C-Cells and their Associated Lesions and Conditions: A Pathologists Perspective

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ABSTRACT

This paper updates the histopathology and cytopathology of thyroid tumors and proliferations derived from the para-follicular or C cells. Beginning with an historical overview, including the recognition of medullary thyroid carcinoma as a distinct histologic entity, its relationship to the hormone, calcitonin, (which was discovered in the same decade) and to thyroid C cells, medullary carcinoma and its variants are reviewed. The molecular biology of the tumors and the associated mutations in the tumors (somatic mutations) are discussed. Additionally the genetic features (germline mutations) including familial clusters and associations with other endocrine and neuroendocrine lesions are reviewed. Screening for the tumor and its precursors is included with a review of the latest American Thyroid Association guidelines for treatment as well as timing and approach to surgery. Tabular data of specific germline mutations and their relationships to tumor virulence, and prognosis are illustrated. Precursor and early C cell lesions such as C-cell hyperplasia and micro-medullary carcinoma are discussed. Difficulties and controversies in the definition of C-cell proliferations which are neoplastic and those which are “reactive” are reviewed. The entity of medullary microcarcinoma or medullary microcarcinoma is illustrated and the distinction between C cell nodules and microcarcinoma is defined using the latest available criteria. Finally the latest approved chemotherapeutic agents and their results in metastatic medullary thyroid carcinoma are included.

Key Words: Medullary thyroid carcinoma, Thyroid cancer, RET, Genetics, Tyrosine kinase inhibitors

INTRODUCTION

The definition and description of medullary thyroid carcinoma is only about half a century old but the importance of this tumor in terms of its recognition for diagnosis, and its separation from follicular derived thyroid tumors has engendered a massive literature. Medullary carcinoma was one of the earliest human malignancies to be associated with a specific tumor marker, calcitonin, which became critically important molecule because it could be used diagnostically and prognostically in patients with this tumor.

The syndromes associated with medullary carcinoma including multiple endocrine neoplasia types IIA, IIB (or III) and familial medullary carcinoma which described early the knowledge in understanding of the tumor itself. The genetic implications were also recognized quickly and led to the development of readily available and reliable genetic testing in the clinical setting. Finding germline mutations in ret proto-oncogene allowed for early diagnosis and intervention before the development of the tumors or when they manifested at very early stage.

The recognition of precursor lesions such as neoplastic C-cell hyperplasia (both diffuse and nodular) and the earliest manifestation of the tumor, that is, micro-medullary carcinoma in prophylactic thyroidectomy has led to the recognition of these entities by histopathologists in thyroid resections and apparently sporadic cases of medullary carcinoma.

The wide variety of patterns which medullary carcinoma can assume, offers a challenge to histopathologists and even more diagnostic difficulty to the cytopathologists. The current authors have noted numerous examples in their consultative practice of thyroid aspiration biopsies of medullary carcinoma, which have been diagnosed as lesions of follicular origin, follicular neoplasms, or follicular lesions of uncertain significance (FLUS). It is likely that the relative rarity of medullary carcinoma of the thyroid complicates the evaluation of thyroid FNAs in the setting of increased frequency of sporadic thyroid nodules of follicular origin.

From the molecular pathology point of view, medullary carcinoma and its related lesions were the first thyroid neoplasms and pre-neoplasms to be extensively studied. This resulted in a) the recognition of genetically determined neoplasms and pre-neoplasms, b) the testing of relatives of patients with apparent sporadic medullary carcinoma leading to the discovery of families with the disease, c) the development of screening tests leading to prophylactic thyroidectomy and d) analytical studies of numerous
families with various germline mutations allowing for tabulated recommended timing (age) for surgical intervention in as yet unaffected relatives of patients with this tumor.

This review will focus on various aspects of medullary thyroid carcinoma and its precursor lesions predominantly from the cytopathology and histopathology view points; the known implications of molecular (genetic) testing in terms of prognosis will be discussed; the difficult area of frank C-cell hyperplasia as compared to borderline lesions will be also reviewed including a brief discussion of so-called reactive C-cell hyperplasia.

HISTORICAL PERSPECTIVE

The hormone, calcitonin, was discovered and characterized in the early 1960s; the thyroid C cell had been described in many animal species by the end of the 19th and early 20th centuries. Nonidez reported the presence of argyrophilic cytoplasmic granules C-cells and also remarked on their para-follicular location; he also hypothesized that these cells may have endocrine function (1). The term “calcitonin” was first coined by Copp and Cheney in as a substance of parathyroid origin as it was responsible for lowering serum calcium levels (2). The thyroid origin of calcitonin was first confirmed by Hirsch in 1963 and Foster in 1964 (3,4). The term “C-cells” was introduced by Pearse and later on with Bussolati demonstrated the presence of calcitonin in these cells by immunofluorescence. Interestingly, parallel to all these discoveries (5,6) in the early 1960s, pathologists were defining the morphology of the tumor now known as medullary thyroid carcinoma (MTC) (7,8). In 1966, Williams proposed that MTC might be derived from the C cell and predicted that if the C cell was the source of calcitonin, the tumors might also produce this hormone (9). Meyer and Abdel-Bari in 1968 demonstrated calcitonin-like activity in extract derived from medullary thyroid;(10) this observation was later confirmed by Bussolati and colleagues by immunofluorescence techniques (11).

The C cells are derived embryologically from the neural crest and migrate into the thyroid along with the ultimobranchial body. Hence, these two elements may be closely associated in the adult thyroid; however, the ultimobranchial body itself does not show calcitonin immunoreactivity and should not be considered as hyperplastic C cells (12,13). In humans, C cells are found along the lateral aspects of the thyroid lobes in the upper two-thirds of the gland. The C cells comprise less than 0.1% of the thyroid mass in humans (14,15). Wolfe and colleagues showed C-cell distribution in the thyroid by immunohistochemistry. The number of C cells in the thyroid differs according to age. They identified larger numbers of these cells in infants and children under the age of 6 than in adults. In children, groups of up to six cells can be seen, with as many as 100 cells noted in a low-power microscopic field. In adult glands no more than 10 cells should be found in a low-power field (14,15).

Clusters of C cells in adults have been described in endocrinologically normal adults. O’Toole and colleagues examined thyroid glands from forensic autopsies and recognized a trend toward increased numbers of these cells in older individuals (over age 60); however, they noted large standard deviations (16). This remains a problem area for pathologists (17).

MEDULLARY THYROID CARCINOMA

Medullary thyroid carcinoma is rare and comprises fewer than 10% of all thyroid malignancies (18-20). This tumor is of great diagnostic importance because of its aggressiveness, its close association with multiple endocrine neoplasia syndromes MEN type 2A (MEN IIA or Sipple’s syndrome); type 2B (MEN IIb, MEN III or mucosal neuroma syndrome) or familial non-MEN MTC (FMTC), and a relationship to C-cell hyperplasia as probable precursor lesion (20-22). While the majority of medullary carcinomas are sporadic, up to 25% are familial (23,24).

In 1985, Masahide Takahashi transfected NIH 3T3 fibroblast cells with sonicated human lymphoma DNA segments, which resulted in transformation of these cells (25). Subsequent studies demonstrated that this new biologically active gene was generated by recombination of two separate normal DNA segments. The name “RET” means “REarranged during Transfection” represents the transforming gene product found in Takahashi’s experiment (25). The proto-oncogene RET is composed of 21 exons located on chromosome 10 (10q11.1) and encodes for a transmembrane receptor tyrosine kinase for members of the glial cell line-derived neurotrophic factor family (GDNF) and associated ligands nurturing, artreminin, and persephin. RET is involved in a number of cellular signaling pathways during development regulating the survival, proliferation, differentiation, and migration of the enteric nervous system progenitor cells, as well as survival and regeneration of neural and kidney cells (26-28).

The genetic linkage analysis performed in 1987 by two separate investigators mapped the MEN2A locus to a region on chromosome 10 (29,30). In 1990 increased expression of the RET gene was found in both familial and sporadic human MTC and pheochromocytomas by Santoro and colleagues (31). Based on mapping of the RET
gene on chromosome 10 these investigators also suggested that this region of chromosome 10 might be involved in the proliferation and differentiation of neuroectodermal tissues (31). At present it is well known that virtually all patients with familial MTC have germline RET mutations, whereas up to 75% of patients with sporadic MTC have somatic RET mutations, a feature of clinical aggressiveness (23,32-35).

**CLINICAL PRESENTATION & GENETICS**

The clinical features are similar in both sporadic and familial cases that are symptomatic (18,20,32,36). Medullary carcinoma can affect patients of any age; however, most affected individuals are adults with an average age of about 50 years (18,19). In familial cases, though, children can be affected; also in these instances the age of diagnosis tends to be younger (mean age: about 20 years) (24). Although sporadic medullary carcinomas are seen more commonly in women, familial cases have an equal sex ratio, since an autosomal-dominant mode of inheritance is present (20,24,37-39).

Most patients with medullary carcinoma will present with a painless and firm thyroid nodule. In up to 50% of cases, obvious nodal metastases will be present at the time diagnosis. Distant metastases to lung, bone, or liver may also be noted initially in about 15% to 25% of cases (40,41). When the tumor produces excess hormone other than calcitonin, the presenting symptoms may be related to that hormone hypersecretion (adrenocorticotropic hormone [ACTH], prostaglandin)(42-45).

In the familial forms there are associated endocrine or neuroendocrine lesions (Table I). To date over 100 mutations (single or multiple), duplications, insertions or deletions involving RET oncogene have been identified in patients with hereditary MTC. Mutations in RET in families with MEN-2A (95% of families) and FMTC (85% of families) have been seen in one of the five-cysteine codons in exon 10 and exon 11 (19,35,46-51).

**Sipple syndrome** (MEN type II or IIA) also known as “Classical MEN2A” consists of medullary thyroid cancer and C-cell hyperplasia, adrenal pheochromocytoma and adrenal medullary hyperplasia, and parathyroid hyperplasia (52,53). Although most affected patients will have the complete syndrome, not every patient will manifest each of these lesions. Even in families with the complete syndrome, parathyroid lesions affect only 16-25% of patients. In MEN-2A RET-mutations usually occur in codons 609, 618, or 620 of exon 10 or codon 634 of exon 11 (19,34,55). The latter is identified frequently (>60%); and has been

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**Table I**: Phenotypic/clinical characteristics of multiple endocrine neoplasia (MEN) type-2 variants
associated with the presence of pheochromocytoma and hyperparathyroidism and rarely cutaneous lichen amyloidosis (56-61). Patients with MEN2a and a unilateral pheochromocytoma usually develop contralateral adrenal lesion within 10-years. RET-germline mutations are present in 50% of patients with Hirschprung’s Disease (HD); which can be seen in approximately 7% of patients with MEN2A. The HD usually manifests shortly after birth; it is important to exclude HD in older MEN2A patients presenting with colonic symptoms. It has been shown that a variety of phenotypic expressions in MEN-2A families can be seen with the same RET mutations (57,59,62). Mutations in specific codons have been correlated with clinical behavior and symptomatology in some families (59,61,63-65).

**MEN type IIB** consists of medullary thyroid carcinoma and C-cell hyperplasia, pheochromocytoma and adrenal medullary hyperplasia, mucosal neuromas, gastrointestinal ganglioneuromas, and musculoskeletal abnormalities (33,60,66-70). These patients may have familial disease (over 50% do); some cases arise apparently as spontaneous mutations. A point mutation at codon M918T (exon 16) has been noted in 95% of cases with MEN-2B (71-73). The other rare mutations reported in MEN2B patients include genotype A883F (exon 15), double mutation V804M/Y806C at codon 804 (exon 14) and 806 in the same allele (72,74-76). Mutations in codons 918 and 883 are associated with MTC presenting at younger age with high risk of mortality and disease-specific mortality (72,77,78). Because MTC is generally considered a lethal tumor, it is recommended that first degree relatives of the affected family member should undergo genetic screen; a positive result should be followed be prophylactic total thyroidectomy (24,79-82). Most endocrinologists and pediatric endocrinologists recommend total thyroidectomy before the age of six in children found to have “aggressive” RET mutations in their germline (19,24,79,83). MEN IIB shows similarity to von Recklinghausen disease since in neurofibromatosis similar lesions are found in the gastrointestinal tract, and pheochromocytomas are common. Nerve growth factor has been identified in some medullary carcinomas of these patients; it has been postulated that this product of the tumor may be responsible for the neural lesions seen in patients with MEN type IIB (84). However, the neural lesions often precede the development of medullary cancer by many years.

**Familial medullary thyroid carcinoma** is characterized by the presence of a RET germline mutation in families with MTC, or in a patient with MTC without known family history of MTC. These both scenarios of MTC occurrence are unassociated with neither pheochromocytoma nor hyperparathyroidism (20,24,85,86).

**Sporadic medullary thyroid carcinomas** can also demonstrate RET mutations; these are usually limited to the tumor cells and rarely in germline (indeed if found in the latter, the possibility of familial disease needs to be considered) (87). In sporadic MTC, somatic mutations have been seen in exon 16 of the RET (M918T) in 11-60% of cases; this variability shows a strong correlation with tumor size. The M918T mutations in sporadic MTC are associated with aggressive clinical course and prognosis. The rare somatic mutations in sporadic MTC are seen in codon 618, 603, 634, 768, 804, 883 and partial deletion of RET gene (77,87-93). It has been shown that 80% of sporadic MTC lacking somatic RET mutations have mutations in RAS oncogene (HRAS, KRAS, or rarely NRAS) (24,94-97).

The pathologist may contribute to the determination of familial rather than sporadic disease if, upon examining a medullary carcinoma of the thyroid, he or she notes multifocal or bilateral tumors and the presence of C-cell hyperplasia (18,20,36).

**PATHOLOGY**

Medullary carcinoma is usually located in the area of highest C-cell concentration (i.e., the lateral upper two-thirds of the gland). In familial cases, multiple small nodules may be detected grossly, and rarely, lesions may be found in the isthmus. The tumors range in size from barely visible to several centimeters. Many medullary carcinomas are grossly circumscribed but some will show infiltrative borders. Gross necrosis and hemorrhage can also be seen (18,20,22,36,98).

The typical medullary carcinoma may be microscopically circumscribed or more likely will be freely infiltrating into the surrounding thyroid. The pattern of growth is of tumor cells arranged in nests separated by varying amounts of stroma. The tumor nests are composed of round, oval, or spindle-shaped cells; there often is isolated cellular pleomorphism or even multinucleated cells. Figure 1,2. The nuclei are uniform, demonstrate characteristic neuroendocrine stippled chromatin with low nuclear/cytoplasmic ratio (Figure 3). Intranuclear cytoplasmic inclusions are commonly noted. Mitoses can be seen. The tumor stroma characteristically contains amyloid although this is not necessary for the diagnosis; about 25% of medullary carcinomas do not contain amyloid (Figure 4,5). The amyloid is most likely derived from procalcitonin and, indeed, immunohistochemical stains for calcitonin often stain the amyloid (Figure 6). Calcifications in areas of amyloid deposition are characteristically present. The tumors commonly invade lymphatics and veins (18,36).
Several medullary carcinoma variants have been described. In the papillary variant, a papillary or pseudopapillary growth pattern is identified. The pseudopapillary variant is more common and probably results from fixation artifact (99,100). The true papillary variant is extremely rare and needs to be differentiated from typical papillary thyroid carcinoma; nuclear morphology is the most important distinguishing feature (101-103). The follicular variant (Figure 7) is characterized by the presence of follicles, glands, or tubules (99,104). Care must be rendered to determine that the follicular structures are not just entrapped normal thyroid within the lesion (99,105). Some medullary carcinomas are grossly and microscopically encapsulated (106,107). Mendelsohn and Oertel reported a series of encapsulated thyroid lesions classified as atypical adenomas and showed that many of these were medullary cancers containing immunoreactive calcitonin. The follow-up in encapsulated medullary carcinomas indicates that they have a more benign prognosis than usual medullary tumors (108). The histologic differential diagnosis for the encapsulated variant includes hyalinizing trabecular adenoma. Immunohistochemistry for calcitonin will be positive in the medullary carcinoma, but not within the hyalinizing trabecular adenoma. In the past, some authors have used the term C-cell adenoma to describe encapsulated variants of medullary carcinoma; however,
this terminology is not favored (109). The small cell variant of medullary carcinoma has also been described (110). These tumors look like pulmonary small cell carcinoma from which they need to be distinguished, if possible. The prognosis is worse than for typical medullary carcinoma. While calcitonin expression may not always be seen, the small cell variant of medullary carcinoma often expresses CEA and calcitonin gene-related peptide just as other types of medullary carcinoma do (110). The giant cell variant is rare and is characterized by large atypical cells admixed with areas of typical medullary carcinoma (111). Because of the presence of large atypical cells, this variant needs to be differentiated from anaplastic thyroid carcinoma, a tumor with a worse prognosis when compared to medullary carcinoma. The clear cell variant is a rare form of medullary carcinoma and is characterized by cells with abundant clear cytoplasm (112). Immunohistochemical stains reveal the presence of calcitonin in the lesional cells. Differential diagnostic consideration for this variant includes follicular-derived neoplasms with clear cell cytoplasm as well as metastatic renal cell carcinoma. Other variants of medullary carcinoma include oncocytic (Figure 8) and squamous variants (113). Immunohistochemical stains are often needed to establish the correct diagnosis.

Up to 40% of medullary carcinomas contain mucin, most of which is extracellular, intracytoplasmic mucin can be seen in about 15% of medullary carcinomas (114). Rare tumor may contain melanin pigment, the significance of which is not

Figure 5: Round to oval spindle cells in medullary carcinoma with typical neuroendocrine nuclear chromatin and amyloid deposition (H&E; 40x).

Figure 6: Calcitonin stain highlighting the tumor cells and amyloid deposits (due to procalcitonin) in medullary carcinoma (H&E; 40x).

Figure 7: Pseudo-follicular or acinar/glandular growth pattern in medullary carcinoma (H&E; 40x).

Figure 8: Tumor cells with abundant oncocytic cytoplasm in a case of medullary carcinoma (H&E; 40x).
known (115,116). By immunohistochemistry, the majority of medullary carcinomas express low-molecular-weight cytokeratin, calcitonin, calcitonin gene-related peptide and TTF-1 (117) (Figure 9,10). In addition, many tumors express CEA, which may also be elevated in the serum (118). A variety of other peptides may be found in tumor cells including somatostatin, vasoactive intestinal peptide, and synaptophysin (119,120). Some studies have also identified polysialic acid (neural cell adhesion molecule) in medullary carcinomas but not in other thyroid tumors (121).

Occasional lesions (and often these are small-cell type) do not contain immunoreactive calcitonin (110,122). In order to accept a calcitonin-free tumor of the thyroid as a medullary carcinoma, it should arise in a familial setting or occur in a thyroid with unequivocal C-cell hyperplasia. Immunoreactivity for calcitonin gene-related peptide would add proof to the histogenetic nature of such a lesion. The existence of a true small cell nonmedullary neuroendocrine tumor of the thyroid is accepted.

**MIXED FOLLICULAR AND MEDULLARY CARCINOMA**

These controversial tumors show thyroglobulin and calcitonin immunoreactivity and ultrastructural evidence of differentiation along two cell lines (123-125) (Figure 11-14). Some of the series of these tumors may have been confusing, with trapping of follicles at the invading edge of the medullary carcinoma and diffusion of thyroglobulin into the medullary carcinoma; this may result in diagnosis of mixed tumors showing immunostaining for both hormones (126-129). Caution should be taken for making the diagnosis of mixed medullary and follicular-derived carcinomas.

**CYTOLOGIC DIAGNOSIS OF MEDULLARY THYROID CARCINOMA**

Medullary thyroid carcinoma (usually sporadic) can be detected during routine radiologic evaluation of thyroid gland either due to multiple nodule or screening of other lesions in the neck. The diagnosis of MTC can be made preoperatively by fine-needle aspiration biopsy (FNAB) (130-133).

By ultrasound MTC are solid, ovoid to round, hypoechoic lesions, which lack the “halo” sign, commonly encountered in follicular lesions. Up to 50% of medullary thyroid carcinoma demonstrate bright echogenic foci; indicative of deposits of calcium surrounded by amyloid. Cystic change is unusual for medullary thyroid carcinoma (134,135).

FNAB specimens of medullary are often cellular and frequently show a heterogeneous morphology as seen in surgical pathology specimens. The tumor cells are arranged in either small cell groups, tissue fragments or as single cells. (Figure 15,16) The tissue fragments are usually of small size; papillary and follicular architecture has been described in aspirates of medullary carcinoma. In some cases the cells may be arranged in small cords and nests. Usually the lesional cells are either round to oval or spindle shaped (Figure 15-18). The cytoplasm is usually granular and up to 20% of cases show prominent eosinophilic granules in Romanowsky stained preparations; these occur in 5-10% of the neoplastic cells and contain calcitonin (Figure 17A,B). Interestingly, these granules are less prominent to absent in Papanicolaou stained preparations. The nuclei of the medullary carcinoma demonstrates coarse clumping of the nuclear chromatin with inconspicuous nucleoli; typical of

**Figure 9:** Calcitonin immunostain demonstrating strong and diffuse expression in medullary carcinoma (Immunoperoxidase, 20x).

**Figure 10:** TTF-1 immunostain highlighting the nuclei of medullary carcinoma; this expression can be variable and can range from focal to diffuse (Immunoperoxidase; 10x).
Figure 11 and 12: A case of mixed follicular variant of papillary thyroid carcinoma and medullary carcinoma demonstrating dual growth pattern i.e. follicular and nesting (H&E; 10x & 20x).

Figure 13 and 14: Calcitonin immunostaining highlighting the medullary component (Figure 13) and thyroglobulin highlighting the follicular variant of papillary thyroid carcinoma (Figure 14) (immunoperoxidase, 20x).

Figure 15 and 16: A fine-needle aspiration specimen of medullary thyroid carcinoma demonstrating cellular specimen consisting of round to oval tumor cells on both Diff-Quik® (Figure 15, 20x) and Papanicolaou (Figure 16, 20x).
neuroendocrine tumors (Figure 18,19). Marked nuclear pleomorphism is not common; however, when present the cases are indistinguishable from aspirates of anaplastic thyroid carcinoma (Figure 19). Cytoplasmic intranuclear inclusions can also be seen in aspirates of medullary thyroid carcinoma (130,132,136-141).

In cases where a majority of the cells are round to oval in shape, the nucleus is usually situated eccentrically giving rise to a plasmacytoid appearance to tumor cells (Figure 17A,B). In cases with the prominent round cell pattern the tissue fragments are rare. Some cases of medullary carcinoma may only contain spindle cells and appears similar to a tumor of mesenchymal origin. These aspirates usually show cohesive groups of spindle cells with nucleus in eccentric location and occupying most of the cell. The cytoplasm of spindle shaped tumor cells is scant and stretched out in thin processes (Figure 18, 20). Medullary carcinoma mimicking small cell carcinoma has been reported in the literature. Intranuclear grooves and inclusions can also be encountered in these cases (136,142). Rarely, medullary carcinoma may also show intracytoplasmic mucin.

Amyloid can be seen in aspirates of medullary thyroid carcinoma as acellular material (easily highlighted with Romanowsky stain) in the form of strings, irregular shaped
or as round to oval fragments. It can be seen either with or without closely associated tumor cells (Figure 15). It can be difficult to distinguish amyloid from thick colloid; which is often encountered in neoplastic follicular proliferations. Amyloid can be highlighted with special stains (Congo-red) in cell block preparations (143,144).

The cytologic impression can be confirmed by performing immunostains for calcitonin, (130) however, in some cases this may not be feasible due to scant specimens. In such cases it is prudent to recommend serum calcitonin levels to establish the diagnosis of medullary carcinoma (138,144,145). Some authors have also suggested measuring calcitonin levels in the FNAB rinse to diagnose primary and recurrent/metastatic MTC as an adjunct to cytomorphology (146,147).

**C-CELL HYPERPLASIA**

The definition of C-cell hyperplasia is difficult. The lower limit of C-cell hyperplasia and the upper limit of normal C-cell mass are not clear (17,36,148). Various studies show C-cell clusters in adults that, taken alone, could fit into the category of C-cell hyperplasia. Yet O’Toole and colleagues (16) and Gibson and colleagues (149) noted these clusters of C cells at autopsy in apparently endocrinologically normal individuals. Conversely, the lower limit of medullary carcinoma and upper limit of C-cell hyperplasia is difficult to define. DeLellis and Wolfe (150) state that C-cell hyperplasia ranges from diffuse increase in the cells to nodules of C-cells replacing follicles, and once the basement membrane of the follicle is breached, medullary carcinoma should be diagnosed. However, Carney and associates (151) point out it is not always obvious that the basement membrane has been crossed.

In the classic case of C-cell hyperplasia, the lesion appears as multifocal areas of increased numbers of amphophilic large cells replacing follicular epithelium and also replacing follicles completely forming nodules (Figure 21,22). More specific definitions of C-cell hyperplasia include more than 50 C cells per low-power field and more than 40 C cells/cm2 to more than 50 C cells per 3 low-power fields (152,153). With these definitions C-cell hyperplasia is not only seen in patients with MEN syndromes or familial medullary carcinoma (154), but also in patients with hyperparathyroidism, chronic hypercalcemia of other

![Figure 20: Spindle cell morphology in a fine needle aspiration specimen of medullary thyroid carcinoma (Alcohol fixed Papanicolaou stained smear, 60x).](image)

![Figure 21: C-cell hyperplasia in a MEN2B patient; notice the readily identifiable C-cells with clear cytoplasm in para-follicular location (H&E; 20x).](image)

![Figure 22: C-cell hyperplasia in MEN2B patients demonstrating hyperplastic C-cells with clear cytoplasm and overtaking the follicles (H&E; 40x).](image)
causes, Hashimoto disease, in residual thyroid tissue following removal of medullary cancer (sporadic type), and even in thyroid tissue adjacent to non-medullary carcinomas (153,155). In the mid-1990s, the term “Neoplastic” C-cell hyperplasia was introduced to define a lesion precursor to familial MTC vs. reactive or secondary hyperplasia (22,156).

Because C-cell hyperplasia may be associated with so many lesions, the exact significance using these various definitions cannot be determined. One of the best ways to distinguish C-cell hyperplasia is by routine histologic examination. C-cell hyperplasia associated with familial medullary carcinoma and MEN syndromes is readily observed on routine hematoxylin and eosin (H&E) stains (157,158). The cells are often large and show significant nuclear atypia as well as occasional features of medullary carcinoma. On the other hand, secondary C-cell hyperplasia is often only observed by immunohistochemical staining for calcitonin and quantitative analysis (Figure 23). The actual diagnosis of C-cell hyperplasia may therefore be made by routine histologic examination for the presence of C cells by H&E stains. medullary microcarcinoma can be found in glands removed prophylactically because of positive genetic testing for RET mutations (159). These are similar to micropapillary carcinomas, in that, they measure equal to or less than 1.0 cm. In the familial setting there is usually associated C-cell hyperplasia (160).

Interestingly, C-cell lesions including hyperplasia and medullary carcinoma can spontaneously occur in rodent species including mice and rats. It has been shown that a daily injection of Glucagon-like peptide-1 (GLP-1) receptor agonist, used for the treatment of adult onset diabetes raises cAMP in thyroid C-cells with increasing levels of calcitonin and upon long-term exposure leading to C-cell hyperplasia and medullary thyroid carcinoma. It is hypothesized that similar effects of GLP-1 agonists such as exenatide, liraglutide and others can occur in humans. At present there is neither firm evidence in favor of this hypothesis nor evidence strong enough to completely rule out this increased risk of C-cell lesions associated with GLP-1 agonist treatment in type 2 diabetes (161,162).

**MEDULLARY MICROCARCINOMA**

Medullary microcarcinoma is defined as sporadic or familial tumors measuring 1.0 cm or less in thyroid lobes or glands removed for benign nodules or for non-medullary cancer (22,163,164) (Figure 24). These lesions show a rounded or focally infiltrative pattern of growth, may contain amyloid, and are not necessarily associated with C-cell hyperplasia (22). The glands frequently show chronic lymphocytic thyroiditis (165). In the absence of symptomatic hypercalcitoninemia, or lymph node metastases, these lesions are cured by their simple removal (160,165).

**PROGNOSIS, AND RISK STRATIFICATION BASED ON GENETIC TESTING**

From the clinical standpoint, stage is the most important variable for prognosis (73,166-169). A tumor confined to the thyroid without nodal or distant metastases is associated with prolonged survival (73,169). Several workers have found that younger patients (under age 40), especially women, fare somewhat better than the whole group of medullary cancer patients (167,170). Patients who are discovered by screening, because they are members of...
affected families often have very small tumors and can be cured by thyroidectomy (19,171-173). Patients with Sipple syndrome tend to have less aggressive tumors than the sporadic group; whereas, the patients with MEN type IIB have aggressive lesions (24,73,173,174). Pathologic features that have been related to prognosis include tumor pattern, amyloid content, pleomorphism, necrosis, and mitotic activity (20,73,106). Medullary carcinomas with small cell features and those tumors with extensive areas of necrosis, marked cellular pleomorphism, and high mitotic activity are associated with poor prognosis (20,24,106). Encapsulated tumors and tumors with uniform cytology and abundant amyloid tend to be indolent tumors. Schroder and colleagues found that aneuploidy was associated with a poor outcome (175).

The predictive testing for MEN2 based on genetic mutation testing was first developed by Chi and Wells.(176) This was further supported by a study of a cohort of 477 kindred affected by any MEN 2 syndrome, performed by the International RET Consortium (IRC).(177) To date, the RET-mutation testing detects nearly 100% of the carriers and is the standard of care for all first-degree relatives of patients with newly diagnosed MTC. (19,177) However, due to the variable clinical effects of RET-mutations, a risk based classification scheme was needed to guide management of MTC. Various international workgroup and societies have recommended evidence-based guidelines to provide strategies in clinical care of patients with MTC. In 2009, American Thyroid Association (ATA) provided guidelines on the clinical workup and timing of prophylactic thyroidectomy and extent of surgery based on 4-risk levels (A-D) developed by employing the genotype-phenotype correlation (178) (Table II). The ATA-level D consists of most aggressive mutations and carries the highest risk of developing MTC. These mutations, which are typically seen in MEN2B, are associated with the youngest age at disease onset and the highest risk of mortality. ATA-level C mutations (codon 634) are associated with a slightly lower risk, yet the MTC in patients with these mutations is still quite aggressive and may present at an early age. ATA-level A and level B mutations are associated with a lower risk as compared to level C and level D mutation carriers. However, the risk of MTC is still substantially higher as compared to the risk in general population and consideration of risk-reducing thyroidectomy is warranted (178).

Recently, the ATA revised these guidelines and recommended in order to avoid confusion among these four risk categories (24) (Table II). The category D is now named as “Highest Risk (ATA-HST)” and C to “High Risk(ATA-H)” (Table). The categories A and B are combined into a new category designated as “Moderate Risk (ATA-MOD)” . The ATA-HST category includes patients with MEN2B and the RET codon M918T mutation, ATA-H consists of patients with RET Codon C634 mutation, and ATA-MOD includes those with RET codon mutations other that M918T and C634. Based on these ATA-risk categories prophylactic thyroidectomy has been recommended in children who have inherited a mutated RET-allele, as most thyroids in these on histologic examination will demonstrate C-cell hyperplasia or medullary carcinoma. It is recommended that prophylactic thyroidectomy be performed in the first year of life in children who have inherited a mutated RET-allele, as most thyroids in these on histologic examination will demonstrate C-cell hyperplasia or medullary carcinoma. It is recommended that prophylactic thyroidectomy be performed in the first year of life in children in the ATA-HST category i.e. RET codon M918T mutation, and at age 5 or earlier based on determination of serum calcitonin levels in ATA-H category. Children in ATA-MOD category should have physical examination, ultrasound of the neck and serum calcitonin measurements commencing around at age 5 to decide the timing of prophylactic thyroidectomy (24).

### Table II: American Thyroid Association Risk Assessment 2015 / 2009

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<tbody>
<tr>
<td>Moderate / A &amp; B exon 10: 533, 611, 620; exon 11: 631, 666; exon 13: 768, 790; exon 14: 804; exon 16: 912</td>
<td>&lt;3-5 y</td>
<td>&gt;3-5 y</td>
<td>&gt;3-5 y</td>
<td>May delay beyond age 5 if normal 6 month or annual Cal &amp; neck US, indolent MTC history, family preference</td>
</tr>
<tr>
<td>High / C exon 11: 634; exon 15: A883</td>
<td>&lt;3-5 y</td>
<td>&gt;3-5 y</td>
<td>&gt;3-5 y</td>
<td>Consider before age 5</td>
</tr>
<tr>
<td>Highest / D exon 16: 918T</td>
<td>Immediately</td>
<td>Immediately</td>
<td>Immediately</td>
<td>Immediately</td>
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TREATMENT

All patients diagnosed with MTC should undergo total thyroidectomy with central compartment lymphadenectomy. It has been shown that up to 45% of the patients have metastatic MTC deposits in central compartment; and excision of these lymph nodes at first procedure can dramatically decrease the recurrence rates. The lateral neck lymph nodes are usually evaluated preoperatively by ultrasound examination and the metastases can be confirmed by FNAB followed by compartment oriented lymph node dissection (24,41,179-181).

Distant metastases are not uncommon in MTC and can occur to lung, liver, bone and rarely brain and other body sites (182-189). However, despite widespread metastases MTC is a slow-growing tumor, which leads to opportunities for targeted therapy. These therapies are based on the fact that all patients with MEN2A and MEN2B and half of sporadic MTC patients have RET mutations; furthermore 18-80% patients with sporadic MTC have RAS mutations. In addition, VEGF receptors are often overexpressed in tumor cells and the vascular endothelium within the tumor. Recently, several investigators have shown varying successes in phase 1, II and III clinical trials employing several tyrosine kinase inhibitors (190-192).

Vandetanib (Caprelsa) and cabozantinib (Cometriq) are tyrosine kinase inhibitors approved by the U.S. Food and Drug Administration (FDA) for progressive, metastatic medullary thyroid cancer. These agents target various tyrosine kinases including MET, RET, and VEGFR-2 (190-195).

The FDA approval of vandetanib is based on the results of a phase III, double-blind trial that showed a statistically significant improvement in progression-free survival (PFS) when compared to those randomized to placebo. The median progression-free survival was 16.4 months in the placebo arm and at least 22.6 months in the vandetanib arm (196,197).

The approval for cabozantinib was based on the results of a phase III, double-blind trial that showed a statistically significant improvement in progression-free survival (PFS) compared with placebo (11.2 vs 4.0 months; p< 0.0001). Partial responses were observed only among patients in the active treatment arm (27% vs 0%; p< 0.0001), and more patients in the cabozantinib group than in the placebo group were alive and free of disease progression at 1 year (47.3% vs 7.2%). Median duration of response was 14.7 months (24,198,199).

It is important to realize that these treatments are not without significant short-term toxicity and no large cohort data is available on long-term toxicity of these agents. Therefore, a majority of these treatments are only restricted to carefully monitored protocols for patients with significant tumor burden and disease progression (24,191,200-202).

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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