months) [24]. In the subgroup analysis, patients with *RET M918T* mutations had significantly lengthened overall survival with cabozantinib versus placebo (44.3 vs. 18.9 months) [25]. However, there was no statistically significant increase in overall survival rates for patients with other *RET* mutations, or for all *RET* mutations combined, compared with *RET*-negative patients.

In the vandetanib phase III trial, researchers were unable to conclusively determine the effect of *RET* mutation status on response to treatment, as many of the patients' tumor samples had insufficient quantity or quality of DNA for complete analysis, and of those with adequate samples, very few were *RET*-negative. A subgroup analysis showed improved progression-free survival in *M918T*-mutated patients [2]. It is important to remember that several specific *RET* mutations confer resistance to therapy—*V804L* and *V804M* mutations confer resistance to vandetanib; *V804M* mutations, to cabozantinib [5].

Ponatinib, a small-molecule TKI that inhibits BCR-ABL, FLT3, KIT, FGFR1, PDGFR, VEGFR, and RET, is FDA-approved to treat chronic myelogenous leukemia (CML) or Philadelphia chromosome positive acute lymphoblastic leukemia (ALL). It showed promise in mouse models of RET-driven MTC during initial studies [26]. Compared to cabozantinib and vandetanib, its inhibition of RET is more potent, with a half maximal inhibitory concentration (IC_{50}) against wild-type RET of 7 nM, compared to 122 nM for vandetanib and 164 nM for cabozantinib. Ponatinib is effective in patients with *RET* mutations, including those with *V804M* mutations whose tumors are resistant to vandetanib and cabozantinib (IC_{50}, 12 compared to >10,000 for vandetanib and 4094 for cabozantinib) [6]. A phase II trial evaluating the efficacy of ponatinib in patients with advanced MTC who either failed or did not tolerate both cabozantinib and vandetanib is planned to be completed in March 2016 [27].

Lenvatinib, a multitarget TKI of VEGFR1-3, fibroblast growth factor receptor (FGFR)1-4, PDGFR-α, RET, and KIT, is FDA-approved to treat radioiodine-refractory differentiated thyroid cancer based on a phase III clinical trial showing a 64.8 % overall response rate and an increase in progression-free survival from 3.6 months in the placebo arm to 18.3 months in the lenvatinib arm [28]. In a Japanese phase II trial of lenvatinib in patients with radioiodine-refractory differentiated thyroid cancer, MTC, and anaplastic thyroid cancer, the patients with MTC had an overall response rate of 25 %, with progression-free survival of 6.5 months [29]. Another phase II trial of lenvatinib in 59 patients with unresectable MTC and evidence of disease progression found an overall response rate of 36 %; *RET* mutation status did not change patients' response to therapy [30]. Therefore, lenvatinib may be a useful drug in cases where cabozantinib and vandetanib are ineffective or when the patient is *RET* mutation negative.

Sunitinib is a small-molecule TKI with inhibitory activity against VEGFR 1-2, PDGFR, c-KIT, FMS-like tyrosine kinase (FLT)-3, and RET. A phase II study examining the efficacy of sunitinib in patients with all subtypes of metastatic thyroid cancer showed a RECIST response in 3 of the 6 patients with MTC, with a disease control rate of 71 % [31]. In a second phase II study in 25 patients with metastatic MTC and evidence of disease progression, patients treated

with sunitinib had an overall response rate of 35 % and a clinical benefit rate (partial response and stable disease) of 91 %. Benefit was seen in patients with and without *RET* mutations [32].

Nintedanib blocks RET, VEGFR1-3, FGFR1-3, and PDGFR-α and is used to treat nonsmall cell lung cancer (approved for use in Europe) and idiopathic pulmonary fibrosis (FDA-approved) [33, 34]. There is currently a trial in progress evaluating nintedanib versus placebo in both differentiated thyroid cancer and MTC [35].

Pazopanib is a small-molecule TKI inhibiting VEGFR1-3, PDGFR-α/β, and c-Kit, currently approved for use in patients with metastatic renal cell carcinoma. It has no inhibitory activity against RET. A phase II trial of pazopanib in 35 patients with advanced MTC and evidence of disease progression in the previous 6 months showed modest response to the drug (5/35 patients attained partial response, median progression-free survival, 9.4 months, overall survival, 19.9 months) [36]. While the drug's activity is not strong enough against MTC to warrant routine clinical use, the evidence of benefit despite the lack of RET activity supports the assertion that RET, while important in the development of MTC, may not be a necessary drug target.

Sequential TKI Therapy

TKIs may be used sequentially as salvage therapy after disease progression on an initial drug—progression on one drug does not guarantee that a drug with similar targets will be ineffective [37]. A retrospective review of 10 patients treated sequentially with 2 commercially available TKIs noted that a subset of patients had a clinical response to a second TKI after evidence of disease progression on the first, but with a shorter time to failure than with the first drug [38]. On the first TKI, 40 % of patients had a partial response, 50 % had stable disease, and 10 % had progression of disease. On the second TKI, 20 % had a partial response, 60 % had stable disease, and 20 % had progressive disease.

There are still many unanswered questions regarding the use of TKIs in MTC. We do not know the most potent combination of targets, or whether patients benefit more from continuous therapy versus drug holidays. Over time, the receptors targeted by newer TKIs will be more specific to the patient's disease, or perhaps we will learn to combine different treatment modalities for maximum effect. Currently, however, patients on TKIs inevitably progress, whether due to resistance to the drug, tumor de-differentiation, or for unknown reasons. Frequently, the drugs must be discontinued (or the dose decreased) due to intolerable adverse effects. As these drugs are not curative, the goal should be to prolong life while maintaining the quality of life; therefore, each drug's side effect profile must be balanced with its efficacy.

Neoadjuvant

In patients initially determined to have unresectable disease (due to extent of disease or location near critical structures), treatment with a TKI may cause enough tumor shrinkage that the disease becomes resectable. In a case report from 2010, a patient presented with a 5.8 × 3.5 cm nodal mass surrounding the carotid artery that was initially biopsied as anaplastic thyroid cancer. The patient did not respond to radiotherapy or cytotoxic chemotherapy. Sunitinib was started, and the patient had a marked decrease in the size and 18F-FDG avidity of his neck mass. After 19 months of therapy with sunitinib, the tumor had shrunk to the point that it had become resectable. Surgical pathology showed MTC. Fourteen months after thyroidectomy and neck dissection, the patient's calcitonin was 106 pg/mL and there was no radiographic evidence of disease [39]. There have been no clinical trials yet examining the use of TKIs as neoadjuvant therapy, only case reports, but this may be a viable treatment option for the patient with unresectable disease. Clinical trials with various therapies should be considered.

Mammalian Target of Rapamycin (MTOR)

mTOR proteins are evolutionarily conserved serine/threonine kinases of central importance in the regulation of cell growth, proliferation, and

survival [40]. The mTOR pathway is upregulated during times of growth and inflammation (tumor formation, angiogenesis, insulin resistance, adipogenesis) and is deregulated in disease states such as cancer and type 2 diabetes [41]. Elevated levels of mTOR have been documented in most human cancers [42]. In the past, mTOR inhibitors were used primarily for their immunosuppressive effects post-transplant, but early studies have shown their efficacy as anticancer therapy. In a phase II study of everolimus monotherapy in patients with metastatic thyroid cancer progressive over 6 months, the subset of 10 patients with MTC had 1 partial response and 9 patients with stable disease, lasting 6–33+ months [43]. A second phase II study of everolimus plus sorafenib in similar patients showed that of the 10 patients with progressive, metastatic MTC, 4 had partial response, 4 had stable disease, and 2 had disease progression [16].

mTOR inhibitors are an especially attractive option for patients who cannot use anti-VEGF therapies—for example, patients with extensive tumor involvement of the neck vasculature or laryngopharynx, or who are unable to tolerate the side effects of VEGF inhibitors. Perhaps the combination of mTOR and VEGF/RET inhibition may be useful in the treatment of patients with tumors resistant to monotherapy. Multiple studies are currently underway examining the efficacy of mTOR inhibitors alone or in combination with TKIs, monoclonal antibodies, or somatostatin analogs, in the treatment of MTC.

Immunotherapy

Rather than continuing the search for a more closely targeted TKI, some researchers are approaching the problem from different perspectives, one of which is immunotherapy. Immunotherapy refers to any treatment that uses the immune system to target a cancer, and has multiple different applications, several of which will be discussed here.

Checkpoint Inhibition

Checkpoint inhibition is an area of research that has made significant progress over the past twenty years. Tumor cells of many different types inhibit or escape from immune regulation of cell growth; by manipulating the immune cell–tumor cell interactions, tumor growth may be slowed or even reversed [19]. Upregulated immune surveillance is associated with decreased metastatic potential, and may help to stabilize disease [19]. One focus of study has been the interaction between PD-1 (programed cell death) and PD-ligand (PD-L1/L2). PD-1 is expressed on T-, B-, and NK-activated monocytes, and dendritic cells [44]. Its ligands PD-L1 and PD-L2 are expressed on antigen-presenting cells, placenta, and nonhematopoietic cells that infiltrate tumors. Binding of PD-1 to PD-L1/L2 induces immune tolerance. Aberrant expression of PD-L1/L2 has been demonstrated in a variety of tumor types [44]. A recent analysis of immune markers in large cohort patients with MTC showed that 27 % of cells in the tumor center expressed PD-L1, indicating that a subset of patients may benefit from anti-PD-L1 therapies [19]. Pembrolizumab and nivolumab are monoclonal antibodies against PD-1 that were recently approved for the treatment of unresectable metastatic melanoma and may have application in the treatment of metastatic MTC. Multiple studies are currently in progress; none are specifically dedicated to patients with thyroid cancer, but several are open to patients with any metastatic carcinoma.

Tumor Vaccines

Tumor vaccines contain tumor antigens and are designed to stimulate the body's immune system to recognize tumor cells as foreign. They represent an attractive therapeutic option for several reasons. First, they specifically target tumor cells while sparing normal tissue. As a result, they have been shown to have minimal adverse effects. Additionally, "off-the-shelf" vaccines are inexpensive, stable and may be used in a variety of different cancers. A recent phase I trial

examined the use of Yeast-CEA (GI-6207), a heat-killed strain of recombinant *S. cerevisiae* engineered to produce CEA, with limited but promising results [45]. Twenty-five patients with progressive metastatic carcinomas expressing CEA were treated with a series of GI-6207 subcutaneous injections (every 2 weeks for 3 months, then monthly). One of the 25 patients had metastatic MTC; 22 had colorectal cancer, one had pancreatic adenocarcinoma, and one had nonsmall cell lung cancer. The injections were well tolerated with few adverse effects. Median survival was 7 months; 7 of 24 patients had decline in serum CEA levels with treatment. The patient with MTC was noted to have radiographically stable disease (pleural-based and pericardial metastases) at three months, and stabilization of serum CEA and calcitonin levels. Shortly afterward, the patient developed nonmalignant pleural and pericardial effusions; culture and biopsy were negative for infection, and the effusions resolved rapidly with empiric high-dose steroids. The etiology of the effusions is unclear, but given their location and prompt resolution with steroids, it is possible that they were the results of a vigorous immune response to her metastatic disease. A phase II trial evaluating the use of GI-6207 in patients with metastatic MTC is currently recruiting and will conclude in December 2017.

Other Potential Targets

HRAS

Tipifarnib is a selective farnesyltransferase inhibitor, which inhibits the rate-limiting step in the post-translational modification of RAS (one of the downstream targets of RET) and therefore its oncogenic activity [46]. This therefore seems like an exciting therapy for *RAS*- or *RET*-mutated MTC. A phase I study was performed to evaluate the efficacy of tipifarnib in combination with sorafenib, a TKI inhibiting RAF-1, PDGFR, RET, KIT, and VEGFR-2 [11]. Thirteen patients with progressive metastatic MTC were enrolled, 10 of whom reached first restaging. Thirty-eight percent of the MTC patients had partial response, and 31 % had stable disease for at least 6 months. A phase II trial of sorafenib monotherapy in MTC showed a much lower response rate (1/10 patients with PR, 4 with stable disease >15 months, 4 with stable disease <6 months, 1 with clinical progressive disease), suggesting that either the tipifarnib or the combination is effective, although the two studies are not directly comparable [47].

Recently, tipifarnib was discovered to specifically inhibit HRAS [46]. Therefore, there is a phase II clinical trial opening for patients with tumors positive for *HRAS* mutations. A specific subgroup will be open for patients with MTC [48].

Activating Transcription Factor 4 (ATF4)

While *RET*'s importance in the development and pathogenesis of MTC is well-known, the specific mechanisms by which it potentially promotes cell survival and inhibits cell death are not well understood. Recent work showed the importance of ATF4, a master transcription factor that regulates stress-induced apoptosis via activation of NOXA and PUMA [18]. RET directly inhibits ATF4 via phosphorylation, leading to decreased expression of proapoptotic genes. Simultaneous targeting of RET and ATF4 may be useful in MTC and other cancer types with *RET* mutations. Eeyarestatin is an endoplasmic reticulum-associated degradation (ERAD) inhibitor that blocks proteasomal degradation of misfolded proteins, causing massive induction of endoplasmic reticulum-mediated stress reaction, upregulation of ATF4, and apoptosis [18]. It has been shown to enhance the efficacy of bortezomib against cervical cancer [49]. Given the interaction of RET and ATF4, eeyarestatin may prove useful in the treatment of MTC.

p18

Approximately 70 % of metastatic MTCs demonstrate loss of *P18* (personal communication). *P18* (also referred to as *CDKN2C*) is a tumor suppressor gene that serves as a gatekeeper to entering the cell cycle; its protein product, P18, along with other members of the INK4 (inhibitors of CDK4) family, binds to cyclin-dependent kinase (CDK) 4 and 6,

preventing the formation of active CD4/6-cyclin D complexes. These activated complexes are necessary for downstream signaling that allows the cell to progress from G1 to S phase. Loss of *P18* keeps the MTC cell in a continual growth phase and contributes to disease progression [17]. Therapy with cyclin-dependent kinase inhibitors has shown potential in treatment of breast cancer [50] and chronic lymphocytic leukemia [51] and may be an exciting new treatment approach for patients with metastatic MTC.

Treating Other Effects of MTC

Diarrhea

Severe MTC-related diarrhea is common, occurring in about 30 % of patients [21]. It has a significant impact on patient quality of life and is often unresponsive to conventional antidiarrheal medications. In patients with stable metastatic MTC, intractable diarrhea is sometimes the only indication for initiation of TKI therapy. Despite the significant burden of MTC-related diarrhea, it remains largely unstudied. Loperamide, diphenoxylate/atropine, tincture of opium, colestipol, and cholestyramine are among the treatments generally used, with variable results and adverse effect profiles.

Calcium aluminosilicate antidiarrheal (CASAD) is a naturally occurring calcium montmorillonite clay that adsorbs inflammatory molecules associated with diarrhea [52]. A recent pilot study of seven patients with metastatic MTC, not on systemic therapy, with greater than three bowel movements per day, showed that 6/7 patients were able to discontinue their prior antidiarrheals, and 5/7 considered the drug a success and chose to continue the drug after the initial one-week study period. Further studies are needed to improve this devastating effect of metastatic MTC.

Quality of Life

In addition to refractory diarrhea, patients with MTC have a significant symptom burden at baseline. Most common are diarrhea and flushing, but bone pain, fatigue, dyspnea, dysphagia, hoarseness, sleep disturbances, emotional distress, and weight loss are also seen. Less commonly seen are ectopic Cushing syndrome or pain from bony metastases. The percentage of patients with moderate-to-severe symptoms is similar to that of patients with metastatic nonsmall cell lung cancer, a patient population known to be highly symptomatic (unpublished data). Patients with metastatic disease are frequently started on TKIs, which themselves have significant adverse effects. Vandetanib commonly causes diarrhea, nausea, anorexia, rash, fatigue, hand-foot syndrome, hypertension, dysgeusia, and headache; additionally, it carries a black box warning as it causes QT interval prolongation in up to 14 % of patients [2]. Cabozantinib commonly causes diarrhea, hand-foot syndrome, weight loss, anorexia, nausea, fatigue, dysgeusia, hair color changes, hypertension, stomatitis, constipation, hemorrhage, vomiting, mucosal inflammation, asthenia, and dysphonia; it carries a black box warning for the risks of VEGF-inhibition-related hemorrhage, gastrointestinal perforation, and fistula formation [1]. It is unclear whether or not initiation of the therapy improves the quality of life. Emerging therapy trials must focus on quality of life, especially given that these drugs are not curative and are given long-term.

Conclusion

MTC is often surgically curable if detected before it extends beyond the thyroid. However, the majority of patients will still have biochemically or radiographically detectable disease postoperatively. The only FDA-approved therapies for patients with progressive metastatic disease are cabozantinib or vandetanib, neither of which is curative. In time, progression ensues due to resistance or dedifferentiation. Furthermore, these drugs have multiple adverse effects, necessitating dose reductions or discontinuation. For these patients, inclusion into clinical trials or treatment with salvage therapies where trials are not available should be considered. With no *RET*

mutations found in about 60 % of sporadic MTC, studies exploring non-*RET*-dependent mechanisms hold promise. In addition, multiple unanswered questions such as the sequence of TKIs, combinations of TKIs, or the role of combination therapies (such as immunotherapy plus TKI) remain, offering opportunity for further studies. Studies that combine therapies in ways that maximize efficacy and minimize adverse effects are of particular interest.

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18 Follow-Up and Surveillance of Patients with Medullary Thyroid Carcinoma

Nazanene H. Esfandiari and Megan R. Haymart

Introduction

Medullary thyroid carcinoma (MTC) accounts for almost 5 % of all thyroid carcinomas, and it is responsible for up to 13.4 % of all deaths related to thyroid cancer [1–3]. MTC is a neuroendocrine tumor and arises from the parafollicular cells or C-cells of the thyroid gland and therefore produces serum calcitonin as a tumor marker [4]. The overall survival of patients with MTC is 86 % at 5 years and 65 % at 10 years. Poor prognostic factors include advanced age, advanced stage, prior neck surgery, and associated multiple endocrine neoplasia (MEN) 2B [2, 3, 5]. The clinical courses of patients with MTC vary, and there are reported patients with long-term survival with persistent hypercalcitoninemia [6].

N.H. Esfandiari (✉)
Division of Metabolism, Endocrinology, & Diabetes, University of Michigan, 24, Frank Lloyd Wright Drive, Domino's Farm, Lobby C, Suite 1300, Ann Arbor, MI 48106-0451, USA
e-mail: nazanene@umich.edu

M.R. Haymart
Division of Hematology/Oncology, University of Michigan Health System, North Campus Research Complex, 2800 Plymouth Rd. Bldg. 16, Rm 408E, Ann Arbor, MI 48109, USA
e-mail: meganhay@umich.edu

M.R. Haymart
Division of Metabolism, Endocrinology, & Diabetes, University of Michigan Health System, North Campus Research Complex, 2800 Plymouth Rd. Bldg. 16, Rm 408E, Ann Arbor, MI 48109, USA

The appropriate staging, risk stratification, and management of patients with MTC still remain challenging. The American Thyroid Association (ATA) guidelines for MTC were published to recommend evidence-based guidelines for the diagnosis and management of patients with MTC [7, 8]. When cancer is confined to the thyroid gland, the prognosis is excellent. However, in patients with clinically palpable MTC, the incidence of microscopically positive nodes is more than 75 % [9]. Therefore, total thyroidectomy with central compartment neck dissection is the treatment of choice in patients with MTC [7, 8]. More than 50 % of patients with MTC have cervical neck metastases, and up to 30 % have distant metastases at the time of diagnosis [2]. Less aggressive surgery in the patients with distant metastasis has been proposed with the goal of palliation [8].

Recent ATA guidelines [8] and Machens and Dralle [10] support the need for incorporating the number of metastatic cervical lymph nodes (N1, 1–10; N2, 11–20; N3, >20), which is an important prognostic factor, into the American Joint Committee on Cancer (AJCC) staging system for MTC, rather than just the presence or absence or lymph node metastases. Additional work demonstrated that age, distant metastases, and the number of cervical lymph node metastases are important and correlate with overall survival [11]. The current AJCC TNM classification for MTC lacks several prognostic factors, such as age and postoperative serum calcitonin, in addition to the number of metastatic lymph nodes.

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Although there has not been extensive research clarifying optimal follow-up of MTC patients, there is expert consensus [8]. In the following text, we will summarize the role of serum calcitonin and carcinoembryonic antigen (CEA) levels, imaging studies, *RET* oncogene testing, and if *RET* positive, plasma metanephrines and calcium levels, in the postoperative follow-up of patients with MTC.

Follow-up After Initial Surgery

Role of Measuring Calcitonin and CEA Levels and Other Neuroendocrine Biomarkers

Total thyroidectomy with central compartment neck dissection remains the standard treatment for patients with MTC without distant metastases. Postoperative follow-up of these patients should include serum calcitonin and CEA measurement to identify persistent disease or early recurrence. Calcitonin is produced almost exclusively by neuroendocrine C-cells and is the most specific and sensitive MTC serum marker both before and after total thyroidectomy [12, 13]. Basal serum calcitonin usually correlates with tumor mass and also reflects tumor differentiation [14]. To detect the presence of residual disease, serum calcitonin levels should be checked 3–6 months after initial surgery [8]. If the serum calcitonin level is undetectable, the patient is considered biochemically cured with excellent prognosis. In this group of patients, the five-year recurrence rate is only 5 % [15]. It is important to remember that serum calcitonin has a long half-life. Therefore, the rate of decline in serum calcitonin can be slow in some patients [16]. There has been controversy regard ing the length of time needed to reach the nadir of the calcitonin level after total thyroidectomy. In some patients who are surgically cured, serum calcitonin levels decline rapidly within the first postoperative hour, achieving undetectable levels with the first few postoperative days [17–19]. However, due to differences in clearance of serum calcitonin levels, many clinical investigators have proposed that three months postoperatively is the optimal time to obtain serum calcitonin levels [13, 17].

If serum calcitonin levels are undetectable at three to six months postoperatively, serum calcitonin should be measured every six months for one year and then yearly thereafter [8]. Persistently elevated serum calcitonin levels six months after surgery indicates persistent disease. The extent of elevation of serum calcitonin levels can also suggest the site of recurrence. When serum calcitonin is <150 pg/ml, this indicates persistent locoregional disease in the neck [8, 20]. If serum calcitonin is >150 pg/ml, this suggests the possibility of distant metastases, although many patients with distant metastases often have a serum calcitonin level >1000 pg/ml [8]. In the face of persistent hypercalcitoninemia, workup with several different imaging studies is required to localize the recurrence or persistent disease.

Of note, serum calcitonin can be falsely elevated in patients with chronic renal failure, autoimmune thyroiditis, large cell lung cancer, prostate cancer, mastocytosis, enteric and pulmonary neuroendocrine tumors, and hyperparathyroidism [21–25]. Heterophilic antibodies can also cause falsely elevated serum calcitonin levels [26]. The "Hook effect" can occur with ICMA assays and should remain a concern in patients with a large tumor burden and surprisingly low serum calcitonin levels [27].

In parallel with serum calcitonin, CEA can be used as another tumor marker to detect persistent or recurrent MTC as neoplastic C-cells also produce CEA. CEA is a non-specific tumor marker for MTC, but it does help predict outcome [28–30]. Due to its prolonged half-life, serum levels of CEA may take even longer to reach a nadir. Serum CEA levels can be falsely elevated from heterophilic antibodies, smoking, gastrointestinal tract inflammatory disease, benign lung tumors, or a host of non-thyroid malignancies [8].

In patients with MTC, simultaneous rising of serum CEA and calcitonin levels indicates disease progression. If MTC patients have elevated CEA levels but stable or declining calcitonin levels, poorly differentiated MTC should be considered [31]. Therefore, it is important that serum calcitonin and CEA levels are measured concurrently.

Assessment of serum calcitonin and CEA doubling times postoperatively provides a valuable tool for assessing progression and aggressiveness of MTC [32, 33]. In addition to imaging studies in patients with persistent and recurrent disease, serum calcitonin and CEA should be monitored every six months to determine doubling times [6]. If the doubling time is less than six months, the 5-year and 10-year survival rates are 25 and 8 %, respectively. If the doubling time is more than 24 months, the 5-year and 10-year survival rates are 100 and 100 %, respectively [32, 33]. The ATA provides a calculator to determine doubling time of serial serum calcitonin and CEA measurements on their Website (http://www.thyroid.org/thyroid-physicians-professionals/calculators/thyroid-cancer-carcinoma/) [8]. It is also important to remember that only a few patients may develop tumor recurrence without an elevation in serum calcitonin levels [34].

Other Neuroendocrine Biomarkers

MTC is able to produce several hormonal and non-hormonal substances in the tumor tissue, but not all are secreted into the serum. Most recently, a study suggested that elevated serum level of carbohydrate antigen 19-9 (CA 19-9) is a poor prognostic factor in advanced MTC patients and can be used to identify patients with a higher risk of mortality [35]. Chromogranin A, a common marker for other neuroendocrine tumors, is typically only elevated in advanced MTC [36].

Role of Imaging Studies

Postoperative serum calcitonin levels can guide the choice of imaging studies after total thyroidectomy with central compartment neck dissection. A variety of imaging studies are available. When calcitonin is measurable but <150 pg/ml, the best imaging modality is neck ultrasound (US), as the most likely location of recurrence is in the neck. Besides neck ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI) of the neck can be performed to evaluate for the presence of metastatic disease [8].

If imaging studies are negative and the serum calcitonin remains elevated but stable, the patient should be followed by physical examination, serum calcitonin and CEA levels, and cervical US every six months [8]. If imaging studies are positive for abnormal finding, appropriate treatment should be recommended based on metastasis location.

When the serum calcitonin level is >150 pg/ml, this indicates the possibility of distant metastases. Therefore, ultrasound of the neck, contrast-enhanced CT of the neck and chest, contrast-enhanced MRI or three-phase contrast-enhanced CT of the liver, bone scan, and MRI of the pelvis and axial skeleton should be ordered [8]. Among all of the different imaging modalities, 18-fluoro-2-deoxyglucose (FDG) positron emission tomography (PET) has less sensitivity to detect metastases in patients with advanced MTC [37]. The use of PET scans is sometimes considered when the serum calcitonin level is >1000 pg/mL, as the sensitivity of PET scan to diagnose metastases is better with serum calcitonin levels that are above 1000 pg/ml [38]. Again, subsequent treatment needs to be tailored to the location of metastases.

Brain imaging should be obtained in patients with metastatic MTC and neurological symptoms. The prevalence of brain metastases is likely underreported due to the infrequent use of brain imaging in patients with advanced MTC. Apparent brain metastases occur in 1–5 % of patients with MTC [39]. Radionuclide imaging with 111 In-octreotide and 99mTc-dimercaptosuccinic acid can sometimes be useful, especially when conventional imaging studies are negative or inconclusive [40]. For patients with MTC, there is no single imaging study which can provide optimal whole body imaging.

Role of RET Oncogene Testing

MTC can happen sporadically or can be part of a hereditary syndrome such MEN2A, MEN2B, and familial MTC (FMTC). Hereditary MTC

is characterized by mutations in the *RET* proto-oncogene. The *RET* (RE-arranged during Transfection) proto-oncogene is expressed in cells derived from the neural crest, the brachial arches, and the urogenital system [41, 42]. Mutations have been recognized as a poor prognostic factor for the outcome of patients with MTC [43]. Because 1–7 % of patients with presumed sporadic MTC actually have hereditary disease, every patient with newly diagnosed MTC should undergo *RET* oncogene mutation analysis [44, 45]. Furthermore, all patients with MEN2A, MEN2B, and FMTC have *RET* germline mutations. Some *RET* codon mutations, such as *RET* codon M918 T, are associated with more aggressive clinical course [43].

Patients with a documented *RET* germline mutation should have their first-degree relatives offered genetic testing and genetic counseling [8]. Romei et al. [46] demonstrated that *RET* genetic screening is highly specific and sensitive and allows for the reclassification of apparently sporadic cases of MTC as hereditary. This genetic testing also allows for the identification of gene carriers who require follow-up.

Role of Plasma-Free Metanephrines and Calcium Level Measurements

If the patient has a hereditary condition such as MEN2A and MEN2B, additional testing is needed. Pheochromocytoma can occur in both syndromes, but primary hyperparathyroidism only occurs in patients with MEN2A and is almost always multiglandular. Plasma-free metanephrines should be measured in MEN2A and MEN2B, and serum calcium levels should be obtained in patients with MEN2A. If the serum calcium is elevated, intact parathyroid hormone (iPTH) should be ordered. In the revised ATA guidelines [8], MEN2 has become two distinct syndromes: MEN2A with 4 variants (classical MEN2A, MEN2A and cutaneous lichen amyloidosis, MEN2A and Hirschprung's disease and FMTC) and MEN2B, which is more rare [8].

After the diagnosis of MTC, and while waiting for the result of *RET* oncogene mutation, plasma-free metanephrines should be checked. If the patient does not have a *RET* mutation and plasma-free metanephrines are normal, there is no need to check yearly plasma-free metanephrines thereafter. In patients with a *RET* mutation, plasma-free metanephrines should be checked yearly in MEN2A and MEN2B and serum calcium levels should be checked yearly in MEN2A patients with codon 634 mutation, as there is a higher propensity toward primary hyperparathyroidism in patients with this mutation [47]. In other mutations associated with MEN2A, serum calcium can be checked every two years [47]. If pheochromocytoma is diagnosed, adrenalectomy should be performed; cortical-sparing adrenalectomy should be considered, particularly in patients with bilateral pheochromocytomas at the time of diagnosis. If primary hyperparathyroidism is diagnosed, the patient should undergo parathyroidectomy.

Conclusions

The mainstay treatment for MTC is total thyroidectomy with central compartment lymph node dissection. Three to six months postoperatively, serum calcitonin and CEA levels should be checked. Based on these levels, the patient either is cured or has persistent disease. If patients have signs of persistent disease, further imaging studies are required to localize the tumors. Additional treatments can be tailored according to the location of these tumors. Calcitonin and CEA doubling times are important prognostic tools in patients with MTC and should be followed regularly. The majority of MTC is sporadic. However, 1–7 % of patients with an apparently sporadic form have a *RET* oncogene mutation. If a *RET* oncogene mutation is found, first-degree relatives should be tested and offered genetic counseling. In addition, there are additional surveillance tests warranted in patients with positive *RET* mutation or unknown *RET* mutation including checking plasma metanephrines and serum calcium levels. Follow-up of patients with MTC should be

tailored based on serum calcitonin and CEA levels, imaging, and genetic mutations.

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Surgical Management of Recurrent Medullary Thyroid Cancer

19

Jason A. Glenn and Tracy S. Wang

Introduction

Medullary thyroid cancer (MTC) is a relatively rare neuroendocrine cancer of the thyroid, accounting for up to 5 % of all thyroid cancers in the USA. Unlike differentiated thyroid cancers, which arise from the follicular cells, MTC arises from the parafollicular, or C-cells, of the thyroid. As a result, MTC is not sensitive to radioactive iodine, and therefore, the initial surgical resection is critical for obtaining biochemical and anatomic control. The recommended initial surgical resection for patients with sporadic MTC is total thyroidectomy and central compartment lymph node dissection; the extent of lateral compartment lymph node dissection is guided by the clinical detection of metastatic lymphadenopathy, either by physical examination or by ultrasonography, and by the extent of elevation of preoperative basal serum calcitonin and carcinoembryonic antigen (CEA) levels [1].

Postoperative normalization of serum calcitonin and CEA levels is associated with a favorable outcome; patients with a biochemical cure, typically defined as a serum calcitonin level <10 pg/mL, have improved survival rates, with reported 10-year overall survival of near 98 % and overall survival decreases by up to 30 % in patients with recurrent disease [2–7]. After initial surgery, it is recommended that serum calcitonin levels be reevaluated at approximately 3 months, given the half-life of serum calcitonin. The presence of postoperative hypercalcitoninemia represents a significant clinical challenge, as does the timing of any reoperation [8–11]. This chapter will review indications and approaches to reoperation in patients with persistent or recurrent MTC.

CASE PRESENTATION Patient AR is a 54-year-old man who initially underwent surgery for MTC at the age of 49. His initial surgery included total thyroidectomy, central compartment lymph node dissection; his preoperative serum calcitonin was 3400 pg/mL. He had no evidence of distant metastases on preoperative imaging. Final pathology demonstrated a 2.7-cm primary tumor and 6/11 resected lymph nodes had metastatic MTC. Postoperatively, his serum calcitonin levels decreased to 59 pg/mL. On routine postoperative surveillance, he was noted to have a serum calcitonin of 256 pg/mL and a right central compartment lymph node, measuring 1.8 cm in diameter (Fig. 19.1). Fine needle aspiration was consistent with MTC.

J.A. Glenn · T.S. Wang (✉)
Section of Endocrine Surgery, Division of Surgical Oncology, Department of Surgery, Medical College of Wisconsin, Milwaukee, WI, USA
e-mail: tswang@mcw.edu

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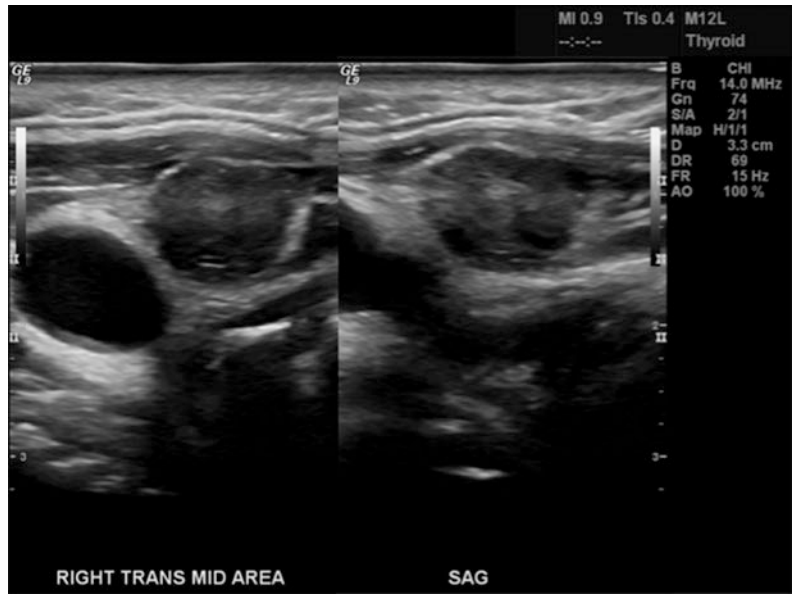

Fig. 19.1 Ultrasound image of an enlarged right central compartment lymph node

Evaluation of Patients Following Initial Surgery for MTC

The strategies for surveillance following initial surgery in patients with MTC have previously been discussed (Chap. 18; Fig. 19.2). Both serum calcitonin and CEA levels may take several months to nadir; previous studies have suggested that serum calcitonin levels may not reach nadir until 3 months postoperatively and that serum CEA levels may take even longer [11, 12]. The postoperative serum calcitonin level which is suggestive of cure is also controversial, with some suggesting that a basal or stimulated serum calcitonin level at or below the limits of the assay is curative, while others suggest that a postoperative stimulated calcitonin level should be <10 pg/mL [12–14]. However, recent studies have suggested that serum calcitonin levels remain detectable in 40–66 % of patients after initial surgery [4–6].

In patients with persistent or recurrent MTC, the degree of elevation of serum calcitonin levels can be used as a guide for the extent of disease, as lymph node metastases have been reported to be present at calcitonin levels of 10–40 pg/mL and serum calcitonin levels of <150 pg/mL suggest that persistent or recurrent disease is limited to the locoregional lymph nodes of the neck [1, 15]. In addition, serum calcitonin and CEA doubling times are typically proportional to the amount of residual tumor burden and may provide insight into the overall growth rate of any persistent or recurrent disease. A recent study by Laure-Giraudet et al. demonstrated that 94 % of patients with serum calcitonin and CEA doubling times of $\leq$24 months had progressive disease, while 86 % of patients with doubling times >24 months had stable disease [16]. Current guidelines suggest that in patients with serum calcitonin levels <150 pg/mL, follow-up can be limited to physical examination and ultrasound of the neck; in contrast, postoperative serum calcitonin levels >150 pg/mL should prompt additional imaging, including computed tomography (CT) of the chest, contrast-enhanced magnetic resonance imaging (MRI) or three-phase contrast-enhanced CT of the liver, and either bone scintigraphy or MRI of the pelvis and axial skeleton [1]. Patients with persistent or recurrent MTC with disease limited to the neck and no evidence of distant metastases are candidates for reoperation.

In contrast, there is limited evidence suggesting that reoperation in patients with persistent

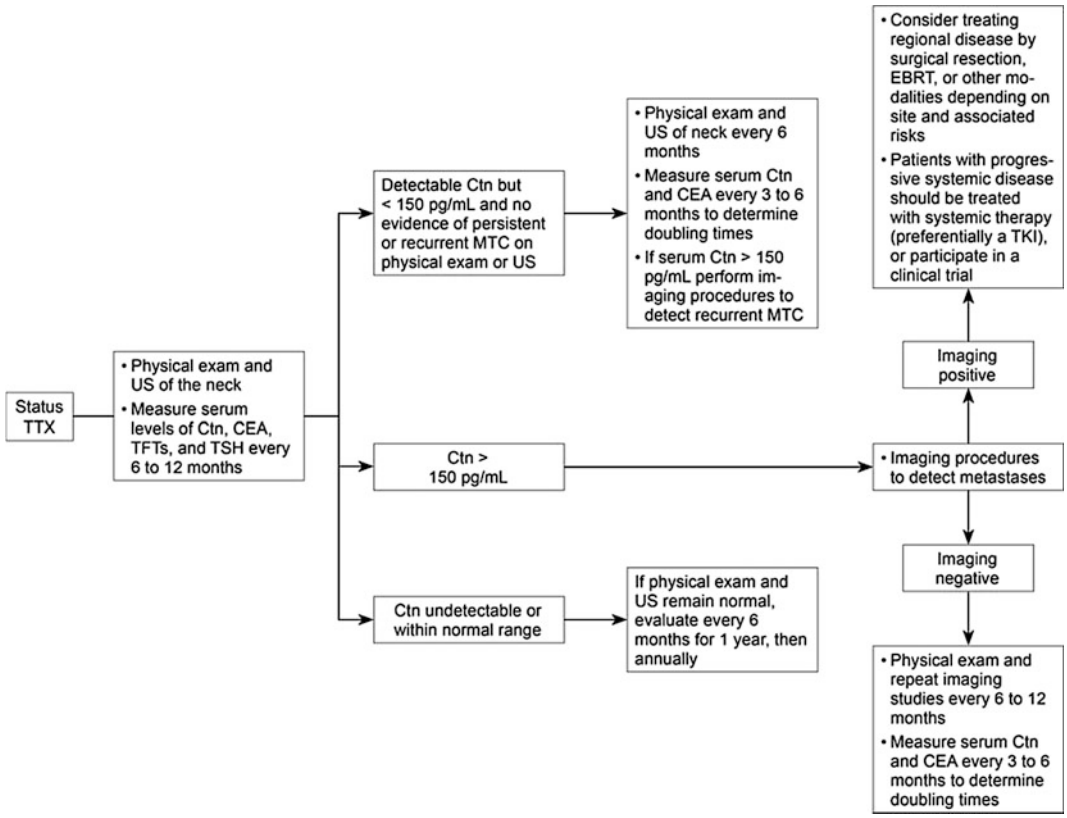

Fig. 19.2 Management of patients following thyroidectomy for persistent or recurrent medullary thyroid cancer. *Ctn* calcitonin; *CEA* carcinoembryonic antigen; *EBRT* external beam radiotherapy; *MTC* medullary thyroid carcinoma; *TFTs* thyroid function tests; *TSH* thyrotropin; *TKI* tyrosine kinase inhibitor; *TTX* total thyroidectomy; *US* ultrasound (Reprinted with permission from [1])

elevation of serum calcitonin levels, but no structural evidence of disease on imaging studies, will benefit from a reoperation. As MTC is often an indolent, slow-growing neoplasm, with prolonged periods of tumor latency, current guidelines suggest that observation may be an acceptable treatment option [1]. This is in part due to evidence that suggests that initial reports of normalization of serum calcitonin levels may overestimate the likelihood of achieving a durable cure [7, 17, 18]. Kebebew et al. reported a cure rate of only 11 % for patients with postoperative hypercalcitoninemia who had negative tumor localization but underwent reoperative neck surgery [17]. Likewise, another study found that the rates of calcitonin normalization might overestimate actual cure rates, because >3 % of MTC patients with initial normalization developed elevated serum calcitonin levels between 1 and 8 years after surgery [9]. A survey of the International Association of Endocrine Surgeons echoed these concerns and found that for MTC patients with postoperative hypercalcitoninemia and negative tumor localization, the majority of surgeons would not recommend reoperation adding to the potential operative morbidity, and it is unclear whether the benefit of removing microscopic (subclinical) disease outweighs the risk of complications associated with reoperation [1, 3, 7, 19].

CASE PRESENTATION: REOPERATIVE PROCEDURE Patient AR underwent a reoperative central compartment neck dissection. The final pathology identified five of seven lymph nodes with metastatic MTC. His

postoperative serum calcitonin levels decreased to 27 pg/mL.

Outcomes Following Reoperation for MTC

While no randomized controlled trials have evaluated the long-term efficacy of reoperation in patients with recurrent MTC, several retrospective series have reported institutional results after reoperation in patients with postoperative hypercalcitoninemia and radiographic evidence of locoregional MTC recurrence. In one single-institution cohort study of 35 patients who underwent reoperative neck dissection for persistent or recurrent MTC, only 4 (14 %) of 29 patients with adequate follow-up had tumor visible on imaging studies; however, the majority (80 %) patients had persistent elevation of serum calcitonin levels [20]. Additional studies have shown that up to 42 % of patients will have a significant reduction in calcitonin levels (a decrease of >35 % from the preoperative level) and up to 38 % of patients had reported normalization of serum calcitonin levels (defined as <10 pg/mL); the findings of these studies are largely dependent on the extent of both the initial surgery and reoperation [10, 20–25].

Given the relatively low rate of biochemical cure following reoperation, careful patient selection is critical to optimize disease-free survival and minimize the risks of procedure-specific complications. Factors previously shown to be associated with long-term eradication of disease following reoperation include a preoperative serum calcitonin <500 pg/mL, age, TNM stage, number of nodal metastases, number of compartments involved, and mediastinal involvement [1, 2, 9, 10]. When appropriate reoperative neck dissection is performed, long-term outcomes can be excellent (reported 10-year disease-free and overall survival rates of 78 and 94 %, respectively); however, due to prolonged tumor latency and an overall lack of published data, the true impact of reoperation on long-term survival remains less clear [1, 26].

A recent meta-analysis was performed of 27 studies and 984 patients who underwent reoperative surgery for MTC, in order to determine the rate of biochemical cure, defined as a normalization of serum calcitonin levels, and further examined based on surgical approach (compartment-oriented vs. non-compartment-oriented) [7]. Overall, reoperation resulted in normalization of calcitonin levels in 0–38 % of patients, while pooled proportion analysis demonstrated normalization of calcitonin levels in 16.2 % of patients (95 % confidence interval [CI] 14.0–18.5 %; Fig. 19.3). When stratified by operative procedure, patients who underwent a compartment-oriented procedure had a higher pooled proportion of calcitonin normalization (18.6 %; 95 % CI 15.9–21.3) than patients who underwent non-compartment-oriented procedures (10.5 %; 95 % CI 6.4–14.7 %). These data suggest that that a compartment-oriented approach to reoperative surgery in patients with MTC offers the best chance for achieving biochemical cure [7].

Role of Palliative Lymphadenectomy in Patients with Persistent or Recurrent MTC

Patients with metastatic MTC often live for years due to the indolent nature of the disease; however, many develop symptoms related to tumor growth or invasion. In comparison with compartment-oriented neck dissections performed for potential cure, palliative resections are often more selective and aimed at reducing symptom-specific tumor burden, thus promoting symptom-free survival and quality of life. This often involves debulking of cervical masses, mediastinal dissections of pre-/paratracheal tumor deposits, or resections of tumors threatening the superior vena cava or the brachial plexus at the thoracic inlet. In general, palliative resection of symptomatic or threatening lesions has been shown to provide long-term relief of symptoms (>8 years in some studies), with minimal operative morbidity in experienced hands; however, reoperation for brachial plexus pain provided only short-term palliation in one retrospective institutional review [23, 27]. Current

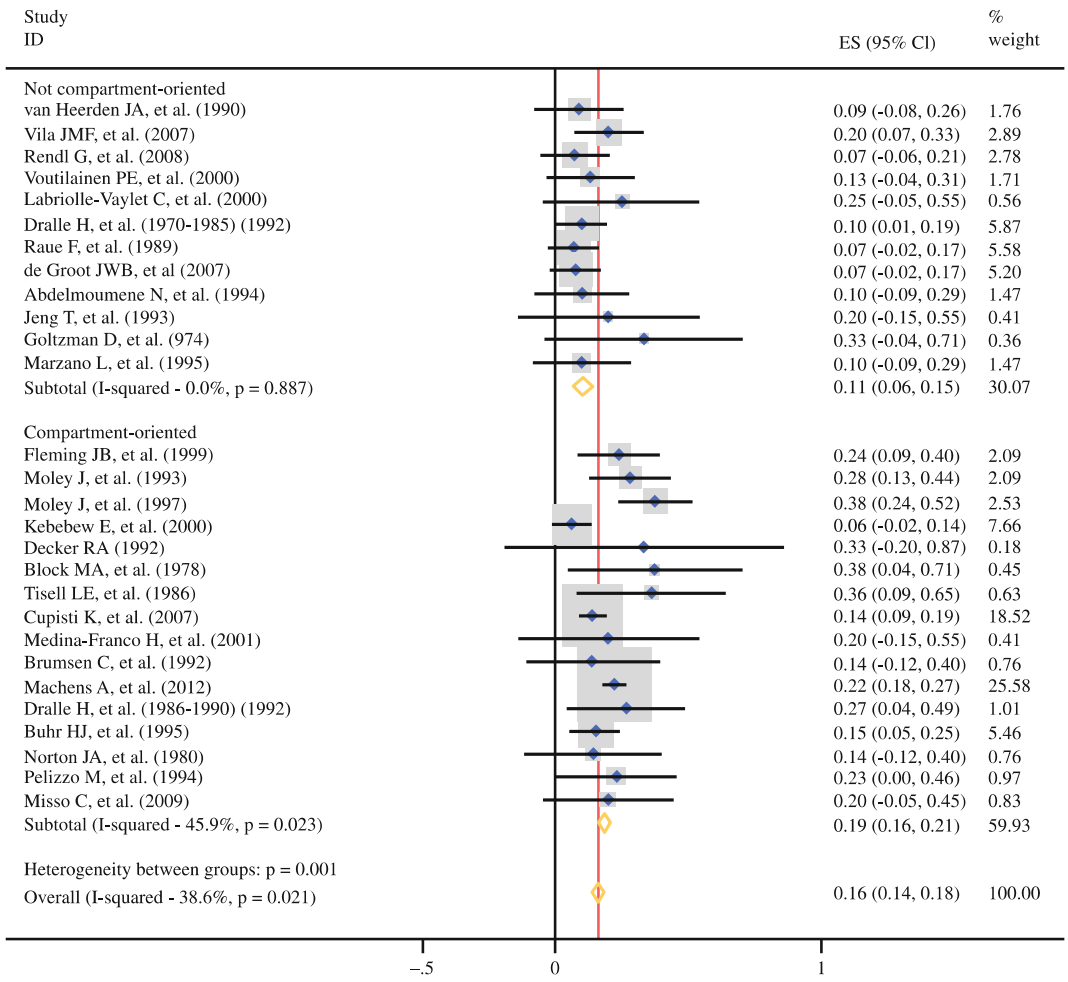

Fig. 19.3 Proportional meta-analysis of calcitonin normalization after reoperative surgery for MTC, stratified by non-compartment-oriented or compartment-oriented procedure (Reprinted with permission from [7])

guidelines suggest, based on expert opinion, that palliative surgery, secondary to space-occupying lesions causing symptoms of mechanical compression (dysphagia, coughing, and dyspnea), pain, or signs and symptoms of hormonal excess should be considered [1].

Procedural Considerations

For reoperative cervical lymphadenectomy, multiple technical factors (i.e., scarring, edema, tissue friability, and neovascularization) often make access to the central compartment difficult, impairing the identification of anatomic landmarks, decreasing tissue compliance/retraction, increasing the amount of tissue manipulation required, and potentiating the amount of subsequent bleeding. Varying degrees of prior dissection and the extent of any new tumor burden make the operative plan for each reoperation unique and difficult to anticipate prior to direct intraoperative visualization. Therefore, in addition to a comprehensive preoperative imaging evaluation, it is very important to review prior operative notes and assess vocal cord function prior to attempting reoperation. Literature on the optimal surgical approach to the reoperative neck

has largely focused on cohorts of well-differentiated thyroid cancer patients, and data regarding MTC reoperative strategy are currently lacking; however, the goals of surgery remain the same. These include (1) maximum exposure of the operative field, (2) meticulous compartment-oriented microdissection, (3) identification of the recurrent laryngeal nerve (RLN) away from areas of prior dissection, and (4) preservation of residual parathyroid tissue (ex vivo if necessary) [28–30].

Several operative approaches to the reoperative neck have been described; however, no studies have demonstrated an improved efficacy/safety of one approach over another. The lateral (back door) approach is considered by many surgeons to be one of the safest approaches, as it gains access to a presumably undissected tissue plane. In this approach, the neck is entered between the anterior border of the sternocleidomastoid and the strap muscles. The dissection is carried down along the carotid to below the level of the sternal notch; the omohyoid muscle can be transected to better expose this region. Here, with gentle retraction of the carotid artery, the RLN can be identified in the paratracheal soft tissue between the carotid artery and the tracheoesophageal groove in the upper mediastinum.

In the low anterior approach, the neck is entered in a similar fashion to the primary operation. The strap muscles are separated down to the sternal notch, and the dissection is carried out in the inferior paratracheal regions, where the RLN can be identified more easily [28–30]. Alternatively, in the anterior superior approach, the RLN is identified as it enters the larynx, and then is carefully traced inferiorly into the thyroid bed. For optimal exposure in any of these approaches, the strap muscles can be transected at their sternal attachments and then bluntly separated off the floor of the thyroid bed and larynx; resection of the strap muscles can typically be performed without significant adverse effects to functional outcome; however, cosmesis remains a concern. Having identified the RLN in the upper mediastinum, the thymus and fibroadipose tissues in this region can be bluntly retracted upward; dividing the fascia above the innominate vessels facilitates this maneuver.

During reoperative procedures, microdissection techniques are essential to preserve critical structures and excise residual disease. Tisell et al. [25] first described this technique in 1986, reporting improved results with a comprehensive compartment-oriented microdissection of all fibroadipose tissue. However, despite meticulous dissection, the majority of patients do not experience calcitonin normalization following reoperation. Due to the perceived risk of complications associated with extensive dissection of a scarred surgical field, some surgeons prefer a more selective approach to reoperative lymphadenectomy, or so-called berry picking. On the other hand, a recent meta-analysis evaluating reoperations for MTC assessed the outcomes of 984 patients from 27 retrospective studies. While the compartment-oriented approach was associated with slightly higher rates of thoracic duct injury (2.8 vs. 1.9 %) and RLN injury (5.7 vs. 1.9 %), it was also associated with lower rates of permanent hypoparathyroidism (1.0 vs. 5.7 %) and higher rates of biochemical cure (18.6 vs. 10.5 %) [7]. Additionally, several recent studies have demonstrated that reoperation may infer no additional morbidity when performed by an experienced surgeon [31, 32]. This suggests that when particular care is taken to minimize operative morbidity, compartment-oriented microdissection may offer the greatest potential for biochemical cure.

As mentioned previously, the most important strategy to preserve the RLN involves identification of the nerve either high or low in the neck in an undissected surgical plane. Once identified, dissection along the RLN can proceed to the level of the inferior thyroid artery [33]. Many surgeons routinely utilize intraoperative neural monitoring in an attempt to assess nerve functional integrity; this is particularly important in reoperative procedures, as the location of the nerve is not always constant and is commonly associated with dense scar tissue in the central neck. Another important consideration of reoperative cervical lymphadenectomy is identification and preservation of the external branch of the superior laryngeal nerve, as injury can

adversely affect the functional outcome. This is sometimes quite difficult, as the superior thyroid vessels are typically ligated during the procedure and previous dissection of the upper pole of the thyroid may result in significant scarring. As such, excision of fibroadipose and residual pyramidal lobe tissue in this region should be performed cautiously.

Preservation of the inferior thyroid artery is recommended to prevent devascularization of the parathyroid glands; the superior glands are left in situ whenever possible. The inferior glands are commonly removed with the lymphadenectomy specimen; therefore, careful examination for parathyroid tissue in the specimen should be performed on the back table. As fibrosis can change the color and morphology of the parathyroid glands, making identification difficult, aspiration for PTH or biopsy with frozen section should be performed to confirm suspected parathyroid tissue. Multiple frozen sections of the surrounding adipose tissue should be performed to rule out the presence of malignancy prior to parathyroid reimplantation into the sternocleidomastoid or non-dominant forearm muscle.

Reoperative LND often poses a unique technical challenge, as scarring from previous procedures can alter normal anatomic relationships, particularly with respect to the spinal accessory nerve and the small branches of the thoracic duct low in level IV and V. Ideally, reoperative LND should follow the same tenants as the primary LND. This includes the compartmental clearance of fibroadipose tissue from nodal levels II, III, IV, and V, with preservation of non-lymphatic structures and minimization of operative morbidity; however, due to varying degrees of fibrosis and invasion, it is not always feasible to preserve these structures. As such, the 2015 Revised ATA Guidelines recommend thorough compartment-oriented dissection of the lateral neck, unless there has been extensive prior dissection of a compartment, in which case a more selective dissection may be appropriate [1, 15]. Highly sensitive cervical imaging often identifies subcentimeter lymph node metastases that can be challenging for surgeons to resect in their entirety, especially in a reoperative neck compartment. As such, many surgeons have chosen ≥ 1 cm as an arbitrary cutoff for the appropriate size to consider reoperation. However, the 2015 ATA Guidelines recommend reoperative neck dissection for image or biopsy-positive disease in the central or lateral neck compartments, without recommended size criteria [1].

CASE PRESENTATION: FOLLOW-UP After reoperative surgery, patient AR underwent continued surveillance with serum calcitonin and CEA levels. He subsequently had an increase in serum calcitonin to 571 pg/mL, and CEA remained undetectable, one year after reoperative surgery. He was found to have metastases in the vertebral spine metastases and left femur. He continued, over the next three years, to have a slow increase in serum calcitonin levels (peak: 7677 pg/mL), with no increase in the number or size of skeletal metastases. He has been treated with systemic tyrosine kinase inhibitors.

Conclusions

The value of calcitonin as a serum marker for MTC recurrence is clear. However, its ability to detect microscopic subclinical disease poses a unique clinical challenge. In the absence of distant metastases, reoperative cervical lymphadenectomy should be considered for patients with clinical, radiographic, and/or cytologic evidence of locoregional recurrence. If no anatomic disease is demonstrated on imaging, patients may not benefit from reoperation. Palliative lymphadenectomy may be considered if there is imminent threat to vital cervical structures or if there are compressive symptoms from bulky nodal disease. Efforts should be made to ensure maximum exposure of the operative field, meticulous compartment-oriented microdissection, identification of the RLN away from areas of prior dissection, and preservation of residual parathyroid tissue. When performed by an experienced surgeon, adherence to these principles will minimize operative morbidity. However, despite meticulous dissection, only about

one-third of patients experience a durable eradication of disease; therefore, the decision to perform reoperative neck surgery in patients with persistent or recurrent MTC should be made to consult with the multidisciplinary team in the context of the overarching goals of treatment.

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